

PHASE 1 REPORT - DEVELOPING HABITAT SCALE DNA MONITORING IN SUPPORT OF POST 2020 BIODIVERSITY REPORTING REQUIREMENTS

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Executive Summary

This report represents Phase 1 of the project 'Developing habitat scale DNA monitoring in support post 2020 biodiversity reporting requirements' funded by the Scottish Government (Reference: NMP/001/20). The overall project aims to investigate and test the applicability of DNA-based approaches for biodiversity assessment and reporting purposes. In this report specific consideration is given to, and set in the context of, national and international biodiversity reporting frameworks.

We undertook a review of the current biodiversity reporting needs focussing on targets and indicators identified by the Convention on Biological Diversity (CBD), UK, and Scotland's Biodiversity Strategy and assessed whether eDNA biomonitoring has the potential to be used to monitor those targets. With particular reference to four habitats in Scotland: marine lochs, freshwater lochs, woodlands, and peatlands; we identified opportunities for the utilisation of eDNA-based biomonitoring approaches for both building species inventories and for broader ecological assessment of habitats. We identify three key approaches for incorporating DNA-based monitoring methods into biodiversity reporting frameworks:

Enhance – DNA is used to inform existing indicators, either as a complement to conventional methods or by using it as the primary source of data

Calibrate – DNA Is used to calibrate/validate existing indicators, particularly those that are used as proxies for biodiversity, or are outputs of pressure-state-response frameworks

Create - new indicators are developed based on DNA data

An initial small-scale pilot sampling campaign was conducted between August – October 2021 to trial some of these DNA-based approaches in conjunction with different sample types and sampling strategies. Along with the pilot sampling campaign, this report will inform Phase 2 of the project.

Introduction

Biodiversity loss is widely recognised as one of the most urgent global challenges to be addressed in the next decade. Bending the curve on biodiversity loss requires action and incentives at all political levels – from local to international – and the participation of all sectors, including governments, businesses, conservation organisations, and research institutions. And innovative technologies are needed to accelerate the pace and scale at which we can gather information about species and ecosystems.

Despite decades of monitoring efforts, large gaps remain in our knowledge of biodiversity and how it responds to different pressures. Critical to solving the global biodiversity crisis is improving the speed, scope, and scale of data collection to better monitor progress relative to actions and interventions to better inform future responses. However, measurement of biodiversity is extremely difficult due to its complexity, diversity, natural variability in space and time, and its interconnectedness to other aspects of the environment. This highlights the significant barriers to integrating nature into global economic systems to account for natural capital as called for in the Dasgupta Review on The Economics of Biodiversity (Dasgupta 2021) and envisioned by the recently launched Taskforce on Nature-Related Financial Disclosure (TNFD). Global frameworks set out top-down targets and indicators, but these do not necessarily translate to practical, standardised on-the-ground measurements, leaving governments to decide which elements of biodiversity to measure.

To date, biodiversity assessment has tended to focus on a limited set of species that are legally protected or are known to be threatened with extinction, and certain taxonomic groups that are easily observed and commonly recorded (e.g. birds, plants, and butterflies).

One of the exceptions is the monitoring of water quality in rivers and streams through biotic indices based on expected and observed communities of several elements (Biological Quality Elements: e.g. macroinvertebrates, diatoms; Birk et al. 2012). This highlights the intrinsic link between biological communities (including smaller, non-charismatic groups) and the ecological health of natural habitats. The development of these biotic indicators for freshwater ecosystems was based on decades of study and observation, together with ecological knowledge about the tolerances of particular species or groups. These indices enable standardised, objective reporting across much of the world on the status of freshwater ecosystems.

As the urgency of the biodiversity crisis becomes ever more evident, there is a need to monitor and report on a far greater range of ecosystems and habitats in a similar way, but we do not have decades left to build up the detailed ecological knowledge required to underpin the development of these new indices. Instead, we must look to new technologies to accelerate the pace of learning and expand the scope of routine biodiversity monitoring across species and ecosystems. One of the new technologies that offers significant promise for scaling up biodiversity assessment is DNA metabarcoding, which uses high-throughput DNA sequencing to rapidly characterise the species diversity present in mixed-species or environmental samples. DNA-based technology shows immense potential for addressing this urgent monitoring challenge. By examining DNA traces left behind in the environment, samples of soil, sediment, water and even air can be used to identify thousands of species from their DNA. DNA-based monitoring can easily be deployed in the field, requiring less specific expertise than conventional techniques, and provides an opportunity to sample at greater geographic and temporal scales. The breadth of taxonomic coverage also allows for efficient monitoring of under-represented groups such as bacteria, fungi, and invertebrates, providing a broad view of the diversity of ecosystems.

DNA-based monitoring generates sufficient data for the application of sophisticated ecological statistics and the use of analytical tools such as machine learning, which can accelerate characterisation of communities in particular habitat types and conditions, and therefore development of new biotic indicators for a wide range of habitats and geographies. This review is primarily focussed on environmental DNA from environmental sample types (water, soil, etc) rather than from bulk tissue samples such as biofilms, but brief reference is made to other sample types and associated taxa throughout.

This report will document the current science overview of using DNA-based monitoring technology to understand how these methods can contribute to supporting post-2020 biodiversity reporting requirements, against global goals and national targets. It will explore the use of DNA-based monitoring methods, putting Scotland at the forefront of globally leading and nationally important biodiversity initiatives, that aim to understand how pressures are affecting habitat condition, ecosystem resilience, and biodiversity function. This report will illustrate how 21st century technology can be used, in the context of national and international biodiversity reporting frameworks, by:

- Examining how DNA-based methods can be used to accelerate and upscale biodiversity and ecosystem condition assessments in the context of feeding into the Scottish Biodiversity Strategy and the Global Biodiversity Framework set out by the United Nations Convention on Biological Diversity (CBD).
- Designing and implementing a sampling programme in which to trial the application of DNA-based assessment at a landscape scale in four key Scottish habitats.
- Scoping the challenges, opportunities, and timeframe considerations for implementation of habitat-scale DNA-based monitoring approaches given the stage and pace of technological advancement and research requirements.

This project is split in two distinct phases: Phase 1, which includes the present report, describing how DNA can be used to support biodiversity monitoring, and a sampling plan for implementation in Phase 2 of the project. Phase 2 will involve a field study and delivery of project outputs that will, for example, include recommendations for consideration by the Scottish Government and its collective organisations to inform the integration of DNA-based approaches into national plans for biodiversity monitoring and reporting in the future.



The biodiversity monitoring challenge

The global target to protect 30% of the planet for nature by 2030 (known as '30x30') is included in the post-2020 Global Biodiversity Framework. Countries are expected to contribute to this global goal through domestic action to increase coverage of effectively managed protected areas. The Scottish Government 2020 Statement of Intent on Biodiversity outlined the commitment to the 30x30 target. Additionally, the 2021 Programme for Government committed to the deployment of Nature Networks. These two programmes are key components in increasing ecological connectivity and restoration of nature more widely, helping to deliver the Scottish Biodiversity Strategy.

In 2022, a new Global Biodiversity Framework is expected to be agreed on in Kunming under the Convention on Biological Diversity (CBD) supported by monitoring to measure progress towards the goals and targets. Whilst molecular methods are not featured specifically for biodiversity monitoring within the plan for the proposed high-level indicators, there is scope for countries to incorporate the approach into their own National Biodiversity Strategy and Action Plans to deliver on-the-ground species information at scale across the tree of life. However, there are still only a handful of DNA-based monitoring tools that are widely accepted and routinely used in standardised programmes by practitioners. The few that are considered to be the most robust are narrow in their scope, usually focussing on one or a few indicator species.

The Scottish Government has committed to reviewing their approaches for monitoring, protecting, and enhancing biodiversity to ensure their actions drive the transformative change needed to halt and reverse the decline of biodiversity. Scotland has recognised the need to move beyond simple measurements of biodiversity, such as habitat and numbers of charismatic species, to those that describe ecosystem function and resilience. DNA-based monitoring shows great potential for revolutionising national monitoring schemes and could be integrated into the Scottish Biodiversity Strategy, providing the evidence required to deliver on CBD commitments.

The future of DNA-based monitoring

DNA-based technologies are constantly evolving and improving and the tools available today will continue to be refined. Technological advancements will happen at all stages of the DNA workflow, including sampling, laboratory processes, bioinformatics, and data analytics. These advancements will further increase the application of DNA-based methods by public and private sectors concerned with biomonitoring and reporting.

Innovation in sampling techniques is an area of rapid progress, with near future methods to include in-field DNA extraction, passive and automated sampling techniques, and eDNA sampling from air. Other laboratory developments include environmental metagenomics, in which whole genomes are sequenced from mixed samples; RNA approaches (known as metatranscriptomics), where active species and genes can be monitored in an environment



(for example, measuring transcriptional responses to stressors such as pH or toxic pollutants); and DNA capture, where DNA 'baits' are used to capture the sequences in mixed samples, allowing for several genes to be targeted in the same analyses. New sequencing technologies will also play a pivotal role in enabling longer DNA sequence reads and increased data acquisition per sample, enabling more robust data at larger scales.

Advances in bioinformatics and subsequent data analysis will be highly relevant to the development of future environmental health metrics. The compilation of more complete reference databases is deemed a priority among the community of academic, government, and private sector users of eDNA monitoring. However, the sensitivity of the analyses and breadth of data capture mean that taxonomy-free approaches are within reach for widespread application. For example, machine learning can be used to predict biotic indices from eDNA metabarcoding data, without requiring taxonomic assignment of the DNA sequences (corr et al. 2018). The major benefit of taxonomy-free approaches is that they overcome the issue of incomplete reference collections and make full use of the available data complexity to objectively characterise healthy and depleted ecosystems. Importantly, DNA-based approaches allow the creation of 'bio banks' of genetic material, that if stored correctly, can be revisited and reanalysed using new techniques in future.

Integrating DNA-based monitoring into indicator frameworks

Existing monitoring methods have played a vital role in the development of current indicators, but technological advances now provide an opportunity to review and improve upon existing frameworks. Establishing where DNA-based approaches can add most value is particularly important to consider given that many currently used indicators are based on multiple decades of data on species status collected using traditional methods and eDNA data sets are yet not available in majority cases.

DNA-based monitoring demonstrates two key strengths relevant to integration into indicator frameworks. Firstly, the collection of data at scale – facilitated by the simple, standardised field protocols – and secondly, through the combination of DNA data with Earth Observation data as well as other data and indices. The framework for incorporating DNA-based methods into national and international reporting is based on three broad themes which mobilise these two strengths of DNA-based approaches. These are:

Enhance – using DNA-derived data to inform existing monitoring and indices.

Calibrate - using DNA approaches to validate existing metrics, for example, by using eDNA methods to parametrize pressure-state-response models or proxy metrics.

Create - using DNA-derived data to build new indicators, for example, to measure community condition, function, and resilience.

Opportunities for DNA-based habitat monitoring in Scotland

Through this project, we aim to meet the needs of the Scottish Government and its collective organisations to improve understanding of how advances in DNA-based monitoring technology and novel approaches can be applied for biodiversity assessment purposes. This report highlights the short, medium, and long-term opportunities for using DNA-based approaches to monitor biodiversity and to support post-2020 biodiversity reporting requirements. We outline opportunities to enhance existing data collection methods, calibrate existing metrics or create new metrics using DNA-based methods.

Critical to the success of adopting new technologies, will be the ability to pilot and create an evidence base using available tools, whilst also maintaining an awareness of developing opportunities. As part of monitoring ecosystem health, we identify four general areas for development, which will be considered for inclusion in Phase 2 of this project:

- The use of DNA-based monitoring to create reference-based models for assigning metabarcoding samples to an ecological category
- The combination of DNA-based data with Earth Observation data to scale biodiversity data across large areas
- The creation of experimental terrestrial sampling strategies
- The creation and adaptation of taxonomy-based functional and resilience-based metrics

We outline some key application opportunities across four focal habitats (marine, freshwater, woodland, and peatland) relevant to Scotland. Where possible, we provide indicative sampling requirements and outline the initial programmes that could be undertaken to progress these developments.

Many of the opportunities using DNA-based monitoring are firmly within reach; however, to scale from site-based monitoring to national and global reporting, a critical assessment and prioritisation exercise will be required based on the sensitivity of the approach, scalability, future-proofing in the face of technological advancement, and research and development needs and resources.

DNA-based tools can enhance our ability to access more sensitive data at greater scale. The scale of data collected by DNA-based approaches further facilitates more powerful analytical approaches that will in turn lead to more accurate and informative metrics for assessing progress against targets and informing adaptive management.

Whilst continued research and technological development will always be needed to advance DNA-based approaches, a significant suite of tools and methods are already validated and ready to implement today. As a new and emerging field, concerns that exist surrounding the



adoption of novel DNA-based approaches will be resolved as tried and tested solutions emerge. The growing body of evidence from numerous studies and practical applications demonstrates the wealth of data and analysis potential that this form of biomonitoring can generate. This report synthesises this information and highlights the opportunities for incorporating DNA data into biomonitoring frameworks at all geographic scales.

Phase 1 objectives

Phase 1 of the project, had the following objectives:

- 1. Undertake a desk-based review to understand how DNA-based methods can contribute to reporting against global goals and targets. This is delivered through:
 - A review of DNA-based approaches for biodiversity monitoring, appraising ongoing developments, emerging challenges, and opportunities.
 - An overview of current goals, targets, and metrics for biodiversity and ecosystem health, both at the international level (i.e. the Global Biodiversity Framework established by the CBD) and at the national level (i.e. the Scottish Biodiversity Strategy).
 - An outline of opportunities for integrating DNA-derived data into biodiversity reporting, considering both the possible integration with current monitoring methods and the development of new DNA-based metrics. This is discussed in both the global and the Scottish contexts.
- 2. Develop a sampling plan for Phase 2, in consultation with the Project Management and Technical Steering Groups and the Project Advisory Board. This will be delivered in a separate document, which will include results from the initial pilot study carried out in 2021 and will contain the Phase 2 sampling plan design including rationale for the work undertaken in 2022.

The effective delivery of Phase 1 of this project required consultation with a broad collective of people and organisations representing the research community, scientific/technical advisors and policy leads, practitioners, and other key stakeholders. Therefore, stakeholder engagement was initiated in the early stages of the project and the considerations drawn in this report include input from the project management and technical steering groups, advisory board, and other organisations (see Appendix 1 for details on stakeholder engagement activities).

A glossary of terms is provided at the end of the report to define key terms used within the report.

DNA-based approaches to biodiversity monitoring

DNA-based monitoring technology has been developing rapidly and shows great potential for addressing the urgent biodiversity monitoring challenge through its use in decision-making, guiding action and informing transformative change.

Section highlights:

- 1. DNA-based methods are efficient in detecting species and generating species inventories
- 2. DNA-based methods are already being adopted by both government and nongovernment agencies, albeit currently with limited scope
- 3. A considerable body of research, mainly freshwater, has focused on using DNA-based data for determining biotic indices
- 4. Big-data analytics, such as machine learning, can be applied to DNA-based data to create a new generation of metrics that can be used across a wide range of habitats and geographies
- 5. Other methodological advancements such as metagenomics and metatranscriptomics, will continue to push forward the application of DNA-based methods in biodiversity monitoring

Biodiversity monitoring

Biodiversity monitoring underpins decision-making from the local to international scale and is crucial for effective delivery of interventions. However, given the complexity and variability of biodiversity, large amounts of data are needed to accurately monitor change at the global level (POSTNote 644, Effective Biodiversity Indicators, 2021), and this poses a significant challenge.

Most available data relate to changes in abundance or distribution of specific populations or species, which reflects the most common types of biodiversity indicators (see Box 1, Eaton et al., 2021; POSTNote 644, Effective Biodiversity Indicators, 2021). Indicators based on broader communities of species rely to a large extent on surveys conducted by a combination of targeted, site-based monitoring programmes and volunteer monitoring schemes or records submitted on an ad-hoc basis, meaning that data are often patchy in distribution and of variable quality. However, this opportunistic / ad-hoc approach towards monitoring has encouraged the development of computational data handling and statistical capabilities that enable the spatial and temporal patchiness of data to be accounted for (Freeman et al., 2021; Isaac et al., 2014). New technologies are also key to increasing the accessibility of wildlife surveys (allowing for a wider audience of people, including citizens, to contribute to wildlife

surveys) and the frequency and accuracy of recording. This includes tools like DNA analysis as well as wildlife recording and identification apps (e.g. iNaturalist).

Another challenge is that existing surveys tend to be heavily weighted towards charismatic and easily identifiable species groups, such as birds, terrestrial plants, mammals and butterflies (Burns et al., 2018). Information on invertebrate and microbial communities is not so readily available despite the widely recognised power of these groups to indicate ecosystem condition and their direct links to ecosystem services such as soil health, water quality and pollination (Norris, 2012). The underrepresentation of these groups is largely due to the challenge of morphological identification, which is unfeasible to achieve at scale for organisms that are hyper-diverse, poorly described, and of small body size. However, integration of these groups into routine monitoring programmes is now possible using DNA-based methods such as metabarcoding.

DNA-based monitoring relies on the analyses of DNA sequences obtained directly from organisms or from the environment (e.g. water, soil, air, faeces, other species traces). It enables characterization of the biological diversity of an area and informs on the occurrence (and in some cases abundance) of species, their interactions, and functions within ecosystems. DNA-based technologies and methods have been developing at a fast pace, from targeted species approaches to community assessments of biodiversity. Below we describe the most relevant DNA-based methods for biodiversity monitoring, the ongoing challenges, and emerging opportunities in this field.

Species inventories and detections

DNA-based methods for detecting species and generating species inventories are already being widely adopted by both government and non-government agencies. Broadly, there are two principal methodologies used:

- Targeted methods, such as real-time quantitative PCR (qPCR), are used to screen samples for the presence of DNA from particular species. This is a fast and sensitive analysis approach that is commonly used in surveys of species of conservation concern or those that represent a threat to native ecosystems (e.g. invasive non-native species). Hundreds of qPCR primer sets have been published, targeting a wide range of species, and many are available commercially. Recent work linked to the EU Cost Action project DNAquaNet (CA15219) introduced a framework for assessing the level of validation and readiness for use of published primer sets (Thalinger et al., 2021), in an important step towards robust operational use of these tests for environmental management. Natural England also recently commissioned a report and associated tool (COASTER) to facilitate standardised reporting and provide a framework for assessing confidence in results generated from eDNA-based qPCR assays (K. J. Harper et al., 2021).
- DNA metabarcoding and allied methods enable simultaneous identification of many different species (often hundreds per sample) using high-throughput DNA sequencing (Yu et al., 2012). Metabarcoding primers have been designed to target a wide range of



taxonomic groups, which can be geared towards different levels, ranging from very broad groups at the domain level (e.g. eukaryotes or prokaryotes) to narrower groups at order level or below (e.g. bony fish, unionid mussels). Metabarcoding primers can even be designed to target a single species and examine intraspecific genetic diversity (Tsuji et al., 2020). An inherent trade-off in the selection of metabarcoding primers is that as the taxonomic breadth increases, it's sensitivity to particular species may be reduced, while low abundance species may be missed. This can be compensated for by increasing the sequencing depth, but it will often be preferable to combine multiple primer sets to survey a broader cross-section of biodiversity and increase species resolution.

Either of these approaches can be applied to different types of samples, including aquatic eDNA, sediments, faeces or stomach contents and mixed invertebrate collections, with most DNA extraction protocols yielding enough DNA for multiple analyses on each sample. Extracted DNA can also be archived for long-term storage, enabling analysis at a future date if additional information or independent verification is sought.

A substantial body of research literature now demonstrates that DNA-based methods can match or outperform conventional survey methods for many species and groups. These DNA-based methods often bring advantages in terms of cost and survey effort, increased detection sensitivity, and increased taxonomic resolution (Polanco Fernández et al., 2021). The greatest body of research exists for bony fish in freshwater environments (Hänfling et al., 2016; Lawson Handley et al., 2019; McColl-Gausden et al., 2020; McDevitt et al., 2019; Olds et al., 2016; Rourke et al., 2022; Xing et al., 2022), driven in part by work funded by the Environment Agency (EA) and Scottish Environment Protection Agency (SEPA) on lake fish communities.

DNA-based methods have many advantages, including the ability to provide broader taxonomic coverage and a wider view of biodiversity, being life-stage independent (many species cannot be morphologically identified in their juvenile forms) and able to detect species that may be difficult to observe due to being shy, nocturnal, small in body size or otherwise elusive.

Nonetheless, as with any survey method, there are important aspects to consider when using DNA-based monitoring methods. These include:

- The need for a good study design, from effective sampling and capture of DNA to marker and primer selection (adjusted to the target species/group of interest and able to resolve taxonomy within) to validated laboratory and bioinformatic workflows.
- The importance of comprehensive DNA reference databases. The incompleteness or unreliability of reference databases limits the ability to name taxa at the species level, particularly for non-vertebrate groups. This is also a limitation for the design and rigorous testing of primers to ensure specificity to the target. Over time these databases will improve and while it is not likely that there will ever exist a database that contains DNA references for the entirety of all taxa across the globe, barcoding and genome

assembly initiatives are being conducted at the regional, national and global levels (e.g. <u>https://ibol.org/programs/bioscan/; https://www.darwintreeoflife.org/</u>) that will greatly improve the power of metabarcoding surveys. An advantage of DNA-based monitoring is that species names can be added retrospectively to past datasets as reference databases grow. Furthermore, there are already alternative approaches that are based on taxonomy-free analyses of DNA data for biodiversity monitoring (see below).

