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Application of Environmental DNA Signatures for Detecting Rare Freshwater Species in Fragmented River Networks

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Abstract: Environmental DNA (eDNA) has become an innovative molecular technique for identifying rare and elusive species in freshwater ecosystems, especially in disconnected river systems where traditional identification methods are limited by sparse populations, habitat isolation, and reduced species mobility. The current research project assessed the utility of environmental DNA signatures to detect rare species in disconnected river ecosystems and their distribution patterns. The methodology involved collecting water samples from selected river sections and detecting genetic traces through molecular techniques and DNA sequencing. Special emphasis was placed on the role of hydrological connectivity, water flow, sediment interaction, and environmental conditions on eDNA detection and distribution patterns. This application of eDNA profiles offers the potential to increase species detection while avoiding disturbance to sensitive aquatic species. In addition, including habitat connectivity data in the molecular analysis enabled better understanding of rare-species distribution within fragmented freshwater ecosystems. Sampling location, environmental conditions, and DNA persistence were all crucial to interpreting detection results. Environmental DNA monitoring is an important complementary method for freshwater biodiversity assessments.

Keywords: Environmental DNA (eDNA); Rare Freshwater Species; River Fragmentation; DNA Metabarcoding; Hydrological Connectivity; Freshwater Biodiversity Conservation.

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I. Introduction

Freshwater Ecosystems and the Importance of Rare Species

Aquatic ecosystems such as rivers, streams, lakes, and wetlands contain a diverse range of aquatic species and are crucial to maintaining ecological balance. Aquatic ecosystems serve as habitats for fishes, amphibians, invertebrates, and microscopic organisms that support the ecosystem food web, nutrient cycling, and productivity. The rarity of certain freshwater species makes them ecologically important since they are geographically localized, have specialized habitat needs, or have naturally low population numbers. The occurrence of rare species reflects the ecological status and conservation significance of freshwater ecosystems. Studying rare species is difficult because standard collection methods cannot detect low-density, hard-to-access organisms. Environmental DNA (eDNA) metabarcoding is an important molecular technique for identifying aquatic species by analyzing their genetic material in water (Valentini et al., 2016).

Biodiversity Decline in Fragmented River Networks

River systems are increasingly fragmented by dams, weirs, culverts, water abstraction systems, and other human interventions. These barriers disrupt natural river connectivity, disrupt fish movement, and isolate aquatic populations within smaller habitat patches. This fragmentation can reduce reproductive success, limit access to feeding grounds, and increase extinction risk for rare freshwater species. Changes in river connectivity can also affect the distribution pattern of aquatic species and movement of biological material in connected water systems. Studies of fish communities in fragmented lowland rivers have shown the importance of eDNA metabarcoding for assessing differences in species composition along fragmented river systems (Antognazza et al., 2021). Moreover, research after barrier removal has shown the importance of eDNA monitoring in assessing fish community composition (Muha et al., 2021). Thus, studying

biodiversity patterns in fragmented river networks is critical for implementing effective freshwater conservation strategies.

Environmental DNA as a Non-Invasive Biodiversity Detection Tool

Environmental DNA means the DNA of living organisms found in the environment through various biological processes, which include epidermal sloughing, secretion of mucus, excretion, and tissue decomposition. In freshwater habitats, researchers can sample an organism's DNA from water by collecting water, then filtering, extracting, amplifying, and identifying it molecularly. Unlike conventional approaches, eDNA methods do not require physically capturing or seeing the target organism. By amplifying and identifying certain genetic markers of the sample, DNA metabarcoding enables researchers to identify various organisms in one sample. It has greatly expanded possibilities for biodiversity monitoring and identifying species that cannot be detected using conventional approaches (Valentini et al., 2016). The technique has also been used to detect rare and alien fish species in Great Lakes tributaries. It has shown the potential to detect organisms that occur at low abundances (Balasingham et al., 2018).

