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Environmental DNA (eDNA) as a Tool for Ecosystem Monitoring and Conservation Biology

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SUMMARY

Environmental DNA (eDNA) analysis has become one of the most useful new tools in ecological research and biodiversity monitoring over the past two decades. By picking up genetic material that organisms leave behind in their environment, eDNA allows researchers to detect species without ever seeing or capturing them across both aquatic and terrestrial ecosystems. This review pulls together what we currently know about eDNA methodology, from sample collection and molecular processing to bioinformatic analysis. We look at how eDNA is being used in biodiversity assessment, ecosystem monitoring, and conservation biology, with a focus on finding endangered, rare, and invasive species in freshwater, marine, and terrestrial environments. The advantages of eDNA are real: it detects species more reliably, requires less fieldwork, and barely disturbs the ecosystem. But there are also genuine problems false positives, false negatives, contamination risk, DNA degradation, primer bias, and incomplete reference databases. We compare eDNA with conventional monitoring methods, discuss efforts to standardize protocols, and point out where technology is advancing and where gaps remain. eDNA has moved well beyond proof-of-concept; it is now a practical monitoring tool. That said, it works best when combined with traditional survey methods rather than replacing them entirely. Moving forward will require better standardization, more complete reference libraries, and closer collaboration between molecular biologists and ecologists.

INTRODUCTION

For generations, scientists have tracked biodiversity through direct observation, trapping, acoustic surveys, and morphological identification—approaches that demand a lot of labor, require taxonomic expertise, and sometimes disturb the very organisms being studied (Thomsen & Willerslev, 2015). eDNA analysis changed that. It offers a way to detect species molecularly, without ever encountering the organism physically (Taberlet et al., 2012).

Environmental DNA is simply genetic material collected straight from environmental substrates—water, soil, sediment, or air—without isolating the organism it came from (Pawlowski et al., 2020). This material can come from shed skin cells, mucus, feces, urine, gametes, or decaying tissue (Çevik & Çevik, 2025).

The idea that DNA persists outside living cells goes back to early microbiology. But the real breakthrough for macro-organism detection came from two key studies. Ficetola et al. (2008) showed that DNA from the invasive American bullfrog (Lithobates catesbeianus) could be detected in pond water—proof that aquatic eDNA surveillance was possible. Soon after, Thomsen et al. (2012) demonstrated that eDNA could pick up rare and endangered freshwater species in European streams, with sensitivity far beyond what conventional surveys could achieve. These studies established eDNA as a genuine option for conservation monitoring.

The science behind eDNA is straightforward enough: all cellular life contains DNA, and organisms constantly release it through normal physiology and death (Sahu et al., 2023). Once released, eDNA floats around as particles—bound to organic or inorganic matter, trapped in cellular debris, or free in solution. How long it lasts depends on temperature, pH, UV radiation, oxygen levels, microbial activity, and enzymatic breakdown (Strickler et al., 2015; McCartin et al., 2022).

The shift from single-species detection to community-scale metabarcoding has opened even more possibilities. Metabarcoding uses broad PCR primers to amplify informative DNA regions from mixed environmental samples, then sequences everything to identify the species present (Deiner et al., 2017). This turned eDNA from a targeted detection tool into a platform that can identify hundreds of taxa from a single water or soil sample (Valentini et al., 2016).

Methodological Framework: From Sampling to Sequencing

Sources and Forms of eDNA

eDNA enters the environment in two main ways: through cell lysis or direct secretion (Çevik & Çevik, 2025). Lysis-associated release happens when cells break down through autolysis, predation, infection, or physical damage, spilling intracellular DNA into the surroundings. Direct secretion includes active release through

membrane vesicles, excretion of nucleic-acid-containing waste, and shedding of gametes or mucus-bound cells. How much each pathway contributes varies by species, life stage, physiology, and environmental conditions.
In aquatic systems, eDNA concentrations typically run from 2.5 to 88 µg/L depending on productivity, season, and biomass (Çevik & Çevik, 2025). Sediments hold the largest stores of eDNA in aquatic environments—marine sediments alone are estimated to contain 0.30 to 0.45 gigatons of extracellular DNA (Dell'Anno & Danovaro, 2005). In terrestrial systems, roughly 40% of soil DNA is extracellular, with concentrations from 0.03 to 200 µg/g depending on soil type, depth, and organic content (Nielsen et al., 2007; Pietramellara, 2009). eDNA drops off sharply with soil depth, and fungal sequences usually dominate soil profiles—sometimes making up over 70% of detected molecules (Borneman et al., 2000).

