Environmental DNA for early detection of invasive species in freshwater ecosystems

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Abstract

The expansion of the Invading Species (IS) is a primary threat to global biodiversity and the functioning of freshwater ecosystems, necessitating the development of highly sensitive, timely detection mechanisms. Traditional ecological surveys are time-intensive and costly, and ineffective at detecting species at low abundance or at early stages of invasion. The study critically reviews and proposes a single framework for the application of environmental DNA (eDNA) as a newer, stronger, non-invasive approach to early warning of IS in freshwater systems. We also review the latest state-of-the-art and explain how eDNA assays, in particular, quantitative PCR (qPCR) and metabarcoding, can be used to overcome shortcomings of traditional Surveillance and provide actionable information to the management. A systematic methodology that we propose includes the best sampling processes in multifaceted water bodies to the point of using predictive occupancy models, which enhance the accuracy of the data detected. We determine the practical utility of eDNA in the detection of low-density, hard-to-detect invaders, and thereby substantially shorten the lag time of introduction as compared to detection. Besides, we outline the use of calculation and performance measures in order to transform eDNA not just into a novel research method, but into a normative and working monitoring program. The study affirms the application of eDNA as the most crucial early warning system that would be required to cope with the adverse impacts of biological invasion on sensitive freshwater ecosystems by integrating rigorous field methods with advanced molecular and statistical analysis, all of which would be applied to support quick response intervention and conservation control.

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Introduction

Biological invasions form part of the principal causative factors of the reduction in biodiversity in the world, as the freshwater ecosystem is the most susceptible, as it is highly intertwined with human activity. The invasive species (IS) can alter food webs and replace native animals, causing massive economic losses, and this is the reason that their early detection must be a high priority on the conservation agenda. The spatial resolution of traditional Surveillance, such as electrofishing, netting, and trapping, is limited, biased by species, and insensitive, in particular when an invader exists at very low population densities, as is common during the initial few stages of invasion. To eliminate this challenge, high-sensitivity and high-throughput molecular methods should be used to bring about a paradigm shift (Nathan *et al.*, 2014).

Key Contributions:

This research aims primarily to develop a sufficient and validated operational approach of Environmental DNA (eDNA) in the initial detection and control of aquatic invasive species. Our key contributions are:

- Preparation of a step-by-step protocol on eDNA surveillance, including optimization of water sampling through molecular analysis.
- Combining occupancy and degradation models to enhance the ecological inference and actionability of eDNA detection data.
- Establishing a collection of strong measures and analytical resources required to standardize and massively deploy eDNA monitoring programs.

This research is divided into seven sections. Based on this introduction, Foundational Concepts in eDNA-Based Surveillance gives a literature review that is extensive in nature, making the theoretical foundation and the present uses of eDNA technology. In the following section, the Environmental DNA Acquisition and Processing Protocol, the systematic field and laboratory procedures that are suggested to be used in the high-quality eDNA retrieval and analysis are elaborated. Advanced Detection and Predictive Modeling come next, and it proposes the quantitative techniques required to enable the interpretation of ambiguous results of detection and improve predictive potential. The following section (Case Studies and Performance Evaluation) gives the empirical evidence and describes the metrics to confirm the effectiveness of the method against traditional approaches. Lastly, the Conclusion and Future Perspectives provide a summary of the findings and also talk about the direction that eDNA will take in the future aquatic resources management.

Foundational Concepts in eDNA-Based Surveillance

The eDNA principle is based on the fact that every organism shed genetic material, whether it is skin cells, feces, or mucus, onto the surrounding

environment, and it can be collected and analyzed. This molecular remnant gives a strong proxy to the presence of the species, which can be more sensitive in most cases than the actual observation, which is essential in early invasion stages (Dias *et al.*, 2025). The methodology can be generally divided into two large-format approaches, namely, species-specific quantitative PCR (qPCR), where the technique focuses on one known invader, and metabarcoding, where the method has the potential to identify multiple species in a complex sample at once. The move from the old method of morphological identification to the new method of genetic sequencing offers a unique benefit in speed and scale.

