Exploring Environmental DNA (eDNA) for Monitoring and Conserving Aquatic Biodiversity

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Abstract

The primary concern and challenge of the twenty-first century is the continuous loss of biodiversity on Earth and there is a worldwide political agreement to limit and stop this reduction. The lack of awareness about the basis and allocation of biodiversity is the major obstacle. The conservation strategies to save aquatic biodiversity depend upon the estimate of population size, distribution patterns, and monitoring of species. Species monitoring depends on physical identification by counting individual or visual surveys but traditional surveys remain problematic to overcome this problem there is an urgent need for effective and innovative methods for monitoring biodiversity on a wide scale. Environmental DNA (eDNA) is also known as organismal DNA it can be found in the surroundings and developed from cellular material of organisms released into the terrestrial and aquatic environment. It may be collected and tracked with molecular technique which is essential for the early recognition of both native or invasive species as well as the discovering of elusive or endangered species. This chapter has shown the methods of eDNA analysis, its application in aquatic biodiversity, the limitations and challenges of eDNA, and its prospects.

Keywords: Environmental DNA (eDNA); Biodiversity; Conservation; Monitoring; Environment

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Introduction

Monitoring biodiversity in the aquatic environment is complicated because of its inconspicuousness as a crucial factor of the biological environment. The successful management of ecosystems and applications of environmental policies depend on precise and authentic assessment of species and biodiversity distribution (Kissling et al., 2018). To monitor aquatic organisms underwater, biologists require novel, non-invasive quick, and inexpensive approaches. Luckily this need can be fulfilled by environmental DNA (eDNA) technique. eDNA term initiated in microbiology (Ogram et al., 1987) and it describes the genetic material (DNA) found in water, air, or mud. Environmental DNA has been extensively used for identifying microbial groups in humans and animals (Karygianni et al., 2020).

For the last few years, eDNA has been extensively used to identify aquatic animals, the researchers collecting water samples to identify taxa all around the world. In 2008 first time eDNA was used to detect the existence of frogs (*Rana catesbeiana*) in natural wetlands and controlled environments, which opened the new gate for identifying aquatic organisms in biological and ecological communities (Ficetola et al., 2008). Methods of eDNA have been effectively verified in marine, freshwater, and estuarine. The methods based on eDNA are appropriate for freshwater biota as they give precise collection and statistics analysis approaches with high possibilities of recognition (Hering et al., 2018). Monitoring eDNA is particularly helpful for the initial identification and monitoring of endangered, rare, invasive, and cryptic species because at low density it gives accurate detection (Beng & Corlett, 2020). In recent years, eDNA metabarcoding which involves designing universal primers of short fragments on the homologous of the whole aquatic population DNA has gained acceptance for monitoring the whole aquatic diversity across a wider taxonomical range due to the progress of high-throughput sequencing (Tsuji et al., 2019).

The Science of eDNA

Definition and Concept

eDNA is a very new and rapidly evolving technique any discussion of it should begin with a clear definition of the term. For the first time it was proposed in 2001, that environmental DNA (eDNA) can be characterized as genomic material taken directly from environmental samples (mainly from water, remains, and soil) without the observation of the target organisms. The basic concept of eDNA depends upon the mechanism by which eDNA is released by the animals. Humans release DNA into their surrounding environment by their natural shed of hair, urine, skin cells, sweating, and feces as well as their entire body tissue degeneration. Fish, which are more prevalent in species that live in freshwater environments exfoliate their epithelial cells to release DNA from their mucus, scales, feces, and eggs or sperm. eDNA can be released through decomposition, predation, or any kind of destruction (e.g., through carcasses, fur, and feathers) (Minich et al., 2020; Guardiola et al., 2022). "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 oviposition 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. Now, eDNA can be simply defined as DNA obtained directly from natural environments (Wang et al., 2021).

Methods for eDNA Analysis

eDNA typically can be identified from varieties of environments but the selection methods and extracting procedure may be altered and adopted which type of sample or particular goal of the research (Bruce et al., 2021a). For aquatic species, methodological standardization, knowledge about the environment of target taxa and eDNA technique are crucial. Here, describes the entire workflow into a few phases which are given blow.

