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Environmental DNA (eDNA): A revolutionary tool for biodiversity assessment and ecosystem monitoring

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Abstract
Environmental DNA (eDNA) has emerged as a transformative molecular approach for biodiversity assessment and ecosystem monitoring, offering a sensitive, non-invasive, and cost-effective alternative to conventional biological surveys. Organisms continuously release genetic material into their surroundings through skin cells, mucus, feces, urine, saliva, reproductive cells, and decomposing tissues. This DNA can be recovered from environmental samples such as water, soil, sediment, snow, and air, enabling researchers to detect species without direct observation or capture. Recent advancements in polymerase chain reaction (PCR), quantitative PCR (qPCR), next-generation sequencing (NGS), metabarcoding, and bioinformatics have significantly enhanced the accuracy and efficiency of eDNA-based monitoring. The technology has found extensive applications in biodiversity assessment, aquatic and terrestrial ecosystem monitoring, conservation of endangered species, detection of invasive organisms, fisheries management, wildlife conservation, environmental impact assessment, and disease surveillance. Compared with traditional survey methods, eDNA requires less field effort, minimizes habitat disturbance, and provides rapid detection of rare, elusive, or cryptic species. Nevertheless, several challenges remain, including DNA degradation, environmental contamination, PCR inhibition, incomplete reference databases, and the need for standardized sampling protocols. Continued improvements in sequencing technologies, computational biology, and international reference databases are expected to further strengthen the reliability of eDNA analyses. This review summarizes the historical development, fundamental principles, sources, analytical workflow, major applications, advantages, and limitations of environmental DNA, highlighting its growing importance as a revolutionary tool for biodiversity conservation and sustainable ecosystem management.

Keywords: Environmental DNA (eDNA), biodiversity assessment, ecosystem monitoring, conservation biology, molecular ecology, metabarcoding; next-generation sequencing; bioinformatics

Introduction

Biodiversity is fundamental to ecosystem stability, ecological resilience, and the provision of ecosystem services that sustain human societies. Accurate assessment of biological diversity is therefore essential for understanding ecosystem dynamics, evaluating environmental change, and developing effective conservation strategies. Traditionally, biodiversity monitoring has relied on field surveys involving direct observation, specimen collection, trapping, and morphological identification. Although these methods have provided valuable ecological information, they are often labor-intensive, time-consuming, expensive, and dependent on taxonomic expertise. Moreover, many organisms are rare, cryptic, nocturnal, or inhabit inaccessible environments, making them difficult to detect using conventional approaches.

Environmental DNA (eDNA) has revolutionized biodiversity monitoring by enabling the detection of organisms through traces of genetic material present in environmental samples. Instead of capturing or observing organisms directly, researchers collect samples from water, soil, sediment, or other environmental matrices and analyze the DNA fragments released naturally by living organisms. This non-invasive approach has significantly improved the sensitivity, speed, and efficiency of species detection while minimizing disturbance to natural habitats.

The rapid development of molecular biology techniques, including polymerase chain reaction (PCR), quantitative PCR (qPCR), digital PCR, next-generation sequencing (NGS), and DNA metabarcoding, has accelerated the adoption of eDNA across diverse ecological disciplines. Today, environmental DNA is widely used for biodiversity inventories, ecological restoration, invasive species surveillance, wildlife conservation, fisheries management, environmental impact assessment, and disease monitoring. Its integration with bioinformatics and computational tools has further enhanced the capacity to analyze complex ecological datasets and identify multiple species simultaneously from a single environmental sample.

As global biodiversity continues to decline due to habitat destruction, pollution, climate change, and biological invasions, efficient monitoring tools have become increasingly important. Environmental DNA provides an innovative solution by supporting rapid, accurate, and large-scale biodiversity assessments. This review highlights the

historical development, principles, analytical workflow, applications, advantages, and challenges of eDNA technology, emphasizing its role in advancing biodiversity conservation and ecosystem monitoring.

Principles of Environmental DNA

Environmental DNA is based on the principle that all living organisms continuously release genetic material into their surrounding environment through natural biological activities. Skin cells, hair, feathers, scales, mucus, feces, urine, saliva, pollen, spores, reproductive cells, and decomposing tissues all contribute DNA to environmental matrices. These DNA fragments persist for varying durations before degrading under the influence of environmental conditions such as temperature, ultraviolet radiation, microbial activity, pH, and water chemistry.

