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The Role of Environmental DNA (EDNA) in Biodiversity Conservation

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ABSTRACT

Environmental DNA (eDNA) represents a transformative approach in biodiversity conservation, enabling non-invasive, efficient, and accurate monitoring of ecosystems. This review explores the definition, applications, and future directions of eDNA in conservation biology. By capturing genetic material shed by organisms into their environments, eDNA offers a powerful tool for detecting species presence, monitoring population dynamics, and assessing ecosystem health. The technique has shown particular promise in identifying rare and elusive species, providing critical data for conservation efforts. Despite its potential, eDNA faces challenges related to interpretation accuracy and methodological standardization. Advancements in eDNA technologies, including metabarcoding and metagenomics, promise to enhance its utility in biodiversity monitoring and conservation planning. This review underscores the significance of eDNA as an innovative tool for sustaining biodiversity and highlights areas for future research and technological improvement.

Keywords: Environmental DNA (eDNA), Biodiversity conservation, Non-invasive monitoring, Metabarcoding, Metagenomics.

INTRODUCTION

Efficient and accurate monitoring is vital in biodiversity conservation. However, traditional survey methods based on direct observations, capturing, and DNA sampling using invasive or destructive methods have limitations in surveying various species simultaneously or on a large scale. With the advancement of molecular technology, we can detect genetic information derived from protists, fungi, plants, and animals in the environment for biodiversity research [1]. This genetic information derived from organisms that are not physically present is called environmental DNA (eDNA). Although it has been used in biology, the concept and definition of eDNA are not unified. Therefore, in this chapter, we define eDNA as "genomic materials derived from organisms or viruses that are directly obtainable from the natural environment other than the organisms or viruses themselves, extracellular DNA, internal DNA transported through any host organisms or consumed foods, DNA momentarily held by soil, water, or other materials through touching with the organism itself, and sedimented DNA. [1, 2]. Environmental DNA (eDNA) is a non-invasive and user-friendly tool for biodiversity and conservation studies. In the previous methods, we had to directly capture the target organisms (e.g., trapping, canopy fogging, underwater/underground observations) or observe the organisms themselves in the natural environment. However, eDNA consists of DNA that leaks into the surrounding environment from the body surface, feces, urine, mucus, blood, embryos, and trace DNA ingested through ingestion of food but does not contain living organisms. Thus, the development of the eDNA analysis is a groundbreaking tool for "detecting phantom organisms" that allows us to monitor a wide variety of organisms simultaneously, leading to the effective monitoring of biodiversity over wide areas from the smallest scale of environmental research to the largest [3, 4].

DEFINITION AND CONCEPT OF EDNA

As a relatively new and fast-developing technique, any discussion about eDNA should set the tone by properly defining the term. First proposed in 2001, environmental DNA (eDNA) can be characterized as genetic material obtained directly from environmental samples (mainly from water, sediment, and soil) without the observation of the target organisms. Therefore, eDNA is capable of reflecting the biological conditions of ecosystems or species inhabiting them [5, 6]. The concept of eDNA is based on the process through which eDNA is released by the organisms. Fish excrete DNA from their feces, mucus/slime, scales, eggs, or sperm and through the exfoliation of their epithelial cells, which are more common in species inhabiting freshwater habitat. Pigs release DNA from their feces, shedding of skin, body hair, and secretion. Humans even excrete their DNA in their surrounding environment through their natural shedding of hairs and skin cells, urine, semen, sweat, and feces as well as through the disintegration of their entire bodies and tissues. Decomposition, predation or any kind of destruction that takes place enables the release of eDNA (e.g., through carcasses, fur, feathers) [7. 8]. The first term used to mention the DNA molecules in natural water was "molecular Ichthyological resources". However, it was strict and also confusing for representing various organisms such as macroinvertebrates, plants (e.g., alga), protozoa, or non-fish animals. "ES-DNA" ("excess of Subcellular-DNA") was also used to describe eDNA since it is considered to belong to portions of the DNA left within the environment after an animal's considers, nests or ovipositions as well as. Ecosystem DNA was also used to represent the diverse organisms within the ecosystem through which the eDNA molecules originate from or within. Currently, eDNA can be simply defined as DNA obtained directly from natural environments [9].

