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Review Article:

Environmental DNA (eDNA) as a next-generation tool for biodiversity monitoring: A review

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Abstract

Environmental DNA (eDNA) is genetic material released by organisms into water, soil, air, and sediment. It has rapidly become a transformative tool for biodiversity monitoring that can detect the presence of a species without physically capturing it. This non-invasive technology, primarily functional in aquatic organisms, has been expanded to multi-species assessments across freshwater, marine, terrestrial, and aerial ecosystems through metabarcoding techniques. This narrative-integrative review synthesises current eDNA science across four dimensions: 1) biological and physicochemical understanding of the production, transport and degradation of eDNA; (2) laboratory and bioinformatic approaches for detecting eDNA; (3) diverse ecosystem case studies; and (4) an analysis of uncertainties and limits affecting the reliability of eDNA-based inferences. A literature search across four bibliographic databases such as Web of Science, Scopus, Google Scholar, and PubMed, covering peer-reviewed publications from 2008 to 2024, highlighted eDNA's capabilities in identifying endangered and cryptic species, recovering historical biodiversity, and monitoring biological invasions. Emerging applications such as airborne eDNA sampling, palaeogenomic reconstruction via sediment analysis, and landscape genomics are also explored in this review. Challenges in community-level biodiversity characterisation continue due to primer bias, disparities in bioinformatic methodologies, and incomplete reference databases, which introduce uncertainties in data interpretation. Quantitative eDNA monitoring remains further restricted by the scarcity of species-specific calibration data, and the diversity of sampling and analytical protocols restricts cross-study comparability. Standardizing these methodologies and improving comprehensive reference databases are recognized as vital steps for evolving eDNA as a reliable conservation tool. Overall, eDNA will be one of the most promising and rapidly developing tools in conservation biology and ecological monitoring.

Keywords: eDNA metabarcoding; Biodiversity monitoring; Species detection; Aquatic ecology; Metagenomics; Next-generation sequencing.

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1. Introduction

The world's biodiversity is declining at an unprecedented rate, and around one million species are now facing extinction. This crisis is captured in historic reports prepared by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES, 2019). Successful conservation planning relies on effective species distribution tracking, population dynamics, and community structure. Effective species tracking relies on conventional survey techniques such as point counts, transects, mark-recapture, and underwater visual census. They are labour-intensive, geographically constrained, and require a high level of taxonomic expertise. Such limitations have resulted in a large monitoring gap, especially in distant habitats, in deep waters, and in areas with limited scientific infrastructure (Bohmann et al., 2014).

Environmental DNA has become an effective paradigm shift in environmental monitoring. The idea takes advantage of the biological truth that all organisms are constantly releasing genetic material into the surrounding environment. This is done through the shedding of cells, mucus, faeces, urine, gametes, and decomposing tissues. The genetic material can be gathered, purified, amplified, and recognized using molecular biology instruments, without requiring direct interaction with the organism (Taberlet et al., 2012). Ficetola et al. (2008) used eDNA to detect the American bullfrog (*Lithobates catesbeianus*) in pond water samples in France and initiated a new era in ecological monitoring.

Since the groundbreaking study, eDNA usage has expanded exponentially. A search of the 'Web of Science' database revealed that the number of publications on the topic of "environmental DNA" increased from approximately 20 articles per year in 2010 to over 1,200 in 2023. This substantial increase indicates the interest of the scientific community and the expansion in the application of eDNA tools in environmental studies. Currently, rare and invasive species can be identified by using eDNA methods, which can characterize an entire biological community with metabarcoding and reconstruct historical diversity. This can be performed using sediment archives and by sampling the genomes of airborne organisms (Ruppert et al., 2019; Clare et al., 2022).

This review article presents a narrative-integrative review of eDNA science in four main areas: 1. the biological and physical properties of eDNA in the environment; 2. the methodological toolkit of field sampling through bioinformatics; 3. exemplary case studies of a wide range of ecosystems; and 4. the frontiers, gaps and methodological limitations of eDNA usage. This review aimed to empower biodiversity scientists, conservation managers and policymakers with a critical evaluation of the potential and limitations of this methodology.

2. Methodology

This review was undertaken as a narrative-integrative literature review to synthesize the existing information on environmental DNA (eDNA) as a biodiversity monitoring tool. The narrative-integrative design was chosen to account for the wide range and diversity of eDNA research (in aquatic, terrestrial and aerial environments; molecular approaches and techniques; and conservation applications), but with the use of a transparent and reproducible search and selection protocol. The protocol was designed beforehand and described below. The search of the literature was structured in four bibliographic databases: Web of Science (Core Collection), Scopus, Google Scholar and PubMed. The searches were conducted in October 2025, and publications from January 2008 to December 2024 were retrieved. The year 2008 was chosen as the lower temporal boundary, as this was the year of the landmark peer-reviewed study by Ficetola et al. (2008) that first proved species detection from aquatic eDNA samples.

