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Environmental DNA (EDNA) Monitoring for Biodiversity and Invasive Species Detection

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Abstract

Monitoring using environmental DNA (eDNA) has become a potent, non-invasive method for identifying invasive species and assessing biodiversity across a range of freshwater settings. This fictitious study used qPCR and metabarcoding studies at 12 sampling sites to assess species richness, community composition, and the presence of invasive species. The findings revealed a distinct regional range in biodiversity, with invasive species found only in a few areas and wetlands showing the highest species richness and marshes the lowest. The sensitivity of eDNA methods in ecological surveillance was strengthened by statistical studies that verified notable habitat-level variations. All things considered, the results demonstrate the effectiveness of eDNA as a quick and accurate tool for conservation planning and ecological threat early detection.

Keywords: Environmental DNA, Biodiversity Monitoring, Invasive Species Detection, Metabarcoding, Qpcr Analysis, Freshwater Ecosystems

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1. INTRODUCTION

Environmental DNA (eDNA) monitoring has become a game-changing technique in biodiversity assessment, providing a very sensitive and non-invasive way to identify invasive and native species in aquatic environments[1]. Due to the extraordinary biological stresses that freshwater environments around the world are facing, such as pollution, habitat fragmentation, and the rapid spread of exotic taxa, standard monitoring techniques frequently fail to capture the entire range of biodiversity that is present[2]. A more thorough and precise depiction of species existence is provided by eDNA-based techniques, which get around many of these restrictions by making it possible to detect trace genetic material released by organisms into their surroundings[3]. This study used species-specific qPCR assays and eDNA metabarcoding to assess biodiversity trends and identify invasive species in a variety of freshwater environments, providing important information about ecosystem health and early detection systems[4].

1.1.Statement of the Problem

Worldwide, freshwater ecosystems are seeing a sharp decline in biodiversity and a rise in biological invasions; nevertheless, conventional monitoring techniques are still time-consuming, labour-intensive, and frequently insufficient to identify uncommon or early-stage invasive species[5]. Because of sample constraints, observer bias, and the cryptic nature of many aquatic animals, traditional field surveys often fall short of capturing the entire range of species richness. More sensitive, scalable, and effective detection technologies are therefore desperately needed in order to enhance conservation and management by offering trustworthy ecological data[6]. Although environmental DNA (eDNA) analysis has become a viable substitute, its use is still restricted in many areas, and there is still a dearth of comparative information on biodiversity gradients and the prevalence of invasive species in various freshwater ecosystems. In order to fill these gaps, our study used targeted qPCR and eDNA metabarcoding to identify invasive taxa in various habitats and evaluate species richness patterns[7].

1.2.Background of the Study

Environmental DNA (eDNA) analysis, which provides a non-invasive, very sensitive method for identifying species presence from genetic material released into the environment, has become a revolutionary tool in biodiversity monitoring[8]. Conventional biodiversity studies frequently depend on physical capture or direct observation, which can be time-consuming, seasonally limited, and biased toward taxa that are easy to identify[9]. On the other hand, rare, cryptic, and early-stage invasive species that could normally go undetected using traditional approaches can be found utilizing eDNA techniques[10]. Rapid and precise biodiversity assessment has become crucial for conservation planning as ecosystems around the world face growing challenges from biological invasions, habitat degradation, and climate change. An effective way to monitor ecological changes, guide management choices, and improve early-warning systems for invasive species detection is to combine eDNA metabarcoding with species-specific qPCR assays. This study expands on previous advancements by employing eDNA-based techniques to assess biodiversity trends and identify possible invasive species throughout freshwater ecosystems.

1.3.Objectives of the Study

- To use environmental DNA (eDNA) metabarcoding at specific sample locations to evaluate the biodiversity of freshwater ecosystems.
- To use species-specific qPCR assays to identify and validate the existence of putative invasive species.

- To assess species richness and community composition in various habitat types, including marshes, ponds, rivers, and wetlands.
- To examine the ecological implications of geographical trends in eDNA-based biodiversity indicators for monitoring and conservation initiatives.

2. METHODOLOGY

Environmental DNA (eDNA) monitoring was used in this work to identify possible invasive species in freshwater environments and assess local biodiversity patterns. High-resolution molecular data that could assist early detection frameworks and ecological assessments was the goal of the research. Standard eDNA monitoring techniques were followed throughout all procedures to guarantee repeatability and dependability.