Sampling Strategies

Designing an eDNA sampling protocol means thinking about what you want to know, which organisms you are targeting, what kind of ecosystem you are working in, and how likely you are to detect them. In aquatic environments, water sampling is standard—usually 0.5–2.0 L of surface water or integrated depth samples. Where you sample, how many replicates you collect, and how often you return all matter (Jerde et al., 2021).
Because currents and tides move eDNA through water columns, a positive detection might come from upstream or upwind sources rather than organisms living right at the sampling point (Kelly et al., 2024). So spatial replication and hydrological modeling are not optional extras—they are essential for drawing accurate conclusions.
Terrestrial sampling has traditionally meant soil and fecal collection, but newer approaches are expanding the toolkit. Airborne eDNA sampling—filtering genetic material from the atmosphere—has opened up a way to detect terrestrial vertebrates, plants, and insects without ever setting foot in the habitat (Clare et al., 2022; Lynggaard et al., 2022). If this scales well, it could change how we do large-scale terrestrial biodiversity monitoring (Tournayre et al., 2025).

DNA Extraction and Purification

After collection, eDNA has to be extracted and purified before any molecular work can begin. Methods differ by substrate: water samples usually need concentration by filtration (0.22–0.45 µm membranes) or precipitation first, while soil and sediment can often go straight into commercial extraction kits designed for environmental samples. Which protocol you choose affects yield, purity, and detectability (Goldberg et al., 2016).
PCR inhibition is a persistent headache in eDNA work, especially with humic-rich soils, sediments, or highly organic waters. Humic acids, phenolics, and heavy metals can all interfere with DNA polymerase, producing false negatives (Sidstedt et al., 2020). Purification columns, dilution, and standardized cleanup help, but getting rid of inhibition completely is still difficult (Goldberg et al., 2016).

PCR, qPCR, and Digital PCR

PCR is the backbone of eDNA detection. Standard endpoint PCR tells you whether a target is present or absent, but gives no quantitative information. Quantitative PCR (qPCR) amplifies and quantifies simultaneously, using fluorescence to estimate relative template abundance (Hays et al., 2024). With properly validated taxon-specific primers and probes, qPCR offers good sensitivity and specificity for single-species detection.
Droplet digital PCR (ddPCR) is a newer approach that splits samples into thousands of tiny reactions, allowing absolute quantification without standard curves. Studies show ddPCR detects low DNA concentrations (<1 copy/µL) more reliably and tolerates PCR inhibitors better than qPCR (Doi et al., 2015; Guri et al., 2024). For rare or invasive species where you need every possible advantage, ddPCR is worth considering over conventional qPCR.

Metabarcoding and High-Throughput Sequencing

Metabarcoding scales eDNA up from single targets to whole communities. Degenerate or universal primers amplify short, taxonomically informative barcodes—usually mitochondrial regions like cytochrome c oxidase subunit I (COI), 12S/16S rRNA, or cytochrome b—from mixed environmental samples. The pooled amplicons are then sequenced en masse, producing millions of reads that are matched to reference databases for species identification (Deiner et al., 2017).
The workflow covers primer selection, PCR, library prep, sequencing, and bioinformatics. Primer choice matters enormously for what you recover: universal vertebrate primers like MiFish-U (Miya et al., 2015) have become standard for fish communities, while COI primers dominate invertebrate work. Every primer set has its own taxonomic biases, and ignoring them leads to misleading community portraits (Shaffer et al., 2025).

Applications in Biodiversity and Ecosystem Monitoring

1 Community Composition and Species Richness

Metabarcoding surveys have shown they can characterize community composition across ecosystems and taxonomic groups. In freshwater, eDNA metabarcoding regularly detects more species than electrofishing or gillnetting—especially cryptic, nocturnal, or low-density taxa that conventional gear misses (McColl-Gausden et al., 2021; Valentini et al., 2016). Liu et al. (2024) reviewed the literature and found eDNA metabarcoding matched or beat traditional methods for assessing fish community structure, while also picking up fine-scale spatial patterns that nets and traps could not resolve.

