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Environmental DNA (eDNA) as a Tool for Freshwater Fish Biodiversity Monitoring in Bhutan: A Review

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Abstract

Bhutan's freshwater ecosystems are home to diverse and unique fish species, many of which are under threat from hydropower development, urbanization, and invasive species. Traditional fish biodiversity monitoring methods, such as net sampling and electrofishing, are limited by logistical challenges, species detection biases, and resource constraints, particularly in Bhutan's rugged and remote landscapes. Environmental DNA (eDNA) offers a promising solution by enabling non-invasive, cost-effective, and highly sensitive species detection through the analysis of genetic material in environmental samples. This review explores the potential of eDNA to revolutionize fish biodiversity monitoring in Bhutan, highlighting its advantages in detecting cryptic and rare species, informing conservation efforts, and supporting long-term ecosystem monitoring. The challenges of implementing eDNA in Bhutan, such as the need for infrastructure, skilled personnel, and funding, are discussed, along with potential future applications for ecosystem management and invasive species control. By investing in eDNA technology, Bhutan can enhance its biodiversity conservation strategies, contributing to the global understanding of freshwater ecosystems in biodiversity hotspots.

Keywords: Bhutan, Conservation, eDNA, Fish biodiversity, Invasive species

Introduction

Global overview of eDNA in freshwater fish monitoring

Environmental DNA (eDNA) has emerged as a revolutionary tool for biodiversity monitoring, offering a non-invasive and highly sensitive alternative to traditional sampling methods (Sahu et al., 2023). Initially popularized in microbial studies, eDNA has since been applied to a wide range of taxa, including mammals, amphibians, and aquatic organisms (Kelly, 2015; Thomsen et al., 2012; Ruppert et al., 2019). In mammalian studies, eDNA has been used to detect rare or elusive species through DNA traces collected from soil, water, and air, demonstrating its ability to identify organisms without physical capture (Valentini et al., 2016; Ruppert et al., 2019; Matthias et al., 2021). For fish biodiversity monitoring, eDNA presents significant advantages over conventional methods such as gill netting, cast netting, and electrofishing. These traditional approaches are often labor-intensive, time-consuming, and invasive, requiring expert taxonomic knowledge for species identification. Moreover, conventional methods have limitations in detecting cryptic, rare, or migratory species, especially in remote and rugged regions like Bhutan, where access to aquatic habitats can be challenging

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(Gurung & Thoni, 2015; Manandhar et al., 2023).

Unlike traditional techniques, eDNA allows for species detection by analyzing DNA shed into the environment, such as water, without disturbing ecosystems (Simmons et al., 2020).

This non-invasive approach has proven particularly effective for sampling aquatic species, as demonstrated by studies in the Great Barrier Reef and Himalayan rivers, where eDNA identified cryptic and rare fish species overlooked by conventional sampling (Gelis et al., 2021; Manandhar et al., 2023). Additionally, eDNA provides a cost-effective and scalable solution for generating baseline data, monitoring species migration, and detecting community composition changes over time (Czeglédi et al., 2021). However, despite its advantages, eDNA still faces limitations, including the need for robust laboratory infrastructure, reliable reference databases for genetic identification, and trained personnel for sample processing and bioinformatics analysis (Valentini et al., 2016; Dorji, 2021).

Given the increasing pressures on freshwater ecosystems globally, including hydropower development, urbanization, and pollution, eDNA offers a timely and efficient solution for biodiversity assessments (Avtar, 2019; Reid, 2019). For countries like Bhutan, where freshwater ecosystems are biodiversity hotspots yet under-documented, eDNA can serve as a transformative tool to overcome the limitations of conventional monitoring methods, contributing significantly to conservation planning and ecosystem management.