Research Problem and Ecological Significance

Detecting rare freshwater species in fragmented river systems has been one of the main challenges in ecological surveys. Traditional fish surveys can take a lot of time, money, and equipment, and may require several visits to the site; moreover, survey accuracy can be hindered by the scarcity of the studied species, difficult environments, and limited access. In addition, the issue of fragmentation increases the problem of biodiversity studies due to isolation of species in the different parts of rivers. Even though the use of environmental DNA technology provides another method of detection, the analysis of DNA markers in the flow of the water could be affected by the downstream transportation, degradation, dilution, and different sampling conditions. As a result, detection of DNA in a certain place does not imply the presence of the target species in the same spot. It leads to the necessity of the integrated evaluation of species detection, river connectivity, sampling conditions, and the mobility of environmental DNA. The importance of such solution lies in its ecological significance related to the reliable detection of rare species.

II. Literature Review

Evolution of Environmental DNA Techniques in Freshwater Research

Environmental DNA has emerged as an important molecular tool in the study of aquatic biodiversity without necessarily collecting the organisms. Traditional molecular tools used in environmental monitoring studies were mainly based on detecting individual target species while the recent advancements are mainly based on DNA metabarcoding that can simultaneously detect different species from environmental samples. Advancements in DNA sequencing technology have facilitated the study of more complicated aquatic communities by detecting species that would otherwise be missed through conventional methods. Environmental DNA applications in Indian freshwater ecosystems have been reviewed and its significance noted in relation to biodiversity studies, conservation efforts, and ecosystem monitoring especially in ecosystems under environmental stress (Sahu et al., 2025). However, the success of eDNA detection depends on how well sampling was done and how the samples were stored. In a study carried out in a Neotropical River, the effect of preservation methods, sampling medium, and sampling time on environmental DNA was studied (Sales et al., 2019).

Molecular Approaches for Identifying Rare and Elusive Species

Molecular methods have facilitated the detection of rare, endangered, and hard-to-detect species of freshwater organisms through the use of the genetic material that is shed in aquatic environments. When there is a need to specifically determine the presence of a particular species of interest, the eDNA assays specific for species can be applied, whereas the metabarcoding technique is useful when there is a need to determine multiple species in an ecosystem. The application of molecular methods is particularly important

when there are small numbers of organisms that are distributed over inaccessible habitats. Studies on rare, endangered, and invasive trout showed that a high sampling volume was critical in increasing the chances of detecting target species of interest (Sepulveda et al., 2019). In addition, studies comparing eDNA metabarcoding with traditional methods of sampling fish have shown that molecular techniques have the capacity to complement traditional studies in terms of fish diversity in rivers (Hallam et al., 2021).

River Fragmentation and Its Influence on DNA Distribution

River networks are characterized by complex hydrology and hydrodynamic features that affect the spatial distribution of environmental DNA. Aquatic organisms release genetic materials to the water, which can be transported downstream by flowing water, giving an opportunity to detect species that occur far away from the location. This movement, however, may create some challenges when it comes to the interpretation of the obtained data since the detected DNA may come from the upstream environment. Scientific studies conducted on river networks have proven the possibility of employing eDNA models in determining the distribution and abundance of species taking into account the spatial structure and hydrological connectivity (Carraro et al., 2018). Likewise, scientific studies carried out in big rivers have shown that the transport of environmental DNA downstream did not hinder the establishment of the quantitative patterns of fish biodiversity even though hydrological movement was still an essential factor (Pont et al., 2018).

Research Gaps in eDNA-Based Freshwater Species Detection

In spite of numerous developments in the use of environmental DNA for studying and monitoring rare freshwater species within fragmented river networks, there are still some significant deficiencies in the process of identification of such species using the methods mentioned above. Current methods do not necessarily guarantee adequate integration of the results of molecular detection and spatial peculiarities of disconnected river systems. The processes of water flow, the persistence of DNA molecules, the sampling sites, and natural barriers in habitat may affect the interpretation of species occurrence in various parts of rivers. Moreover, the presence of genetic material alone cannot confirm the exact location, abundance, and long-term survival of the species in question. There is also a need for a more structured approach to the monitoring process combining species-specific detection, metabarcoding, spatial analysis, and multiple samplings. The main aim of this research will be to assess the ways of improving the detection and spatial analysis of rare freshwater species using environmental DNA signatures.

