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Environmental DNA Metabarcoding for Assessing Freshwater Alien Species Invasions in Under-Monitored Tropical River Basins

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Abstract

Often biological invasions are mounting to freshwater ecosystems in tropical areas but the lack of monitoring because of the high species richness, lack of expertise to track the taxonomy and logistical hurdles of traditional surveying techniques. Environmental DNA (eDNA) metabarcoding has been adopted as a revolutionary molecular methodology that is capable of identifying aquatic life via DNA fragments

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released into water bodies that are non-invasive, sensitive and scalable as an alternative technique to biodiversity. This research paper is an evaluation of the applicability and efficacy of eDNA metabarcoding in the detection and evaluation of freshwater alien species invasion in under-monitored tropical river basins. Water samples were taken in a systematic manner and were taken at the upstream, midstream and downstream parts of the river which represented a diverse ecological as well as anthropogenic setting. EDNA was extracted and its amplification was done with standardized universal barcode markers and subjected with high throughput sequencing and powerful bioinformatic pipelines. These findings indicate that eDNA metabarcoding is significantly more efficient in comparison to the traditional monitoring methods as it will show the diversity of a greater number of alien taxa, such as cryptic, rare, and pre-immune species that usually cannot be observed with the help of traditional methods. Through spatial analysis, clear invasive hotspots were found that were closely linked to anthropogenic processes, change in land-use, and hydrologic connection of river networks. The results indicate that eDNA metabarcoding can offer fast, affordable, and precise evaluations of freshwater biological invasions in information void tropical systems. The introduction of eDNA-based surveillance into the current conservation and biosecurity systems can play a big role in the early detection, evidence-based management choices and long-term protection and sustainable management of vulnerable tropical freshwater ecosystems.

Keywords

Environmental DNA, metabarcoding, alien species, freshwater invasions, tropical rivers, biodiversity monitoring.

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Introduction

Threats to Freshwater Ecosystems in Tropics

Among the most affected ecological systems in the world are freshwater ecosystems which are constantly degraded through of habitat changes, pollution, climate change and growing invasions of biological species (Yeonjin, 2025; Suresh kumar, & Rajan, 2026). River basins sustain tremendously large proportions of biodiversity and endemism in tropical settings, but are growing threatened by human pressures in the form of land-use shift, agricultural intensification, urban growth, and hydrological transformation (Dahlan Abdullah, 2024). Alien invasions are another important cause of ecological conflict, which causes competition with the local species, change in the trophic relationships, habitat, and drastic economic losses (Nathan et al., 2014). Although there is a high ecological value, tropical freshwater systems are poorly monitored, which restricts the invasive species to be detected on time before managing them.

Weaknesses of Traditional Monitoring Solutions

Conventional approaches to biodiversity monitoring of freshwater such as netting, electrofishing, trapping and visual survey are generally resource consuming and require knowledge in taxonomy (Dahlan Abdullah, 2024). Such methods are often incapable of detecting rare, cryptic or low abundance species, especially at the initial stages of invasion when such management interventions have the greatest impact (Khan, 2025; Rahim, 2025). The lack of infrastructure, complexity of habitats, richness in species, seasonal hydrological similarity and complexity of habitats in tropical river basins in addition limit effectiveness and dependability of traditional monitoring programmers. Harvest, the consequence of such invasions is that, in such regions, the biological invasions are usually noticed when they are too well-established and ecologically harmful.

Environmental DNA Metabarcoding as A New Tool

The environmental DNA (eDNA) metabarcoding has become a highly effective molecular method to conduct assessment of aquatic biodiversity. Biological beings release genetic material in their environment in skin cells, mucus, faeces, and reproductive products and these can be collected and determined by examining water samples. eDNA metabarcoding is a method of genetically marking different taxa using universal genetic markers with a scaleable technique called high-throughput sequencing, which brings multiple taxa under observation without any physical capture and visualization. It is cost-effective, has great sensitivity, and is scalable and hence is more effective in managing invasive species and identifying biodiversity in data-limited tropical freshwater systems.