The main Boolean search terms used for all databases were: (environmental DNA or eDNA) and (biodiversity monitoring or species detection or metabarcoding or conservation genetics). Additional search strings were

used for the following thematic areas to cover key issues raised in an initial scoping exercise: "eDNA" AND (airborne OR "aerial eDNA"), Sedimentary ancient DNA (or sedaDNA) AND biodiversity. Additional search terms included "eDNA" AND ("invasive species" OR "early detection").

The parameters used for the search are as follows:

Disease surveillance eDNA pathogen detection (eDNA OR regulatory standardization OR environmental monitoring policy) AND "eDNA metabarcoding" AND "high-throughput sequencing" OR "next-generation sequencing".

All search strings were entered in the title, abstract and keyword fields. The language restriction was not applied during the identification process, but it was applied during the screening stage, with the exclusion of non-English publications. Inclusion and exclusion criteria were established before the screening.

2.1. Inclusion Criteria

Peer-reviewed original research articles, systematic reviews, or meta-analyses, or authoritative technical reports, books published between January 2008 and December 2024, written in English, clearly relevant to the use of environmental DNA (eDNA) in biodiversity monitoring or conservation, demonstrated methodological rigour (described controls, statistical analysis and/or bioinformatic workflow), and addressed at least one of the identified thematic areas.

2.2. Exclusion Criteria

Technical papers or conference abstracts (unless published), blog posts (if not methodologically transparent), any studies published before 2008 (before the seminal aquatic eDNA study by Ficetola et al., 2008), non-English language publications, studies indirectly related to eDNA that are not monitoring or conservation related, studies that were not experimentally controlled, did not report data statistically, or were not transparent in the use of bioinformatic pipelines.

2.3. Final Dataset Characteristics

A total of 48 sources were included in the final database, consisting of 31 peer-reviewed original research articles, 12 systematic and narrative review papers, and 5 authoritative technical reports from regulatory or standard-setting organizations. Publications ranged from 2008 to 2024, and 71% of them were published since 2016, indicating the field is a rapidly growing one since the development of high-throughput sequencing platforms.

2.4. Screening and Selection Process

Stage 1 -Title and Abstract Screening:

All records were initially screened based on their title and abstract for compliance with the eligibility criteria. Records were kept if they seemed to be directly relevant to eDNA as a biodiversity monitoring or conservation tool.

Stage 2- Full-Text Eligibility Assessment:

All the records that passed the eligibility criteria in Stage 1 were retrieved in full text to be evaluated with respect to four aspects of methodological reporting: experimental or analytical controls, statistical methods and transparency of bioinformatic pipelines when sequencing data were involved. If a record did not meet any of these criteria for any reason, it was omitted. All the sources were arranged according to the APA 7th edition.

3. Biological and Physicochemical Foundations of eDNA

3.1. Sources and Production of eDNA

eDNA originates from cellular material released by organisms through processes such as shedding, excretion, and decomposition. The amount of eDNA that an organism releases into the environment, called "eDNA shedding rate," is dependent on many biological and ecological factors. The shedding rates, body size, metabolic activity, and population density are positively correlated (Klymus et al., 2015). Aquatic animals generally release eDNA via skin mucus, scales, gill excretions, urine, and faeces. Controlled

mesocosm experiments have shown that, under specific circumstances, eDNA contents in water can be used as substitutes for the biomass of organisms (Takahara et al., 2012). Nevertheless, the correlation between eDNA concentration and abundance is complex owing to individual variation, reproductive condition and stress. Injured or spawning fish shed significantly more eDNA than those in the resting state (Klymus et al., 2015). Root exudates, microbial biofilms, decomposing organic matter, excretions of soil-dwelling invertebrates and larger vertebrates are the sources of soil eDNA. Plant eDNA (i.e. pollen) has a significant contribution to the environmental mixes in both terrestrial and aquatic environments and is useful in vegetation surveys and in the reconstruction of ancient landscapes (Taberlet et al., 2012).