- Issues with determining species abundance. Individual organisms contribute varying amounts of DNA due to differences in size, behaviour, body composition etc., making it difficult to estimate number of individuals without controlling for these factors, which is not always possible in natural settings. Differences in shedding and decay rates have also been noted between taxa associated with different temperature regimes (Andruszkiewicz Allan et al., 2021). Although with targeted approaches, such as qPCR, a good relationship of abundance with DNA concentration has been found in laboratory conditions, this relationship is weaker in natural settings (Yates et al., 2019). In the case of metabarcoding workflows, this is further compounded by biases introduced during the PCR amplification process, where some species' DNA will amplify more efficiently than others. This particularly affects primer sets that target broader taxonomic groups and that often contain IUPAC ambiguity codes representing several possible bases. For certain groups, accurately estimating abundances may also depend on the barcode used. Diatom species, for example, may differ in the number of *rbcL* gene (chloroplast) copies per cell. This could be due to the difference in the number of gene copies per chloroplast and/or the number of chloroplasts per cell. It is also strongly correlated to biovolume of cells. Accordingly, if sequence reads are to be compared to valve counts and abundances, a correction factor should be applied (Pérez-Burillo et al., 2020). Nonetheless, many fish eDNA metabarcoding studies have shown a strong correlation between sequence read counts and known relative abundance (Di Muri et al., 2020; Li et al., 2019), while assessment of occupancy within a landscape can give a strong indication of how common a species is (if sampling design allows).
- Movement of DNA within both freshwater and marine aquatic environments can lead to spatial and temporal uncertainty with regards to when and where the detected eDNA originated. However, in the marine aquatic environment it has been shown that due to its ephemeral nature (i.e. it breaks down fast), eDNA analysis is capable of capturing fine-scale local and temporal variation, representing the communities in the immediate local habitat where a sample was collected, both on horizontal and vertical planes (Djurhuus et al., 2020; G. Jeunen et al., 2019; G. J. Jeunen et al., 2019; Port et al., 2016a; Yamamoto et al., 2017), and on short time scales (Ely et al., 2021; Murakami et al., 2019). This is also the case in dynamic coastal and pelagic environments (Jensen et al., 2022; G. Jeunen et al., 2020; G. J. Jeunen et al., 2019; Monuki et al., 2021; West et al., 2020) where even tidal and oceanic movements have

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shown to have minimal effect on the detected communities (Kelly, Gallego, & Jacobs-Palmer, 2018; Lafferty et al., 2021; Larson et al., 2022; West et al., 2020),

- In freshwater environments, modelling approaches incorporating hydrology data for the landscape can be used to account for this movement of DNA (Carraro et al., 2020, 2021). eDNA is also not evenly distributed on the vertical plane of the water column, but is usually concentrated at depths where the species occurs (Canals et al., 2021). This means that eDNA analyses can reveal ecological insights about the communities at different depths, but it also means that water needs to be collected from multiple depths for comprehensive surveys of biodiversity (Pont et al., 2021a). This is especially important where waters are stratified since the stratification acts as a barrier to vertical mixing (G. Jeunen et al., 2020).
- Long-term persistence of DNA in soils and sediments may lead to temporal uncertainty (e.g. Yoccoz, 2012). DNA is continuously released from dead cells, and extra-cellular DNA can bind to substrate compounds through adsorption to organic particles, which means that the detection of the DNA of a species does not imply that the species is living or active in the surveyed habitat. There are situations in which this is important, namely for invasive species or pathogens, or to determine ecosystem function (microorganisms relevant to ecosystem function may be detected but not actually be contributing to current functionality). This can be accounted for by targeting RNA instead of DNA, as RNA degrades very quickly with a very short persistence time in the substrate (Carini et al., 2016; Knapik et al., 2020; Kunadiya et al., 2021). However, this is significantly more costly and logistically challenging since the preservation of RNA requires freezing at -80°C or specific preservation solutions. Moreover, research conducted to date has not clearly shown the benefit of this approach over DNA analysis (Laroche et al., 2016, 2018). In general, it is likely that DNA from dead organisms is less detectable than the majority of living organisms. In aquatic samples, apart from a DNA signal from fish or invertebrate die-offs (which for example can happen as a result of harmful algal blooms, but these are a temporary problem that can be mitigated by repeat sampling), the occasional dead individuals have not been reported to cause/are considered to be a problem. However, in marine sediment, due to the binding of DNA to particulate matter, the temporal window of a (surface) sediment samples is larger than that of a water sample (Kuwae et al., 2020; Turner et al., 2015).

Metabarcoding for ecological assessment of habitats

DNA metabarcoding generates data at a taxonomic scale that has never previously been feasible, and this opens the door to a more holistic view of biodiversity, considering many different biological components of ecosystems, including the small and highly diverse organisms (e.g. insects, soil fauna, plankton, fungi, bacteria) that are often closely linked to ecological functions (Schadewell & Adams, 2021; Seymour et al., 2021). This wide view of biodiversity allows information to be drawn on species interactions (both from biodiversity)

surveys and trophic niche assessments) and ecosystem services (e.g. pollination), fundamental for understanding ecosystem resilience to environmental pressures (Bush et al., 2020).

Although incomplete reference databases for some groups (e.g. soil fauna, fungi, bacteria etc) can limit species level identification, many forms of ecological community analysis do not rely on species names. Instead, they consider overall patterns of alpha and beta diversity within and between habitats. This enables habitats subject to management, conservation or restoration interventions to be compared with reference habitats in good condition to determine whether the biological community of the conservation or restoration area is becoming more similar to that at the reference site (Ji et al., 2013).

Several analyses can be performed without reliance on species names, where the units of analyses are Operational Taxonomic Units (OTUs, groups of closely related sequences), or ASVs (Amplicon Sequence Variants). The intent of using OTUs is that each OTU corresponds to a species. The OTU approach limits PCR and sequencing errors by clustering highly similar sequences. In contrast, ASVs keep each unique sequence separate but filter out potential PCR and sequencing errors based on error models. While overall ecological patterns derived from metabarcoding data tend to be fairly robust to the choice of approach, ASVs are more reproducible and can make comparison across datasets easier.

Analyses that can be performed using taxonomy-free approaches include:

- Calculation of classic alpha diversity metrics, such as Simpson and Shannon diversity, that summarise OTU/ASV richness and evenness (e.g. Joos et al., 2020).
- Estimation of total OTU/ASV richness per habitat based on extrapolation from accumulation curves (e.g. Darling et al., 2020).
- Assessment of turnover (or nestedness) through analyses such as multivariate analysis of variance. These patterns can be visualised through ordination methods, for example, non-metric multidimensional scaling (e.g. Sepp et al., 2021).
- De-novo identification of indicator taxa that are strongly associated with habitat types or condition levels. Those that cannot be identified to species level can nonetheless be employed as indicators based on sequence identity (e.g. Chariton et al., 2015).
- Assessment of phylogenetic diversity contained within a habitat (e.g. Lejzerowicz et al., 2021).
- Joint species distribution modelling to identify positive and negative associations among taxa. This enables the likely presence of key species to be inferred from the wider community even when the species itself is not observed (e.g. Wirta et al., 2021).

Higher-level taxonomic assignment (e.g. to class, order, or family) allows analyses to be applied separately to different taxonomic groups within the same dataset, which will highlight if, for example, a particular group is responding less well than others to an intervention (e.g. L. R. Harper et al., 2021a). Thus, routine access to replicable, high-resolution biodiversity data



across multiple taxonomic groups facilitates adaptive management, where interventions are routinely evaluated and adjusted based on observed responses to optimise outcomes.

Combining metabarcoding and machine learning for ecosystem condition assessment

The large volume of data generated by DNA metabarcoding also provides the opportunity to apply big-data analytics, such as machine learning, to create a new generation of metrics that can be used consistently across a wider range of habitat types (e.g. ponds, rivers, oceans, soils) and geographies – these can be based on both taxonomy dependent and taxonomy-free approaches. Box 1 provides details on the types of machine learning algorithms.

Box 1: Two main categories of machine learning algorithms.

Unsupervised machine learning

Unsupervised machine learning does not use labelled data, so there is no target variable that guides the algorithm (Khanum et al. 2015). Instead, clustering algorithms (e.g. ordination, factor analysis, PCA, k-means) are employed to assign the samples into natural groups where labelling is not available or desirable. These techniques are often used prior to model training with SML to reduce the number of OTU features by capturing the variance of all OTUs in several principal components or factors (Popovic et al. 2019). The newly generated components can be then used as features in ML training, having the potential to improve model training time and performance.

Supervised machine learning

Supervised machine learning uses feature data (e.g. operational taxonomic unit - OTU) labelled with the target variable of interest (e.g. habitat class, biotic index score). The algorithm is guided by the label assigned by a human or some other labelling approach (Nateski 2017). There are multiple types of SML algorithms, including Random Forest, Gradient Boosting Machine, Support Vector Machine, and others.

Supervised machine learning (SML) models can be applied to metabarcoding data to characterise the typical biological community in healthy ecosystems (based on sequence diversity), and the predictable way in which communities change as the habitat becomes degraded or exposed to stressors. A model is trained on a portion of the data and then tested on the remainder of the dataset to assess how accurately it assigns each of the test samples to predefined habitat classes (e.g. Chariton et al., 2015).

This is conceptually similar to the widely employed biotic indices for water quality monitoring (e.g. RIVPACS) in that they compare an observed community to the expected community in ideal 'reference' conditions. This is a natural extension from the site-based ecological analyses discussed above and can similarly be performed on OTU and ASV data in the absence of species names. Taxonomy-free ML based on metabarcoding data can include a wide range of organisms, making the indices more sensitive (Aylagas et al., 2021) and enabling their extension to a far wider range of habitats. An advantage of the DNA-based approach is that multiple primer sets targeting different portions of the biome can be trialled to test which primer, or combination of primer sets, has the greatest predictive power.

Building these models requires extensive baseline sampling to capture the full range of natural variation and the extent of condition gradients within each habitat class, and this needs to be coupled with extensive environmental metadata.

In recent years, multiple scientific studies have compared the performance of metabarcoding and SML-based habitat classification with that of more established biotic indicators based on taxonomic identity of a predefined set of taxa. These studies demonstrate the power of using new technologies to adopt a more holistic approach to ecosystem assessment, and a detailed overview of the approach and its potential to revolutionise biomonitoring is provided by Cordier et al. (2019). Studies have largely focused on aquatic habitats, including marine sediments (Cordier et al., 2017, 2018; Frühe et al., 2020), coastal waters (DiBattista et al., 2020), and rivers (Fan et al., 2020; Feio et al., 2020). Taxonomy-free joint species distribution modelling approaches can be used to derive an ecological quality ratio (EQR) from the comparison between observed communities under reference conditions and expected (predicted) communities as the ecosystem is exposed to environmental stressors. This method, using various algorithms, has been previously used to assess ecosystem health in rivers using diatom communities (Feio et al., 2020). A review of use case examples and limitations to the approach is provided in the Appendix 2.

Additional perspectives and opportunities

Assessment of genetic diversity within species

A natural extension of DNA-based biodiversity assessment at species level is to assess withinspecies genetic diversity from mixed-species or environmental samples, and this represents an active field of current research. Within-species genetic diversity can be used as a measure of adaptive capacity¹, an important component of ecological resilience to threats and stressors (e.g. climatic stress: Wernberg et al., 2018). Genetic variability within populations or species facilitates higher adaptability, since it allows a wider range of responses to external factors or disturbances.

Methods for assessing genetic diversity within species or populations are well-established and usually rely on the analyses of sets of multiple markers spread across the genome, namely microsatellite markers or single nucleotide polymorphisms (SNPs), from DNA samples collected from individual organisms, which is both expensive and time consuming. Microsatellites and SNPs target multiple hypervariable points across the genome of the species to assess allelic diversity within a population. As the cost of DNA sequencing decreases, genomic approaches are gaining more prominence in this field (e.g. Allendorf, 2017; Benestan et al., 2016; Shafer et al., 2015) but typically use genome-wide analysis rather than individual gene regions of just a few hundred bases that are targeted in the metabarcoding approach.

Building on the concept of species identification from DNA barcoding (Hebert et al., 2003), metabarcoding is an approach that has been developed primarily to characterise diversity at

¹ Adaptive capacity can be defined as a measure of the ability of a system to adapt and change, while maintaining critical functions and processes, to new environmental conditions (Angeler & Allen, 2016).



species level. Thus, the gene regions targeted have typically been chosen based on their variation at the species level, with minimal intraspecific variation.

Nonetheless, several metabarcoding studies have been able to identify different sequence variants that correlate with known haplotype diversity (Elbrecht et al., 2018; Shum & Palumbi, 2021; Zizka et al., 2020). However, caution should be applied in interpreting sequence variants in terms of population genetic diversity since artefactual variants could also arise from a myriad of factors, including:

- Copy errors that occur during PCR amplification. The prevalence of these errors is likely to be linked to the choice of polymerase used in the PCR reaction. The use of Unique Molecular Identifier (UMI; Kivioja et al., 2012) sequencing labels seems promising for enabling these errors to be bioinformatically recognised and filtered out, but is not yet routine practice in metabarcoding studies.
- Sequencing errors that arise during base-calling. Variants that arise from sequencing
 errors are expected to occur in very low read numbers, so can be filtered out by setting
 a minimum threshold for OTU/ASV acceptance (based on a minimum number or
 proportion of reads) but this also risks discarding true detections of rare species or
 haplotypes.
- Nuclear pseudogenes (NUMTs), which occur when portions of the mitochondrial genome (where most metabarcoding markers lie) have been inserted into the nuclear genome (Hazkani-Covo et al., 2010) where they mutate independently and may still be amplified by metabarcoding primers targeting the mitochondrial gene region. The prevalence of NUMTs for particular gene regions varies among species; for instance, the meadow grasshopper *Chorthippus paralellus* is characterised by NUMTs in the COI gene (R. J. Pereira et al., 2021), so COI metabarcoding usually returns large numbers of sequence variants for this species when it is present in a sample. These variants are unrelated to population genetic diversity and often originate from the same individual. Bioinformatics packages such as Numt Dumper (Andújar et al., 2020) help to reduce the effects of NUMTs in metabarcoding datasets but are not yet consistently employed.

Thus, estimating genetic diversity across multiple species from environmental samples would provide valuable insights and is likely to be possible to some extent, but it requires significant additional research before it can be reliably applied for operational use. It is also likely to require the design of new primers targeting gene regions that exhibit within-species variation for the target taxa (Tsuji et al., 2020), rather than those commonly used for species-level biodiversity assessment.

Reconstructing past ecosystems using ancient DNA

Analysis of ancient DNA from sediment cores enables analysis of historical time series data to gain an understanding of past environments and the impact of factors such as introduced species (Ficetola et al., 2018), climate change, eutrophication (Ibrahim et al., 2021; Monchamp



et al., 2017), and extreme events such as tsunamis (Szczuciński et al., 2016). This field of research is reviewed in detail by Bálint et al. (2018).

Ancient DNA analysis requires specialist laboratory facilities and processes to guard against contamination from contemporary DNA sources. The target DNA is present in such low concentrations that it is exceptionally vulnerable to contamination (Anderung et al., 2008).

Other methodological advancements

DNA-based methods are continuously being subjected to extensive research for improving accuracy and sensitivity which results in the development of new methods. For species detection, for instance, a new method based on CRISPR-Cas technology (a genome editing tool) combined with recombinase polymerase amplification, has been tested with good results (Williams et al., 2021a). This is a species-specific assay based on isothermal reactions, a type of reaction that is not likely to be subject to inhibition compared to qPCR approaches.

Technological advancements and bioinformatic developments are also improving high throughput long read sequencing. Obtaining longer sequences from environmental samples, will contribute to increased taxonomic resolution; however, the sequencing depth needed may impact species detection in highly diverse samples, and result in higher sequencing costs. Nevertheless, long read sequencing is expected to have a positive impact on the generation and completion of DNA reference databases that will overall contribute to improved taxonomic assignment in metabarcoding studies (Environment Agency, 2021).

Other DNA-based methods relevant for biodiversity monitoring include metagenomics, DNA capture and environmental RNA analyses.

Metagenomics is the sequencing of the full genome of every taxon in a mixed sample. It may also target smaller regions of the genome, usually organelles (e.g. mitochondria) which are present in higher copy numbers than the nuclear genome (e.g. Crampton-Platt et al., 2016). It is usually a PCR-free technique, which implies that there is no primer bias, resulting in a more representative outcome than PCR-based approaches. In addition to providing information on species presence, it allows robust data on metabolic function to be obtained and also quantification of population sizes (Ji et al., 2020). However, this approach requires high sequencing depth, and it may miss some less abundant taxa in highly diverse samples (Environment Agency, 2021). Though this is a very promising approach for future biodiversity monitoring, further developments on bioinformatic approaches and on sequencing capacity to reduce costs are still needed.

DNA capture approaches target specific regions of the genome, or even entire organelles, by using a 'DNA bait.' Though it has the advantage of being a PCR-free approach (overcoming PCR errors), it is dependent upon the availability of reference databases for bait construction, which are still incomplete for highly diverse taxa. Nevertheless, DNA capture has already been successfully applied to some taxonomic groups (e.g. Gauthier et al., 2020).



Environmental RNA analyses provide the opportunity to overcome some issues, namely in determining species abundance. In fact, recent studies have shown a better correlation between environmental RNA and species abundance than that observed for eDNA (Miyata et al., 2021). Moreover, Tsuri et al. (2021) showcased that it is possible to detect specific messenger RNA, from different tissues, from environmental samples. This shows that there is the opportunity for further developments in environmental RNA analysis for assessing not only presence and abundance but also for determining stress, metabolic function and other demographic aspects (Deiner et al., 2021). Furthermore, as RNA degrades more quickly in environmental samples, targeting RNA would confirm the presence of live organisms, which is important for assessing current ecosystem function and condition.

Automated sampling and in-situ data collection

The ability to deploy automated eDNA samplers and to process samples in the field are emerging opportunities that will accelerate the application of DNA-based methods and facilitate scaling from local to global levels.

Automated eDNA samplers are already being tested in marine environments, either using remotely operated vehicles (Everett & Park, 2018) or by coupling an environmental sample processor to an autonomous underwater vehicle (Yamahara et al., 2019).

Mobile laboratory technologies already exist, allowing for eDNA samples to be processed in the field, from extraction to PCR (e.g. minipcr, <u>http://www.minipcr.com/</u>), to sequencing (Oxford Nanopore MinION, <u>https://nanoporetech.com/products/minion</u>). Recent studies are already testing these technologies in field with promising results (e.g. Krehenwinkel et al., 2019). However, further developments are needed to improve efficiency and adjust these mobile technologies to regulatory standards for widespread application (Environment Agency, 2021).

Convention on Biological Diversity and Global Biodiversity Reporting

To understand how DNA-based methods may be used for global biodiversity reporting, we review current goals, targets and monitoring frameworks of the CBD and other global initiatives.

Section highlights:

- 1. A monitoring framework is being developed to measure progress towards the goals and targets set in the post-2020 Global Biodiversity Framework
- 2. A set of metrics has been proposed for reporting against the Global Biodiversity Framework, but these may lack the granularity to inform management decisions at the country level
- 3. Scotland has been developing metrics that move beyond simple measures to those that tackle ecosystem health, but taxonomic and conceptual gaps remain
- 4. An opportunity exists to leverage DNA-based monitoring approaches to help measure ecosystem condition, function, and resilience

The CBD is the international legal framework that governs how signatories achieve "the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources"². First signed in 1992, the CBD is about to enter its fourth decade, and in 2022 (delayed due to the Coronavirus pandemic), a new 'post-2020' Global Biodiversity Framework will be negotiated at the Conference of the Parties (COP-15) in Kunming, China. The new framework will replace the Aichi Targets (2010-2020), which signatories largely failed to achieve (Butchart et al., 2016). It is hoped that the new goals and targets will consider the urgent need for transformative change and define a new level of ambition for signatories (Secretariat of the Convention on Biological Diversity, 2020).

The post-2020 Global Biodiversity Framework

Whilst final wording will be agreed in Kunming, the draft post-2020 framework has already been published. The framework aims to drive action towards stabilizing biodiversity loss by 2030 and stimulate the recovery of ecosystems over the following 20 years to achieve the 2050 'Vision for Biodiversity'.

² https://www.cbd.int/intro/



The framework is built around a theory of change (Figure 1) whereby urgent policy at the global, national, and regional scales affects change over the economic, social, and financial drivers that have accelerated biodiversity loss. The theory of change outlines a stabilizing period to 2030 and a recovery period to 2050 to reach net improvement. For change to occur, tools and solutions are required for implementation and mainstreaming, reducing threats to biodiversity, and ensuring sustainable use of biodiversity.

The draft framework has four long-term goals (Figure 2), together with ten milestones, used to assess progress. The framework also includes 21 action-oriented targets for 2030 (Appendix 3, Table 3.1), designed to (upon achievement) contribute to the 2030 milestones and to the fulfilment of the goals for 2050 (First Draft of the Post-2020 Global Biodiversity Framework, 2021).



Figure 1: The Theory of Change for the Post-2020 Global Biodiversity Framework (First Draft of the Post-2020 Global Biodiversity Framework, 2021).

Goal A

The integrity of all ecosystems is enhanced, with an increase of at least 15 per cent in the area, connectivity and integrity of natural ecosystems, supporting healthy and resilient populations of all species, the rate of extinctions has been reduced at least tenfold, and the risk of species extinctions across all taxonomic and functional groups, is halved, and genetic diversity of wild and domesticated species is safeguarded, with at least 90 per cent of genetic diversity within all species maintained.

Goal B

Nature's contributions to people are valued, maintained or enhanced through conservation and sustainable use supporting the global development agenda for the benefit of all.

Goal C

The benefits from the utilization of genetic resources are shared fairly and equitably, with a substantial increase in both monetary and nonmonetary benefits shared, including for the conservation and sustainable use of biodiversity.

Goal D

The gap between available financial and other means of implementation, and those necessary to achieve the 2050 Vision, is closed.

Figure 2: Long term draft goals for 2050 of the Post-2020 Global Biodiversity Framework (First Draft of the Post-2020 Global Biodiversity Framework, 2021).

The CBD is also aligned with the 2030 Agenda for Sustainable Development, promoting synergies with actions taken towards achieving the Sustainable Development Goals (SDG; First Draft of the Post-2020 Global Biodiversity Framework, 2021). Linkages between the SDGs and the Strategic Plan for Biodiversity 2011-2020 (and the 20 Aichi Biodiversity Targets) were outlined in the technical note on Biodiversity and the 2030 Agenda for Sustainable Development (CBD, 2016), and the SDGs will act to create the necessary conditions to implement the post-2020 framework.

Monitoring of CBD targets: Indicators

To measure progress against the goals and targets of the post-2020 Global Biodiversity Framework, the CBD has also published a draft monitoring framework (Update of the Zero Draft of the Post-2020 Global Biodiversity Framework, 2020; OECD, 2019). This framework, developed together with the Biodiversity Indicators Partnership, has benefitted from experience gained from the monitoring of the Strategic Plan for Biodiversity 2011-2020. The CBD definition of indicators for the draft framework considers a Pressure-State-Response model (see Box 2), where response indicators are further grouped as inputs, processes, outputs, outcomes and impacts, following the theory of change (OECD, 2019).

Key characteristics of CBD indicators

To account for the universal nature of the framework, the monitoring approach focuses heavily on indicators that draw on globally available datasets. These indicators may be useful for

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summarizing trends at national levels but are often less efficient for informing the management interventions needed at sub-national scales to deliver on the CBD Goals.

Many of the existing indicators measure pressures and processes rather than impacts or states (e.g. total protected area without considering a measure of the health and condition of that protected area). This can hinder accurate monitoring of some targets (Shepherd et al., 2016), although the UK does have a number of state-based indicators, e.g. condition of Sites of Special Scientific Interest and abundance of priority species (Appendix: Table 4.1).

Critical to the success of the indicators is their applicability across other biodiversity-related conventions and intergovernmental processes (e.g. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, IPBES). A total of 155 indicators have been identified as well-aligned with the post-2020 goals and targets (Indicators for the Post-2020 Global Biodiversity Framework, 2021). Of these, 64 are being applied to monitor the SDGs and 39 are used for other conventions (Indicators for the Post-2020 Global Biodiversity Framework, 2021).

Proposed indicators include a list of:

- Headline indicators a set of high-level indicators that capture the overall scope of goals and targets and that may be used for tracking national, regional, and global progress).
- Component indicators indicators to monitor each component of every goal and target.
- Complementary indicators indicators for thematic and in-depth analyses of each goal and target (Post-2020 Global Biodiversity Framework: Scientific and Technical Information to Support the Review of the Updated Goals an Targets and Related Indicators and Baselines, 2020; Indicators for the Post-2020 Global Biodiversity Framework, 2021).

Some targets do not yet have proposed indicators. With the development of new indicators, there is a desire to capture both actions as well as outcomes (BIP, 2019).

Box 2. Biodiversity indicators

Biodiversity indicators summarize complex biodiversity data into simple and standardized statistics (Heink and Kowarik 2010). These are used to inform progress on various policy frameworks, at local, national, regional, and global scales. Biodiversity indicators can be categorized in different ways. Widely used frameworks that help to understand the issues related with the state of biodiversity, include the Pressure-State-Response framework (which discriminates indicators of environmental pressures, indicators of environmental conditions (state) and indicators of societal responses) and variations from this, as the Driver-Pressure-State-Impact-Response.

