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Environmental DNA (eDNA) as a Transformative Tool for Biodiversity Monitoring: Applications, Methods, Challenges and Future Directions

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ABSTRACT

Every organism leaves a trace of itself behind, through mucus, waste, gametes, shed skin cells, or decaying tissue, in the form of genetic material known as environmental DNA (eDNA). Over the past decade, this genetic residue has become a valuable, non-invasive, and relatively inexpensive tool for tracking biodiversity across aquatic, terrestrial, and even airborne ecosystems. This review examines the core principles behind eDNA-based research, along with the methods used to collect and analyse it, its growing range of applications, the challenges that still limit its use, and where the field is likely headed next. Samples taken from water, soil, sediment, or air can be processed through techniques such as quantitative PCR (qPCR), droplet digital PCR (ddPCR), and next-generation sequencing (NGS)-based metabarcoding, all of which allow researchers to detect and track species without ever having to see or capture them directly. These tools have opened up a wide range of practical uses, from catching invasive species early, to tracking rare and endangered wildlife, surveying fish and amphibian populations, conducting large-scale marine biodiversity assessments, supporting fisheries management, monitoring pathogens in aquaculture systems, and even studying disease spread through wastewater. Still, the technology comes with its own set of hurdles. DNA can break down quickly depending on environmental conditions, samples can produce false positives when genetic material drifts downstream from its source, reference databases are often incomplete, and estimating how many individuals of a species are actually present remains difficult. Even so, as molecular techniques continue to improve, reference libraries grow more comprehensive, and eDNA data gets combined with machine learning, remote sensing, and ecological modelling, this approach is steadily becoming one of the most important tools available for biodiversity assessment, ecological monitoring, and conservation planning.

INTRODUCTION

Monitoring biodiversity is essential for successful ecosystem management, for conservation of threatened habitats and for early detection of biodiversity loss. Traditional survey techniques, such as electrofishing, visual census, and mark recapture and morphological identification can be effective, but they can be time consuming, costly, and potentially destructive of organisms and habitat, particularly in remote or sensitive areas [1]. Environmental DNA (eDNA) is the sum of all DNA obtained from environmental materials without capturing or isolating organisms themselves, and is an alternative that is very effective, non-invasive and inexpensive [1, 2, 3]. Scientists can detect and analyse a molecular trail left behind by organisms when they shed their skin cells, excrement, gametes and decaying tissues [1].

The use of mitochondrial DNA amplification using PCR to detect invasive American bullfrog (*Rana catesbeiana*) in pond water was the first step in modern eDNA monitoring, followed by groundbreaking conceptual and methodological advances that set the stage for current eDNA studies [3, 5]. The advent of next-generation sequencing and high throughput metabarcoding allowed multiple communities of organisms to be screened from a single environmental sample [6], and a recent study showed that fish, amphibian and invertebrate communities could be screened from a variety of environments with a standardised eDNA workflow [7].

Despite these advances, eDNA faces challenges including rapid DNA degradation [8], false positives arising from downstream transport of DNA [9], and limitations imposed by incomplete reference databases and unstandardised protocols [10, 11]. Nevertheless, eDNA applications continue to expand across aquatic, terrestrial and aerial habitats, encompassing biodiversity assessment, invasive species detection, rare taxa monitoring, fisheries management, pathogen surveillance and conservation planning. This review synthesises the principles, methodologies, applications, limitations and future directions of eDNA research, highlighting both its strengths and ongoing challenges in biodiversity monitoring.

Sources And Distribution Of eDNA

Sources of eDNA

Environmental DNA originates from shed epithelial cells, mucosal secretions, faeces, urine, gametes and decomposing tissue, released through lysis-associated pathways driven by bacterial endolysins and prophages, and through lysis-free pathways including membrane vesicles and active secretion [1]. Shedding rates vary with body size, metabolic rate, reproductive condition, stress and parasite load, with osmotic stress and fish handling markedly increasing eDNA output, whilst spawning events generate seasonal concentration peaks through simultaneous gamete release and heightened activity [1, 12]. eDNA concentrations can reach as high as 88 μg/L in eutrophic lakes, though typical values vary considerably across systems [1]. Sediments represent the largest eDNA reservoir, containing more than 90% of total extracellular DNA, with deep-sea sediments estimated to hold 0.30 to 0.45 Gt globally, whilst soil eDNA constitutes around 40% of total soil DNA at concentrations of 0.03 to 200 μg/g dry weight in upper horizons [1, 13].