1. Sampling Approaches

In aquatic ecosystem, generally a sterilized DNA free bottle and one-time use sampler usually appropriate from the surface collection of water (e.g for surface plankton), while a sampler has structure that resembles a pole or rope-like structure is utilized for underwater (Berry et al., 2019). Though, as expertise are developing to make easier sample collection and enhancing proficiency, sterility and replicability of water sampling and it is also employing a fully integrated sampling system (Thomas et al., 2018). Additionally, for sampling flexibility, mobile polymerase chain reaction (mPCR) and field research amplification of eDNA has been established to allow quickly on-site eDNA identification (Doi et al., 2021), thus quickly scaling-up biomonitoring. Since not all species can be benefit from every approach for the collection of eDNA samples (Bruce et al., 2021a).

2. Preservation

After collection, samples are frequently stored on ice or at 4 °C temperature, frozen at 20 to 80 °C, dry preserved using absorbents (like silica gel) and liquid preserved with refined preservatives (like ethanol, Benzalkondium chloride (0.01 %)) (Jo et al., 2021) and lysis agents (e.g. Longmire's buffers) (Kumar et al., 2020; Bruce et al., 2021a).

3. Removal or Extraction of eDNA

Further processing techniques for accumulating eDNA including filtration, centrifugation, ultracentrifugation and precipitation approaches (Tsuji et al., 2019) however, can be extracted directly when they are not put through an accumulation process. Purification technique uses narrow porous membrane (e.g. 0.22 l, 0.45 l) to catch DNA; filtration technique utilizes salt and ethanol precipitate DNA, while DNA (Bruce et al., 2021a). The filtration process utilizes large volume of water it is more frequently used (commonly 0.5–2 μm) (Tsuji et al., 2019). At present commercial eDNA filtering apparatus are also offered for both off-site and on-site uses (like EnviroDNA). Nonetheless a variety of DNA extraction techniques have been used to obtain DNA template results. The tests are important to ensure that DNA extraction method is reliable for the upcoming DNA application (Deiner et al., 2017).

4. Sequencing and Amplification

Target species identification emphasize on a single or small number of species and using species-specific primers to replicate specified targets by using traditional PCR (cPCR) for 'presence and absence', and quantitative PCR (qPCR) for quantification of DNA and it is also utilized for more precise identification when DNA fragments are uncommon (Wineland et al., 2019). To confirm that they don't cross amplify with their similar taxa specific primers for target species must be created and evaluated (Rowney et al., 2021). Alternative types of PCR, the droplet digital PCR (ddPCR), has also established for accurate and highly sensitivity species (Nathan et al., 2014) and the CRISPR-Cas technique for species detection has also been utilized (Williams et al., 2019).

5. Bioinformatics

The variety of data generated by environmental DNA and metabarcoding research needs computerized process for the curation of classifications and taxonomy assignment (Deiner et al. 2017). Selecting the ideal bioinformatic pipelines is crucial for achieving accurate results. Recently created pipelines in addition to pre-existing (like Braque, QIIME 2) may be used rendering to study (Mathon et al., 2021). Moreover, optimal uses between the OTU (operational taxonomic units) and ASV (amplicon sequence variant) can also affect systematic obligation. While ASVs detect distinct sequence changes and PCR filtration and sequencing errors, offering further accurate and exact assessments of only one nucleotide variants, OTUs that overcome these faults are often grouped sequences based on a threshold resemblance (Callahan et al., 2017). In general, the selection of these limits ultimately rely which indicator is utilized, reference data base and goals of research.

Applications of eDNA in Biodiversity Conservation

The application of eDNA has provoked a lot of interest from researchers since it broke ground a little over a decade ago. This pioneering approach serves several purposes: it provides a new insight for understanding the biology and ecology of a specific species and provides the means to conduct non-invasive monitoring. It can also be used to assess biodiversity and create a predictive framework (Fonseca et al., 2023: Sahu et al., 2023). First and foremost, the greatest amount of research pertaining to eDNA conservation has been given to terrestrial animals. Since eDNA facilitates the monitoring of uncommon species that raise conservation concerns.

Besides, it is not sustainable to use typical census or survey techniques due to time and expensive. The cryptic or solitary nature of certain species like elusive carnivores and bats makes detection more complicated. Consequently, eDNA has been promoted as rapid, cost-effective and precise alternative tool. An additional challenge facing these species is their sparse distribution, coupled with their cryptic behavior, something particularly relevant in forest and mountain habitats. Supposed fugitives from the domestic animal trade, on the other hand, may live relatively close to one another. That being said, avoiding misconceptions is crucial for maintaining conservation prospects that can be used elsewhere (Piaggio et al., 2021).

Monitoring Rare and Elusive Species

Monitoring rare and elusive species, which are of specific conservation concern, it usually takes a lot of resources and management input.

