

Environmental DNA (eDNA) for Monitoring Aquatic Biodiversity: Methods, Applications, Challenges and Conservation Implications

Dr. C. Jyothsna

Associate Professor, Department of Zoology, Nagarjuna Government College (A), Nalgonda, Telangana

ABSTRACT

Environmental DNA (eDNA) is a novel molecular tool for assessing aquatic biodiversity. It is the genetic material that organisms release into the environment around them through their skin cells, mucus, feces, urine, gametes and other biological materials. In aquatic ecosystems, this DNA can be extracted from water samples and analyzed using molecular techniques to identify species without the need to capture, observe or disturb the organisms. Traditional approaches to monitoring biodiversity (e.g., netting, electrofishing, visual surveys, and trapping) can be laborious, costly, and ineffective for detecting rare, elusive, nocturnal, or low-abundance species. Other methods for detecting aquatic organisms such as fish, amphibians, aquatic invertebrates and invasive species include environmental DNA metabarcoding and species-specific quantitative polymerase chain reaction (qPCR). This review addresses the basic principles of eDNA analysis, the sampling protocols, laboratory procedures, applications, benefits and limitations and the implications for conservation. It also looks at the role of eDNA in freshwater, riverine, wetland, estuarine, and marine environments. Contamination control, degradation of DNA, water movement, completeness of reference databases and interpretation of species detection results are all given much attention. The article concludes that eDNA can be a valuable complementary tool to assess aquatic biodiversity if sampling designs, laboratory procedures, and statistical interpretation are carefully standardized.

Keywords: Environmental DNA, aquatic biodiversity, DNA metabarcoding, molecular ecology, qPCR, freshwater ecosystems, marine biodiversity, invasive species, conservation biology.

INTRODUCTION

Aquatic ecosystems support a wide range of organisms and provide essential ecological services, including nutrient cycling, water purification, fisheries production, and maintenance of food webs. Rivers, lakes, ponds, reservoirs, wetlands, estuaries, and oceans contain diverse communities of fish, amphibians, crustaceans, molluscs, plankton, and microorganisms. However, these ecosystems are increasingly affected by habitat degradation, pollution, overexploitation, invasive species, climate change, and changes in water flow.

Reliable biodiversity assessment is necessary to understand ecological changes and develop effective conservation strategies. Traditional survey techniques remain important, but they can have limitations. Some species are difficult to capture or identify, while others occur at low densities or inhabit inaccessible locations. Repeated field surveys can also require considerable time, trained personnel, and financial resources.

Environmental DNA offers an additional method for detecting organisms through genetic traces present in water. By collecting and analysing water samples, researchers can obtain evidence of species presence without directly observing or capturing the organisms. The development of DNA sequencing technologies has further expanded the use of eDNA for detecting multiple species simultaneously.

The application of eDNA is particularly relevant to biodiversity monitoring because it can improve the detection of rare and elusive species, facilitate the early identification of invasive organisms, and support long-term ecological surveillance. Nevertheless, the method requires careful interpretation because DNA detection does not always establish the exact location, abundance, reproductive status, or viability of an organism.

This article reviews the scientific principles, methods, applications, and limitations of eDNA-based aquatic biodiversity monitoring, with emphasis on its role in zoological research and conservation management.

Objectives of the Study

The major objectives of this review are:

1. To explain the concept and scientific principles of environmental DNA.
2. To examine the applications of eDNA in monitoring aquatic biodiversity.
3. To compare eDNA-based approaches with conventional biodiversity-survey methods.
4. To discuss the relevance of eDNA for aquatic conservation, particularly in freshwater ecosystems and Indian biodiversity management.
5. To suggest future research directions for improving eDNA-based biodiversity assessment.

RESEARCH METHODOLOGY

This article adopts a narrative review methodology based on established scientific literature concerning environmental DNA, molecular ecology, aquatic biodiversity, and conservation biology.

The review synthesises published research on eDNA sampling, DNA extraction, molecular identification, metabarcoding, species-specific detection, and ecological interpretation. Foundational studies and methodological papers are used to explain the development and applications of the technique.