2.1.Description of Research Design

The study employed a field-based, cross-sectional molecular survey design. DNA metabarcoding and species-specific qPCR assays were used to analyze water samples that were gathered from several pre-selected locations. The design placed a strong emphasis on comparing biodiversity indicators spatially across environments with different levels of human impact.

2.2.Sample Details

The study didn't use any human subjects. Twelve freshwater locations, including ponds, rivers, and wetlands, provided the "samples" of water. Three replicate 1-L samples were provided by each site in order to account for regional variation. The selection of sites was predicated on historical species records, ecological significance, and accessibility.

2.3.Instruments and Materials Used

The investigation used 0.45-µm cellulose nitrate filter membranes, a portable vacuum filtration equipment, sterile sampling bottles, and nitrile gloves. A high-throughput sequencer (Illumina MiSeq), species-specific primers, universal metabarcoding primer sets (COI, 12S), qPCR chemicals, gel documentation systems, DNA extraction kits (Qiagen DNeasy), and UV-sterilized PCR workstations were among the supplies used in the lab. Sample locations were georeferenced using field GPS equipment.

2.4.Procedure and Data Collection Methods

Water samples were promptly placed on ice after being gathered at each location in sterile containers. Filters were kept in 95% ethanol prior to being transported to the laboratory, and samples were filtered on-site to minimize DNA degradation. Spin-column procedures were used to extract DNA from filters. Vertebrate and invertebrate markers were the focus of established PCR amplification procedures used to create metabarcoding libraries. Additionally, recognized invasive taxa were found using species-specific qPCR tests. To keep an eye on contamination, field blanks, laboratory blanks, and negative controls were used throughout.

2.5.Data Analysis Techniques

Chimera removal, operational taxonomic unit (OTU)/amplicon sequence variant (ASV) clustering using DADA2, and quality trimming were all part of the typical bioinformatics pipeline used to treat raw sequencing reads. Utilizing curated reference databases and BLAST searches, taxonomic assignments were carried out. To evaluate patterns of biodiversity, measures for species richness at the site level and relative read abundance were computed. Replica consistency and positive control performance were used to validate detections after qPCR data were analyzed using threshold cycle (Ct) values. Multivariate ordination (NMDS) and geographical distribution mapping were used for comparative studies.

3. RESULTS

Comprehensive molecular data from all 12 freshwater sampling sites were obtained through the environmental DNA (eDNA) survey. Across replicates, amplification success was constant, while contamination controls showed no change. While species-specific qPCR assays detected the presence of two possible invasive species at specific places, metabarcoding indicated significant biodiversity gradients. The findings shed light on the composition of communities, patterns of regional distribution, and species richness.

As a result of varying ecological complexity and environmental variation, sequencing yield and taxonomic recovery varied among sites. For each sampled location, the total reads, ASVs, species richness, and invasive species detections are shown below to provide an overview of the overall detection pattern.

Table 1: Sequencing and Detection Summary Across Sites

Site Code	Total Reads Obtained	ASVs Detected	Species Richness (Total Taxa)	Invasive Species Detections
R1	92,450	148	34	0
R2	101,233	162	37	1 (Species A)
R3	85,674	139	29	0
P1	76,554	121	26	0
P2	88,902	150	32	1 (Species B)
P3	72,331	115	24	0
W1	110,112	170	41	1 (Species A)
W2	95,881	158	36	0
W3	82,343	130	28	0
M1	67,904	108	22	0
M2	70,550	112	23	0
M3	75,100	118	25	0

According to the summary, marsh locations displayed somewhat lower biodiversity, whereas wetlands and river sites yielded the highest species richness and ASV counts. Only isolated sites were found to have invasive species, indicating localized establishment as opposed to extensive dissemination.

To verify the existence of high-risk invasive taxa, species-specific qPCR assays were conducted. Quantitative information regarding detection strength and possible abundance was provided by the Ct values acquired across sites. The table below provides a summary of these assay results.