Study Objective and Study Significance

The current research project will compare the performance of eDNA metabarcoding as a method to determine the invasion of alien species of freshwater in unexplored tropical river basins. In particular, it is aimed at the comparison of detection efficiency with traditional monitoring processes, the determination of the patterns of distribution of invasive species in space, the role of anthropogenic factors and hydrological processes in invasion processes. This study aims at providing contributions to the current research in producing viable, early-detection systems that enable the biodiversity preservation, treatment of invasive species and the regulation of freshwater systems.

Related Work

Environmental DNA (eDNA) is a fast-developing potent tool of conducting molecular ecology to track biodiversity in water, which has serious benefits over conventional methods of performing the survey. Alterations preliminary research indicated that it was possible to discover uncommon and endangered aquatic animals via eDNA that was extracted out of water samples, emphasising its level of sensitivity and its non-invasive character (Cantera et al., 2019; Fukaya et al., 2021). This method later became extended to general biodiversity indices, and eDNA metabarcoding was shown to be a useful system in order to monitor multi-species presence in freshwater environments at the same time (Nayak, 2025; Gillet et al., 2018; Tandi, 2025).

Comparative analyses done of temperate river systems have uniformly indicated that eDNA metabarcoding demonstrated superiority over other standard techniques like electrofishing and netting in species richness capturing and early detection of invasive taxa (Goldberg et al., 2013; Karthik et al., 2014). Multispecies methods models also supported the idea that eDNA offers a greater detection probability, especially with low-abundance and cryptic species which go undetected with other distribution surveys (McColl-Gausden et al., 2021). Such results promote the possibility of using eDNA as a biological invasion early-warning system (Senapati et al., 2019).

Regardless of all these developments, the implementation of eDNA metabarcoding in freshwater systems in the tropics is also facing its own peculiar challenges. By using high temperatures, DNA can be degraded faster, whereas complex hydrology can affect the eDNA transportation and preservation (Muha et al., 2017; Sindhu, 2025). Moreover, partial data on reference databases of tropical species hinder the identification of taxa and the degree of confidence on species identification (Nasir et al., 2020; Sigsgaard et al., 2015). However, according to recent researches carried out in southeast Asian and tropical river systems, it has been showed that marker selection and optimization of sampling techniques can lead to dramatic improvement in detection rates (Bosco & Pavalam, 2025; Kavitha, 2025).

Ecological and hydrodynamic models have also been integrated with environmental DNA to make the species distribution and abundance more successful in providing more information about the invasion processes (Madhanraj, 2025). Moreover, the integration of eDNA data with species distribution modelling has been also very useful in predicting the risks of invasion and that there might be hotspots of freshwater invaders (Arvinth, 2025; Usun & Ait Fares, 2025). Nevertheless, full, basin-wide evaluation of alien species invasion with eDNA metabarcoding of under-monitored tropical rivers has been limited. This work contributes to the available information by reviewing the suitability of eDNA metabarcoding as a tool to identify and monitor invasion of freshwater alien species in tropical river basins (Thomsen et al., 2015).

Materials and Methods

The selection of a study area and the design of the sampling were performed using a convenience or quota sampling method.

Tropical River Basin Selection

The ecological significance of the basins of under-monitored tropical rivers was taken into consideration, as well as their susceptibility to biology invasions and absence of historical biodiversity data. Focus was directed at river systems that are facing growing anthropogenic pressure such as land-use change, agricultural growth, urbanization and altered hydrological regimes since these are known to encourage anthropogenic introductions and propagation of alien species. The sampling was conducted in such a way that it represented a combination of a variety of environmental factors and invasion risks that are often realized in the tropical freshwater environment, and in the general sense, the results would apply to similar areas that lack data.