The article does not present newly collected field samples, original laboratory experiments, or primary statistical results. Accordingly, tables and figures are provided as literature-based comparisons and conceptual illustrations rather than as original experimental findings.

A systematic review would require a documented database search, explicit inclusion and exclusion criteria, and a reproducible screening procedure. Those procedures have not been claimed for this narrative review.

Concept and Scientific Principles of Environmental DNA

Definition of eDNA: Environmental DNA is genetic material recovered directly from environmental samples rather than from an organism collected specifically for DNA analysis. In aquatic ecosystems, this material may occur in suspended cells, mucus, scales, faeces, gametes, tissue fragments, and extracellular DNA. When an organism enters a water body, it releases biological material into the surrounding environment. DNA can then be collected from the water, extracted in a laboratory, and analysed to identify the organisms associated with the sample.

The foundational studies by Ficetola et al. (2008) demonstrated the use of environmental DNA for detecting an invasive amphibian, establishing an important application of this approach in ecological monitoring.

Sources of eDNA in aquatic ecosystems:

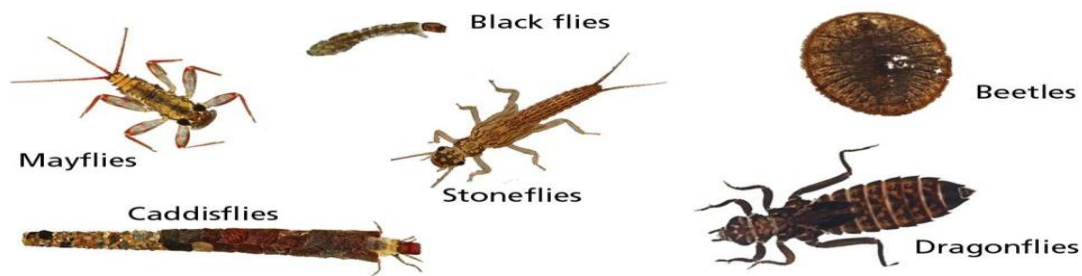


Fish and other vertebrates: Fish release DNA through mucus, scales, faeces, urine, reproductive products, and damaged cells. These traces can help researchers detect fish species in rivers, lakes, reservoirs, and coastal waters.

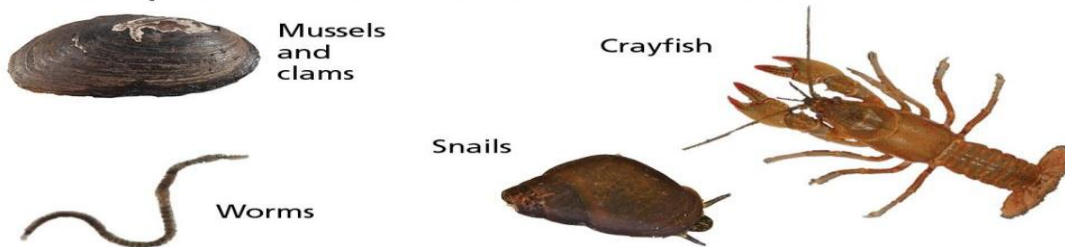


Amphibians: Frogs, salamanders, and other amphibians release DNA from skin cells, mucus, eggs, and other biological materials. eDNA can help detect species that are difficult to observe directly.

Examples of insect macroinvertebrate larvae



Examples of non-insect macroinvertebrates



Aquatic invertebrates: DNA from insect larvae, molluscs, crustaceans, and other invertebrates can enter the water through cells, excretion, and tissue fragments. Detection depends on the sampling method and molecular markers used.

and movement of eDNA

After release, DNA may remain inside cells or become associated with suspended particles and sediments. Its persistence depends on environmental conditions, including temperature, ultraviolet radiation, microbial activity, pH, water chemistry, and particle interactions.

Water currents can transport DNA away from its source. Consequently, a positive result may indicate that an organism is present nearby or upstream, or that its DNA was transported from another location.