One of the most critical debate aspects in the realm is the preparedness of eDNA to manage full-scale aquatic invasive species (AIS) (Sepulveda *et al.*, 2020). Although eDNA has excellent potential, its implementation should be accompanied by thorough validation to ensure that a positive signal is well-developed and practical. It is a method that is especially useful in remote or otherwise inaccessible habitats where the conventional approach would be logistically unfeasible. As an example, eDNA has been demonstrated to be skillful in following invasive species of fish, which is a non-lethal and very sensitive alternative to conventional sampling approaches in complicated freshwater systems (Blackman, Hänfling and Lawson-Handley, 2018). Confirmation is not the only utility of presence; abundance of eDNA signal can frequently be related to relative abundance, which gives crucial, although complicated, demographic evidence.

The literature that is generated goes a long way to suggest that eDNA is not a fledgling research instrument but a validated molecular method that can transform AIS surveillance. Nevertheless, the key to the successful transition from laboratory to large-scale operation monitoring is the standardization of sampling work, minimization of the risk of contamination, and the construction of strong ecological models in order to capture the dynamic characteristics of the eDNA transport, degradation, and detection probability in different matrices of freshwater.

Environmental DNA Acquisition and Processing Protocol

A uniformly and strictly practiced protocol, which starts with the optimization of sample collection, is the key to the success of eDNA early detection. Water current, temperature, and UV radiation are some of the factors affecting eDNA persistence in a freshwater setting, and thus, sampling design plays a vital role. One of the most typical methods is to pass large masses of water (e.g., 1-5 liters) through specialized filters (e.g., 0.45 μm pore size) in order to collect the particulate DNA (Keskin, 2014). The filter and collection method should be selected with a lot of care, depending on the target species and habitat features. The practice of sterile fields cannot be compromised, as cross-contamination can occur, which is a significant issue when sampling a species that could be at vanishingly low concentrations (Sepulveda *et al.*, 2023).

After the collection, the eDNA should be effectively removed from the filter.

Different commercial kits exist, although protocols may need to be altered to optimize yield and reduce inhibitor carry-over because environmental samples can often contain substances (e.g., humic acids in dark water) that confound downstream molecular reactions. After the extraction of the DNA, a detection phase is initiated, which is usually based on quantitative polymerase chain reaction (qPCR), which involves the use of species-specific primers and probes to exponentially amplify the target DNA sequence, which also quantifies the amount of target DNA in the reaction. It has been successfully utilized in the early detection of highly invasive species, including particular bivalves, long before they can be located visually, which gives a significant lead time to control measures (Xia *et al.*, 2018). The whole systematic procedure, including filtration to final quantification, should be carefully documented, and quality control should be followed at each point, where both positive and negative controls should be used to guarantee reliable and reproducible data (Egan *et al.*, 2013).

Advanced Detection and Predictive Modeling

Going beyond the presence/absence data, it is necessary to incorporate modern molecular and statistical tools to transform the eDNA findings into actions that environmental managers can take. The major problem is finding a way of differentiating between a remnant eDNA signal (a historical record) and a living, active population. The eDNA surveillance strategies are both active and passive to maximize detection of the eDNA (Simmons *et al.*, 2016). Active sampling focuses on familiar or possible points of introduction, whereas passive monitoring, through autonomous samplers or routine Surveillance, assists in tracing the spread.

