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Applying DNA barcoding and ecological DNA approaches for biodiversity monitoring in aquatic ecosystems

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Abstract. Molecular ecology is reshaping traditional perspectives on natural systems by integrating advanced molecular techniques into ecological research. This integration offers novel insights into long-standing ecological questions and supports the development of more effective conservation strategies and sustainable resource management. The advent of DNA barcoding, which enables precise species identification using short, standardised genomic regions, has significantly improved taxonomic resolution, particularly for taxa with ambiguous or unresolved classifications. In parallel, environmental DNA (eDNA) analysis is revolutionising biodiversity monitoring by facilitating the detection of organisms directly from environmental samples, without the need for physical specimen collection. Together, DNA barcoding and eDNA represent a powerful, non-invasive, and efficient toolkit for studying biodiversity, especially in aquatic ecosystems, where traditional survey methods are often constrained. This review synthesises recent advances in the application of these molecular approaches to aquatic biodiversity monitoring, with a focus on their underlying principles, practical applications, methodological challenges, and prospects.

Key words: DNA barcoding, eDNA, biodiversity monitoring, aquatic ecosystems.

Principles of DNA Barcoding

DNA barcoding is a rapid, non-invasive, and reliable method for species identification using short, standardised DNA sequences (Antil et al., 2023). It is based on sequencing a standardised genomic region that varies between species but is conserved within a species. In animals, the cytochrome c oxidase I (COI) gene is most commonly used; in plants - rbcL and matK; and in fungi - the internal transcribed spacer (ITS) region. These sequences are compared with curated reference databases such as the Barcode of Life Data Systems (BOLD) and GenBank for species identification (Meiklejohn et al., 2019). The standard workflow includes specimen collection, DNA extraction, amplification of the barcode region by PCR, sequencing, and comparison with reference databases (Fig. 1). This approach is particularly effective for identifying cryptic species, monitoring invasive species, and supporting taxonomic research. Limitations include varying taxonomic resolution, especially among closely related species, and gaps in existing reference libraries (Weigand et al., 2019). Rapid species identification through this method finds applications in forensic science, endangered species monitoring, and disease research. Although any nucleotide sequence capable of distinguishing species can serve as a DNA barcode, there is no universal marker for all organisms - a challenge, especially for plants, where single loci do not yield reproducible results. Unlike phylogenetic markers, barcode regions do not require mutational homology, which broadens their applicability. The effectiveness of a barcode locus depends on curated reference databases and robust statistical validation. Key criteria for optimal barcode regions are sufficient genetic diversity for species discrimination, conserved flanking sequences for universal primer design, and short length to facilitate PCR and work with degraded DNA (Antil et al., 2023). As with any analytical tool, DNA barcoding requires careful application, methodological rigour, and awareness of its limitations to ensure accuracy and universality.

Integrating modern sequencing technologies with high-coverage reference libraries significantly increases the accuracy, speed, and reliability of DNA barcoding. Besse et al. (2021) emphasise the critical importance of selecting taxon-specific barcode markers tailored to different taxonomic groups and discuss common methodological challenges such as PCR inhibition and sequence ambiguities. Their work highlights the need for well-curated reference databases for successful DNA barcoding. Unlike animal barcoding, where COI is widely accepted, the authors underscore the difficulty of finding a universal plant barcode due to genomic complexity. To overcome this limitation, combining chloroplast loci (such as matK and rbcL) with nuclear markers is recommended; adding the ITS region improves resolution for closely related species, subspecies distinction, and hybrid identification.

DeSalle & Goldstein (2019) stress the importance of combining DNA barcode data with morphological and ecological information for robust taxonomic conclusions. Riza et al. (2023) highlight the potential of integrating machine learning with DNA barcoding to improve taxonomic classification, especially in complex plant groups like legumes. Their study shows that the Pearson method is most effective for clustering ITS sequences in plants and helps resolve taxonomic ambiguities.