Table 2: qPCR Ct Values for Invasive Species Targets

Site Code	Species A Ct (Mean ± SD)	Species B Ct (Mean ± SD)	Detection Status
R2	31.4 ± 0.3	ND	Detected A
P2	ND	29.8 ± 0.5	Detected B
W1	33.2 ± 0.4	ND	Detected A
All others	ND	ND	Not Detected

The Ct values revealed comparatively low DNA concentrations, and only three sites had detectable quantities of invasive species. The dependability of detection patterns was supported by the fact that no amplification was produced at any of the remaining locations or negative controls.

Species richness estimates for rivers, ponds, wetlands, and marshes were averaged in order to evaluate biodiversity across habitat categories. The following table displays the average richness and variability across these habitats.

Table 3. Mean Species Richness by Habitat Type

Habitat Type	Mean Species Richness	SD
River	33.3	3.7
Pond	27.3	3.2
Wetland	35.0	4.6
Marsh	23.3	1.5

Marsh habitats had the lowest mean richness values, whereas wetlands had the greatest, followed by river sites. The hypothesis that more diversified communities are sustained by structurally complex habitats was validated by this pattern.

3.1.Statistical Analysis

To assess species richness across the four habitat types (Marsh, Pond, Wetland, and River), a one-way ANOVA was performed. We verified that the assumptions of normality and homogeneity of variance were met. The species richness of the various ecosystems varied significantly.

The observed differences in species richness among habitat categories were tested for statistical significance using a one-way ANOVA. Below are the ANOVA table, post-hoc comparisons, and descriptive statistics.

Table 4. ANOVA – Species Richness by Habitat Type

Habitat Type	N	Mean	Std. Deviation
River	3	33.33	3.786
Pond	3	27.33	3.215
Wetland	3	35.00	4.583
Marsh	3	23.33	1.528
Total	12	29.75	5.753

ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	312.167	3	104.056	10.086	.004
Within Groups	103.333	8	12.917		
Total	415.500	11			

Post-Hoc Test (Tukey HSD)

Comparison (I–J)	Mean Difference (I–J)	Std. Error	Sig.
River – Pond	6.00	2.94	.231
River – Wetland	-1.67	2.94	.945
River – Marsh	10.00*	2.94	.033
Pond – Wetland	-7.67	2.94	.124
Pond – Marsh	4.00	2.94	.563
Wetland – Marsh	11.67*	2.94	.017

The analysis showed that the richness of the various habitat types varied significantly, with marsh sites being the most different from wetlands and rivers. The reduced richness in marsh habitats was found to be a significant contributor to overall variation by post-hoc tests.

4. DISCUSSION

The present study used environmental DNA (eDNA) monitoring in rivers, ponds, wetlands, and marsh habitats to assess freshwater biodiversity and the presence of invasive species. The results show that both native taxa and invasive species may be detected with sensitivity and reliability using eDNA techniques, with a noticeable geographical difference in species richness and community composition. Early detection of invasive species at particular locations was made possible by qPCR-based detection and biodiversity patterns that matched recognized ecological gradients.

4.1.Interpretation of Results

According to the study, rivers, ponds, marshes, and wetlands had the highest species richness. Given that wetlands usually sustain complex, nutrient-rich ecosystems that are favorable to a variety of organisms, this gradient is ecologically coherent. ANOVA-confirmed statistically significant differences in richness show that habitat structure and environmental factors have a major impact on the distribution of biodiversity. The discovery of invasive species at just three locations points to restricted introduction events or early-stage invasions. Overall, the findings demonstrate how well eDNA metabarcoding and qPCR produce high-resolution ecological insights.

4.2.Comparison with Existing studies

The results of this work complement and build upon earlier research on eDNA-based invasive species detection and biodiversity assessment. Our identification of two invasive taxa, even at low abundance levels, is supported by Larson et al.'s (2020)[11] comparable demonstration of the sensitivity of eDNA procedures for early invasive species surveillance. Variable detection efficiency across habitats was noted by Sepulveda et al. (2020)[12], and our results showed that rivers and wetlands had higher species richness than ponds and marshes. In line with the somewhat reduced species diversity found in our pond and marsh sites, Harper et al. (2019)[13] noted that small and shallow water bodies frequently exhibit inconsistent eDNA signatures. According to Beng and Corlett (2020)[14], eDNA metabarcoding accurately depicts wide biodiversity gradients, which is in line with the distinct regional patterns found in our sequencing data. Last but not least, Xia et al. (2018)[15] verified that eDNA can help with early detection of invasive bivalves and other cryptic species, supporting the efficacy of the qPCR-based detections found in this investigation. When taken as a whole, these comparisons show that our findings provide fresh spatial insights across a variety of freshwater habitat types while also being highly consistent with the body of scientific knowledge.

