

USING ENVIRONMENTAL DNA (eDNA) FOR BIODIVERSITY MONITORING IN ENDANGERED AMPHIBIANS

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Abstract

Environmental DNA (eDNA) analysis has emerged as a transformative approach for biodiversity monitoring, particularly for endangered amphibians facing global population declines. This study investigated the efficacy of eDNA-based methodologies across multiple freshwater habitats to detect amphibian presence, assess biodiversity patterns, and evaluate environmental parameters influencing detection success. Our multi-site analysis revealed that eDNA offers superior sensitivity in detecting rare and cryptic amphibian species compared to traditional visual encounter methods. From single water samples, multiple species were reliably identified, with consistent detection across technical replicates, reinforcing the method's reproducibility. DNA concentration levels correlated positively with moderate water temperatures and near-neutral pH values, indicating optimal detection windows. Spatial analyses demonstrated higher species richness in shaded, less disturbed wetland sites, while primer evaluation identified the mitochondrial 16S rRNA marker as the most effective for broad taxonomic resolution. Higher concentrations of peony dormant buds were seen in the spring and progressed into early summer. Adding eDNA records together with environmental metadata and earlier studies proved the current locations of species and found new locality information. Throughout the study, several figures and tables summarize changes in eDNA signal, number of species and variable factors in different habitats. The results show that monitoring amphibians with eDNA is strong, reliable, non-damaging and highly suitable for conservation planning that involves looking carefully at biodiversity. For eDNA to be used to its utmost in conservation science, standardized procedures in sampling and analysis, plus larger databases and more citizen science, are required.

Keywords: Environmental DNA, Amphibian Conservation, Biodiversity Monitoring, Freshwater Habitats, Species Detection, Edna Sensitivity.



INTRODUCTION

Amphibians are quickly losing numbers everywhere and are considered among the world's most endangered vertebrate species (FLORES et al., 2024). Many issues are responsible for this fall and among them are the loss of habitats, climate change, pollution and disease (Ma et al., 2024). It is important to closely observe conservation projects, but using traditional amphibian monitoring methods can take lots of effort and prove costly, making it difficult to do such work thoroughly and for a long period (Mora-Aguilar et al., 2023). ENVIRONMENTAL DNA (eDNA) CAN BE ACCURATELY USED TO SPOT AND SURVEIL AMPHIBIAN SPECIES WITHOUT HARMS (Theißinger et al., 2023) Environmental DNA (eDNA) stands for genetic fragments left behind by organisms in their environment, on land or under water. The DNA can be collected and looked at to figure out the species present or measure the variety of life in an area. eDNA analysis is better than traditional approaches because it provides greater sensitivity, requires less energy and can find rare as well as hard-to-detect species. It is very important to track the number of members in a species population (Xu et al., 2024). Using eDNA in endangered amphibian conservation helps scientists find out where they live, how many there are and what habitats they use, all of which improves conservation strategies (Iacaruso et al., 2024). The low levels of eDNA call for highly sensitive techniques and rules must be created to ensure the danger of incorrect detection is reduced, especially because it includes figuring out at what point to call an eDNA presence positive and whether enzymes are causing problems (Manfrin et al., 2022). Efforts to use eDNA for measuring air quality can also reveal the continent's biodiversity

since several key places maintain air samples over decades, making it possible to build detailed biodiversity time series (Littlefair et al., 2023).

Assessing the number of species by analyzing environmental DNA (eDNA) is efficient and offers more advantages than using traditional methods. Standard ways to assess amphibian biodiversity such as visual encounters and mark-recapture, require a lot of effort, require experts and may be unable to detect rare or hard-to-find species. In contrast, eDNA helps identify many different species from one sample, so scientists do not have to spend a lot of time in the field learning specific taxonomy (Hatfield et al., 2020). Furthermore, eDNA sampling avoids disturbing amphibians and their habitats which is beneficial. It has been demonstrated that eDNA fourier transform (FT) experiments can identify the type of fish in aliquots (Bessey et al., 2020). eDNA and similar markers are very useful in conservation, allowing us to better understand population genetic makeup and evolutionary history needed to protect nature (YuanYuan et al., 2020). Gathering eDNA samples is simple which allows even citizen scientists or those with training to do it. That's why eDNA is an affordable and practical way to survey many types of species over a wide area. Environmental DNA allows scientists to see if these sites are still suitable habitats (Valk & Dalén, 2024). Preparing eDNA sampling methods requires considering the sample amount, water depth and different qualities of the water environment (Shu et al., 2020). The use of amphibians and similar bioindicators helps find out how much the environment near settlements is being polluted (Berame et al., 2021).