DNA barcoding is a valuable tool for species identification due to its speed, accuracy, and non-invasiveness, but definitive species assignment often requires integration with traditional taxonomic methods combining genetic, morphological, and ecological data. While DNA barcoding is especially effective for well-characterised taxa, it has limitations for poorly studied or novel groups. Both the advantages and limitations of this technology are critically reviewed by Krishnamurthy et al. (2012). They analyse challenges such as mitochondrial DNA inheritance patterns, introgression, recombination, heteroplasmy, and the presence of nuclear mitochondrial pseudogenes (numts), which can complicate accurate species identification and hinder efforts to conserve genetic diversity. Furthermore, the authors emphasise that genetic thresholds for species delimitation vary significantly among taxonomic groups and do not always correspond to ecologically or evolutionarily significant differences. They highlight the need for reliable reference databases, flexible analytical approaches, and integration of multiple complementary techniques to improve identification accuracy and reliability.

What is environmental DNA (eDNA)?

Environmental DNA (eDNA) is genetic material released by organisms into the environment through skin cells, mucus, faeces, gametes, and other biological materials. In aquatic ecosystems, eDNA can be collected directly from water samples, making it a non-invasive tool for biodiversity assessment (Huang et al., 2022). The standard work process involves water sample collection, filtration, DNA extraction, and amplification via targeted qPCR or broad-spectrum metabarcoding approaches (Ruppert et al., 2019) (Fig. 1). Metabarcoding uses universal primers and high-throughput sequencing to simultaneously identify multiple taxa from a single sample, often relying on the same barcode regions used in traditional DNA barcoding (Deiner et al., 2017; Taberlet et al., 2012).

Compared to traditional methods, eDNA can detect rare, elusive, or low-abundance species and enables community monitoring with less effort and cost (Deiner et al., 2017; Thomsen & Willerslev, 2015). However, challenges such as DNA degradation, contamination, and variable species detection thresholds require careful management (Barnes & Turner, 2016; Ficetola et al., 2016).

Over the past decade, methodological inconsistencies have posed major obstacles to reproducibility and comparability in environmental nucleic acid (eNA) research. Bunholi et al. (2023) note that the lack of standardised procedures leads to significant variations in key elements such as sample volume, filter types/sizes, DNA/RNA extraction methods, genetic marker selection, and bioinformatic tools. Limited coverage in eDNA /

eRNA reference databases also hampers accurate species identification and biodiversity analysis. A further challenge is the absence of detailed methodological descriptions, especially in eRNA studies, which compromises reproducibility. To advance eNA-based monitoring in aquatic environments, the authors advocate for improved reference databases and standardised, transparent protocols.

The article "What is environmental DNA?" by Power et al. (2023) provides an in-depth analysis of the physical composition and variability of eDNA in marine environments. Using serial filtration with different pore sizes, metabarcoding with universal/taxon-specific primers, and electron / confocal microscopy, the study reveals that eDNA is structurally diverse, comprising free DNA fragments, whole cells, tissue particles, whole organisms, and complex aggregates in microbial biofilms. Physical characteristics and particle size influence both DNA capture efficiency and detected biodiversity. Notably, larger-pore filters (e.g., 5 µm or 10 µm) often yield greater diversity for certain groups than commonly used 0.45 µm filters. Additionally, eDNA in biofilms serves as a significant genetic reservoir for diverse organisms. These findings challenge the widespread assumption that finer filters are universally optimal and suggest filter choice should align with target taxa and research objectives. Advances in DNA sequencing and expanded reference databases continue to enhance eDNA techniques' accuracy and scope, solidifying their role in biodiversity conservation and environmental monitoring (Ruppert et al., 2019). E-BIOM, for instance, is a European laboratory specialising in eDNA services to support biodiversity and ecological conservation (https://www.e-biom.com/).