The detection of eDNA involves collecting environmental samples containing these genetic traces and extracting the DNA using specialized laboratory protocols. Species-specific primers or universal genetic markers are then used to amplify target DNA regions through PCR-based techniques. The amplified DNA sequences are compared with reference databases to identify the organisms present in the sampled environment.

The sensitivity of environmental DNA analysis depends on several factors, including DNA shedding rates, environmental persistence, sampling strategy, laboratory procedures, and analytical methods. Although eDNA primarily provides information on species presence or absence, advances in quantitative molecular techniques are improving the ability to estimate relative abundance and biomass. Consequently, eDNA has become an effective and reliable approach for detecting rare, endangered, invasive, and cryptic species that may otherwise remain undetected using traditional ecological surveys.

Sources and Types of eDNA

Environmental DNA can be recovered from a wide variety of natural and artificial environments. Water is the most commonly sampled medium because aquatic organisms continuously release DNA into rivers, lakes, ponds, reservoirs, wetlands, estuaries, and marine ecosystems. Soil represents another important source, containing DNA from plants, fungi, microorganisms, insects, and vertebrates. Sediments often preserve DNA for longer periods than water and provide valuable information on both present and historical biodiversity.

Recent research has demonstrated that environmental DNA can also be collected from snow, ice, air, biofilms, wastewater, and even cave environments. Airborne environmental DNA has emerged as a promising technique for monitoring terrestrial biodiversity, allowing the detection of mammals, birds, insects, and plants through airborne genetic particles.

Based on its origin, environmental DNA can be classified into intracellular DNA and extracellular DNA. Intracellular DNA remains enclosed within intact cells, whereas extracellular DNA exists freely in the environment following cell rupture or decomposition. Another classification distinguishes mitochondrial DNA from nuclear DNA. Mitochondrial DNA is widely used in eDNA studies because it occurs in multiple copies per cell and offers greater detection sensitivity. Nuclear DNA, although less abundant, provides valuable information for population genetics and species discrimination. Ancient environmental DNA preserved in sediments, permafrost, and ice cores has also become an important resource for reconstructing historical ecosystems and understanding long-term ecological change.

Workflow of Environmental DNA Analysis

The effectiveness of environmental DNA (eDNA) analysis depends on a carefully designed workflow that ensures the accurate detection and identification of target organisms. The process involves systematic sampling, DNA preservation, extraction, molecular amplification, sequencing, and computational analysis. Each stage is critical because errors introduced during sample collection or laboratory processing may compromise the quality and reliability of the results. Standardized protocols and stringent quality control measures are therefore essential to minimize contamination, maximize DNA recovery, and ensure reproducibility across different studies.

Sample Collection

Sample collection is the first and one of the most important steps in eDNA analysis. The choice of sampling strategy depends on the study objectives, target organisms, habitat characteristics, and environmental conditions. Water samples are widely used for aquatic biodiversity assessments, whereas soil, sediment, snow, air, and wastewater samples are collected for terrestrial or specialized ecological studies. Sterile bottles, filtration units, and sampling equipment are used to prevent contamination from external DNA sources. In aquatic environments, water is typically filtered through membranes that retain suspended DNA particles, while soil and sediment samples are collected using sterile corers or spatulas. Multiple samples are generally collected from different locations and time intervals to improve detection probability and account for spatial and temporal variation in DNA distribution. Proper field documentation, replication, and contamination control are essential for generating reliable eDNA datasets.

DNA Preservation

Environmental DNA begins to degrade soon after its release due to microbial activity, ultraviolet radiation, temperature fluctuations, and enzymatic processes. Therefore, preserving DNA immediately after sample collection is essential to maintain sample integrity. Water filters are commonly preserved using ethanol, specialized preservation buffers, or freezing at low temperatures. Soil and sediment samples are stored in sterile containers under refrigerated or frozen conditions until laboratory processing. Maintaining a cold chain during transportation minimizes DNA degradation and preserves the original genetic composition of the sample. The use of sterile gloves, disposable equipment, and field blanks further reduces the risk of contamination and ensures the authenticity of molecular analyses.

