E&P **ENVIRONMENTAL GENOMICS RESEARCH** PROGRAMME

An IOGP programme

International Association of Oil & Gas Producers

DRAFT | November 2024

Efficacy of eDNA vs Conventional Monitoring Methods

This Guidance is publicly available as a draft version for readers for review and trial its use. Any comments that improve the content or workability of the draft guidance are welcomed by the JIP.

Please send comments to **info@iogp-edna.org**.

We will endeavour to address comments, if received by Feb 28th 2025, in the Final (edited and formatted) version of the guidance which will be available early in 2025. Any comments received after this date or any comments that cannot be addressed within this timeframe will be considered for future updates to the guidance. Updates are planned every 1-2 years to capture the rapid advances in this field.

Prepared by NatureMetrics and ID-Gene for the International Oil and Gas Producers' Association Joint Industry Program on Environmental Genomics.

REVISION HISTORY

RELEASE	NAME	COMPANY	DATE OF ISSUE	COMMENTS
R1	Ed Wort	NatureMetrics	2024-Jan-26	First release.
R2	Jan Pawlowski	ID-gene	2024-Nov-03	Revision of initial release to improve relevance, completeness, accuracy and readability.
R3	Ed Wort	NatureMetrics	2024-May-28	Revisions following JPs comments
R4	Nicolas Tsesmetzis	Shell	2024-Oct-11	Addition of Disclaimer.
R5	Michael Marnane	Chevron	2024-Nov-21	Addition of Instructions to readers

Executive summary

Environmental DNA (eDNA) based methods are increasingly used for biodiversity monitoring across a range of habitats in which the energy industry operates. This publication reviews eDNA and conventional biomonitoring methods, compares their efficiency, and discusses their advantages and limitations. It highlights that, although eDNA methods are still developing, there are many potential applications throughout the life cycle of an energy project, either as stand-alone or complementary monitoring tool.

This guidance consists of seven sections: (1) an introduction to the importance of environmental monitoring in the energy industry, (2) an overview of conventional monitoring methods, (3) and overview of eDNA-based methods, (4) a comparison of the efficacy of conventional and eDNA-based methods, using a selection of case studies, (5) the current application of eDNA-based methods in the energy industry, (6) considerations when deciding whether to use eDNA-based methods, and (7) future directions for eDNA-based technology. The guidance considers different habitats (freshwater, marine, terrestrial) and wide range of taxonomic groups, from vertebrates to microbes.

Based on a critical review of more than 200 scientific papers, the guidance shows that eDNA methods are mature enough to be considered as an integral part of biomonitoring in several energy industry applications. Their efficacy is particularly high in the domain of detection and identification of individual species. They also perform well in holistic biodiversity surveys, as they can detect small-sized and inconspicuous taxa, that are difficult to identify using conventional methods. On the other hand, the eDNA approach is currently less successful in inferring the abundance of organisms, especially larger-sized taxa. The taxonomic composition of communities inferred from eDNA and conventional methods is often different. This makes the eDNA approach more challenging when applied to morphology-based ecological indices, but solutions have been proposed in the literature to overcome these limitations.

The main advantages of an eDNA approach over conventional monitoring methods are its lower level of effort and cost, its ability to detect species when they are difficult to observe directly, and to identify them when their morphological features are indistinct. Moreover, eDNA sampling is non-invasive, and from the safety point of view, it is less labour intensive, employs less hazardous materials, and reduces HSE exposure. Processing eDNA samples can be implemented using existing laboratory pipelines , and applied to a broad range of taxa without any special taxonomic expertise. Although there are some biological data that cannot be easily inferred from eDNA data, such as biomass, age or health, the recent attempts to obtain at least some of these data from eDNA or eRNA are promising.

The overall consensus that comes out of this guidance is that the eDNA-based methods have the potential to be fully integrated into biomonitoring of energy industry activities. The extent of their application depends on monitoring objectives as well as habitats and target taxa. The eDNA-based methods can be used as stand-alone tools for the detection of particular species (invasive, endangered, or pollution indicators). They could also be applied to assess environmental impacts and ecological status, although further validation of currently available eDNA-based metrics might be necessary. A combination of eDNA and conventional

methods (such as imagery and/or acoustics) is appropriate for many applications. eDNA analyses provide a different type of information that is often complementary to what conventional biomonitoring can provide. The use of multiple methods, including eDNA-based techniques, will make future biomonitoring more powerful and comprehensive, which will help inform business decisions and aid in sustainable development within the energy industry.

Table summarising different eDNA and conventional methods (**bold** = most frequently used at present for that target community) that can be used for various habitats and target communities, at each major phase of operation for the energy industry. For eDNA method status: CA = community analysis; SA = stand-alone. For phase for eDNA application: B = Baselining; E&P = Exploratory and production drilling; OSR = Oil spills and remediation; D = Decommissioning; R = Restoration

Habitat	Target community	eDNA methods	Conventional methods	eDNA application	
				Recommended use	Phase of operation
Marine	Benthic sediment	**Sediment sample;** Macrofauna sample	**Macrofauna sample; Benthic imagery;** Active acoustics	CA with further validation	B; E&P; OSR; R
	Benthic hard substrate	**Surface scrape;** Surface swabs; Surface suction	**Surface scrape; Benthic imagery;** Active acoustics	CA in combination	B; E&P; OSR; D; R
	Fish	**Water filter**	**Netting; Benthic and aerial imagery;** Active acoustics	Stand-alone CA & SD	B; E&P; OSR; D; R
	Broad vertebrates	**Water filter**	**Visual observations; Passive acoustics;** Aerial imagery	CA & SD in combination	B; OSR; R
	Zooplankton & Phytoplankton	**Water filter;** Bulk net sample	**Water sampling; Netting;** Active acoustics; Aerial imagery	CA in combination and SD stand-alone	B; OSR; D
Freshwater	Fish	**Water filter**	**Netting; Electrofishing**	CA in combination and SD stand-alone	B; E&P; OSR; D; R
	Aquatic Invertebrates	**Water filter;** Bulk sampling	**Kicknet;** Traps	CA with further validation and SD stand-alone	B; E&P; OSR; R
	Phytobenthos	**Biofilm sampling**	**Biofilm sampling;**	CA with further validation	B; E&P; OSR; R
Terrestrial	Broad vertebrates	**Water filter;** Air DNA; Surface swabs; iDNA	**Visual and auditory; Camera traps; Vertebrate traps; Artificial cover**	CA and SD in combination	B; OSR; D; R
	Invertebrates	**Invertebrate traps;** Air DNA; Surface swabs	**Invertebrate traps**	CA in combination	B; OSR; D; R
	Soil	**Soil sampling**	**Soil sampling**	CA with further validation	B; E&P; OSR; R

Key

Phase of operation: B = Baselining; E&P = Exploration and production drilling; D = Decommissioning; OSR = Oil spills and Remedation; R = Restoration

Recommended use: CA = Community analysis; SD = Species Detection

Contents

Glossary

Assay	Analysis used for DNA samples, usually referring to PCR reaction conditions and primers.
ASV	Amplicon Sequence Variant – metabarcoding datasets of individual high-quality sequences because sequences that contain errors are typically filtered out using denoising algorithms. Often used as an alternative or in combination with clustering (OTUs) for taxon delimitation.
AUV	Autonomous Underwater Vehicle
Benthic	Organisms living at the bottom of a water body (seafloor, river or lake bottom)
Bioinformatics	The application of genomics, computational biology, statistics, and programming in which DNA sequenced or other biological data are processed, analysed and integrated for research or communications.
Bulk sample	Sample composed of unsorted organisms, mainly referring to invertebrates
Conventional	For the purposes of this document, any survey method that is not DNA-based
COI	Mitochondrial gene commonly used for barcoding. For metabarcoding, it is frequently used for analysis of invertebrate taxa.
ddPCR	Digital Droplet PCR - This method is similar to qPCR in application but is more robust to inhibitors, does not require standards, and offers greater precision for quantifying DNA concentration (e.g. Baker et al. 2018).
DEM	Digital Elevation Model – model of topography
Diatom	Single-celled algae (typically with a silica frustule) frequently used for water quality assessments.
DSM	Digital Surface Model – DEM with any additional structures (vegetation, anthropogenic)
eDNA	Short for 'environmental DNA'. Refers to DNA in the environment, including DNA released from organisms through excretion, shedding, mucous 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 sampled in low concentrations and can be degraded (i.e. broken into short fragments), which limits the analysis options.
Elasmobranch	Taxonomic group of cartilaginous fish (sharks, rays).
Eukaryote	Organism with a nucleus (animals, plants, fungi, algae).
GCN	Great Crested Newt
iDNA	Insect-derived DNA; DNA sourced from insects (e.g. mosquitoes and leeches) which is from target organisms (e.g. vertebrates) that the insects feed on.
INNS	Invasive and Non-Native Species
Malaise trap	Trap commonly used to sample flying insects
Metabarcoding	Refers to identification of multiple species from DNA using barcode genes. Target sections of DNA are amplified with primer pairs and PCR, followed by high-throughput sequencing and bioinformatics

	processing. Can identify hundreds of species in each sample, and 100+ different samples can be processed in parallel to reduce cost.
Metagenomics	Non-targeted sequencing of DNA within a sample compared to metabarcoding which sequences a specific gene region (e.g. Bista et al. 2018)
Metatranscriptomics	Study of the transcriptome, the sequences of RNA within a sample. Also used to identify the "active" community (e.g. Knapik et al. 2020).
Morphology/Morphological analysis	Assessment of organisms within a sample based on visible physical characteristics (commonly used identification).
Omics	Colloquialism used to encompass transcriptomics, genomics, metabolomics etc.
OTU	Operational Taxonomic Unit – sequences clustered above a given similarity threshold to assign quasi-species status.
PAM	Passive Acoustic Monitoring – bio-acoustic surveying
Passive sampler	eDNA sampler without any moving (active) components, typically using a material that adsorbs eDNA.
PCR	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.
Pelagic	Adjective for describing biology in the marine water column
PLFA	Phospholipid fatty acid – conventional analysis used for assessing microbial communities
Pitfall trap	Trap used to sample surface-dwelling invertebrates
Primer	Short sections of synthesised DNA that bind to either end of the DNA segment to be amplified by PCR. Can be designed to be totally specific to a particular species (so that only that species' DNA will be amplified from a community DNA sample), or to be very general so that a wide range of species' DNA will be amplified. Good design of primers is one of the critical factors in DNA-based monitoring.
Prokaryote	Organism without nucleus (bacteria, archaea)
PSD	Particle Size Distribution – the weight distribution of different grain sizes (mud, sand, gravel) of a sediment
qPCR	Quantitative PCR - A fluorescent signal is emitted as DNA is amplified which allows DNA concentration to be quantified, thus this approach can be used to quantify the concentration of targeted DNA.
Reference database	Publicly available database of DNA sequences with taxonomic assignments from many species around the world. These databases serve as a reference against which unknown sequences can be queried and identified to a taxa. The most commonly accessed database is NCBI, which is maintained by the US National Institute of Health. Anyone can search for DNA sequences at https://www.ncbi.nlm.nih.gov.
ROV	Remotely Operated Vehicle
(DNA) Sequence	A section of DNA. A DNA sequence is made up of four nucleotide bases represented by the letters A, T, C & 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), identical copies of the same sequence are obtained for each species in the sample. The

	number of copies obtained per species is known as the number of sequence reads, and this can be related to the relative abundance of species.
Spike-in	Addition of DNA to a sample. This artificially added DNA can be used to assess and correct for biases in PCR and bioinformatics pipelines.
Thermocline	A water layer within which there is a rapid change in temperature (typically marine and lakes).
18S (rRNA)	Gene coding for small subunit ribosomal RNA. Commonly used in metabarcoding for targeting eukaryotes/metazoans.

1 Introduction and Overview

1.1 Background

The energy industry undertakes a variety of ecological assessments, frequently as a regulatory requirement for proposed, existing or decommissioned assets. Conventional biodiversity survey methods, which usually have standardised protocols based on direct observations or collection of organisms, are broadly accepted by industry and regulators. However, these conventional methods can be labour-intensive, expensive, limited in taxonomic scope and require experts with taxonomic training.

eDNA-based methods can complement, match and in many cases outperform conventional biodiversity survey methods (Keck, Blackman, et al. 2022; Valentini et al. 2016; Deiner et al. 2017). Their other advantages include reduced cost and survey effort, and increased taxonomic resolution. A recently published literature review identified that eDNA performed better than conventional methods in the majority of 194 comparative studies in terms of sensitivity (ability to detect species at low abundance), overall species richness detected and cost-effectiveness (Fediajevaite et al. 2021). The ease of field sampling for eDNA and its sensitivity has already made it a useful tool for species detection by professionals and citizens (Biggs et al. 2015). Environmental DNA-based methods can generate measures of ecosystem health (Pawlowski et al. 2018; Cordier et al. 2020). However, the current limitations of eDNA analysis in being able to quantify abundance and discrepancies between molecular and morphological identification can impede the uptake and acceptance by regulators of eDNA-based methods for biodiversity surveys.

The application of eDNA-based methods to in the energy industry is at a relatively early stage. Of the studies that have been conducted, their results show the immense potential of using DNA as an alternative to conventional monitoring. The detection of marine mammals during baseline assessment, the assessment of environmental impacts on sediment fauna during operational phase, or the detection of invasive non-native species (INNS) during the decommission phase are only few examples of potential applications of eDNA-based monitoring at different phases of energy industry projects.

1.2 Scope and Objectives of this Guidance

This document is intended to provide industry environmental practitioners with guidance on when and where to apply eDNA methods. Recommendations within this guidance are based on a comprehensive literature review of conventional and eDNA-based biodiversity monitoring methods, with the aim to guide the selection of the most suitable methods for a given application, research objectives, study taxa and habitat. The document provides a critical overview of current applications of eDNA-based methods and, for a range of industry applications, evaluates where these methods:

- can be used as stand-alone tools to obtain the desired output.
- should be used in combination with conventional methods to provide all the information required.
- are technically feasible, but further testing and validation is required for industrial application.
- are not appropriate for use.

This report focusses primarily on the topics of field sampling and eDNA data processing and analysis. Components such as the regulatory acceptance, preservation, sample preparation and DNA extraction are addressed where pertinent, but are covered to a greater extent in Guidance Sections 2 and 3.

2 Overview of conventional biological monitoring methods

2.1 Applications

A range of biological data are collected and analysed using conventional methods in the energy sector. These can include:

- species presence (and to a lesser degree, absence), temporal and spatial distribution
- species richness and evenness (alpha diversity)
- community composition and structure (beta diversity)
- abundance and biomass (absolute and relative; also using coverage as an abundance indicator)
- condition/health (frequently based on community composition or indicator species presence/absence)
- physiological response to pollution (mussels and fish tissue analysis commonly used in energy industry)
- behaviour (less frequently recorded for the energy industry, but can influence other biological data metrics)

For the purposes of this document, we have focused on biological data that are commonly used in biomonitoring associated with energy industry operations that could potentially be obtained using eDNA-based methods. Conventional methods used for their collection are described below for marine, terrestrial and freshwater environments (Table 2.1).

Table 2.1 – Overview of Conventional Biological Monitoring Methods

Marine	Freshwater	Terrestrial	Others
Aerial imagery	Netting	Visual and auditory	Remote sensing
Visual observations	Electrofishing	Passive acoustics	Acoustic tagging
Passive & active acoustics	Kick net	Camera (infrared & thermal) traps	Crustacean trap
Underwater imagery	Sediment sampling	Vertebrate traps	Tissue sampling
Trawls/netting	Biofilm sampling	Artificial cover	Citizen science
Sediment sampling		Invertebrate traps	Box surveying
Plankton sampling		Soil sampling	Hand searches
		Microbial communities	

2.1.1 Marine

Main taxa targeted in the marine environment are marine mammals, reptiles, seabirds, fish, benthic invertebrates and zooplankton. Aerial and vessel observer surveys are used for marine mammals, reptiles and seabirds, with passive acoustic monitoring also being applied to marine mammals. Fish data are most frequently derived from video and acoustic imagery and occasionally trawls. Benthic communities (those living at the bottom of the water body) are monitored either using video imagery targeting fish and sessile fauna or using sediment grabs or cores to study benthic macro- and meiofauna, with active acoustics (sidescan sonar, MBES) used for identifying biotopes. Planktonic biota (those living in the water column) include zooplankton (ichthyoplankton and invertebrate plankton) and phytoplankton are monitored

using trawled plankton nets and filtered water samples. Acoustic and satellite methods can also be used for considering the distribution of phytoplankton.

Aerial surveys, Visual Observation and Passive Acoustic Monitoring. Marine vertebrates, such as mammals, seabirds, reptiles and elasmobranchs (cartilaginous fishes like sharks, rays, and skates), often include protected species, and monitoring them is required by current regulations. Conventional methods include aerial surveys, visual observations and passive acoustic monitoring to detect the target species in the areas of energy industry activity.

Dedicated aerial surveys, either via manned aircraft or drone, are frequently done prior to environmental baselining or even geophysical surveying. Aerial surveys typically use high-resolution imagery to detect species of interest, often combined with machine learning tools to reduce the cost of manual video analysis. However, this method is dependent on the target taxa being visible in the imagery which is affected by multiple environmental factors such as cloud cover and water turbidity.

Real-time visual observations are often required at fixed intervals during offshore operations because protected species such as marine mammals can be impacted by the noise from operations (such as geophysical surveys, pile-driving or drilling operations). Observations are often conducted from a survey vessel or platform. This requires constant observations throughout vessel deployment/offshore activity, with several marine mammal observers (MMO; UK) or protected species officers (PSO; USA) working in shifts (depending on the size/scope of the project this entails quite large field teams). Observations and vessel activity are recorded and submitted to the relevant national authority, with QA/QC usually carried out on land following project completion. Visual observations are generally limited by distance, sea state, and time spent on the surface by targeted species.

Simultaneously to visual observations, sound can be monitored using passive acoustic monitoring devices (PAMs). Detections from PAMs of marine mammal sounds can be carried out on board a vessel in real time or deployed on moorings for periods of time. However, lengthy data analysis is frequently required to first pick out an acoustic signal and then to identify the species (Palmer et al. 2019).

Underwater imagery. Data extracted from video imagery are commonly used for investigating fish and sessile benthic fauna communities. Video imagery allows species identification and assessment of species diversity. It can also be used to count protected benthic species and measure the percentage cover and area of protected habitats (e.g. corals, sponge cover). Laser pointers or a fixed frame are commonly used to measure seabed features. Baited remote underwater video (BRUV) can be used for some mobile fauna, such as elasmobranchs (Boussarie et al. 2018).

The deployment of underwater video cameras requires trained personnel to oversee their use and depends on water turbidity and weather conditions. A high amount of effort is required to obtain video imagery results with a high field sampling cost (equipment rental and deployment cost). There is also a long turnaround time as data are analysed over a period of weeks from the date of sampling until processed data are available. Although there is the potential to reduce human effort from the identification and observation process with advancing machine-

learning technology (Montereale Gavazzi et al. 2021), the video imagery remains a relatively expensive and time consuming method. Moreover, the imagery data may fail to detect cryptic taxa that are camouflaged, buried within the sediment or hidden in crevices.