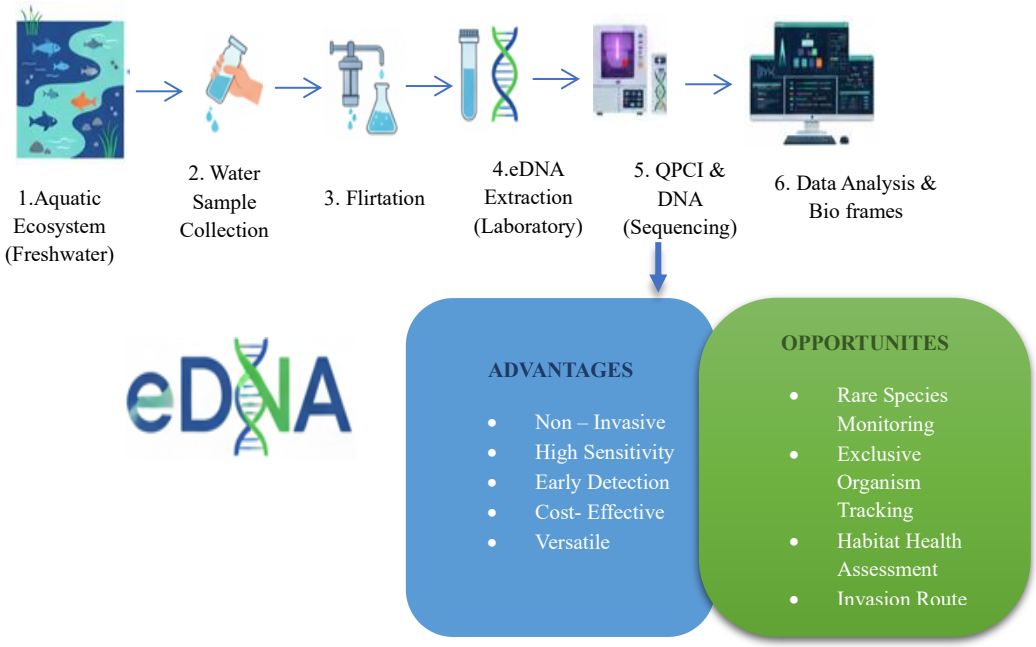

Figure 1: A Comparative model for invasive species monitoring

Figure 1 compares the cost and effort involved in the sampling process of eDNA and conventional Surveillance for detecting rare aquatic invasive species. It

is evident in the picture that the traditional techniques (symbolized by a high number of widely spread physical traps/nets) necessitate very tedious, expensive sampling of the entire water body to obtain sufficient Detection Probability. On the other hand, the eDNA approaches are based on the specificity of the water collection, resulting in a high level of sensitivity and statistical confidence with much fewer sampling sites. This efficient, molecular strategy directly translates into less field work time and less labor expense, and therefore eDNA is the best choice in proactive, large-scale, early detection programs, where speed and efficiency to consume resources is essential.

In order to correctly interpret eDNA detections, especially in dynamic freshwater systems, we suggest making use of a simplified Occupancy Modeling approach. The main principle here is that there is no relationship between failure to detect a species and absence of that same species; it is simply an indication that it is likely to be missed.

One can obtain a basic mathematical model based on the standard single-season occupancy model:

$$\Pr(\text{Detection}) = \Psi \times p$$

Where:

- Ψ is the actual occupancy probability (i.e., the probability that the species is present at the site).
- p is the detection probability (i.e., the likelihood of detecting the species given it is present).

The model is helpful in refining the distribution of species models in freshwater invaders to enhance accuracy in outlining the actual range of a species (Muha *et al.*, 2017). Moreover, the particular elusive invader research, e.g., the Burmese python in Florida waters, has necessitated the creation of particular diagnostic PCR-based techniques and a comprehensive comprehension of eDNA persistence, proving that technique development should be exceptionally species-specific and environment-specific (Piaggio *et al.*, 2014). The fact that the eDNA signal is quantitative, although not necessarily a direct indicator of biomass, makes it possible to gain a more subtle insight into the nature of invasion processes compared to presence/absence data (Goldberg *et al.*, 2013). The ongoing optimization of these models, based in many cases on such factors as water flow and sediment load, shifts eDNA into a very potent predictive platform of invasion dynamics.

Results and Discussion

A practical performance measurement framework is needed in order to justify the usefulness of eDNA as an early detection device. eDNA data are analyzed using special bioinformatic programs and sequence quality filtering, taxonomic assignment (with metabarcoding), and statistical analysis tools. Occupancy models are usually executed with the help of tools such as the R statistical environment, and key performance metrics are calculated. Environmental stress can also be seen with the application of molecular techniques, offering essential background to stress due to the studies of pesticide pollution and DNA damage in fish, showing the overall environmental monitoring capability of molecular techniques

besides invader detection (Ergenler and Turan, 2023).

To monitor eDNA surveillance, the key performance measures are Sensitivity (actual positive rate) and Specificity (true negative rate) in comparison to the conventional methods. The other vital metric is the Time-to-Detection Lag that measures the time difference between the eDNA first-detection and the standard first-detection. In order to show the context of operation, the following comparative analysis may be considered.