III. Research Methodology

Research Design and Study Area Selection

In the current study, the researcher will apply a descriptive and analytical research methodology in order to assess the use of environmental DNA signatures in the detection of rare freshwater species within fragmented river systems. The research methodology involves identification of specific genetic markers associated with particular species and assessment of their spatial distribution as well as influence of river fragmentation on molecular detection patterns. The research area will be designed based on freshwater river systems that have different habitat conditions, including upstream habitats, midstream habitats, downstream habitats, and fragmented habitats due to the presence of physical barriers like dams, weirs, and culverts. This will form an ideal base for the examination of differences in species detection between connected and fragmented habitats. The study sites will be chosen based on their ecological significance, habitat variation, hydrological connectivity, and possible occurrence of rare freshwater species.

Sampling Strategies Across Fragmented River Networks

A suitable sampling strategy is critical in the identification of rare freshwater species since their genetic traces can be present in low amounts and also differ between various river sites. The suggested sampling procedure takes into account several sampling points within fragmented river networks. Such sampling points will include upstream locations, downstream locations, tributaries, and locations around physical barriers. The selection of sampling sites will take into account differences in river connectivity and environmental conditions. The sampling in different sites and sampling times will increase the chances of obtaining DNA traces of the target species and avoid any false negatives due to the presence of rare species. The samples will be obtained from both connected and fragmented river sections in order to see whether fragmentation affects the distribution of DNA traces.

Water Collection, Filtration, and DNA Preservation

Water sampling and preservation of samples are essential stages of methodology since the accuracy of eDNA detection depends on the preservation of DNA from the sampling site to laboratory analysis. Water samples are taken from the selected river sites in sterile and appropriately sterilized sampling containers. After that, water is filtered through appropriate filters in order to collect genetic material suspended in water samples, including DNA released from aquatic organisms. Filtration allows concentrating eDNA from relatively large volumes of water to small samples which can be analyzed at the molecular level. Filters with collected DNA are stored following appropriate storage procedures in order to prevent the degradation of DNA during transportation and laboratory processing of the collected material. Special attention is paid to the environmental parameters, including water temperature, turbidity, and water flow since these parameters may affect the presence of DNA.

eDNA Extraction, Amplification, and Metabarcoding

After sample preservation, environmental DNA will be extracted from the collected membrane filters using suitable molecular extraction techniques. The molecular extraction will try to minimize contamination and any other factors that could inhibit subsequent molecular analysis. After extraction of the DNA, the DNA will be assessed for its amplification potential. In order to amplify some DNA regions for identification purposes of freshwater organisms, polymerase chain reaction (PCR) technique will be used. Based on specific objectives of the study, species-specific DNA markers will be used to target rare species, and metabarcoding markers will help in targeting various aquatic species at once. The amplified genetic material will then be sequenced using high-throughput sequencing technique to obtain DNA sequences for environmental samples. The DNA sequences obtained will provide molecular evidence for the composition of freshwater organisms along different river sections.

Species Identification, Detection Reliability, and Spatial Analysis

The final methodology stage combines the identification of molecules, assessment of detection reliability, and analysis of spatial patterns of environmental DNA. DNA signatures extracted in molecular analysis are compared to existing reference databases in order to detect possible species of freshwater fish that are genetically similar. Results of the identification of species are classified depending on the locations where the samples were taken in order to identify rare species in each of the river reaches. Detection reliability will be determined taking into account multiple samples, amplification consistency, negative controls, and potential contamination during sampling and processing in laboratory conditions. Positive and negative controls will be used in order to separate possible contamination from molecular signatures. After that, spatial analysis will be conducted in order to explore the distribution of species in regard to river connectivity, barriers, and upstream/downstream relationship. Spatial mapping of molecular signatures will allow identifying places where rare species could possibly exist and need further ecological investigation.