Trawling. Fish and mobile pelagic and benthic macrofauna data are occasionally collected via midwater or bottom trawling. Such data are valuable because they can directly assess the abundance and age of target species. However, the method is highly invasive and bottom trawling can have negative impacts on the benthic community (Jac et al. 2021), as well as the surrounding ecosystem. The use of trawls can also entangle with cables and physical infrastructure. Therefore, trawling is not recommended for surveying the offshore energy sector.

Sediment sampling. Sediment samples from benthic grabs or cores are commonly used for the investigation of benthic macrofauna and meiofauna. Benthic macrofauna-based indices such as AMBI (AZTI Marine Benthic Index, Borja, Franco, and Pérez 2000), are currently used for the assessment of impacts associated with offshore platforms activity. There are also indices that are tailored for particular groups of benthic meiofauna (e.g. The Maturity Index, Bongers 1990; foram-AMBI, Alve et al. 2016). All these indices are calculated based on a list of species assigned to particular ecological categories and their abundance. Particle size distribution (PSD) and physicochemical variables (heavy metals, hydrocarbons and organic carbon) can be obtained from the same sediment samples. A combination of imagery data, benthic macrofauna and PSD can be used to assign a biotope, such as when using the EUNIS habitat classification system (https://mhc.jncc.gov.uk/). Habitat classification can then be used to assess environmental change and impact.

Collecting benthic macro- and meiofauna data, however, requires substantial effort in the field with sampling equipment deployment and retrieval, on-board sieving, and preservation in potentially hazardous solutions such as formaldehyde or industrial denatured alcohol. Laboratory time required to analyse samples from a project can be many weeks to months, with turnaround times often dependent upon the increasingly limited availability of taxonomists and sample complexity. Industrial standards such as the NMBAQC (NE Atlantic Marine Biological Analytical Quality Control) give clear guidance on the taxonomic identification, procedures for reference collections and sample reanalyses (Cooper and Rees 2002). Nevertheless, such standards are usually limited to a certain biogeographic area or particular habitats.

Plankton sampling. Planktonic data used for biomonitoring consist mainly of marine zooplankton, including various taxa of invertebrates (mostly copepods and cnidarians) and ichthyoplankton. Marine phytoplankton comprising various groups of algae are less commonly used. The samples are obtained from water either using plankton nets or by water filtering. They can be fixed in buffered formalin or with Lugol's solution.

The main challenge of planktonic data analysis is taxonomic identification of generally small sized and often inconspicuous morphotypes. Automated analysis of plankton with imagery, such as the ZooCAM In-Flow imaging system allowing fast onboard counting, sizing and classification (Colas et al. 2018) is becoming more prevalent to assess this community. Other

systems have also been developed for microscopic plankton (Pollina et al. 2022). Nevertheless, the accuracy of such systems in term of species identification is still far from being optimal.

2.1.2 Freshwater

Fish, benthic invertebrates, and benthic diatoms are the main biological quality elements to be assessed during the monitoring of water bodies (e.g. EU WFD, Directive 2000/60/EC). Freshwater monitoring can also include macrophytes and phytoplankton, but these elements are less commonly used and will not be discussed here.

Netting and electrofishing. Freshwater fish populations are commonly monitored using netting or electrofishing. A range of different nets can survey fish, with the most popular being gill netting, seine netting and fyke netting. Fish captured through netting can be identified, measured, sometimes sexed and any other relevant biological information taken (e.g. tissue samples). Several different netting methods can be used to ensure representative biological sampling, which may still reveal limited diversity (Hallam et al. 2021). This is in part due to the different conditions under which the netting techniques are effective and associated biases such as mesh size selectivity. This can result in species of interest not being assessed by netting. Logistically, a high sampling effort is required, especially in larger rivers and reservoirs, where entanglement of nets and trawls can be problematic.

Another widely used method for collecting fish data is electrofishing. The method consists of creating an electric field in a small area of water to immobilize fish, which then float to the surface and can be collected. This is a relatively comprehensive form of fish sampling, as it is less selective than most nets. The upstream wading electrofishing is used in streams and rivers, while a boating method is used in larger waterbodies that are too deep or wide to wade effectively. Once the sampling is completed, the crew records data on the catch, identifying each fish to species level, recording weights, and often noting any signs of external diseases or parasites. Fish are released back into the water once the data is recorded.

Both netting and electrofishing are invasive methods that can be harmful to fish. They also present a high risk from an HSE perspective and require trained personnel. As each method is relatively selective in the capture of species not all species are surveyed equally. A risk of fish mortality can be minimised through careful equipment design and protocols which allows for catch and release. However, fish may not always released unharmed due to unforeseen conditions or events(D. E. Snyder 2003).

Aquatic invertebrates sampling. Several groups of aquatic invertebrates are used as indicators of water and sediment quality and ecosystem health. Most commonly used are EPT insects representing orders Ephemeroptera (mayfly), Plecoptera (stonefly) and Trichoptera (caddisfly), which are known to be particularly sensitive to water pollution. Another group of aquatic insects widely used as bioindicators of water quality in lakes are chironomids (non-biting midges) (Kranzfelder et al. 2015). Biological quality of sediment in rivers and lakes is commonly assessed using oligochaetes (Lafont et al. 2012) or nematodes (Höss et al. 2011).

Kick net sampling is the most popular method to collect aquatic insects and other invertebrates from rivers and streams. It is carried out by disturbing the river or stream bottom by kicking or shuffling and collecting organisms in a net downstream. Sweep net sampling is conducted in ponds and lakes to capture different taxa that inhabit aquatic vegetation. Sediment samples for oligochaetes and nematodes are collected using different types of nets, grabs or piston drills. The sediments are sieved and fixed with formaldehyde, and the specimens are sorted and morphologically identified under microscope.

To ensure that the sampling is representative of the site, it is important to sample different microhabitats (e.g. slow water, weeds, tree roots) and sampling effort can be allocated based on microhabitat prevalence. Identification to family level and taxon counts can then be carried out in the field and assigned an abundance category. The identification to species level is carried out in a laboratory under a microscope and usually necessitates an experienced taxonomist. Difficulties associated with the identification based on morphological features are the main factor limiting the use of aquatic invertebrates for ecological diagnostics.

Biofilm sampling for benthic diatoms. Monitoring water quality in rivers and streams using benthic diatoms (phytobenthos) is prescribed in Europe and recommended in other countries. Because diatoms are very diverse and grow rapidly, they respond quickly to changes in environmental conditions due to natural or anthropogenic impacts. As certain diatom species have preferences for different water quality conditions (nutrients, salinity, pH, heavy metals, temperature, and flow) the analysis of species composition in a sample can be used as a biological indicator of organic pollution, eutrophication, acidification and metal pollution. Among various groups of diatoms, those living as epilithic phytobenthos in rivers and streams are commonly used as bioindicators (Rimet and Bouchez 2012).

Sampling for diatoms is usually carried out by scrubbing the biofilm from upper surfaces of stones, placed in a sampling tray with stream water (Taylor et al. 2007; Gonzalo and de los Reyes Fernandez 2012). The samples are preserved in Lugol's iodine or ethanol and processed at a laboratory using a range of hazardous substances (Taylor et al. 2007; M. G. Kelly et al. 1998). Slides are prepared for microscopy and the diatom valves are counted to measure the relative abundance of different species. The identification of freshwater diatoms requires expert taxonomic knowledge. Yet, intraspecific variability can be very high, and the morphological differences between species can be so subtle that even trained taxonomists may come to different conclusions.

2.1.3 Terrestrial

Terrestrial biodiversity surveys typically combine a range of survey methods targeting different taxonomic groups. Methods include (among others) visual surveys for field signs of terrestrial vertebrates, the use of camera traps for large mammals and other vertebrates, mist netting for birds, mist netting and harp trapping for bats, acoustic surveys for amphibians, birds and bats,

a variety of trapping approaches for small mammals, reptiles and invertebrates, and detailed plot surveys for vegetation. There is increasing use of remote sensing for initial assessments of habitat suitability and vegetation, but this is limited in its ability to deliver species specific data.

Visual and auditory surveys. Visual and auditory field surveys such as walkovers, transects and point counts are commonly used for species surveys in terrestrial habitats The surveys are carried out by trained ecologists and can encompass a broad range of taxa including mammals, birds, amphibians, reptiles, and insects. Typically, surveys involve searching an area for field signs indicating the presence of the target species or directly observing the species. In the case of bird surveys, these are usually based on the identification of bird song rather than visual observations. Visual surveys may also include determining habitat suitability for the species of interest. Surveys are carried out at different times of day or night to capture the activity of different species groups and targeting the optimum season or spread across multiple seasons. They can be combined with other survey methods such as for example hand or dip netting, rock-turning for amphibians or searching buildings or trees for bat roosting features.

Acoustic surveys. Acoustic monitoring is widely used for bats but also for other terrestrial vertebrates (see Sugai et al. 2019 for a review). Acoustic surveying can capture more aerial insectivorous bat species than any other combination of sampling methods (Appel et al. 2022). Acoustic surveys may involve the use of handheld detectors during bat emergence surveys or activity transects or static detectors may be deployed to record passively. These passive acoustic recorders do not require the presence of the researcher for the whole period and can be programmed to operate on a species-targeted schedule. Static detectors are deployed within the survey area, whereby ideally different habitats should be sampled and the microphones positioned to ensure optimal chances of detection. Typically these are deployed for at least 5 nights in a row, often over a period over several months or across several seasons (Collins, 2023). Recordings are then processed and analysed using call analysis software in order to identify calls to species or genus level where possible (there are large regional variations in the availability of reference libraries which may limit the possibility of identifying the species).

Camera trapping. Monitoring of mammals, reptiles and ground-dwelling birds can be carried out non-invasively using camera traps. These are widely used in wildlife monitoring projects worldwide, typically using remotely activated devices that capture images or video when a motion and/or heat sensor is triggered. The majority of studies focus on mammals, considerably fewer on birds and very few targeting reptiles or amphibians (Burton et al. 2015). They are subject to a range of detection biases, such as animals not entering the detection zone, habitat characteristics (e.g. dense vegetation), attraction to a camera or even camera trap positioning and settings. These can be overcome to some degree by statistical modelling such as occupancy modelling (Wevers et al. 2021). As with all imagery, the camera traps images

are reliant on human identification and counting of species by trained taxonomists, which is time consuming, although automated identification is increasingly being used (Schneider et al. 2020).

Traps for capturing mammals, reptiles and amphibians. Small mammals, reptiles and amphibians can be captured using a variety of traps. Ground-dwelling small mammals are commonly sampled using a baited box with a trapdoor, with slightly different designs depending on the size of target species (Torre et al. 2016). Pitfall traps using buckets placed in a line with drift fences can outperform the detection rates of other trapping methods for some species (Thompson and Thompson 2007; Mena et al. 2021). Trapped mammals can be tagged, allowing for the number of different individuals trapped to be assessed, and population size can be estimated (Slade and Blair 2000). However, these methods have been found to have a lower detection rate for multiple species than camera traps (M. L. Thomas et al. 2020).

Artificial Cover Objects (ACOs) are regularly used for surveying reptiles, whereby ACOs create favourable refugia and can be accessed to count species and number of individuals on a frequent basis (e.g. Wilson, Mulvey, and Clark 2007).

Mist nets and harp traps are regarded as one of the most effective methods of sampling flying vertebrates (Trevelin et al. 2017). When targeting bats, they are deployed near roost entrances, in areas with vegetation, or water where bats fly low (Ferreira et al. 2021) and in areas where they are more likely to be caught such as along commuting routes or at swarming sites. In addition, harp traps are widely used to capture bats. Thicker mist nets are usually used to capture birds in low visibility environments to complement visual and auditory methods (Marques et al. 2013). Birds in mist nets have been found to have a low (<1%) risk of injury and mortality (Spotswood et al. 2012).

Traps for insects and other arthropods. Insects with different modes of life are sampled using a variety of trapping methods and subsequently identified in a laboratory. As discussed below, the same sampling methods can also be adapted for DNA-based identification. These almost always kill the specimens sampled (regardless of if they are processed using DNA-based or morphological methods), which can be problematic when considering protected taxa. It can be illegal to trap if there are protected invertebrates in the area (e.g. rusty patched bumble bee in the USA; Franz 2020).

Flying insects are commonly collected using Malaise traps or pan traps. The Malaise trap is placed in a natural flyway, where hundreds of insects can be directed to the apex of the trap where they fall into a collection bottle of preservative solution. The trap can be deployed indefinitely, with only the bottle replaced periodically (Kirse et al. 2021), making it a very low maintenance and low-cost sampling technique. As it can be deployed in a highly standardized way, the Malaise trap can be used for biomonitoring programs that seek to track long-term trends, including the impacts of development or restoration activities on species diversity. Pan traps use containers that are partly filled with soapy water. The water is usually dumped in a

net and transferred to a preserving agent (usually ethanol) (Anderson et al. 2013). Other methods for sampling flying insects include: flight intercept and window traps, light traps, sweep nets for Hemiptera and Orthoptera and aerial nets for Lepidoptera and Odonata, and sticky traps primarily for agricultural pest monitoring (Böckmann et al. 2021).

Ground surface-dwelling arthropods (particularly beetles and spiders) are commonly collected using pitfall traps, which are containers sunk in the soil and filled with a preservative solution such as a mixture of water and ethylene glycol. This allows for high field replication due to the low handling time. Features such as funnels and guidance barriers can also be included to increase capture efficiency. However, there is not a uniform method for sampling surface-dwelling arthropods, which can lead to variable results (Boetzl et al. 2018).

Collecting soil invertebrates. For smaller invertebrate fauna such as nematodes and microarthropods, soil sampling and subsequent sorting is the most commonly applied method, albeit rarely used at commercial scale due to the inherent time and effort. The sorting process involves a density-based separation (Schenk et al. 2020), and/or a filtration based sorting step. Sorting can be a labour-intensive and equipment-heavy process. When sample numbers are high, this requires substantial lab space. Invertebrate indicator species identified in soils can include earthworms, nematodes, mites and springtails. The metrics derived from these indicators generally incorporate abundance data of the target species group.

Monitoring microbial communities. Traditionally, soil bacteria and fungi have been assessed through culture-based methods (e.g. Janssen et al. 2002) or phospholipid fatty acid (PLFA) measurements (Frostegård, Tunlid, and Bååth 2011). A combination of culturing and molecular techniques are common approaches for identifying and monitoring microbial communities (Wani et al. 2022). Culture-based methods are generally considered selective and biased as a relatively small percentage of bacteria species can be cultured in the lab (e.g. F. O. P. Stefani et al. 2015). The varying concentrations of PFLAs are used as a rapidly obtained proxy for different microbial communities and biomass, particularly for the fungal to bacterial ratio (Frostegård, Tunlid, and Bååth 2011). However, PFLA analysis has largely been superseded by DNA-based methods for community characterisation.

2.1.4 Remote sensing and other survey methods

Currently, remote sensing is gaining attraction as a method for non-invasive surveys of aquatic and terrestrial environments over large areas using aircraft (including drones) or satellites, with minimal deployment of personnel. In terrestrial environments, remote sensing can produce high level 3D images of vegetation and measure plant biomass. In freshwater and marine environments, remote sensing is mainly used to estimate algae abundance (particularly chlorophyll concentration) and sediment load. It is an ideal tool to assess water extent and catchment hydrology, which are generally required and are highly relevant to water eDNA sampling (Carraro, Stauffer, and Altermatt 2021). Whilst habitat assessments derived from remote sensing are not measures of biodiversity, they can provide metrics such as Habitat

Extent, Habitat Connectivity, NDVI (a ratio between the red and near infrared values, useful in understanding vegetation density and assessing changes in plant health), and soil type and health. The use of remote sensing as an environmental/ecological tool for habitat suitability and vegetation have been extensively discussed in a previous IOGP report (IOGP 2020).

Besides remote sensing, there are other biological survey methods that are frequently applied to biomonitoring with governmental targets or are led by conservation and academic research organisations. These other conventional methods include:

- Acoustic tagging of individuals
- Trapping (e.g. crustaceans, decapods)
- Tissue sampling for monitoring health or population genetics
- Active searches for terrestrial invertebrates (under microhabitats such as dead logs, under stones or plants associated with particular invertebrates)
- Citizen science (visual) observations
- Bird nest, bat and small rodent box surveys
- Feeder box surveys
- DNA analysis of faecal samples for species identification

Some of these methods, such as tissue sampling are used in energy industry monitoring to survey the accumulation of pollutants in wild caught fish or blue mussels. However, as these methods cannot be replaced or complemented by eDNA, they are not presented here.

2.2 Challenges and limitations of conventional methods

The main challenges and limitations of conventional biomonitoring methods are summarized below:

- High sampling costs: conventional survey techniques are labour intensive and sometimes rely on complex logistics (especially in the marine environment).
- Slow sample processing and lengthy data analysis: specimen sorting and morphological species identification is usually time-consuming, greatly increasing the period from sampling to data analysis; in some cases, such as video imagery and acoustic data, the turnaround time of data analysis can also be very long.
- Low frequency of sampling: the variations between samples can be very high, but repeated sampling is cost-prohibited
- Limits of visual observations: the accuracy of observations can be limited by environmental factors (weather conditions, water turbidity, etc.) and behaviour of species (e.g. those buried in the sediments or hidden in crevices, etc); observer skills can also be insufficient due to short training and limited taxonomic knowledge.
- Limits of morphological identification: using morphological characters to identify species can often be challenging, and for many taxa, an identification to family or genus level is accepted; in general, morphological identification of small sized and often inconspicuous morphotypes, including immature stages of life cycles requires highly skilled experts; moreover, some common taxa comprise a multitude of cryptic species that can only be distinguished by genetic data.
- Lack of taxonomic expertise: accurate identification of sampled organisms requires good knowledge of morphological taxonomy; yet, the number of experienced taxonomists is rapidly decreasing, and the training of new taxonomists is rare.
- Limited range of taxa sampled: conventional methods are based on large size mega- or macrofauna that are relatively easy to observe and identify; small-sized taxonomic groups (e.g. meiofauna and microbiota) are generally ignored, even if they are highly sensitive to environmental impacts.
- Invasiveness or destructiveness of sampling: Fishing or trapping of vertebrates, as well as collecting bulk invertebrate samples, may have negative effects on populations of protected and endangered species. Some sampling methods such as bottom trawling can destroy habitat.
- Hazardous products: solutions used for preservation of collected specimens (formaldehyde, denatured alcohol) are potentially hazardous; this is also the case of substances used for processing biofilm samples (HCL, KMnO4, hydrogen peroxide).
- Pathogen risks: conventional sampling procedures may increase the risk of pathogen transfer among sites on sampling equipment if not sterilised.