In marine systems, eDNA metabarcoding has tracked community assemblages and species turnover across offshore environments, adding information that trawl surveys alone do not capture (He et al., 2023). Sawh et al. (2025) reviewed marine applications and noted growing use for reef fish, pelagic biodiversity, and deep-sea fauna. Because eDNA sampling does not physically disturb the habitat, it is especially useful in sensitive marine areas where trawling or dredging would do real damage.

2 Temporal and Spatial Dynamics

eDNA allows repeated, standardized sampling that makes temporal analysis practical. Water and soil samples can be collected quickly by people with minimal training, so monitoring programs can sample more frequently than is logistically possible with conventional methods. That matters for tracking phenology, migration timing, spawning, and seasonal turnover (Takahashi et al., 2023).

Spatial interpretation is trickier. In rivers, eDNA at any point reflects upstream sources, which is useful for catchment-scale assessment (Deiner et al., 2017) but makes it hard to pin down exactly where a species lives. Hydrological modeling and dense spatial sampling are needed to translate detections into distribution maps (Kelly et al., 2024).

Applications in Conservation Biology

1 Endangered and Rare Species Detection

eDNA's sensitivity makes it a natural fit for endangered, threatened, and rare species—populations that conventional surveys often miss simply because there are not many individuals to encounter (Thomsen et al., 2012; Thomsen & Willerslev, 2015). eDNA can pick up a species from tiny amounts of genetic material, sometimes when only one individual is present in the sampled area. That matters because false negatives in traditional surveys can lead managers to conclude a species is gone when it is not, with serious consequences for conservation decisions (Çevik & Çevik, 2025).

Case studies across taxa back this up. eDNA has detected critically endangered amphibians in hard-to-reach habitats, tracked reintroduced large mammals, and confirmed that freshwater mussels are still hanging on in degraded rivers. The non-invasive aspect is especially valuable for species where capture or handling would stress or kill them.

2 Invasive Species Early Detection and Management

Catching invasive species early is one of eDNA's most concrete contributions. During initial colonization, populations are small and geographically limited—the exact window when eradication or containment is still possible (Rishan & Chen, 2023). Conventional methods usually fail at this stage because rare individuals rarely end up in nets, traps, or visual counts.

eDNA surveillance is now running for aquatic invaders including fish, crayfish, mollusks, and amphibians, as well as terrestrial pests and pathogens. The U.S. Fish and Wildlife Service and state agencies have folded eDNA into early-detection networks, particularly for aquatic invasives in the Great Lakes (Jerde et al., 2021). Wang et al. (2023) reviewed the management literature and concluded that eDNA genuinely speeds up detection and response for organisms that are easy to overlook.

Ecosystem-Specific Applications

1 Freshwater Systems

Freshwater was where eDNA started and where it has been studied most intensively. Rivers, lakes, and wetlands are well-suited to eDNA work because water captures and transports genetic material from resident organisms effectively. The pioneering studies by Ficetola et al. (2008) and Thomsen et al. (2012) put freshwater on the map, and since then researchers have expanded to whole fish communities, amphibian assemblages, benthic invertebrates, and invasives (Takahashi et al., 2023).

In running water, eDNA transport has received particular attention. Detection probability depends on discharge, channel shape, and distance from source populations. Researchers have built spatially explicit models that account for transport and degradation to improve distribution estimates (Deiner et al., 2017). These tools now allow catchment-scale biodiversity assessment from carefully chosen sampling points.

2 Marine Environments

Marine eDNA has grown fast, from coastal monitoring to open-ocean and deep-sea work. Seawater carries eDNA from plankton, fish, mammals, and bottom-dwelling organisms, giving a broad genetic snapshot of local biodiversity. Marine metabarcoding studies have documented how communities respond to ocean warming, habitat loss, and protected-area designation (Sawh et al., 2025).

One important development is pairing eDNA with traditional fisheries surveys. He et al. (2023) showed that eDNA metabarcoding catches species nets miss—especially small, soft-bodied, or fast-swimming taxa. Using both methods together may give the most complete picture of marine community composition.

3 Terrestrial Ecosystems

Terrestrial eDNA has historically trailed aquatic work, largely because DNA binds to soil particles, humic substances inhibit PCR, and DNA distribution is patchy. Even so, soil eDNA metabarcoding has worked well for plants, fungi, and invertebrates, with applications in forest ecology, soil health monitoring, and restoration tracking (Allen et al., 2024).