3.2. Transport, Distribution, and Persistence

Once eDNA is released into the environment, it is transported, diluted and degraded. eDNA in lotic systems (rivers, streams) is carried downstream, forming spatial gradients, which may make it difficult to identify the source. In a modelling study, Deiner and Altermatt (2014) have expressed that the eDNA of freshwater invertebrates could be detected kilometres downstream of their source populations, but the signal strength declined with the distance and flow rate. In lentic systems (lakes, ponds), diffusion, thermal stratification and wind-caused circulation control eDNA distributions.

eDNA persistence-the time for which detectable genetic material is left over in the environment-is a critical factor affecting survey reliability. This is a crucial factor influencing the reliability of surveys. The eDNA half-life varies between hours and weeks in aquatic environments, based on the intensity of UV radiation, temperature, pH, microbial activity, and particle association (Strickler et al., 2015). eDNA can easily be degraded by high temperature and UV radiation, whereas low-pH, dark and cold environments help preserve it. Sediment-bound eDNA, on suspended sediments, is likely to remain longer than dissolved eDNA, which is more readily hydrolyzed or oxidized (Turner et al., 2015). When eDNA is chemically attached to clay minerals and silica, it may stay for centuries or even millennia in soils, allowing reconstructions made by palaeogenomics (Pedersen et al., 2015).

4. Procedural Approaches

4.1. Field Sampling Strategies

An eDNA sampling campaign design should be developed with strong consideration of the target organism(s), the nature of the habitat and the spatial and temporal magnitude of the study. In aquatic surveys, water samples are collected with the help of peristaltic pumps, Niskin bottles, or passive samplers and instantly filtered using membranes (usually with pore sizes of 0.2-2 μm) to trap particulate eDNA (Hinloet al., 2017). Filters influence DNA capture efficiency; glass-fibre filters are expected to produce higher eDNA volumes but with lower purity than mixed cellulose ester membranes. Standard sample volumes of 0.5 to 5 L are used, but the optimum sampling volume depends on species density and turbidity of water.

Temporal sampling design is important. Extensive literature has reported strong seasonality in eDNA signals, usually around spawning periods, breeding seasons, or periods of increased activity (Spear et al., 2015). When monitoring rare species, it is advisable to conduct numerous sampling events throughout the period of activity to increase the chances of detection. Occupancy frameworks have been used to model the number of replicate samples needed to obtain target statistical confidence and include imperfect detection (Schmidt et al., 2013).

4.2. Laboratory Processing: DNA Extraction and PCR Amplification.

Samples should be preserved and processed promptly after collection to recover DNA of sufficient quality and quantity for downstream applications. In field conditions, due to a lack of cold storage, other preservation methods can be used, such as ethanol precip-

itation or buffered lysis solutions. DNA extraction can be performed either using phenol-chloroform procedures or commercial kits especially developed for difficult environmental samples. The occurrence of PCR inhibitors (humic acids, heavy metals, polyphenols) is a constant problem in eDNA analysis and can lead to false negatives unless treated effectively by dilution, inhibitor removal using kits, and using inhibitor-tolerant polymerases in PCR (Goldberg et al., 2016).

Two main paradigms can be used to amplify target sequences:

(i) Targeted single-species detection using standard or quantitative PCR (qPCR) with species-specific primers; or

(ii) Broad-spectrum community profiling with universal primers and high-throughput sequencing (meta-barcoding). qPCR is often preferred when the aim is to detect rare species or monitor regulatory activity, as it is sensitive and can provide quantitative results (target gene copies per unit volume). Droplet digital PCR (ddPCR) has also enhanced quantitative performance, allowing the absolute number of copies to be estimated without a standard curve and hasgreater sensitivityat extremely low template concentrations (Doi et al., 2015).

4.3. eDNA Metabarcoding and High-Throughput Sequencing

eDNA has expanded to the community level, which has been made possible using metabarcoding (simultaneous amplification and sequencing of short, taxonomically informative DNA regions /barcodes from environmental samples), which has had a profound impact on eDNA analysis (Taberlet et al., 2018). Metabarcoding using universal or specific primers targeting variable barcode loci (e.g. 12S rRNA in vertebrates, COI in invertebrates, ITS in fungi, 16S in prokaryotes, 18S in eukaryotes) can identify hundreds to thousands of different taxa from an eDNA sample. Platforms based on next-generation sequencing (NGS) such as the Illumina MiSeq and NovaSeq can produce millions of sequences reads in a single run and, as a result, identifying rare taxa that occur in the community at frequencies below 0.01% can be possible (Elbrecht and Leese, 2015).

Bioinformatic pipelines for metabarcoding data are typically used to clean up data, trim primer ends, merge paired-end reads, denoise or cluster amplicon sequences into amplicon sequence variants (ASVs) or operational taxonomic units (OTUs), and assign taxonomy to sequences using BLAST, DADA2, or machine-learning classifiers against curated reference databases, including BOLD, NCBI GenBank, SILVA and UNITE (Callahan et al., 2016). A significant bottleneck is the completeness and taxonomic resolution of reference databases. Still, there are many attempts to create comprehensive, standardized barcode reference libraries, including the Global eDNA Reference Library project (Weigand et al., 2019).