3 Overview of DNA-based Methods

3.1 General Outline

eDNA-based methods can be applied to a vast range of habitats and regions targeting biota wide range of taxa, from bacteria to blue whales. This section reviews the main eDNA methods in use, particularly those successfully applied within the energy industry.

eDNA methods can be broadly divided into two groups: single species and multiple species applications Each group of applications can be addressed with specific analysis methods:

- Single species
  - Quantitative PCR (qPCR): A fluorescent signal is emitted as DNA gets amplified by PCR. This allows for DNA concentration to be quantified. This approach can be used to assess species presence/absence and its abundance, if a qPCR assay is validated.
  - Digital PCR (dPCR): This method consists in partitioning the DNA into thousands of droplets (ddPCR) or microfluidic chambers and quantifying the number of positive amplifications in each partition. It is similar to qPCR in application but is more robust to inhibitors and offers greater quantitative precision of DNA concentration
- Multiple species
  - Metabarcoding: a selected DNA fragment (DNA barcode) is amplified by PCR and sequenced on a high-throughput sequencing platform.
    - eDNA metabarcoding refers to processing environmental samples of water, soil or sediment.
    - Bulk metabarcoding refers to processing a mixture of specimens sorted from samples (by sieving, filtering or elutriation)
  - Metagenomics: the DNA of an environmental sample is sequenced directly without a prior PCR amplification of a specific gene region
  - Metatranscriptomics: the RNA from environmental samples is processed to assess the “active" community.

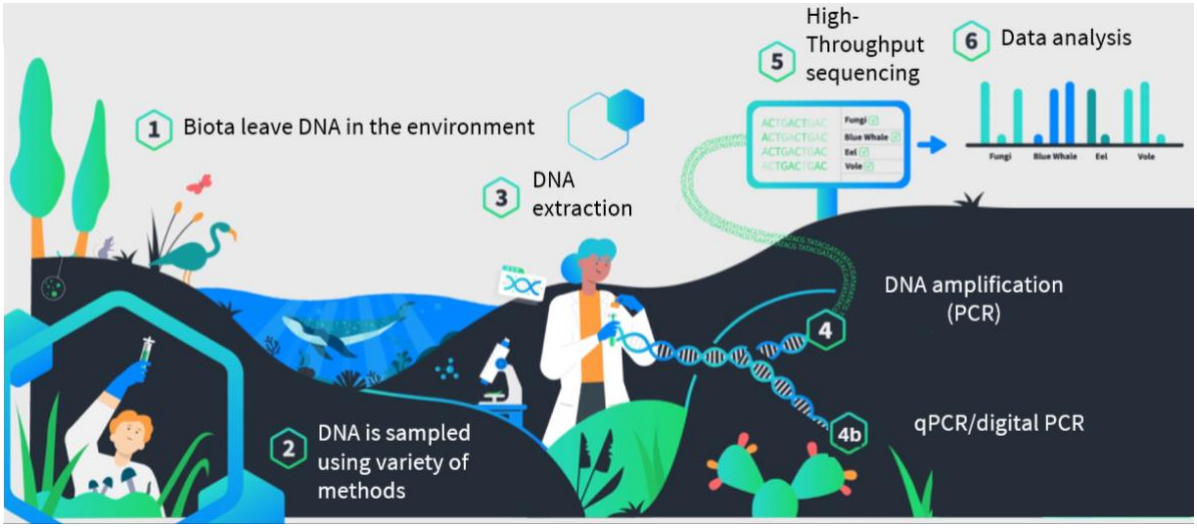


Figure 1: Workflow of eDNA analysis for (4-6) metabarcoding and (4b) single-species detection.

The most popular eDNA-based methods employed in biomonitoring are metabarcoding and qPCR. Metabarcoding is commonly used when measuring diversity, whilst qPCR is mainly applied when targeting one or a few species (e.g. protected, commercially important, or invasive, non-native species). Metabarcoding and qPCR can both be considered cost-effective analyses where less than 10 species are of interest. However, for larger number of species, metabarcoding is the preferred approach.

Validated qPCR can:

- be more sensitive for detecting individual species at low population levels, outperforming metabarcoding for a given taxon (Moss et al. 2022)
- have known limits of detection and the potential for quantification (see Thalinger et al. 2021 for a more in depth discussion of qPCR validation),
- have a greater propensity for facilitating in-field detection due to the lower complexity of steps and equipment involved (A. C. Thomas et al. 2020)

From a practical perspective, single species assays are cheaper, easy to standardize and provide a faster turnaround time, which can be essential when rapid action is recommended. However, the time and effort required to develop and validate qPCR assays is considerable compared to the use of standard metabarcoding methods,. For a more detailed discussion of the merits metabarcoding and single species assays, see Bruce et al. (2021). The advantages of each method are summarized in Table 3.1 below.

Table 3.1 – Comparison of single species assays and metabarcoding

qPCR / dPCR	Metabarcoding
More sensitive for detection of single species	Many species can be detected simultaneously
Detection limits can be established	Detection can be improved through increase of sequencing depth
Target DNA can be quantified	Relative abundance can sometimes be inferred from number of reads (sequences)
Lower complexity and rapid analysis can facilitate application	Cost per sample can be reduced through multiplexing

When preparing to carry out an eDNA-based biodiversity survey, clear objectives must be set before selecting eDNA approach, sampling methods, location and number of samples (Figure 2). These objectives in the context of energy industry activity might be:

- baselining to describe biodiversity status and to identify spatial and seasonal variation in species occurrence
- monitoring species introduction to a location
- monitoring changes in community composition associated with an impact

- monitoring the recovery of initial state during decommission and restoration

Next step is to decide whether qPCR or metabarcoding fit are the best for the project objectives and which species or taxonomic group are to be targeted. Depending on the objectives and target taxa, the environment (e.g., water or soil) to be sampled and a sampling method need to be defined. Final design of the survey should also account for local conditions and selection of sampling sites in order to ensure the validity of obtained data.

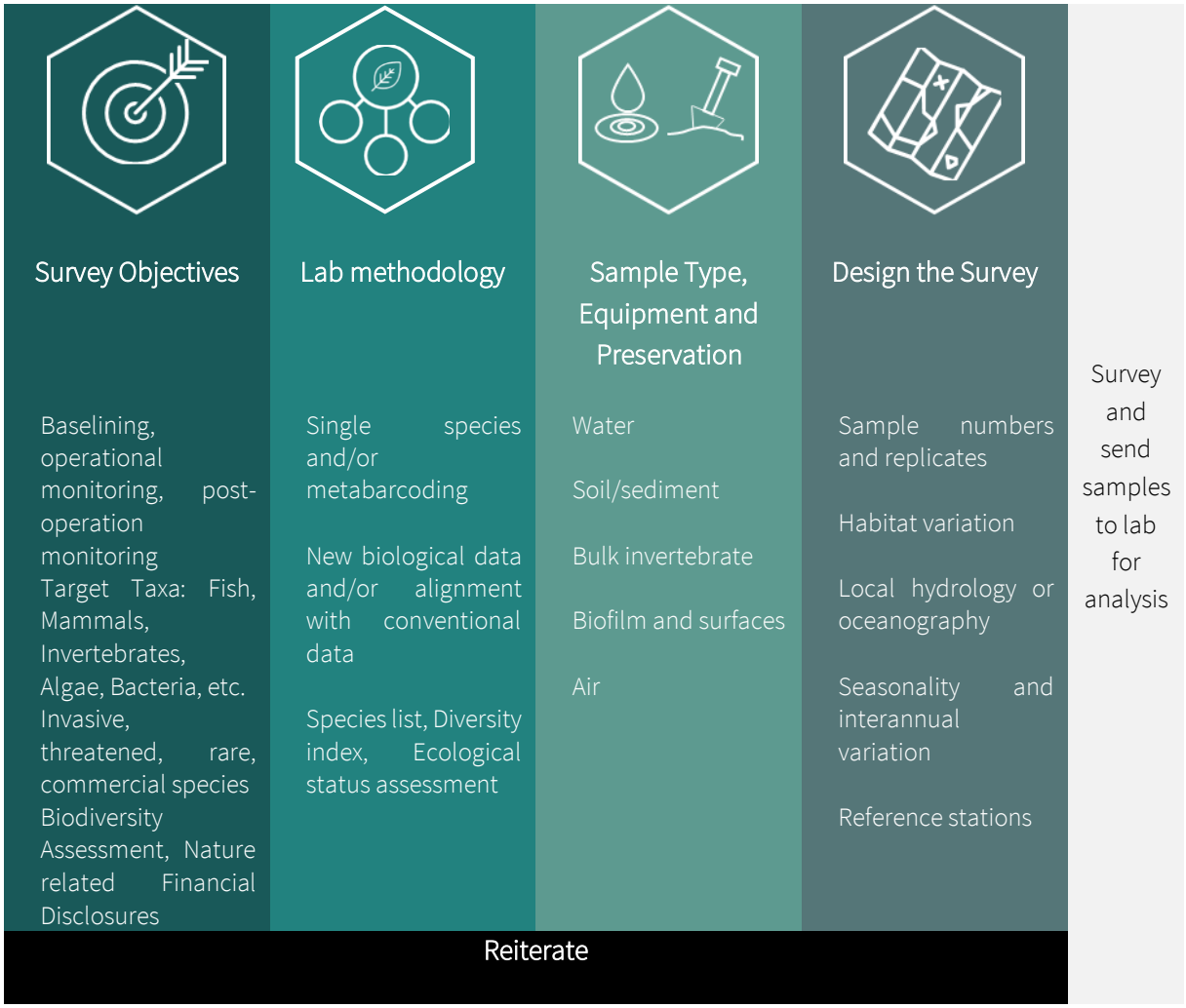


Figure 2. Steps for consideration when carrying out eDNA-based biodiversity surveys

Once a project execution plan is determined, the eDNA biomonitoring workflow can be broken down into the following steps:

- Field sampling and preservation
- Sample preparation and DNA extraction
- DNA quality checks (concentration and/or purity)
- PCR and quality checks
- Library preparation and sequencing for metabarcoding
- Bioinformatics and reporting

The detailed description of this workflow is provided in the IOGP publication Environmental Genomics Applications for Environmental Management Activites in the Oil and Gas Industry

Chapter 2, with further detail regarding regulatory acceptance and application and opportunities and needs in Chapters 3 and 4.

3.2 Applications

This section addresses the common applications of eDNA-based methods used to sample different environments and taxonomic groups.

eDNA is commonly isolated from water, sediment or soil. Additionally, in the case of freshwater environments, biofilm DNA samples provide a good material for analysis of phytobenthos (mainly diatoms) and small-sized invertebrates. Recent studies show also increasing evidence for the usefulness of airborne DNA, in particular to detect terrestrial vertebrates, flying insects and plants.

A more comprehensive list of taxa (mainly large-sized) that are not well represented (i.e., differ from traditional samples) in water or sediment eDNA samples (aquatic insects, marine benthic invertebrates, zooplankton), can be obtained through bulk DNA extracted from a pre-processed mix of specimens. The material for bulk DNA is collected in the same way as for morphological studies and only the processing of samples is different.
Below, we present the different types of samples used for DNA analyses for different environments and target taxa (Figure 3).

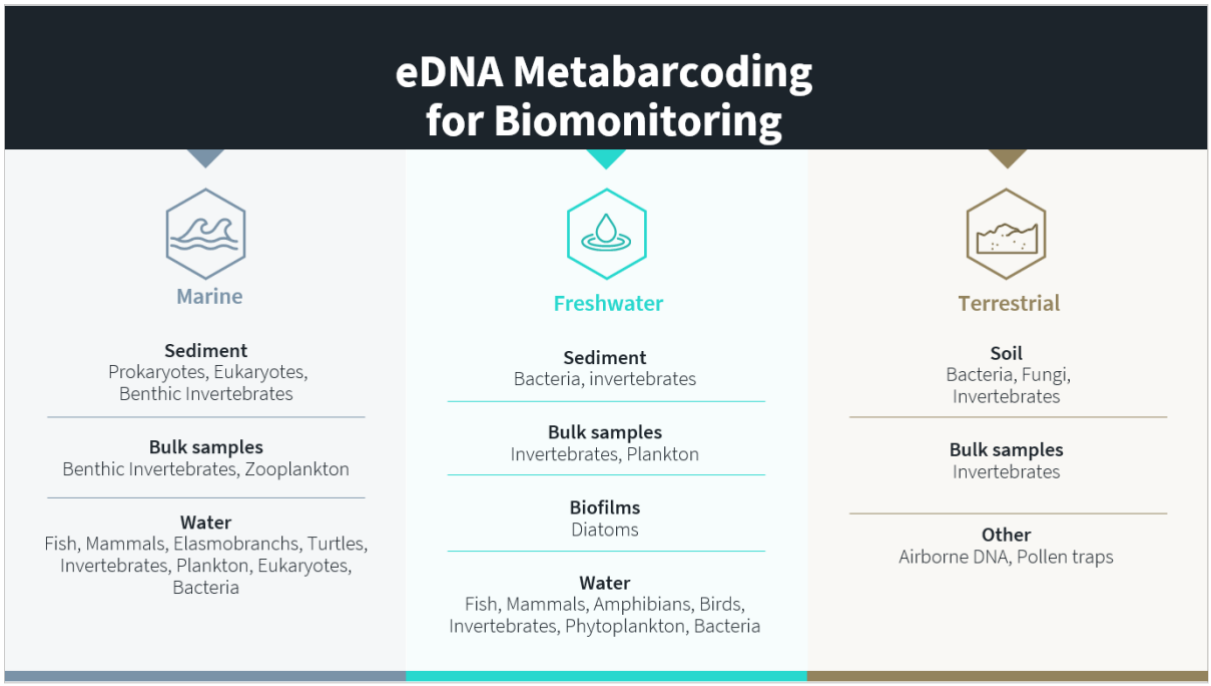


Figure 3. Potential applications of eDNA-based methods for different environments, sample types and target taxon groups.

3.2.1 Marine

DNA-based monitoring of marine biodiversity is mainly applied through analysis of sediment and water samples. Sediment eDNA is generally used to assess changes in the benthic macro- and meiofauna community, while water eDNA tends to be used to assess a broad range of vertebrates as well as planktonic taxa. Soft bottoms are preferentially sampled for eDNA, either

through analysis of sediment samples or bulk samples. eDNA analysis of hard bottoms is more challenging.

Soft bottom sediment samples from grabs or cores are commonly collected during conventional monitoring activities as well as during geotechnical surveys. These samples can be used for eDNA-based monitoring provided that the surface is undisturbed. Sediment is subsampled, frequently to a depth 2-3 cm, where most of epibenthic species are found. However, if the focus is on infauna, subsurface layers down to 10 cm should be subsampled. These subsamples can then be analysed separately or pooled and subsampled. For reviews of the methods of sediment sampling for eDNA and its preservation, see Pawlowski et al. (2022) and Wort et al. (2022).

Sediment eDNA samples can be used for monitoring a wide range of taxonomic groups. Prokaryotes have been used for the functional composition before and after contamination events (Krolicka et al. 2014), identify potential resilience, track site remediation (Wang et al. 2013), and to monitor restoration activities (e.g. Laroche et al. 2018). Microbial eukaryotes, meiofauna and traces of benthic macrofauna present in sediment eDNA samples have been extensively used for environmental assessment of impacts associated with marine aquaculture (Pawlowski et al. 2014), and O&G activity (Lanzén, Mendibil, et al. 2021).

Vertebrate groups such as teleost fish and elasmobranchs can also be detected in sediment DNA, but this analysis typically recovers less diversity than water eDNA samples (Koziol et al. 2019). Furthermore, there is a high degree of temporal uncertainty regarding eDNA preservation in sediments as the DNA often has a longer residence time than in water (Kuwae et al. 2020; Sakata et al. 2020). This means that baselining fish communities from sediment may not reflect the community at the time of sampling or show community changes associated with acute impacts.

Marine hard substrates are rarely sampled for eDNA due to difficulty in using grab or core samplers (but see Alexander et al. 2022; Alexander, Marnane, McDonald, et al. 2023). This may be resolved by a suction based sampling approach as proposed by Keeley et al. (2021) (Figure 4). The substrate type can influence the communities detected, hence any sampling must be representative of the local heterogeneity and be appropriate for the survey objectives (Koziol et al. 2019). For shallow or intertidal hard bottom samples, scrape or swab samples obtained by divers or ROVs can be used (Wangensteen, Cebrian, et al. 2018; Wangensteen, Palacín, et al. 2018; Alexander, Marnane, Elsdon, et al. 2023). For deep-sea sites, the bottom water can be used as a source of eDNA (Günther et al. 2018), although there is low taxonomic overlap between benthic water and substrate communities (Laroche et al. 2020a).

Bulk samples of benthic macroinvertebrates can be used as an alternative of sediment sampling. In this case, sediment samples are sieved on-board as for the conventional benthic monitoring and then preserved for subsequent DNA extraction and metabarcoding. If the sediments contain large amount of mineral debris (shells, coral skeletons) it is preferable to

extract DNA from preservative solution (ethanol) rather than from homogenized material. Analysis of bulk DNA enables identification of specimens using both morphology and DNA and provides data that are more similar to conventional methods compared to extracting eDNA from sediment. However, the taxonomic composition of bulk DNA samples are often different from conventional observations (Cowart et al. 2015; Loos and Nijland 2021), not to mention that smaller components of the benthic community (meiofauna and microbiota) are lost, reducing the range of community level data that can be obtained from eDNA analysis. Moreover, the relative abundance inferred from bulk samples can be biased by biomass variations of mixed species.

Bulk samples can also be used for zooplankton DNA analysis. In this case, plankton nets are used to collect specimens from surface waters. Samples of mixed specimens can be stored in ethanol or eDNA can be extracted directly from the mixed specimens. However, as the zooplankton communities can fluctuate due to environmental and seasonal changes, weekly or monthly repeated sampling is recommended (Song et al. 2021).

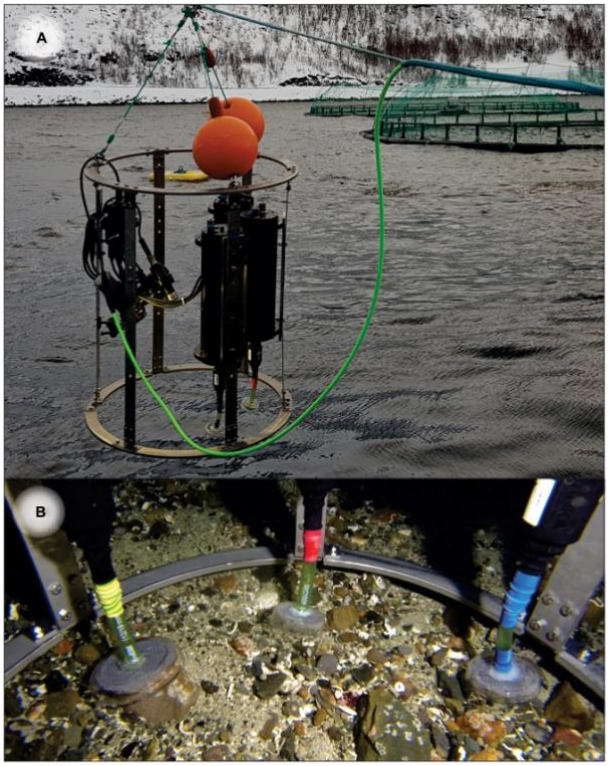


Figure 4: Suction based substrate sampler for hard substrate. Image and publication credit to Keeley et al. (2021).