Consequently, monitoring effort may be hindered by under representation of populations, inadequate coverage of time and space and limited ability to evaluate relationships between environmental change and population dynamics. Relative abundance estimates may be sufficient for control programs for highly invasive species, they could not be useful in providing the fine-scale biological data often needed to support management and conservation efforts. eDNA present a number of alternatives to overcome current conservation challenges, its persistent and long-term presence in aquatic and terrestrial environments, independence from direct detections, and abilty for detecting rare and cryptic species (Huerlimann et al., 2020; Duarte et al., 2023). 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. However, more than 800 studies testing the application of eDNA to conservation purposes include some connection with rare or elusive species (Shu et al., 2020).

Monitoring the Health of Aquatic Ecosystem

The aquatic microbiota is vibrant for aquatic environments, which provide crucial services to the ecosystem in both marine and freshwater ecosystems. The composition of aquatic microbial community is fluctuating, moving with time, geography and change with ecological circumstances (Sehnal et al., 2021). Though, it is suggested that water ecosystem depend on fundamental microbial processes while different functions of aquatic microbiota are conserved worldwide. Based on eDNA microbiota biodiversity analysis can offer significant information on the function and health of aquatic environment (Shaw et al., 2016). The eDNA study may recognize the optimum bacterial groups (like bacteria, algae, fungi and protozoa, etc.) in culture water in aquatic culture habitats to achieve long-term stability and maximum ongoing output (Flegel et al., 2019; Liu et al., 2021; Sehnal et al., 2021). An instant monitoring approaches for pathogens, vectors and hosts is crucial for evaluating present and upcoming threats to human health since patterns of disease transmission are impacted by changes in the global environment. For survival, both humans and animals rely significantly on aquatic and other nutrient bases and on aquatic bases have an enormous effect on both human and animal public health. The eDNA methods have been extensively used to monitor major public health hazards, particularly in schistosomiasis and vector-borne diseases, directly from the field 396 environment, thus providing a less expensive, non-invasive, less difficult and effective observation method for human health hazard monitoring (Huver et al., 2015; Sengupta et al., 2020; Amarasiri et al., 2021). Epidemiological data combined with eDNA surveys conducted in aquatic environments can yield vital information for formulating plans to reduce and mitigate threats to human public health.

Holistic Ecosystem Health through eDNA

Environmental DNA (eDNA) appeared as a prevailing method for evaluating and monitoring the health of ecosystem in a holistic manner. The researcher can determine whether species are present in the ecosystem, monitor changes in biodiversity, and assess ecosystem dynamics with little interference from the environment by analysing genetic material. Conventional survey methods may neglect rare or elusive species, while eDNA offers thorough insight into species richness and abundance. Finding biodiversity hotspots and evaluating ecosystem resilience depend heavily on this information (Deiner et al., 2016). Changes in species abundance and composition brought on by invading species, pollution, climate change, or habitat loss can be detected using eDNA. These alterations frequently act as early warning indicators of ecological deterioration (Yates et al., 2021). The eDNA sampling is non-invasive, scalable, and economical, which makes it appropriate for long-term monitoring programs across a variety of ecosystems, in contrast to older techniques that may be used for catching or disturbing species (Bohmann, 2014). Through the integration of eDNA data with ecological, hydrological, and remote sensing models, researchers can acquire a comprehensive understanding of ecosystem functions and processes. This holistic approach aids in making informed conservation and restoration decisions (Taberlet et al., 2018). A revolutionary method for evaluating the health of ecosystems, eDNA allows for a comprehensive analysis of biodiversity, ecosystem services, and environmental change.

Role of eDNA in Understanding Ecosystem Services

Research using these non-destructive eDNA-based techniques has provided useful results in both terrestrial (soil and air) and aquatic (marine, estuaries and freshwater) ecosystem (Berry et al., 2019). From last eras, based on eDNA approaches has been effectively used to recognize several perilous perceptions of ecosystem (like migration, species interaction and chosen habitat) (Wu et al., 2019), comprising the discovering and tracking important and rare organisms for which samples collection is essential for management efforts (Stewart et al., 2017). The initial recognition of invasive species at small population (Muha et al., 2019) or whole communities from unexplored regions (Ritter et al., 2020) has also been carried out for several taxa. However, based on eDNA techniques has been effectively used for detection a variety of taxonomic group, from microbes (Abdelfattah et al., 2018) to invertebrates.