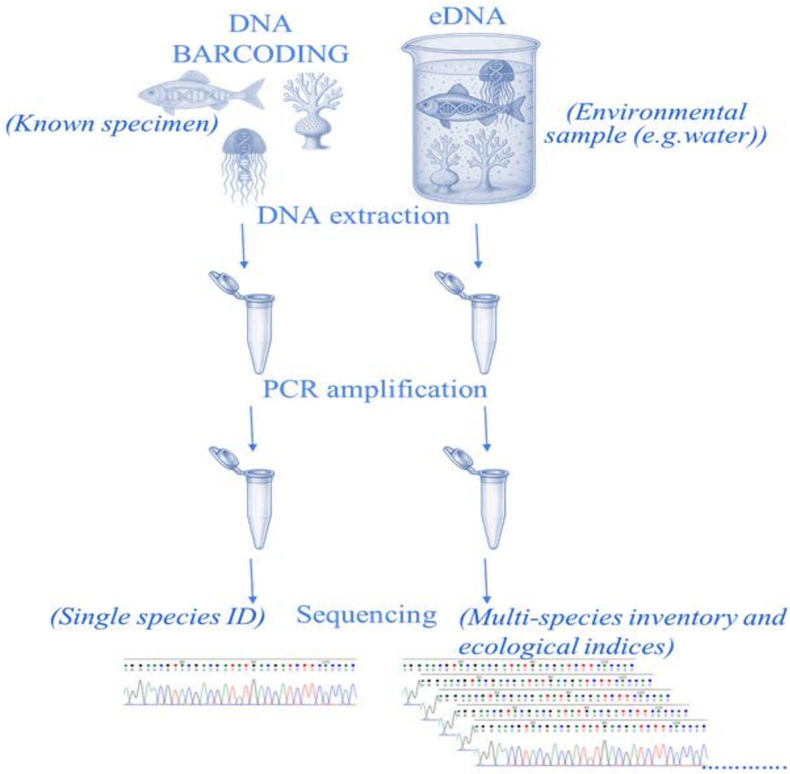

Fig. 1. Procedure for DNA barcoding and eDNA analysis.

Synergy between DNA barcoding and eDNA

DNA barcoding provides high taxonomic resolution through sequencing standardised, short genome fragments for precise species identification (Deiner et al., 2017; Beng & Corlett, 2020). Environmental DNA, in turn, enables non-invasive collection of genetic material from the environment (water, soil, sediment), facilitating the detection of multiple organisms, including rare and hard-to-access taxa (Thomsen & Willerslev, 2015; Beng & Corlett, 2020) (Table 1).

Table 1. Similarities and differences between DNA barcoding and eDNA analysis.

Step	DNA Barcoding Procedure	eDNA Barcoding Procedure	Similarities / Differences
Sample Collection	Collect tissue or specimen from a single organism (e.g., leaf, insect leg).	Collect environmental samples containing DNA traces (e.g., water, soil, air).	Both start with sample collection, but DNA barcoding uses individual specimens, while eDNA uses mixed environmental DNA.
DNA Extraction	Extract DNA from the collected specimen using kits or protocols.	Extract DNA from environmental samples, often requiring more sensitive protocols due to low DNA concentration.	Both require DNA extraction, but eDNA extraction is more challenging due to dilute and mixed DNA.
PCR Amplification	Amplify a specific barcode region (e.g., CO1 for animals) using primers targeting conserved flanking regions.	Amplify barcode regions from the mixed DNA sample using universal primers targeting the same barcode region.	Both use PCR targeting barcode regions; eDNA PCR may amplify multiple species simultaneously (metabarcoding).
Sequencing	Sequence the amplified barcode region from the single species.	Sequence the mixed PCR products, often using high-throughput sequencing for community analysis.	Both rely on sequencing, but eDNA often uses next-generation sequencing for multiple species.
Sequence Alignment	Align sequences (e.g., using software like MUSCLE or BIOEDIT) to compare and check quality before analysis.	Align sequences from multiple species obtained from eDNA sequencing to identify species present.	Both require sequence alignment to ensure sequences are homologous and comparable before identification.
Species Identification	Compare aligned sequences against reference databases (e.g., BOLD, GenBank) to identify species.	Compare aligned sequences against databases to identify species in the environmental sample.	Both use reference databases for species identification.
Data Management	Store specimen metadata and sequence data in databases like BOLD with voucher specimen linkage.	Store sequence data and environmental metadata, often without physical voucher specimens.	DNA barcoding links to physical specimens; eDNA barcoding usually lacks physical vouchers.

The integration of these two approaches combines their strengths: the specificity and accuracy of DNA barcoding with the broad, non-invasive scope of eDNA analysis. eDNA metabarcoding uses DNA barcoding principles to analyse mixed samples, simultaneously detecting multiple species by comparing sequences to reference libraries created through classical barcoding (Deiner et al., 2017; Porter et al., 2018). The effecttiveness of this approach depends on the completeness and quality of reference databases—without well-curated databases, taxonomic identification of eDNA sequences is limited and may lead to false negatives or ambiguous results.