Seawater sampling design should consider that eDNA can be highly localised (both vertically and horizontally), particularly in low-energy, slow-movement environments and when the water column is stratified (Port et al. 2016; Yamamoto et al. 2017; Jeunen et al. 2020; Djurhuus et al. 2018; Ely et al. 2021). Samples from the surface, at the thermocline, and near the bottom

can have different biological communities. Knowing oceanographic conditions is key to ensuring samples are taken at appropriate depths for interpretation of results. Niskin or GoFlo bottles are often used in an offshore setting (e.g. Closek et al. 2019), although nearshore, surface water samples can be taken using similar methods as sampling in freshwater.

Autosamplers can also be deployed for eDNA sampling at fixed intervals at a given location (Mynott 2019; Formel et al. 2021), or deployed on autonomous underwater vehicles (AUVs) (Hansen et al. 2020; Yamahara et al. 2019b; Truelove et al. 2022). An alternative is to use passive eDNA samplers which do not filter water (Bessey et al. 2021). These may be suitable for time-sensitive biological surveys, because they can collect eDNA over an extended duration if the adsorbent material is deployed successively. Research of this particular sampling method has developed rapidly in the past 2 years, testing a wide variety of materials and protocols (Jeunen et al. 2022; Verdier et al. 2022; Kirtane et al. 2020).

Seawater eDNA samples are usually filtered and preserved with a buffer (e.g. Longmire's solution) and/or frozen once recovered on the vessel (Bruce et al. 2021). Mechanical filtration using a pump is recommended to reduce the time and effort processing and allowing a greater volume of water to be filtered. This is key for marine sampling where the water typically has a lower concentration of eDNA than freshwater. Sampling can be carried out by personnel with minimal training in most conditions. Furthermore, fewer personnel are required than conventional sampling, resulting in less hours on deck and hence less HSE risks. For further detail on water eDNA sampling, refer to IOGP JIP34 Project 2 publication on 'Development of Industry Guidance on eDNA Sampling Standards and Guidelines'.

Seawater eDNA can be used to detect a wide range of pelagic taxa, from vertebrates to bacteria. While marine mammals and fish are successfully detected with eDNA, elasmobranchs, reptiles, and seabirds have been more problematic (although see Farrell et al. 2022; Boussarie et al. 2018; Liu et al. 2022). In the case of elasmobranchs and reptiles, this is thought to be due to their relative low abundance and low concentration of eDNA shed by these organisms (Adams et al. 2019). In these cases, sampling a greater volume of water can increase the detection probability of these rare species (McClenaghan et al. 2020; Schabacker et al. 2020).

Among other organisms, bacteria are frequently analysed from marine water samples following oil spills and in the subsequent remediation phase (Brakstad et al. 2015; Dubinsky et al. 2013; Krolicka et al. 2019; Zhang et al. 2018), with some studies also applying metatranscriptomics to show the active bacterial community (Knapik et al. 2020). These studies consistently use a finer filter mesh (0.22 µm) to enhance capture of these microorganisms.

3.2.2 Freshwater

DNA-based methods for freshwater biodiversity monitoring principally use a filtration kit and a standard sampling protocol. Bulk invertebrate samples as well as biofilm samples can be used

to target selected groups of freshwater bioindicators (aquatic insects, oligochaetes, nematodes, diatoms) when investigating ecological status of water bodies.

Freshwater samples contain DNA of organisms that spend part or all their lives in water as well as those from the surrounding areas, due to surface runoff of biological material and animals using it for drinking and bathing. This means that freshwater samples can be analysed for a broad range of aquatic, semi-aquatic and terrestrial species, allowing for rapid surveying of both freshwater and terrestrial environments (see also section 3.2.3.).

Freshwater eDNA is commonly used to assess the diversity of fish and amphibians. Numerous papers report using eDNA to assess fish diversity or to detect particular species (Hänfling et al. 2016; Bradley et al. 2022). The utility of eDNA for amphibian monitoring is best illustrated by the example of a great crested newt assay used since 2014 in the UK that has broadly replaced habitat suitability surveys with a qPCR test (Biggs et al. 2014; Rees et al. 2014).

Freshwater eDNA has also been used successfully to detect aquatic molluscs (Blackman et al. 2020) and some groups of insects used as bioindicators (e.g EPT; Brantschen et al. 2021). The water eDNA analysis of microbial communities (prokaryotes and eukaryotes) has been shown to have a broad range of applications, such as detecting pathogens and parasites (Bass et al. 2023), as well as the assessment of agricultural and human waste pollution impacts (Jin et al. 2018; Green et al. 2014; Malayil et al. 2020). More specifically, bacteria from water samples have also been analysed for assessing the impact of oil spills and hydrocarbon degradation (Irfan Ali Phulpoto 2021; Jurelevicius et al. 2013; Tiburcio et al. 2021).

During field sampling, water eDNA samples are commonly filtered *in situ*, using manual or mechanical pumping (A. C. Thomas et al. 2020; Bruce et al. 2021). In some cases, water samples are frozen to minimize DNA degradation and subsequently thawed and filtered in a lab, or water samples are kept cool for transport back to a lab for filtering. During the early adoption of eDNA, other methods of eDNA capture were also used, such as ethanol precipitation (Biggs et al. 2014) and centrifugation (Klymus et al. 2017). Both methods are currently considered as inefficient, as only a limited volume of water can be processed, resulting in a low DNA yield (Bruce et al. 2021). Nevertheless, ethanol precipitation is still used in the UK for one of the few regulated eDNA survey methods (detection of great crested newts, Biggs et al. 2015).

The DNA breaks down within hours to days in water, thus eDNA analysis focused on macrofauna usually captures fine-scale local (horizontal and vertical) and temporal communities. The persistence of eDNA in water depends on a range of factors, including:

- Water transport and mixing (flow, stratification, wave action)
- Temperature
- Water chemistry (e.g. pH, salinity, conductivity)
- Microbial activity
- UV-B radiation

- Substrate (the underlying layer – in rivers, streams, oceans etc.)

Some of these factors can be addressed by sampling strategies or analyses that consider seasonality, waterbody size, type, depth, and substrate. In particular, for rivers and streams, currents can result in downstream transport of eDNA (Spence et al. 2021; Pont et al. 2018; Wacker et al. 2019). For enclosed ponds and lakes, eDNA can be highly localised due to lack of flow and wave action (Brys et al. 2021; J. Li et al. 2019; Shu et al. 2022). Similar to marine systems, vertical eDNA transport may be limited in deeper lakes with thermal stratification (Littlefair et al. 2020).

Bulk invertebrate samples are an alternative method for eDNA analysis of invertebrates living on the surface or in the sediments and are often used as bioindicators of ecological quality status of freshwater bodies. Bulk DNA samples can be collected using the same procedures as for conventional methods as outlined in section 2.3.1. The principal differences are the preservation solution (usually ethanol) and the method for sample preparation depending on the target taxa. The most common method for freshwater invertebrates collected using kick-nets is homogenisation of the bulk sample (e.g. Elbrecht et al. 2017; Pereira-da-Conceicoa et al. 2020). However, the eDNA extraction from sample preservative also provides good results (Zizka et al. 2019). Similar bulk eDNA protocols apply for aquatic oligochaetes and nematodes, yet in the case of oligochaetes the best results were obtained using high-throughput sequencing of sorted and genetically tagged specimens (Vivien et al. 2020).

Biofilm samples can be used as a source of eDNA for monitoring the ecological status of rivers and streams using diatoms. Biofilm sampling methodology for eDNA is the same as for conventional monitoring outlined in section 2.3.1, following standard protocols (NF EN 13946 – April 2014) as set out under the Water Framework Directive (WFD). A Technical Report from the European Committee for Standardisation (CEN/TR 17245, 2018) provides recommendations to maintain compatibility of eDNA samples. This can be obtained by preserving biofilm samples in 70% ethanol (e.g. Pérez-Burillo et al. 2020) or by using commercial DNA preservation buffers (Visco et al. 2015; Apothéloz-Perret-Gentil et al. 2017). Biofilms can also be passive samplers for freshwater fish and macroinvertebrates eDNA, although their capacity to detect rare species was rather limited (Rivera et al. 2021; 2023).

3.2.3 Terrestrial

Terrestrial taxa can be surveyed using eDNA-based methods in a variety of ways. The most common is the analysis of soil, water and bulk eDNA samples. There are also several alternative DNA-based approaches to monitor terrestrial communities that include:

- faeces (Guillerault et al. 2017)
- insect-derived DNA (Gogarten et al. 2020)
- airborne DNA (Lynggaard et al. 2022)
- snow (Franklin et al. 2019)

- floral swabs (Newton et al. 2023)
- rollers on hard substrates (Kyle et al. 2022; Allen et al. 2023; Valentin et al. 2020)

Soil eDNA can be used to assess the diversity of bacteria (Nacke et al. 2016), fungi (Danielsen et al. 2021), invertebrates (Bienert et al. 2012) and terrestrial mammals (Leempoel, Hebert, and Hadly 2020). Soil microbial communities are commonly used for terrestrial restoration studies (van der Heyde, Bunce, and Nevill 2022). Soil fungi and bacteria responses to environmental change (e.g. pollution) are often more rapid than changes in the plant communities, what makes them excellent proxies for monitoring shifts in ecosystems (Bahnmann et al. 2018).

Soil sampling is usually carried out using composite subsamples (combining several small soil samples) within a given area to reduce small-scale variability. Sampling depth must be kept constant between samples. Subsamples can be collected using a mini-corer, trowel/scoop or where the ground is particularly hard, a metal corer. Samples are frozen or preserved in a buffer, and small subsamples (1 – 10 g) are used for final DNA extraction (Bruce et al. 2021).

Water samples can be used to detect terrestrial vertebrates, which leave their DNA traces when drinking, wading, swimming, urinating or defecating in water (Ushio et al. 2017; K. E. Williams et al. 2018; Harper et al. 2019). However, detection of terrestrial taxa can be sporadic as their contact with water is not constant. Consequently, their DNA is likely to be present at lower concentrations than aquatic or semi-aquatic taxa which may result in an absence of detection for some samples. Detection of terrestrial taxa is highly dependent on level of sampling effort, sample replication, sample representativeness, and species either directly interacting with water and/or their DNA being transported from other waterbodies/via runoff. Furthermore, eDNA sampling from water is not often possible in arid or frozen conditions.

Bulk DNA samples derived from different types of traps are frequently used for DNA analysis of larger mobile terrestrial invertebrates (isopods, myriapod, insects). The bulk samples are collected using the same procedures as for conventional means (e.g. Piper et al. 2019; Oliverio et al. 2018). The differences for eDNA-based methods include using ethanol for preservation and the bulk samples are often homogenised (Oliverio et al. 2018). However direct DNA extraction from the preservation buffer is being used more often to allow for concomitant morphological identification (Drummond et al. 2015).

3.3 Challenges and limitation of eDNA-based methods

To conclude, the main challenges and limitations of eDNA-based methods are listed below.

- Persistence of eDNA in water, sediment and soil depends on a range of environmental and biological factors, which should be taken in consideration when designing the eDNA-based survey.
- eDNA disperses in water over long distances making it difficult to determine exact location where species occurred.

- Species detectability in water eDNA (freshwater or marine) depends on DNA excretion (how much of DNA is shed by target species to the environment), degradation, and transport or diffusion (e.g. elasmobranchs and reptiles are difficult to detect due to their relative low abundance and low concentration of eDNA shed by these organisms).
- Taxonomic resolution of closely related species (ability to distinguish them) can be difficult with commonly used DNA barcodes.
- DNA accumulation over time in sediment and soil may impede temporal analysis of collected data.
- Small volume of sediment or soil (1-10 g) used for DNA extraction make it difficult to obtain a representative sample of larger size organisms such as benthic macroinvertebrates.
- Hard bottoms are difficult to sample for eDNA.
- Collecting bulk DNA samples can be labour intensive (no difference compared to conventional methods).
- Bulk DNA provides a reliable inventory of species, but their relative abundance is difficult to infer due to biomass and methodological biases.
- Substrate availability may be limited (e.g.no water for a site), resulting in sub-optimal eDNA methods being feasible for given taxa at a location

4 Comparison of Conventional and eDNA Methods

4.1 General Outline

For each biological group in marine, terrestrial and freshwater habitats, this section compares conventional methods and eDNA-based methods. This comparison is based on literature review presented in the Appendix. A total of 138 publications were selected. These were reviewed and summarised in tables (appendices) detailing:

- Location and habitat
- Conventional sample type
- DNA sample type and target
- Whether more taxa were detected with eDNA-based methods vs. conventional methods
- Whether more useful community analyses were obtained from DNA samples
- Taxon overlap between sampling methods
- Correlation of abundance/biomass and DNA (read numbers, qPCR or ddPCR outputs)
- Whether authors recommend eDNA-based methods as a replacement

Results were divided into broad taxonomic and ecological categories (biological groups), as in the previous sections. The following groups are considered:

Marine:

- Vertebrates (fish, marine mammals, reptiles; birds and elasmobranchs)
- Benthic invertebrates
- Zooplankton and phytoplankton

Freshwater:

- Fish
- Aquatic invertebrates (molluscs, arthropods)
- Phytobenthos (diatoms)

Terrestrial:

- Vertebrates, including semi-aquatic taxa such as amphibians
- Invertebrates

This section focuses on the 'target taxa' that were directly compared in reviewed papers to provide concise and clear comparisons. This results in a more conservative assessment of eDNA-based methods relative to conventional methods, as it does not consider the whole spectrum of taxa which can be detected using the same sample (e.g. vertebrates where mammals are targeted). This comparison downplays one of the inherent advantages of eDNA metabarcoding of targeting a broad range of taxa and recovering non-target taxa from an analysis at the same time. For this report, taxon/taxa will be used for comparing number of OTUs or ASVs with the number of morphospecies.

4.2 Marine

4.2.1 Vertebrates

A total of 13 publications were reviewed for marine vertebrate comparisons. 8 papers focussed on fish, 2 on aquatic reptiles, 2 on marine mammals and 1 on broad vertebrates. Studies were done on different habitats, from coral reefs and kelp forests to subarctic waters. In 11 studies, the eDNA and conventional data were obtained simultaneously, with conventional data obtained through underwater visual surveys, video ROV, trawl catch, and surface visual observations. In two studies, the comparison was done based on previous observations. Most studies used eDNA metabarcoding, with only two that used qPCR/dPCR for single species detection.

At a broad taxonomic level, the authors highlight a good congruence between eDNA and conventional methods, especially for fish and marine mammals. For example, 26/31 families of deep-water fish were found with both eDNA and trawling (Thomsen et al. 2016). However, at species level, taxa identified by eDNA only partially overlapped with those found through trawling or visual surveys (Closek et al. 2019). In general, the eDNA methods detected a significantly greater species number (up to 3 times as many) as reported for visual surveys (Alexander et al. 2022; Lamy et al. 2021). This difference was particularly high in tropical waters, where more taxa were detected in eDNA samples than in underwater visual surveys. However, in tropical waters, fewer taxa were identified to species level (Polanco Fernández, Marques, et al. 2021; Valdivia-Carrillo et al. 2021), likely resulting from gaps in DNA barcoding reference libraries which will likely improve over time. By comparison, the publicly available reference database for marine mammals and northern hemisphere fish species is relatively complete (Miya, Gotoh, and Sado 2020) making the eDNA studies much more effective.

Compared to fish and marine mammals, the effectiveness of eDNA was lower for aquatic reptiles, as indicated by the inconsistent detection of turtles, snakes and skinks in Australian estuaries (West et al. 2021). These species were semi-aquatic or terrestrial which could explain the difficulties in detecting them in water eDNA. Turtles and elasmobranchs may shed less DNA material than other taxa, leading to a lower concentration of eDNA, and therefore greater difficulty in detection (Closek et al. 2019). These limitations can be overcome through use of targeted primers and including sequences with low counts for elasmobranchs (Boussarie et al. 2018), or use of qPCR assays targeting turtles (Farrell et al. 2022).

eDNA detects a broader phylogenetic diversity and is more effective at detecting rare species known to be in the area but not observed visually within the study (Port et al. 2016; Polanco Fernández, Marques, et al. 2021). It also allows identification of fish and marine mammals as part of a broad vertebrate assessment. eDNA outperforms diver counts to capture spatial patterns at fine scales with less effort (Lamy et al. 2021). Finally, eDNA contributes with independent data that help understand and conserve threatened species such as sea turtles (Farrell et al. 2022) and whales (Baker et al. 2018). However, authors agree that the

combination of eDNA and visual surveys provides the most comprehensive survey approach to capture the broadest range of taxa (e.g. Alexander et al. 2022).

4.2.2. Benthic community

Twenty four publications were reviewed to compare the efficacy of eDNA versus conventional methods for assessing benthic biota. Conventional benthic biomonitoring is often based on an assessment of macroinvertebrates (and less frequently meiofauna), obtained through grab or core sediment sampling. A few of the reviewed papers directly compared the results obtained through analysis of sediment DNA with morphological identification of macrofauna (Aylagas et al. 2018; Cowart et al. 2015; Steyaert et al. 2020; Klunder et al. 2020; Cahill et al. 2018). Other studies used the indices based on benthic macrofauna as a benchmark, against which the eDNA detections of macrofauna as well as other groups were compared. The authors tested whether DNA data provided the same ecological quality assessment as conventional benthic macrofauna surveys. In addition, a few papers compared molecular and morphological data for meiofauna (foraminifera and nematodes).

The papers generally reported a low taxon overlap between conventional and eDNA-based methods. Metabarcoding was found to detect only 36% and 50% of macrofauna species identified by morphological surveys in seagrass (Cowart et al. 2015) and estuarine habitats (Aylagas et al. 2018), respectively. Both methods were unable to identify the same set of species, even when the number of taxa was similar (Steyaert et al. 2020; Cahill et al. 2018). In most of the studies examined, sediment metabarcoding detected more taxa (OTUs/ASVs) than conventional methods. For example, the number of benthic taxa revealed by metabarcoding was on average three times higher in sediments near offshore gas platforms in the North Sea compared to conventional surveys (Klunder et al. 2020). This was attributed to the inclusion of broader and cryptic taxon groups within smaller size classes, particularly notable when comparing metabarcoding results from sieved and unsieved samples where fewer taxa were identified in sieved samples (Steyaert et al. 2020).