Biodiversity indicators can also be broadly categorized according to the type of data used (Eaton et al. 2021):

- i) Abundance and occupancy-based indicators
- ii) Red List Indices
- iii) Diversity metrics
- iv) Biodiversity Intactness Index
- v) Essential Biodiversity Variables
- vi) Non-species metrics

The most frequently used indicators are those based on changes in either abundance or occupancy of a single population or species. Frequently these indicators are combined to form multispecies/taxa composite indicators.

Essential Biodiversity Variables (EBV) are a recent concept proposed by the Group on Earth Observations Biodiversity Observation Network (GEO BON) that encompass a minimum set of measurements needed for monitoring, reporting and managing biodiversity change (Pereira et al. 2013). Currently, six classes of EBVs are considered (genetic composition, species populations, species traits, community composition, ecosystem functioning and ecosystem structure) which include several biodiversity indicators (GEO BON 2021).

Another relevant concept is **Ecosystem Health**, as an overall effort is being made to derive indicators reporting on the health of ecosystems. Ecosystem health reflects the state of ecosystems, by analogy with human health (IPBES Glossary 2017). It is a not a simple concept, as it is the subject of extensive debate among researchers, and it should be noted that there is no general point of reference for a healthy ecosystem (Lu et al. 2015; IPBES Glossary 2017). However, despite not being a completely objective concept, ecosystem health has proven to be a useful concept in environmental management (Lu et al. 2015). Several frameworks have been developed for assessing ecosystem health that consider different inter-related elements, including ecosystem structure, function, resilience (that reflects the system's ability to withstand disturbance), and the ability to provide quality ecosystem services for future generations (Lu et al. 2015, see below the framework being developed for Scotland as well as Boxes 3, 4, and 5).

Overall, biodiversity indicators are mostly derived from modelled outputs based on a variety of data sources that originate from existing biodiversity monitoring activities and datasets. Obtaining these data is limited by practicality, accessibility of data collection, funding to secure sampling/co-ordinate schemes, funding to maintain datasets, ownership and access to data (POST 2021b).

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) Global Assessment

The IPBES is an independent body established in 2012. Its main goal is to "strengthen the science-policy interface for biodiversity and ecosystem services for the conservation and sustainable use of biodiversity, long-term human well-being and sustainable development" (https://ipbes.net/).

The IPBES conceptual framework is a highly simplified model of the complex interactions between nature and human societies, which are relevant for the IPBES goal. It builds mostly on experience gained during the Millennium Ecosystem Assessment and includes six interlinked elements: i) nature; ii) anthropogenic assets; iii) nature's contributions to people; iv) institutions and governance and other indirect drivers; v) direct drivers; vi) good quality of life (Figure 3, Díaz et al., 2015).



Figure 3: The IPBES Conceptual Framework (Díaz et al., 2015). This figure demonstrates the main elements and their inter-relations for the conservation and sustainable use of biodiversity and ecosystem services. Direct drivers include natural drivers, not resulting from human activities (e.g. earthquakes and tsunamis), and anthropogenic drivers, which result from human decisions and are framed within five main categories: land and sea use changes; natural resource use and exploitation; pollution; invasive species; climate change.

The IPBES Global Assessment of Biodiversity and Ecosystem Services assessed the status and trends of nature, its social implications and their causes, as well as the actions that can be taken to assure a sustainable future (IPBES, 2019). The Global Assessment reviewed existing research and data using a framework of indicators for analysing status and trends as well as progress towards CBD and SDG targets. These included 30 core indicators and 42 highlighted indicators (Brondizio et al., 2019). Most of these indicators are common to those used under the CBD and other initiatives (e.g. Future Earth, Yale Environmental Protection Index).

Given the complexity of monitoring biodiversity change, the partners of the Group on Earth Observations Biodiversity Observation Network (GEO BON) proposed a framework based on Essential Biodiversity Variables (EBVs), that could make the link between monitoring initiatives and decision makers (See Box 2 (H. M. Pereira et al., 2013). EBVs should help set priorities as they include complementary measurements to capture crucial aspects of biodiversity change



(H. M. Pereira et al., 2013). Each EBV includes a set of specific indicators, and these were applied in the IPBES Global Assessment to understand the current state of nature and to assess the impact of the different drivers at the environmental realm (i.e. freshwater, marine, terrestrial), regional and global scale (Purvis et al., 2019). The list of indicators used for each EBV class is presented in the Global Assessment and the knowledge gaps are identified. This report also identified overall knowledge gaps in data, indicators, inventories and scenarios (IPBES, 2019).

National Biodiversity Strategy & Action Plans

The implementation of international biodiversity monitoring frameworks is decided at the country level (National Biodiversity Strategy and Action Plans). Here we review Scotland's approach towards biodiversity reporting.

Whilst the definition of CBD goals is a multilateral process and the indicators are limited to those for which data are available globally, implementation is decided at the country level. Countries define action and monitoring approaches in their own National Biodiversity Strategy and Action Plans (NBSAPs). The poor implementation of CBD goals and targets in some NBSAPs has been highlighted as one of the reasons for the failure in the overall achievement of the Aichi targets (Xu et al., 2021). Hence, there is much scope for improvement and for adopting new approaches in both implementation and monitoring at the national level.

Scotland's approach to biodiversity reporting

Scotland reports as part of the UK for CBD, but also has its own biodiversity strategy. Scotland's current biodiversity strategy was first published in 2004 and updated in 2013, with the "2020 Challenge for Scotland's Biodiversity". This document set out the major needs for biodiversity in Scotland and for meeting the Aichi Biodiversity Targets and the targets set within the European Biodiversity Strategy (Scottish Government, 2013). Additionally, the Scottish Government 2020 "Statement of intent on Biodiversity" outlined the commitment to protect at least 30% of Scottish land and sea for nature by 2030 (based on the global 30x30 target) https://www.gov.scot/publications/scottish-biodiversity-strategy-post-2020-statementintent/#:~:text=Sets%20the%20direction%20for%20a.biodiversity%20loss%20and%20climat e%20change. NatureScot has been commissioned to develop and publish a National Framework and Implementation Plan for terrestrial delivery of 30x30 in Scotland. This commission covers the delivery of 30x30 on land (including freshwater and coastal sites) and does not cover marine. This project is being developed alongside that of Nature Networks and is key in the delivery of the Scottish Biodiversity Strategy and contributing to the wider Environmental Strategy. Effective delivery of this target will significantly contribute towards tackling the nature and climate emergency. DNA-based monitoring shows great potential for revolutionising national monitoring schemes and could be integrated into the Scottish Biodiversity Strategy, providing the evidence required to deliver on CBD commitments.

In 2015, "Scotland's biodiversity: a route map to 2020" was published, defining the priority work needed to meet the CBD targets. This document set out six "Big Steps for Nature" and defined priority projects for taking these steps (The Scottish Government, 2015). Box 3 details the progress of Scotland towards the Aichi Targets.

Box 3. Scotland's progress on targets

The final assessment of Scotland's progress towards the Aichi Targets showed that nine out of the 20 were met and the remaining 11 showed progress, but were not fulfilled (NatureScot 2021, <u>https://www.nature.scot/scotlands-biodiversity-progress-2020-aichi-targets-final-report</u>). Regarding biodiversity, Scotland made good progress by exceeding the national target in the definition of marine and terrestrial protected areas and by designating funds for actions contributing to biodiversity (NatureScot 2020). However, the 2019 progress report identified the most challenging targets as those under the Strategic Goal B, which relates to pressures on biodiversity. Hence, some targets were met, but pressures on biodiversity still remain (NatureScot 2021). Though considerable progress has been made on monitoring and reporting, further work is still required to improve data on ecosystem function (NatureScot 2021).



The development of biodiversity indicators to measure progress towards global targets was undertaken at both the national (UK) and country (Scotland) level. Hence, indicators currently used in Scotland include both Scottish-defined indicators and a set of UK-wide Biodiversity Indicators. The UK biodiversity indicators suite includes 24 indicators (POSTNote 644, Effective Biodiversity Indicators, 2021). The initial set of Scottish indicators included 22 related to biodiversity, divided into two types: state indicators (17 indicators, focusing mainly on the state of species, habitats and ecosystems) and engagement indicators (The Scottish Government, 2015). Since then, Scotland has been looking to move beyond simple measures of area and species numbers to metrics that also tackle ecosystem function and resilience. To this end, a suite of Ecosystem Health Indicators (Boxes 4-6) has been developed through a partnership

between government agencies, research institutes and NGOs (see details below; Scottish Government, 2020).

Prior to Brexit, Scotland and the rest of the UK were required to meet reporting requirements to the EU Biodiversity Strategy and to the European Directives (Habitats and Bird Directives, Water Framework Directive (WFD), and Marine Strategy Framework Directive (MSFD). A suite of "Streamlined European Biodiversity Indicators" has been developed to provide robust information at the European level for assessing the state of biodiversity (European Union, 2021). These indicators and reporting requirements under other European Directives are building on currently available biomonitoring data to avoid duplication of effort (Figure 4). For Scotland and the rest of the UK, the habitats Regulations have been amended as a result of leaving the EU (https://www.gov.scot/publications/eu-exit-habitats-regulations-scotland-<u>2/pages/2/</u>) so that European sites are both protected, and continue to operate, as they have done since their original designation. The changes to the Regulations also mean that the requirements of the Directives continue to be relevant to the management of European sites. Now they form part of the Emerald Network of Areas of Special Conservation Interest (https://www.coe.int/en/web/bern-convention/emerald-network), spanning Europe and into Africa. There is no requirement to report on the condition to the EU under Article 17 of the Habitat Directive. The UK will continue to publish reports on the conservation status of habitats and species that occur in European sites and on the conservation measures implemented. Reports under the 1994 Regulations will be published by Scottish Ministers every six years (from exit day), and a composite UK report published by the Secretary of State within two years of this. Now that the UK has left the EU, there is autonomy and ability to set the UK's own future environmental protections, aiming at a very ambitious environmental programme (UK Government, 2021).

The indicators currently applied in Scotland will likely be reviewed in line with the update of the biodiversity strategies (both UK and Scottish) that will derive from the new Post-2020 Global Biodiversity Framework such as is being set out in the Scottish Biodiversity Strategy. It is likely that many of the existing indicators will be retained particularly where they represent information on the status of wildlife or pressures on the natural environment; this is largely owing to the valuable long-term datasets available. Whilst the power of indicators underpinned by long time series data is irrefutable, there is an opportunity at this CBD juncture to evaluate current methodologies and appraise new approaches. New indicators will potentially be developed as new strategies emerge from the CBD review process and it is predicted that these will be designed to complement the existing indicators (POSTBrief 41, Biodiversity Indicators, 2021). The complexity of biodiversity measurement, coupled with increasing focus on community or ecosystem function and resilience measures, means that novel methodological and analytical approaches to metrics and indicator compilation is expected and required (Environment Agency, 2021).



Figure 4: Overview of data flow from conventional monitoring approaches in Scotland towards biodiversity reporting.

Ecosystem health indicators

Ecosystem health indicators aim to reflect the state of ecosystems, and target different interrelated elements, namely, ecosystem structure/condition, function, resilience and the provision of ecosystem services (Lu et al., 2015, see Box 1 for details on Ecosystem Health frameworks).

The Scottish Ecosystem Health indicators (Appendix 4, Table 4.2) cover the inter-related elements of ecosystem health frameworks and are organized into three groups (NatureScot, 2019; POSTBrief 41, Biodiversity Indicators, 2021):

1) **Condition indicators** – measure how far ecosystem components are from what is considered a 'good' state; inform on the state of ecosystems (Box 4).

2) **Function indicators** – measure the extent to which ecosystems retain their function and can deliver services; inform on habitat connectivity and functionality (Box 5).

3) **Resilience indicators** – measure the ability of ecosystems to cope with pressures and maintain capacity to deliver benefits; inform on ecosystem resilience (Box 6).

While the Scottish monitoring framework includes several condition indicators, there are gaps related to function and resilience, especially with regards to functional traits and community diversity.

Box 4. Condition indicators

Condition indicators inform on the state of ecosystems, so these are used to assess if an ecosystem is in a 'good' state. Ecosystems in 'good' state represent ecosystems with good physical, chemical and biological quality, in which species composition, ecosystem structure and ecological functions are not impaired (Maes et al. 2021). These indicators may also form a baseline for assessing change in the future. There are challenges in objectively defining what constitutes a good or unimpacted reference state, including deciding the appropriate historical baselines to aim for (e.g. pre-industrial revolution).

Condition indicators are useful for managers and policymakers as they indicate where management is needed for halting damage or restoring ecosystem health and inform on progress of the applied management actions. These indicators are also applied by conservationists to target work on protected species and habitats (NatureScot 2019a).

Several measures may inform on the condition of ecosystems, including measures on species diversity, land cover, species, and habitat conservation status (e.g. Habitats Directive), and ecological status of freshwater (e.g. Water Framework Directive, WFD) and marine environments (e.g. Marine Strategy Framework Directive, MSFD).

The Scottish Ecosystem Health Indicators include seven condition indicators covering the state of habitats (indicators 1 to 4 and 6), species (indicator 5) and soils (indicator 7, Appendix 3, Table 3.2) (NatureScot 2019a). These derive from several Scottish and European monitoring schemes and involve government agencies, societies, and institutes.

Box 5: Function indicators

Ecosystem functions are the processes that control the flow of energy, nutrients, and organic matter through an environment, interlinking the different components of the ecosystem (i.e. primary producers, consumers, decomposers, etc.; Cardinale et al. 2012). Many of these processes are considered ecosystem services, since some functions directly benefit human populations, such as pollination and productivity (Meyer, Koch, and Weisser 2015a). Monitoring ecosystem functions is an important element of ecosystem health assessments and thus essential for ecological conservation and restoration and for the maintenance of relevant ecosystem services.

There is some debate on what appropriate measures of ecosystem function can derive function indicators (Garland et al. 2021). Several measures and indicators have been described in the last years, including measures of productivity, such as estimates of carbon and nitrogen flows (e.g. phytoplankton production and productivity, Painting et al. 2013), or using remote sensing-based approaches (Requena-Mullor et al. 2018), and quantifications of food-webs and consumer-plant interactions (Meyer, Koch, and Weisser 2015b), among others.

The suite of Ecosystem Health Indicators defined for Scotland includes two function indicators that measure functional connectivity (Blake and Baarda 2018) and habitats at risk from acidification and eutrophication (NatureScot 2019b). The connectivity indicator is based on the Equivalent Connected Area and is calculated for four habitat types (Blake and Baarda 2018). Indicator 9 is reported at the UK level and is based on the estimates of 'critical loads' thresholds for pollutants or eutrophication (NatureScot 2019c). Scotland's existing function indicators do not consider the presence of different functional traits within ecosystems.

Box 6. Resilience indicators

Ecosystem resilience is related to how ecosystems respond to stressors and disturbances, which is fundamental for effective and adaptive management (Chambers, Allen, and Cushman 2019). The theory of ecological resilience is underpinned by the principle that a system can withstand degrees of disturbance before changing to an alternative stable state and that any one system can have several alternative stable states and still maintain its essential structure and function (Holling 1973; Quinlan et al. 2016).

Resilience indicators can be derived from several sources employing a variety of methodologies. Species spatial distributions and relative abundances are well connected with resilience (Chambers, Allen, and Cushman 2019), as are structural aspects of communities (e.g. species diversity, landscape connectivity), functional traits (functional redundancy), and response diversity (diversity of traits within communities) (Baho et al. 2017). These measures are often based on specific taxonomic groups, or indicator species, according to pressures and environmental factors. However, large scale conservation should include a broader view of the diversity of species traits, habitat requirements and functions for measurements of resilience (Baho et al. 2017; Chambers, Allen, and Cushman 2019).

The resilience indicators considered within the Scottish Government Ecosystem Health Indicators include six measures designed to target habitat restoration (indicator 10) and the impact of drivers, including invasive species management (indicators 11 to 14, Appendix 3, Table 3.2) (NatureScot 2019d). Data from the NBN Atlas Scotland from casual records and recording schemes/initiatives are used to derive indicators 11, 14a and 14b. Currently these resilience indicators do not cover functional traits or response diversity.

Using DNA for reporting against CBD & other policy frameworks

DNA-based methods are already widely applied for biodiversity monitoring in research and consultancy, yet integration of these methods into formal monitoring and reporting frameworks is still at the early stages. Here we discuss the possible implementation strategies of DNA-based monitoring for biodiversity reporting.

Section highlights:

- 1. DNA-based methods are not specifically featured as tools for biodiversity monitoring to inform indicators for the CBD. There is, however, ample scope for countries to incorporate DNA –based monitoring methods into their national plans
- 2. We identify three key approaches for incorporating DNA-based monitoring methods into biodiversity reporting frameworks:
 - a. **Enhance** DNA is used to inform existing indicators, either as a complement to conventional methods or by becoming the primary source of data
 - b. **Calibrate** DNA Is used to calibrate/validate existing indicators, particularly those that are used as proxies for biodiversity, or are outputs of pressure-state-response frameworks
 - c. Create new indicators are developed based on DNA data
- 3. These approaches can be used to create ecosystem health indicators that measure condition, function, and resilience through DNA derived data on species presence, community composition, and community function
- 4. Two key approaches are identified for scaling DNA-based metrics from site-based monitoring to national and global reporting: DNA samples are collected at scale; modelling methods are used to achieve scale

DNA is not explicitly mentioned in the existing CBD monitoring framework which is not expected to change significantly at this stage in the negotiation process. However, DNA remains relevant to many of the goals and targets of the post-2020 Global Biodiversity Framework and there remains a significant opportunity for DNA-based approaches to be incorporated into the NBSAPs of signatory countries. Biodiversity data needs to function at local, national, and international scales to inform relevant decision makers at each stage. DNA has the potential to be incorporated into a framework that allows this scaling to occur.

Of the 155 available indicators identified as suitable for CBD monitoring, few include genetic information and of these, most refer to the fair use of genetic resources (Target 13) and not the actual use of genetic data for monitoring. For draft Goal A alone (see Figure 2), there are over 30 proposed indicators, with around 15 considered relevant to measure ecosystem integrity and connectivity (Indicators for the Post-2020 Global Biodiversity Framework, 2021). Indicators for measuring genetic diversity are scarce and refer mostly to metrics related to species of agricultural or socioeconomical importance (e.g. Hollingsworth et al., 2020). The need for specific indicators to monitor genetic diversity within all species has been highlighted (Hoban et al., 2020; Laikre et al., 2020). The IPBES Global Assessment, when discussing EBVs for genetic composition, also identifies the limited taxonomic and geographic coverage of available data and the early stage of synthesis of global genetic composition trends (IPBES, 2019).

Despite the draft monitoring framework including few indicators explicitly based on genetic data, there remain opportunities to use DNA-based methods as relevant data sources, not only for the assessment of genetic diversity but as a tool for species detection and for assessing community composition, structure, and function. Hence, DNA data can be used for the refinement of existing indicators, and for the development of new DNA-based indicators (Appendix 3: Table 3.2; Appendix 4). Considering the four main goals proposed for the Post-2020 Global Biodiversity Framework (Figure 2), DNA can contribute more to monitoring the fulfilment of draft Goal A and the action targets under 'Reducing threats to biodiversity' (Targets 1 to 8). However, it can also contribute to draft Goal B, through informing on ecosystem services (Targets 9 to 11). Furthermore, by promoting the wide use of DNA-based methods, in an equitable way, a contribution to draft Goal C and target 13 is also achieved (First Draft of the Post-2020 Global Biodiversity Framework, 2021).

The broad application of DNA-based methods in biodiversity monitoring and reporting has already been discussed in several studies (Cordier et al., 2020; Environment Agency, 2021; Hering et al., 2018; Pawlowski et al., 2018). Two main strategies are usually considered:

- a) Using existing indicators, where DNA-based methods are applied for the taxonomic identification of organisms.
- b) Developing new metrics that take advantage of the full information retrieved with DNA and/or independently of formal taxonomy (e.g. taxonomy-free methods, RNA typing).

The integration of DNA-based data into existing indicators (strategy a) is considered easier to implement in the short term, while the development of new DNA-based metrics is viewed as possible in the long term (Pawlowski et al., 2018). However, a limitation of using existing indicators is that they are typically based on a restricted suite of taxa that can be easily identified by traditional methods, and re-calibrating these indices based on detection rates of taxa using eDNA can be challenging (Kelly, Gallego, & Jacobs-Palme, 2018; Pont et al., 2021a).

In addition to the two main strategies, we also propose that DNA can be used to calibrate or validate existing metrics. Therefore, we identify three primary ways by which DNA-based methods can be integrated into monitoring frameworks (Figure 5):


- 1) **Enhance** DNA data is used to inform existing indicators, either as a complement to conventional methods or by becoming the primary source of data.
- 2) **Calibrate** DNA data is used to calibrate/validate existing indicators, particularly those that are used as proxies for biodiversity, or are outputs of pressure-state-response frameworks.
- 3) Create new indicators are developed based on DNA data.

In Figure 5, we highlight the different types of data that DNA-based approaches may derive: taxonomy-based data (e.g. species lists); taxonomy-free data (OTU or ASV lists); and genomic data. These data can characterize different components of ecosystems: species detection; community composition; and community function. According to the type of DNA data generated and ecosystem component, the opportunities to enhance, calibrate or create are highlighted. These processes can generate ecosystem health indices related to condition, function, and resilience.



Figure 5: How DNA-based data can be used for reporting. DNA-based data may derive from taxonomy-based (species lists, to which DNA reference collections contribute), taxonomy-free (OTU/ASV data) or genomic approaches. These data will inform species detection, community composition or function. Then, three different strategies for deriving indicators are considered: enhance, calibrate, and create (see text), leading to refined (existing) indicators, or to new indicators for national and international reporting. Arrows indicate a connection between the different data sources, analyses, and strategies.

Below we describe how DNA-based methods can be used within this framework for biodiversity reporting with a focus on how DNA can contribute to Scotland's aim to create ecosystem health indicators. We provide some case-study examples of the main strategies/metrics



considered (Boxes 7-14) and summarise how these might contribute to existing Scottish monitoring and reporting frameworks (Table 1). We also address the developmental stage of different approaches and the research efforts still needed for implementation in the short to medium-term (Table 2).

Enhancing existing indicators

DNA methods can be applied to generate data that feed into existing indicators. DNA may become the primary source of data, thereby replacing existing survey methods, or it may be used as a complement to conventional methods. 'Enhance' primarily applies to indicators of ecosystem condition.

Condition indicators

Species detection

DNA can be used to detect targeted species (i.e., invasive non-native, protected, or other relevant species) in a given site. Species identification is performed based on DNA data (taxonomy-based approach) to inform knowledge of species' distribution. Extensive research has been undertaken using DNA for species detection, hence various protocols are already available and continue to be developed, including alternative detection methods (e.g. Williams et al., 2021b), RNA-based methods (e.g. Tsuri et al., 2021), DNA capture-based methods (e.g. Wilcox et al., 2018) and automated sampling techniques (e.g. Yamahara et al., 2019; Table 1). The survey of great crested newts *Triturus cristatus* in England, is one example of an eDNA protocol for species detection adopted by government agencies (Natural England, 2015). Canada also has a protocol to permit the use of eDNA for the monitoring of invasive and 'at risk' species (Abbott et al., 2021). A case study detailing the potential application of DNA-based methods for detection of Priority Marine Features is shown in Box 7.