DNA Extraction

DNA extraction involves isolating genetic material from environmental samples while removing contaminants that may interfere with downstream molecular analyses. Commercial extraction kits and standardized laboratory protocols are widely used because they provide high-quality DNA with minimal contamination. The extraction process generally includes cell lysis, removal of proteins and

inhibitors, purification of nucleic acids, and DNA recovery. Environmental samples often contain substances such as humic acids, clay particles, and organic compounds that inhibit PCR amplification. Consequently, purification steps are incorporated to eliminate these inhibitors and improve DNA quality. The concentration and purity of extracted DNA are subsequently assessed using spectrophotometers or fluorometers before molecular analysis.

PCR and qPCR

Polymerase Chain Reaction (PCR) is the cornerstone of environmental DNA analysis because it enables the amplification of minute quantities of DNA recovered from environmental samples. Species-specific primers are used when detecting individual target organisms, whereas universal primers amplify conserved genetic markers suitable for multiple species. Quantitative PCR (qPCR) extends this capability by measuring DNA concentration in real time through fluorescent signals, providing greater sensitivity and allowing semi-quantitative estimation of target DNA abundance. More recently, digital droplet PCR (ddPCR) has further improved detection accuracy by partitioning DNA samples into thousands of microscopic reactions, enabling absolute DNA quantification even at extremely low concentrations. These molecular techniques have greatly enhanced the sensitivity, precision, and reliability of eDNA-based biodiversity monitoring.

DNA Sequencing

DNA sequencing determines the nucleotide sequence of amplified genetic fragments and forms the basis for species identification. Early eDNA studies frequently employed Sanger sequencing for individual DNA fragments; however, modern investigations predominantly utilize next-generation sequencing (NGS) technologies because they can analyze millions of DNA sequences simultaneously. High-throughput sequencing enables DNA metabarcoding, allowing researchers to identify multiple species from a single environmental sample. Sequencing platforms generate extensive genetic datasets that reveal species richness, community composition, and biodiversity patterns across ecosystems. Advances in sequencing technologies have reduced analytical costs while significantly increasing sequencing speed, accuracy, and throughput.

Bioinformatics Analysis

Following DNA sequencing, bioinformatics tools are employed to process and interpret the large volume of genetic information generated. Raw sequence data undergo quality filtering, removal of sequencing errors, trimming of low-quality regions, and elimination of contaminant sequences. The processed sequences are then compared with reference databases such as GenBank and the Barcode of Life Data System (BOLD) to assign taxonomic identities. Statistical analyses and ecological modeling are subsequently performed to evaluate species diversity, community structure, distribution patterns, and temporal changes in biodiversity. Modern bioinformatics pipelines integrate machine learning algorithms, cloud computing, and advanced computational methods, enabling rapid processing of complex eDNA datasets and improving the accuracy of biodiversity assessments.

Applications of Environmental DNA

Environmental DNA has transformed biodiversity research by providing a rapid, highly sensitive, and non-invasive approach for detecting organisms across diverse ecosystems. Its broad applicability has made it an indispensable tool in ecological research, conservation biology, environmental management, and natural resource monitoring.

Biodiversity Assessment

Environmental DNA has become one of the most effective methods for biodiversity assessment because it enables simultaneous detection of multiple species from a single environmental sample. DNA metabarcoding allows comprehensive inventories of plants, animals, fungi, and microorganisms without extensive field surveys. This approach improves species detection efficiency, reduces sampling effort, and facilitates long-term biodiversity monitoring at local, regional, and global scales. Environmental DNA is particularly valuable for documenting species richness in inaccessible habitats where conventional surveys are difficult to conduct.

Aquatic Ecosystem Monitoring

Aquatic ecosystems are among the most extensively studied environments using eDNA technology. Water samples collected from rivers, lakes, ponds, wetlands, estuaries, and oceans contain DNA released by fish, amphibians, mollusks, crustaceans, aquatic plants, and microorganisms. Environmental DNA enables rapid monitoring of aquatic biodiversity, assessment of ecosystem health, and detection of changes in community composition resulting from pollution, habitat degradation, or climate change. The technique has become an important component of freshwater and marine conservation programs worldwide.