The differences in taxonomic composition between metabarcoding and morphological methods might be explained by the small volume of sediments analysed for eDNA-based methods, subsequently resulting in a small fraction of the macrofauna community being detected (Aylagas et al. 2016; Pawlowski et al. 2022). Some papers also raise the issue of the incompleteness of reference databases, which leaves numerous sequences unassigned (Cowart et al. 2015; Steyaert et al. 2020). Another reason may be primer bias that can impede detection of some common genera of nematodes (Rzeznik-Orignac et al. 2017). More specifically, the difference between molecular and morphological data in the case of foraminifera was explained by the fact that conventional methods consider only shelled taxa, while eDNA analysis detects shelled and soft walled taxa (Frontalini et al. 2020).

Despite the differences in richness and taxonomic composition, the papers univocally show that metabarcoding data provide similar ecological quality status (EQS) as conventional benthic macrofauna surveys. This can be partly related to a correlation between biomass of dominant macrofauna indicators and their relative abundance in eDNA data (Lejzerowicz et al. 2015; Klunder et al. 2020). However, when macrofauna was not well represented in sediment DNA, taxonomy-free approaches have been proposed to overcome the absence of macrofauna eDNA data. A supervised machine learning approach was introduced to predict the EQS from bacteria or microbial eukaryotes, using macrofauna indices as benchmark (Cordier et al. 2017; 2018; Frühe et al. 2020). In addition, a multi-trophic metabarcoding biotic index was created for monitoring benthic communities (Keeley, Wood, and Pochon 2018).

The authors of reviewed papers agree that metabarcoding shows great promise for benthic monitoring of soft and hard bottoms. Although the use metabarcoding data comes with many challenges (reviewed in Duarte et al. 2021), several studies show that macrofauna-based indices can be efficiently replaced by metabarcoding analysis of other benthic taxa (meiofauna, protists, or bacteria). Depending on whether the aim of monitoring is to provide the inventory of soft-bottom community or to assess its ecological status, metabarcoding data can be used as a complement to, or replacement of, conventional methods. For hard-bottom fauna the combined approach (eDNA and video imagery) is recommended to detect the most taxa, because any single method will miss some species.

4.2.3. Zoo- and phytoplankton

A total of 5 publications were reviewed for pelagic non-vertebrate community comparisons. Three papers compare taxonomic composition of zooplankton community identified by microscopy to metabarcoding data obtained from seawater eDNA and/or bulk plankton samples collected using plankton trawls or a Continuous Plankton Recorder (CPR) (Deagle et al. 2018; Djurhuus et al. 2018; Suter et al. 2021). One paper estimates phytoplankton relative abundance compared to quantitative optical data (Pierella Karlusich, Pelletier, et al. 2022). The fifth paper analysed the efficiency of qPCR assay for the detection of pelagic larvae of the invasive European green crab (Roux et al. 2020).

The eDNA-based zooplankton community studies consistently found higher number of species compared to microscopic analyses, up to 1.6 times in the case of eDNA metabarcoding and even higher for bulk samples (Suter et al. 2021). Although significant differences in taxonomic composition were observed, all dominant taxa were detected by both molecular and microscopic analyses (Djurhuus et al. 2018; Kim et al. 2019). Regarding zooplankton quantitative data, the results of eDNA and bulk metabarcoding would require considerably more calibration to obtain relative abundance data (Deagle et al. 2018). Yet, it has been shown that biomass calculations for some zooplankton species can be derived from qPCR assays, where the gene copy number has been found to correlate with biomass (Jungbluth et al. 2022).

Similarly, a robust metagenomic approach was proposed to estimate relative abundance of phytoplankton targeting a photosynthetic gene (Pierella Karlusich, Pelletier, et al. 2022).

The papers conclude that eDNA metabarcoding is a promising technique for plankton biomonitoring. However, they also indicate several challenges related to the use of eDNA-based methods, including cross-contamination issues during sampling for DNA, gaps in reference database, and biases in quantitative data (Deagle et al. 2018). Further refinement and standardization seem necessary to make the methods more reliable (Suter et al. 2020). A combined approach of conventional (netting and more recently developed imagery approaches) and eDNA-based methods is recommended (Ibarbalz et al. 2019).

4.3 Freshwater

4.3.1 Fish

A total of 26 publications were reviewed for freshwater fish comparisons between conventional and eDNA-based methods. The papers report the eDNA data from different water bodies, including lakes, rivers, streams and ponds. The majority of papers characterize fish community, comparing taxonomic composition and relative abundance inferred from eDNA to data obtained using various conventional methods. Few papers focus on single species detection important for conservation or management of invasions.

In most publications, more taxa were found using eDNA-based analyses than with conventional methods, with the exception of emptied/drained ponds, where essentially all fish were captured (Blabolil et al. 2022; Di Muri et al. 2020). There was also one study where a combination of different conventional methods obtained a greater number of taxa than the eDNA-based survey alone (Nakagawa et al. 2018). Overall, eDNA appears to be the optimal method for assessing fish diversity, identifying a greater species richness with equal or lower sampling effort than conventional methods. This frequently led to more informative community analyses (Hayami et al. 2020; Hervé et al. 2022; Lawson Handley et al. 2019; J. Li et al. 2019; M. R. Snyder and Stepien 2020).

The reviewed literature indicated that a high proportion (60 - 100%) of fish species identified using conventional methods were also detected using eDNA surveys. In addition, eDNA-based surveys can have a lower risk of false negatives relative to conventional methods (Blabolil et al. 2022; Bradley et al. 2022). However, identification to species level of eDNA sequences can be challenging due to incomplete reference databases, especially in the tropics (Doble et al. 2020). Another challenge is the lack of taxonomic resolution of common DNA barcodes between some closely related species.

Fish abundance and biomass data generally correlated with the number of sequence reads or qPCR results (e.g. Pont et al. 2022), but this relationship also varied depending on the species, environmental conditions and life history traits (see Appendix for more details). Very few publications considered eDNA-based analysis to be a complete replacement for conventional methods, particularly with regard to abundance (Di Muri et al. 2020; 2022; Boivin-Delisle et al.

2021), but considered eDNA-based sampling best used in combination with conventional methods. In this scenario, fish size and abundance data can be collected in accordance with regulations such as the Water Framework Directive, whilst the use of eDNA analysis led to an overall reduction in sampling effort and cost.

An important difference between eDNA-based and conventional freshwater fish surveys is that multiple conventional sampling methods are commonly used in parallel as each method is selective, meaning only part of the spectrum of biodiversity can be detected (Hallam et al. 2021; Golpour et al. 2022; Pukk et al. 2021). By contrast, eDNA surveys frequently show a capacity for detecting a broad range of species, including elusive taxa that were previously undetected by conventional methods (Hallam et al. 2021).

4.3.2 Aquatic Invertebrates

A total of 16 publications were reviewed to compare eDNA-based methods and conventional methods used for assessment of freshwater invertebrates. The eDNA-based invertebrate community data were compared to kick-net sampling and other conventional methods used for the assessment of water and sediment quality. The targeted taxa included aquatic insects, molluscs and oligochaetes (the latter isolated from sediment and Surber samples). Some papers also used eDNA methods to detect invasive macroinvertebrate species (e.g. quagga mussels, Blackman et al. 2020).

Most of the studies used eDNA from filtered water, although in some cases bulk invertebrate samples were analysed. Bulk invertebrate samples often produced a higher diversity of target taxa (Harper et al. 2021b; Pereira-da-Conceicoa et al. 2020), but were more localised in their representation compared to eDNA from water samples. This may reflect incompleteness of sampling using kick nets, resulting in low taxonomic overlap between water eDNA and kick net data from the same locations (Keck, Hürlemann, et al. 2022). Furthermore, one of the advantages of eDNA over bulk sample DNA is that eDNA can also be used for other taxa (e.g. invertebrates initially, followed by vertebrates, (Harper et al. 2021a), allowing for more efficient ecological surveys.

Overall, the eDNA-based methods detected a higher diversity than conventional sampling (double number of genera in Uchida et al. 2020). However, both methods do not always detect the same taxa (62% overlap between genera at regional scale, Mächler et al. 2019). Moreover, the target taxa are often a minor fraction in water eDNA datasets. Their proportion can increase by selecting the optimal primer pair (Leese et al. 2020, Brantschen et al. 2022; 2021) or by using multiple assays (Elbrecht and Leese 2017). On the other hand, the eDNA-based methods detected taxa such as small-size invertebrates and protists which were not detected by kick-net or other types of conventional sampling. eDNA methods result in a more holistic overview of freshwater biodiversity and more accurate assessment of ecological status at different trophic levels than conventional methods.

Despite the differences in community composition, both eDNA and conventional methods generated similar scores for freshwater biotic quality indices (Fernández et al. 2019; Seymour et al. 2020; Vivien et al. 2020). Some authors used machine learning to predict ecological status of rivers from eDNA data, when the number of traditional indicator taxa was low (Brantschen et al. 2021). A good congruence between metabarcoding data and morphology-based indices was also found for aquatic oligochaetes that are often used as bioindicators of sediment quality in rivers and lakes (Vivien, Lejzerowicz, and Pawlowski 2016). However, in this case the high throughput sequencing of individuals one by one was considered as more reliable to infer the biotic indices because it provided abundance data (Vivien et al. 2020).

Few studies used qPCR rather than metabarcoding to detect invasive non-native (INNS) invertebrates (Dougherty et al. 2016; Blackman et al. 2020; Rice, Larson, and Taylor 2018) . eDNA-based surveys using qPCR often outperform conventional kick-net sampling in detecting INNS, especially when the target species is at low density. In contrast, eDNA-based surveys using metabarcoding sometimes detect fewer INNS than conventional sampling (Blackman et al. 2021). However, both approaches are complementary, as water eDNA analysis can detect species that are overlooked by traditional sampling.

4.3.3 Phytobenthos (diatoms)

A total of 9 publications were reviewed for benthic diatoms used as bioindicators of water quality in rivers and streams. Some of the papers focused on comparing diatom community composition in molecular and morphological datasets, others compare the diatoms indices inferred from biofilm eDNA and microscopic data.

Several studies found discrepancies in taxonomic composition and abundance of diatom species inventories generated by microscopy and metabarcoding (Nistal-Garcia et al. 2020). These discrepancies have been explained by the incompleteness of the reference database, primer bias or inaccuracy of bioinformatic pipelines (Bailet et al. 2019). The presence of cryptic genetic diversity in many diatoms also complicates the taxonomic assignment of sequences. These discrepancies result in a mismatch between species found using morphological and eDNA-based methods. Species misidentifications may in turn influence the disparity between sequence derived relative abundance and actual abundance, becoming a source of differences in ecological assessments (Zimmermann et al. 2014; Visco et al. 2015).

Solutions have been proposed to overcome these discrepancies in order to enable using diatoms metabarcoding data to assess water quality. Molecular indices were inferred from diatoms data and their values were compared to morphological indices obtained from the same samples and used as a benchmark. The best results were obtained using taxonomy free approaches to predict molecular index or to assign indicator values to metabarcodes (Apothéloz-Perret-Gentil et al. 2021). A cell biovolume correction factor was proposed to overcome quantification biases (Vasselon et al. 2017). Its application generally improved the

correlation between the two methods, suggesting that relative abundance estimates derived from sequence reads are feasible (Borrego-Ramos et al. 2021).

All reviewed studies generally agree that diatom metabarcoding has the potential to replace conventional microscopy-based analyses. Nevertheless, given the important differences of diatoms molecular and morphological data, this cannot be done by a simple replacement of specimens by sequences. Further research is needed to develop a novel metric derived directly from diatom metabarcoding data (M. Kelly et al. 2020).

4.4 Terrestrial

4.4.1 Vertebrates

A total of 33 publications were reviewed for terrestrial and semi-aquatic vertebrate comparisons between conventional and eDNA-based methods. The majority of these papers used eDNA captured via water filtration followed by qPCR detection of target species. Soil eDNA, airborne DNA and iDNA (invertebrates derived DNA) were also used to detect vertebrate species, although only a few comparative studies were available.

Amphibians and reptiles are common targets of eDNA-based surveys. For amphibians, the success rate of detection compared to the conventional surveys can be very high (84% for newts, Rees et al. 2014). However, the number of taxa identified varies between the methods, with a large number of unique observations for both eDNA and conventional methods (Svenningsen, Pertoldi, and Bruhn 2022; Bálint et al. 2018). For some amphibians, the visual and audio techniques outperform metabarcoding, especially as individuals metamorphosed (Moss et al. 2022). Therefore, a combination of eDNA-based and conventional methods is recommended to correct for the detection biases inherent in surveys, especially taking into account different life histories.

Regarding reptiles, the performance of eDNA-based methods varies between studies (Lacoursière-Roussel et al. 2016; Davy, Kidd, and Wilson 2015; Piaggio et al. 2014). Conventional trapping outperformed qPCR assays for semi-aquatic snakes (Rose et al. 2019), but eDNA performed better for the detection of invasive pythons (Hunter et al. 2015). Thus, eDNA-based methods are useful for reptile surveys as part of general scoping, but not as a stand-alone method. The majority of studies targeted aquatic reptiles using eDNA from water samples, with less success in detecting reptiles from water eDNA than mammals (Nordstrom et al. 2022). This is widely considered to be due to the hard outer layer of reptile skin resulting in lower rates of DNA shedding (Adams et al. 2019). Studying terrestrial reptiles using soil eDNA or cover boards has not been extensively explored, but shows promise (Nordstrom et al. 2022; Kyle et al. 2022; Ryan et al. 2022; 2020). This has been shown to generate data for reptiles not identified using camera traps, thus providing a complementary dataset (Ryan et al. 2022).

The effectiveness of eDNA surveys was also tested for mammal and bird monitoring. Mammalian eDNA was usually obtained from water or soil samples, but an alternative source was invertebrate-derived (iDNA) (Abrams et al. 2019, Gogarten et al. 2019) from insects pr other

invertebrates, such as mosquitos, flies, leeches or ticks, that have consumed or have attached mammalian DNA (see review by Carvalho et al. 2021). Bird eDNA is most commonly detected with broader vertebrate assays from water samples (Polanco Fernández, Mutis Martinezguerra, et al. 2021) even though there are bird-specific primers (Ushio et al. 2017). Birds have also been detected from eDNA collected from soils (Ryan et al. 2020), air (M. D. Johnson et al. 2023) and flowers (Newton et al. 2023).

Substrate plays an important role in eDNA surveys of terrestrial fauna. Sampling soil for vertebrate eDNA is limited in part by the unknown degradation timeframes of eDNA in soils (Leempoel, Hebert, and Hadly 2020) and has lower detection probability than sampling water (Sales et al. 2020). This difference may be caused by the water mixing and spreading eDNA over a wider area than eDNA deposited in soils, although targeted sampling of soil around shelter locations may improve detections of particular species (Ryan et al. 2020). This targeted sampling approach may be particularly viable when considering species distributions in arid areas (Egeter et al. 2018) or for species with a limited use of waterbodies (Harper et al. 2019).

In general the eDNA-based mammal surveys pick up a similar number of taxa to conventional methods. Yet, the composition of assemblage detected by eDNA differed from camera traps or mist nets. Camera traps generally detected larger taxa, while eDNA data was more effective in detecting smaller-sized species (Mena et al. 2021; Harper et al. 2019; Sales et al. 2020; Lyet et al. 2021). The same trend was observed in the case of iDNA surveys, which preferentially detected larger-bodied species compared to camera traps (Gogarten et al. 2020; Abrams et al. 2019).

In some environments, such as tropical forests, the reference database was a limiting factor for eDNA-based vertebrate surveys (Mena et al. 2021). The tropics are characterized by a high species diversity but there is relatively low financial support for barcoding projects to increase the quality of reference databases. As reference databases expand to match those in temperate regions, so too will the capacity of eDNA-based metabarcoding for species level identification. A combination of eDNA-based and conventional methods is currently recommended in the tropics. The complementarity of both approaches for vertebrate monitoring in temperate regions is also recommended (Svenningsen, Pertoldi, and Bruhn 2022).

4.4.2 Terrestrial invertebrates

A total of 12 publications were reviewed for comparisons between conventional and eDNA-based methods applied to soil and above ground invertebrate communities. The papers focused mainly on soil arthropods and nematodes and compared their diversity in soil eDNA and bulk samples split for metabarcoding and morphological analysis.

The eDNA-based methods generally performed as well as or better than conventional methods when comparing terrestrial invertebrate communities. Most of the papers show that metabarcoding detected a higher diversity of invertebrates than trapping or other

conventional methods. However, the efficacy of soil eDNA metabarcoding depends on the taxonomic groups targeted in the study. eDNA-based methods outperformed morphological identification when targeting nematodes in terrestrial habitats (Kitagami and Matsuda 2022). Yet, another study of nematodes claims that metabarcoding over- or underestimated the prevalence of some nematode families (Treonis et al. 2018). Morphology and metabarcoding of soil and bulk samples yielded well-correlated estimates of diversity and community composition of arthropods within soil (Oliverio et al. 2018). However, specific terrestrial invertebrate species considered as focal ecosystem service providers in orchards had very few taxa identified to a species level by metabarcoding of soil eDNA (Todd et al. 2020). Similarly, metabarcoding revealed more species of termites in soil eDNA, but less species of ants and springtails compared to the morphological data of tropical soil fauna (Basset et al. 2022). Bulk sample metabarcoding can reproduce ecological patterns of morphologically identified beetle community from the same pitfall and malaise traps, but soil eDNA detected different beetle community and greater diversity of invertebrates than conventional methods (Watts et al. 2019). Multiple sampling methods are recommended for insect biodiversity monitoring when carrying out DNA-based methods, as each has merits for particular ecological niches (Chua et al. 2023; M. Li et al. 2023),

4.5 Summary of method comparison

4.5.1 Fieldwork (ease of use, sampling effort, cost, safety)

Compared to the conventional methods, eDNA samples can be very simple to collect. This is particularly true in the case of water eDNA samples used to monitor fish and other aquatic (and semi-aquatic) vertebrates as well as aquatic invertebrates and microbiota. The main advantage of water eDNA sampling is that it can be done with far less expertise and effort and hence at much lower cost. Comparatively, the conventional methods take longer to obtain samples and often require trained ecologists or taxonomists to be present.

Water eDNA sampling is completely non-invasive and usually less hazardous, compared to trawling, netting or electrofishing. It can be more effective compared to visual observation and aerial surveys of marine vertebrates, especially when animals dive, or compared to PAMs (passive acoustic monitors), which detect mammals only when they generate noise. By contrast, for taxa shedding sufficient eDNA (e.g. mammals), they can be detected throughout the water column regardless of their activity (Booth, Sinclair, and Harwood 2020), provided the sampling strategy accounts for the dispersion and degradation of target eDNA.

There are also some practical advantages of using water eDNA in the case of marine industries monitoring. For example, the towed equipment used to collect conventional samples is often impractical to use near marine infrastructure such as oil rigs, jetties, cables, pipelines, manifolds, or offshore windfarms due to loss of gear, safety considerations and exclusion zones. These methods are therefore mainly applicable prior to construction of such

infrastructure during the baselining phase. If a follow-up survey is required once the facilities are in place as part of ongoing monitoring, conventional methods introduce additional HSE exposure, hazards and overall execution risk. For continued monitoring of the impact of an offshore development, conventional imagery and eDNA methods present the best combination of options. In addition, eDNA will often detect elusive taxa that are living in/on marine structures, infrastructure, or artificial reefs, and cannot be or are difficult to be detected using imagery.