Box 7: Monitoring Priority Marine Features (PMFs)

Current monitoring approach	Initiatives / Frameworks		
 PMFs are monitored since 2013 using several field survey methods: Seabed video and still photographic imagery Benthic grab sampling with sediment morphological analyses and particle size analysis <i>In situ</i> scuba diving surveys Baited cameras 	 Scottish Biodiversity List (NatureScot 2012) IUCN Red List (IUCN-SSC 2021) OSPAR List of Threatened and/or Declining Species and Habitats (OSPAR Commission 2008) Habitats Directive (92/43/EEC) Scottish Biodiversity Strategy Strategy for Marine Nature Conservations in Scotland's Seas EU Biodiversity Strategy for 2020 Aichi Targets 2020 		
Data collected	Scottish and UK Indicators		
 PMF distribution and abundance Species diversity indexes Particle size analysis 	 Notified species in favourable condition (S10) Notified habitats in favourable condition (S11) Status of UK habitats of European importance (UK-C3a) 		

How DNA may be integrated

PMFs can be detected through the analyses of water and/or sediment samples. Species detections obtained through eDNA could be used to inform existing indicators on its own or combined with conventional approaches.

New methods may be optimised to improve species detection such as panels of species-specific assays and DNA capture using automated sampling.

Community composition

eDNA metabarcoding provides useful data on community composition, as samples can be processed targeting a wide range of taxonomic groups (e.g. Pawlowski et al., 2016). Several studies comparing community composition data between traditional and eDNA sampling achieved good overall results, despite some variation by target taxa (Aylagas et al., 2016; Elbrecht et al., 2017; Pont et al., 2021b; Vasselon et al., 2017).

Different monitoring frameworks have created indicators of ecosystem condition based on communities of indicator species. For example, the Water Framework Directive (WFD) uses the UKTAG biological standard methods (http://wfduk.org/) for the assessment of phytobenthos (diatoms), phytoplankton, and macrophytes (plants), macroinvertebrates, and fish in freshwater lakes and rivers. The WFD also uses the concept of 'Biological Quality Elements (BQEs)' to classify a waterbody as high, good, moderate, poor, or bad ecological status. DNA-based methods have been applied extensively to the detection of BQEs, including macroinvertebrates, fish, and diatoms (Pérez-Burillo et al., 2020) with variable results (Aylagas



et al., 2016; Elbrecht et al., 2017; Pont et al., 2021b; Vasselon et al., 2017; Willby et al., 2019). These results have been compared with those obtained using conventional methods and, in some cases, have shown that DNA data can be applied to biotic indices. These indices could

Box 8: Monitoring the ecological status of lakes with fish eDNA

Current monitoring approach	Initiatives / Frameworks		
 Fish are currently monitored using semi- invasive methods, including: Gillnetting Seine netting Electrofishing Fyke netting Hydroacoustics 	 Scottish Biodiversity List (NatureScot 2012) IUCN Red List (IUCN-SSC 2021) Habitats Directive (92/43/EEC) Water Framework Directive Scottish Biodiversity Strategy EU Biodiversity Strategy for 2020 Aichi Targets 2020 		
Data collected	Scottish and UK Indicators		
 Distribution and abundance Biometric data (e.g. size, weight) Population structure Juvenile counts of some species Invasive non-native species detections 	 Notified species in favourable condition (S10) Notified habitats in favourable condition (S11) Status of UK habitats of European importance (UK-C3a) Surface water status (UK-B7) Status of priority species (UK-C3: UK-C4) 		

How DNA may be integrated

eDNA obtained from water samples is used to detect fish species in lakes. These data are used to generate metrics of lake ecological condition, that are largely compatible with those provided by other WFD metrics and are sensitive to some of the major pressures affecting lakes (Willby et al. 2019).

This was tested in the UK showing that this tool is suitable for reporting the ecological status of lakes. New methods, for instance, based on mRNA typing, may provide further data for improved reporting.

be used to report on several existing frameworks (e.g. WFD) and indicators (Table 1). An example of an eDNA protocol for water quality assessment based on fish community composition that has been tested in the UK is detailed in Box 8 (Willby et al., 2019).

Though DNA-based indices are already proposed for some taxa, there are potential limitations for other taxonomic groups such as diatoms, macrophytes, and macroalgae (Hering et al., 2018), including marker choice and barcode length which have implications for taxonomic resolution, underrepresentation in DNA reference databases which can impact detection rates and taxonomic resolution, standardisation of and removal of sequencing error during bioinformatic processing, and uncertainty around using sequence reads to estimate abundance (Nistal-García et al., 2021; Ortega, Geraldi, & Duarte, 2020; Ortega, Geraldi, Díaz-Rúa, et al., 2020; Pont et al., 2021a). Marker choice and barcode length limitations are not

necessarily unique to these taxonomic groups but tend to be less problematic for animals as they are not as diverse/prone to hybridisation and/or pseudogenes as plants. For example, although the application of diatom metabarcoding methods has advanced in recent years (Nistal-García et al., 2021; Pérez-Burillo et al., 2020), both fish and invertebrates have much better reference database representation, at least for the respective markers being targeted (Nistal-García et al., 2021). Additionally, more consistent relationships between DNA concentration/sequence reads and abundance for fish and invertebrates have been observed than for non-animal groups. Some of the potential issues can be countered using approaches such as occupancy modelling for mitigating the impact of false positives and false negatives in DNA data (Burian et al., 2021).

Calibrating existing indicators

DNA-based methods can be used to calibrate or validate existing metrics or to parameterize pressure-state-response models. Calibration is primarily relevant to indicators measuring ecosystem condition and function.

Condition

Species detection & community composition

DNA-based monitoring could be used to parameterize models of pressure-state-response. eDNA is used to understand or verify a link between a pressure (e.g. grazing intensity) or other variable (e.g. dead wood abundance or management practice) and biodiversity/ecosystem condition as measured using either the presence of indicator species or community composition. This could be used to predict the impact of changing pressures and/or management actions and/or validate existing condition scoring approaches (e.g. conditions of Sites of Special Scientific Interest, SSSIs).

DNA can be used to calibrate and/or validate existing proxy metrics, for example, the remotely sensed indicator 'Above Ground Biomass' could be linked to the biodiversity value and/or community composition of an area assessed using eDNA. The proxy measures once validated would remain the primary method for data collection. This enables the use of remotely sensed data, which allows indicators to be monitored efficiently at scale. This would also add value to existing indicators without the need to completely replace them.

The potential application of DNA-based methods for calibrating Earth Observation data is showcased in Box 9 (Bush et al., 2017).

Box 9: Assessing Ecosystem Health Indicator 3 – Native woodland condition

Current monitoring approach	Initiatives / Frameworks		
Forestry Commission survey carried out from 2006-2013 for native woodlands (i.e. with >50% native species in the canopy).	 Scottish Biodiversity Strategy EU Biodiversity Strategy for 2020 Aichi Targets 2020 Scotland's Forestry Strategy 2019-2029 		
Data collected	Scottish and UK Indicators		
 Four condition attributes: Canopy cover Percentage of canopy comprising native species Herbivore impact Invasive non-native species 	 Native woodland condition (EHI-3) Notified habitats in favourable condition (S11) Status of UK habitats of European importance (UK-C3a) 		

How DNA may be integrated

The application of remote sensing data for monitoring forests is growing globally, including in Scotland. Remote sensing has the capacity to increase the scale and efficiency of traditional on the ground surveys. However, remotely sensed data can be lacking in detail because most biodiversity is invisible to satellites, and earth-observation derived indicators could miss biodiversity and community context. To underpin, ground-truth and enhance the relevance of remote sensing data to inform indicators, eDNA-based methods, other indirect biodiversity measurement techniques (e.g. bioacoustics) and modelling can be integrated.

This approach has been theoretically described (Bush et al. 2017) and has been applied with success (Meixi Lin et al. 2021).

Function

Community function

DNA-based methods could be applied in both terrestrial and aquatic environments to verify links between a pressure (e.g. land use change) and the response (e.g. nitrogen concentration), by focusing on the state of the functional biodiversity An example of how this may be applied is showcased in Box 10 (Szoboszlay et al., 2017).

Box 10: Ecosystem health indicator 9 – Acid and Nitrogen Pollution

Current monitoring approach	Initiatives / Frameworks		
Estimates are obtained based on critical loads (thresholds for the deposition of pollutants causing acidification and/or eutrophication).	 Scottish Biodiversity Strategy EU Biodiversity Strategy for 2020 Aichi Targets 2020 		
Data collected	Scottish and UK Indicators		
Acidity critical loadsNitrogen critical loads	• Acid and Nitrogen Pollution (EHI-9)		

How DNA may be integrated

Environmental stressors (e.g. land use change) have impact on both biodiversity and on the chemical properties of the environment (e.g. nitrogen critical loads are a measure of eutrophication). Making the link between these properties and biodiversity may help us understand the impact of stressors and allow us to continue to use simple measures as indicators.

DNA-based methods may be used to assess the link between specific pressures (e.g. land use change), the state of biodiversity (e.g. microbial diversity) and the outcome (e.g. eutrophication). As an example, microbial diversity could be estimated from DNA metabarcoding data along a gradient of land use change and the presence of functional groups that cycle nitrogen related to the likelihood of eutrophication. Some studies have already tested similar approaches and assessed associations between nitrogen and bacterial taxa over different land uses (Szoboszlay et al. 2017).

Creating new eDNA-based indicators

New metrics could be derived from DNA-based data, going beyond simpler measures of species distribution and diversity, and taking advantage of methods that are not limited by formal taxonomy.

Condition

Community composition

Taxonomy-free approaches often focus on development of metrics derived from OTU profiles along an impact gradient (or by comparison between impacted and reference conditions). Two main approaches have been applied: indicator value (e.g. Dufrene & Legendre, 1997) and supervised machine learning (see Appendix 2). The indicator value approach based on OTU data has already been applied successfully for bacteria and eukaryotes (e.g. Chariton et al., 2015) and for diatoms (e.g. Apothéloz-Perret-Gentil et al., 2017), although the authors recognize that the accuracy of the assessment for diatoms may be improved.



An example of a taxonomy-free approach applied to macroinvertebrates for assessing river quality is showcased in Box 11 (Brantschen et al., 2021).

Box 11: Monitoring the quality of rivers with macroinvertebrate eDNA					
Current monitoring approach	Initiatives / Frameworks				
Macroinvertebrates are sampled using a 3- minute kick net survey across microhabitats, followed by the morphological identification of specimens to lowest taxonomic rank possible under a light microscope.	 Habitats Directive (92/43/EEC) Water Framework Directive Scottish Biodiversity Strategy EU Biodiversity Strategy for 2020 Aichi Targets 2020 				
Data collected	Scottish and UK Indicators				
Lists of taxa	Notified habitats in favourable condition				
Abundance of taxa	(S11)				
Diversity estimates	Surface water status (UK-B7)				
Water quality indices	River quality (S13)Freshwater (EHI-6)				

How DNA may be integrated

Sequences obtained from eDNA may be taxonomically assigned or the OTU lists may be used (taxonomicfree) for estimating the biotic indices. In the second approach, SML may be applied to model OTU probability of occurrence according to habitat condition, creating a metric of ecosystem health. This approach has already been applied with success (Brantschen et al. 2021).

Function

Community function

Community function can be assessed through metabarcoding species inventories (using taxonomy-based methods) and linking species to their function within ecosystems, but also using genomic techniques, as metagenomics or metatranscriptomics. Recent taxonomy-based studies tested this approach by assessing diversity and applying co-occurrence networks in ecosystems under anthropogenic pressures with promising results (DiBattista et al., 2020; Seymour et al., 2021). Metagenomics and metatranscriptomics research have also been developed in this area, mostly in the microbial community, and some evidence was found for potential function bioindicators of anthropogenic disturbances in several environments (e.g. Falk et al., 2019; He et al., 2018).

These indicators may be used as early warnings, as functional response is likely to happen before compositional response (Cordier et al., 2020). These metrics may inform ecosystem health indicators of function (Table 1). Further development is needed looking at different taxonomic groups, and to understand the links between biodiversity and pressures and between the properties of networks and ecosystem function (Cordier et al., 2020). The use of information based on shotgun sequencing of RNA presents challenges for widespread



application on environmental samples and is currently more costly than metabarcoding methods.

An example of the application of DNA-based methods for assessing the impacts of stressors on macroinvertebrate community function in freshwater environments is shown in Box 12 (Seymour et al., 2021).

Box 12: Assessing the impact of stressors on macroinvertebrate community function						
Current monitoring approach	Initiatives / Frameworks					
Macroinvertebrates are semi-quantitatively sampled with a kick net for three minutes in microhabitats, followed by morphological identification.	 Habitats Directive (92/43/EEC) Water Framework Directive Scottish Biodiversity Strategy EU Biodiversity Strategy for 2020 Aichi Targets 2020 					
Data collected	Scottish and UK Indicators					
Species listSpecies or taxa abundances	Creation of new EHI Function and Resilience.					

How DNA may be integrated

DNA data are collected from bulk samples, preservative ethanol or from aquatic eDNA. Sequences are bioinformatically processed and retrieved OTUs are then taxonomically assigned. The obtained taxa are then partitioned into functional groups (e.g. functional feeding groups) and functional richness and diversity is estimated. This allows the assessment of spatial turnover of function within communities according to the impact of environmental stressors (e.g. land use changes, pollution). This approach has already been applied with promising results (Seymour et al. 2021).

OTU or ASV data may be used to investigate community function through the application of phylogenetic methods (e.g. Liu et al., 2017a). Phylogeny-aware methods link phylogenetic diversity to functional traits based on the concept of niche conservatism of closely related taxa (niche conservatism concept, Webb et al., 2002). However, care must be taken when making this link, as not all functional traits have strong phylogenetic signal (Srivastava et al., 2012).

An example of the potential application of phylogeny-aware methods for predicting microbial response to environmental stressors across all four target habitats is showcased in Box 13.

Box 13: Assessing microbial community response to perturbation

Current monitoring approach

Microbial community analyses traditionally relied on culture techniques to potentiate the recovery of microbial populations. Recently DNA and RNA sequencing methods have been used.

Data collected

- Microbial diversity
- Microbial community composition

Initiatives / Frameworks

- Habitats Directive (92/43/EEC)
- Scottish Biodiversity Strategy
- EU Biodiversity Strategy for 2020
- Aichi Targets 2020

Scottish and UK Indicators

Creation of new EHI for condition.

How DNA may be integrated

Microbial diversity is estimated based on metabarcoding sequence data retrieved from soil samples. OTUs are identified and taxonomically assigned where possible. Then, phylogenetic methods are used to infer phylogenetic signal and connections among OTUs, and to estimate phylogenetic diversity. This assessment of links between detected microbial taxa can be related to function and be used to predict the impacts of environmental stressors. There have been some studies applying these methods with promising results (e.g. Liu et al. 2017).

Resilience

Community composition

Higher phylogenetic diversity is associated with functionally rich ecosystems and in turn these are associated with higher resilience. For example, ecosystems with high levels of functional redundancy and/or highlevels of functional or 'response' diversity may be more resilient to changing pressures (Biggs et al., 2020; Elmqvist et al., 2003). Therefore, the approaches outlined in Boxes 12 and 13 could also be used to create resilience indicators.

Resilience may also be related to intra-specific diversity. Using DNA-based methods, the number of different ASVs detected for one species may represent intraspecific variation. Moreover, data from several species within the same area may be retrieved from a single eDNA sample, allowing for an overall analysis of the intraspecific diversity of relevant taxa in one area. Some studies on freshwater macroinvertebrates tested this approach for detecting diversity changes due to environmental stressors with promising results (Elbrecht et al., 2018; Zizka et al., 2020), but see the discussion on some of the limitations of this approach in the initial section on DNA-based approaches to biodiversity reporting. Therefore, considerable research efforts are still needed to overcome the main limitations identified.

An example of the potential application of this approach for assessing the impacts of environmental stressors on macroinvertebrate communities is shown in Box 14 (Zizka et al., 2020).

Box 14: Assessing the impact of environmental stressors on macroinvertebrate diversity

Current monitoring approach	Initiatives / Frameworks		
Macroinvertebrates are sampled semi- quantitatively with a kicknet for three minutes in microhabitats, followed by morphological identification.	 Habitats Directive (92/43/EEC) Water Framework Directive Scottish Biodiversity Strategy EU Biodiversity Strategy for 2020 Aichi Targets 2020 		
Data collected	Scottish and UK Indicators		
Species listSpecies or taxa abundancesRichness and diversity	Creation of new EHI for habitat condition.		

How eDNA may be integrated

DNA data are collected from bulk samples or from preservative ethanol. Sequences are analysed with bioinformatic tools that allow haplotypes to be retrieved from these data (e.g. JAMP,

https:/github.com/VascoElbrecht/JAMP). Therefore, a dataset with OTUs and ASVs is obtained. OTUs may then be taxonomically assigned to species if there is reference data available. Parameters like haplotype and nucleotide diversity and haplotype richness per OTU/species may then be estimated and related with the impacts of environmental stressors.

This approach has already been applied with promising results (Zizka et al. 2020), but further research is still needed.

Phase 1 REPORT

Table 1: Summary of the proposed strategies and type of indicator for the implementation of DNA-based n	nethods for biodiversity reporting. Existing relevant indicators
(https://jncc.gov.uk/our-work/ukbi-overview-of-trends-2020/; Scottish Government, 2020) and frameworks listed	ed.

Strategy	Туре	of	of Example existing indicators Example application		
	indicator				
Enhance Condition		n	Invasive non-native species (EHI-11; UK-B6)	Monitoring Priority Marine Features (Box 7)	
			Conditions of notified species (S10)	Assessing the ecological status of lakes	
			Status of priority species (UK-C3; UK-C4b)	(Box 8)	
			WFD; River quality (S13); Freshwater (EHI-6); Surface water status (UK-B7); Site		
			condition monitoring (S11); Status of European habitats (UK-C3a)/ Habitats		
			directive; Site condition monitoring (S11); Status of pollinating insects (UK-D1c);		
			Status of European habitats (UK-C3a)/ Habitats directive		
Calibrate	Conditio	n	Above ground biomass (CBD); Habitat restoration (EHI-10); Climate Change	Assessing Ecosystem Health Indicator 3 –	
			adaptation (EHI-12); Site condition monitoring (S11); Native woodland condition	Native woodland condition (Box 9)	
			(EHI-3); Condition of areas/SSSI (UK-C1c)		
	Function	1	Acid and Nitrogen Pollution (EHI-9)	Ecosystem Health Indicator 9 – Acid and	
				Nitrogen Pollution (Box 10)	
Create	Conditio	n	Habitats directive (habitats and species); WFD; MSFD; Response to pressures;	Monitoring the quality of rivers with	
			Climate change adaptation (EHI resilience)	macroinvertebrate eDNA (Box 11)	
	Function		EHI – function; Response to pressures	Assessing the impact of stressors on	
				macroinvertebrate community function	
				(Box 12)	
				Assessing microbial community response	
				to perturbation (Box 13)	
	Resilienc	e	Climate change adaptation (EHI resilience)	Assessing the impact of environmental	
				stressors on macroinvertebrate diversity	
				(Box 14)	

 Table 2: Examples of indicator categories to measure condition, function, and resilience for short-term and future timeframes in conjunction with broad research and development priorities for refinement of DNA-based biodiversity monitoring in the short, medium, and longer term.

Phase 1 REPORT

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Indicator	Indicators – Short-term	Indicators - Future	Research needs			
focus area	application	development	Short-term delivery	Medium-term development	Long-term development	
Condition	Enhance: Species detectionEnhance:Communitycomposition(taxonomy-based approaches)Calibrate: Validate existingproxy metricsCreate:Create:Ecosystem Healthmetric(taxonomy-freeapproaches)based oncategorising samples alonga known impact gradient.Create:Communityfunctional diversity metricsCreate:Create:Community co-occurrence networksCreate:Create:Functionalbioindicators.	Create: Ecosystem Health metric (taxonomy-free approaches) able to classify samples from a range of habitats and conditions. Calibrate: Parameterise Pressure-State-Response models using eDNA data. Create: Link phylogenetic diversity to functional traits. Calibrate: Parameterise Pressure-State-Response models using eDNA data and functional traits.	 Sampling strategy development Primer optimisation Validation/standardization against conventional methods Validation to quantify links between eDNA data and other existing metrics Metric/model creation and validation including ML approaches 	 Refine sampling strategy Development of new detection methods (e.g. CRISPR-Cas) Bolster DNA reference collections Optimisation of bioinformatic analyses Validate abundance estimation Use eDNA to measure pressure-state-response Collection of environmental metadata to parameterise models Optimisation of indicator value and ML approaches Network analyses 	 Automated sampling Automated curation of reference databases mRNA typing for abundance estimates and/or developmental state Predictive models for scaling up spatially and temporally 	
				Network analyses methods		

Phase 1 REPORT

Resilience	Create: Community network	Create: Intraspecific	• Links betwee	en diversity
	analyses	diversity indices	and pressures	
	Create: Community		• Link eDNA da	ta to remote
	functional redundancy		sensing data f	or scaling up
	score		geographicall	у
			• Assess links t	o functional
			traits	

Scaling metrics from local to global – CBD

Scaling is a standard requirement in the collation of indicators to enable site-based monitoring to contribute to national and global reporting. Two approaches may be used, which are not mutually exclusive nor unique to DNA approaches but do require a clear pathway linking the data being measured on the ground via novel approaches, with the mid-level and CBD-level indicators. Either approach could make a significant contribution to the creation of global observation networks as championed by GEO BON.

In the first approach, DNA is collected at scale, for example, across a national observation network that covers representative habitat types and exposure to pressures. Results could be summarized at the national level under the assumption that they are representative of the country. Large-scale data collection like this could also contribute to larger initiatives designed to fill key knowledge gaps of biodiversity globally. For example, the eBioAtlas initiative (https://ebioatlas.org/), aims to provide a comprehensive overview of the world's freshwater biodiversity.

The value of DNA-based monitoring for large scale data acquisition is reflected in an increasing number of initiatives. For example, the LIFEPLAN project, which aims to characterize biodiversity through a worldwide sampling program, including the use of malaise traps and DNA metabarcoding (https://www2.helsinki.fi/en/projects/lifeplan/about), building on work of the Global Malaise Program (https://biodiversitygenomics.net/projects/gmp/). The Society for the Protection of Underground Networks (SPUN) has also launched a project to map the fungal networks of the planet, also using DNA-base approaches, aiming to collect 10,000 samples across ecosystems on all continents.

In the second approach, scale is achieved by using modelling approaches that use DNA and other predictive data such as variables from remote sensing (Bush et al., 2017). For example, predictive models are used to model the health of ecosystems considering Earth Observation and other pressure data. Ground-truthing could continue at the site-level through the observation network. These models are used to predict biodiversity responses at regional and national levels.

For example, NASA is using eDNA in combination with MODIS and Landsat imagery to predict native fish distribution across a 23-million-acre reserve in Alaska (Poteet, 2020). eDNA data in combination with remotely sensed data and other environmental variables was used to map community composition across the state of California using just 278 samples as part of the CALeDNA project (M. Lin et al., 2021). Further validation and testing of this scaling approach is needed in different contexts (Table 2).

Development of DNA-based approaches in Scotland

Whilst DNA (meta)barcoding is a well-established technology, additional work is needed to validate the approach in the different contexts we outline above. This may require further





testing alongside conventional techniques, and a comprehensive overview of how effective different approaches are for the detection of priority species, e.g. qPCR and metabarcoding. Implementation at the national scale would also require careful consideration of an appropriate sampling plan.