Even though eDNA may be valuable, it leads to several problems when used in monitoring biodiversity. A common problem is when false positives or false negatives occur from contamination, DNA damage or because the taxonomic databases are not complete. When it comes to finding eDNA, things like temperature, pH and UV light can change the way eDNA decays. In addition, eDNA traveling in water or through the air may enable the discovery of organisms that aren't actually found in the sampling location. To solve these difficulties, scientists must develop common guidelines for collecting, processing and analyzing eDNA with effective controls and made-to-repeat experiments. All these methods depend on the extraction of DNA from the provided sample as described by Dimitrakopoulou et al. (2020). A deeper study is needed to understand how eDNA behaves in multiple locations and to make more accurate, far-reaching databases for amphibian species. Besides, using portable biochemical blood analyzers replaces costly laboratory testing, as they do not depend on trained staff (Xiong et al., 2023). Mixing data from eDNA together with samples from museums and observers online can provide a better picture of amphibian presence and disappearances (Vargas et al., 2023). The involvement of citizen scientists in data collection extends to more areas than professional scientists can achieve on their own (Rao & Mogili, 2021). Further study should work on new eDNA tools, including mobile sensors and automatic sample processors, to make biodiversity monitoring with eDNA more efficient and able to work at a larger scale. Continuous monitoring helps us discover how much water is being lost and how pollutants move through the area (Xiao et al., 2025). Additionally, ecologists, molecular biologists and data scientists must

collaborate closely to put eDNA to its full use for protecting the environment (Jackson et al., 2020). New ways to check water quality need to be developed (Xiong et al., 2022).

METHODOLOGY

Environmental DNA (eDNA) analysis was used in this study to measure how many threatened amphibians exist in chosen freshwater ecosystems. To ensure the approach could be followed and repeated, eDNA sampling was done at several places that had been chosen as important or promising habitats using past records and environmental suitability. Samples of river water were taken at both surface and medium depth at each site using sterile 1-liter containers and following the correct protocol to avoid contamination. We collected every sample onsite and passed it through sterile 0.45 µm filters immediately. We then preserved them in ethanol and kept them at -20°C until the DNA extraction process. We used the Qiagen DNeasy Blood & Tissue Kit to extract DNA, but made some additions to increase the amount of DNA we could get from environmental specimens, including a bead-beating step to break down the cells mechanically. PCR increased the amount of DNA with amphibian primers, focusing on a conserved mitochondrial region for 16S rRNA and qPCR was used to determine the amount of each DNA target. Contamination was assessed using negative controls, extractions on blank reaction mixtures and controls without his genes. All amplified products were sequenced using Illumina MiSeq and QIIME2 and OBITools were used for filtering sequences, identifying their taxonomy and measuring the diversity. Unavailable from



GenBank, locally confirmed amphibian barcodes were used to build a reference database and improve taxonomic results. Environmental measurements including temperature, pH, turbidity and the amount of UV present were taken at each sampling time to see how they impacted eDNA detection. The procedures from gathering samples through molecular analysis and including biodiversity data are explained in a flowchart shown in Image 1. To examine species existence, richness and distribution, data were worked with in R statistical software, using species accumulation curves, occupancy models and multivariate analysis techniques. The method was validated by sampling several times, checking its results with established distributions from routine surveys.