Distribution Across Ecosystems

In lotic systems, eDNA is carried downstream from source organisms, sometimes over several kilometres. Invertebrate eDNA has been detected several kilometres from its source in a Swiss river, a finding with clear implications for the spatial interpretation of survey data [9]. In marine systems, eDNA persistence has been shown to vary with salinity, temperature and UV exposure, with decay rates typically between 10 and 50 hours [14]. Seawater eDNA has further been confirmed to reliably characterise diverse marine fish fauna [15]. In soils, adsorption onto clay minerals slows degradation, enabling palaeoecological reconstruction over centuries to millennia [1]. Aerial eDNA, collected through high-volume air samplers, now reliably yields detectable DNA from vertebrates, insects, plants and fungi [1]. Filtration of 0.22 to 0.45 □m for water, PCI/DNeasy extraction for soils, and MD8 air samplers for atmospheric collection have been proposed in previous studies [6].

eDNA SAMPLING METHODS

There is no standardized protocol; the most effective depends on the goals of the study, the species of interest, the ecosystem where the study will take place, and the method for detection [1]. In aquatic systems, filtration of water with 0.2–0.45 □m glass fibre membranes is the most common method and a minimum of three replicates should be taken per site to minimize the risk of a false negative [1, 6]. The varying detection rates that can be obtained from different combinations of capture and extraction devices underscore the need for method validation prior to widespread use [16]. Thermal stratification, anoxia and high organic

content sometimes lead to adapted protocols for pond habitats [17]. Extreme acidity (pH as low as 3.5) and turbidity were observed in Malaysian blackwater peat swamp to complicate the process of eDNA recovery, and middle stream stations with 480 mL per replicate filtering appeared to be the best stations for eDNA recovery [18]. Although integrated spatial sampling covers a larger area, it has been shown to consistently underestimate the concentration of eDNA compared to point sampling, a consideration of importance for fisheries assessment programmes [19].

An innovative alternative using Mediterranean sponges as passive biological samplers has been proposed, demonstrating that different sponge species accumulate fish community eDNA through their natural filter-feeding and offer a low-effort complement to conventional water filtration [20].

Table 1: Comparison of eDNA Sampling Methods Across Different Ecosystems

Sampling Medium	Matrix	Target Organisms	Documented Characteristics	Reference
Water (Aquatic)	0.2–0.45 □m filtration	Fish, amphibians, macroinvertebrates	High sensitivity; non-invasive; widely validated	[1,4,5]
Sediment	Core and surface samples	Benthic fauna; microbial communities; historical biota	Long-term DNA preservation; palaeoecology	[5,6]
Soil (Terrestrial)	DNeasy/PCI extraction	Mammals, reptiles, soil microbiota, plants	Wide taxonomic coverage; no water needed	[1,8,13]
Air (Aerial)	MD8 high-volume air sampler	Insects, birds, plants, fungi, vertebrates	Broad terrestrial coverage; passive collection	[1,6]
Marine Sponges	Sponge tissue extracts	Marine fish, eukaryotes	Natural large-volume filtration; passive and continuous	[20]

eDNA Detection and Analysis Techniques

The analytical methods used in eDNA studies fall broadly into two groups: targeted single-species assays that confirm the presence or abundance of a known taxon, and community-level approaches that characterise the full diversity of organisms present in a sample [1]. The right choice depends on the ecological question and available resources.

PCR and Quantitative PCR (qPCR)

Conventional PCR remains widely used for single-species detection, amplifying short mitochondrial or nuclear DNA fragments with species-specific primers [4, 21]. Quantitative PCR (qPCR) extends this by estimating DNA copy numbers per unit volume of water, providing semi-quantitative population data. A positive relationship between qPCR eDNA concentrations and larval amphibian density has been demonstrated in natural streams [22], whilst qPCR has also been shown to track relative fish abundance across seasons in lakes [23]. qPCR has further been applied to guide targeted minnow trap deployment for invasive loach removal, achieving better outcomes with less survey effort [24].