Stratification of Sampling locations (Spatial)

In order to measure spatial variability of spatial species distribution and invasion patterns, a stratified sampling across upstream, midstream and downstream river segments was used to capture these patterns. These upstream locations were generally considered to be relatively undisturbed or forested, the middle part was affected by agricultural incidents, and the lower ones were denoted by urban and industrial influences. It was possible to evaluate the invasion gradients along the river continuum with this spatial stratification and to identify the possible invasion hot spots related to human activity, as well as hydrological connections. The scientific distribution of sampling sites provided extensive space coverage and better identification of the known and the emergent alien species.

Design Temporality and Seasonal Design

Sampling within various seasons was done to manage the temporal difference in hydrology and its effects on the environmental DNA (eDNA) movement, dilution, and retention. The variation of rivers, rainfalls and temperature of water with the season may lead to changes in eDNA concentration and probability of detection. The study design further increased the reliability of the species detection method by using seasonal sampling as well as the ability to assess the invasion patterns more precisely by the year to assess the results Figure 1. This time scale made sure that the structure of the monitoring took into account dynamic ecological processes that are inherent to the tropical river systems.

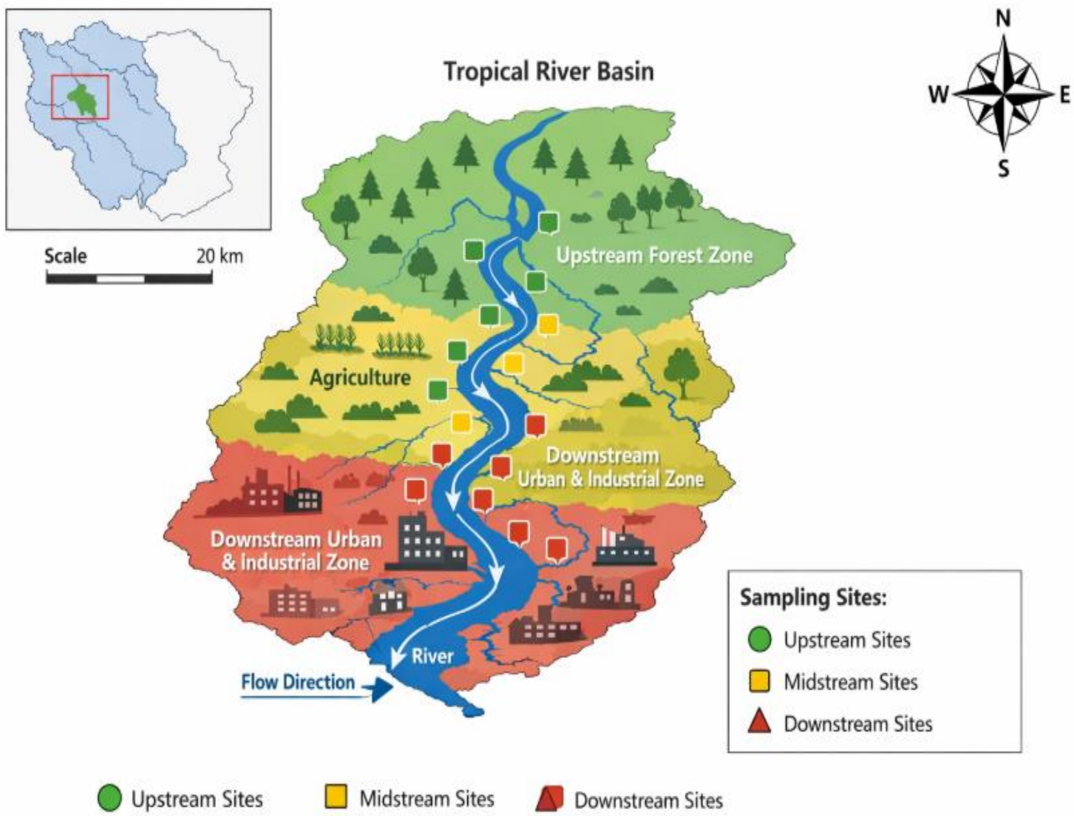

Figure 1. Study area and spatial distribution of sampling sites across the tropical river basin