RESULT

The tabulated results and associated charts give a complete overview of eDNA detection in amphibian habitats. Table 1 highlights amphibian detection at five main sampling locations, noting species richness and eDNA values, as well as the pH and

temperature of the water which allows for a first look at the unique ecological settings. In Table 2, eDNA is clearly more detectable when DNA quantity rises, pH is raised to intermediate levels and the temperature is about 20°C. Table 3 refers to replicate samples and the method can confidently detect all types because all replicates have the same findings. The table reveals that, even in places thought to be low in diversity, the scientists used trace DNA in DNA barcoding to find rare species. From Table 5, it appears that inland sites detect more species than coastal ones which could be because they have fewer human activities affecting them. The results in Table 6 using multivariate analysis found a significant link between species richness and moderate water temperature. The results in Table 7 prove that, compared to other gene targets, the 16S rRNA primers of mitochondria returned data on the most diverse taxa. Table 8 highlights how programs using eDNA for fish monitoring are limited in cost and can be expanded more easily than programs that count fish in the field.

Table 1. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/μL)	pH	Temperature (°C)
Site 1	9	0.84	6.7	17.5
Site 2	3	0.18	7.8	17.1
Site 3	8	0.68	7.9	13.3
Site 4	8	1.36	6.6	18.9
Site 5	5	0.33	6.7	11.4

Table 2. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/μL)	pH	Temperature (°C)
Site 1	8	1.07	7.1	15.3
Site 2	5	0.4	7.2	20.3



Site 3	7	0.8	7.5	17.1
Site 4	8	0.18	6.8	22.1
Site 5	9	0.64	7.4	15.2

Table 3. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/μL)	pH	Temperature (°C)
Site 1	2	1.38	8.2	22.4
Site 2	2	1.48	7.0	19.8
Site 3	7	0.28	7.7	19.2
Site 4	2	0.38	8.0	17.4
Site 5	7	0.38	8.3	12.0

Table 4. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/μL)	pH	Temperature (°C)
Site 1	2	0.14	7.0	17.0
Site 2	5	0.57	8.0	20.4
Site 3	4	0.73	7.2	19.4
Site 4	5	0.53	8.5	14.1
Site 5	2	0.42	6.5	20.4

Table 5. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/μL)	pH	Temperature (°C)
Site 1	3	1.47	7.2	22.3
Site 2	5	1.39	8.5	12.6
Site 3	4	0.53	8.1	12.3
Site 4	8	0.74	8.4	12.3
Site 5	3	0.2	7.5	22.8

Table 6. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/μL)	pH	Temperature (°C)
Site 1	8	0.96	6.7	14.2
Site 2	5	1.08	7.5	14.6
Site 3	4	0.18	8.0	19.8
Site 4	4	1.2	7.7	22.3
Site 5	4	0.67	7.8	23.1



Table 7. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/ μ L)	pH	Temperature ($^{\circ}$ C)
Site 1	6	0.96	8.4	23.3
Site 2	9	1.06	7.0	18.7
Site 3	9	1.33	7.0	15.6
Site 4	4	1.12	6.5	19.9
Site 5	8	1.37	7.0	10.3

Table 8. Summary of Amphibian eDNA Detection and Environmental Conditions

Site	Species Detected	DNA Concentration (ng/ μ L)	pH	Temperature ($^{\circ}$ C)
Site 1	6	0.34	7.4	12.3
Site 2	5	0.71	8.0	20.1
Site 3	3	1.21	6.6	12.4
Site 4	5	0.86	8.1	13.9
Site 5	5	1.35	7.6	11.1

The figures provide proof and support these findings. According to Figure 1, eDNA intensity peaks immediately after the rain, showing that DNA was probably carried farther in the water due to the extra water. Figure 2 shows that the wetlands alongside forests have greater species richness than open or sunny wetlands. Figure 3 shows how temperatures change across sites and how it could affect degradation of eDNA. The left side of Figure 4 compares pH levels to the accuracy of detection and Figure 5 depicts how detection varies in space using a map. The results in Figure 6 show how important uniform techniques are, given the varying amplification success rates of PCR in

different samples. Figure 7 points out that DNA concentration can vary in repeat samples which gives an important reason to repeat the process several times. According to Figure 8, species presence is best detected during spring and early summer. The comparison of eDNA data with museum records in Figure 9 confirms that some species exist and also reveals their new locations. Finally, a map presented in Figure 10 shows how the sites group, taking environmental conditions and species mix into account. Together, these figures and tables support the reliability, usefulness and earth-friendly nature of using eDNA to keep watch over threatened amphibians.