Scientific implication: eDNA detection provides evidence of genetic material in a sample, but it does not automatically prove that a living organism is currently present at the exact sampling point.

Major Techniques Used in eDNA Analysis

Three major molecular approaches are used in aquatic biodiversity monitoring.

Species-specific quantitative PCR (qPCR): Quantitative polymerase chain reaction detects DNA from a selected target species using specific primers and a fluorescent probe or another detection chemistry. It is particularly useful when researchers want to determine whether a rare, threatened, or invasive species occurs in a water body. For example, a monitoring programme may develop a validated qPCR assay to detect a particular invasive fish in a reservoir.

Advantages: High sensitivity, targeted detection, and the possibility of estimating target DNA concentration.

Limitations: It usually detects only the selected species and cannot independently provide a complete inventory of the aquatic community.

Digital PCR (dPCR): Digital PCR partitions a sample into many individual reactions and estimates the quantity of target DNA from the number of positive and negative partitions. It can be useful when target DNA concentrations are low or when precise quantification is required. However, assay design, inhibition, contamination, and sample quality remain important considerations.

DNA Metabarcoding: DNA metabarcoding uses broad-range primers to amplify selected genetic markers from a mixed environmental sample. High-throughput sequencing then generates many DNA sequences, which are compared with reference databases to identify multiple taxa. Common markers include mitochondrial 12S rRNA for many vertebrates, cytochrome c oxidase subunit I (COI) for many animals, and selected 16S rRNA or other markers for particular taxonomic groups.

Metabarcoding is useful for community-level biodiversity assessment because a single sample may contain DNA from numerous species.

It does not necessarily identify every species in a community. Detection depends on primer performance, DNA quality, reference-sequence coverage, and bioinformatic procedures (Zhang et al., 2023; Takahashi et al., 2023).

Applications of eDNA in Aquatic Biodiversity Monitoring

Assessment of fish diversity: Fish diversity can be difficult to assess in deep lakes, large rivers, turbid waters, and habitats with complex vegetation. Conventional netting and electrofishing may not detect every species, especially those occurring at low densities. eDNA metabarcoding can provide supplementary information about fish-community composition. Repeated surveys can help document changes in species detections across seasons or between impacted and relatively undisturbed sites. However, differences in DNA release, primer efficiency, and water movement mean that sequence counts should not be interpreted directly as fish abundance.

Monitoring threatened and endangered species: Rare or threatened aquatic species may be difficult to detect through conventional surveys. A validated species-specific eDNA assay can support surveys of their potential habitats. This approach may reduce disturbance during monitoring, especially where capture is difficult or undesirable. Nevertheless, a negative result does not conclusively establish that a species is absent.

Detection of invasive species: Early detection of non-native species is important because established invasive populations may be difficult or expensive to control. eDNA can be used to screen reservoirs, lakes, canals, and rivers for target invasive organisms. Positive detections can trigger confirmatory sampling, targeted field surveys, or management assessments. False-positive detections must be considered carefully because they could lead to unnecessary control measures.

Monitoring aquatic macroinvertebrates: Aquatic macroinvertebrates, including insect larvae, snails, mussels, and crustaceans, contribute to nutrient cycling and food-web functioning. Their community composition is also used in ecological assessments of freshwater quality. Metabarcoding can complement conventional macroinvertebrate identification. However, a review of riverine macroinvertebrate monitoring found that eDNA metabarcoding did not consistently detect as many taxa as morphological methods. This supports the use of combined approaches rather than assuming that eDNA alone captures all invertebrate diversity.

Assessment of ecological change and pollution: Researchers can compare eDNA-based species detections across locations exposed to different environmental pressures, such as wastewater discharge, agricultural runoff, habitat alteration, or industrial pollution. Changes in detected community composition may indicate ecological differences that warrant further investigation. However, eDNA species detection alone is not a direct measure of pollution concentration or ecosystem health. It should be combined with water-quality measurements and ecological indicators.