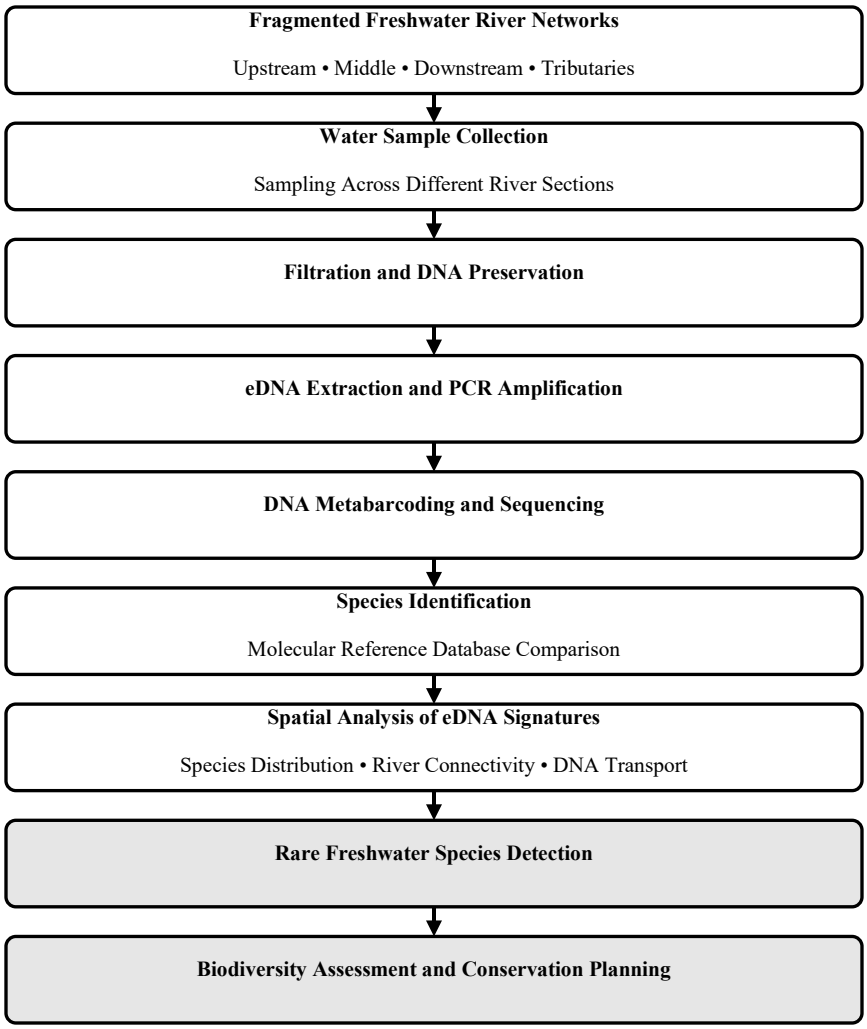


Figure 1: Architecture Flow Diagram for eDNA-Based Detection of Rare Freshwater Species in Fragmented River Networks

The entire eDNA process is shown in figure 1 from water collection along fragmented river networks to molecular processing, species identification, and spatial analysis of the results, which can help in monitoring rare freshwater species.

IV. Results and Discussion

Detection Patterns of Rare Freshwater Species

The eDNA markers have opened a window to identify rare freshwater biodiversity using the eDNA signals which would not be identified using traditional methods of identifying the biodiversity. The detection signals could be influenced by factors such as the abundance of species, the type of habitats, the rate of DNA shed, among others. Species with low abundances are likely to show weak signals making their identification possible depending on the correct sampling method used. Through sampling of the water several times and using molecular methods targeting specific species, the chances of detecting rare species in various river stretches increases. The identification of rare and invasive fish species using eDNA provides an example of how the molecular technique can be used to detect elusive species. Hence, the detection frequency and molecular signals can provide important information about the rare biodiversity found in freshwaters.