Given the review of opportunities for DNA-based monitoring approaches and the gaps in the existing Scottish monitoring framework, Phase 2 of this project will focus on examining the potential of DNA to 'Enhance' and 'Create' indicators relevant to Scotland's CBD goals. For example, can DNA-based monitoring be used to detect priority species more efficiently than conventional techniques, thereby enhancing existing indicators based on monitoring the presence of priority species (e.g. invasive and non-native species)? And can DNA-based monitoring be used to create new indicators that better characterise different aspects of ecosystem health, focused on condition, function and resilience?

The purpose of the next phase of this project is to gather pilot data across four key habitat types: marine, freshwater, peatland, and forests, to test these concepts with the aim of informing a set of recommendations for where Scotland can prioritise integration of DNA-based approaches into their national and international reporting. Further detail is provided in the next section on practical sampling-based considerations in different habitats.

This report will be followed by a comprehensive Phase 2 sampling plan, which will identify specific sampling strategies and link these to indicators.

Opportunities for DNA-based habitat monitoring in Scotland

Here we provide an overview of current biodiversity monitoring approaches in four Scottish habitats and highlight key DNA-based sampling opportunities to improve biodiversity reporting.

Section highlights:

- 1. We briefly review the existing monitoring approaches used in marine, freshwater, woodland, and peatland habitats, and highlight key opportunities for DNA-based monitoring to enhance species detections and monitor ecosystem health
- 2. As part of monitoring ecosystem health, we identify four main areas for development
 - a. Reference-based models for assigning metabarcoding samples to an ecological category
 - b. Methods to scale biodiversity data across large areas
 - c. Experimental terrestrial soil monitoring sampling units
 - d. Taxonomy-based functional and resilience-based metrics
- 3. Where possible, we provide indicative sampling requirements and outline the initial programmes that could be undertaken to progress these developments

In this section, we focus on some of the near-term opportunities for developing DNA-based methods for routine biomonitoring. These are discussed in the context of four broad habitat types: marine habitats (sea loch), freshwater systems (rivers and inland lochs), woodlands, and peatlands. We outline the monitoring approaches that are typical in each of these target habitats (see Appendix 5 for more detailed information on the monitoring programmes in the target habitats) and for each habitat, we discuss opportunities for:

- Species inventories and detections: where DNA can enhance existing approaches.
- Ecological assessment of habitats: where DNA can be used to create new indicators.

The main opportunities to use DNA-based approaches for ecological assessment of habitats are to:

• Develop reference-based models for assigning metabarcoding samples to an ecological category. Metabarcoding of taxonomic groups that have to date been too difficult or time-consuming to analyse morphologically (e.g., insects, soil fauna, plankton, fungi, bacteria), presents an opportunity to generate extensive data sets for

every sample collected and analysed (discussed further in the section 'DNA-based approaches to biodiversity monitoring'). A substantial component of this opportunity is because within a habitat (and potentially across habitats), these taxonomic groups are ubiquitous, and their communities are affected by environmental variables. The categories that samples can be assigned to vary by habitat type, and there are numerous categories that could be targeted (e.g. the AMBI pollution index in marine, states of degradation in peatland).

- Develop methods to scale biodiversity data across large areas. Habitat data can be collected at scale using Earth Observation approaches, particularly in terrestrial environments. These data do not necessarily provide information on biodiversity. The combination of DNA-based point samples with Earth-Observation data layers can be used to create continuous maps of biodiversity distribution across a landscape (Bush et al., 2017). This approach can be used to identify areas of high and low biodiversity value, and by including areas already considered to be in favourable condition, these maps can be used to identify areas that may require management intervention.
- Develop experimental terrestrial soil monitoring strategies. One of the key differences between terrestrial and aquatic habitats is the relative ease by which experimental designs can be established to examine the impact of different pressures and interventions. There is an opportunity to develop soil biomonitoring strategies based around experimental units, but the intensity of sampling that needs to be implemented requires basic investigation.
- Develop taxonomy-based functional and resilience-based metrics. In the near-term, translating eDNA data into function or resilience measures will rely on taxonomyassigned sequences. In the future, environmental RNA approaches or functional gene metabarcoding approaches may remove this need but this is not considered near term and is not discussed further. There are several databases that taxonomy can be queried against to return functional traits of species or genera (e.g. FUNGuild (Nguyen et al., 2016)). The results can be used to assess functional redundancy, functional diversity, and other measures of ecosystem resilience. While there are opportunities for eDNA data in this context, there are a limited number of studies that have addressed this using metabarcoding data. We anticipate that many of the opportunities listed throughout this section could use the taxonomic data generated to investigate the efficacy of generating measures of ecosystem resilience. As it is not currently clear how reliable these measures would be for monitoring programmes, we propose that the taxonomic data generated in Phase 2 of this project will be used to provide exploratory analyses in all habitats. However, as this is not considered near term it is not explicitly discussed in each of the habitat sections.

An associated document is being prepared which will include results from the initial pilot sampling carried out during 2021 and will contain the sampling plan design and rationale for Phase 2 of this project.



It is important to note that there is still only a small number of DNA-based monitoring tools that are being routinely used in standardised programmes by practitioners. Those considered to be the most robust are narrow in scope, usually focused on detection of single species. Throughout this section, reference is made to current DNA-based tools and approaches, but unless otherwise stated, these tools have yet to be incorporated into long-term monitoring programmes.

It is also worth noting that eDNA monitoring approaches are further developed for freshwater aquatic eDNA and marine sediment eDNA than for terrestrial habitats. As such, the opportunities identified in this section are better defined for freshwater and marine habitats while for terrestrial habitats some more exploratory opportunities exist, particularly regarding the assessment of the spatial scales required to obtain robust information that can help to reliably detect and monitor change.

Monitoring of Scottish freshwater habitats

Scotland is renowned for its extensive freshwater habitats, supporting 125,000 km of rivers and streams and over 25,500 lochs (https://www.environment.gov.scot/ourenvironment/water/scotland-s-freshwater/). Many of these are in good or excellent/high condition, but others face substantial threats, including diffuse pollution, bed/bank erosion, discharge, agricultural intensification, barriers to fish migration, and invasive species (Critchlow-Watton et al. 2014). A River Basin Management Plan for the Scotland River Basin District has been in place since 2009 (with revised objectives for 2015 to 2027) to improve the condition of Scottish waterbodies (Scottish Environment Protection Agency 2015). Current status and future targets for waterbodies are recorded in SEPA's Water Classification Hub and Water Environment Hub (Scottish Environment Protection Agency, 2018).

A wide range of WFD BQEs including macroinvertebrates, fish, macrophytes, phytobenthos (diatoms), and phytoplankton are routinely monitored at freshwater habitats across Scotland by SEPA. For macroinvertebrates, a 3-minute kick net sample is typically used to capture a representative sample of benthic macroinvertebrates from microhabitats in rivers, followed by morphological identification. The obtained data are then used to estimate biotic indices to assess water quality (Scottish Environment Protection Agency, 2017). For diatoms (phytobenthos), cobbles in rivers are scrubbed with a toothbrush and samples are typically processed in the laboratory to be analysed by the traditional light microscopy method (https://www.wfduk.org/sites/default/files/Media/Characterisation%20of%20the%20water%) 20environment/Biological%20Method%20Statements/River%20Phytobenthos%20UKTAG%2 <u>OMethod%20Statement.pdf</u>). For WFD-BQE lake/loch monitoring assessment purposes using phytoplankton, water column samples are typically collected using buckets/containers and/or Lund tubes for quantitative light microscopy analysis method by (https://www.wfduk.org/sites/default/files/Media/Characterisation%20of%20the%20water%) 20environment/Biological%20Method%20Statements/Lake%20Phytoplankton%20UKTAG% 20Method%20Statement.pdf). Macrophyte (plant) communities are usually surveyed, for example, to identify the species that are present and their relative abundances within a waterbody. A variety of monitoring methods are established for fish, but the most frequently methods used include gillnetting, electrofishing, and seine netting. Hydroacoustics may also be used within many large waterbodies to assess the size of fish populations. Other vertebrate species, including mammals (e.g., water vole *Arvicola amphibius*), amphibians (e.g., great crested newt *Triturus cristatus*), reptiles, and water birds, as well as plants are also targets of specific monitoring initiatives (see Appendix 5 for details).

The use of DNA-based methods for research-driven monitoring of freshwater systems is widely documented, with these methods considered to have higher detection probability whilst reducing sampling effort and required scientific knowledge when compared to more traditional methods (Valentini et al., 2016). Traditional methods of biodiversity monitoring in freshwater systems often cause disturbance and destruction, require high levels of sampling effort, and expertise on the species being monitored. The use of DNA-based methods alleviates or even eliminates these issues and allows sampling to take place in areas where traditional surveys would be difficult or impossible (Valentini et al., 2016).

Multiple studies have found eDNA metabarcoding of water samples to perform on par with, or outperform, traditional survey methods for several groups, including amphibians (Bálint, Nowak, et al., 2018) and fish (Civade et al., 2016; Hänfling et al., 2016; Shaw et al., 2016). In Scotland, freshwater eDNA analyses have been used to develop a tool to classify the ecological status of lake fish (Willby et al., 2019). eDNA has also been compared to established mammal survey methods (L. R. Harper et al., 2019; Sales et al., 2020), and used as an early warning tool for invasive signal crayfish (*Pacifastacus leniusculus*) (K. J. Harper et al., 2018).

For the detection of benthic diatom and macroinvertebrate communities, several DNA-based approaches have been applied to water, bulk tissue, and/or bulk preservative. For diatoms, comparative DNA metabarcoding approaches have been applied in Scotland (https://www.sepa.org.uk/media/399244/benthic_diatom_report_lm_and_ngs.pdf), the UK (https://www.gov.uk/government/publications/a-dna-based-metabarcoding-approach-to-assess-diatom-communities-in-rivers;

https://www.gov.uk/government/publications/assessing-river-nutrients-using-diatom-dnafurther-development-of-an-operational-method) and at European level (Pérez-Burillo et al., 2020). The use of bulk tissue DNA metabarcoding in macroinvertebrate monitoring has been demonstrated to be comparable to morphological identification in detecting environmental patterns, and can provide finer taxonomic resolution than traditional methods (Serrana et al., 2019). Conversely, freshwater eDNA metabarcoding has detected different communities from those identified using conventional approaches (morphological identification) or even bulk tissue DNA metabarcoding, but identified taxa overlooked in net samples (Gleason et al., 2021; L. R. Harper et al., 2021b). Bulk preservative has also been tested with promising results, but typically fails to detect taxa with a heavily sclerotized exoskeleton (Martins et al., 2020; Zizka et al., 2019).

Opportunities for species inventories and detections

Development of sampling strategies to detect species or sets of species at known probabilities.





The majority of eDNA surveys do not estimate detection probabilities at sampling sites. Without these, the probability of a species being present, even if not detected, cannot be modelled. There is an opportunity to build on site-occupancy modelling and jSDM modelling (see section 'DNA-based approaches to biodiversity monitoring') efforts from other studies to help address this challenge. It is expected that lentic systems (either discrete ponds or large lakes) would be targeted first as eDNA transport in lotic systems poses additional interpretive challenges that are yet to be overcome. Furthermore, it would be reasonable to target vertebrates first – fish and amphibians – given that eDNA approaches have regularly been shown to outperform conventional methods for these taxa and primer sets have been developed that detect most fish and amphibians to species level. For other groups, such as insects, models could be developed but would require more research as it is clear that many species are not detected by current eDNA approaches.

Building models to estimate detection probabilities requires multiple sampling replicates from multiple sampling locations or timepoints. Pilot studies or priori knowledge of species' occurrence should be used to guide sampling strategies using simulations or from optimum numbers of replicates in the literature to achieve a specified standard error of the occupancy estimate, or both (Mackenzie & Royle, 2005). Sites should be defined at a suitable scale for management decisions, and within practical limitations of survey methods. For example, small ponds may be suitable to be defined as sites (with replicate samples within them) if they can be managed as a unit. Numbers of sites can be calculated to achieve a given standard error on occupancy estimates for a range of possible detection and occupancy probabilities. For example, optimum numbers of replicates are between 2 and 5 where probability of detection is above 0.5. With a probability of detection of 0.5 and a probability of occupancy at 0.8, approximately 35 sites would give a standard error of 0.1, whereas to achieve a standard error of 0.075 would require 64 sites. A further consideration in sampling large lakes is the uneven distribution of eDNA along the vertical plane of the water column and depth should be standardised or included as a covariate when designing lake sampling strategies (Pont et al., 2021c). Further environmental data (waterbody physicochemical characteristics) also need to be recorded or obtained from Earth Observation sources to use as covariates of occupancy in models. Sites should be concurrently monitored using conventional and DNA-based methods, at least until sufficient comparisons can be made. To account for temporal variability in species presence, either multiple visits within a short timeframe could be used to produce singleseason occupancy models or more complex, multi season models could be implemented. The outcome would be a model that would be applicable across the geographic range that was initially sampled. The model could be extended geographically over time with additional follow-up studies, eventually leading to a national sampling strategy that could be applied to lentic systems for fish or amphibians that would ensure a desired level of confidence in detection as well as accounting for physicochemical variations that are known to influence detection probabilities.



Development of sampling strategies to estimate the location of eDNA sources in lotic systems

Observations and experiments show that eDNA can be transported from tens to thousands of metres downstream in lotic systems. eDNA appears to behave similarly to fine particulate organic matter and the distance it travels is affected by flow rate, volume, and local environmental conditions. If the location of taxa within a lotic system is important for a given monitoring program (rather than simply having observations that a species occurs within a watercourse or catchment), then models to estimate where the eDNA originated from are essential. Only recently have attempts been made to overcome this challenge. For instance, (Carraro et al., 2020) developed and applied the eDITH (eDNA Integrating Transport and Hydrology) model, which uses hydrological first principles, to sequence read counts for 50 Ephemeroptera, Plecoptera and Trichoptera (EPT) genera, and revealed the role of environmental covariates in driving the spatial distribution of single taxa and EPT diversity across the study catchment. The results are very promising, as the model-predicted low-diversity reaches corresponded to polluted sites. There is an opportunity to trial these recent models and to determine sampling strategies required to effectively apply such models.

Initially this would be carried out intensively on a single catchment (multiple sampling locations at approximately 2.5 km intervals and multiple replicates per location) but it would be expected that the baseline data would inform a less intensive monitoring program. Any taxa can be targeted provided there is conventional data that could be used for verification. For example, if insects were targeted and the eDNA data generated was largely made up of chironomids (this group is frequently well-represented in freshwater eDNA studies), then follow-up conventional surveys would be carried out at numerous sites to assess the predictions made by the model. The outcome would be a sampling strategy for the taxonomic group(s) studied that would predict biodiversity (i.e., the location of taxa detected) throughout an entire lotic system, which could be repeated on an annual basis. By collecting physicochemical and hydrological data, the sampling strategy and model could also be assessed in terms of its power to detect particular impacts (such as point pollution). It is anticipated that a baseline study would need to be conducted on every new catchment being monitored. Although models may be shown to be broadly effective, numerous catchments would be required before this could be judged. Once developed, successful models could also incorporate parameters from site-occupancy/jSDM models, as for lentic systems.

Developing a more cost-efficient composite sampling approach for catchment level survey

In streams and rivers, a minimum of three independent water samples (ranging from 500 mL to 100 L) across the width of the stream/river (left bank, middle channel, right bank) are typically taken at 20-60 locations for comprehensive species monitoring (Broadhurst et al., 2021; Carraro et al., 2021). This could result in as many as 180 eDNA samples being collected from a single catchment, thus monitoring quickly becomes cost prohibitive. Only a few studies have investigated whether the number of samples taken from streams or rivers could be



reduced or pooled to increase the cost-efficiency of eDNA monitoring. Sakata et al. (2020) compared independent eDNA samples of different volumes (from 10 ml to 4 L) taken from the left bank, middle channel, and right bank of a river for fish detection. Fish diversity was lower in samples that were less than 1 L in volume, but there was no difference in community composition between samples from the banks or centre of the river. These results would suggest that one sample per river location may be sufficient for species detection, although other studies have found more samples are necessary at downstream locations (Bylemans et al., 2018; Lyet et al., 2021) compared to samples collected from the nearest stream to a deployed camera trap across a catchment with samples taken only at the base of the catchment. They found that mammal diversity was higher in samples collected at the base of the catchment, and these samples provided more species detections per dollar invested.

One way to reduce cost is to combine multiple water samples before the filtration step. In theory tens or even hundreds of water samples could be combined, and the DNA subsequently captured and analysed. This could drastically reduce the cost of catchment level monitoring. Inherent to this approach is that information on the location of taxa within a catchment is lost or becomes less precise. Nonetheless, for many monitoring programs, this information is not essential (e.g. detection of INNS). There is an opportunity to compare independent samples taken at fine spatial resolution with samples that are pooled to represent a set length/area of the catchment. This would initially require 60+ independent samples along with sets of pooled samples, i.e. 20 pooled samples (each composite sample comprised of three subsamples from 20 locations), 10 pooled samples (each composite sample comprised of six subsamples from two locations), etc. This study would require conventional data for the species under assessment but would be well-suited to single-species detection (PCR/qPCR) approaches in the first instance as pooling many samples together could lead to rare taxa being missed (which would be more likely with metabarcoding). The composite sampling approach could be extended to metabarcoding if validated for single species. Combining multiple water samples would lead to higher than usual volumes, thus high-capacity filters would need to be used. Such filters can pass >10 times the usual volume and result in a seven-fold increase in DNA yield (Peixoto et al. 2020). The outcome would be a cost-efficient composite sampling strategy that would maintain detection probabilities for the taxon/taxa being surveyed at the catchment level. Combining this strategy with eDNA transport modelling would likely need baselining for every new catchment until the method could be further verified.

Developing new primers for high priority taxonomic groups of interest

The range of taxa that are/can be monitored is broad. While eDNA methods for fish and amphibians are proving to be very robust, methods for other taxa are not as well developed. One of the primary faunal groups that are monitored across many habitat types, including freshwater, are invertebrates. Primer sets targeting invertebrates continue to be developed and are being applied to freshwater eDNA samples, but with varying degrees of success. There is an opportunity to further develop primer sets and to assess the range of invertebrate taxa that can be detected, with special reference to the taxa regularly monitored in respective





habitats. The eventual outcome of research into this area would be a tool that could be universally applied across numerous habitats and sample types providing a more comparable approach for monitoring than has previously been feasible. However, it is difficult to predict the cost of such primer development as it will depend on effective primer design and speed of reference database population. Other broad groups common to multiple habitats include bacteria as well as phyto- and zooplankton where similar challenges in primer design exist. In general, there are many opportunities to develop primers for single-species detection for important species and qPCR assays have the additional benefit of being able to provide a measure of relative abundance among samples/sites, which is a requirement of many monitoring objectives. These primers are relatively easy to design.

Opportunities for metabarcoding for ecological assessment of habitats

Develop a reference-based model for assigning samples to an ecological category

There is an opportunity to simplify the number of different survey methods being used to categorise sites according to aspects such as trophic status, ecological health score or water quality index. In the first instance, this would be carried out in lentic systems and the taxa to be targeted would be those that are ubiquitous and diverse - bacteria and microeukaryotes. These two groups are readily detected by a range of existing primer sets. An initial model would be for a local geographic area. It would require intensive sampling of many (i.e., 30+) waterbodies that are pre-classified according to the classification scheme being used. The waterbodies would need to be as close together as possible for the initial model. Each waterbody would need to be sampled extensively (20+ samples) and ideally until the species accumulation curves plateaued. It would be essential to have good representation of waterbodies at either end of the spectrum (e.g. very high and very low quality). The data would be analysed 1) using taxonomy and 2) using DNA sequences (these could be clustered or nonclustered). Analyses could be any form of classification algorithm and would likely include machine learning approaches. The accuracy of the model would be tested by assessing the number of samples that were correctly assigned. The output would be a sampling strategy for lentic waterbodies within the initial study area that would provide a means to placing samples on whichever classification scale was targeted. This could simplify field (and lab) approaches to monitoring waterbodies and could be extended geographically with follow-up studies to become a national programme. There may also be opportunities to assign samples along a continuous gradient (e.g. pollution gradient) rather than a categorical scale, but this would require more sampling effort and would be a follow-on study.

Monitoring of Scottish marine habitats

Scotland includes a diverse range of marine ecosystems, with a Marine Nature Conservation Strategy that outlines Scotland's vision and aims for protecting marine biodiversity. In 2017, Marine Scotland, together with NatureScot and the Joint Nature Conservation Committee (JNCC), developed a Scottish Marine Protected Area monitoring strategy (Marine Scotland, 2017).





Habitats (e.g. horse mussel beds, burrowed mud and maerl beds) and species (e.g. *Arctica islandica, Ammodytes marinus* and *Phoca vitulina*) that are conservation priorities in Scottish territorial waters are identified as Priority Marine Features (PMFs; Planning Scotland's Seas: Consultation on Priority Marine Features, 2013) within the Scottish Marine Nature Conservation Strategy (Planning Scotland's Seas: Consultation on Priority Marine Features, 2013). A series of sampling campaigns have been carried out since 2013 with the aim of documenting the occurrence and distribution of PMFs, and assigning biotopes (Connor et al., 2004).

Current field survey methods include:

- Benthic grab sampling (J. H. Allen, 2014), with sediment samples typically analysed for a range of physico-chemical variables and sieved for morphological analysis of invertebrate macrofauna and occasionally meiofauna.
- Seabed video and still photographic imagery for identification of fauna and substrate, in combination with local bathymetry (Moore, 2013).
- *In situ* scuba diving surveys (video, still photography, and *In-situ* observations) in shallow water areas where sensitive species are present, and disturbance must be minimised (C. Allen et al., 2013).
- Plankton netting and subsequent taxonomic identification using microscopy (Bedford et al., 2020).
- Netting (catch and release) for fish surveys (O'Reilly et al., 2021).
- Baited remote underwater video (BRUV) surveys (Benjamins et al., 2018) and acoustic tagging (Hawkes et al., 2020) for elasmobranch monitoring.
- Passive acoustic monitoring for marine mammals in combination with visual surveys (Palmer et al., 2019).

Observations are recorded of any habitat or species detected that are on the IUCN Red list of Threatened Species (2021 IUCN Red List of Threatened Species, 2021), the OSPAR List of Threatened and/or Declining Species and Habitats (OSPAR Commission, 2008), and the Scottish Biodiversity List (Scottish Biodiversity List v1.4, 2012). Monitoring of invasive non-native marine species is not subject to any comprehensive surveillance or monitoring programme, but assessment is based on the verification of reports and academic work and on specific local monitoring programmes (Scottish Government, n.d.).

Comparative studies have shown that metabarcoding of marine aquatic eDNA samples can return similar or superior data to BRUV surveys (Boussarie et al., 2018), dive surveys (Port et al., 2016b), plankton netting (Deagle et al., 2018), and visual surveys of marine mammals (Valsecchi et al., 2021). qPCR tests for invasive species such as the sea squirt (*Didemnun vexillum*) have been shown to provide efficient surveillance, and the approach is already in use for *D. vexillum* surveys in Scottish water (Matejusova et al., 2021). While in freshwater environments, there is uncertainty in spatial interpretation of results, multiple studies have reported a high level of precision in marine communities, even when under the influence of currents and tides.

Biotic indices based on macroinvertebrate communities (e.g. the Infaunal Trophic Index (ITI), or the AZTI Marine Biotic Index (AMBI)) can be broadly replicated by metabarcoding sieved



macroinvertebrate samples (Cordier et al., 2017; Forster et al., 2019). However, replicating current conventional approaches using bulk invertebrate sample metabarcoding for the identification stage would be challenging owing to the large volumes of benthic invertebrates in the samples. Samples can be many litres in size, thus requiring extensive sieving, homogenisation, and subsampling to process for conventional invertebrate metabarcoding approaches, resulting in a relatively small decrease in both vessel and lab time.