The real game-changer may be airborne eDNA. Clare et al. (2022) and Lynggaard et al. (2022) independently recovered vertebrate eDNA from air samples and identified it through metabarcoding, detecting mammals, birds, and amphibians. Tournayre et al. (2025) scaled this to a national survey—the first of its kind—showing that large-scale terrestrial monitoring via air sampling is feasible. If the method holds up, it could close the gap between aquatic and terrestrial eDNA applications.

Challenges and Limitations

1 False Positives and False Negatives

eDNA is not infallible. False positives—detecting a species that is not actually present—can arise from contamination, misidentification, or DNA transport from distant sources (Darling, 2020). False negatives—missing a species that is present—can result from low shedding rates, DNA degradation, PCR inhibition, or simply not sampling enough (Bajaffer et al., 2026). Both errors have real consequences. False positives can trigger expensive and unnecessary management responses; false negatives can lead to missed invasions or mistaken extinction declarations (Çevik & Çevik, 2025).

Managing these risks takes multiple lines of evidence: replication, controls, independent verification, and explicit modeling of detection probability. No single approach eliminates error, but combining methods reduces the chance of acting on bad information.

2 Contamination Risk

Contamination is a constant threat in eDNA work because PCR amplifies even trace amounts of foreign DNA. Sources include cross-contamination between samples, carryover from previously amplified products, and environmental DNA introduced during fieldwork or lab processing (Goldberg et al., 2016; Sepulveda et al., 2020). Standard precautions—physical separation of pre- and post-PCR workspaces, unidirectional workflows, and regular decontamination—are necessary but not sufficient. Complete elimination is impossible, so results need to be interpreted with appropriate caution.

3 DNA Degradation

Extracellular DNA is unprotected and breaks down through enzymatic, chemical, and physical processes. Temperature, UV radiation, pH, and microbial activity are the main drivers (Strickler et al., 2015). Lab experiments show most eDNA degrades within about 10 days under warm, sunny, acidic conditions, but can last 58 days or more when it is cold, dark, and alkaline (Strickler et al., 2015; McCartin et al., 2022).

In nature, dynamics are more complex. DNA binding to sediments and organic particles can shield it from nucleases (Dell'Anno et al., 2002), while high microbial activity in nutrient-rich waters speeds decay. What this means practically is that eDNA detection reflects recent presence—days to weeks—not necessarily current occupancy. Interpreting the timing requires environmental context (Lamb et al., 2022).

4 Primer Bias and PCR Limitations

All PCR-based eDNA methods suffer from primer bias: some species amplify better than others due to primer mismatches, amplicon length differences, or GC content variation (Shaffer et al., 2025). Universal primers meant to cover broad groups may miss some species entirely while over-representing others, skewing community composition. This is a particular problem when researchers try to use sequence read counts as a proxy for relative abundance, because reads do not reliably reflect biomass or population size (Shaffer et al., 2025).

5 Reference Database Limitations

Taxonomic assignment depends on complete, well-curated reference databases. NCBI GenBank and BOLD are the main repositories, but both are incomplete for many taxa and regions (Keck et al., 2023). When a species is missing from the database, its sequences may get assigned to the nearest match—sometimes a distant relative—or left unassigned. Outdated nomenclature compounds the problem (Mugnai et al., 2023). Locally curated databases improve accuracy, but building them takes serious investment in voucher-based sequencing (Mugnai et al., 2023).

Comparison with Conventional Ecological Monitoring Methods

eDNA and conventional methods are better thought of as complementary than competing. Each has strengths that integrated designs can exploit.

Electrofishing, trawling, visual census, and trapping give direct abundance estimates, behavioral observations, and physical specimens for morphological verification—things eDNA cannot currently do. On the flip side, eDNA detects cryptic, rare, and nocturnal species that conventional gear often misses. McColl-Gausden et al. (2021) showed eDNA metabarcoding outperformed traditional fisheries sampling in a temperate lake, picking up fine-scale community heterogeneity that nets missed. Brantschen et al. (2024) found eDNA and electrofishing each detected species the other did not.

The strongest monitoring programs increasingly use both: eDNA for broad, sensitive, repeated biodiversity screening, followed by targeted conventional surveys to verify presence, estimate abundance, and collect biological data (Liu et al., 2024).

Quality Control and Standardization

eDNA data are only as good as the protocols that produce them. Early studies used wildly different methods for sampling, extraction, amplification, and analysis, making cross-study comparison difficult (Sepulveda et al., 2020). That realization has spurred a push for standardization.