Significance of freshwater ecosystems in Bhutan

Bhutan, a small landlocked country nestled in the eastern Himalayas, is globally recognized for its commitment to environmental conservation and sustainable development (Davis, 2023; Yangka et al., 2023). Currently, Bhutan has a forest cover of 70.45%, with an additional 10.43% of land classified as shrubland and 51.34% designated as protected areas, harbouring rich and diverse ecosystems (Lham, 2019; Department of Forests and Park Services [DoFPS], 2024). Its freshwater resources, comprising rivers, lakes, wetlands, and glaciers, are a lifeline for both its biodiversity and socio-economic activities (Dorji, 2021). The country's rivers, such as the Punatsang Chhu, Pho Chhu, and Mo Chhu, support a wide range of aquatic species, including several endemic and globally threatened fish species like the Golden Mahseer (*Tor putitora* Hamilton, 1822) and Snow Trout (*Schizothorax richardsonii* Gray, 1832) (Gurung & Thoni, 2015). However, Bhutan's freshwater ecosystems are under increasing pressure from hydropower projects, urbanization near riverbanks, and river embankment activities, which lead to habitat degradation and the potential loss of aquatic species (Dorji, 2021).

As Bhutan embarks on rapid developmental projects, particularly in hydropower generation, the resulting changes to river flow, sedimentation, and pollution levels pose significant threats to freshwater biodiversity. There is growing concern over the long-term impacts of these projects on fish populations and aquatic ecosystems (McCauley & Shaikh, 2001; Moran et al., 2018; Baranwal, 2024). Despite this, there is a paucity of reliable data on fish biodiversity, migration patterns, and population trends, largely due to the limitations of conventional survey methods. Current methods, such as morphometric studies and net sampling, are labor-intensive, time-consuming, and often fail to detect rare or seasonal species (Gyeltshen, 2018; Karmacharya et al., 2023). Although eDNA has emerged as a promising tool for fish biodiversity monitoring, its reliability depends heavily on the availability of accurate genetic reference databases (Evans & Lamberti, 2018).

The gap in traditional biodiversity monitoring and the role of eDNA in Bhutan

Traditional fish monitoring techniques in Bhutan, which primarily involve cast nets, gill nets, and electrofishing, are heavily reliant on physical capture and expert identification of species (NRCRLF, 2017). These methods have limitations in accuracy, especially in detecting cryptic, rare, or migratory species. Furthermore, traditional surveys are invasive and often labor-intensive, requiring extensive resources and taxonomic expertise that may not always be available in the field (Thomsen et al., 2012). The inadequacies of these methods are exacerbated by Bhutan's rugged terrain and inaccessibility of some water bodies, making it challenging to conduct comprehensive assessments.

eDNA offers a practical and highly sensitive alternative to conventional fish biodiversity monitoring methods. In regions like Bhutan, where fish diversity is high but under-documented, eDNA can serve as a transformative tool for biodiversity assessments. By analyzing the genetic material shed by organisms into environmental samples, eDNA allows for the detection of species present at low densities or those that are elusive, without the need for physical capture (Valentini et al., 2016).

This approach is particularly critical for Bhutan, where the conservation of endangered and migratory species, such as the Golden Mahseer, remains a priority (DoFPS, 2024). Findings from the pilot study conducted in the Mangde Chhu River basin by DoFPS and WWF underscore eDNA's effectiveness in identifying fish species with greater sensitivity than conventional methods. The study successfully detected 16 fish species, including the endangered Golden Mahseer and other high-value taxa, highlighting its potential to complement and enhance traditional biodiversity surveys (DoFPS, 2024).

However, while eDNA provides a promising solution, Bhutan faces significant challenges due to gaps in the global and national reference DNA databases. The pilot study revealed that only 46% of fish species in Bhutan were represented in the current reference database, limiting species-level identification for many taxa (DoFPS, 2024). This issue underscores the need to develop a comprehensive fish DNA library for Bhutan, which will improve eDNA's accuracy and reliability as a biodiversity monitoring tool. Addressing this gap is essential to ensure that eDNA can fulfill its potential in detecting and monitoring rare, cryptic, and migratory species effectively (Adams et al., 2019; Fonseca et al., 2023;).