Droplet Digital PCR (ddPCR)

Droplet digital PCR (ddPCR) partitions reactions into thousands of nanolitre droplets, giving absolute copy-number quantification without calibration curves [5, 25]. ddPCR has been shown to outperform qPCR for fish abundance estimates when target DNA is scarce, which is precisely the situation for rare or newly introduced species [25]. ddPCR is recommended where eDNA concentrations are near detection limits, though its higher cost limits it to single or few targets per reaction [6].

eDNA Metabarcoding and Next-Generation Sequencing

Metabarcoding through next-generation sequencing (NGS) is one of the most powerful approaches for community-level biodiversity assessment. Universal PCR primers amplify a standardised barcode region (typically 12S rRNA, 16S rRNA, or COI) from all organisms in the eDNA extract [3], and millions of resulting sequences are assigned to taxa using databases such as GenBank and BOLD [1]. Entire animal and plant communities have been detected from single river samples [10], and fish, amphibians and invertebrates have been surveyed simultaneously using a unified workflow [7]. Application of 12S rRNA metabarcoding to rivers in French Guiana detected markedly greater fish species richness than electrofishing and netting combined [26]. However, metabarcoding is affected by primer bias and database gaps, particularly for tropical and endemic taxa [1, 6]. eDNA metabarcoding has further been shown to match conventional acoustic and visual surveys for Mediterranean amphibians whilst detecting additional species missed by traditional methods [27].

Table 2: Comparison of eDNA Detection and Analysis Techniques

Technique	Primary Target	Sensitivity and Resolution	Key Limitations	Reference
PCR (Conventional)	Single species; presence/absence	High specificity; binary output only	Cannot quantify; labour-intensive	[1,4,21]
qPCR (Real-time)	Single or few species; quantitative	Quantitative; high sensitivity; fast results	Species-specific primers required; may miss new taxa	[1,6,22,23]
ddPCR (Digital)	Rare or endangered species; absolute quantification	Ultra-sensitive; absolute copy-number; no standard curve	High reagent cost; few target taxa per reaction	[5,6,25]
eDNA Metabarcoding (NGS)	Multiple species; community-level	Broadest coverage; detects entire communities	Database gaps; PCR amplification bias	[1,5,6,7,10]
Metagenomics (Shotgun NGS)	All taxa without PCR amplification	Maximum diversity; no amplification bias	Very high cost; complex analysis required	[5,6,28]

Applications of eDNA in Biodiversity Monitoring

Aquatic Biodiversity Assessment

In Danish rivers and lakes, eDNA demonstrated higher detection sensitivity for certain endangered fish and amphibian species compared with conventional netting methods in the study conditions reported by Thomsen et al. [29]. Targeted PCR assays have successfully detected five of six target macroinvertebrate species, including rare gastropods and amphipods [30]. eDNA metabarcoding combined with landscape genetics has been shown to reveal population connectivity and cryptic genetic lineages in riverine fishes missed entirely by electrofishing [31]. In marine systems, 70 MOTUs have been identified from Maltese coastal seawater, including loggerhead turtle (*Caretta caretta*), striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops*

truncatus) [32]. eDNA has been noted as especially useful in threatened river habitats affected by dams and pollution, where non-destructive water sampling avoids the barriers of physical capture [33].

Invasive Species Detection and Management

Invasive Asian carp (*Hypophthalmichthys* spp.) eDNA was detected in Great Lakes tributaries before any fish was physically captured, providing an early warning that enabled timely containment [34]. eDNA barcoding can detect invasive American bullfrog at far lower densities than visual surveys [21]. qPCR has been applied to guide invasive loach removal, whilst repeated eDNA monitoring after eradication has been shown to provide early warning of reinvasion events [24, 35]. eDNA is noted to be most cost-effective at low invasion densities, when early intervention matters most [36].

Endangered and Cryptic Species Monitoring

eDNA has successfully detected species at sites that extensive physical surveys had missed [37]. eDNA is valuable for generating occurrence data for threatened taxa to inform IUCN Red List assessments [28]. The endangered Japanese freshwater crayfish (*Cambaroides japonicus*) has been detected in streams where decades of conventional surveys had failed [38]. In contrast, Kirtland's Snake (*Clonophis kirtlandii*), which uses water only incidentally, was rarely detected by eDNA, illustrating that sensitivity is context-dependent and species ecology must inform survey design [39].

Fisheries, Aquaculture, and Ecosystem Health

eDNA has numerous applications in fisheries and aquaculture, covering population assessment, pathogen detection and water quality evaluation [40]. Early detection of salmon pathogens such as ISAV and SAV allows preventive management before outbreaks occur [1]. eDNA metabarcoding has been shown to characterise microbial community structure, extending monitoring to ecosystem functional health [6]. Realising eDNA's management potential has been argued to require embedding it within established regulatory frameworks [2]. Non-invasive spawning monitoring of the endangered Macquarie perch (*Macquaria australasica*) has been demonstrated through tracking reproductive activity from eDNA concentrations without harmful netting during the spawning season [12].