APPLICATIONS OF EDNA IN BIODIVERSITY CONSERVATION

The possibilities arising from the application of environmental DNA (eDNA) in the framework of biodiversity conservation are numerous. The application of eDNA has received considerable attention among researchers since it broke ground a little over a decade ago. This innovative approach serves multiple purposes: it offers a new tool for understanding the biology and ecology of a particular species and provides the means to perform non-invasive monitoring. It can also be used in a predictive framework and as a means of evaluating biodiversity, to say nothing of the ethical, legal, and social implications it has in store. What follows is an overview of the approaches that have been described in the literature, ordered by their decreasing pragmatic applicability [10, 1]. First and foremost, terrestrial vertebrates have received the most eDNA conservation-related attention. The reason stands to logic, since eDNA aids in the monitoring of rare species that arouse conservation concern. Furthermore, given the expense and time constraints involved, traditional census or survey techniques are impractical to apply. For some species, such as bats and elusive carnivores, their cryptic and solitary nature complicates detection further still. As a result, eDNA has been displayed as a quick, cost-effective, and accurate alternative tool. An additional challenge facing these species is their sparse distribution, coupled with their cryptic behaviour, something particularly relevant in forest and mountain habitats. Supposed escapees from the pet animal trade, on the other hand, may live relatively close to one another. That being said, avoiding misapprehensions is crucial for maintaining conservation horizons that can be used elsewhere [11].

MONITORING RARE AND ELUSIVE SPECIES

Monitoring rare and elusive species, which are of particular conservation concern, generally requires substantial resources and management input. As a result, monitoring effort may suffer from underrepresentation of populations, poor spatial and temporal coverage, and limited capacity to assess relationships between environmental change and population dynamics. Although relative abundance estimates may already be sufficient for control programs for highly invasive species, they may not be as helpful in providing the fine-scale biological data often needed to support management and conservation efforts. In this context, eDNA offers several solutions for addressing current conservation challenges, thanks to its persistent and long-term presence in aquatic and terrestrial environments, independence from direct detections, and capacity for detecting rare and cryptic species [12, 13]. Several researchers have focused on detecting rare and elusive species in freshwater, the environment where eDNA studies began to take off. They developed a laboratory method to detect bigheaded carps in water samples. Since then, several studies have assessed the effectiveness of eDNA methods to detect different species of rare and elusive fish, such as the white shark, Pyrenean desman, Florida panther, Assam roofed turtle, and Japanese giant salamander. In addition, more than 800 studies testing the application of eDNA to conservation purposes include some connection with rare or elusive species [14].

CHALLENGES AND LIMITATIONS OF EDNA

1. Introduction Containing direct evidence of the organisms present in an environment, eDNA methods are increasingly used to support biodiversity research and conservation policy. However, eDNA, with its

promise of increased sampling efficiency, also poses challenges and calls for critical reflection on whether, when, and how it can support biodiversity monitoring and assessments [15].

2. Interpreting eDNA Results All eDNA contains both technical and biological variation that can affect its reliability for a given purpose. Recent work shows that different methods, primers, and algorithms in combination with the environment (e.g., sediment, water) and the different temporal scales of study (from minutes to millions of years) considered can make important differences in the results obtained. Furthermore, additional biological factors (samples have limits of detection), spatial factors (dispersal of DNA, inactivation rates of DNA), and methodological factors (e.g., DNA extraction, contamination) must be considered when interpreting results. Overall, this complexity calls for great caution in interpreting the absence of eDNA as absence of target species, particularly when data are not collected systematically and are neither collected nor analyzed in ways that are appropriate for the detection of cryptic and rare species [16].

INTERPRETATION OF RESULTS

Understanding the ability of eDNA analysis to draw accurate conclusions regarding the presence or absence of species is necessary if eDNA studies are to provide data for management decisions and strategic biodiversity conservation plans. However, this is not always straightforward. There are several factors that can lead to flawed or misconstrued conclusions drawn from eDNA results. One of these is the potential for free-living eDNA to give a positive result even when the target species is not present [17]. The quantity of eDNA detected can also provide additional layers of complexity to the interpretation of results. In both field-based and laboratory-based methodologies, a positive eDNA result is not necessarily proportional to the biomass of the target species. For example, the eDNA of a species present at a low density could be detected in quantities exceeding those of a species present at a higher density. A range of factors such as behavior, physiology, and eDNA detection method can account for this discrepancy. It is not impossible for negative results to arise from the presence of the target species, resulting in a type II error. This can occur where the detection method used is not sensitive enough to detect low quantities of eDNA, where there are problems to do with amplifying the unique region of the target species' DNA (false negative), or when eDNA fails to enter the target area. Moreover, it is not possible for failing to detect eDNA to be exclusive to the absence of the target species (type I error). A range of factors such as hydrology, weather conditions, eDNA degradation, and particle disintegration affect eDNA degradation [18].