Marine and estuarine biodiversity: In marine and estuarine environments, eDNA can support monitoring of fish, plankton, benthic organisms, and other aquatic taxa. It is particularly useful for repeated sampling across habitats that are difficult to survey directly. Interpretation can be complicated by tides, currents, dilution, and the transport of DNA across habitat boundaries. Combining water sampling with conventional observations and habitat data can improve ecological interpretation.

Tables for Comparative Analysis

Comparison of major biodiversity-monitoring methods

Parameter	Traditional surveys	eDNA qPCR	eDNA metabarcoding
Primary purpose	Direct observation or collection	Detect a target species	Detect multiple taxa
Organism capture	Often required	Usually not required	Usually not required
Rare-species detection	May be difficult	Often sensitive for validated targets	Can detect rare taxa if assay and sampling are suitable
Community assessment	Yes	Limited to selected targets	Yes, subject to marker coverage
Abundance estimation	Possible with suitable survey design	Target DNA quantity, not necessarily organism abundance	Read counts are not direct abundance estimates
Species identification	Morphological identification	Target-specific identification	Reference-based sequence identification
Main limitation	Effort, accessibility and detectability	Requires a target-specific assay	Primer bias, reference gaps and analytical complexity

Source: Author's synthesis based on Deiner et al. (2017), Goldberg et al. (2016), and Takahashi et al. (2023).

Major applications of eDNA in aquatic ecosystems

Application	Target organisms or indicators	Potential contribution	Important limitation
Fish biodiversity	Freshwater and marine fishes	Community inventory and distribution screening	DNA transport may obscure the exact location
Threatened species	Rare fish and amphibians	Non-invasive detection	Negative results may reflect imperfect detection
Invasive-species surveillance	Non-native fish, molluscs and other taxa	Early warning and targeted follow-up	Contamination may cause false alarms
Macroinvertebrate assessment	Aquatic insects, molluscs and crustaceans	Supplementary community assessment	Some taxa may be missed by primers
Ecological monitoring	Multiple aquatic taxa	Comparison of communities across sites	Detection differences may reflect sampling or laboratory effects
Marine biodiversity	Fish, plankton and benthic organisms	Repeated surveys in complex habitats	Tides and currents transport DNA
Restoration monitoring	Habitat-associated communities	Track changes following restoration	DNA detection alone cannot establish restoration success

Source: Author's synthesis from Zhang et al. (2023), Takahashi et al. (2023)

Factors Affecting the Accuracy of eDNA-Based Monitoring

Water temperature: Temperature influences DNA degradation and microbial activity. DNA may persist for different periods under different temperature conditions, so samples collected in different seasons may not be directly comparable without considering these effects.

Water flow and hydrological conditions: In flowing rivers, DNA may travel downstream from its original source. Floods, heavy rainfall, and changes in discharge can redistribute DNA and alter its concentration. In standing water, local circulation, sediment disturbance, and water-column mixing can influence the distribution of DNA.

DNA degradation: Ultraviolet radiation, microbial enzymes, chemical conditions, and other environmental processes can break down DNA. Degraded DNA may not contain an intact target region suitable for amplification.

Sampling effort: The number of sampling locations, replicate samples, water volume, sampling depth, and timing influence the probability of detection. A single negative sample may be insufficient to establish species absence. Repeated samples and repeated surveys can improve detection reliability.

Primer specificity and reference databases: Molecular primers may amplify some taxa more effectively than others. Reference databases may also lack sequences for locally occurring species. These limitations can result in missed species or identification only at a higher taxonomic level. Researchers should document the marker, primers, reference database, sequence-assignment thresholds, and quality-control procedures.

False positives and false negatives: A false positive occurs when a method reports a target that is not genuinely represented in the intended sample. Contamination, misidentification, and transported DNA can contribute to misleading interpretations. A false negative occurs when the target is present but is not detected. Low DNA concentration, poor sampling, DNA degradation, PCR inhibition, or primer mismatch can contribute to this outcome. Goldberg et al. (2016) and subsequent reviews emphasise the importance of validating the entire eDNA workflow and accounting for uncertainty in ecological interpretation.