4.3.Implication for Existing Studies

These findings align with, extend, and support existing work in the field of eDNA-based ecological monitoring:

- They support earlier findings that eDNA metabarcoding accurately captures biodiversity at the community level in a variety of environments;
- The species richness gradient reflects trends found in ecological studies that indicate more diversity in habitats with complex structural features.
- Research showing that eDNA can provide quick, inexpensive surveillance in comparison to traditional nets or visual techniques is supported by early invasive species detection.

- The work adds to the increasing number of studies confirming eDNA as a tool for managing invasive species and conservation planning.

4.4.Limitations of the Study

Despite its strengths, the study had several limitations that should be acknowledged:

- While sufficient for an exploratory survey, the sample size might restrict generalizability to larger ecological regions.
- The lack of direct measurements of environmental conditions that affect DNA degradation, such as temperature, turbidity, and UV exposure, may have an impact on detection rates.
- Some local species may still not be fully covered by reference databases used for taxonomic assignment.
- There was a chance that invading taxa would go undetected because qPCR detections depended on a small number of species-specific assays.

4.5.Suggestions for Future Work

To advance the scope and reliability of eDNA monitoring, future studies should consider the following directions:

- To capture seasonal variation in biodiversity dynamics, use more extensive geographical and temporal sampling.
- In order to improve species-environment correlations, incorporate environmental metadata, such as water chemistry, temperature, and hydrological conditions.
- To enhance invasive species surveillance, broaden the range of species-specific qPCR assays.
- To improve taxonomic resolution and find rare or cryptic species more efficiently, use long-read sequencing technology.
- Create hybrid monitoring frameworks by combining eDNA data with conventional ecological surveys.

5. CONCLUSION

This study proved that environmental DNA (eDNA) monitoring is a sensitive and all-encompassing method for identifying invasive species and evaluating freshwater biodiversity. Strong insights into community composition, regional changes, and early warning indicators for ecological disturbances were obtained through the use of species-specific qPCR assays and metabarcoding. The results confirm eDNA's expanding function as a non-invasive genetic technique that can assist programs for ecosystem observation and biodiversity conservation.

5.1.Summary of Key Findings

The study yielded several important findings:

- Species richness differed greatly among habitat types, with marsh sites exhibiting the lowest biodiversity and wetlands the highest.
- The ability of eDNA to capture fine-scale ecological variation was confirmed by the successful identification of different taxa across all 12 sites by metabarcoding.
- Two invasive species (Species A and B) were found at particular places by qPCR assays, demonstrating the potential of eDNA to assist early detection systems.
- ASV counts and sequencing depth were uniform across sites, suggesting dependable laboratory procedures and sampling.
- The validity of the molecular data was supported by the absence of contamination in the field or lab controls.

5.2.Significance of the Study

The study adds to the increasing body of evidence demonstrating the effectiveness and scalability of eDNA monitoring as an ecological evaluation method. The results highlight the usefulness of eDNA in real-time ecosystem monitoring, biosecurity control, and conservation planning by demonstrating distinct variations in biodiversity across habitat types and accurately identifying invasive species. Molecular techniques like eDNA provide an effective way to get precise biodiversity data without endangering natural habitats as environmental problems grow more complicated.

5.3.Recommendations

Based on the study findings, the following recommendations are proposed:

- In order to discover invasive species early, establish regular eDNA surveillance at high-risk locations.
- Combine eDNA monitoring with traditional ecological surveys to improve programs for assessing biodiversity.
- Create reference DNA databases tailored to certain regions to increase the precision of taxonomy assignment.
- Promote eDNA-based monitoring as an addition to national biodiversity monitoring frameworks for conservation organizations and policymakers.
- Perform long-term seasonal eDNA investigations to record changes in species occurrence and environmental circumstances throughout time.

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