Table 3: Applications of eDNA in Biodiversity Monitoring

Application Area	Ecosystem	Key Finding / Example	Reference
Foundational species detection	Freshwater ponds, France	First demonstration that eDNA in pond water detects an invasive vertebrate (American bullfrog) without direct physical observation	[4]
Invasive species detection	Great Lakes, North America	Asian carp eDNA detected in water samples before physical netting confirmed its presence, providing a sight-unseen early detection capability that enabled timely containment	[34]
Endangered species monitoring	Freshwater rivers and lakes	eDNA detected rare, threatened fish and amphibians with higher sensitivity than conventional electrofishing and netting	[22,29]
Marine fish diversity	Coastal seawater, Denmark	Seawater eDNA metabarcoding characterised a diverse marine fish fauna comparable to traditional netting in richness	[15]

Fish community assessment	Rivers, French Guiana	eDNA metabarcoding detected greater fish species richness than electrofishing and netting surveys combined	[26]
Macroinvertebrate monitoring	Rivers and lakes, Switzerland	eDNA detected five of six target macroinvertebrate species, including rare gastropods, isopods, and amphipods	[30]
Amphibian community survey	Mediterranean ponds, Spain	eDNA metabarcoding matched acoustic and visual surveys for amphibians and detected additional species at low density	[27]
Marine vertebrate detection	Coastal waters, Maltese Islands	eDNA detected 70 MOTUs including loggerhead turtle, striped dolphin, and bottlenose dolphin from filtered seawater	[32]
Invasive fish management	Freshwater canals, USA	qPCR-guided minnow trap deployment achieved targeted removal of invasive loach with improved catch-per-unit-effort	[24]
Fisheries and aquaculture	Aquaculture systems	eDNA enabled early detection of salmon pathogens (ISAV, SAV) before clinical symptoms, allowing preventive management	[40]

Factors Affecting eDNA Persistence and Detectability

Abiotic Factors

Temperature is among the most influential abiotic drivers of eDNA persistence, as elevated water temperatures accelerate microbial enzymatic activity and chemical hydrolysis, shortening eDNA half-life [8]. Temperature, salinity, UV radiation and microbial community composition have each been shown to significantly affect eDNA degradation rates [41]. eDNA concentrations in alkaline lotic mesocosms have been found to be one to two orders of magnitude higher than in acidic ones, in one experimental study, half-lives were reported below 1.2 hours under the most extreme acidic conditions tested [42]. UV-B radiation has been shown to cause significant photochemical DNA damage at water surfaces during daytime sampling [43]. Precipitation can simultaneously dilute eDNA through increased flow and introduce terrestrial DNA through surface runoff [1], whilst adsorption onto clay minerals in sediments can extend DNA persistence over geological timescales, enabling palaeoecological applications [1].

Biotic Factors

Body size, metabolic rate, reproductive condition and parasite load all affect how much eDNA an individual sheds [1]. Acute stress from predator encounters, handling, or osmotic change has been associated with increased eDNA shedding rates in laboratory studies [1], though the ecological significance of this variation in natural settings warrants further investigation [1]. Microbial degradation of extracellular eDNA is modulated by temperature, dissolved oxygen and organic matter availability [8]. Spawning creates seasonal peaks in eDNA concentration through simultaneous gamete release, increased adult activity and larval contributions [12]. These biological rhythms must be factored into the design of time-sensitive monitoring programmes.

Challenges and Limitations of eDNA Monitoring

Both false-positive and false-negative detections carry serious consequences for biodiversity monitoring and management. Insufficient PCR replication may generate false positives through contamination and false negatives through degraded or dilute

eDNA, with at least three positive PCR replicates per site recommended to improve detection reliability [44]. Detection probability, occupancy modelling and statistical power remain important considerations for accurate interpretation of eDNA data [11]. Downstream transport can cause eDNA detected at a sampling site to originate from organisms kilometres upstream, complicating spatial interpretation of species distributions, particularly for invasive species management [9], with tidal oscillations creating similar challenges in coastal and estuarine environments [14].