Future Prospects of eDNA Research

Integration with conventional methods: Combining eDNA with visual surveys, nets, electrofishing, acoustic methods, and morphological identification can provide complementary evidence. This is particularly important for groups that may be poorly represented in existing molecular databases or inadequately detected by particular primers.

Automated and high-frequency sampling: Automated samplers and field-based molecular instruments may allow more frequent monitoring and quicker detection of selected species. Such systems could be useful for surveillance at drinking-water reservoirs, conservation sites, and locations where invasive species pose a concern.

Improved reference databases: Regional barcode libraries covering native fish, amphibians, molluscs, aquatic insects, and other organisms would improve species identification. Voucher specimens and verified taxonomic records are important for constructing reliable reference datasets.

Statistical models for imperfect detection: Occupancy models and related statistical methods can account for the possibility that a species is present but goes undetected during sampling. Such methods are particularly relevant when comparing sites, seasons, or management interventions.

Integration with ecological and environmental data: Combining eDNA data with water temperature, pH, dissolved oxygen, turbidity, nutrient concentrations, habitat information, and hydrological measurements could help researchers investigate relationships between aquatic communities and environmental conditions.

Standardisation and open data: Consistent reporting of sampling protocols, controls, laboratory procedures, sequence-processing steps, and metadata will improve reproducibility and comparisons among studies. Publishing sequence data and analysis workflows in accessible repositories can also support future research. Recent reviews identify method standardisation, improved reference databases, and integration with complementary monitoring approaches as continuing priorities in aquatic eDNA research.

DISCUSSION

Environmental DNA represents an important development in molecular ecology because it enables researchers to investigate aquatic biodiversity using genetic material collected directly from the environment. Its major contribution is the ability to detect organisms without requiring their direct capture or observation in every instance. The reviewed literature indicates that eDNA has applications in fish-community assessment, rare-species detection, invasive-species surveillance, and aquatic ecosystem monitoring. DNA metabarcoding is particularly useful when the research objective is to investigate

multiple taxa simultaneously, whereas qPCR and digital PCR are more appropriate for questions involving selected target species.

However, the reliability of eDNA results depends on the complete analytical process. Sampling design, water conditions, filtration, DNA preservation, molecular markers, laboratory contamination, and reference-database coverage all influence the final outcome. An important distinction is that species detection, population abundance, and ecosystem health are different ecological measurements. Detecting a species' DNA does not necessarily establish the number of individuals present or demonstrate that an ecosystem is healthy. Likewise, the absence of a detection does not prove the absence of the species. The 2023 review by Takahashi et al. and the 2024 review by Poyntz-Wright et al. highlight the need to assess methodological suitability for different taxonomic groups and to avoid treating eDNA as a universally complete replacement for conventional monitoring. For zoological research, eDNA is therefore most useful when the research question is clearly defined and the molecular results are interpreted alongside field observations, habitat information, and appropriate quality-control procedures.

Major Findings of the Review

The principal findings from the reviewed literature are summarised below.

- ❖ Environmental DNA provides a non-invasive or minimally invasive approach to detecting aquatic organisms.
- ❖ Species-specific qPCR and digital PCR are useful for targeted surveillance, whereas metabarcoding can support community-level biodiversity assessment.
- ❖ eDNA may improve the detection of rare, elusive, and invasive species, subject to assay sensitivity and sampling conditions.
- ❖ Environmental conditions and water movement affect DNA persistence, distribution, and interpretation.
- ❖ Contamination, incomplete reference databases, primer bias, and imperfect detection remain important methodological challenges.
- ❖ DNA sequence-read counts cannot automatically be interpreted as organism abundance.
- ❖ Combining eDNA with conventional ecological surveys can provide complementary evidence and improve biodiversity assessment.
- ❖ Standardised protocols, regional reference libraries, adequate replication, and transparent data reporting are important for reproducible results.

These findings represent a synthesis of published literature, not original experimental results obtained from a new field investigation.

Suggestions and Recommendations

Based on the review, the following recommendations are proposed for researchers and biodiversity-management authorities.