Terrestrial Ecosystem Monitoring

Recent advances have expanded the use of eDNA beyond aquatic environments to terrestrial ecosystems. Soil, sediment, leaf litter, and airborne samples provide valuable information about plants, mammals, birds, reptiles, insects, fungi, and microbial communities. Environmental DNA allows researchers to investigate species distributions, ecological interactions, and habitat quality without disturbing wildlife. The integration of soil and airborne eDNA has significantly enhanced biodiversity monitoring in forests, grasslands, deserts, and agricultural landscapes.

Endangered Species Detection

Environmental DNA offers exceptional sensitivity for detecting endangered, threatened, and rare species with minimal disturbance to their habitats. Even when population sizes are extremely small, traces of DNA released into the environment can be identified using sensitive molecular techniques. Conservation organizations increasingly employ eDNA to monitor species recovery programs, evaluate habitat suitability, and identify critical conservation areas. Early detection supports informed management decisions and contributes to the protection of vulnerable populations.

Invasive Species Surveillance

Early detection of invasive species is essential for preventing ecological and economic damage. Environmental DNA enables the identification of invasive organisms before they become well established, allowing rapid implementation of management and eradication strategies. Because eDNA can detect species at very low population densities, it is particularly useful for surveillance programs in freshwater systems, ports, reservoirs, and protected natural areas where invasive species pose significant threats to native biodiversity.

Fisheries Management

The fisheries sector increasingly utilizes environmental DNA for monitoring commercially important fish species, evaluating spawning habitats, assessing stock distributions, and detecting illegal or unreported species. Compared with conventional netting and electrofishing, eDNA provides a less invasive and more efficient method for estimating species occurrence across large aquatic systems. These data support sustainable fisheries management, resource conservation, and ecosystem-based management strategies.

Wildlife Conservation

Environmental DNA has become an important tool in wildlife conservation by enabling non-invasive monitoring of mammals, birds, reptiles, amphibians, and other vertebrates. DNA recovered from waterholes, soil, caves, and air can reveal the presence of elusive wildlife without direct observation. Such information assists in mapping species distributions, evaluating habitat connectivity, monitoring protected areas, and assessing the effectiveness of conservation programs. The technology is particularly valuable for monitoring cryptic and nocturnal species that are difficult to detect using conventional methods.

Environmental Impact Assessment

Environmental DNA is increasingly incorporated into environmental impact assessments associated with infrastructure development, mining, hydropower projects, urban expansion, and industrial activities. Baseline biodiversity data generated through eDNA surveys enable accurate evaluation of ecological conditions before project implementation. Subsequent monitoring helps identify changes in species composition and ecosystem health, allowing mitigation measures to be implemented where necessary. The rapid and non-invasive nature of eDNA makes it a practical addition to environmental assessment programs.

Disease and Pathogen Monitoring

Environmental DNA also plays an important role in detecting disease-causing organisms and monitoring pathogen distribution in natural and managed ecosystems. Molecular analysis of environmental samples enables the identification of bacteria, viruses, fungi, and parasites that may threaten wildlife, livestock, aquaculture, or human health. Wastewater-based eDNA surveillance has become particularly valuable for monitoring infectious diseases at the community level and supporting public health interventions. Early pathogen detection contributes to improved disease management, biosecurity, and environmental health monitoring.

Advantages of eDNA Technology

Environmental DNA (eDNA) has transformed biodiversity research by providing an efficient, sensitive, and non-invasive alternative to traditional ecological survey methods. One of its greatest advantages is the ability to detect organisms without direct observation, capture, or handling, thereby minimizing disturbance to wildlife and their habitats. This feature is particularly beneficial for monitoring endangered, rare, elusive, or cryptic species that are difficult to detect using conventional sampling techniques.

Another significant advantage of eDNA is its high detection sensitivity. Even minute quantities of DNA released into the environment can be amplified using advanced molecular techniques such as PCR and qPCR, allowing researchers to identify species present at very low population densities. This capability supports the early detection of invasive species, enabling timely management interventions before populations become established.