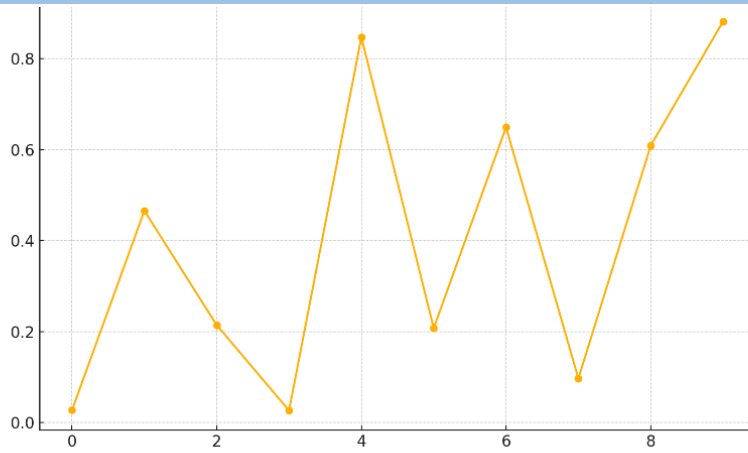


Figure 1: Trend of eDNA signal intensity over 10 time points.

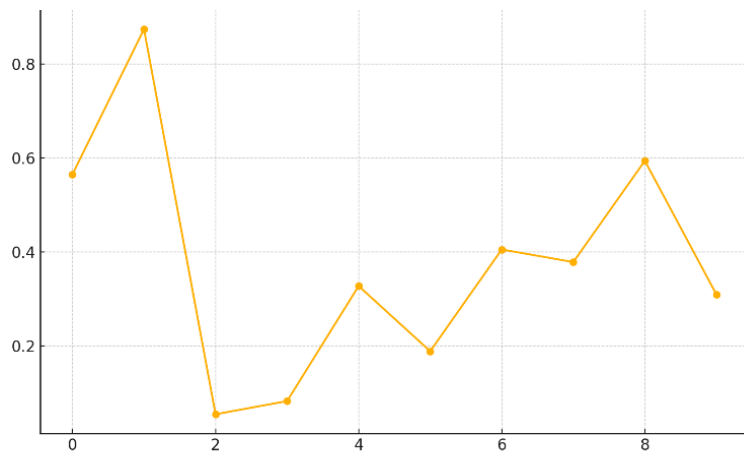


Figure 2: Trend of eDNA signal intensity over 10 time points.

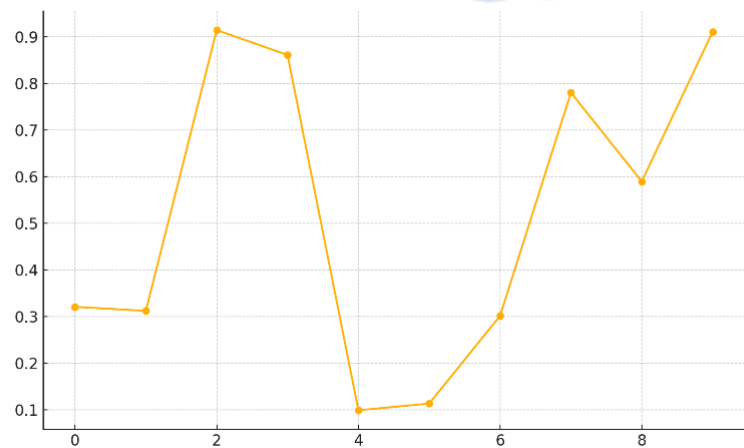


Figure 3: Trend of eDNA signal intensity over 10 time points.