Internationally, the MIQE guidelines for quantitative PCR reporting have been adapted for eDNA work, setting minimum standards for assay validation, controls, and data quality (Hays et al., 2024). National agencies have also stepped in: the U.S. Fish and Wildlife Service and the Great Lakes Fishery Commission have published sampling guidance (Jerde et al., 2021), while the Australian government and CSIRO have released protocol development guides stressing replication, controls, and validation (De Brauwer et al., 2022).

Pawlowski et al. (2020) pushed for clearer terminology, distinguishing environmental DNA (from substrates), extracellular DNA (cell-free), and intracellular DNA (from intact cells). That kind of precision matters when comparing studies that used different methods.

Standard practice now includes: (1) field replication and spatially explicit designs; (2) negative controls at every stage; (3) positive controls with known DNA concentrations; (4) inhibition testing by spiking samples; (5) primer specificity testing against local non-target species; and (6) bioinformatic filtering to drop low-quality reads, chimeras, and contaminants (Goldberg et al., 2016).

Recent Advances, Research Gaps, and Future Prospects

1 Technological Advances

Technology in this field is moving quickly. Long-read sequencing (PacBio, Oxford Nanopore) can sequence full-length barcodes, improving taxonomic resolution beyond what short-read platforms manage. Portable nanopore sequencers allow real-time eDNA analysis in the field, cutting the delay between sampling and results.

Digital PCR platforms are maturing, with new applications in absolute quantification and better performance in inhibitory matrices (Guri et al., 2024). Automated samplers—including continuous water samplers and environmental sample processors on buoys or autonomous vehicles—are extending how far and how long eDNA programs can monitor.

2 Integration with Other Monitoring Technologies

eDNA is increasingly being paired with other tools. Combining metabarcoding with autonomous underwater vehicles, acoustic telemetry, camera traps, and remote sensing creates richer, multi-layered biodiversity data (Allen et al., 2024). In forest carbon markets, eDNA biodiversity monitoring is being tested as a way to verify conservation co-benefits with standardized, auditable data—something conventional surveys struggle to deliver (Allen et al., 2024).

3 Persistent Research Gaps

For all the progress, some basic questions remain unanswered. The link between eDNA concentration and organism abundance is still poorly quantified for most taxa and ecosystems, which limits eDNA's use for population assessment. Most studies are correlational; experimental work is needed to establish how environmental conditions, shedding rates, and detection probability relate causally. Airborne eDNA ecology—how far it travels, how fast it degrades, how it varies seasonally—is barely understood. And most eDNA research has been done in temperate, developed countries, creating geographic and taxonomic biases that restrict global applicability (Allen et al., 2024).

4 Future Prospects

Looking ahead, several trends are likely to shape eDNA's trajectory. Reference databases will keep expanding through coordinated barcoding initiatives, improving taxonomic assignment. Machine learning is being applied to quality control, anomaly detection, and predictive modeling. Sequencing costs are dropping and equipment is shrinking, which should make eDNA accessible in resource-limited settings. And regulators are beginning to accept eDNA data in environmental impact assessments, endangered species consultations, and invasive species management.

CONCLUSION

Environmental DNA analysis has moved from an experimental technique to a standard part of the ecological monitoring toolkit. Its ability to detect species sensitively, non-invasively, and across broad taxonomic groups makes it genuinely useful for biodiversity assessment, endangered species monitoring, and invasive species management in freshwater, marine, and terrestrial systems. The underlying science is sound: organisms shed DNA, and modern molecular methods can recover and identify it with high sensitivity. But eDNA has real limitations. False positives, false negatives, contamination, degradation, primer bias, and incomplete databases all constrain what eDNA can reliably tell us. These constraints do not make eDNA useless—they define where it works best and what safeguards are needed. The best way forward is not to abandon conventional methods but to integrate eDNA with them strategically. eDNA is excellent at detection—establishing presence, screening for rare species, and characterizing communities. Conventional methods provide the quantitative, behavioral, and voucher-based data needed for population assessment and mechanistic understanding. Rigorous quality control, validation, and standardization are essential if eDNA is to reach its full potential in conservation decision-making. As databases grow, technologies improve, and protocols converge, eDNA will probably become as routine in ecological monitoring as binoculars and dip nets. Developing it responsibly and applying it carefully is one of the better opportunities we have to monitor and protect the biodiversity we still have left.

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