This synergy is particularly evident in studies combining both methods. For example, coupling specimen barcoding with eDNA metabarcoding provides complementary information on species distribution and diversity. While specimen collection and barcoding yield high-confidence identifications and populate reference libraries, eDNA metabarcoding reveals broader community structure and detects hard-to-access or rare taxa that may be missed by traditional sampling (Porter et al., 2018).

Moreover, eDNA metabarcoding often demonstrates higher sensitivity in detecting species diversity compared to conventional methods, especially in marine and terrestrial environments where sampling is difficult or destructive. However, the reliability of identifications depends on the availability of barcode sequences for relevant organisms in the studied environment, underscoring the importance of ongoing barcoding efforts (Stat et al., 2017; Weigand et al., 2019).

The synergy between DNA barcoding and eDNA is expressed through their mutual complementarity: barcoding builds the reference framework for accurate eDNA analyses, while eDNA expands the scale and scope of biodiversity monitoring. Together, they enable more efficient, comprehensive, and non-invasive assessment of ecosystem health, species distribution, and community dynamics, crucial for conservation and natural resource management.

Applications in aquatic biodiversity monitoring

eDNA analysis is emerging as a transformative tool for rapid and comprehensive biodiversity monitoring in aquatic ecosystems. eDNA metabarcoding effectively captures both vertebrate and invertebrate communities, including rare and endangered species, and reflects habitat-specific community composition (Chang et al., 2025). In marine settings, eDNA analyses have successfully characterised fish assemblages at regional scales, revealing biodiversity patterns shaped by habitat. Applications in freshwater environments are particularly promising for monitoring threatened species and assessing ecological impacts, such as detecting community changes following environmental disturbances. Compared to traditional methods, eDNA techniques offer greater efficiency, non-invasiveness, and sensitivity, enabling the detection of cryptic species with minimal sampling effort. In both marine and freshwater ecosystems, eDNA has been successfully used for early detection of invasive species, mapping the distribution of threatened taxa, and tracking seasonal changes in biodiversity. In some cases, eDNA analysis has demonstrated higher sensitivity and detected more species than traditional techniques such as electrofishing or trawling (Rees et al., 2014).

Recent research demonstrates that high-throughput sequencing of eDNA from seawater samples has successfully characterised vertebrate communities across diverse marine habitats, revealing habitat-specific species compositions and detecting rare or endangered species with high sensitivity (Thomsen et al., 2016). In freshwater systems, targeted qPCR assays have shown linear relationships between eDNA concentration and species biomass, and mesocosm experiments confirm that eDNA degrades within one to two weeks after organism removal, supporting its use for detecting recent species presence (Cantera et al., 2019). Innovations such as low-cost filtration systems and genome skimming for reference database development further enhance scalability and taxonomic resolution. Species-specific assays have improved detection of invasive, cryptic, or endangered taxa, as shown by Droplet Digital PCR assays for invasive fish in New Zealand lakes (Picard et al., 2023) and qPCR protocols for Asian paddle crab and common musk turtle (Westfall et al., 2021; Davy et al., 2015). However, challenges persist in turbid waters or low-abundance contexts, where inhibitor management and replication are critical (Harrison et al., 2019).

Cortez et al. (2025) employed eDNA metabarcoding in an 800 km² reservoir, identifying 29 previously unreported species and advocating for eDNA as a robust and cost-effective tool. Xie et al. (2021) applied eDNA to study zooplankton and fish communities in dynamic freshwater ecosystems, emphasising habitat heterogeneity. Macher et al. (2024) compared eDNA and eRNA signals in river systems to infer species habitat preferences, noting eRNA's localised signal due to faster degradation. This aligns with the concept of eRNA reflecting active processes but focuses on freshwater habitats, not coastal marine zones.

Bista et al. (2022) conducted an annual time-series analysis of aqueous eDNA, revealing ecologically relevant dynamics of lake ecosystem biodiversity. Thomsen & Willerslev (2015) provided a foundational review outlining the state of the field, methodological considerations, and the strengths of eDNA, including non-invasive detection and sensitivity to rare or elusive species. Cerrillo-Espinosa et al. (2025) demonstrated the use of eDNA metabarcoding in marine biodiversity hotspots, revealing distinct community structures across depth gradients.