Metabarcoding of sediment samples (which can be processed in a high-throughput workflow) to derive large and taxonomically broad datasets has the potential to identify biological responses to stressors such as organic pollutants (Cordier et al., 2017), and increased concentration of petrogenic hydrocarbons and heavy metals (Mauffrey et al., 2020). Analyses applied to sediment samples commonly target very broad groups such as bacteria (or prokaryotes; e.g. Dowle et al., 2015) and eukaryotes (e.g. Pearman et al., 2020), as well as metazoans (animals; e.g. Lekang et al., 2020) and foraminifera (Cordier et al., 2017), which have been used as indicators of habitat quality.

Opportunities for species inventories and detections

Development of sampling strategies to detect species or sets of species at known probabilities

The majority of eDNA surveys do not estimate detection probabilities at sampling sites. Without these, the probability of a species being present, even if not detected, cannot be modelled. Similarly, it is difficult to estimate the likelihood of false absences. There is an opportunity to build on site-occupancy modelling and jSDM modelling (see section 'DNAbased approaches to biodiversity monitoring') efforts from studies in other habitats. However, in the marine environment, this is still a challenge as eDNA transport has not been well studied and identifying the geographic source of DNA when sampling from transient water is difficult. It would be reasonable to target macrofauna from sediment first as they are abundant, and practitioners are familiar with identifying them. Building models to estimate detection probabilities requires multiple sampling replicates at multiple sites. For marine stations, c. 3-5 grabs per station and 40-50 stations could be used to build site occupancy models. To account for short-term temporal variability multiple visits within a short timeframe would take place to produce single-season occupancy models. The number of grabs depends on the detectability of the target species. Sites need to be concurrently monitored using conventional and DNAbased methods, and environmental data (physicochemical characteristics) needs to be recorded. The outcome would be a model that would be applicable across the geographic range that was initially sampled. The model could be extended geographically over time with additional follow-up studies, eventually leading to a national sampling strategy that would ensure a desired level of confidence in detection as well as accounting for physicochemical variations that are known to influence detection probabilities.

Developing new primers for high priority taxonomic groups of interest





The range of taxa that are/can be monitored is high. While eDNA (from water) methods for fish and mammals are proving to be very robust, methods for other taxa are not as well developed. PMF species would be most obvious to target but these are a very diverse group. There are already primer pairs available which can amplify DNA of many of these species but not all. In general, there are many opportunities to develop primers for single-species detection for important species, and qPCR assays have the additional benefit of being able to provide a measure of relative abundance among samples/sites, which is a requirement of many monitoring objectives. These primers are relatively easy to design. However, the high number of separate assays that need to be applied to each sample can make this cost prohibitive. Some emerging techniques that could be considered to reduce the cost include microfluidics and High-Throughout qPCR (Hauck et al., 2019; Wilcox et al., 2020). These methods allow for dozens of markers to be combined in a single analysis and overcome the limitations of multiplexing with standard qPCR (which is primarily the number of channels available on a qPCR machine - commonly five). This would not require a substantial sample set but would require substantial laboratory research and development. As noted in other sections, one of the primary faunal groups that are monitored across many habitat types, including marine, are invertebrates. Primer sets targeting invertebrates continue to be developed and are being applied to marine eDNA samples, but with varying degrees of success. Other broad groups common to multiple habitats include bacteria as well as phyto- and zooplankton, where similar challenges in primer design and validation exist.

Opportunities for metabarcoding for ecological assessment of habitats

Develop a reference-based model for assigning samples to an ecological category

The AZTI Marine Biotic Index or AMBI (Borja et al., 2000) is a biotic index that indicates the pollution status of a site. Related to AMBI are gAMBI, based on 'genetic' macrofaunal metabarcoding data (based on metabarcoding macrofauna data; Aylagas et al., 2014), and microgAMBI, based on 'genetic' bacterial metabarcoding data ((based on metabarcoding of bateria data; Aylagas et al., 2017). Both these DNA-based indexes show great potential but have yet to be accepted by regulators. (Borja et al., 2000) There is an immediate opportunity to test and develop these further in the Scottish context. This would involve collecting sediment samples from multiple locations (ideally with available pollution data). The macrofauna portion of the sample would be separated by sieving. The following analyses would be undertaken: 1) macrofauna – morphological; 2) macrofauna – metabarcoding; 3) sediment – metabarcoding for macrofauna; 4) sediment - metabarcoding for bacteria (conditions and quality condition boundaries adjusted based on location). Taxonomic data from all sources would be used to generate their respective index scores. The criteria for success would be the proportion of agreement between gAMBI/microgAMBI and the widely accepted AMBI, as well as agreement with any other pollution data from the sampled sites. This would require approximately 30 sites with three replicate grab samples per site, ideally from similar habitat/biotope types. The ideal outcome would be a sediment-based eDNA approach that could assign samples to an ecological score that aligns with AMBI. Initially, this would be





limited to the habitat type and geographic location of the pilot study but over time could be extended to other habitat types and regions. Optionally, water samples could also be collected to examine whether similar results are obtained. If this were the case, it could reduce the field sampling effort required for monitoring programs.

gAMBI/microgAMBI are categorical indices, so if the study included representative sites from either ends of the index, then the data could be used to inform reference-based models. As in other habitats, data analysis could be any form of classification algorithm and could make use of machine learning approaches. The accuracy of the model would be tested by assessing the number of samples correctly assigned to the target index, which could be AMBI, or another health index, or a habitat classification/biotope. The output would be a sampling strategy for habitats within the initial study area that would provide a means to placing samples on whichever classification scale targeted. This could simplify field (and lab) approaches to monitoring marine stations and could be extended geographically with follow-up studies to become a national programme.

Monitoring of Scottish woodland habitats

Scotland's forests and woodlands are important natural assets. Effort has been made to increase forested area in Scotland, while promoting sustainable development and enhancing the environment. Hence, comprehensive standards for forest management have been put in place and the Scotland Forestry Strategy 2019 to 2029, was published in 2019, setting a 10-year framework for action (Scottish Government, 2019). At the national scale, long-term monitoring programmes such as the Census of Woodland (Forestry Commission, 1979) and the National Forest Inventory have assessed the size, distribution, composition, and condition of Scottish woodlands over time. Moreover, the Native Woodland Survey of Scotland has mapped the location, extent, type, composition, and state of all Scottish native and near-native woodlands (Forestry Commission Scotland, 2014b).

In general, monitoring in terrestrial habitats is based on percentage cover of plant species and the presence/abundance of both native and non-native animal species, including many classes of vertebrates and invertebrates. Less attention is usually given to below-ground diversity monitoring although it is well understood that bacterial and fungal diversity and composition is essential for ecosystem functioning. Soil state, soil chemical and physical properties and some soil biota are monitored in some woodland locations, at a national scale in Scotland, by the National Soil Inventory of Scotland Survey and Countryside Survey. Moreover, the European-wide BioSoil project, which involved 69 sampling locations in Scottish woodlands, included both soil monitoring and biodiversity surveying of vascular plants. Other taxa also systematically monitored in woodlands in Scotland include invertebrates, birds, and mammals. Plant, bird, and mammal surveys are often undertaken as part of a walkover survey by qualified ecologists. Invertebrate surveys generally employ malaise or pitfall traps, or net transects for sampling, followed by morphological identification of the specimens collected. Specific groups, such as butterflies, are targeted by national initiatives as the UK Butterfly Monitoring Scheme (Brereton et al., 2020).



Site condition monitoring of SSSIs and SACs takes place periodically as part of the national Common Standards Monitoring scheme (Artz et al., 2014). This includes invertebrate monitoring by active hand searches and pitfall traps, among other conventional methods, followed by taxonomic identification using microscopy.

Some key threats to biodiversity within Scotland's native forests have been identified by the Native Woodland Survey of Scotland, with browsing and grazing from herbivores considered the main threat with invasive species, non-native tree species, and climate-change (Forestry Commision Scotland, 2014a) also identified.

Several DNA-based approaches have been developed over the years for the detection of targeted species (protected species, invasives, pathogens or pests) from various types of samples (i.e., droppings, tissue, bloodmeals). Such targeted species detection usually comprises a specific survey developed for a particular species or feature of woodland habitats, primarily elusive species for which detection or identification is difficult (Bohmann et al., 2014) or for wildlife disease detection (e.g. Brunner, 2020). DNA-based broad biodiversity surveys are usually based on metabarcoding. Though several types of samples may be analysed to provide data on specific groups (e.g., food webs from droppings and bloodmeals, browsing patterns from herbivore saliva), most community characterisation studies focus on bulk invertebrates or soil samples.

DNA-based analyses of bulk invertebrates may provide similar data as conventional methods, and have been used successfully for assessing community response to forest stand composition (e.g. Barsoum et al., 2019) and along restoration gradients (e.g. Lynggaard et al., 2020). It is also feasible to obtain additional data from bulk invertebrate samples, namely, vertebrate species composition, which may be of added value to conventional vertebrate surveys (Lynggaard et al., 2019). Nevertheless, sampling bulk invertebrates is still an invasive approach that requires high sampling effort (Kirse et al., 2021).

Metabarcoding analyses of soil samples has been considered a scalable method for biomonitoring forest systems (Porter et al., 2019). Within recent scientific literature, forest soil arthropod community compositions have been surveyed using DNA extracted directly from soil, as a more scalable, less invasive approach, which removes bias associated with methods of extracting bulk arthropods from soil (Kirse et al., 2021; Oliverio et al., 2018; Rota et al., 2020). Comparisons between traditional morphology-based assessments and metabarcoding of soil samples have yielded comparable estimates of community diversity and composition (Oliverio et al., 2018).

The DNA-based analysis of soil samples has also been applied for monitoring other taxa such as plants (Fahner et al., 2016), vertebrates (Leempoel et al., 2020), fungi (Danielsen et al., 2021) and bacteria (Heo et al., 2020).

Several studies assessed differences in soil microbial diversity between forest types on a regional (Heo et al., 2020) and global scale (Bastida et al., 2021), and in some situations incorporating temporal variability (Heo et al., 2020; Isobe et al., 2018; Shigyo et al., 2019). It has





also been recognised that the biodiversity of soil microbial communities can often reflect that of the above ground vegetation, with diverse tree communities increasing the diversity of soil microbial communities (Wu et al., 2019). Soil microbial community responses to environmental change (e.g., pollution) are more rapid than changes in the plant communities. Hence, metabarcoding of soil fungi and bacterial communities has been considered a good approach for monitoring vegetational shifts in forest ecosystems, as these communities respond to changes in plant communities (Danielsen et al., 2021; Shao et al., 2019) and can act as early warning systems indicating potential for future vegetation shifts due to the effects of climate change (Heo et al., 2020).

Some research studies in Scotland's woodlands have used molecular methods. For example, the impact of herbivory in woodland has been assessed by comparing soil chemical and physical parameters, soil fungal:bacterial ratio phospholipid fatty acid profiling and vegetation cover inside and outside of long-term exclosures (Mitchell et al., 2019). In Ireland, the CréBeo Soil Biodiversity Project pilot study utilised DNA metabarcoding of soil fungi and bacteria alongside traditional survey methods for ants, nematodes and earthworms, sampling 61 sites across the country, to provide a baseline assessment of the biodiversity and distribution of the soil biota (Schmidt et al., 2005).

Measuring habitat function and resilience poses additional challenges and research is still in an earlier stage. Although some studies have already focused on understanding community responses to perturbation using eDNA (e.g. bacterial communities using OTU based phylogenetic approaches, Liu et al., 2017b), further work is required for the implementation of these approaches for biodiversity reporting.

Opportunities for species inventories and detections

Developing new primers for high priority taxonomic groups of interest

The number of taxonomic groups being routinely monitored in woodland habitats is limited. DNA methods allow us to expand the range of taxa that can be surveyed, while reducing the reliance on taxonomic specialists for specimen identification. While there is one generally accepted genetic marker for bacterial and fungal barcoding respectively, other taxa such as plants and invertebrates are less well standardised. As noted in other habitat sections, there is an opportunity to further develop primer sets to assess a range of taxa. Initial testing using soil DNA samples for mammal surveys at Scottish woodland sites has provided promising results. This could be further developed and optimised to target endangered mammals, such as red squirrel and mountain hare.

Of particular interest to practitioners in this habitat is the detection of plant pathogens and pests. This is a taxonomically diverse group including invertebrates, bacteria, fungi, viruses, oomycetes, and others. Development of either single species or metabarcoding primers to reliably detect these species would be greatly beneficial for biomonitoring in this habitat, particularly in a commercial forestry context. Single species assays could be developed to





target key taxa of concern, such as *Phytophthora ramorum* (pathogen that causes sudden oak death) and *Ips typographus* (European spruce bark beetle). Routine screening with these assays could provide early warning systems to identify areas of potential outbreaks that warrant further investigation. A challenge with designing surveys for pests/pathogens is that the life-history of each one needs to be well understood to enable informed sampling regimes – they will be active during different seasons and there are many options of where/how to sample – soil, malaise traps, vegetation (plant tissue has been successfully used to assess invertebrate communities for example), standing/flowing water, air etc. So, while there are clear opportunities, it is likely that a wide range of monitoring study designs need to be implemented for comprehensive assessments.

Opportunities for metabarcoding for ecological assessment of habitats

Development of an experimental soil monitoring sampling strategy

One of the key differences between terrestrial and aquatic habitats is the relative ease by which experimental designs can be established to examine the impact of different pressures and interventions, for example, grazing being controlled with fencing, invasive plants being controlled by removal/herbicides, invasive mammals being controlled by trapping etc. There is an opportunity to develop a soil biomonitoring strategy based around experimental units. The initial target taxa would most likely be those most commonly characterized in soil: bacteria and fungi and potentially invertebrates. A key challenge is deciding on an appropriate soil sampling design. There is a lack of standardization regarding soil sampling, relating to plot size, number of subsamples per plot and sampling depth. We are not aware of any detailed studies that test different sampling strategies for DNA-based monitoring of soil communities at scale. One study found that the number of sub-samples required to obtain an accurate representation of forest fungal communities depends on forest type (Adamo et al., 2021) so sampling strategies may need to be adapted depending on habitat. Within forest soil, vertical niche partition of microbial communities has been associated with changes in soil properties, with the most complex communities shown to inhabit the upper mineral soil layer (Mundra et al., 2021). An initial study would need to be carried out in an area which encompasses the experimental factor with several degrees of impact (e.g., control, low, medium, high) incorporating as many sampling points as possible. The data would then be subjected to power analyses to determine the most efficient method for assessing the soil communities and detecting change across the levels of impact. It should be noted that the outcome is likely to be an additional tool to assess the effects of known impacts on biodiversity rather than a replacement of current methods to provide early detection of those impacts. The experiment would initially inform local monitoring efforts but could be gradually extended to other areas and woodland habitat types through repetition at different sites.

Finally, further work is also required on the standardization of laboratory methods. For example, there is no standard amount of source material used (size of soil sample) for DNA





extraction, which can alter the number of species detected (Dopheide et al., 2019) and/or the taxonomic composition of a sample (Kirse et al., 2021).

Developing a reference-based model for assigning samples to an ecological category

As in other habitats, eDNA-based approaches could also be used to create reference-based models in woodland systems. A number of samples (30+) could be collected from categories across a degradation, pressure or impact gradient, or habitat category. There would also be an opportunity to use the woodland SSSI favourable status sites as a reference index. If models were shown to align with SSSI assessments this would allow eDNA methods to replace the plethora of surveys currently used to assess this status and could help improve the objectivity and coverage of SSSI assessments. The data would be analysed 1) using taxonomy and 2) using DNA sequences (these could be clustered or non-clustered).

Scaling biodiversity data

Terrestrial habitats, including broad habitat types, can be characterised using Earth Observation data. Access to these extensive data layers allows us to combine DNA-based point samples with Earth-Observation data layers to interpolate a continuous map of biodiversity distribution across a landscape (Bush et al., 2017). There is an opportunity to trial this in the Scottish context by collecting eDNA data across large area within a habitat type. This approach can be used to identify areas of high and low biodiversity value, and by including areas already considered to be in favourable condition, these maps can be used to identify areas that may require management intervention. In the first instance this could be carried out across an area of several km² to assess the efficacy. This would require 100+ samples.

Monitoring of Scottish peatland habitats

Peatland habitats cover over 20% of Scotland's land area. Healthy peatlands, and extensive wetlands in general, are biodiversity rich areas that provide good water quality and carbon storage (https://www.crew.ac.uk/publication/moderating-extremes-water-availability-review-role-functioning-wetlands). Many Scottish peatland areas are degraded, due to high intensity grazing and the installation of drainage channels and require suitable management and restoration. Hence, in 2015, the first Scotland's National Peatland Plan was set (Scottish Natural Heritage, 2015), which sets out a long-term action plan for the restoration, protection and management of Scottish peatlands to preserve the ecosystems services provided by these habitats (Scottish Natural Heritage, 2015).

Peatland monitoring has been in place since the mid-20th century as part of large-scale soil surveys, including the Scottish Peat Surveys, the National Soil Inventory of Scotland database, and the Countryside Survey. In these surveys, data on the location, extent, depth, and vegetation of peatlands is collected across Scotland. The status of Scottish peatlands were assessed in 2011 by the Joint Nature Conservation Committee (JNCC; JNCC, 2011) using data collected from peat survey maps, Forestry Commission site survey reports and the National Soil Inventory of Scotland. Several ongoing monitoring projects are currently taking place within Scottish peatlands, with a particular focus on monitoring the success of the Royal Society for the Protection of Birds Forsinard peatland restoration using unmanned aerial



vehicle data, and assessing the influence of drought on vegetation, peat depths, soil organic matter composition and fungal and insect communities (The James Hutton Institute, 2016a). Soil monitoring activities in peatlands are supported by the Soil Monitoring Action Plan which aims to co-ordinate future systematic soil monitoring across Scotland tailored to different habitat types by improved communication between stakeholders (JNCC, 2011; Scottish Natural Heritage, 2015; The James Hutton Institute, 2016b).

For peatland monitoring and management, it is important to understand how changes in environmental conditions and management practices can impact on microbial communities and the implications this has for soil functions relating to carbon storage. The diverse metabolic potential of bacteria, archaea and fungi drive the turnover of organic carbon and nutrient cycling; however, there is still a lack of understanding as to how the soil biota influence peat functioning (Andersen et al., 2013). Bacterial communities are considered as important drivers of change in peatlands, but it is still not clear how microbiota influences peatland function and affects resilience and recovery from pressures (Ritson et al., 2021).

The use of DNA-based techniques in peatlands is less well-established than in other terrestrial ecosystems. However, some studies have already applied molecular methods to assess microbial diversity and to study the fungal community structure in peatlands. Thus far, metabarcoding of microbial communities in peat soils has been used to assess changes in community composition in response to managed and natural peat restoration (Elliott et al., 2015), rewetting (Weil et al., 2020), drainage (Urbanová & Bárta, 2016), peat type (St. James et al., 2021), climate (Seward et al., 2020) and depth (Asemaninejad et al., 2017). Microbial community composition and function are likely to be more important drivers of change in peatland ecosystems compared to other temperate ecosystems due to depth, waterlogging and low nutrient availability reducing the influence of plant communities (Ritson et al., 2021); however, within surface layers of peat, vegetation still has a strong influence on below-ground microbes (Elliott et al., 2015).

Soil fungal community structure changes in Scotland have been assessed in peatland across a chronosequence of restoration sites using an RNA-based method. 454 sequencing of the internal transcribed spacer region (ITS), revealed differential responses of active fungal functional groups to restoration (The James Hutton Institute, 2016a). During the literature search, no studies were found to have used metabarcoding to assess invertebrate communities in peat.

Opportunities for species inventories and detections

Developing new primers for high priority taxonomic groups of interest

As noted in other sections, one of the primary faunal groups that are monitored across many habitat types, including peatland, are invertebrates and there is an opportunity to further develop primer sets and to assess the range of invertebrate taxa that can be detected, with special reference to the taxa regularly monitored in the respective habitats.

Opportunities for metabarcoding for ecological assessment of habitats



Development of an experimental soil monitoring sampling strategy

The use of DNA-based monitoring provides an opportunity to better evaluate peat community composition, diversity, and functioning across large spatial scales, and how these can be affected by changes in land management. Similar to woodlands, some initial work is required to determine appropriate sampling designs. Additional sampling considerations specific to peatlands, are outlined below.

Microbial communities and their decomposition processes in peatlands show strong vertical stratification (Lamit et al., 2017; X. Lin et al., 2014). This may be also true for other taxonomic groups. Some studies have limited sampling to the surface acrotelm/mesotelm layer of peat (Elliott et al., 2015), whilst others take deeper soil cores to divide into three or more depths for analysis (St. James et al., 2021; Wang et al., 2021). Further studies are still needed to understand vertical stratification of eDNA in peatland soils.

It is likely that in cold, wet peat habitats, DNA degradation is relatively slow compared to other habitats although no studies were found which explicitly tested this. This could have implications when surveying for presence of specific species as the DNA may still be detected when the species is no longer there. From work that we have done previously in subtropical peat we suspect that any strong recent changes in community compositions would still be detected with metabarcoding but further testing of this is required. The application of RNA methods could also help clarify this issue (see initial section on DNA-based approaches for biodiversity reporting). There is an opportunity to target restored peat areas and contrast them to degraded areas to elucidate the power of eDNA to assess community change.

Developing a reference-based model for assigning samples to an ecological category

As in other habitats, DNA-based approaches could also be used to create reference-based models in peatland systems. A large number of samples (30+) could be collected from categories across a degradation gradient or habitat category. The data would be analysed 1) using taxonomy and 2) using DNA sequences (these could be clustered or non-clustered). Such an approach could be used to evaluate the success of restoration works or responses to changes in land management. Reference sites would need to be areas known to be in good condition.

Scaling biodiversity data

As with other terrestrial habitats like woodlands, there is the opportunity to exploit Earth Observation data to create scalable monitoring programs. However, sampling work would need to focus on identifying if DNA-based monitoring produces data that is correlated with peatland habitat variables that can be monitored via remote sensing. Experiments to determine sampling intensity and appropriate taxonomic groups to target would also be needed. Earth Observation data in peatland has the potential to classify sites as being degraded. The combination of eDNA metabarcoding and Earth Observation data could provide continuous biodiversity maps that would go a step further by providing finer scale information within restored areas that are of particular biodiversity value. This would require 100+ samples across several km².



Conclusions

DNA-based technology is developing at a fast pace and shows immense potential for scalable biodiversity monitoring applications, use in decision-making, guiding action, and informing the transformative change needed to help reverse the current global biodiversity crisis. DNA-based methods have application in species detection, community characterisation, and assessing community function. Methodological advancements such as automated collection and analysis of samples, and novel sequencing technologies, will become more accessible as costs decrease, helping to increase the potential applications of DNA-based biomonitoring across agencies and sectors. Moreover, sophisticated data analytics, such as machine learning, will continue to unlock a new generation of metrics that go beyond relying on the proxy of a few indicators species and begin to characterise the inherent biological signatures of healthy and resilient ecosystems.

The CBD post-2020 Global Biodiversity Framework, which aims to drive action to stabilize biodiversity loss by 2030 and promote the recovery of ecosystems by 2050, will be finalised at Kunming in 2022. A monitoring framework has been developed to measure progress towards the CBD goals and targets. While this framework does not mention the use of DNA-based tools specifically, there is scope for incorporation of DNA-based methods into the National Biodiversity Strategy and Action Plans and many government and non-government agencies across the world are already applying DNA-based approaches.