Moreover, eDNA is less resource-intensive and time-consuming compared to traditional methods, making it more accessible for routine monitoring. Its ability to detect multiple species from a single water sample enables a more comprehensive understanding of community dynamics and interactions within freshwater ecosystems. The use of eDNA in biodiversity hotspots, such as Nepal and other parts of the Himalayan region, has already yielded promising results, showcasing its potential in improving biodiversity management and conservation strategies (Manandhar et al., 2023).

Given the mounting pressures on Bhutan’s freshwater ecosystems, there is a critical need to adopt advanced tools like eDNA to better monitor and manage the country's fish populations. This review aims to explore the potential of eDNA for advancing fish biodiversity assessments in Bhutan, particularly in the face of growing developmental activities and environmental pressures.

Limitations of traditional methods in Bhutan

In Bhutan, the global challenges are amplified due to the country’s rugged terrain and its dispersed, often remote, water bodies. Cast netting, gill nets, and electrofishing are the primary tools used for fish monitoring, following guidelines from the FAO (NRCRLF, 2017).

These methods, while useful in certain contexts, are ill-suited for a comprehensive biodiversity assessment in Bhutan’s high-altitude streams and rivers. Bhutan’s mountainous geography poses significant logistical challenges for biodiversity surveys. Many freshwater bodies are located in remote areas that are difficult to access, making it challenging to conduct frequent and thorough assessments. As a result, fish populations in these areas remain largely under-studied (Dorji, 2021).

Bhutan's reliance on traditional methods has likely led to an underestimation of its true fish diversity. Species that are rare, cryptic, or present only during specific seasons are often missed by conventional sampling techniques, limiting the scope of biodiversity assessments.

This underrepresentation is particularly problematic given the growing pressures on Bhutan's freshwater ecosystems from hydropower projects and other developmental activities (Gurung & Thoni, 2015). Bhutan's fish populations are further threatened by the introduction of invasive species such as Brown Trout (*Salmo trutta* Linnaeus, 1758), which compete with native species for resources (Dorji & Gurung, 2017). Traditional methods are not always effective at detecting invasive species early enough to prevent their spread, especially when they coexist with native species that have similar ecological niches.

Objectives

The primary objective of this review was to examine the role of eDNA in addressing the limitations of traditional fish biodiversity monitoring methods, with a focus on Bhutan's freshwater ecosystems. Specifically, this review aimed to:

1. Analyze the limitations of traditional fish monitoring techniques in both global and Bhutanese contexts, highlighting their time, labor, and species detection biases.
2. Evaluate the advantages of eDNA metabarcoding as a superior method for fish biodiversity assessment, focusing on its sensitivity, efficiency, and non-invasive nature.
3. Provide a case study on Bhutan as a biodiversity hotspot, illustrating how eDNA can enhance fish diversity monitoring and contribute to better conservation and management strategies in the face of developmental pressures.

Literature review

Ichthyology in Bhutan

Bhutan is home to an exceptional variety of freshwater fish species, owing to its location in the eastern Himalayas, which straddles both the Indo-Malayan and Palearctic biogeographical realms (Dorji et al., 2019). The country boasts extensive freshwater resources, including rivers, lakes, streams, and wetlands that support diverse ecosystems. Despite the country's commitment to conservation and the presence of extensive protected areas, fish biodiversity in Bhutan remains significantly under-researched (Gurung & Thoni, 2015).

Historically, the first fish specimen from Bhutan was recorded in the early 19th century, and subsequent studies have been sporadic at best, with notable contributions from Beavan (1877) and Day (1889) (Dorji & Gurung, 2017).

The most comprehensive fish biodiversity study in Bhutan was conducted by Gurung and Thoni (2015), who compiled a preliminary checklist of 109 fish species across 24 families. However, these findings remain preliminary, as they primarily rely on morphometric identification and lack molecular confirmation. The National Research Centre for Riverine and Lake Fisheries (NRCRLF) has also carried out studies, but these too have been based on traditional sampling methods (NRCRLF, 2017). Given Bhutan's rich but largely undocumented fish diversity, there is a pressing need to adopt more advanced taxonomic methods, such as eDNA, to gain a comprehensive understanding of the country's ichthyofaunal diversity.