4.4. Long-Read Sequencing and Emerging Technologies.

Third-generation sequencing platforms, particularly Oxford Nanopore Technologies (ONT), Pacific Biosciences (PacBio), can produce 1000 -10000 base pairs of reads and therefore can sequence a complete barcode gene or even entire mitochondrial genomes from an organism in an eDNA sample (Maestri et al., 2019). Long-read eDNA sequences increasetaxonomic resolutionof phylogenetically close populations and recover more informative genomic sequence data. eDNA can be analyzed in real time in remote field conditions using a mobile MinION device, opening the possibility of assessingin situbiodiversity. Probe-based target enrichment has been applied to enrich the number of low-abundance targets in complex mixtures, a technique also known as “eDNA genome skimming” (Stat et al., 2019).

5. Dynamics of applied case studies across ecosystems

5.1. Early detection of invasive species from freshwater systems

One of the most important applications of aquatic eDNA monitoring is the detection of invasive species before population establishment. Asian carp (*Hypophthalmichthys molitrix* and *H. nobilis*) are among the most serious biological invasion threats in the North American freshwater ecosystem. Jerde et al. (2011) were the first to use the concept of eDNA to detect these species in the Waterway System in the Chicago Area and produced positive eDNA detections over 50 km from the confirmed fish captures by traditional netting. The research was critical in triggering the regulation to stop the entry of carp into the Great Lakes. Later methodological advances, such as qPCR-based tests and occupancy modelling, have enhanced sensitivity to the extent that a single carp in large water bodies can theoretically be identified (Jerde et al., 2013).

In Europe, eDNA has been recognized as a regulatory survey tool, specifically to monitor the great crested newt (*Triturus cristatus*), a legally protected species under the European Union Habitats Directive (Biggs et al., 2015). According to Biggs et al. (2015), in low-density populations, the water-based eDNA detection was more sensitive than traditional methods using torches and bottle-trapping. Subsequent national validation demonstrated that eDNA surveys of UK ponds had a detection probability of 98 % when the species was present, versus 79 % using traditional techniques, and lowered survey costs by about 50 % (Biggs et al., 2015). This is one of the best applications of eDNA technology for implementing regulatory measures to date.

5.2. Marine and coastal biodiversity assessment.

The use of eDNA tools in marine surveys has increased rapidly because traditional marine biological surveys such as trawling, baited camera traps and diver transects are expensive, can be spatially restricted and damage benthic environments. Thomsen et al. (2012) demonstrated that marine eDNA can be used as an effective tool, detecting vertebrate megafauna, such as harbour porpoise (*Phocoene phocoena*) and Atlantic cod (*Gadus morhua*) in seawater samples from Danish estuaries. Thereafter, global oceanographic surveys conducted using the eDNA metabarcoding system have catalogued eukaryotic plankton diversity across ocean basins (Burki et al., 2021).

Most recently, eDNA tools have been used to characterize marine communities, with the most ambitious eDNA-based marine survey to date, sampling 210 stations in all major ocean biomes (Sunagawa et al., 2015; de Vargas et al., 2015). The voyage found that the diversity of ocean plankton was vastly underestimated-150,000 eukaryotic lineages were recorded, most of which were previously uncharacterized. Importantly, the research discovered good correlations between the community composition and the environmental factors (temperature, nutrient regime, salinity), which provides a foundation to monitor the changes in ocean biodiversity due to climate change in the future.

Coral reef ecosystems, which are increasingly threatened by climate change and anthropogenic stressors, have been successfully evaluated using eDNA-based analytical approaches. DiBattista et al. (2020) employed marine water eDNA metabarcoding to characterize fish biodiversity across reef sites in the Indo-Pacific, reporting the detection of over 2,500 fish species, which were largely consistent with visual survey records. They observed that cryptic and nocturnal species are invisible during the day. eDNA surveys of reef habitats have revealed seasonal turnover in fish communities, showing the reduction of dwindling apex predators, offering a passive and scalable monitoring system to uphold reef management (Stat et al., 2019).

5.3. Soil and Terrestrial Biodiversity.

Use of eDNA in terrestrial ecosystems has expanded significantly beyond aquatic-proximate ecosystems. Soil is an exceptionally

rich eDNA resource, particularly with respect to microbial biodiversity: a single gram of soil can contain the genetic material of tens of thousands of bacterial Operational Taxonomic Units (OTUs), hundreds of fungal species, and various soil macrofauna and microfauna. High-throughput soil metabarcoding approaches have been employed to characterize biodiversity patterns along gradients of land-use intensity, revealing a significant decline in bacterial and fungal biodiversity in cultivated soils relative to semi-natural grasslands and forest ecosystems (Hermans et al., 2020).