Table 1: Comparative Analysis of Environmental DNA (eDNA) vs. Conventional Surveillance for Early Detection of Aquatic Invasive Species

Performance Metric	Environmental DNA (eDNA) Surveillance	Conventional Survey Methods (e.g., Netting, Trapping, Visual)	Relevance to Early Detection
Sensitivity	Very High. Able to detect species at trace levels and low abundance (e.g., a few individuals) (Nathan *et al.*, 2014).	Low to Moderate. Highly dependent on population size, species behavior, and habitat accessibility.	Crucial for identifying species during the earliest, pre-establishment phase of invasion (Blackman, Hänfling and Lawson-Handley, 2018).
Time-to-Detection Lag	Minimal. Provides the earliest warning signal, shortening the lag time between introduction and discovery (Sepulveda *et al.*, 2023).	Significant. Lag exists due to the need for individuals to reach a detectable size or density.	Short lead time is essential for the success of rapid response and eradication efforts (Xia *et al.*, 2018).
Spatial Coverage/Effort	High efficiency. One small water sample can survey a large, connected area (e.g., entire stream section) (Simmons *et al.*, 2016).	Low efficiency. Requires direct effort across many specific points or prolonged deployment time (Piaggio *et al.*, 2014).	Simplifies field logistics, especially in remote or difficult-to-access sites.
Non-Target Impact	Zero. Completely non-invasive, no stress or harm to aquatic organisms (Muha *et al.*, 2017).	Varies. It can cause stress, injury, or mortality, particularly with electrofishing or destructive trapping.	Supports conservation ethics and allows monitoring of endangered co-occurring species (Weigel *et al.*, 2014).
Identification Accuracy	High. Relies on species-specific primers (qPCR) or reference databases (Metabarcoding) (Goldberg *et al.*, 2013).	Variable. Relies on expert taxonomic knowledge; prone to misidentification for cryptic or juvenile species (Keskin, 2014).	Essential for confirmation, avoiding false positives, and ensuring that management actions are targeted correctly.
Applicability to Cryptic Species	Excellent. Highly effective for organisms that are rare, nocturnal, or possess cryptic life stages (e.g., larvae) (Egan *et al.*, 2013).	Poor. Cryptic species are inherently difficult to observe or capture (Ergenler and Turan, 2023).	Extends surveillance capacity to historically difficult-to-monitor invaders.
Risk of False Positive	Moderate. Risk primarily from cross-contamination during sampling or lab processing (Sepulveda *et al.*, 2020).	Low. Primarily from misidentification or reporting of historical data.	Requires rigorous quality control and standardized protocols to ensure data actionability (Dias *et al.*, 2025).
Management Integration	Quantitative Signal. Potential for integration into Occupancy Modeling and resource management simulations (Majdanishabestari and Soleimani, 2019).	Presence/Abundance Data. Often provides counts/biomass, but typically less frequent/extensive than eDNA data.	Enables data-driven decisions on resource allocation and intervention strategy.

Table 1 gives a direct comparison of critical performance measures between the eDNA surveillance procedures and traditional, direct-capture approaches (e.g., netting, trapping, or electrofishing) in freshwater environments. The primary goal is to prove the greater ability of eDNA in early detection in a quantitative and qualitative way, which is crucial in the early low-density phase of an invasion. Such metrics as Sensitivity and Time-to-Detection Lag are emphasized to demonstrate that eDNA significantly reduces the threshold to discovery and gives the environmental managers the most critical lead time to conduct a successful rapid response action. All the comparisons are supported by references to the given literature, which highlights the evidence base of the operational readiness of eDNA. This review is the empirical basis of promoting the extensive use of molecular monitoring in the management of aquatic resources.

This comparison chart shows the operational benefits of eDNA. The effectiveness of the method should also be discussed in the context of water resource management, where it can be combined with other environmental data, including the models of simulation-optimization applied to consider environmental issues (Majdanishabestari and Soleimani, 2019). eDNA can complement the efficacy of defensive strategies, including the design and zoning of marine safety regions, by providing correct and prompt data, and by improving the focus of surveillance activities (Weigel *et al.*, 2014). Performance Evaluation. It is common to use the Receiver Operating Characteristic (ROC) curves, or other graphical representations, to provide a graphical representation of the trade-off between actual positive rate and false positive rate to ensure the optimal threshold of the assay to use to define a positive result.