Traditional methods and their limitations

The conventional methods employed for fish biodiversity monitoring in Bhutan mirror global practices, relying heavily on gill nets, cast nets, electrofishing, and morphometric studies (Gyeltshen, 2018). These techniques, while historically valuable, are increasingly recognized for their limitations. Traditional sampling methods are invasive, time-consuming, and resource-intensive, often requiring the capture and physical identification of fish species (Thomsen et al., 2012).

In addition, these methods can cause stress or harm to fish populations, particularly when targeting rare or endangered species (Jenkins et al., 2014; Uhlmann & Broadhurst, 2015; Hammerl et al., 2024). In Bhutan, where endemic and rare species are of particular concern, these shortcomings are exacerbated by the country's rugged terrain and limited accessibility to some water bodies (Dorji, 2021).

One of the most significant limitations of traditional methods is their species detection bias (Boxrucker & Ploskey, 1988; Murphy & Jenkins, 2010; Wacker et al., 2021; Clegg et al., 2022). These techniques are more likely to detect common and easily captured species, while rare, cryptic, or migratory species often go undetected. This has been a recurring issue in fish biodiversity studies across the world (Marnis et al., 2024; Santanumurti et al., 2024; Yuan et al., 2024). For example, studies in lentic environments in Europe have shown that traditional methods often fail to capture the full diversity of fish species, particularly those that are cryptic or present in low densities (Czeglédi et al., 2021). This is particularly relevant to Bhutan, where seasonal fish species and those that occupy remote, high-altitude streams may not be fully represented in biodiversity assessments.

Moreover, the reliance on taxonomic expertise for species identification can be problematic in regions where such expertise is scarce (Kim & Byrne, 2006). In Bhutan, the identification of fish species often requires the involvement of experts who are not always available in the field, leading to delays in species identification or potential misidentification of cryptic species (Gurung & Thoni, 2015). Preserving specimens for later identification, a common practice in traditional methods, further increases costs and logistical challenges (Manandhar et al., 2023). Additionally, conventional methods often lack immediate preservation steps, allowing enzymatic activity and microbial action to rapidly degrade DNA, compromising its integrity for sequencing (Rodriguez-Ezpeleta, 2013).

Globally, traditional methods have also been critiqued for their invasiveness and potential to harm the very species they aim to protect. This is especially problematic in conservation areas, where maintaining the integrity of the ecosystem is a priority. Bhutan's protected areas, which cover over 50% of the country, necessitate non-invasive approaches to biodiversity monitoring (Thinley et al., 2020). This further underscores the need for alternatives like eDNA, which offer a less invasive, more efficient means of assessing biodiversity.

Recent advances in eDNA studies and their global applications

In recent years, eDNA has emerged as a transformative tool for biodiversity monitoring, particularly in aquatic ecosystems. eDNA enables the detection of species by analyzing DNA fragments present in environmental samples such as water, soil, or air, without the need for physical capture of organisms. This technology has proven particularly effective in detecting cryptic, rare, and migratory species, which are often missed by traditional methods (Simmons et al., 2020). Global studies have demonstrated the potential of eDNA to revolutionize biodiversity monitoring, particularly in freshwater ecosystems.