Environmental DNA Collection and Preservation

Sampling and Control of Contamination of Environmental DNA

The environmental DNA samples were collected at the sites through single use, sterile containers to reduce chances of cross contamination. The surface water samples were taken in accordance with the eDNA field protocols and the handling of the samples was done gently not to come in contact with non-sterile surfaces. Biological replicates were also obtained at every site to enhance the probability of detection and examines the heterogeneity in the distribution of eDNA across space. The sampling process was done with the inclusion of field blanks and negative controls, which were aimed to determine the presence of possible contamination by equipment or handling, as well as environmental exposure, and therefore data integrity and reliability of the collected samples were guaranteed.

Sample Preservation and Transfer

To avoid degradation of DNA samples, water samples were filtered immediately on the sampling site or it was preserved at chilled conditions pending it could be filtered. The extracellular and intracellular DNA was trapped and filtered using sterile membrane philtres and stored in sterile tubes. All the samples were also taken to the lab with controlled temperature in order to preserve the DNA integrity Figure 2. The suitable preservation and handling conditions played an important role in reducing the loss of DNA and also effective downstream molecular analysis especially in the tropical region where elevated temperatures may hasten deterioration of DNA.

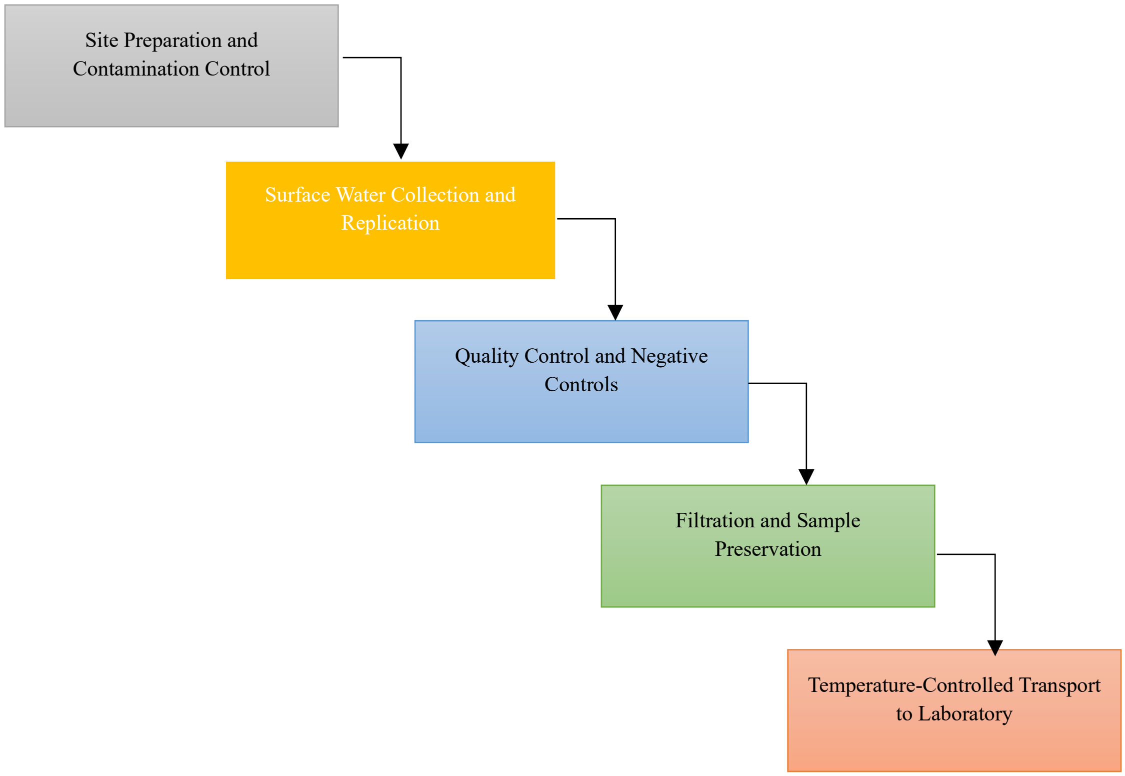

Figure 2. Workflow for environmental DNA collection, contamination control, and sample preservation