Xie et al. (2021) applied eDNA to study zooplankton and fish communities in dynamic freshwater ecosystems, emphasising the role of habitat heterogeneity. Macher et al. (2024) compared eDNA and eRNA signals in river systems, noting that eRNA provides a more localised signal due to faster degradation, which is useful for inferring active biological processes. Future advancements should prioritise methodological refinements, expanded reference databases, and machine learning applications to unlock the full potential of eDNA for guiding conservation strategies and ecosystem-based management (Chang et al., 2025).

Species-specific assays improve the detection of invasive, cryptic, or endangered taxa. ddPCR assays for invasive fish (*Perca fluviatilis, Scardinius erythrophthalmus*) in New Zealand lakes optimised sampling design (Picard et al., 2023), while qPCR protocols for Asian paddle crab (*Charybdis japonica*) and common musk turtle (*Sternotherus odoratus*) achieved high specificity for biosecurity and distribution mapping (Westfall et al., 2021; Davy et al., 2015). Methodological refinements - such as optimised filtration, targeted assays, and machine learning integration—enhance detection accuracy for conservation and management (Ficetola et al., 2019). Future efforts should prioritise standardised protocols, eRNA applications, and stakeholder engagement to maximise ecological insights and policy impact (Bohmann et al., 2022; Rourke et al., 2022).

Despite its advantages, eDNA monitoring also has limitations. These include difficulties in quantitatively estimating species abundance, the potential for false positive or negative results, DNA degradation in aquatic environments, sample contamination, and a lack of standardised protocols. Standardisation of sampling, extraction, and bioinformatics is essential for comparability across studies (Beng & Corlett, 2020). Challenges remain in turbid waters or low-abundance contexts, where inhibitor management and replication are critical (Harrison et al., 2019). Thomsen & Willerslev's (2015) address key methodological considerations - sampling design, DNA degradation, marker choice, primer bias, and taxonomic assignment. Future progress should prioritise methodological improvements, expanded reference databases, and machine learning applications to unlock the full potential of eDNA for guiding conservation strategies and ecosystem-based management (Chang et al., 2025).

Additionally, the dynamics of eDNA are influenced by factors such as temperature, light, microbial activity, and hydrology, which can complicate the interpretation of results over time and space. Despite these challenges, technological advances - including high-throughput sequencing, portable sequencers, real-time analysis capabilities, and the integration of artificial intelligence and remote sensing - are greatly expanding the possibilities for eDNA monitoring. Improving accuracy and reproducibility will require the development of standardised protocols, expansion of genetic reference databases, and the promotion of international collaboration (Deiner et al., 2017). Despite current limitations, eDNA technology offers significant potential for effective, accurate, and large-scale monitoring of aquatic biodiversity and ecosystem health. It is expected to play a key role in addressing global challenges such as biodiversity loss and climate change (Ruppert et al., 2019). Ethical frameworks for data ownership and equitable implementation must also evolve alongside technological adoption (Pochon et al., 2017).

Conclusions

The synergy between DNA barcoding and eDNA represents a significant advancement in biodiversity monitoring and conservation. Combining the highly specific and reliable species identification offered by DNA barcoding with the broad, non-invasive scope of eDNA analysis enables more efficient, comprehensive, and sensitive detection of biological diversity across ecosystems, particularly in aquatic environments. This integration facilitates not only the detection of rare, elusive, or endangered species but also the tracking of whole-community dynamics, the early detection of invasive taxa, and the assessment of ecosystem health.

However, realising the full potential of these technologies requires overcoming several challenges: standardizing protocols, expanding and curating genetic reference databases, minimizing false positive/negative results, and better understanding ecological/technical factors influencing eDNA dynamics. Contemporary technological innovations—including high-throughput sequencing, portable sequencers, artificial intelligence integration, and remote sensing—further enhance the accuracy and applicability of these methods.

In conclusion, integrating DNA barcoding and eDNA analysis establishes a new standard for biodiversity monitoring and natural resource management. This synergy will play an increasingly pivotal role in global efforts to conserve biodiversity, adapt to climate change, and promote sustainable ecosystem management.

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