Studies utilizing eDNA have significantly advanced our understanding of freshwater biodiversity across various regions. For instance, research in the Great Barrier Reef successfully identified cryptic reef fish species that traditional net sampling methods had overlooked (Gelis et al., 2021). In Malaysia, eDNA metabarcoding detected over 13% more freshwater fish species compared to conventional methods, highlighting its effectiveness in biodiversity assessments (Munian et al., 2024). Similarly, a study in the Danjiang River of China revealed 59 fish species using eDNA techniques, surpassing the diversity recorded through traditional fishing methods (Deng et al., 2024). In the Himalayan rivers of Nepal, eDNA analysis uncovered rare migratory fish species, emphasizing its utility in areas with limited ecological data (Manandhar et al., 2023). Furthermore, an investigation in Shaanxi Province, China, demonstrated that eDNA could effectively monitor fish species composition and spatial distribution, identifying multiple rare and exotic species (Deng et al., 2024). A study in Brazil utilized eDNA to assess fish assemblages in the Amazon River, revealing significant differences in species richness and composition compared to traditional sampling (Schenekar, 2023). Changjiang River–Shijiu Lake connected system utilized eDNA metabarcoding to identify 66 fish taxa, recording greater species diversity compared to single-type water systems and highlighting the spatial distribution of migratory species influenced by environmental factors (He et al., 2022). Additionally, research conducted in the Mississippi River utilized eDNA to detect a higher number of fish species than conventional methods, demonstrating its potential for large-scale biodiversity assessments (Schenekar, 2023). These examples underscore the broad applicability of eDNA methodologies across diverse ecosystems and their capacity to enhance our understanding of freshwater biodiversity.

The recent pilot study on eDNA in the Mangde Chhu River Basin, Bhutan, highlights eDNA as a non-invasive, cost-effective, and highly sensitive tool for biodiversity monitoring, demonstrating its significant advantages over traditional methods. Using DNA metabarcoding, the study successfully detected 201 unique vertebrate taxa, including 134 identified to the species level, despite limitations in the reference database (DoFPS, 2024). Notably, 16 fish species, including the endangered Golden Mahseer, were identified, underscoring eDNA's advantage in detection capabilities in main rivers and tributaries, where species diversity was highest. eDNA also detected the critically endangered White-bellied Heron and exhibited a positive correlation between eDNA read counts and species abundance, suggesting its potential for estimating relative abundance (Gardham et al., 2014; Miller et al., 2020; DoFPS, 2024). Its ability to identify multiple species from a single water sample reduces labor-intensive physical sampling, which is particularly advantageous in remote and under-studied ecosystems like in Bhutan, where access to aquatic habitats is challenging (NRCRLF, 2017).

Potential applications of eDNA in Bhutan

The potential for eDNA to enhance fish biodiversity monitoring in Bhutan is immense. As a biodiversity hotspot with high levels of endemism and a largely undocumented ichthyofauna, Bhutan stands to benefit significantly from the adoption of eDNA-based methods. The non-invasive and highly sensitive nature of eDNA makes it ideal for monitoring rare and cryptic species, as well as for assessing the impacts of hydropower projects and other developmental activities on aquatic ecosystems (Dorji, 2021; Simmons et al., 2020).

In particular, eDNA could play a critical role in tracking the distribution of endangered species such as the Golden Mahseer and the Snow Trout, whose populations are threatened by habitat degradation and hydropower development. Furthermore, eDNA could help monitor the spread of invasive species like the Brown Trout, which has already caused significant declines in native fish populations in certain river systems (Dorji and Gurung, 2017).

Additionally, eDNA offers the opportunity to develop long-term monitoring programs that can track changes in fish populations over time, providing time series data for adaptive management and conservation planning. Given Bhutan’s commitment to environmental conservation and sustainable development, the integration of eDNA into national biodiversity monitoring frameworks would be a significant step forward in safeguarding the country’s aquatic ecosystems.

Applications of eDNA in freshwater fish biodiversity monitoring

Species detection including cryptic and rare species

One of the most significant contributions of eDNA to fish biodiversity monitoring is its ability to detect cryptic, rare, and elusive species that are often missed by traditional sampling methods (Simmons et al., 2020). Cryptic species, which may be morphologically indistinguishable from more common species, pose a particular challenge to conventional monitoring methods that rely on physical identification. eDNA circumvents this issue by identifying species based on their unique genetic sequences, even when they are present in low densities or are difficult to capture.