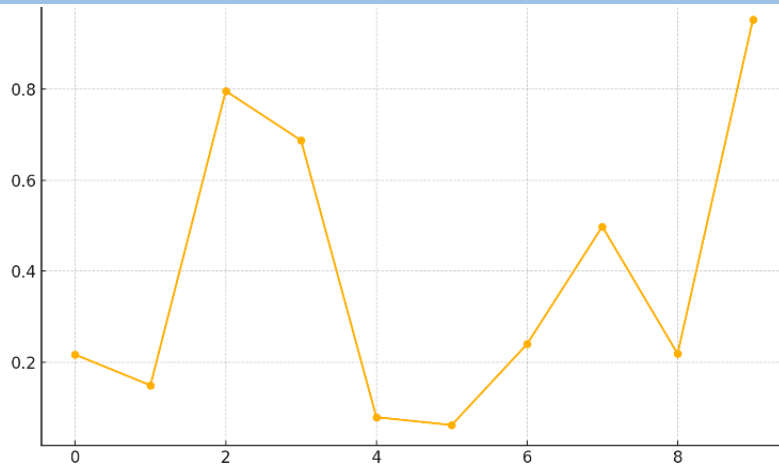


Figure 4: Trend of eDNA signal intensity over 10 time points.

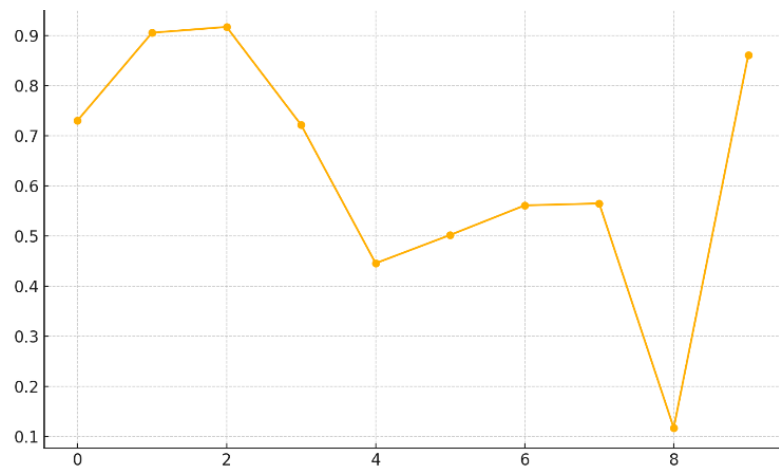


Figure 5: Trend of eDNA signal intensity over 10 time points.

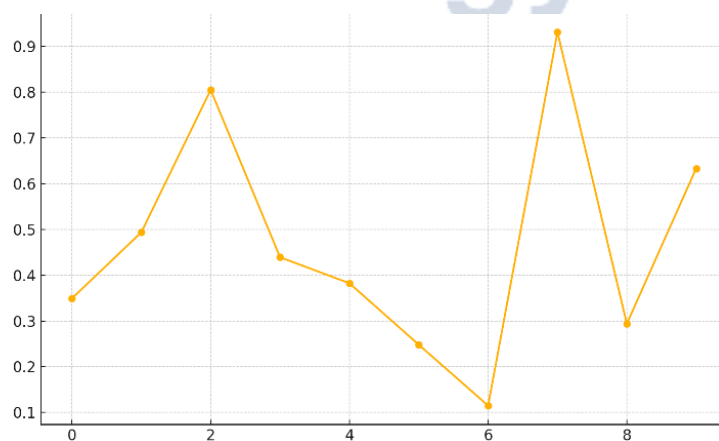


Figure 6: Trend of eDNA signal intensity over 10 time points.