Environmental DNA also improves the efficiency of biodiversity surveys by allowing multiple species to be identified simultaneously from a single environmental sample through DNA metabarcoding and next-generation sequencing. Compared with traditional surveys, eDNA requires less field effort, reduces labor costs, and shortens the time needed for data collection. Furthermore, standardized laboratory protocols enhance reproducibility and facilitate comparisons among different studies and geographical regions.

The versatility of eDNA is another important strength. It can be applied across freshwater, marine, terrestrial, and even atmospheric environments using samples collected from water, soil, sediment, air, snow, or wastewater. Its integration with bioinformatics, geographic information systems (GIS), remote sensing, and artificial intelligence has further expanded its applications in ecological research, conservation planning, environmental impact assessment, and long-term ecosystem monitoring. Collectively, these advantages make environmental DNA one of the most promising molecular tools for modern biodiversity assessment and sustainable ecosystem management.

Limitations and Challenges

Despite its remarkable advantages, environmental DNA technology faces several limitations that must be carefully considered during study design and data interpretation. One of the primary challenges is the degradation of DNA under environmental conditions. Factors such as temperature, ultraviolet radiation, microbial activity, pH, salinity, and water flow influence the persistence of DNA in the environment, thereby affecting detection success and the accuracy of species identification.

Environmental contamination represents another significant concern. DNA from researchers, laboratory equipment, sampling instruments, or previously processed samples may contaminate environmental samples and produce false-positive results. Consequently, strict contamination control measures, including sterile sampling techniques, negative controls, and laboratory quality assurance protocols, are essential throughout the analytical process.

Another limitation is the inability of eDNA to consistently estimate species abundance or biomass. Although quantitative PCR methods provide information on DNA concentration, the amount of environmental DNA released by an organism varies depending on species biology, physiological condition, life stage, behavior, and environmental factors. Therefore, DNA concentration does not always accurately reflect population size.

Incomplete reference sequence databases also limit the accuracy of species identification. Reliable taxonomic assignment depends on the availability of validated DNA barcode sequences for target organisms. Many microorganisms, invertebrates, fungi, and rare species remain poorly represented in existing databases, leading to uncertain or incomplete identifications. Additionally, PCR

inhibitors commonly present in soil and sediment samples may interfere with DNA amplification and reduce detection sensitivity.

The absence of universally accepted sampling protocols remains another challenge. Variations in sample volume, filtration methods, DNA extraction procedures, primer selection, sequencing platforms, and bioinformatics pipelines can produce inconsistent results among studies. Continued international efforts to standardize methodologies, expand reference databases, and improve computational analyses are essential for maximizing the reliability and broader application of environmental DNA technologies.

Conclusion

Environmental DNA has emerged as one of the most significant innovations in molecular ecology, revolutionizing biodiversity assessment and ecosystem monitoring. By enabling the detection of organisms through genetic material present in environmental samples, eDNA provides a rapid, sensitive, and non-invasive alternative to conventional biological survey methods. The integration of advanced molecular techniques, including PCR, qPCR, next-generation sequencing, and DNA metabarcoding, has greatly enhanced the efficiency and accuracy of species detection across freshwater, marine, and terrestrial ecosystems.

The wide range of applications of eDNA—including biodiversity assessment, aquatic and terrestrial ecosystem monitoring, conservation of endangered species, surveillance of invasive organisms, fisheries management, wildlife conservation, environmental impact assessment, and disease monitoring—demonstrates its immense value for ecological research and environmental management. The technology enables early detection of ecological changes, supports evidence-based conservation strategies, and contributes to sustainable management of natural resources.

Although several challenges remain, including DNA degradation, contamination risks, incomplete reference databases, and the need for methodological standardization, continuous advances in molecular biology, sequencing technologies, bioinformatics, and computational ecology are steadily improving the reliability and applicability of environmental DNA analyses. The integration of eDNA with remote sensing, artificial intelligence, geographic information systems, and automated environmental monitoring platforms is expected to further expand its role in global biodiversity conservation.

In conclusion, environmental DNA represents a revolutionary approach to ecological monitoring that complements traditional field-based surveys while providing unprecedented opportunities for rapid, accurate, and large-scale biodiversity assessment. As technological developments continue and international standardization efforts progress, eDNA is poised to become an indispensable component of future environmental monitoring programs, conservation planning, and sustainable ecosystem management worldwide.

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