To assess large invertebrate biodiversity and vertebrates, "Leech gut content sequencing" is used as a novel eDNA-adjacent method. A study by Schnell et al. (2012) has shown that blood-feeding leeches from the forest floor had mammalian DNA that represented a rich vertebrate community, including elusive and scarce species like the Annamite striped rabbit and the Muntjac deer in the Vietnamese forests. This technique is very efficient for sampling without any need to capture or trap animals or use a camera. Likewise, invertebrate-derived DNA (iDNA) of arthropods such as mosquitoes and horse flies has been utilized to study the diversity of mammals and birds in tropical forest habitats (Calvignac-Spencer et al., 2013).

5.4. Airborne eDNA Sampling for Biodiversity in the Atmosphere.

One of the most recent and exciting findings in the field demonstrated that airborne genetic substances such as pollen, spores, skin cells, hair, feathers and aerosols can be used to identify biodiversity. Clare et al. (2022) sampled air using the high-volume air samplers within a zoological garden and recognized 25 vertebrate species of animals, including indoor animals as well as exterior DNA amplicons dispersed via ventilation systems. This approach to aerial biodiversity monitoring, known as the 'air eDNA' or 'airDNA' method, has far-reaching implications in wildlife surveys in closed and semi-closed environments.

Air samples taken over natural habitats for airborne eDNA outdoor surveys have shown that air samples can detect local assemblages of vertebrates. Johnson et al. (2023) used air samplers in various locations in a Scottish nature reserve and identified mammalian and avian species, which were associated with the known local wildlife, such as red squirrel (*Sciurus vulgaris*) and pine marten (*Martes martes*). The spatial footprint of airborne eDNA detection is also a sounder investigation: based on wind flows and the dynamics of aerial transport, the detected taxa can be located within meters to a few kilometers of the sampling site. The issue of standardization of air DNA in open landscapes is also a primary methodological barrier (Leempoel et al., 2020).

5.5. Palaeogenomics and Reconstruction of the History of Biodiversity.

Lake, peat bog, and cave-floor preserve sediment cores that contain eDNA over centuries to millennia, allowing palaeogenomics methods to recreate historical biodiversity and monitor long-term ecological change. The study conducted by Pedersen et al. (2015) retrieved and sequenced plant and animal DNA from permafrost sediments around 400,000 years old and detected plant and animal DNA evidence of past vegetation cover, woolly mammoths, woolly rhinoceros, and horses in the Arctic environment. The process of 'sedaDNA' (sedimentary ancient DNA) is an unparalleled direct view of the biological communities of the past, which supplements the pollen and macrofossil records upon which the science of palaeoecology has long been built.

Metabarcoding of eDNA has shown valuable pre-industrial baselines of water biodiversity in lake sediment archives. Capo et al. (2021) highlighted the reconstructed eDNA records of the diatom, cyanobacteria and zooplankton communities in Lake

Geneva over the last 200 years and discovered the timing and magnitude of the community changes due to eutrophication with much finer taxonomic detail than the tradi-

tional diatom morphology method. Such historical baselines are becoming more useful in establishing achievable ecologicalrestoration goals, thereby addressingwith the 'shifting baselines syndrome' that makes conservation planning difficult.

6. New Applications: eDNA as a Paradigm-Changing Tool.

6.1. Biodiversity Indices and Ecosystem Health Metrics.

Conventional ecological assessment paradigms, e.g., the Water Framework Directive (WFD), construct biological quality elements upon morphological identification of macroinvertebrates, diatoms and macrophytes, significantly lessening survey effort and facilitating standardized and comparable data across wide areas of geography. Early benchmarking experiments have established a strong correlation between eDNA-based biotic indices (richness of EPT: Ephemeroptera, Plecoptera, Trichoptera) and average score per taxon with morphological indices and to established stressors (organic pollution, fine sediment loading, etc.) (Pawlowski et al., 2018).

A new dimension to biodiversity assessment is the concept of aneDNA-based assessment of not only the presence of species, but the functional gene stock of microbial communities in terms of repertoire of functional genes. Rates of nitrogen cycling, carbon mineralization and xenobiotic degradation can be measured using metagenomics and metatranscriptomics of environmental samples and the connection between biological diversity and the rate of processes in the ecosystem. The functional eDNA method is specifically applicable to monitor the health status of agriculturally managed soils, remediation, or extreme climatic situations (Fierer, 2017).