DNA Extraction, Amplification, and Metabarcoding

Environmental DNA Extraction

The DNA was also obtained on the membrane philtres as an environmental DNA which was extracted in the form of environmental DNA using commercially available assays that were specially designed to recover low-concentration DNA in aquatic samples. The extraction was done in a clean laboratory environment to reduce crossover contamination and the negative extraction controls were done to cheque any possible laboratory-based contamination. The extraction protocol was formulated so as to acquire efficient intracellular and extracellular DNA fragmentation that offers total acquisition of genetic material that is shed off by aquatic organisms, in the sampled environment, in a thorough manner.

PCR Amplification and Assay Probe

The amplifications were performed with universal metabarcoding primers and were aimed at the standardised primers of the mitochondrial genetic markers, such as cytochrome c oxidase subunit I, (COI) and 12S ribosomal RNA genes. They have been chosen as these markers have been tested to have good taxonomic and ideal performance in freshwater biodiversity assessment. The optimization of PCR conditions was aimed to minimise the amplification bias along with the reproducibility, and technical replicates were introduced in order to increase the detection reliability of a wide range of taxonomic groups including fish, amphibians, mollusks, and crustaceans.

Preparation of Libraries and Metabarcoding Workflow

Amplified PCR products were differentiated into indexed sequencing libraries to permit multiplexing of a number of samples in a single high-throughput sequencing run. Each sample was given a unique molecular index to make sure that each sample has been properly demultiplexed in the downstream analysis. Standardised metabarcoding procedures were used to prepare library samples to ensure that consistency and minimise sequencing artefacts Figure 3. The method facilitated large scale sequencing and provided an effective means of measuring biodiversity of freshwater and alien species presence in all the sites of sampling.