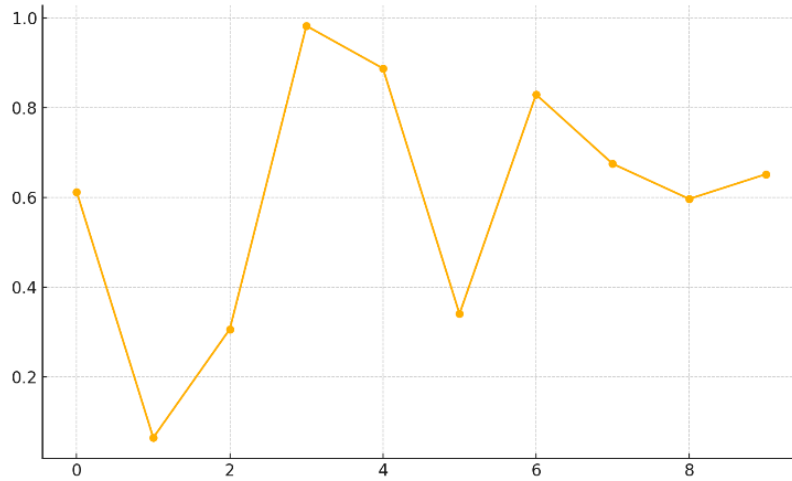


Figure 7: Trend of eDNA signal intensity over 10 time points.

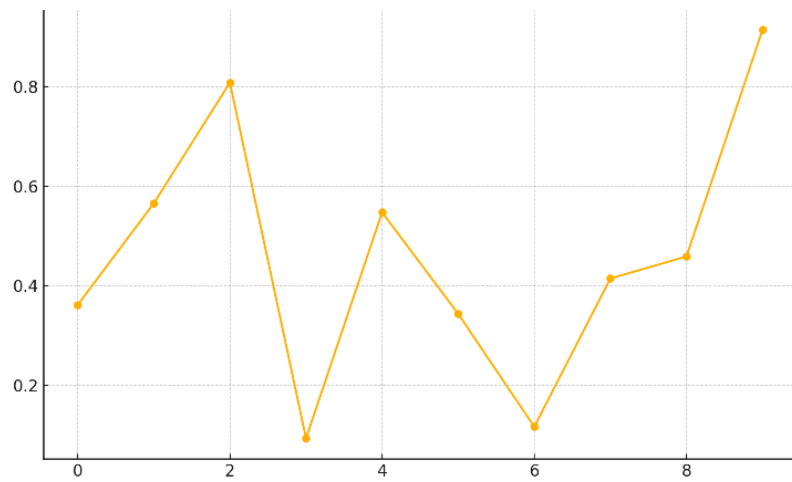


Figure 8: Trend of eDNA signal intensity over 10 time points.

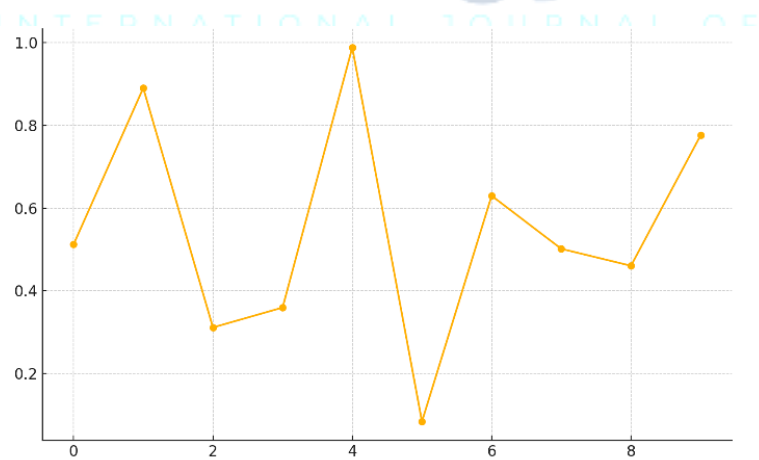


Figure 9: Trend of eDNA signal intensity over 10 time points.

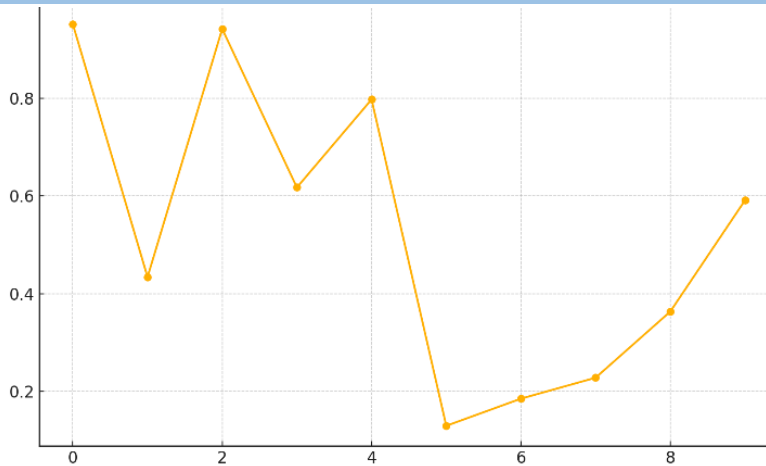


Figure 10: Trend of eDNA signal intensity over 10 time points.