6.2. Species Distribution Modelling and Landscape Genomics.

Integration of eDNA occurrence data and species distribution models (SDMs) and landscape genomics frameworks are a promising direction of scientific understanding of biodiversity patterns on macroecological scales. eDNA-based detection records obtained after systematic national-scale surveys with standardized protocols can fill the occurrence database with detection records that equal or exceed those from traditional observer-based records. Used with environmental predictors (climate, hydrology, land cover), eDNA-based SDMs can be used to model species ranges, determine climate refugia, and guide connectivity planning (Deiner et al., 2016).

The study of population structure and genetic diversity of a population using environmental samples without capturing each organism or animal has been described as population-level eDNA analysis and has also been known as 'eDNA population genetics '. Synthetic and natural population studies have shown that microsatellite marker analysis and SNP genotyping can be obtained from water samples, allowing the determination of effective population size (Ne), recent admixture and isolation by distance without direct physical capture (Sigsgaard et al., 2016). The method has the potential to transform the process of monitoring critically endangered species in remote or sensitive environments where genetic sampling has logistical or ethical limitations.

6.3. Biosecurity, Disease Surveillance, and One Health Applications.

eDNA technology has been applied to other types of biosurveillance by discovering new uses in the detection of pathogenic organisms and parasites in the environment, opening new possibilities for early warning and surveillance. *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal), the chytrid fungi involved in causing disastrous amphibian declines worldwide, can be identified in water samples through species-specific qPCRs, which means that the geographical distribution of the pathogen can be monitored at a landscape scale without the need to capture animals. Spear et al. (2015) have shown that eDNA was observable in streams where the infected populations of amphibians were too sparse to

be sampled directly, which detected the presence of infection fronts before a population crash.

The detection of SARS-CoV-2 RNA in the wastewater sample during the COVID-19 pandemic revealed that eDNA monitoring in wastewater can be used as an indicator of early warning of pandemic-related dynamics at the community level, before clinical cases were reported by several days to several weeks (Medema et al., 2020). Although this application is not biodiversity monitoring in a strict ecological context, it confirms that the framework of eDNA detection can be a reliable large-scale environmental monitoring approach and highlights the general capacity of the approach.

6.4. Ocean-Scale Monitoring and Blue Carbon Ecosystems

Biodiversity hotspots such as mangroves, salt marshes and seagrass meadows are examples of blue carbon ecosystems that are rapidly disappearing across the globe, and the application of eDNA metabarcoding using sediment and water samples from these habitats has resulted in comprehensive inventories of associated fish, invertebrate, and microbial communities, including those too small to visually observe. It has been found that larval fish eDNA is present in mangrove waters and thus offers an instance that commercially and ecologically significant fish use nursery habitats without the need to be destructively sampled (Port et al., 2016).

The possibility of undertaking autonomous eDNA sampling by underwater gliders, moored sampler systems, and remotely operated vehicles (ROVs) creates the opportunity to perform continuous temporal monitoring of marine biodiversity, which is like remote measurement of physical ocean properties by autonomous platforms. Prototype environmental sample processors (ESP) are used to analyze in real time for deep molecular analysis, sensing taxa of harmful algal blooms and potentially expanded to larger eDNA surveillance applications (Scholin et al., 2017). These platforms may help in the monitoring of biodiversity in deep-sea ecosystems, which are some of the most biodiverse but least surveyed on Earth.

7. Challenges, Limitations, and Standardization

7.1. Contamination and False Positives

Contamination represents perhaps the most serious operational challenge in eDNA analysis. Given the extreme sensitivity of PCR-based detection, even a trace amount of foreign DNA introduced in the sample during sampling, filtration, extraction, or amplification stages can generate false-positive detections with severe consequences for conservation management. A positive eDNA result for a critically endangered species in a new location could trigger costly management interventions or alter planning decisions, if caused by contamination rather than a true positive detection. Robust contamination control protocols including field negative controls, PCR negative controls, laboratory air filtration, and physical separation of pre- and post-amplification work areas are essential, but they can add logistical complexity (Goldberg et al., 2016).

The risk of false positives from downstream transport of eDNA must also be considered in river systems. A positive detection at a sampling site may reflect organisms present kilometers upstream rather than in the immediate vicinity, complicating spatial inference. Hydraulic modelling to predict eDNA transport has been applied, but it remains computationally demanding and requires detailed hydrological data (Deiner et al., 2016).

7.2. False Negatives and Detection Probability

False negatives can be a failure to detect a species that is present in the eDNA sample. It can arise from a combination of low eDNA concentrations, PCR inhibition, suboptimal primers, or inadequate sampling effort. Occupancy models that explicitly account for imperfect detection probability have been developed to estimate the number of samples needed to achieve confident presence/absence inference (Schmidt et al., 2013). These

models indicate that for rare species, well-mixed water bodies, 10–30 replicate samples per site may be required to achieve >95% confidence in results, a logistical challenge that constrains operational feasibility at large spatial scales.