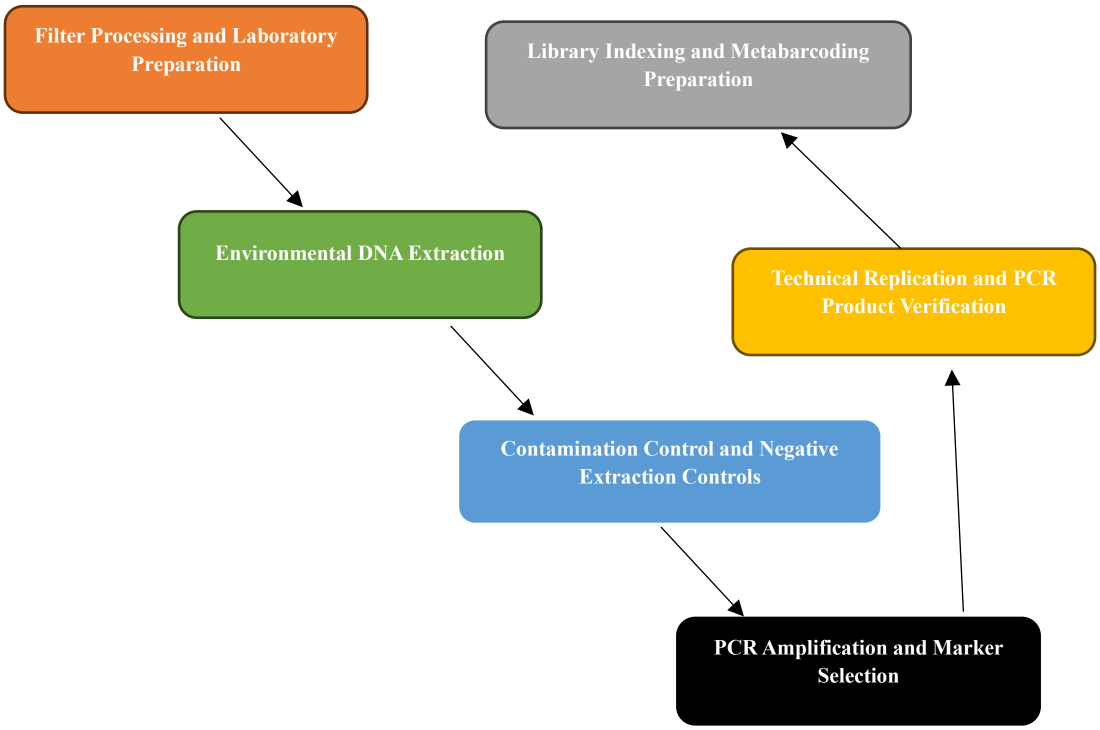

Figure 3. Laboratory workflow for environmental DNA extraction, amplification, and metabarcoding

High-Throughput Sequencing and Bioinformatic Analysis

High-Throughput Sequencing

Next-generation sequencing specifications were likewise undertaken using readable sequencing libraries that had been prepared, operated in vast amounts and generated significant amounts of high-quality sequence reads on a single run. These allowed extensive reporting of amplified genetic markers to allow both abundant and low-abundance taxa to be identified in the environmental samples. Each sequencing run involved the appropriate controls to keep track of the sequencing performance and possible index cross-talk. The sequencing depth was high and needed to capture the ordinary and early-stage species of aliens in complicated freshwater places.

Pipeline Bioinformatics Processing

The data were rigorously processed under a bioinformatic workflow in order to ensure data accuracy and reliability. The first step was to philtre quality reads and eliminate poor quality reads, primer sequences and sequencing artefacts, then chimaera sites were detected followed by chimaera elimination. The sequences of a high quality were then grouped into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) using known bioinformatic pipelines. It was a standardized method that reduced the possible effects of analytical bias and could assure reproducible representation of samples with respect to taxonomic diversity.

Spatial Analysis and Taxonomic Assignment

Taxonomic classification was done through the process of matching the processed sequences with pre-existing reference databases of verified freshwater taxa. Particular focus was placed on the precise determination of known alien and invasive species in order to aid in invasion evaluation. The patterns of spatial distribution of detected alien species were then compared to define areas of invasion hotspots and test how they relate to environmental variables, as well as anthropogenic factors, including land use and hydrological connexions Figure 4. The combined analysis framework presented information about the dynamics of invasion and facilitated the use of evidence-based management advice.