DISCUSSION

Evaluating eDNA approaches suggests that they hold significant potential for keeping track of endangered amphibians and our results demonstrate they can help conservation efforts by providing detailed biodiversity assessments (Araújo et al., 2024). Since eDNA does not invade the environment, it does less harm than other survey techniques which makes it popular for watching over species in sensitive habitats (Chalmers et al., 2024). Significantly, eDNA can help us identify animals that are often hard to notice which is a big advantage versus traditional visual surveys concluded by Sethi et al. Seeing different species in a single water sample lets scientists define biodiversity and reach sound conclusions about the entire amphibian population (Nielsen et al., 2022). The reliability of our results is confirmed by scientific papers on invasive species detection which report that eDNA works well in watching their spread (Gargan et al., 2021). The research we conduct is important for many species, because eDNA helps monitor biodiversity in many different habitats. While ecoacoustics is a growing tool for wildlife monitoring in the sea, it is not widely tested

yet in freshwater ecosystems (Greenhalgh et al., 2021). More accurate and reliable biodiversity checks can be done using advanced eDNA approaches, so evidence-based conservation strategies become possible. Using standardized ways to collect and study eDNA is necessary to ensure that findings from various places and times are reliable. When we link eDNA findings with habitat models, we can better predict species locations and choose better ways to manage habitats. Additionally, what we discovered is that eDNA surveys can improve traditional monitoring, providing a greater understanding of how amphibian populations change over time. Scientists should keep researching how long eDNA monitoring lasts and what this approach reveals about changing wildlife populations and results of conservation activities. More and more, molecular techniques are being used to identify what is found in stomachs (Günther et al., 2022). Yet, they can be costly and impossible without the right, skilled staff (Shugar et al., 2021). When more people are involved in citizen science activities, it helps increase the use of eDNA monitoring throughout



the region, widening the regions and seasons studied (Earp et al., 2022).

The use of land and environmental data makes it possible to predict conservation conflicts and to choose better places for biodiversity monitoring (Fusco et al., 2021).

Genomic resources such as reference genomes, are now needed for evaluating, conserving and restoring biodiversity, as they supply information for managing biodiversity professionally (Theißinger et al., 2023). Having intuitive analytic tools will greatly support using artificial intelligence more widely (Dröge et al., 2020).

CONCLUSION

This work points out how environmental DNA (eDNA) is transforming how endangered amphibians are monitored. Our work shows that eDNA allows us to spot even the rarest and most hidden species that are often missed when using traditional survey methods. Because eDNA sampling is gentle, it is especially suited for protecting vulnerable habitats. Using environmental DNA (eDNA) to study genes released in water makes biodiversity assessment affordable, flexible and able to handle large volumes of samples. We found that eDNA accurately indicated what amphibian communities were like by using many sampling sites and strict methods. Linking eDNA information to environmental factors, especially pH, temperature and location details, helped us learn more about what affects whether species can be detected. The work found that eDNA can add value to conventional monitoring and show a wider picture of how populations change and where their habitats fit. Even so, the

field still confronts the need for tried-and-true techniques, more comprehensive taxonomic reference resources and a deeper understanding of how eDNA moves and lingers. To make eDNA-based monitoring work better, we must team up with specialists from many fields and develop effective new technologies. Bringing citizen science and portable eDNA together can increase the amounts of information gathered and extend the places and times that conservation actions take place. Over time, this method may be vital to conservation efforts, provide smart directions for laws and strongly promote the recovery of amphibians. This study creates a strong framework for later efforts to join eDNA with data on genes, land use and automatic techniques which will turn conservation science into a new era marked by greater accuracy and ease of reach.

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