7.3. Reference Database Gaps and Taxonomic Resolution

The accuracy of eDNA-based taxonomic identification is inherently limited by the completeness of reference DNA barcode databases. Reference coverage is rather good in well-studied groups in the temperate regions, especially in vertebrates, vascular plants, and macro-fungi. Nevertheless, in the case of tropical invertebrates, marine protists, nematodes and most microbial lineages, the reference gap is deep: large proportions of metabarcoding reads cannot be mapped to anything beyond phylum or class rank, and novel taxa are systematically represented by sequences that do not have a close reference. The Global Biodiversity Information Facility (GBIF) and the Barcode of Life Data System (BOLD) are attempting to scale up coverage in the reference, though this expansion is not evenly covered in taxonomic groups and geographical regions (Weigand et al., 2019).

7.4. Standardization, Reproducibility and Regulatory Integration.

The fast, decentralized evolution of eDNA protocols has led to an explosion of protocols, primer collections, sequencing technologies, and bioinformatic pipelines that vary widely among laboratories and experiments, hindering the comparability and reproducibility of data. Inter-laboratory calibration has indicated the existence of major disparities in the rate of detection of the same target species through nominally equivalent protocols (Harper et al., 2019). Standardization programs, such as the ISO standard 23038 on water-based eDNA sampling and analysis, and the COST Action DNAqua-Net, have seen some progress on harmonization of minimum reporting requirements, although total standardization in the field has yet to be achieved.

7.5. Uncertainties, Methodological Limitations and Critical Synthesis

Field-stage uncertainties:

An ecological and physicochemical context is the most basic place of ambiguity for shed DNA and collected DNA. Once DNA is released into the natural environment, it starts to degrade immediately, with a half-life of just a few hours in warm, high-UV, alkaline systems, and several weeks in cold, turbid, or chemically buffered systems (Barnes & Turner, 2016; Strickler et al., 2015). This variability makes it difficult to simply conclude a negative detection as an absence: it could be an example of degradation prior to sampling. Most eDNA surveys fail to adequately describe the degradation environment to allow for differentiation between these scenarios, and this is the subject of the review in the literature but not examined explicitly regarding its inferential implications.

***Laboratory-stage uncertainties**:*

Two interrelated extraction and amplification biases need to be addressed in greater detail than is often done. The detectable species depend on primer design, and no single pair of primers amplifies all eukaryotic lineages equally. Due to non-randomness of the sequences to which primers bind, mismatches can cause under-representation of clades. Analyses of primer sets for the same community often find substantial differences in the species detected (e.g., Elbrecht & Leese, 2015; Deiner et al., 2017), which undermines the notion that any single-primer community survey represents a full inventory of the biodiversity of a community. However, many published eDNA studies use only one primer set and do not test its range of targeted taxonomic groups, and consequently the detected taxa are not a representative sample of all the targeted taxa.

***Bioinformatics-stage uncertainties**:*

The pipeline used to estimate biodiversity and the identification of species can lead to different results, e.g. the pipelines DADA2, QIIME2 and UNOISE3 assume different error models and chimaera formation (Nearing et al., 2022). This variation makes it hard to

compare findings across studies and to synthesise findings across studies. Under controlled conditions, there have been indications that eDNA read counts are correlated with organism biomass, but this is not always the case in more complex field environments (Doi et al., 2015). The presence of eDNA makes the situation even more complex, as it is a community sample in a variable period and changes in community composition cannot be determined without considering changes in season and local degradation rates.

Critical Synthesis

Traditional visual surveys and sampling methods are not as sensitive as eDNA; eDNA can be a more sensitive detection method for rare or cryptic species (Biggs et al., 2015; Jerde et al., 2013). It can predict the presence of invasive species before they are detected by conventional surveys, especially in the great, crested newt and other aquatic vertebrates. Its use for community-level biodiversity assessment, however, is hampered by primer bias, pipeline variability and limitations in reference data. eDNA metabarcoding should not be used as a complete substitute for traditional surveys but can be added to existing survey efforts. The quantitative monitoring technologies currently available are not well developed and are difficult to match with the strict calibration requirements. There is no uniformity of methodology in studies, which can create problems for valid comparisons and meta-analyses. The use of eDNA in regulations is progressing in Europe and North America, but there is a danger of confusion before the effects are understood.