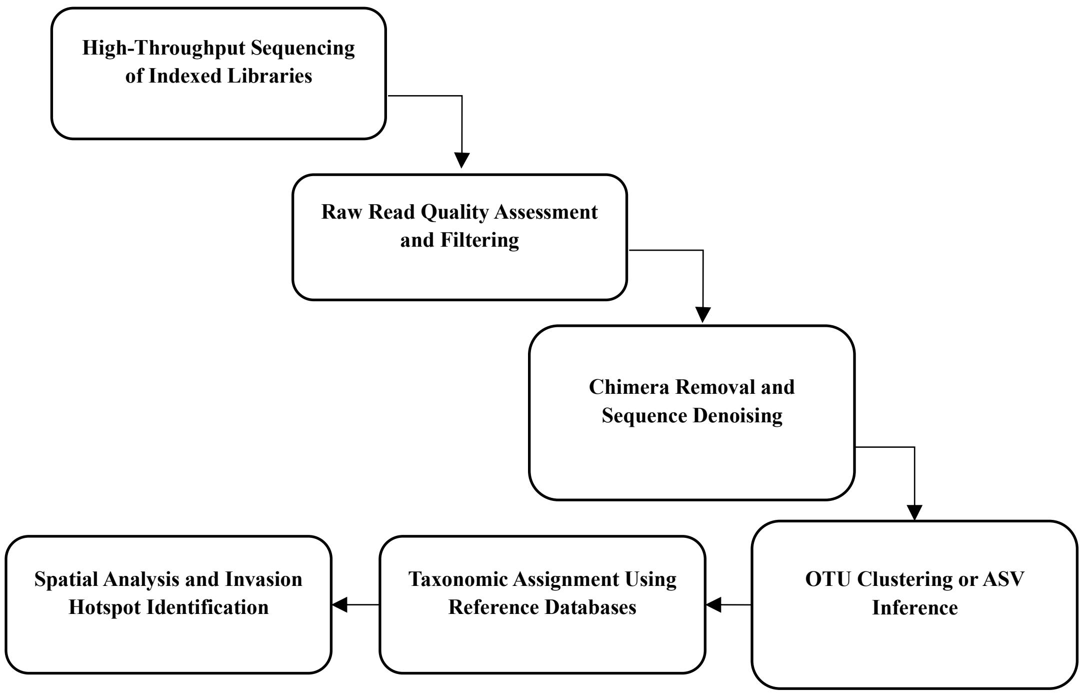

Figure 4. Workflow for high-throughput sequencing and bioinformatic analysis of environmental DNA data

Results and Discussion

Improved Detection of Freshwater Alien Species with the help of eDNA

The use of environmental DNA metabarcoding has led to a significantly better identification of freshwater taxa than traditional ways of monitoring. The study was able to identify several alien taxa that were not previously documented in the investigation areas such as low-abundance and cryptic species. There was high sensitivity in the detection of eDNA at early stages of invasions and this showed that eDNA was capable of exposing biological invasions at the initial stages before the population could be established. These findings indicate higher detection efficiency of eDNA metabarcoding and its usefulness as an effective invasion surveillance monitoring in information-gap tropical river ecosystems.

Invasion Hotspots and spatial patterns

The analysis of the spatial distributions has shown that there are definite trends of alien species representation in the continuum of rivers. The areas where invasion was overwhelmingly dominant were the midstream and downstream areas, which are heavily impacted by agricultural discharge, urban discharge, as well as augmented hydrological inter-relationships. Such regions are likely to provide the entry and distribution of alien species by anthropogenic transfers and disturbed habitats. Conversely, upstream forested sites had a lower abundance of alien species implying that less anthropogenic disruption and environmental obstacles to natural environment inhibit successful invasion. The significance of factors in the landscape level influenced the freshwater invasion dynamics is emphasized by this spatial differentiation.

Time Changeability and Hydrological Effect.

ANOVA revealed that the detection of alien species was significantly variable during the sampling season and strongly linked to the seasonal factors of river flow and the hydrology. Increased discharge periods also seem to affect eDNA transport, dilution, as well as persistence, and its subsequent influence on detection probability. These results highlight that sampling at any individual time can incorrectly estimate the extent of invasion and instead, the repetitive seasonally informed sampling can preferably offer the correct estimates. Integration of temporal replication will improve reliability of eDNA-based surveillance especially in tropical river systems that have high seasonal variability.

Consequences to Surveillance and Monitoring Systems

The aggregated findings confirm that eDNA metabarcoding is a highly appropriate, non-invasive and scalable method in monitoring freshwater alien species in under-monitored tropical river basins.