FUTURE DIRECTIONS AND INNOVATIONS IN EDNA RESEARCH

eDNA science is working closely with innovative computer science to develop novel technologies for future aquatic ecosystem demographic analysis. In the case of eDNA from vertebrates, traditional approaches are evolving into a sensitive cross-disciplinary approach between classical methods of molecular techniques and bioinformatic analysis. Finding rare and elusive indicator species at a landscape level using field-based molecular genetic techniques is becoming a possibility. Complex mixtures of eDNA can be analyzed either using the taxonomy-based metabarcoding approach of sequencing dominant organisms for broad estimates of community composition, which has to date been the main focus of the eDNA research papers [19]. One of the major challenges of our current analytical frameworks is how to find the extremely low abundance species in the DNA mix that are too shy, frightened, or difficult to capture. Metabarcoding and metagenomics of eDNA will become pioneering approaches in ecological niche analysis that were previously difficult to study at this metazoan population scale. In an era of declining invertebrate taxonomy expertise scaling down to molecular taxonomy studies of the metazoa will set the pace for future approaches to invertebrate genetics and biodiversity assessment. It is these types of molecular signatures that will provide a trail of data that our ancestors could potentially use to trace our evolutionary pathways as all explored species have been there before us [20].

METABARCODING AND METAGENOMICS

While the ability to reconstruct species inventories from bulk samples may sound futuristic, a major advantage of concentrating eDNA from the environment is its capacity "to cover all components of biodiversity simultaneously". Consequently, eDNA allows us not only to remotely collect information on biodiversity without the need for targeting, trapping or capturing individuals, but to detect highly cryptic, slow-moving, rare or short-lived species, which would ordinarily be overlooked by observation and field surveys. It is, in this vein, whether eDNA sequencing leads to taxonomically appropriate species detection that most concerns biologists assessing metabarcoding and metagenomic surveys of such bulk samples. But the ability to detect shallower branches of the zoological tree of life belies powerfully meta-analytical discriminatory power: the joint detection of dozens of species within cladistic groups, or biodiversity as a composite whole [21]. In summary, the ability to generate comprehensive, top to

bottom inventories of species from bulk samples can be highly meaningful. In recent years, biologists have tended to popularize the notion that metabarcoding and metagenomics will quickly come of age and reshape the ways in which biodiversity can be studied and reported. Certainly, while the debate rumbles on over empirically best practices in applying eDNA sequence and estimates of bias, this innovative methodology argues the most exciting future trends in eDNA for biodiversity conservation [22]. Environmental DNA (eDNA) has rapidly progressed from earliest demonstrations of its capacity to detect cryptic and elusive species through shed genetic material, to development of metabarcoding and shotgun sequencing techniques of eDNA to assess entire communities. The accessibility of eDNA from water, soil, and air enables applications in diverse ecosystems, including marine, freshwater, terrestrial, and aerial realms. Applications of eDNA are increasingly used in biodiversity biomonitoring, including in assessments of rare and endangered vertebrates, invertebrates, and plants. Highly effective eDNA detections can allow less effort and disturbance than traditional surveys, enabling improved frequency and distribution of monitoring, as well as detection when animal and plant populations are small and non-breeding [23]. The extra attention paid to eDNA in biodiversity conservation will likely address many applications to date and in the future. Currently, applications of eDNA in conservation and monitoring are increasing and developing rapidly. As eDNA techniques continue to improve in sensitivity, power, and applicability, they will help to re-imagine and re-innovate conservation and monitoring in ways we are yet to fully appreciate. A spectrum of remaining theoretical and practical challenges will help to guide ongoing development of these technologies and their optimal applications. Finally, demonstrations of how eDNA has supported conservation and restoration will help to increase the uptake and importance of eDNA as a monitoring and research approach. These will require the thorough evaluation of eDNA's performance against other tools, as well as evaluations of its effectiveness toward positive conservation outcome [24].

CONCLUSION

Environmental DNA (eDNA) has emerged as a revolutionary tool in biodiversity conservation, offering significant advantages over traditional methods. Its ability to non-invasively capture and analyze genetic material from a wide array of organisms facilitates comprehensive biodiversity assessments, particularly for rare and elusive species. While challenges remain in terms of data interpretation and methodological consistency, ongoing advancements in eDNA technologies, such as metabarcoding and metagenomics, are poised to enhance its accuracy and applicability. As eDNA techniques continue to evolve, they will play a crucial role in reimagining and improving conservation strategies, providing robust data to support the preservation and restoration of biodiversity across diverse ecosystems. Future research should focus on addressing current limitations and standardizing methodologies to fully realize the potential of eDNA in global conservation efforts.

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