The water eDNA can be used to supplement other activities (ROV collecting data or visual inspection). To decrease vessel time and costs, the industry is using ROVs and AUVs for collecting a range of data, including visual inspection of the sub-sea infrastructure. Whilst equipment hire or purchase still represents a significant cost, ROVs and AUVs can also be used to collect eDNA samples (Closek et al. 2019; Scholin et al. 2017), imagery data (Alexander et al. 2022), and ichthyoplankton data (Pierella Karlusich, Lombard, et al. 2022). Furthermore, multiple eDNA samples can be collected over a temporal range for longer term monitoring using an automated sampler compared to sampling during a single vessel deployment. For longer term surveys, autonomously sampled eDNA can be used (Searcy et al. 2022), which uses equipment similar to a fish trap and does not require regular field visits.

The sediment eDNA and bulk DNA sampling methods are broadly the same as conventional methods. However, a smaller volume of sediment is required for eDNA-based analyses, which makes it more suitable for sampling collection by AUVs and ROVs compared to conventional macrofaunal analysis. Also, less time is required for on deck processing (taking small cores or spooning subsamples from sediment grabs) saving considerable vessel time (Laroche et al. 2020a). The preservation of sediment and bulk eDNA samples has a significantly lower HSE risk, with minimal hazards compared to a formalin solution typically used for conventional sample preservation.

Regarding terrestrial sampling, the time and resources needed for a conventional field survey will vary significantly according to the size, accessibility and features of the site, as well as depending on the target taxonomic group. Camera surveys and some vertebrate trapping methods require the labour and resources for mobilising a large amount of equipment, resulting in significant upfront and analysis costs (Mena et al. 2021). eDNA sampling of terrestrial fauna typically takes less than half an hour per sample, not accounting for reaching the sampling location. Furthermore, this does not require a taxonomic expert for sampling in the field, but does require trained personnel for the subsequent analysis (Deiner et al. 2017). These advantages are less evident in the case of bulk DNA samples that are collected using the same methods as in conventional monitoring, whereby invertebrates are trapped, stored in buffer and then analysed via metabarcoding. Invertebrate communities can also be directly detected via soil eDNA sampling. An advantage of using soil samples is being able to survey for multiple taxonomic groups such as bacteria, fungi, and invertebrates simultaneously.

4.5.2. eDNA samples analysis (processing effort, data obtained, reference sequence library, turnaround time)

The turnaround time is the main difference between conventional and eDNA analysis. Conventional methods can generate data instantly and *in situ*, for example during visual observations of marine megafauna or morphological identification of fish. Rapid identification can be critical when trying to adaptively manage industrial activities in the presence of a protected species, or when providing an early warning of the presence of INNS (Whitaker, Brower, and Janosik 2021). Although eDNA-based detections are becoming more rapid (Besson et al. 2022), they cannot generate immediate species observation data, hence conventional methods may be more appropriate where immediate detections are required.

On the other hand, eDNA-based methods are automated and high-throughput, making them considerably less labour intensive and requiring less specialised taxonomist expertise compared to morphological analysis. This means that biological data can often be generated more rapidly and at a lower cost, especially when large number of samples is to be analysed or when a large number of species need to be identified.

eDNA-based methods have been demonstrated to be comparatively cheaper than conventional methods with regards to labour and laboratory costs, and requiring fewer in-field resources for the information gained. However, in some cases the eDNA analysis costs can be similar or even higher. For example, the costs of eDNA-based analysis per sample, can double the cost of camera trapping or small mammal traps due to the high unit costs. Yet, when comparing cost per species detected, eDNA-based methods have been shown to be a third of the price of camera trapping and just above half the cost of small mammal traps (Mena et al. 2021).

The ability to use multiplexing in the analysis of eDNA samples means that efficiencies and cost-savings are gained as the number of samples increases, whereas processing of imagery or collected and sorted specimens increases proportionately to the total number of samples taken. Moreover, different taxa can be detected from the same eDNA samples, which reduces cost of sampling because DNA extraction is only done once per sample regardless of how many separate assays are run. Although reporting results from eDNA-based surveys may take longer, compared to conventional surveys, they are more likely to include species of particular interest (e.g., INNS). The eDNA can also be used to examine ecosystem health more holistically and thereby may detect changes earlier than conventional methods, allowing for more timely management of industry activities.

4.5.3. Output data

eDNA sampling consistently detects more taxa than conventional sampling, albeit with fewer identifications to species level, especially when using metabarcoding in marine and/or tropical environments. This is mainly due to the incompleteness of reference databases. The need to complete the reference database is highlighted as a priority by most of reviewed papers.

The gaps in reference databases may also explain some of the disparity in species detected between eDNA and conventional methods. Taxonomic composition of metabarcoding data is broadly similar to morphology-based data, but their match largely depends on targeted taxa. The congruence is generally low for aquatic macroinvertebrates that are present in water or sediment eDNA samples only as DNA traces. The analysis of bulk samples provides more consistent results allowing detection of the majority of species present in the sample. The taxonomic overlap of data from eDNA-based and conventional sampling can also increase with better primers.

On the other hand, an advantage of conventional methods is that abundance is usually included in the outputs in terms of counts of individuals, which is more difficult to obtain with eDNA data. If population biological data such as size and abundance are required, conventional methods should be used. Although, advances are being made to overcome some of these challenges (Luo et al. 2022), for example, both qPCR and metabarcoding results frequently correlate with abundance and/or biomass for fish, this relationship is highly variable and direct inference is not robust. A cautious approach and regular calibration of eDNA-based methods against conventional methods is still needed when using eDNA for estimating abundance or biomass.

As remote and automated biological surveys become more prevalent, a combination of imagery (benthic and aerial), acoustic and eDNA data are likely to dominate environmental assessments. Broad species detections and community analyses from eDNA-based methods can be complemented by the quantitative measurements generated by imagery data such as underwater video.

4.6 Advantages of eDNA-based methods vs conventional methods

As shown in section 4.5, the eDNA-based and conventional methods have each their strengths and limitations. The latter have been already listed in sections 2.2 and 3.3. The advantages are summarized in Table 4.1 with reference to the fieldwork, sample analysis and output data.

Table 4.1

Table 4.1 – Advantages of eDNA-based biomonitoring and conventional methods at different stages

	Advantages eDNA	Advantages conventional
Field work	• Samples easy to collect, require less time and limited expertise • Sampling more effective, independent of species activity and habitat • Samples can be obtained in parallel to other monitoring activities • No need to use towed equipment near industrial installations • Sampling non-invasive	• Visual observations, netting or trapping provide instant, real-time data • Camera traps and other trapping devices have comparable low unit costs

	• Samples preservation using non-hazardous solutions	• Sampling protocols are standardized for different habitat and substrate types
Sample analysis	• High-throughput automated sample processing • Reduced time and costs when many samples processed simultaneously • No need for taxonomic expertise • Same samples can be used for surveying multiple taxonomic groups	• Turnaround time of sample processing and storage can be very short • Sample processing requires a relatively simple equipment
Output data	• More taxa consistently detected • More sensitive to detect rare species • Broader taxon range data available • Possible identification of inconspicuous and cryptic taxa • Species identification less subjective, depending solely on taxonomic coverage of reference database • Retroactive analyses possible	• Species Identification possible if taxonomic expertise available • Abundance data obtained through counting specimens • Biomass, age and health state can be reported • Biotic indices well established

4.7 Recommendations

Based on the reviewed papers, the status of eDNA-based methods compared to conventional methods was established. The criteria taken into consideration were the complexity, requirements and H&S of field sampling, the turnaround time and costs of sample processing and congruence of output data. For each habitat and target taxa, the eDNA-based methods were evaluated as:

- Stand-alone tools
- Preferentially used in combination with conventional methods
- Technically feasible but requiring further testing and validation

When appropriate, the single species detection and community analysis were evaluated separately. We assume that the appropriate markers for species detection are available.

Table 4.2 – eDNA method status for different taxa and data analyses

Habitat	Taxon	eDNA analysis	eDNA method status
Marine	Fish	Community analysis	Stand-alone
		Species detection	Stand-alone
	Other vertebrates	Community analysis	In combination
		Species detection	In combination
	Soft bottom benthos	Community analysis	Further validation
	Hard bottom benthos	Community analysis	In combination

	Zooplankton/Phytoplankton	Community analysis	In combination
		Species detection	Stand-alone
Freshwater	Fish	Community analysis	In combination
		Species detection	Stand-alone
	Aquatic invertebrates	Community analysis	Further validation
		Species detection	Stand-alone
	Phytobenthos (diatoms)	Community analysis	Further validation
Terrestrial	Vertebrates	Community analysis	In combination
		Species detection	In combination
	Soil invertebrates	Community analysis	Further validation
	Above-ground invertebrates	Community analysis	In combination

5 Application of eDNA-based surveys in Energy Industry Operations

5.1 Review of existing literature

Numerous studies explored the potential of eDNA-based technology to assess the environmental impact of energy industry activity. The topics of these studies include:

- Monitoring the impacts of exploratory drilling and oil and gas offshore platforms
- Monitoring the impacts associated with hydraulic fracturing
- Monitoring the impacts of oil spills
- Assessing long-term effects of decommissioning

Among 29 papers reviewed in this section, ten papers used sediment metabarcoding to assess the environmental impact of offshore oil and gas platforms. The focus of these studies was to find new bioindicators among microbial and meiofaunal communities that could potentially replace macrofauna currently used in routine benthic monitoring (Lanzén et al. 2016; Mauffrey et al. 2020). Laroche et al. (2017) showed that bacterial communities exhibited the strongest response to drilling and extraction impacts, followed by foraminifera, while macroinvertebrates (identified using morphology) were the least responsive. The microbial community was also a focus of the study by Krolicka et al. (2020), who found that composition and abundance of bacterial taxa correlate with the level of petroleum-related compounds such as barium and PAH. Correlation with environmental parameters (barium, THC and heavy metals) was demonstrated in most of benthic eukaryotic communities present in the vicinity of offshore oil and gas platforms (Laroche et al. 2016; Frontalini et al. 2020). Both water and sediment metabarcoding show that the impact is limited to the area less than 200m from the platform (Cordier 2019). Eukaryotic metabarcoding data were also used to calculate biotic indices, which were shown to be consistent with those inferred from benthic macrofauna (Mauffrey et al. 2020).

eDNA-based methods were also extensively used to assess the effects of oil spills and oil pollution on marine coastal habitats. Metabarcoding, metagenomic and metatranscriptomic studies analysed the impact of Deepwater Horizon spill on bacterial and eukaryotic communities (Bik et al. 2012; Mason et al. 2012). Long-term ecological effects of residual oil on benthic communities were analyzed in contaminated area at South Korean coast (Xie et al. 2018), while Oladi et al. (2022) analyzed the impact of frequent crude oil spills on coral reef ecosystem in the Persian Gulf. The mesocosm experiments conducted by Krolicka et al. (2017; 2019) allow identification of key microbial indicators of crude oil pollution, while Knapik et al. (2020) used metatranscriptomics to identify bacterial functional genes that could be used as target for oil-related genosensing.

Compared to many studies applying eDNA to the marine environment, much less was done on monitoring energy industry activity in freshwater and terrestrial environments. Few studies

were conducted on the impact of hydraulic fracturing on microbial communities living in the streams, however, they were limited to one geographic area (Northwestern Pennsylvania) and bacterial community only (Ulrich et al. 2018). Bacterial metabarcoding was also used to test different soil covers used for the decommissioning of oil sands mining sites (F. Stefani et al. 2018). On the other hand, freshwater zooplankton metabarcoding was used to assess the efficiency of oil spill remediation activities in a boreal lake (Ankley et al. 2021). The water eDNA was also used to evaluate the impact of gasoline spill on population of giant salamander in Pennsylvania (Perelman et al. 2021).

Table 5.1 – eDNA studies of energy industry activity and related impacts

O&G activity	Target taxa	eDNA method	Project objective	Reference
Exploratory drilling	Bacteria	Sediment metabarcoding	Microbial bioindicators of drilling waste discharge	Nguyen et al. (2018)
Exploratory drilling and gas production field	Bacteria	Sediment metabarcoding	Bacterial community metabolic response	Laroche et al. (2018)
Oil platforms (New Zealand)	Bacteria and eukaryotes	Sediment eDNA and eRNA	Benthic monitoring of offshore drilling sites	Laroche et al. (2017)
Oil platforms (New Zealand)	Benthic foraminifera	Sediment metabarcoding	Macrofauna and foraminifera response	Laroche et al. (2016)
Oil platforms (North Sea)	Eukaryotes	Phylogenetic microarray	Benthic monitoring of oil-drilling sites	Lekang et al. (2020)
Oil platforms (North Sea)	Eukaryotes	Sediment metabarcoding	Benthic monitoring of oil-drilling sites	Lanzen et al. (2016)
Oil platforms (North Sea)	Bacteria and eukaryotes	qPCR and metabarcoding	Identification of microfaunal benthic indicators	Krolicka et al. (2020)
Oil platforms (North Sea)	Eukaryotes (3 markers)	Sediment metabarcoding	Biotic indices inference	Mauffrey et al. (2020)
Oil platforms (Persian Gulf)	Elasmobranch	qPCR, water metabarcoding	Whale shark aggregation at an oil field	Thomsen et al. (2016)
Gas platforms (Adriatic)	Eukaryotes (5 markers)	Water and sediment metabarcoding	Response of planktonic and benthic communities to platforms activity	Cordier et al. (2019)
Gas platforms (Adriatic)	Benthic foraminifera	Sediment metabarcoding	Comparing morphology and metabarcoding data	Frontalini et al. (2020)
Oil spill (Gulf of Mexico)	Eukaryotes	Sediment metabarcoding	Deepwater Horizon oil spill impact on coastal habitats	Bik et al. (2012)
Oil spill (Gulf of Mexico)	Bacteria	Metabarcoding, Metatranscriptomics	Oil spill impact on beach microbial communities	Lamendella et al. (2014)
Oil spill (Gulf of Mexico)	Bacteria	Metagenomics, Metatranscriptomics	Response of bacterial communities to aliphatic hydrocarbons	Mason et al. (2012)
Oil spill (Gulf of Mexico)	Nematodes	Bulk DNA metabarcoding	Oil spill impacts on intertidal meiofauna	Brannock et al. (2014; 2017)

Oil spill (Persian Gulf)	Bacteria and eukaryotes	Sediment metabarcoding	Oil spill Impact on coral reef sites	Oladi et al. (2022)
Oil spill (South Korea)	Bacteria and eukaryotes	Sediment metabarcoding	Microbial and metazoan response to oil spill	Xie et al. (2018)
Gasoline spill (river)	Amphibians	Water eDNA	Impact on endangered giant salamander	Perelman et al. (2021)
Oil spill remediation	Freshwater zooplankton	DNA and RNA metabarcoding (bulk samples)	Natural vs active shoreline cleaning in freshwater ecosystems	Ankley et al. (2021)
Oil spill remediation	Freshwater zooplankton	DNA and RNA metabarcoding (bulk samples)	Experimental testing of zooplankton response to environmental stressor	Ankley et al. (2022)
Oil pollution	Bacteria	qPCR and metabarcoding	Identification of microbial key indicators	Krolicka et al. (2019)
Oil pollution	Bacteria	Water metabarcoding	Mesocosm experiment	Krolicka et al. (2017)
Oil pollution	Bacteria	Meta-transcriptomics	Development of genosensors	Knapik et al. (2020)
Hydraulic fracturing	Bacteria	Water, biofilm and sediment metabarcoding	Response of stream microbial communities to environmental impacts	Trexler et al. (2014)
Hydraulic fracturing	Bacteria	Biofilm metabarcoding	Chemical pollution impact on streams microbiota	Johnson et al. (2017)
Hydraulic fracturing	Bacteria	Water and sediment metabarcoding	Response of stream microbial communities to environmental impacts	Ulrich et al. (2018)
Decommission oil sands	Bacteria and fungi	Soil metabarcoding	Impact of soil covers on oil sands mining sites	Stefani et al. (2018)
Decommission oil platforms	Fish and elasmobranch	Water metabarcoding	eDNA vs ROV stereo-video observations	Alexander et al. (2022)
Biocorrosion control	Bacteria	Water metabarcoding	Monitoring microbiota in produced water	Dutra et al. (2023)

5.2 Conventional vs eDNA-based monitoring using offshore platforms as a model

Here, we present current conventional methods used for biomonitoring of offshore platforms and discuss the potential of eDNA analyses to replace and/or complement these methods as the most frequently considered model. Our selection of conventional methods is based on the *Guidelines for environmental monitoring of petroleum activities on the Norwegian continental shelf* (Norwegian Environmental Agency 2020), to which we added acoustic and visual observation of marine mammals from the UK JNCC Guidelines during geophysical operations (JNCC 2017).

Currently, the only biological component included in water column monitoring of offshore platforms are ecotoxicological analyses of contaminants present in tissues of wild caught fish

and caged blue mussel. Monitoring the communities of fish and other pelagic species is not comprised in the current regulations. However, the Guidelines consider using the eDNA methods as part of water column monitoring in the near future. Such potential application could be the use of eDNA in combination with acoustic and visual surveys to detect cetaceans during baseline surveys. In addition, water eDNA metabarcoding could also be used to periodically survey the impact of energy industry activity on pelagic biota.

Regarding seabed monitoring, the conventional surveys include both hardbottom and softbottom macro- and megafauna diversity. The softbottom meiofauna (e.g., foraminifera, nematodes) is also mentioned, though considered as an additional activity. The visual surveys using ROV video footage are proposed to monitor hardbottom fauna (e.g., corals, sponge and sea-pen communities). The softbottom macrofauna is analysed based on sediment grab samples. In both cases, the morphological species identification could potentially be replaced by eDNA, either by analysis of DNA extracted from bulk samples or eDNA metabarcoding of water or sediment samples. Both methods can be used to provide species list and taxonomic composition of benthic communities. As shown by papers in this section, the sediment metabarcoding could also be used to assess ecological status of benthic communities focusing on microbial or meiofaunal bioindicators.

Table 5.2 – Conventional monitoring methods of energy industry activity in marine environment and corresponding eDNA-based solutions.

Conventional monitoring (operation phase)	Potential eDNA solution
Water column monitoring	
Marine mammals (cetaceans): acoustic and visual observation	Water eDNA metabarcoding or qPCR
Wild caught fish tissue ecotoxicological analysis	Not applicable
Blue mussel contaminants monitoring	Not applicable
Benthic monitoring	
Hardbottom benthic megafauna: visual survey, mapping of vulnerable species habitats	Bulk DNA or water eDNA metabarcoding
Softbottom benthic macrofauna: morphology-based identification, counting, diversity indices	Bulk DNA or sediment eDNA metabarcoding
Softbottom benthic meiofauna: morphology-based identification, diversity indices	Sediment eDNA metabarcoding

5.3 Prospective eDNA applications to monitor energy industry activities

Here, we propose some eDNA applications that could be relatively easy to implement at different phases of energy industry project lifecycle. We focus on marine environment where most of the reference studies relative to energy industry activities were conducted, but these applications could be easily transferred to the freshwater and terrestrial habitats. The current

development status of each eDNA method is provided based on the reviewed papers (section 5.1).