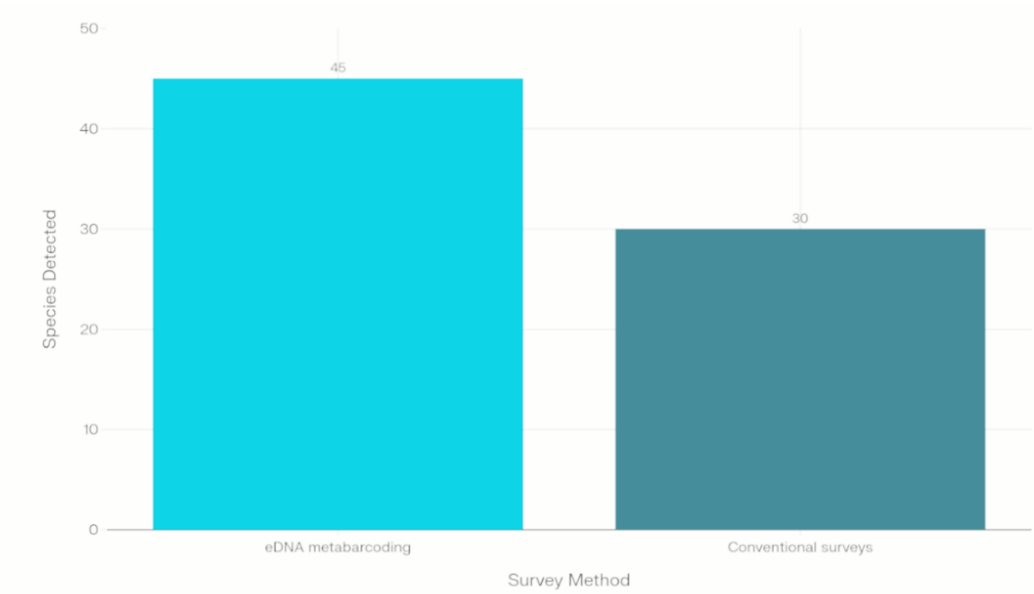

Figure 5. Comparison of freshwater species detection using eDNA metabarcoding and conventional survey methods

The fact that the system was able to detect previously unknown invaders demonstrates its promising features as an early warning system, giving the ability to conduct timely management interventions before ecological and economic consequences of the issue become serious. Nevertheless, the issues like incomplete reference databases and uncertainty issues concerning the eDNA transportation dynamics need to be overcome in order to enhance taxonomic resolution and spatial interpretation. By complementing freshwater invasion surveillance systems with eDNA metabarcoding deployed together with hydrological modelling, ecological risk assessment, and traditional surveys, freshwater invasion surveillance could be enhanced and aid in making evidence-based decisions in conservation and policy.

Table 1. Summary of freshwater alien species detection and invasion patterns using eDNA metabarcoding

Assessment Aspect	Observation	Key Interpretation
Detection method comparison	eDNA metabarcoding detected a higher number of freshwater taxa compared to conventional surveys	Demonstrates superior sensitivity of eDNA for detecting alien, rare, and cryptic species
Alien species detection	Several confirmed alien species identified, including previously unreported taxa	Indicates strong potential of eDNA for early invasion detection
Early-stage invasions	Low-abundance and cryptic species detected at initial invasion stages	Supports eDNA as an effective early-warning monitoring tool
Spatial distribution	Higher alien species richness in midstream and downstream river sections	Invasion hotspots associated with anthropogenic pressure and hydrological connectivity
Upstream sites	Lower alien species richness in forested upstream regions	Suggests natural barriers and reduced disturbance limit invasion success
Temporal variability	Seasonal variation in alien species detection linked to flow conditions	Highlights influence of hydrology on eDNA transport and persistence
Sampling strategy implication	Single-time sampling may underestimate invasion extent	Repeated, seasonally informed sampling improves accuracy
Management relevance	eDNA enables rapid, scalable, and non-invasive monitoring	Supports integration into freshwater biosecurity and conservation frameworks

Conclusion

The research findings in this project affirm that environmental DNA metabarcoding is a robust, sensitive and scalable method of evaluating the invasion by alien species in freshwater rivers in remote tropical river basins. eDNA metabarcoding surpasses several of the main challenges of traditional ways of monitoring, such as low rates of detection, large quantities of logistical and technical effort, and taxonomic restrictions, thus making it possible to conduct a global outlook of biodiversity and prevent multiple invasive taxa. This capacity to detect cryptic and early invasions helps on its weaknesses of being a proactive monitoring tool that can provide timely management interventions. The use of eDNA-based monitoring, which can be integrated into freshwater biosecurity and conservation models, can have a significant positive impact on the monitoring of invasions, evidence-based decision outcomes, and sustainable management and long-term preservation of vulnerable tropical freshwater ecosystems to rising anthropogenic stresses.

Author Contributions

All Authors contributed equally.

Conflict of Interest

The authors declared that no conflict of interest.

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