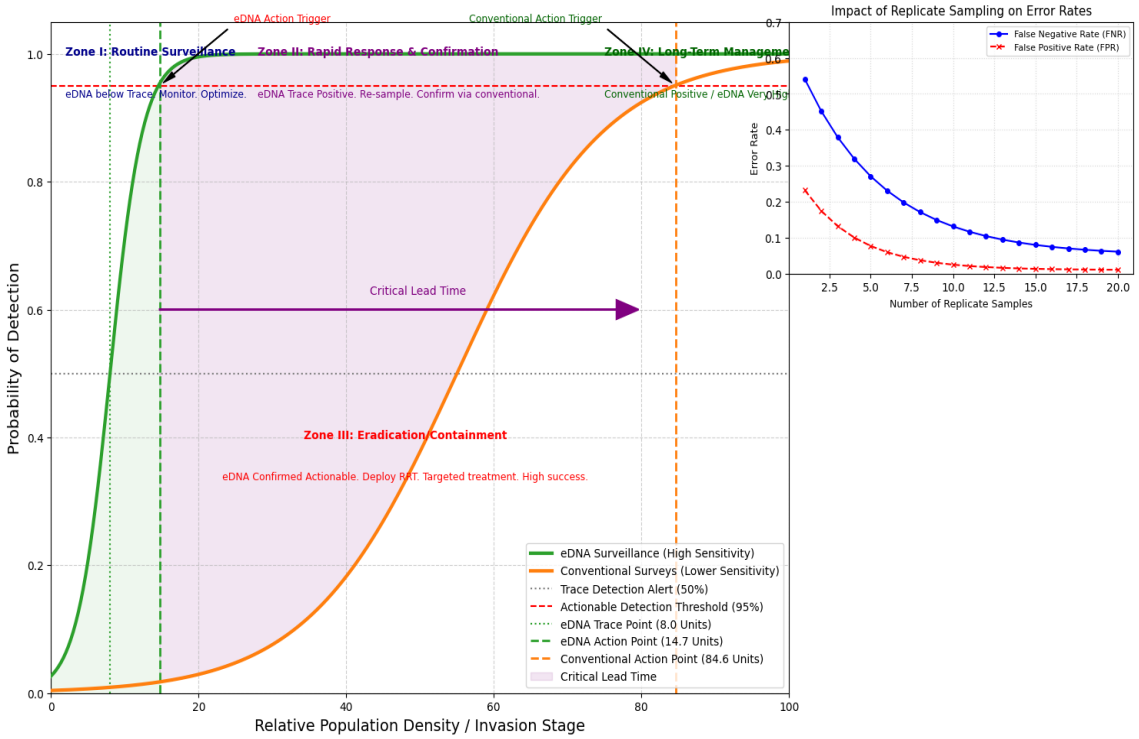

Figure 2: Risk-Based surveillance - linking eDNA detection to intervention thresholds

Figure 2 is a Decision support tool that depicts that eDNA surveillance has a greater operational edge compared to traditional Early Detection, Rapid Response (EDRR). The key plots are the Probability of Detection versus Population Density, which illustrates that eDNA has a higher analytical capability at low invasion stages. This delicacy causes a Critical Lead Time, the time interval during which eDNA is known to be actionable (95 percent sure) when conventional techniques fail. This lead time is quite crucial because it determines the transition of the expensive Long-Term Management (Zone IV) to the success of Eradication (Zone III). The inset graph (which demonstrates the fallacy of replicate sampling on error rates) and the integrated management zones offer the risk-based framework upon which conservatory intervention will be affected successfully and in a timely manner.

Conclusion

This study has illustrated the vast potential of the Environmental DNA (eDNA) as a sensitive, affordable, and fast method of early detection of invasive species in freshwater environments. Through the following systematic protocol outline, the combination of a progressive statistical occupancy model and the identification of key performance indicators, we have developed the basis to translate eDNA off the research bench into an operationalized, healthy surveillance platform. The main result is that eDNA can significantly decrease the critical time-to-detection lag that gives environmental managers the decisive lead time in developing practical, quick response strategies before the population of an invasive species becomes specialized and becomes established and contagious. The method is a cornerstone of next-generation aquatic monitoring programs due to the non-invasive nature of the technique and its potential to be used on a large scale. The future of eDNA surveillance will become more automated as in-situ autonomous eDNA samplers and microfluidics technologies will be utilized to carry out sample processing and analysis in the field. It is also a constant requirement to optimize quantitative models to include more complex environmental factors, including the eDNA degradation rates in a wide range of water chemistries and flow regimes. With the decrease in sequencing prices, eDNA metabarcoding will be the method of choice to monitor the community level, and the presence of known and unknown invaders, with a single process, will be possible. Finally, the effective control of aquatic invasives depends upon the combination of this potent molecular tool with the traditional ecological experience and effective management practices, which will guarantee the health and biodiversity of our freshwater in the long term.

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