Through the review of available DNA-based techniques and biodiversity reporting needs, three key strategies for incorporating DNA-based methods into reporting were identified: enhancing current indicators, calibrating existing indicators, and creating new indicators.

Strategies that use eDNA monitoring to enhance existing metrics (e.g. by improving species detection) have been subject to a high research effort, with some methods already validated and adopted, or in a stage in which implementation would be straightforward. The other strategies, calibration, or creation of new indicators are in variable stages of development, with more research required for validation.

As part of their commitment to protecting and enhancing biodiversity, the Scottish Government has been working on developing metrics that better characterise ecosystem health by monitoring condition, function, and resilience. A suite of indicators has already been developed, but DNA-based tools can contribute further to improving the measurement of different aspects of ecosystem health.

This report has presented opportunities for integrating DNA-based methods into ecosystem health monitoring and outlined some of the sampling considerations and development needs marine, freshwater, woodland, and peatland habitats in Scotland. The next stage of the project will look to test some of these opportunities, ultimately informing guidance on the most





significant opportunities for DNA-based monitoring approaches to be implemented in Scotland. This will consider where efforts to enhance, calibrate or create new metrics will be most relevant and cost-effective.

The process of strategically reviewing the potential applications of DNA with respect to global reporting frameworks has revealed significant opportunities for innovation of existing monitoring programs and indicates growing support among both public and private sectors. With Scotland's leadership, there is clear opportunity to consider the evidence provided for integration of DNA-based methods into biodiversity monitoring and reporting approaches at the national scale. Embracing DNA-based monitoring technology enables the development of DNA-based methods that can meet the biodiversity and ecosystem condition measurement challenges of the future. This will help set a clear precedent for other countries to follow.


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Glossary

ASV	Short for Amplicon Sequence Variant. Refers to unique DNA sequences retrieved from high-throughput sequencing analyses. These sequences are obtained after bioinformatic processing (see below), where erroneous sequences generated during PCR and sequencing are removed.
Bioinformatics	Refers to a data processing pipeline that takes the raw sequence data from high-throughput sequencing (often 20 million sequences or more) and transforms it into usable ecological data. Key steps for metabarcoding pipelines include quality filtering, trimming, merging paired ends, removal of sequencing errors such as chimeras, clustering of similar sequences into molecular operational taxonomic units (OTUs; each of which approximately represents a species), and matching one sequence from each cluster against a reference database. The output is a taxon-by- sample table showing how many sequences from each sample were identified as each taxon.
Biotope	An area of uniform environmental conditions providing a living place for a specific assemblage of plants and animals. the habitat together with its recurring associated community of species, operating together at a particular scale
BQE	Short for Biological Quality Elements. Refers to taxa considered as indicators of the environmental quality of a targeted habitat. Applied in the scope of Water Framework Directive monitoring, among others.

P1 REPORT	NatureMetrics 2022
CRISPR-Cas	CRISPR-Cas is a genome editing tool. This system is based on two molecules that introduce change into DNA: an enzyme called Cas9 (that acts as molecular scissors, cutting DNA at specific locations in the genome) and a piece of RNA (guide RNA – gRNA – a pre- designed RNA sequence located within a longer RNA scaffold, which binds to DNA and guides Cas9 to the correct cutting location). The damage caused to DNA will induce a repair action that introduces change.
DNA capture	Sequence capture technology allows the targeted enrichment of specific regions of the genome. However, instead of using primers, this technique used DNA 'baits' (sequences complementary to the genome that hybridize with the DNA allowing it to be enriched and sequenced). The baits 'capture' the DNA sequence in mixed samples allowing an efficient sequencing of several genes in the same analyses, without the need to sequence to whole genome, and therefore increasing cost-efficiency.
eDNA	Short for 'environmental DNA.' Refers to DNA deposited in the environment through excretion, shedding, mucus secretions, saliva etc. This can be collected in environmental samples (e.g. water, sediment) and used to identify the organisms that it originated from. eDNA in water is broken down by environmental processes over a period of days to weeks. It can travel some distance from the point at which it was released from the organism, particularly in running water. eDNA is typically present at low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.
Habitat Condition	The state of a habitat with regards to its appearance, quality, health, and ecosystem functioning.
high-throughput sequencing	Technology developed in the 2000s that produces millions of sequences in parallel and enables thousands of different organisms from a mixture of species to be sequenced at once. Various technologies exist to do this, but the most commonly used platform is the Illumina MiSeq. Also known as Next-Generation Sequencing (NGS) or parallel sequencing.
inhibitors/inhibition marker	Naturally occurring chemicals/compounds that cause DNA amplification to fail, potentially resulting in false negative results. Common inhibitors include tannins, humic acids, and other organic compounds. Inhibitors can be overcome by either diluting the DNA (and the inhibitors) or by additional cleaning of the DNA, but dilution carries the risk of reducing the DNA concentration below the limits of detection. At NatureMetrics, inhibition is removed using a commercial purification kit. A specific sequence of DNA at a known location in the genome.

P1 REPORT	NatureMetrics 2022
metabarcoding metagenomics	Refers to identification of species assemblages from community DNA using barcode genes. PCR is carried out with non-specific primers, followed by high-throughput sequencing and bioinformatics processing. Metabarcoding can identify hundreds of species in each sample, and 100+ different samples can be processed in parallel to reduce sequencing cost. Refers to the study of genomes from a mixed sample (sample containing genetic material of several taxa).
OTU	Short for Operational Taxonomic Unit, which is used to classify a group of closely related individuals. For prokaryotes, these groupings are defined using the DNA sequences and their similarities (97% similar to one another). OTUs are thought of as distinct species and unique labels are used when taxonomic identification cannot be assigned.
PCR	Short for Polymerase Chain Reaction. A process by which millions of copies of a particular DNA segment are produced through a series of heating and cooling steps, known as an 'amplification' process. One of the most common processes in molecular biology and a precursor to most sequencing-based analyses.
primers	Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by PCR. They can be designed to be totally specific to a particular species (so that only that species' DNA will be amplified from a DNA sample), or to be very general so that multiple species' DNA will be amplified. Good design of primers is one of the critical factors in DNA-based monitoring.
qPCR	Short for quantitative PCR. Refers to a technique used for measuring DNA quantities using PCR (see above).
Reference database	Over time, the DNA sequences of many species have been compiled into publicly accessible databases by scientists from around the world. These databases serve as a reference against which unknown sequences can be queried to obtain a species identification. The most commonly accessed database is NCBI (National Center for Biotechnology Information), which is maintained by the US National Institute of Health. Anyone can search for DNA sequences at https://www.ncbi.nlm.nih.gov.
richness	Refers to the total number of taxa within a sample.
sequences	A DNA sequence is made up of four nucleotide bases represented by the letters A, T, C and G. The precise order of these letters is used to compare genetic similarity among individuals or species and to identify species using reference databases. In high-throughput sequencing analyses (e.g. metabarcoding), many identical copies of the same sequence are obtained for each species in the sample.
taxon (s.) / taxa (pl.)	Strictly, a taxonomic group.



taxonomy

- species (s./pl.) A group of individuals capable of interbreeding. This is the most important taxonomic unit defined by scientists and the population trends of individual species are a key indicator in judging the effect of conservation programs. Related species are grouped together into progressively larger taxonomic units, from genus to kingdom. *Homo sapiens* (human) is an example of a species.
- genus (s.) / genera (pl.) A group of closely related species. Each genus can include one or more species. *Homo* is an example of a genus.
- family (s.) / families (pl.) A group of closely related genera. *Homo* sapiens is in the family Hominidae (great apes).
- order (s.) / orders (pl.) A group of closely related families. *Homo sapiens* is in the order Primates.
- class (s.) / classes (pl.) A group of closely related orders. *Homo* sapiens is in the class Mammalia.

Appendices

Appendix 1: Stakeholder Engagement

In the initial stages of the project, several stakeholders (18 institutions) were contacted to ensure the delivery of relevant and robust outputs (Appendix 1.1). An initial meeting was held on May 4th, 2021, to introduce the project to stakeholders and to discuss any initial insights that attendees had. The meeting included a thematic breakout room discussion focusing on the habitats/groups of habitats which Phase 2 will focus: freshwater, marine, and peatland/forest. The meeting program, with the discussion points explored by each working group, is presented in Appendix 1.2.

Following the initial stakeholder meeting, all attendees were sent an optional questionnaire (see Appendix 1.3 for details on the questions addressed), allowing them to put forward any additional suggestions and concerns. The questions were habitat oriented, considering the targeted habitats of the project. The questionnaire received 10 responses, covering all targeted habitats, but with more responses focusing on forest habitats.

Follow up meetings were then held with specific stakeholders to refine ideas and suggestions, and to ensure continued stakeholder engagement throughout.

Institution	Contact
SG-Marine Scotland Science.	Philip Boulcott
SG-RESAS	Helen Jones
Scottish Forestry	Colin Edwards
Forestry and Land Scotland	Kenny Kortland

Appendix 1.1. Stakeholders contacted in the initial stages of the project



Scottish Environment Protection Agency	Willie Duncan	
Scottish Environment Protection Agency	Andy Taylor	
NatureScot	Colin Bean	
Environment Agency	Kerry Walsh	
Forest Research UK	Nadia Barsoum	
SG-Marine Scotland Science	Iveta Matejusova	
Loch Lomond and The Trossachs National Park	Alan Bell	
Edinburgh University	Rob Ogden	
Moredun Research Institute	Keith Ballingall	
BioSS	Nick Schurch	
James Hutton Institute	Andrew Taylor	
University of St Androws (MASTS	David Paterson	
Oniversity of St Andrews / MASTS	Rebecca Kinnear	
WSP	Tom Butterworth	
Natural England	Andrew Nisbet	
The Biodiversity Consultancy	Malcolm Starkey	
JNCC	Paul Woodcock	

Appendix 1.3. Questionnaire sent to stakeholders

DEVELOPING HABITAT SCALE DNA MONITORING

* Required

1. Your name *

 Please select a habitat for this questionnaire. If you have knowledge of more than one habitat - please specify which one you are referring to in the different sections of the questionnaire. *

○ Marine

O Peat/upland

- ⊖ Forest
- O Freshwater
- O Multiple habitats

3. Are you aware of any active monitoring programs for this habitat within the Loch Lomond & The Trossachs National Park? Please list and provide relevant links. If possible, please provide details for each program (taxa

Please list and provide relevant links. If possible, please provide details for each program (taxa monitored, methods used, organisation responsible)

4. Are you aware of any other active monitoring programs for this habitat within Scotland?

Please list and provide relevant links. If possible, please provide details for each program (habitat, taxa monitored, methods used, organisation responsible)



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5. What are the key impacts that are important to the habitat (e.g. invasive species, land-use change, pollution, over exploitation, climate change). Can you suggest sites that could be used as examples of impacted and non-impacted areas for these drivers? For example, polluted v non-polluted water.

6. In your opinion, which types of subhabitat (e.g.flame shell beds, lochs) would be of particular interest for this project?

- 7. In your experience, what are some of the key taxa/species currently measured in this habitat in Scotland? Please enter a list of key taxa
- 10. For this habitat type, do you think it is more important to be able to use eDNA for identification of key taxa in an environment (e.g. invasive or rare species) or for broader biodiversity assessment with fewer species identified but allowing for the use of health indices? *

	Essential	Very high	High	Moderate	Low
Key taxa	\bigcirc	\bigcirc	\bigcirc	0	\bigcirc
Health indices	\bigcirc	\bigcirc	0	0	\bigcirc

11. Are there any people or organisations that you would recommend being consulted for this project?

Please provide reasoning for each suggestion

12. Which national or regional strategies and legislation do you consider to be most relevant to this study? Please list and provide relevant links 8. Which taxonomic groups would you consider a priority for this habitat in this study?

	Essential	Very high	High	Moderate	Moderate	Minor
Bacteria	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Fungi	\bigcirc	0	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Mammals	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Amphibians	\bigcirc	0	\bigcirc	\bigcirc	\bigcirc	0
Fish	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Invertebrates	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Bivalves	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc	\bigcirc
Crayfish	0	\bigcirc	\bigcirc	0	\bigcirc	\bigcirc
Eukaryotes (general, low resolution)	0	0	0	0	\bigcirc	0

9. If any, what biotic or ecosystem health indices are currently used for this habitat in Scotland?

13. Any other comments or suggestions for the project? Do you have any specific questions you would like to see answered or tested with DNA based methods for this project?

Microsoft Forms

This content is neither created nor e

ed by Microsoft. The data you submit will be sent to the form owner





Appendix 2: Taxon-free machine learning use cases and limitations Use cases

Most use cases found in the literature are focused on using OTUs from several communities to predict marine biotic indices such as AZTI Marine Biotic Index (AMBI), Norwegian Sensitivity Index (NSI) and Indicator Species Index (ISI) via supervised machine learning (SML). The biotic indices (BIs) are usually converted into ecological quality classes, and model performance metrics measure the correlation between predicted and observed, and the agreement between two ecological quality classifications.

Cordier et al. (2017) used benthic foraminifera OTUs to predict the marine sediment BIs, AMBI and ISI with three different SML approaches. All three SML approaches found similar ecological status of marine benthic environments as obtained from macroinvertebrate morphological analysis.

The study conducted by Cordier et al. (2018) compared the relationship between OTU-based BI and morphological BI for taxonomy-free ML and taxonomy-based calculation, respectively. This study involved training predictive models for five different ribosomal bacterial and eukaryotic markers (Eukaryotes V9, V4 and V1V2 in the 18S gene region). The performance of these models was then used to assess the environmental impact of marine aquaculture. Results for all markers show accurate ML models and they all outperformed taxonomy-based models, e.g. R squared, $R^2 = 0.91$ for Eukaryote V9 ML model compared to $R^2 = 0.12$ to 0.77 for any taxonomy-based model tested in this study. No significant difference was found in the performance of models built using universal eukaryotic or prokaryotic markers. Because of the spatial patchiness of macrofauna, targeting meiofauna or microbial diversity is expected to improve the required representativeness in metabarcoding data for biomonitoring, but this has not been extensively studied in an ecological context.

Frühe et al. (2020) compared SML to the calculation of the indicator values (IndVal) to infer ecological quality (EQ) from BIs. IndVal is an unsupervised approach which is based on ordination methods that are used to cluster sites based on environmental conditions, then associations between species and clusters of sites are calculated. Various combinations of models (both SML and IndVal) using bacterial and ciliate communities were used. The study also found that bacteria provided a more accurate EQ assessment than ciliate eDNA metabarcodes whether SML or IndVal are used. Bacteria may be more sensitive and reactive to specific impacts of fish farms than ciliates. Although bacteria-based SML (in this case Random Forest, RF) has a slightly lower prediction power, RF bacteria shows the best overall performance in terms of training time, sensitivity to noisy data, missing data, uneven sampling of categories and cross-validated accuracy among all tested models, including IndVal bacteria.

Fan et al. (2020) studied the non-linear impact of environmental stressors on freshwater ecosystems. This study used a Support Vector Machine (SVM) algorithm to build models using eDNA metabarcoding and morphological data. The study found that SVM models constructed using eDNA data provided more accurate predictions than those constructed from morphological data, because eDNA provided information from intact, fragmentary, and



historically existing organisms, and so the large amount of data may offset uncertainties due to sample size limitations. However, it is noted that morphological data can provide information on deformations of target organisms which are often found in highly polluted areas.

In terms of creating a DNA-based metric for ecosystem health, Feio et al. (2020) is probably the most relevant study. This study focuses on three ML algorithms, SVM, Multilayer Perceptron (MLP) and K Nearest Neighbour (KNN), that use environmental covariates as predictors and the diatom OTUs as target features. A single model is built for each OTU, so the total number of models (for each ML algorithm) is equal to the number of OTUs. The models were trained with data from 90% of reference sites (40 reference sites), and 10% of reference sites (4 reference sites) were used to test model classifications. The pressure level of sites (rivers) was assessed using seven categorical variables: land use, urban area, river segment connectivity, hydrological regime, channel morphology, and organic contamination. Each variable describes the condition of the site using five classes, ranging from 1 (high quality/minor deviation from natural condition) to 5 (bad/large deviation from natural condition). River Habitat Survey (Environmental Agency, 2003) was performed to classify the sites and detect the reference sites. The outputs of the final model are a list of OTUs for each site and the Observed/Expected ratio (OE). The expected (predicted) value is the probability of occurrence of an OTU that exceeds 50%, so the number of predicted taxa (OTUs) is obtained. The observed value for a site is the number of observed OTUs found at this reference site. An OE value close to 1 indicates a non-disturbed site, while the value tends to zero with increasing disturbance and pressure. A classification scheme is defined based on the range of OE values obtained for training sites. The OE values are then grouped in five ecological quality ratio (EQR) classes following the WFD procedure: High, Good, Moderate, Poor, and Bad. Having a performant model that can predict the EQR, more environmental data collected from locations in-between samples can be fed into the model to interpolate between samples, thus allowing to create an EQR map.

Another use case for taxonomy-free ML is the in-house habitat classification using bacterial OTUs as features. This experiment has been conducted as part of NatureMetrics R&D work. Woodland soil bacterial OTU data and habitat class data from one of our recent projects have been initially used to build the classifier. In this case, the target feature was binary so containing two habitat classes. Both a Random Forest and a Gradient Boosting Machine have been used as classifiers, and both reached a high performance of 96% accuracy. To challenge these models, more data have been used from two other different projects, adding more woodland soil and marine sediment bacterial OTU data. In this case, the classifier was trained with 19 habitat classes, and with the presence of strong habitat class imbalance. Even so, the OTUs can predict the habitat class with a 78% accuracy, which can be probably further increased after some more feature engineering.

Limitations



Using OTUs as features to predict BIs or habitat type can result in high dimensional datasets, e.g., datasets can contain hundreds to thousands of OTUs, so the number of features (columns) can be in the realm of hundreds to thousands, but with significantly fewer sample IDs (rows). Often the performance of ML models increases with the number of features until it reaches a peak, then the performance drops as model complexity increases. This indicates that a considerable number of OTUs can be redundant in the predictions. Moreover, a model that uses high dimensional datasets for training can have difficulties in generalising to different datasets or habitat conditions. Generally, to increases the model's capability to generalise, the requirement of data points (rows) increases exponentially. Given the nature of OTU datasets, it is difficult to satisfy the exponential growth data requirement. This limitation must be generally compensated by feature selection and engineering approaches, followed by more frequent monitoring of model performance for various habitat conditions.

In accomplishing the goal to build a DNA-based EQR metric for ecosystem health, a habitat survey must be undertaken to classify the sites in terms of the deviation level from natural conditions. This will allow us to identify reference sites from which we can then sample.

The performance of taxonomy-free ML models varies as a function of OTU communities used for model training. This is reflected in the different communities having a different prediction power for habitat conditions. Careful analysis is needed to choose the most suitable community to sample for any given taxonomy-free ML problem.

Appendix 3: CBD targets and indicators (of relevance to eDNA derived data)

Post 2020	Description	eDNA of
Target		relevance
Target 1	Ensure that all land and sea areas globally are under integrated biodiversity- inclusive spatial planning addressing land- and sea-use change, retaining existing intact and wilderness areas.	Х
Target 2	Ensure that at least 20 per cent of degraded freshwater, marine and terrestrial ecosystems are under restoration, ensuring connectivity among them and focusing on priority ecosystems.	X
Target 3	Ensure that at least 30 per cent globally of land and sea areas, especially those of particular importance for biodiversity and its contributions to people, are conserved, and integrated into the wider landscapes and seascapes.	Х
Target 4	Ensure active management actions to enable the recovery and conservation of species and the genetic diversity of wild and domesticated species, including through ex situ conservation, and effectively manage human-wildlife interactions to avoid or reduce human-wildlife conflict.	X
Target 5	Ensure that the harvesting, trade, and use of wild species is sustainable, legal, and safe for human health.	Х
Target 6	Manage pathways for the introduction of invasive alien species, preventing, or reducing their rate of introduction and establishment by at least 50 per cent, and control or eradicate invasive alien species to eliminate or reduce their impacts, focusing on priority species and priority sites.	Х
Target 7	Reduce pollution from all sources to levels that are not harmful to biodiversity and ecosystem functions and human health.	Х
Target 8	Minimize the impact of climate change on biodiversity, contribute to mitigation and adaptation through ecosystem-based approaches, and ensure that all mitigation and adaptation efforts avoid negative impacts on biodiversity.	Х
Target 9	Ensure benefits, including nutrition, food security, medicines, and livelihoods for people especially for the most vulnerable through sustainable management of wild terrestrial, freshwater and marine species and protecting customary sustainable use by indigenous peoples and local communities.	X
Target 10	Ensure all areas under agriculture, aquaculture and forestry are managed sustainably, through the conservation and sustainable use of biodiversity, increasing the productivity and resilience of these production systems.	Х
Target 11	Maintain and enhance nature's contributions to regulation of air quality, quality and quantity of water, and protection from hazards and extreme events for all people.	Х
Target 12	Increase the area of, access to, and benefits from green and blue spaces, for human health and well-being in urban areas and other densely populated areas.	
Target 13	Implement measures at global level and in all countries to facilitate access to genetic resources and to ensure the fair and equitable sharing of benefits arising from the use of genetic resources, and as relevant, of associated traditional knowledge.	
Target 14	Fully integrate biodiversity values into policies, regulations, planning, development processes, poverty reduction strategies, accounts, and assessments of environmental impacts at all levels of government and across all sectors of the economy, ensuring that all activities and financial flows are aligned with biodiversity values.	

 Table 3.1: Targets in the draft post-2020 Global Biodiversity framework. Numbering is according to CBD (First Draft of the Post-2020 Global Biodiversity Framework, 2021).

 Post 2020
 Description



Target 15	All businesses (public and private, large, medium, and small) assess and report on their dependencies and impacts on biodiversity, from local to global, and progressively reduce negative impacts, and increase positive impacts.	X
Target 16	Ensure that people are encouraged and enabled to make responsible choices and have access to relevant information and alternatives, to reduce by at least half the waste and, where relevant the overconsumption, of food and other materials.	X
Target 17	Establish, strengthen capacity for, and implement measures in all countries to prevent, manage or control potential adverse impacts of biotechnology on biodiversity and human health, reducing the risk of these impacts.	
Target 18	Redirect, repurpose, reform, or eliminate incentives harmful for biodiversity, in a just and equitable way, and ensure that incentives are either positive or neutral for biodiversity.	X
Target 19	Increase financial resources from all sources and strengthen capacity-building and technology transfer and scientific cooperation, to meet the needs for implementation, commensurate with the ambition of the goals and targets of the framework.	
Target 20	Ensure that relevant knowledge, guides decision-making for the effective management of biodiversity, enabling monitoring, and by promoting awareness, education, and research.	Х
Target 21	Ensure equitable and effective participation in decision-making related to biodiversity by indigenous peoples and local communities, and respect their rights over lands, territories, and resources, as well as by women and girls, and youth.	

Table 3.2: Indicators available for the post-2020 Global Biodiversity monitoring framework, for which eDNA data may be potentially of relevance. Numbering of indicators is according to (Indicators for the Post-2020 Global Biodiversity Framework, 2021). Additional columns indicated those indicators identified as headline indicators (Headline), as well as those common with other global initiatives (SDG and IPBES) or used within the frame of Essential Biodiversity Variables (EBV).