Contribution of eDNA to Holistic Conservation Strategies

Environmental DNA (eDNA) is emerging as a transformative tool in biodiversity monitoring and conservation strategies. It offers several advantages that complement traditional conservation methods, enabling more holistic approaches.

- eDNA enables the identification of species from environmental samples, such as water, air and soil, deprived of the need for physical capture or close observation. This is especially helpful for cryptic, elusive or rare species that are hard to track with traditional techniques. The evaluation of biodiversity in both aquatic and terrestrial environments is made possible by eDNA, which increases the effectiveness and precision of species inventory and population monitoring. For example, eDNA has proven useful in identifying endangered aquatic animals, like the Great Crested Newt (*Triturus cristatus*), which helps with conservation planning and habitat management (Biggs et al., 2015).
- Early intervention to stop establishment and spread is made possible by eDNA's quick and sensitive detection of invasive species at low population densities. Targeted eradication efforts made possible by early diagnosis provide cost-effective management. For instance,

management plans to safeguard native fish populations have been influenced by the identification of invasive Asian carp species in North American waters using eDNA analysis (Jerde et al., 2011).

- eDNA data provides insights into ecosystem resilience and the development of adaptive conservation strategies by tracking changes in species distributions brought on by climate change. Finds climate refugia and tracks alterations in the demographics of the community over time. For instance, eDNA monitoring of fish and amphibian range shifts shows evidence of migrations caused by climate change (Valentini et al., 2016).
- eDNA provides information on food webs and ecosystem health by simultaneously recognizing several species, allowing for ecosystem-level studies.supports the design and assessment of marine protected areas (MPAs) and other integrated management techniques. For instance, the efficiency of MPAs in preserving marine biodiversity has been evaluated using eDNA metabarcoding (DiBattista et al., 2020).

eDNA Approaches over Conventional Survey Techniques

Non-invasive surveys techniques like eDNA analysis may offer significant benefits over conventional approaches where it is necessary to disturb the species of interest and captured to obtain an optimistic identification, invading on animal welfare. The eDNA analysis has been demonstrated to be a precise identification technique and seems to associate with traditional survey findings (Dejean et al., 2012) described that the environmental DNA of target species was located in all aquatic areas where the species has been previously detected by conventional techniques. Furthermore, eDNA was also found in regions where the targeted species were not existing, indicating that it is more complex technique for detection. Nevertheless, it is important to note that in certain instance, the eDNA analysis success rate has not constantly 100% although the species of interest was known to exist (Thomsen et al., 2012b). Expected that with ongoing enhancements to sampling and laboratory techniques, there will be further progresses on the success rates of eDNA-based detection systems.

Traditional survey windows for migratory animals like the great crested newt (GCN) could be extended with this technique. Great crested newts (GCN) typically reside on terrestrial environment, hibernating from October to March and mate in ponds and pools during their prime breeding season, which lasts from March to May. This creates a survey "window" where the GCN can be found using eDNA analysis in addition to traditional survey techniques (egg counts, torchlight surveys, and trapping) (Thomsen et al., 2012b). It is hoped that eDNA analysis will be able to expand this survey window to include the period that larvae and juveniles occupied ponds when conventional sampling techniques are not ideal.

In terms to standard survey approaches, eDNA analysis can save a significant amount of time and money on sample effort, particularly when examining the dispersal of erratic or endangered species. In a study on invasive Asian carp in Chicago, Illinois, electrofishing at a site acquired 93 days of human effort to find an only one silver carp, yet eDNA analysis only took 174 days to obtain a optimistic results (Jerde et al., 2011).

An area of effort with eDNA analysis is the nature of the water body to be sampled. The type of water body to be sampled is a challenge for eDNA analysis. While eDNA identification in flowing water is more challenging, certain situations, such ponds and lakes, are easier to analyses. Numerous investigations have effectively eDNA-surveyed stream species (Goldberg et al., 2011; Jerde et al., 2011, Mahon et al., 2013). Large amounts of water (2–10 L) were used by all to make up for the eDNA that was lost due to water flow. These studies highlight how important it is to use distinct approaches for various contexts rather than a "one size fits all" strategy.

Conventional surveying techniques although have significant benefits besides eDNA approaches, ultimately the conventional technique can differentiate between live and dead animals. Furthermore, eDNA approaches cannot discriminate between the different life cycles of species of attention such as larvae (tadpoles) juveniles, and adults in amphibians which can be done with traditional methods. eDNA techniques are unable to offer comprehensive information on the potential locations of target species or any microorganism ecosystem variation, in contrast to traditional approaches that allow you to know in "real-time" when and where a target species was present.