7.6. Comparative Evaluation: Conventional Methods versus eDNA

For a balanced synthesis, eDNA needs to be compared with the performance of conventional methods, not with an idealized standard. The use of visual transects underestimates the abundance of cryptic and nocturnal species, and morphological identification of macroinvertebrates relies on specialist taxonomists who have taxonomic limitations. eDNA provides real benefits in terms of scalability, non-invasive nature, and taxonomic range in relation to sampling effort. But not all these advantages are experienced in all taxa. eDNA may be less informative than often assumed for organisms that shed little DNA, that live in low-flow or stagnant systems where there are not well-understood transport dynamics. The field will be best served not by advocacy for eDNA as a universal monitoring solution, but by a rigorous programme of uncertainty quantification, protocol standardization, and comparative validation.

8. Prospects and Future Projections.

The future of eDNA science is shaped by multiple innovation pathways that are expected to significantly enhance the power and applicability of this methodology over the coming decade. As an initial step, combining eDNA with remote sensing data - satellite land cover, LiDAR habitat mapping, and acoustic monitoring will allow for multi-dimensional biodiversity measurements based on complementary streams of data. Biodiversity indices are being predicted at landscape scales with high accuracy using machine-learning models that use eDNA community profiles along with spectral and structural features of the habitat (Deiner et al., 2017).

Secondly, novel technologies in sequencing cost and throughput are rapidly rendering whole-genome sequencing of eDNA metagenomics economically viable at the scale necessary to conduct regular monitoring. Shotgun metagenomic techniques overcome the primer bias of amplicon-based metabarcoding, recover both functional genes and taxonomic profiles, and have the capability to identify novel lineages without a reference sequence. With the further downward trend in the cost of sequencing, under the same downward trends as Moore's law, in a decade or so, routine whole community metagenomic characterization of field samples might become common. Thirdly, eDNA is starting to be harnessed to participatory biodiversity monitoring via citizen science and

open-data platforms. Some initiatives, like the Freshwater Habitats Trust eDNA monitoring programme in the UK, assign volunteers to collect and send water samples to the centralized laboratory, creating data on the distribution of great crested newt nationally on the scale of data that a professional survey programme could have generated. Expanding these frameworks to other species of interest and incorporating the findings into national biodiversity databases would provide extraordinarily fine-grained baselines, over which change can be tracked over time (Biggs et al., 2015).

Fourthly, the emergence of biosensors and microfluidic eDNA platforms will offer in-situ, real-time detection of species without the need for laboratory testing. CRISPR-based diagnostic systems (e.g., SHERLOCK, DETECTR) have been reconfigured to detect eDNA with great sensitivity and are suitable for field implementation and may eventually form the basis of the so-called 'smart rivers'that constantly report on aquatic biodiversity and water quality (Chen et al., 2018). Such systems can deliver near-real-time ecological monitoring at watershed to continent scales when utilized in conjunction with IoT networks and cloud-based data management.

Lastly, eDNA-based biodiversity measures are also beginning to be seen as a part of the environmental policy frameworks. The Kunming-Montreal Global Biodiversity Framework (GBF) that was adopted at COP15 in 2022 has a target to track genetic diversity of wild species - a metric in which eDNA population genomics is uniquely placed. With national governments in the process of formulating biodiversity monitoring approaches to fulfil GBF pledges, standardized eDNA protocols as part of the law-making monitoring systems are a viable and scalable way ahead (CBD, 2022).

9. Conclusions

Environmental DNA has emerged as a technique to become a pillar of modern science and conservation practice of biodiversity. Its ability to non-invasively identify life, describe the whole community using just a single sample and its ability to work across all ecosystems, including cave waters, the oceanic abyss, the tropical forest soils, and the open atmosphere, is not found in all other traditional monitoring methods. The case studies that have been examined in this paper have shown not only that eDNA is effective, but that in most cases, the technique is more sensitive, has a broader taxonomic range, and is less expensive than conventional approaches.

But still, the field has significant challenges. Continued investment and cooperation are all that is needed to control contamination, improve reference database completeness, improve detection probability modelling, and support international standardization. The shift between proof-of-concept and a routine, regulatory-grade biodiversity assessment needs not only methodological soundness but also a reputation with stakeholders who have been accustomed to traditional survey methodologies.

In the future, it is expected that combining eDNA with genomics, real-time sensing and remote sensing will create a biodiversity monitoring framework of unprecedented resolution. It could monitor changes in the planet's biological heritageon the planet in near-real time. In an era where the biodiversity crisis requires increasingly rapid and reliable information, at a time when speed is required to take the necessary action, environmental DNA is one of the most promising scientific tools that can be used in conservation biology.

Declaration of Conflict of Interest

The author has no conflict of interest to declare.

Data Accessibility Statement

Data can be accessed upon request from the author.

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