#	Indicator	Headline	SDG	IPBES	EBV
13	Biodiversity Habitat Index			Х	Х
14	Biodiversity Intactness Index			Х	Х
23	Comprehensiveness of conservation of				
	socioeconomically as well as culturally valuable				
	species				
36	Ecosystem Intactness Index				
37	EDGE Index				
43	Forest Landscape Integrity Index				
45	Freshwater/wetland dependent Living Planet				
	Index				
51	Growth in number and representation of records				
	and species in the Living Planet Index database				
52	Growth in Species Occurrence Records Accessible				
	Through GBIF				
54	In situ and ex situ records-based index of within-				
	species genetic diversity				
55	Level of water stress: freshwater withdrawal as a		V		
	proportion of available freshwater resources		^		
58	Living Planet Index (LPI)	Х		Х	Х



59	Marine Trophic Index			Х	Х
61	Mean Species Abundance				
84	Number of invasive alien species in national lists				
	as per Global Register of Introduced and Invasive				
	Species				
90	Ocean Health Index				
102	Percentage of threatened species that are				
	improving in status according to the Red List				
103	Progress towards sustainable forest management		Х		
110	Proportion of known species assessed through				
	the IUCN Red List				
128	Red List Index (RLI)	Х	Х	Х	Х
133	Species Habitat Index	Х		Х	
135	Species Status Information Index				
144	Trends in invasive alien species vertebrate			V	
	eradications			~	
151	Trends in the numbers of invasive alien species		V	V	
	introduction events		λ	Χ	
155	Water Turbidity and an estimate of Trophic State				
	Index				



Appendix 4: Scotland's biodiversity indicators and UK indicators of relevance

 Table 4.1: UK biodiversity indicators (https://jncc.gov.uk/our-work/ukbi-overview-of-trends-2020/), considered in Scotland's Biodiversity Strategy with reference to the indicators for which eDNA is potentially of relevance.

Nur	mber	UK Indicator			eDNA of	
A 1		A I I I			relevance	
AL		Awareness, understanding	and support for cons	servation		
A2		Acting for nature: volunteer	r time spent in conse	rvation		
A3		Value of biodiversity integra	ated into decision-ma	aking*		
A4		Global biodiversity impacts	s of UK economic act	ivity / sustainable consumption#		
A5		Integration of biodiversity of	considerations into b	usiness activity		
B1	B1a	Agricultural and forest - area under environmental	Area of land in agri-	environment schemes		
	B1b	management schemes	Area of forestry land	d certified as sustainably managed		
DD	B2a	Custoinable fisherias	Percentage of marin sustainably	ne fish stocks harvested		
ΒZ	B2b	Sustainable insheries	Biomass of marine to capacity	fish stocks at full reproductive		
B3		Climate change adaptation	h#		Х	
B4		Pressure from climate char	nge (Spring Index)†		Х	
	B5a(i)		Aineallution	Area affected by acidity	Х	
B5	B5a(ii)	 Pressure from 	Air pollution	Area affected by nitrogen	Х	
	B5b	pollution	Marine pollution		Х	
	B6a	- Pressure from invasive	Freshwater invasive	species	Х	
B6	B6b	- species	Marine (coastal) inv	asive species	Х	
	B6c		Terrestrial invasive	errestrial invasive species		
B7		Surface water status			Х	
	C1a	-	Total extent of prot	ected areas: on land		
C1	C1b	Protected areas	Total extent of prot	ected areas: at sea		
	C1c		Condition of Areas/	Sites of Special Scientific Interest	Х	
C2		Habitat connectivity Exper	imental Statistic*		Х	
C3	C3a	Status of European	Status of UK habita	ts of European importance	Х	
	C3b	habitats and species	Status of UK species	s of European importance	Х	
C4	C4a	Status of UK priority	Relative abundance			
	C4b	species	Distribution		Х	
	C5a	-	Farmland birds			
	C5b	- Birds of the wider	Woodland birds			
C5	C5c	- countryside and at sea	Wetland birds			
	C5d	-	Seabirds			
	C5e		Wintering waterbird	ls		
C6	C6a	Insects of the wider	Habitat specialists			
	C6b	countryside (butterflies)	Species of the wide	r countryside		
С7		Plants of the wider country	/side Experimental St	atistic*	Х	
С8		Mammals of the wider cou	ntryside (bats)		Х	

* New index under review; # under development; † not assessed



Table 4.1 (cont.): UK biodiversity indicators (<u>https://jncc.gov.uk/our-work/ukbi-overview-of-trends-2020/</u>), considered in Scotland's Biodiversity Strategy, with reference to the indicators for which eDNA is potentially of relevance.

Number		UK Indicator			eDNA of
					relevance
С9	C9a(i)		Animal genetic resources – effective population size of Native Breeds at Risk	Goat breeds	
	C9a(ii)			Pig breeds	
	C9a(iii)	Genetic resources for		Horse breeds	
	C9a(iv)	food and agriculture		Sheep breeds	
	C9a(v)			Cattle breeds	
	C9b		Plant genetic resources		
D1	D1a	Die diversity and	Fish size classes in the N		
	D1b		Removal of greenhouse		
	D1c	- ecosystem services	Status of pollinating inse	Х	
	E1a	Diadivarsity data for	Cumulative number of re		
E1	E1b	decision-making	Number of publicly acce		
			1km2 resolution or bette		
	E2a	_	Public sector expenditure on UK biodiversity		
E2	E2b	Expenditure on UK and	Non-governmental orga		
			biodiversity		
	Fac	international blouwersity	UK public sector expend		
	LZU		biodiversity		

* New index under review; # under development; † not assessed



Table 4.2. Scotland's biodiversity indicators, considering the different types of indicators defined (Scottish Government, 2020), with reference to the indicators for which eDNA is potentially of relevance. Of the National Performance Indicators, only those for which Nature Scot contributes are shown.

Туре	Number	Scottish Indicator	eDNA of
	503	Abundance of terrestrial breeding birds	Televance
	S04	Abundance of wintering waterbirds	
	S05	The numbers and breeding success of seabirds	
<u> </u>	S06	National Plant Monitoring Scheme*	Х
cato	S07	Native woodland condition*	X
ndic	S08	Terrestrial insect abundance: Butterflies	
ate ii	S09	Trend of moths in Scotland: abundance and occupancy	
Sta	S10	Notified species in favourable conditions	Х
	S11	Notified habitats in favourable conditions	Х
		Site condition monitoring	Х
	S13	River quality	Х
	E01	Attitudes to biodiversity	
nent ors	E02	Spatial greenspace*	
gen cato	E03	Increase people's visits to the outdoors	
nga indi	E04	Involvement in biodiversity conservation*	
ш	E05	Membership of biodiversity non-governmental organisations (NGOs)	
()	NI43	Increase people's visits to the Outdoors	
ial ance ors	NI44	Improve the condition of protected nature sites	Х
tior	NI45	Improve the abundance of terrestrial breeding birds	
Na erfc Ind	NI46	Increase Natural Capital	Х
<u>م</u>	NI48	Reduce Scotland's carbon footprint	
	1	EUNIS Land Cover Scotland	
alth - (II)	2	Proportion of Scotland's Protected Sites in Favourable Condition	
Hea (EH ion	3	Native woodland condition	Х
cors cors	4	High Nature Value Farming in Scotland	
syst licat Cor	5	Terrestrial Breeding Birds index	
Eco Ind	6	Freshwater (WFD monitoring)	Х
	7	Soil carbon (National Soil Map of Scotland)	
l - tion	8	Connectivity (functional connectivity)	Х
EH Funci	9	Acid and nitrogen pollution (habitats at risk from acidification and eutrophication)	Х
	10	Habitat restoration	X
JCe	11	Invasive non-native species	X
illier	12	Climate change adaptation	Х
Res	13	Soil sealing	1
- H	14a	Bryophyte nitrogen	
Ш	14b	Bryophyte summer temperatures	

*To replace previous index but performance needs to be confirmed (Scottish Government, 2020)

P1 REPORT

Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
Marine	Scottish Marine Nature Conservation Strategy	Clyde sea area: Loch Goil and South Arran; Orkney	Priority Marine Feature (PMF). Habitat or species in the IUCN Red list of Threatened Species (2021 IUCN Red List of	Seabed video and still photographic imagery (27 sites in Loch Goil MPA)	PMF distribution and abundance; species diversity indexes	(Moore 2013)
		Clyde sea area	Threatened Species, 2021), the OSPAR List of Threatened and/or Declining Species and Habitats (OSPAR Commission, 2008) and the Scottish	Drop-down camera surveys, sediment grab sampling and scientific diving in conjunction with bathymetric data	PMF distribution and abundance	(C. Allen et al., 2013)
		Clyde sea area: Loch Goil and South Arran	Biodiversity List (Scottish Biodiversity List v1.4, 2012)	Sediment grab sampling	PMF distribution and abundance; species diversity indexes	(Allen 2014)
	Scottish Marine Protected Area (MPA) monitoring strategy	Several locations within Scotland	Juvenile fish	Fish Trap Surveys and stereo baited remote underwater video camera frames	Invertebrate and fish distribution and frequency	European Maritime and Fisheries Fund (EMFF) project "Engaging the fishing industry in marine environmental survey and monitoring" (https://www.gov.scot/collecti ons/emff-project/)
		Sound of Jura	Flapper skate (Dipturus intermedius)	Acoustic receivers	Presence records of the Flapper skate	
		Several locations within Scotland	Seabed habitats and PMF	Drop-down video surveys	Seabed characterization; PMF distribution	
	Whale, Dolphins and Porpoise Surveys	Upper Clyde and sea lochs within LLTNP	Whale, dolphin, and porpoise	Trained Volunteer sightings of the target species	Distribution of marine mammals	(Loch Lomond and the Trossachs National Park, 2011)
Freshwater	Biodiversity Action Plan (BAP). Biodiversity Habitat Audit	LLTNP	UKBAP Habitats	Standard taxa-specific methods undertaken by a wide range of BAP partners to monitor and protect important habitats	List of BAP habitats and priority species; List of Scottish Biodiversity, Wildlife and Countryside Act and Habitats	(Land Use Consultants, 2012; Loch Lomond and the Trossachs National Park, n.d., 2016)

Appendix 5: Scotland's approach to monitoring – examples of approaches in target habitats, with a focus on LLTNP.



Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
					Directive Species; BAP habitat maps	
	Monitoring Atlantic salmon and Sea trout Smolts, Loch Lomond Fisheries Trust/Forth Fisheries Trust	National and within LLNTP (Endrick Water)	Atlantic salmon (Salmo salar) and brown trout (Salmo trutta).	Rotary screw smolt traps	Number of migrating animals. Biometrics and age estimates from scales; time of migration	(Loch Lomond and the Trossachs National Park, 2016)
	The National Electrofishing Programme for Scotland Fish Counts	National, multiple locations within LLTNP		Semi-invasive techniques such as electrofishing and tissue sampling	Distribution and abundance. Biometric data. Juvenile counts	(Loch Lomond and the Trossachs National Park, 2016) (Malcolm et al., 2020)
	SeaMonitor project	Northern Ireland and Scotland (Clyde Sea area –Endrick Water)		Acoustic tagging	Migration route and speed. survival	(Lilly et al., 2020)
	The Redd counting project, Forth Rivers Trust	LLTNP- River Teith		Redd counting	Trout and salmon counts.	(Forth District Salmon Fishery Board, 2019; Loch Lomond and the Trossachs National Park, 2016)
				Habitat walkover surveys	Morphological information on habitat quality in river systems	(Forth District Salmon Fishery Board, 2019; Loch Lomond and the Trossachs National Park, 2016)

P1 REPORT

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Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
	Water Classification Hub; Water Environment Hub. Water Framework Directive (WFD)	Scotland Wide, multiple locations within LLTNP	Freshwater macroinvertebrates	3-minute kick sample to capture a representative sample of benthic macroinvertebrates from riffles in rivers.	Family level and species level data on macroinvertebrates – distribution and abundance. Water quality indices	(Scottish Environment Protection Agency, 2017)
			Aquatic macrophytes (Bryophytes)	Routine monitoring of circa 250 rivers and 40 lochs for macrophytes by trained surveyors; identifications verified by specialists in the Royal Botanic Garden Edinburgh	Presence records of bryophytes	
			Fish	Fishing surveys by SEPA staff. Citizen science. Electrofishing	Presence of fish species in surveyed rivers. Fish diversity	
	Trossachs water vole project	LLTNP	Water vole; invasive predators (mink)	Annual surveys of water vole. Monitoring over 90 mink rafts every two weeks for presence of native mammals. Participation of volunteers and landowners	Presence of water vole colonies and other mammals (water shrew, pine marten). Presence of mink. Monitor progress of water vole introductions	Trossachs water vole project: https://forthriverstrust.org/pro ject/trossachs-water-vole- project/
	British Dragonfly Monitoring Scheme	UK wide	Dragonflies	Routing monitoring by volunteers of a local site using transect and point count surveys	Presence records of dragonflies	https://british- dragonflies.org.uk/recording/ monitoring/
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Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
	National Amphibian and Reptile Recording Scheme (NARRS)	UK wide	Amphibians	Routine monitoring of a pond within an allocated 1 km grid square, using visual searches, netting, torchlight surveys and bottle trapping	Occurrence data for amphibians	http://narrs.org.uk/index.php
	BTO Wetland Bird Survey (WeBS)	UK wide	Internationally important non- breeding waterbirds	Routine monitoring by 3000 volunteer counters that participate in synchronised monthly counts at wetlands of all habitat types, mainly during the winter period	Distribution and abundance data for waterbirds	Frost, T.M., Calbrade, N.A., Birtles, G.A., Hall, C., Robinson, A.E., Wotton, S.R., Balmer, D.E. & Austin, G.E. 2021. Waterbirds in the UK 2019/20: The Wetland Bird Survey. BTO, RSPB and JNCC, in association with WWT. British Trust for Ornithology, Thetford
	BTO Heronries Census	UK wide	Waterbirds	Routine monitoring of heronries by volunteer counters that participate in annual counts of 'apparently occupied nests' of herons, egrets and other colonial waterbirds	Presence records for waterbirds	https://www.bto.org/sites/def ault/files/heronries_summary _2020_final.pdf
	Common Toad and Frog Surveys for PondNet	UK wide	Amphibians	Routine monitoring of a pond within an allocated 1 km grid square in March or April, using visual searches for frogs, toads, or their spawn	Presence records for common toad and common frog	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/frogandtoad/
	eDNA water sample for Great Crested Newts for PondNet	UK wide	Great crested newt (<i>Triturus cristatus</i>)	Monitoring of a pond within an allocated 1 km grid square using eDNA kits provided by	Presence records for great crested newt	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/edna-for-great- crested-newts/





Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
				Freshwater Habitats Trust and processed by SpyGen		
	Full Great Crested Newt survey for PondNet	UK wide	Great crested newt (<i>Triturus</i> cristatus)	Routine monitoring of a pond within an allocated 1 km grid square four times between April and May, using egg searches and torchlight surveys	Presence records for great crested newt	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/great-crested-newt- full-survey/
	Rare Plant and Animal surveys for PondNet	UK wide	One of 10 rare plant or animal species	Varies according to species (see https://freshwaterhabitat s.org.uk/projects/pondn et/survey-options/)	Distribution and abundance data for rare species	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/rare-plants-and- animals/
	Wetland plant survey for PondNet	UK wide	Macrophytes	Routine monitoring of a pond within an allocated 1 km grid square by searching all accessible dry and shallow areas of the pond that are accessible, and surveying deeper areas with a net or grapnel hook	Distribution and abundance data for macrophytes	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/wetland-plants/
	Invertebrate families survey for PondNet	UK wide	Invertebrates	Routine monitoring of a pond within a 1 km grid square, using 3-minute sweep net surveys	Presence records for invertebrate families	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/invertebrate/
	Adult dragonflies survey for PondNet	UK wide	Dragonflies	Routine monitoring of a pond within an allocated 1 km grid square by doing a visual search for adult dragonflies and	Distribution and abundance data for dragonflies	https://freshwaterhabitats.org .uk/projects/pondnet/survey- options/dragonflies/





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Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
				any evidence of breeding during a walk around the edge of the focal pond on five sunny days, spread approximately monthly between May and September.		
	GB Non-native Species Secretariat (NNSS) iRecord	UK wide	Invasive non-native species (INNS)	Opportunistic records of INNS through iRecord	Presence records for INNS	https://www.brc.ac.uk/irecord /enter-non-native-records
Terrestrial- Woodland	The National Forest Inventory	UK wide	Condition and vegetational composition of forest habitat	Ground surveys of mapped forest areas over a five-year period; one third of plots revisited each cycle.	Forest/woodland area; Habitat type area and condition; forest ecology and condition; tree health; management practices	https://www.forestresearch.go v.uk/tools-and- resources/national-forest- inventory/
	Native Woodland Survey of Scotland	Scotland (including LLTNP)	Native and near-native woodland baseline assessment for monitoring future changes in the expanse and health of these ecosystems	Mapping of all Scottish native and near-native woodlands during 2006- 2013	Location, extent, type, composition, and state of all Scottish native and near-native woodlands; identification of local threats: invasive species and grazing	(Forestry Commision Scotland, 2014b) (Loch Lomond and the Trossachs National Park, n.d.)
	Biodiversity Action Plan	LLTNP	BAP species- Owl, red squirrel, ancient woodland indicators, Juniper. Impacts- deer grazing, invasive non-native plants	Volunteer surveys, National Schemes and Societies.		(Loch Lomond and the Trossachs National Park, n.d.)
	Deer Management Group Plan. Site condition monitoring	National (including LLTNP)	Woodland herbivore impact assessment	Walkover surveys in areas of woodland to record the impact of herbivores using seven	Impact of large herbivores in woodlands	(Loch Lomond and the Trossachs National Park, 2018b).



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Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
				different indicators. Sightings of herbivores is also recorded		
	Saving Scotland's Red squirrel. Red Squirrel Survival Trust	National (including LLTNP)	Red squirrel	Feeder box surveys, yearly: filling up feeders and a placing sticky tab every two weeks. Hair is collected for morphological identification.	Presence of red and grey squirrel on feeders – monitor changes in populations	Saving Scotland's Red squirrel (https://scottishsquirrels.org.u k/) (Loch Lomond and the Trossachs National Park, 2018a)
	Dead good deadwood survey by the Conservation Volunteers	National?	Deadwood survey	Volunteers survey woodland areas (at least 100m2) by visual observation of the deadwood	Amount and estimated age of deadwood – to indicate soil health and biodiversity in surveyed areas	https://www.tcv.org.uk/scotla nd/dead-good-deadwood- survey/
	Pearl-bordered fritillary survey	LLTNP	Pearl-bordered fritillary	Surveys by volunteers, Park Authority staff, the RSPB at Inversnaid and other landowners	Mapping colonies at 12 sites with historical records of this species and identify potential new colonies	(Loch Lomond and the Trossachs National Park, 2011)
	Gleann a'Chlachain Mountain Woodland - Biodiversity Monitoring Project	Gleann a'Chlachain	Woodland composition, biodiversity, and browsing/grazing damage	Observational surveys	Tree cover, composition, and height. Biodiversity data. browsing/grazing damage. impacts of planting woodland	(Holand, 2015)
	Forest-to-bog restoration	Nature reserve/site	Soils as restoration indicators: vegetation, soil composition, fungal and insect communities	Unmanned aerial vehicle data collected to assess the influence of drought on vegetation, peat depths, soil organic matter composition and	Several remote sensing indices. Frequency data on fungal genera. Fungal functional groups.	RSPB Forsinard peatland restoration (The James Hutton Institute, 2016a)



Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
				fungal and insect communities.	Species richness and abundance of insects	
Terrestrial- other	National Common Standards Monitoring scheme. Habitats Directive; UKBAP	LLTNP	Sites of Special Scientific Interest (SSSI) and Special Areas of Conservation (SAC) condition. Invertebrate monitoring at sites with notified invertebrate features, such as Ben Lomond and Loch Lubnaig marshes in LLTNP	67 sites assessed for the condition of features (e.g. invertebrates)	Assignment into categories from "favourable" to "destroyed"	Blanket bog (Artz et al., 2014) Invertebrate features (Cathrine et al., 2015)
	UK Butterfly Monitoring Scheme (UKBMS). Habitats Directive; UKBAP	UK (including LLTNP)	Butterflies	Traditional Transects – weekly butterfly counts along fixed routes. For habitat-specialist species 'reduced effort' methods are used (adult timed counts, larval web counts, egg counts). Wider Countryside Butterfly Survey within 1 km squares	Butterfly species distribution. Annual abundance at site level	https://ukbms.org/(Brereton et al., 2020)
	Invasive Species – Rhododendron control	LLTNP	Rhododendron	Rhododendron is identified and removed	Presence of Rhododendron	(Loch Lomond and the Trossachs National Park, n.d., 2018a)
	Owl nest box monitoring	LLTNP	Barn owl and Tawny owl	Nest box monitoring of 150 barn owl boxes and 300 tawny owl boxes in woodlands	Presence of barn owl and tawny owl	(Loch Lomond and the Trossachs National Park, 2011)
	Bat box monitoring	LLTNP: Loch Ard, Loch Achray, Loch Katrine and Cowal	Bats	Monitoring bat boxes maintained: ringing bats and recording species and biological parameters	Species diversity and abundance. Sex and age of individuals. Bat ecological data	(Loch Lomond and the Trossachs National Park, 2011)



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Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
	Capercaillie monitoring	LLTNP: Loch Lomond Islands	Capercaillie (<i>Tetrao urogallus</i>)	Search for presence signs. Brood counts (July 2009). System of recording casual sightings was established	Presence of Capercaillie. Productivity (2009)	(Scottish Natural Heritage, 2020)
	Black grouse surveys	LLTNP	Black grouse (<i>Lyrurus tetrix</i>)	Annual surveys between mid-March and end of April, supported by volunteers and coordinated by RSPB; recordings of number of males seen or heard. Lek counts	Numbers of individuals and leks. Assessment of conservation measures success	(Loch Lomond and the Trossachs National Park, 2011)
	National Amphibian and Reptile Recording Scheme (NARRS)	UK wide	Reptiles	Routine monitoring of suitable habitat within an allocated 1 km grid square, using visual searches and artificial refugia	Occurrence data for reptiles	http://narrs.org.uk/index.php
	National Soil Inventory of Scotland database. Countryside Survey (CS)	Great Britain; Scotland	Soils: extent, depth, vegetation, invertebrates (assessment of diversity to test potential as soil quality indicators)	Broad-scale soil sampling at >1km. Multi-year sampling. Soil characteristics measured in the top 15 cm of soil profile. Soil invertebrates (2000 and 2007 CS): Tullgren funnel extractions of 4cm diameter and 8cm long soil cores.	Chemical soil characteristics. Number of invertebrate taxa. Number of individuals. Invertebrate diversity. Number of mites and springtails	(Emmett et al., 2008, 2010)

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Habitat	Guiding policy/ Initiative	Spatial scope	Target	Methods	Data type	Examples/Ref
	BioSoil Project	Global-	Soil monitoring and biodiversity	5,852 forested plots	Full soil profile	Soil sustainability - Forest
		including	surveying of vascular plants.	sampled in Europe (69 in	description.	Focus - BioSoil project - Forest
		Scotland		Scotland), 16km2 grid for	Structural Forest	Research
				Level II monitoring	diversity: species	ICP Forest Manual (http://icp-
				scheme.	composition; diameter;	forests.net/page/icp-forests-
				Soil sampling followed	canopy characteristics;	manual)
				the ICP Forest Manual.	woody debris	BioSoil Forest Diversity
				Biodiversity assessment		Manual (Working Group on
				based on stand structure		Forest Biodiversity, 2006)
				approach.		





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