Data on species abundance and distribution might be gathered reasonably quickly and cheaply using environmental DNA analysis. This is especially important if the sampling region is quite wide. The information gathered might be used to target particular aquatic species for comprehensive biological studies.

Challenges and Limitations

Despite clear viewpoint and practical use of eDNA, there are number of serious difficulties associated with the method needs to be considered.
Contamination: The utmost significant drawback of environmental DNA (eDNA) is the potential for contamination and false positive findings. The contamination may be occur taking the samples in the field or any stage analyses in the laboratory. While PCR is frequently used in eDNA studies, creating billions of DNA copies that can quickly spread across the lab, lab contamination is particularly harmful. The contamination problem has become much more complex with the rise of NGS approaches. The contamination will be greatly reduced by a rigorous clean-lab routine that uses decontamination techniques and physically separates the labs for PCR work before and after (Willerslev & Cooper, 2005; Champlot et al., 2010).
Inhibition: inhibition when humic compounds or humic acids are co-extracted with DNA from environmental materials, they significantly block enzymes like Taq Polymerase, which amplified the DNA by using PCR process (Matheson et al., 2010). This clearly indicates partiality in eDNA research, which is present in water samples as well as soil ones, however it is most probably to be more pronounced in soil samples (McKee et al., 2015; Sigsgaard et al., 2020). The occurrence of a species may be overestimated or underestimated as a result of false positive or false negative results, which may affect subsequent conservation efforts.
Errors: Errors can occur during PCR or sequencing or prior sampling in long-term conserved DNA (Willerslev and Cooper, 2005; Hansen et al., 2006). Point mutation and formation of chimeric molecules are generated by PCR error (Acinas et al., 2005). Consequently, raw sequence data necessary to rigorously filter in order to reduce false positives or produce a precise taxonomic classification.
Interpretation of results: The careful interpretation of final results is another significant challenge in eDNA investigations. In contrast to traditional surveys, eDNA detection has significant limitations, such as the inability to differentiate between dead and living organisms, definite

life periods (eggs, juveniles and adults) and mixed-breed species. The latter is associated with the widespread use of markers found in mitochondria. Although mtDNA is abundant in excreted cells and useful as a DNA barcode, it can only identify the maternal lineage of a hybrid species. Lastly, eDNA, like any other monitoring method, will only identify a percentage of the total sites occupied by a particular species. Using site occupancy models, Schmidt et al. (2013) illustrated the significance of employing thorough examinations of presence/absence data to produce more accurate eDNA-based species occupancy estimates.

Future Perspectives

eDNA science are collaborating with advanced computer science to create new technologies foe demographic studies of aquatic ecosystem in future. With field based molecular genetics approaches it becoming possibility to finding elusive and rare indicator species at the landscape level. Complex mixtures of eDNA can be examined either using the taxonomy-based metabarcoding method of sequencing dominant organisms for broad estimation of community composition, which has to date been the primary focus of the eDNA analysis (Huang et al., 2022). Finding the incredibly low abundance species in the DNA mix that are too hesitant, afraid, or difficult to catch is one of the main problems with our present analytical frameworks.

In ecological niche analysis, metagenomics of eDNA and metabarcoding will emerge as revolutionary techniques that were previously challenging to investigate at this metazoan population scale. Scaling down to molecular taxonomy studies of the metazoa will set the standard for future methods of invertebrate genetics and biodiversity assessment in an era of declining invertebrate taxonomy knowledge. These kinds of molecular markers will leave a trail of information that our ancestors may be able to use to track our evolutionary paths because every creature that has been studied has been there before us (Compson et al., 2020).

Conclusion

In biodiversity conservation environmental DNA (eDNA) has become an innovative tool with significant advantages over conventional techniques. The capacity to non-invasive capture and analysis of genetic material from a diverse range of creatures facilitate comprehensive assessment of biodiversity, especially for elusive and rare species. In aquatic ecosystem eDNA barcoding illustrated massive potential for tracking specific biodiversity over time and location. Unlike conventional monitoring, monitoring of aquatic biodiversity by using eDNA can assist precisely distinguish fresh water animals without being invasive. This method has changed the basic research technique foe gathering ecological data, tracking and handling ecosystem health. As eDNA technologies develop, they will play a vital role in reconceptualize and enhancing conservation strategies, and offering robust data for preservation and restoration of biodiversity over diverse ecosystem.

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