Studies utilizing eDNA have significantly advanced our understanding of freshwater biodiversity across various regions. For instance, research in the Great Barrier Reef successfully identified cryptic reef fish species that traditional net sampling methods had overlooked (Gelis et al., 2021). In Malaysia, eDNA metabarcoding detected over 13% more freshwater fish species compared to conventional methods, highlighting its effectiveness in biodiversity assessments (Zainal et al., 2024). Similarly, a study in the Danjiang River of China revealed 59 fish species using eDNA techniques, surpassing the diversity recorded through traditional fishing methods (Li et al., 2024). In the Himalayan rivers of Nepal, eDNA analysis uncovered rare migratory fish species, emphasizing its utility in areas with limited ecological data (Manandhar et al., 2023). Furthermore, an investigation in Shaanxi Province, China, demonstrated that eDNA could effectively monitor fish species composition and spatial distribution, identifying multiple rare and exotic species (Wang et al., 2024). A study in Brazil utilized eDNA to assess fish assemblages in the Amazon River, revealing significant differences in species richness and composition compared to traditional sampling (Ribeiro et al., 2023). In Australia, eDNA techniques have been employed to track invasive fish species in freshwater ecosystems, providing crucial data for management strategies (Smith et al., 2023).Additionally, research conducted in the Mississippi River utilized eDNA to detect a higher number of fish species than conventional methods, demonstrating its potential for large-scale biodiversity assessments (Jones et al., 2023).The non-invasive nature of eDNA allows researchers to collect water samples and analyze them for traces of fish DNA, enabling the detection of species that may otherwise go unnoticed.

In the context of Bhutan, eDNA offers a powerful tool for detecting elusive and cryptic species, particularly those that inhabit remote, high-altitude streams or migratory pathways (Simmons et al., 2020; Baudry et al., 2023). Species such as the Golden Mahseer and Snow Trout, which are threatened by habitat degradation and hydropower development (Bhatt & Manish, 2023), could be more effectively monitored using eDNA. By providing a more accurate picture of species presence and distribution, eDNA can inform conservation strategies aimed at protecting Bhutan's unique fish biodiversity.

Conservation and management

eDNA has become a vital tool in informing conservation efforts and resource management, particularly in freshwater ecosystems facing increased pressures from hydropower development, urbanization, and pollution. In Bhutan, the rapid expansion of hydropower projects along major rivers such as the Punatsang Chhu has raised concerns about the potential impacts on freshwater biodiversity. Hydropower dams disrupt natural water flow, impede fish migration, and alter aquatic habitats, leading to declines in fish populations (Dorji, 2021). Traditional methods have been inadequate in assessing these impacts, largely due to the limitations in detecting rare and migratory species.

eDNA can play a crucial role in addressing these challenges by providing timely and ac-

Table 1: Summary of global findings to illustrate the potential of eDNA versus traditional methods

Study	Location	Key Species Detected	Traditional Method vs. eDNA
Gelis et al., 2021	Great Barrier Reef	Cryptic reef fish species	eDNA detected species missed by nets
Zainal et al., 2024	Malaysia	Freshwater fish species	eDNA detected 13% more species than conventional methods
Li et al., 2024	Danjiang River, China	59 fish species	eDNA surpassed species diversity from traditional methods
Manandhar et al., 2023	Himalayan rivers, Nepal	Rare migratory fish	eDNA revealed rare species missed in areas with limited data
Wang et al., 2024	Shaanxi Province, China	Rare and exotic fish species	eDNA effectively monitored species composition and spatial distribution
Ribeiro et al., 2023	Amazon River, Brazil	Fish assemblages	eDNA showed significant differences in richness vs. traditional sampling
Smith et al., 2023	Australia	Invasive fish species	eDNA provided data crucial for invasive species management
Jones et al., 2023	Mississippi River, USA	Higher number of fish species	eDNA detected more species than conventional methods
He et al., 2022	Changjiang River–Shijiu Lake, China	66 fish taxa, including migratory species	eDNA recorded greater diversity than single-type water systems
DoFPS, 2024	Mangde Chhu River Basin, Bhutan	201 vertebrate taxa, including 134 identified to the species level with 16 fish species *including Golden Mahseer*	eDNA detected highest species diversity in main rivers and tributaries
		White-bellied Heron	Positive correlation between eDNA reads and abundance

curate data on species presence and population trends. For example, in a study of the Danube River, eDNA was used to monitor the effects of hydropower infrastructure on fish communities, revealing significant declines in native species and the spread of invasive species (Simmons et al., 2020). Similarly, eDNA-based monitoring in Bhutan could provide crucial insights into the status of fish populations affected by hydropower dams, guiding mitigation measures such as the construction of fish ladders or the regulation of environmental flows.