Table 5.3. Prospective eDNA methods to assess ecological impacts of energy industry activities in marine environment

Table 5.3 – Prospective eDNA methods to assess ecological impacts energy industry activities in marine environment

eDNA method	Target taxa	Aims and Expected results	Current status
Baseline surveys			
Water eDNA metabarcoding/qPCR	Marine mammals (cetaceans)	Avoid disturbance and inference with migratory routes for selected sites	Ready to use
Water eDNA metabarcoding	Fish/pelagic macrofauna	Detect sensitive species / avoid fishing spots	Ready to use
Bulk DNA metabarcoding	Zooplankton	Surface water plankton taxonomic composition	Development phase
	Benthic macrofauna	Benthic macrofauna taxonomic composition	Development phase
Sediment eDNA metabarcoding	Benthic meiofauna	Meiofauna taxonomic composition	Development phase
	Benthic microbial community	Microbial taxonomic composition	Ready to use
Exploratory and production drilling			
Water eDNA metabarcoding	Bottom fish and other fauna	Assess bottom water biological quality	Development phase
Sediment eDNA metabarcoding	Benthic meiofauna and microbial community	Assess sediment biological quality	Development phase
Oil spills and remediation			
Water/sediment eDNA metabarcoding	Microbial community	Assess the impact of contaminants	Ready to use
Water eDNA metabarcoding/qPCR	Fish/pelagic macrofauna	Detect sensitive species	Ready to use
Decommissioning			
Bulk DNA metabarcoding	Hardbottom invertebrates	Assess epibenthic diversity	Development phase
Water eDNA metabarcoding/qPCR	Invasive non-native species (INNS)	Detection of INNS	Ready to use
Restoration			
Bulk DNA metabarcoding	Benthic macrofauna	Congruence with baseline studies	Development phase

Water eDNA metabarcoding/qPCR	Fish/pelagic macrofauna	Detect sensitive species, reintroduction of species	Ready to use
Paleogenomics – sediment ancient DNA	Macrophytes, macrofauna	Reconstruct reference conditions of impacted ecosystems	Development phase

5.3.1 Baseline surveys

Baseline assessments provide a starting point from which to measure potential environmental change. A baseline often consists of a survey of an existing habitat or ecosystem, which may experience subsequent ecological change from energy industry activities. These are static baselines that can be used to illustrate change over time. For determining if changes are specific to the location, a dynamic baseline which accounts for change over time is generally recommended. Although this is reliant upon information over a wider area, particularly reference stations, this design allows any change to be given a broader context.

The eDNA-based methods are particularly useful at the stage of baselining because they offer a rapid and relatively complete overview of what is living in the area of prospective oil and gas activities. Compared to the conventional methods, the eDNA analyses do not require extensive knowledge of each taxonomic group but provide inventory of wide range of taxa, which identification depends on their representativity in reference database. This unique insight into global biodiversity can be obtained based on relatively few water and sediment samples, as the diversity of different taxonomic groups can be explored using the same eDNA samples, saving time and costs of sampling and samples processing.

The eDNA can also be used to detect key species, such as protected or sensitive species, which is critical during baselining. It can inform actions such as the complete avoidance of particular areas, or avoidance of activity during certain times of year, the first stage of the mitigation hierarchy. The monitoring of marine mammals and their migratory routes is particularly important regarding the location of offshore energy activities and infrastructure. This is currently done primarily using acoustics, aerial imaginary and visual observations but could be easily complemented by eDNA analysis. Such broad baselining approach using multiple methods is non-invasive and covers a range of temporal and spatial scales. The eDNA could also be used for the survey of particularly sensitive habitats, such as spawning areas or coral reefs. The method is well established to detect pelagic species in water column eDNA samples. However, it could also be applied to hard bottom fauna in combination with ROV video imagery-

It is worth mentioning that there are already examples of using eDNA for baseline surveys of marine industrial activities other than those associated with the energy industry. For example, eDNA was used to explore deep-sea biodiversity in the area of prospective polymetallic nodule mining (Laroche et al. 2020a; 2020b). The studies show that benthic metazoan diversity is positively correlated with nodules density and sea bottom topography and that the pelagic

eDNA does not affect surveys of benthic community at the deep-sea floor. This type of water and sediment eDNA metabarcoding analyses are technically ready to use and could be easily implemented also during the pre-production baselining of oil and gas activities.

5.3.2 Operations phase

As shown in section 5.1, several studies applied eDNA-based methods for biodiversity monitoring during the operational phase of energy projects. Most of them focus on the assessment of impacts associated with the activity of offshore platforms on benthic diversity. Traditionally, this is done based on benthic macrofauna sorted from sediment samples, morphologically identified and counted. The macrofauna data are used to infer biotic indices, such as AMBI (Borja, Franco, and Pérez 2000) or BQI (Benthic Quality Index, Rosenberg et al. 2004), that serve to assess the ecological status of a benthic community.

Inferring macrofauna-based indices from bulk or sediment eDNA metabarcoding is possible (Lejzerowicz et al. 2015) but not always reliable due to the issues of abundance, biomass variation and low representativity of macrofauna in small sediment eDNA samples (Pawlowski et al. 2022; Wort et al. 2022). These limitations can be overcome by predicting biotic indices from bacterial or eukaryotic eDNA metabarcoding data using machine learning approach (Cordier et al. 2018). Yet, to make this approach successful an extensive training dataset are needed. Alternatively, the macrofauna can be replaced by meiofauna or microbial indicators and indices based on sensitivity to drilling-related contaminants (Laroche et al. 2016; T. T. Nguyen, Cochrane, and Landfald 2018; Krolicka et al. 2019)

5.3.3. Oil spills and remediation

Monitoring oil spills is of key importance for the sustainability of the marine environment. When an unplanned event has occurred, such as a chemical/waste leakage or an oil spill, an environmental survey may be required to comply with a regulatory requested environmental impact assessment to establish a post impact baseline (Hinz et al. 2022). Different sensor technologies are available to detect and monitor oil spills. Remote sensing data can be used to characterize oil spill pollution types (Yang et al. 2023). The environmental impact of oil spills can also be assessed based on microbial communities, particularly bacteria and fungi, as these organisms have rapid responses to environmental changes. The protocols for eDNA metabarcoding of bacterial and eukaryotic microbiota are well-established, and the methods are ready to be applied.

Monitoring microbial community plays also an important role during the remediation process. Selected bacterial groups can be used as indicators of oil pollution (Krolicka et al. 2019) and some of bacterial genes are considered as promising genosensors (Krolicka, Gomiero, and Baussant 2020). Furthermore, the eDNA metabarcoding and metagenomic data could also help in remediation by targeting microorganisms that support biodegradation and assimilation of hydrocarbons as well as screening for hydrocarbon-degrading genes. Bacterial community changes can indicate the progression of oil spill degradation.

On the other hand, the eDNA-based surveys can be used to rapidly obtain samples for a snapshot of the ecosystem condition, potentially even between the spill and the impact. These samples can be biobanked to provide a reference for future remediation work, or simply be used to monitor the recovery of the impacted site (Yergeau et al. 2015).

5.3.4. Decommissioning

At the end of production phase, decommissioning is the first step towards returning of the site to its natural pre-production conditions. Depending on the regulations, the installations can be entirely removed or part of them can be left in place. If the installation is not completely removed it continues to act as an artificial substrate. Such substrate can be rapidly colonized by various epibenthic species and become a hotspot of diversity that impact the whole benthic community in platform vicinity. The species living on such hard substrate belong to various groups of sessile invertebrates, whose identification using visual observation and morphology-based taxonomy is often problematic.

eDNA metabarcoding appears as the best solution to survey a huge diversity of sessile organisms living on hard substrates (Obst et al. 2020). Several studies demonstrate its efficiency in the case of Autonomous Reef Monitoring Structures (ARMS) (Pearman et al. 2020; Ip et al. 2023). The significant increase of benthic diversity was also demonstrated using morphological and metabarcoding analyses in the case of a partly decommissioned gas platform in the North Sea (Klunder et al. 2018). The impact of decommissioned platforms on marine diversity in the Gulf of Thailand was analysed using water eDNA metabarcoding to target fish and elasmobranchs (Alexander et al. 2022), as well as to explore broad range of eukaryotic phyla using water, bio-foul and sediment eDNA samples (Alexander, Marnane, McDonald, et al. 2023). Finally, eDNA metabarcoding has also been used to detect marine invasive non-native species (INNS) (Zaiko et al. 2020). The partly decommissioned platforms could act as steppingstones for INNS dispersal and it is of great ecological importance to include their detection in routine monitoring (Macreadie, Fowler, and Booth 2011).

5.3.5. Restoration

Ecological restoration is the practice of renewing or restoring degraded, damaged, or destroyed ecosystems and habitats via active human intervention. Monitoring diversity before, during and after restoration efforts is used to evidence changes in these ecosystems. Conventional approaches for monitoring restoration include visual surveys, trapping/netting, tissue sampling, and sampling for chemical analysis, such as heavy metals and hydrocarbons.

The eDNA-based methods offer a possibility to assess changes to the biological communities compared to the original baseline. One of the advantages is that the eDNA sampling method can remain constant throughout the changes to the habitat, whereas most forms of conventional sampling would have to be altered due to changes in the habitat type. For example, new artificial reef structures make net tows for plankton or fish prone to

entanglement. Similarly, saltmarshes restored on bare substrate make deployment of underwater cameras for fish community analyses more problematic, whilst a water eDNA sample remains feasible. Furthermore, eDNA-based methods generally have higher detection rates for cryptic and elusive species than conventional methods, and the capability to sample a broader community. This makes eDNA-based methods very useful when tracking the reintroduction of rare species (Riaz et al. 2020; Rojahn, Gleeson, and Furlan 2018) or monitoring any changes to the local biodiversity and ecosystem condition following restoration (Armbruster et al. 2021; van der Heyde, Bunce, and Nevill 2022; Heyde et al. 2020). These benefits of eDNA-based monitoring can also be achieved at a lower effort, increasing overall project efficiency in restoration monitoring.

The use of eDNA-methods applied to restoration can be limited by the lack of genetic data for pre-construction baselining. Various technical and biological factors can also bias the comparison of baseline surveys recorded using conventional and eDNA methods. However, this can be overcome using eDNA data from closely situated, non-impacted reference sites. Alternatively, it is possible to recover reference conditions through paleogenomic analysis of sedimentary archives. Recently, environmental paleogenomics was used to investigate the impact of industrialization, urbanization and agriculture development during Anthropocene (e.g. Barrenechea Angeles et al. 2023; Siano et al. 2021). Such eDNA studies conducted on decadal scale could be of great interest to recover reference conditions in the decommission sites and other areas where industrial activity has stopped but the past biodiversity has not been well documented (N.-L. Nguyen et al. 2023).

6 Elements to be considered when planning eDNA-based surveys

This section discusses elements that should be considered when planning to include eDNA in biomonitoring surveys, as replacement or complement to conventional methods. As shown in the preceding sections, the potential of the eDNA approach to be used for biomonitoring energy industry activities is high. Yet, eDNA analysis and its results may not always meet the criteria and requirements of biomonitoring procedures. Hence, it is important to carefully assess the values of an eDNA approach compared to conventional methods before its practical application. For each element described below, we provide some questions to be asked when selecting the best approach.

6.1 Suitability

The first element to be taken in consideration is the capacity of eDNA to fulfil the purpose of a specific biomonitoring activity. In general, eDNA provides information about the presence/absence or relative abundance of organisms in the sampled environment. It allows the identification of the species, or a group of species based on their DNA barcodes. Hence, it is a valuable tool for detecting target species, for example when monitoring the presence of marine mammals during baseline surveys or searching for invasive non-native species (INNS) during the operational phase. The use of eDNA as replacement or complement to conventional methods is also possible if the goal of the survey is to analyse community response to potential impacts associated with energy industry activities, for example in the case of benthic monitoring around offshore platforms. It is possible to replace morphotaxonomy by eDNA-based community analysis and this could be beneficial for ecosystem health assessment. However, this remains dependent upon a good understanding of DNA sources, biases and error levels as well as sample representativity, as demonstrated in Lanzén et al., (2021).

On the other hand, eDNA cannot usually be used to biologically characterize the populations, to inform directly about the age (although see Zhao, van Bodegom, and Trimbos 2022), the biomass or metabolic activity of target taxa. Some of this information could be obtained indirectly by analysing the eRNA data but the interpretation of such data remains problematic (Cristescu 2019). The eDNA approach can neither contribute much to ecotoxicological tests that are commonly used to assess the impact of pollutants associated with oil and gas activities. This also concerns the analysis of DNA adducts that can be used for the detection of genotoxic chemicals in the environment (Pampanin et al. 2017). In all these cases, the conventional methods are more suitable.

- Questions:
    - How suitable is the eDNA method to fulfil the objectives of the survey?
    - How well will the eDNA-based biodiversity assessment inform about the environmental changes?
    - What kind of biological data is required by the survey?

6.2 Feasibility

The second element is to ensure that the processing of eDNA samples can be easily done and does not present any technical problems. Compared to the conventional methods, the eDNA-based approach is often viewed as technically more complex, and this could discourage its potential users. Indeed, eDNA sampling can be faster and easier, but some precautions have to be taken which are usually not required by conventional biomonitoring. Processing of eDNA samples requires access to laboratory infrastructure and a sequencing facility. Therefore, it is important to ensure that the eDNA sampling can be conducted in the optimal conditions and that molecular facilities are available without triggering issues around logistic or regulatory constraints to sending samples.

The feasibility also depends on how easy it is to detect the particular species or group of species using eDNA. The gaps in reference databases may impede taxonomic assignment of sequences as well as designingspecies-specific assays (Weigand et al. 2019). Moreover, some taxa may be difficult to detect if they only shed small amounts of DNA into the environment. If this is the case, it might be easier to achieve monitoring goals using conventional methods. Alternatively, eDNA could be used as to complement visual observations or other monitoring tools.

- Questions:
    - Are the eDNA protocols well established and is the necessary equipment available?
    - Are the results of eDNA surveys easy to interpret?
    - Are the reference sequences of target species present in the database?

6.3 Reliability

The third element to be considered when selecting the method is how trustworthy the eDNA analysis is. Most conventional methods are based on visual observations and morphological identification. The efficiency and accuracy of field observers and laboratory staff depends on their personal skills and taxonomic knowledge, which is often insufficient to correctly identify specimens to the species level.

The eDNA approach, especially when based on traces of macro- and megafauna, provides an indirect proof of the presence/absence of a target species. There are some technical and biological factors (transportation, degradation, etc.) that can influence the results of eDNA analysis. It is important to ensure that the interpretation of eDNA results takes into account these potential biases. On the other hand, eDNA analysis is more reliable in term of species identification, which is based on automatic assignment of the sequences to the reference database. Its efficiency depends on the completeness of the database and the taxonomic resolution of DNA barcode. These two factors have to be taken into consideration when eDNA analyses are planned.

- Questions:

- Do the eDNA results match those of conventional methods for target species?
- Are the potential biases of eDNA analyses taken into account?
- How good is the taxonomic resolution of the selected marker?

6.4 Acceptance

The validation and acceptance of eDNA methods is another factor to be carefully appraised. In general, conventional biomonitoring is based on well-known bioindicator taxa, whose ecology has been extensively studied. Most of the conventional methods have been standardized and are accepted by official regulations (e.g. WFD, MSFD, Water Act, etc).

As for the formal acceptance of eDNA-based methods, their implementation in routine biomonitoring is still a challenge. Although many eDNA applications have been scientifically tested, their validation in rigorous conditions has rarely been accomplished. The analysis of 546 published single-species assays show that the majority of investigated assays are incomplete (Thalinger et al. 2021). Similarly, the eDNA-based assessments of ecological quality status often lack rigorous validation, such as the ring-tests conducted for benthic diatoms monitoring (Vasselon et al. 2021).

Nevertheless, although only formally accepted for limited applications, the eDNA approach is usually considered a valuable alternative by regulators and environmental protection agencies. Therefore, when choosing between conventional and eDNA methods it might be important to check whether the results of eDNA analysis could be accepted even if the method is not formally recognized. This is often not a problem if the eDNA approach presents a significant improvement of conventional methods in term of sensitivity and quality. The acceptance can be more difficult if the eDNA approach directly competes with the conventional methods, often due to the reluctance of those that use these methods (e.g. diatoms-based assessment of the ecological quality of rivers and streams). To overcome this resistance to change, more convincing demonstration of the effectiveness of particular eDNA applications might be needed.

- Questions:
  - Is the planned eDNA method scientifically supported?
  - Is the method accepted by regulators?

6.5 Economic advantages

The eDNA approach presents some important economic advantages compared to conventional methods. It is generally expected that the eDNA approach will reduce costs considerably. Indeed, a comparative study of costs related to deep-sea biodiversity assessment indicate that eDNA metabarcoding is about 10 times less expensive than conventional methods based on morphological taxonomy (Le et al. 2022). It is anticipated that these costs will rapidly decrease as a result of further development of sequencing technologies. However, it is important to keep in mind that the costs of eDNA analyses are

reduced mainly due to multiplexing, which consists of processing large numbers of samples at the same time. The conventional approach may still be a valid option for small-size projects, which require the analysis of few samples. It could also be a solution in countries where the labor costs are low, and taxonomic expertise is available.

The eDNA approach also offers important time savings. In the case of marine monitoring, the sampling can be done more rapidly saving shipping time and reducing costs considerably. Sediment sampling for eDNA is by far faster than sieving and sorting benthic macrofauna. Similarly, collecting water samples for eDNA analysis takes less time than fishing or zooplankton sampling. Another aspect concerns the rapid turnaround of analyses. While it can take up to 3 months to identify and count all the members of a pelagic or benthic community, the eDNA analyses of water or sediment samples can take less than 2 weeks. Such fast analysis can be useful if an unplanned event occurs or a rapid certification is needed. On the other hand, the time factor may not be as important in the case of routine monitoring that occurs yearly or every second year, and the results of which have no direct impact on energy industry operations.

- Questions:
    - Does the use of eDNA allow for cost reduction?
    - Does it allow for time savings?
    - Is time an important factor for the study objectives, operational considerations or decisions?

6.6 Added value

Despite financial considerations, it is also important to consider what the added value would be if eDNA analysis is selected instead of a conventional method. First, the sensitivity should be considered. There is a general agreement that the eDNA method is more sensitive because it can detect traces that organisms left in the environment. This is particularly important when the species are rare and difficult to observe, as it is often the case for the detection of endangered species or for early warning of biological invasions.