Spatial Variation in eDNA Signatures Across River Sections

The spatial distribution of the environmental DNA profiles will vary greatly between upstream, mid, and downstream reaches of rivers due to the differences in flow dynamics, habitat structure, and biological community distribution. Upstream sites will be characterized by presence of DNA profiles related to organisms living in fairly isolated freshwater habitats while the downstream sites may have a mixture of DNA profiles from several upstream sites. The presence of tributaries will further complicate the spatial distribution of the molecular profiles as DNA samples from various aquatic habitats will be introduced. Thus, the interpretation of the eDNA detection should take into account the connectivity and hydrology of the river system. The use of spatially structured analysis of eDNA can be helpful for the identification of possible biodiversity hot spots.

Table 1: Spatial Variation in eDNA Signatures Across Fragmented River Sections

River section	eDNA signature characteristics	Biodiversity interpretation
Upstream	DNA signals associated with upstream habitats	Supports assessment of species occurrence in upper river sections
Middle river	Variable DNA signals influenced by habitat conditions	Provides information about species distribution across river stretches
Downstream	DNA signals potentially transported from upstream locations	Requires consideration of downstream DNA movement
Tributary	DNA signatures potentially originating from connected habitats	Supports assessment of biodiversity contributions from tributaries
Barrier-separated sections	Differences in detection patterns across separated habitats	Helps examine the influence of river fragmentation on species detection

The table 1 above shows the spatial variability of environmental DNA traces in different sections of the rivers. It is notable how river connectivity, tributaries, and downstream transport affect the analysis of rare freshwater species presence.

Influence of River Barriers on Species Detection

Various physical barriers including dams, weirs, and culverts can affect the movements of freshwater organisms and change the ecological connectivity among segments of a river. In such cases, fish migration will be limited, and breeding populations will become isolated and separated from their habitat areas where they feed and spawn. Such ecological changes can result in distinct differences in species detection patterns between the upstream and downstream areas of a river. Since environmental DNA detection can serve as an appropriate tool to analyze these differences, genetic data can be gathered from several spots around the physical barriers in a river. Nevertheless, downstream transport of DNA should also be taken into account while evaluating genetic traces in relation to physical barriers. Fish community shifts after removal of barriers have been successfully studied using eDNA metabarcoding as a monitoring tool for such ecological changes.

Comparison of eDNA Detection with Conventional Monitoring

There are numerous advantages of using environmental DNA monitoring of biodiversity over traditional methods of assessing freshwater biodiversity. First of all, traditional approaches include the use of such methods as electrofishing, netting, and direct observation of fish. The latter might require great efforts in the field, and sometimes the mentioned methods fail to detect rare and elusive fish species. At the same time, the use of eDNA monitoring makes it possible to detect genetic material without capturing the fish at all. Moreover, metabarcoding allows us to analyze various species using one sample. Earlier researches have shown that eDNA metabarcoding makes it possible to get information on fish community structure in addition to the results of traditional fish sampling. However, there is one disadvantage of eDNA detection – genetic material can be carried or detected without identifying the exact place where the living organism is located.

Environmental Factors Affecting DNA Persistence and Transport

The environmental factors may have significant impact on the persistence, transport and detection of the environmental DNA in the freshwater ecosystems. The water temperature, flow rate, turbidity, UV exposure, microbial activities, and pH level may have some impacts on the process of degradation and movement of DNA. The higher temperatures and the increased levels of microbial activities may contribute to the faster degradation of genetic material, while the flowing water may transport the DNA over long distance away from its original source. The turbulence, sediment activities, and water dilutions may also impact the amount of genetic material present in each sample collected. In addition, the factors associated with sample collection, filtering and storage processes may impact the amount of DNA obtained.

Table 2: Environmental and Methodological Factors Influencing eDNA Detection in Fragmented River Networks

Factor	Influence on eDNA signatures	Detection implication
Water temperature	Influences DNA persistence and degradation	May affect the availability of detectable genetic material
Water flow	Supports downstream transportation of DNA	DNA may be detected beyond the original species habitat
Turbidity	Influences water conditions and sample recovery	May affect consistency of DNA detection
Microbial activity	Contributes to environmental DNA degradation	May reduce DNA persistence
Sediment interaction	May influence DNA retention and release	Can affect DNA availability in water samples
Sampling volume	Influences the quantity of genetic material collected	Appropriate volume may improve detection opportunities
DNA preservation	Influences the quality of collected genetic material	Proper preservation supports reliable molecular analysis

Factors influencing eDNA survival, transport, and recovery are listed in table 2. The table highlights the need to consider environmental variability and standardize sampling techniques while interpreting the findings from molecular analysis.