Contamination can occur at any workflow stage and requires strict separation of field collection, DNA extraction and PCR amplification areas, alongside routine negative controls [10, 37]. Reference database incompleteness, particularly for tropical, endemic and marine taxa, restricts accurate species identification [1], whilst database gaps, absent standardised protocols and unequal access to technology remain key barriers to global eDNA adoption, especially in biodiverse developing countries [45]. Abundance estimation from eDNA concentrations remains challenging due to variable shedding and degradation rates and the significant influence of sampling strategy on concentration estimates [19]. Adoption of FAIR (Findable, Accessible, Interoperable, Reusable) data principles has been proposed to improve standardisation and cross-study comparability [46].

Table 4: Key Challenges in eDNA Monitoring and Recommended Mitigation Strategies

Challenge	Description	Mitigation Strategy	Reference
eDNA Degradation	Temperature, UV radiation, pH, and microbial activity degrade eDNA rapidly; half-life can fall to less than 1.2 h in acidic lotic environments	Cold storage; rapid processing; Longmire buffer or ethanol preservation	[8,41,42,43]
False Positives	eDNA transported by river currents, ballast water, or predator excretion may produce detections far from actual source populations	Spatial replicates; replicate PCRs; occupancy modelling frameworks	[1,11,44]
False Negatives	Low-density species may go undetected owing to dilute or degraded eDNA, or PCR inhibition by humic acids in water	Increased filtration volume; inhibitor-removal kits; multi-tube parallel approaches	[1,4,44]
Contamination Risk	Cross-contamination from equipment, reagents, or aerosols can generate spurious detections at any stage of the workflow	Dedicated clean labs; negative field and extraction controls; bleach decontamination	[1,10,37]
Database Gaps	Incomplete reference libraries prevent taxonomic assignment of sequences, especially for tropical, endemic, and marine invertebrate communities	Expand GenBank and BOLD; prioritise barcoding of endemic species; build regional libraries	[1,6,10,45]
Abundance Quantification	Translating eDNA concentration into species abundance is confounded by variable shedding rates, environmental degradation, and sampling design	ddPCR for absolute quantification; standardised point-sampling; controlled calibration per taxon	[5,6,19,25]

FUTURE DIRECTIONS

The ongoing development of eDNA monitoring will rely on the development of reference databases, especially for tropical and endemic taxa, as well as on the development of bioinformatics pipelines for detecting rare sequences, and on further standardisation of eDNA barcode regions [1]. Efforts to strengthen research capacity and build open access data infrastructure in biodiverse developing countries will also be crucial for broader adoption of eDNA technologies [45]. As the volume of eDNA data and the complexity of the samples continue to grow, advances in machine learning are likely to enhance taxonomic assignment and enable analysis of data on a much larger scale [6]. Combining eDNA with satellite derived remote sensing and ecological modelling would further improve species distribution assessments at large spatial scales that can be difficult to obtain using traditional survey techniques [1,6]. Further, aerial eDNA provides novel opportunities to monitor terrestrial biodiversity by screening for mammals, birds and reptiles that may leave little DNA in soil/water samples [1]. In addition to biodiversity conservation, the use of eDNA is growing. For instance, the use of wastewater eDNA in the surveillance of SARS-CoV-2 during the pandemic showed that this approach can be used for real-time monitoring of the population level, opening up new possibilities for environmental and public health monitoring [1]. It will also need to implement internationally standardised protocols [36] and reporting on data following FAIR data principles [46] and integrate eDNA into the global initiatives on biodiversity like the Kunming–Montreal Global Biodiversity Framework [2].

CONCLUSION

Environmental DNA (eDNA) is a relatively novel, non-invasive method that shows great potential to be a fast, large-scale and cost-effective way to identify the presence of a species in a wide range of ecosystems. It has several properties that make it more appealing than traditional survey methods in many ecological contexts, particularly for locating elusive and rare species and invasive species; but in some cases it may not be as effective as traditional survey methods depending upon the species and conditions. While it has a lot of potential, there are also some challenges, such as degradation of the DNA due to environmental factors, uncertainty related to eDNA movement downstream, contamination potential, variability in DNA shedding, incomplete reference libraries to restrict the accuracy of metabarcoding, and challenges in determining a relationship between eDNA concentration and species abundance. Future advances in molecular methods, increasing reference databases and improved standardization of sampling and analytical methods will further improve the reliability and usefulness of eDNA. eDNA will not replace traditional survey techniques but rather be a useful supplemental survey tool that will help in providing biodiversity assessment, ecological monitoring and ecological conservation planning.

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Conflict of interest

The authors declare no conflicts of interest.

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