Another important added value is the holistic nature of eDNA data. The eDNA approach provides an insight into the global biodiversity encompassing a wide range of taxa, including the most inconspicuous components of biological communities that could never be explored using conventional methods. In principle, all taxa can be surveyed using eDNA, while morphology-based identification is generally limited to a few generally large-sized taxa. It is also important to highlight that the eDNA approach can generate a huge amount of data that can be easily integrated into a global network of information. This can considerably improve biomonitoring by giving access to comparable data in temporal and spatial dimensions, allowing potential retroactive analysis across the life cycle of the project. Further automation of eDNA methods will allow the monitoring of the status of the environment in real time and worldwide.

- Questions:
  - Is the eDNA-based approach more sensitive than conventional methods regarding target taxa?
  - Can the holistic assessment of biodiversity make monitoring more efficient?
  - Can the retroactive analysis of eDNA data be useful for the project?

6.7 SWOT analysis

In this section we propose a SWOT analysis comparing Strengths, Weaknesses, Opportunities and Threats of eDNA projects versus conventional biomonitoring methods. Table 6.7 summarizes the pros and cons that should be taken in consideration when selecting the survey method.

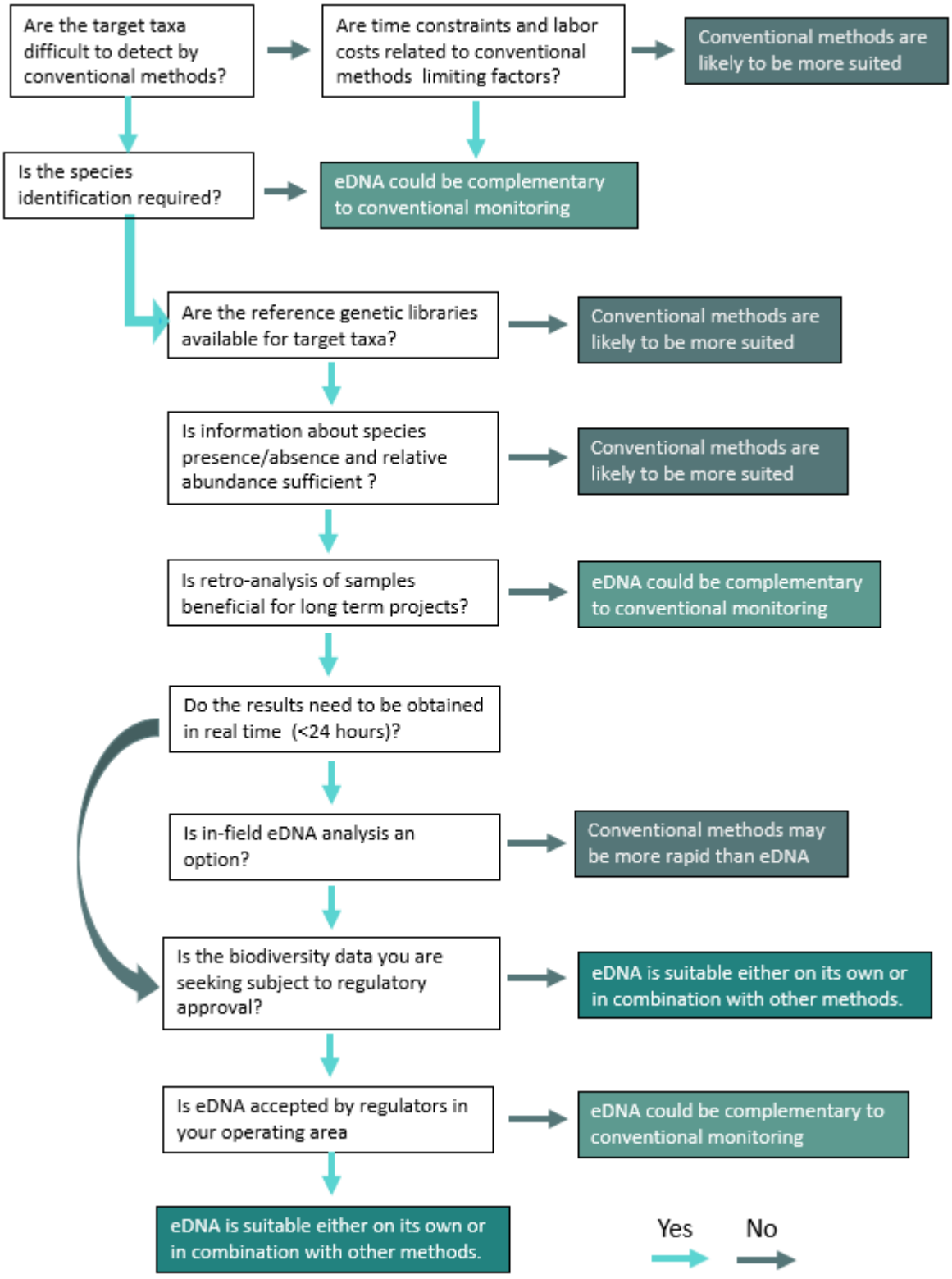
Table 6.7 – SWOT analysis of eDNA-based biomonitoring vs conventional methods

Strengths	Weaknesses
○ **Easier sampling logistics:** short training time, reduced hands-on time, less specialized equipment ○ **Non-invasive sampling** ○ **Automation**: the samples are processed automatically following established standards ○ **Rapidity:** large number of samples can be processed in a short time ○ **Low cost**: processing of eDNA samples can be less expensive ○ **Objective results:** personal taxonomic expertise is not required ○ **High sensitivity:** allows detection of rare species	○ **Biological data:** Some biological data such as population age, biomass, abundance, abnormal growth cannot be obtained from eDNA ○ **Localization:** it may not be possible to obtain a precise location for species due to eDNA movement within the environment ○ **Gaps in barcoding database** can impede species identification ○ **Limiting DNA shedding** can make some species difficult to detect ○ **Technical and biological biases** are not always well understood, limiting quantitative interpretation of eDNA data ○ **Sampling method** that would be optimal for species detection is not always feasible (e.g.no water at a site)
Opportunities	**Threats:**
○ **Environmental regulations**: allows to respond to constantly increasing demand for biomonitoring. ○ **Global biodiversity changes**: wide range of taxa can be identified ○ **Innovative technology**: the methods are constantly improving ○ **Retroanalysis:** the eDNA samples can be stored and analysed several times and with different markers	○ **Acceptance:** new technology can take long to be validated and adopted by regulators ○ **Regulations:** biomonitoring requirements are highly diversified between countries

6.8 Decision tree for selecting eDNA-based methods for biomonitoring

To conclude, we propose considering the following decision tree when planning a biomonitoring project and choosing between eDNA and any conventional methods.

Figure 5: Decision tree for choosing between eDNA and conventional species identification/biodiversity assessment methods



7 Look Ahead – What does the Future hold?

7.1 General

The eDNA-based methods have the potential to make environmental surveying safer and easier, make the processing more efficient and provide more actionable results. This section gives a brief overview of how developments in key areas may allow adoption of eDNA-based methods alone, or in parallel with, conventional methods.

7.2 Sampling Techniques, Equipment, and surveying

Automated sampling

The deployment of automated eDNA samplers will accelerate the application of eDNA-based methods in parallel with other surveying techniques (environmental and geophysical). Automated eDNA samplers are already being used in marine environments, attached to a fixed point for monitoring over time (Mynott 2019), with the ROVs (Everett and Park 2018) or coupling an environmental sample processor with an AUV (Yamahara et al. 2019a). Automated environmental sampling has also been applied to freshwater monitoring (Searcy et al. 2022).

For industrial applications, the development of new autonomous sampling equipment will reduce offshore vessel and staff time, particularly if deployed from infrastructure rather than from a vessel (which is frequently required for ROV/AUV deployment). The lower survey costs from decreased vessel time may allow for enhanced environmental monitoring if the saving is used to collect more samples (McLean et al. 2020). The same ROV/AUV used to collect eDNA can also be used to obtain conventional biological samples as well as other types of data.

Automated and drone-based sampling of freshwater and soil samples can also be considered as part of environmental surveying (Robinson et al. 2022). The application of drone technology has been relatively limited to date (Doi et al. 2017), although the weight of samples or the drone technology do not limit its use. The advantage of this approach would be that remote sensing could obtain broad environmental data for an area, followed by subsequent individually selected eDNA sampling stations for soil and water, to calibrate the habitat data. This would significantly reduce the time and effort of field ecologists.

In-field analysis

Mobile laboratory technologies already exist, allowing for eDNA samples to be processed from extraction to sequencing in the field within marine and freshwater environments (Chang et al. 2020; Hansen et al. 2020; Urban et al. 2021). However, further developments are needed to improve efficiency and adjust these mobile technologies to regulatory standards for widespread application (Environment Agency 2021). If made widely available to industry, automated sampling and sample processing of eDNA could be deployed on buoys and be used together with other systems used for passive acoustic monitoring for marine mammals (Kowarski et al. 2020) or plankton sampling in the marine environment (e.g. Pitois et al. 2021).

In-field methods for rapid single-species detection are also under development, including methods using CRISPR (M. Williams et al. 2019) and various other isothermal amplification methods (Fast et al. 2020). Systematic application of these tools in the field requires investment in the development of reliable methods for simple, fast, in-field extraction of high-quality DNA in a contamination-secure manner. This will reduce the time between sampling and obtaining results from days to hours or even minutes. This makes such methods ideal for rapid detection of INNS, harmful taxa or pollution indicator species. However, this is unlikely to replace visual and PAM observations for marine mammals and reptiles, where a response is required immediately after the observation. For example, a visual observation might lead to immediately stopping high-decibel activity at offshore sites.

Other substrates for DNA sampling

Sampling eDNA from air is in its infancy compared to sampling eDNA from water or soil. However, over the past 2-3 years, there have been an increasing number of studies using airborne DNA for assessing plant communities (M. D. Johnson et al. 2021; Banchi et al. 2020), fungal communities (Rosa et al. 2020), vertebrates (Lynggaard et al. 2022), invertebrates (Roger et al. 2022), and eukaryotes (Aalismail et al. 2021). Analysing DNA from the air offers a totally new approach to assess biodiversity in terrestrial environments. Its use for future baselining of industrial projects and their environmental impact assessment is to be considered. However, some further methodological development may be necessary before the approach is mature and ready to use.

7.3 Laboratory Work and Analysis

These components are primarily addressed in JIP-34 RFP3 and RFP4. They are addressed briefly here from a perspective of how the future eDNA-based methods can deal with the current challenges of data generation and analysis.

Technological innovations

Since the beginning, eDNA-based technology has been in rapid and constant evolution and it is unlikely that this process will stop anytime soon. The high-throughput sequencing platforms are developing continuously to produce more sequences more rapidly (e.g. Illumina NovaSeq) or to propose a portable real-time DNA/RNA sequencing device for an affordable price (Oxford Nanopore MinION). The development of nanopore-like technology is of particular interest for biomonitoring as it responds to a need for a rapid and cost-effective tool to generate sequencing data. Moreover, the long reads produced by nanopore technology enables more accurate recovery of taxonomic and phylogenetic diversity than short amplicon sequencing (Krehenwinkel et al. 2019). Although this technology has long been struggling with technical issues, most of them seem to be solved now and its potential application to biodiversity monitoring and ecosystem health assessment is being recognized (Urban et al. 2021).

Taxonomic reference database

Building a comprehensive reference database is another important challenge for the future of eDNA-based monitoring. The accuracy of biodiversity surveys depends on whether the species can be correctly identified. This is particularly important when eDNA-based methods are proposed to replace the morphology-based identifications. Unfortunately, many morphospecies, especially among the invertebrates and microbial eukaryotes used as bioindicators, have not been sequenced yet. There are a lot of efforts to fill the gaps in the current DNA barcode databases (e.g. Diat.barcode, Rimet et al. 2019). However, these efforts usually focus on one marker that has been selected based on current research limiting the possibility to change the marker. It is expected that the future development of reference databases will use long-read sequencing technologies (e.g. PacBio) to sequence whole genomes (e.g. mitochondrial genomes) or entire operons (e.g. rRNA genes) which will provide better taxonomic resolution and enable selection of more convenient markers for a given taxon (Jamy et al. 2020).

Species detection

The reference database is essential for designing better primers for metabarcoding and single-species detection. New primers can largely increase the proportion of target taxa within the metabarcoding datasets, as demonstrated with marine vertebrates (Valsecchi et al. 2020) and freshwater aquatic invertebrates (Brantschen et al. 2022). Optimizing primers and probes is particularly important for the development of single species assays. This will likely increase the use of eDNA-based methods if their detection capability is improved for the target species, especially with regards to INNS, protected and indicator taxa.

Abundance measures

Future eDNA-based biomonitoring will also need to overcome the limitations related to the semi-quantitative nature of DNA sequence data (Luo et al. 2022). In most of the cases, there is no direct relationship between number of specimens and number of sequences which impedes the comparison of eDNA and conventional abundance data. Calibration against conventional data can be used to correct biases between eDNA concentration and biomass or abundance, although this should consider a range of environmental variables that affect eDNA decay rate as well as the variable biological relationship between shedding rate and biomass or abundance. However, all survey methods have biases and biases of conventional methods should also be considered in this situation. Recently, several studies proposed to correct different biases that impact the relationship between biomass, eDNA concentration and number of sequences through:

- Using of multiple markers with different target regions and biases (Bucklin et al. 2021).
- Including a “spike-in” or internal control of known concentration of DNA in samples (Stoeckle, Ausubel, and Coogan 2022).

- Sequencing mock samples that include known concentrations of target species (McLaren, Willis, and Callahan 2019; Shelton et al. 2022)
- Sequencing the same sample that has been amplified using different number of cycles during PCR (Silverman et al. 2021).

Routine use of these methods, while more expensive, will result in better quantification of each species' eDNA concentration (Gold et al. 2023). This ultimately can result in eDNA-based methods being used to estimate biomass data, allowing for fewer conventional samples to obtain population level data (Jungbluth et al. 2022; Di Muri et al. 2020).

Consistency of results

Automation of eDNA analysis is burgeoning, including sample processing, bioinformatics and data analysis. The use of robotics will minimise risk of any subjectivity or human errors and enhance consistency of results (Tegally et al. 2020). At the same time, the progress of bioinformatic tools is expected to solve some problems related to the interpretation of sequence data, in particular to improve taxonomic assignment of sequences and correct technical errors. The complementarity of eDNA-based and conventional methods requires better fit between molecular and morphological identifications, which remains a challenge for the future developments of eDNA-based biomonitoring.

7.4 Metrics of ecosystem health

To date, the majority of indices of ecological quality status are based on the presence and abundance of particular species and using these to generate a score. Only species that are assigned to an ecological category or an indicator value are included in the calculation of biotic indices (Pawlowski et al. 2018).

The future eDNA-based metrics will overcome this limitation by promoting a more holistic approach to ecosystem condition assessment using a taxonomy-free approach. This can be done either by assigning indicator values directly to sequence data (Apothéloz-Perret-Gentil et al. 2017) or by using machine learning to create models based on the full metabarcoding dataset, without any taxonomic criteria (Cordier et al. 2020). These taxonomy-free models work by learning the biological community signature that defines a healthy ecosystem and predicting how that signature changes as the ecosystem degrades or improves. Once trained, the model can then assign unknown samples to an ecosystem health class or a position on the degradation gradient. Machine learning models have been used to predict the impacts of marine aquaculture (Cordier et al. 2017; 2018; Frühe et al. 2020), as well as to assess the ecological status of coastal marine habitats (DiBattista et al. 2020), and rivers using diatoms (Feio et al. 2020) and phytoplankton (Fan et al. 2020).

The machine learning approach has been shown to be more powerful for ecosystem assessment than taxonomy-based indices (Cordier et al. 2018), but it is not yet clear how

transferable these models are between habitats and locations. Future developments in this direction will require much more extensive training datasets that will take in consideration a broad range of environmental parameters, including seasonality, biogeography, etc. Building a comprehensive database of metabarcodes associated with industrial impacts is essential to develop novel metrics for eDNA-based monitoring.

7.5 Standardisation

To improve management and conservation efforts and enhance the implementation of eDNA-based monitoring a broad standardization of environmental genomics workflows is needed. The eDNA sampling process is discussed in greater detail in IOGP JIP34, Project 2 and the laboratory and bioinformatics components will be considered by the IOGP later (e.g. MIQE guidelines for reporting information on qPCR experiments (Bustin et al. 2009)). Here, we will report the efforts that have already been done in this field and indicate its further development.

Governmental and intergovernmental regulators consider the relative merits and calibration stages of eDNA-based methods against conventional methods prior to recommending them as a monitoring method (Aylagas et al. 2020; Bruce et al. 2021; Hering et al. 2018). Therefore, it is of crucial importance to promote a formal acceptance of eDNA-based methods. This can be done by publishing eDNA guidelines supported by governmental agencies, as it has been done in Japan, Canada and Switzerland (The eDNA Society 2019; Pawlowski et al. 2020; Abbott et al. 2021). Recent initiatives to incorporate eDNA methods into regulatory monitoring and acceptance in legal national and international instruments include:

- a technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses, published by the European Committee for Standardization (FD CEN/TR17245:2018)
- publication of a protocol on metagenomic analysis of meiofauna in marine environments by the International Standardization Organization (ISO 23732:2021(E)
- creation of a working group on DNA and eDNA methods (CEN/TC230/WG28) and publication of the first standard on “Collecting and preserving samples for capture of environmental DNA in aquatic environments from water samples” in 2023 (SN EN 17805:23).

Still, these efforts are at a very early stage. Most of the research on eDNA-based biomonitoring present in this report and elsewhere remains at academic level. To be routinely applicable, the methods used in this research need to go through a long process of standardization and optimization. This process already started and will certainly continue in the future.

7.6. Summary

To conclude, the future of eDNA-based biomonitoring depends on three main factors: technological advances, scientific evidence and regulatory acceptance. Technology is

advancing rapidly, offering a vision of future biomonitoring which relies on automated eDNA samplers and robotics which generate a continuous flow of sequence data that are analysed using machine learning or other AI tools. However, this vision might not be easily accepted by those practicing conventional biomonitoring. For many, eDNA-based methods should help to detect and identify species but not necessarily to change the way the ecological status is assessed and the bioindicator used for that assessment. eDNA-based methods should be calibrated against conventional methods to ensure that results are comparable under a range of conditions allowing interoperability between different datasets. Scientific evidence is needed to demonstrate the accuracy of eDNA-based technology, but its implementation cannot take place without regulatory acceptance. Hence, the combination of these three factors is essential for the success of eDNA-based monitoring to be successful.

As shown by this report, there is a consensus that eDNA-based methods should be integrated into future biomonitoring. Whether this will be done by replacing or in combination with conventional methods will depend on the habitat, target taxa, data required and survey objectives. It should be noted that many eDNA-based methods are still at the level of academic research. Further development and optimization may be required before these methods can be used in routine biomonitoring.. However, the benefits of using eDNA will certainly outweigh these efforts, making future biomonitoring of industrial operations more efficient and beneficial for ecosystem health and biodiversity conservation.

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