- ❖ Develop standardised protocols: Establish consistent procedures for sampling, filtration, preservation, extraction, and molecular analysis.
- ❖ Increase spatial and temporal replication: Sample multiple locations and seasons to account for variation in DNA detection.
- ❖ Strengthen quality assurance: Use field blanks, extraction blanks, PCR controls, and replicate analyses.
- ❖ Improve regional reference libraries: Prioritise molecular reference sequences for native and threatened aquatic species.
- ❖ Combine molecular and conventional surveys: Use morphological identification and direct observations to validate and complement eDNA findings.
- ❖ Avoid unsupported abundance estimates: Interpret DNA concentration and sequencing reads cautiously and validate relationships with organism abundance where required.
- ❖ Promote Indian aquatic eDNA research: Develop pilot studies in rivers, reservoirs, wetlands, and estuaries, including selected sites in Telangana.
- ❖ Support conservation decisions with multiple indicators: Integrate eDNA detections with habitat assessments, water-quality measurements, and established biodiversity indicators.

CONCLUSION

Environmental DNA is a valuable molecular tool for monitoring aquatic biodiversity and supporting conservation research. By analysing genetic material present in water, researchers can detect aquatic organisms and investigate community composition without depending exclusively on direct capture or visual observation. The approach offers considerable

potential for freshwater, riverine, wetland, estuarine, and marine research. It can contribute to the monitoring of fish diversity, threatened species, invasive organisms, and changes in aquatic communities. Its sensitivity and capacity for repeated sampling make it particularly useful for research in habitats where conventional surveys are difficult. Nevertheless, the reliability of eDNA-based monitoring depends on appropriate sampling designs, contamination control, validated molecular assays, suitable reference databases, and careful interpretation of DNA transport and persistence. It should not be treated as a complete substitute for conventional ecological surveys. Future research should focus on methodological standardisation, regional reference libraries, improved statistical approaches, and integration with traditional biodiversity assessment. In India, carefully designed eDNA studies in the Godavari and Krishna river basins, reservoirs, lakes, wetlands, and coastal ecosystems could help expand knowledge of aquatic species distributions and support evidence-based conservation planning. Overall, eDNA should be regarded as a complementary molecular approach that strengthens aquatic biodiversity monitoring when used alongside established ecological methods.

REFERENCES

The following references provide a foundation for the article and further reading. The journal details and DOI links are included where available.

1. Deiner, K., Bik, H. M., Mächler, E., et al. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, 26(21), 5872–5895. <https://doi.org/10.1111/mec.14350>
2. Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4(4), 423–425. <https://doi.org/10.1098/rsbl.2008.0118>
3. Goldberg, C. S., Turner, C. R., Deiner, K., et al. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution*, 7(11), 1299–1307. <https://doi.org/10.1111/2041-210X.12595>
4. Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270(1512), 313–321. <https://doi.org/10.1098/rspb.2002.2218>
5. Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. *PLOS ONE*, 9(1), e86175. <https://doi.org/10.1371/journal.pone.0086175>
6. Roussel, J. M., Paillisson, J. M., Tréguier, A., & Petit, E. (2015). The downside of eDNA as a survey tool in water bodies. *Journal of Applied Ecology*, 52(4), 823–826. <https://doi.org/10.1111/1365-2664.12428>
7. Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. *PLOS ONE*, 7(4), e35868. <https://doi.org/10.1371/journal.pone.0035868>
8. Thomsen, P. F., Kielgast, J., Iversen, L. L., et al. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, 21(11), 2565–2573. <https://doi.org/10.1111/j.1365-294X.2011.05418.x>
9. Yoccoz, N. G. (2012). The future of environmental DNA in ecology. *Molecular Ecology*, 21(8), 2031–2038. <https://doi.org/10.1111/j.1365-294X.2012.05505.x>
10. MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, J. A., & Langtimm, C. A. (2002). Estimating site occupancy rates when detection probabilities are less than one. *Ecology*, 83(8), 2248–2255. [https://doi.org/10.1890/0012-9658\(2002\)083%5B2248:ESORWD%5D2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083%5B2248:ESORWD%5D2.0.CO;2)