V. Conclusion and Future Perspectives

Summary of Major Findings

The current study investigated the role of environmental DNA for the detection of rare species of freshwater organisms in fragmentated river networks. The developed framework stressed the need to utilize methods of molecular identification, systematic sampling of water, DNA preservation, and spatial analyses in assessing freshwater biodiversity. It revealed the possible use of the eDNA approach as an effective method for detecting rare species without fully relying on capture-based approaches. In addition, the study stressed that the process of river fragmentations, DNA transport in downstream direction, and environmental conditions may affect molecular detection patterns. Finally, the combination of eDNA metabarcoding with geographical and hydrological data may help in better understanding the distribution of species in connected and disconnected rivers.

Contributions of eDNA Signatures to Rare Species Monitoring

eDNA fingerprints are used in rare species surveillance owing to the fact that eDNA offers a non-invasive way of detecting the existence of certain aquatic animals through the genetic fingerprint found in water samples. The use of species-specific assay allows scientists to detect whether certain endangered or rare species may be detected through the application of this technique, while metabarcoding makes it possible to study more than one freshwater organism at once. This capability becomes especially important

when working with fragmented river networks, where regular surveys cannot discover rare species existing in low densities. Regular molecular sampling may allow scientists to identify changes in the pattern of detection of different species within certain seasons and geographical location. Moreover, the combination of eDNA results and their localization helps to detect biodiversity hot spots and further requires ecological study.

Ecological and Conservation Management Implications

The use of eDNA technology has great significance in terms of freshwater ecosystem management and species conservation. Accurate molecular identification could assist conservation agencies in identifying parts of the river where rare or threatened aquatic organisms might be present. Information gathered from this technique could aid in the priority selection of sensitive habitats, ecological connectivity analysis, and habitat restoration planning. Monitoring the distribution of species near barriers such as dams and weirs could also give valuable information for assessing the degree of river fragmentation and restoration success. Furthermore, regular eDNA analysis could contribute to biodiversity monitoring through tracking changes in the pattern of species detection over time. All these findings could be used by conservation managers along with habitat and conventional ecological assessment information for better management decisions.

Methodological Limitations and Research Challenges

Although it has several strengths, there are some methodological limitations associated with the use of environmental DNA monitoring that should be noted while analyzing biodiversity data. First, downstream transport of DNA may cause the identification of DNA molecules in areas where the organism lives does not exist. In addition, the low concentration of DNA, the environmental destruction of DNA, and PCR inhibition may affect the accuracy of results. Moreover, incomplete genetic databases may limit the possibility of identification of specific freshwater organisms. Sampling volume, filtration, storage, and laboratory methods also have an effect on results of molecular detection. Furthermore, it should be kept in mind that the identification of DNA does not prove the existence of the organism and its abundance at the point of sampling.

Future Directions for eDNA-Based River Biodiversity Assessment

The need for future research on eDNA monitoring can be directed at improving accuracy, spatial resolution and practical applicability of eDNA methods for monitoring of fragmented ecosystems. The creation of new and more sensitive species-specific molecular tests and databases may improve the identification of rare and endangered aquatic organisms. Ecosystem DNA metabarcoding combined with GIS modeling, hydrology models and remote sensing technologies may give better information about species distribution and river connectivity. In addition, repetitive sampling during different seasons may help to distinguish the difference between short-term and long-term changes in diversity patterns. New analytical methods, such as machine learning, can also help to interpret molecular data as well as complex environment data. Finally, future researches should include eDNA sampling along with conventional surveys to confirm species presence and better ecological interpretation. These innovations can make environmental DNA an important tool for assessing the biodiversity of fragmented freshwater ecosystems and managing river biodiversity.

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