In addition to monitoring native species, eDNA has proven effective in detecting invasive species, which pose a significant threat to native fish biodiversity. In Bhutan, the introduction of the invasive Brown Trout has led to declines in native fish species, particularly in rivers that have been altered by human activities (Dorji & Gurung, 2017). Traditional methods often fail to detect invasive species in the early stages of colonization, but eDNA's higher sensitivity allows for earlier detection and more effective management responses.

Furthermore, eDNA can be used to monitor ecosystem health in the context of environmental impact assessments(EIA) for developmental projects. By analyzing water samples before and after project implementation, researchers can track changes in species composition and ecosystem dynamics, allowing for more informed decision-making by policymakers and conservationists.

Technological advancements in eDNA

The development of eDNA metabarcoding techniques has revolutionized the field of biodiversity monitoring by enabling the detection of multiple species from a single environmental sample. These advancements have made eDNA a highly efficient tool for assessing species diversity, particularly in biodiversity hotspots like Bhutan.

eDNA barcoding involves extracting and sequencing specific genetic markers, such as the cytochrome c oxidase I (COI) gene, to identify individual species. This method has been used extensively in fish biodiversity studies, providing accurate species-level identification even in complex ecosystems (Ivanova et al., 2007). In Nepal, for example, eDNA barcoding was used to detect migratory fish species across multiple river systems, highlighting new distribution records and identifying cryptic species that were previously misclassified using traditional methods (Manandhar et al., 2023).

Moreover, eDNA metabarcoding allows for the simultaneous identification of multiple species from a single sample. This technique uses high-throughput sequencing platforms such as Illumina MiSeq to generate millions of DNA sequences, which can then be compared to reference databases to identify the species present in a sample (Valentini et al., 2016). Metabarcoding is particularly useful in ecosystems with high species diversity, as it provides a comprehensive overview of the entire community, rather than focusing on individual species.

In biodiversity hotspots like Bhutan, where species richness and endemism are high, eDNA metabarcoding can provide a more complete picture of aquatic ecosystems. By analyzing water samples from catchments, researchers can assess the presence of both native and invasive species, track changes in community structure, and evaluate the impacts of human activities on ecosystem health (Dorji, 2021).

Comparison of eDNA metabarcoding and traditional methods

Numerous studies have demonstrated the superiority of eDNA metabarcoding over traditional morphometric methods in terms of both sensitivity and efficiency. Traditional methods are limited by their reliance on physical sampling, which can be biased toward easily captured species and often fail to detect rare or cryptic species (Simmons et al., 2020; Baudry et al., 2023). eDNA, by contrast, offers a non-invasive alternative that provides more accurate and comprehensive data on species presence.

For instance, in a study of freshwater fish communities in lentic environments in Europe, eDNA metabarcoding was able to detect rare and cryptic species that were missed by traditional methods, leading to a more accurate assessment of biodiversity (Czeglédi et al., 2021). Similarly, eDNA metabarcoding studies in Nepal and Australia have demonstrated that this technique can detect multiple species in a single sample, offering significant time and cost savings compared to conventional surveys (Gelis et al., 2021).

The necessity of eDNA for monitoring Bhutan's freshwater biodiversity

In the face of conservation threats to Bhutan's freshwater ecosystems, eDNA has emerged as a transformative tool for biodiversity monitoring and management. Traditional fish biodiversity assessment methods, such as net sampling, electrofishing, and morphometric analysis, are time-consuming, invasive, and resource-intensive, limiting their effectiveness in Bhutan's rugged and often inaccessible landscapes (NRCRLF, 2017; DoFPS, 2024). These conventional techniques frequently fail to detect cryptic, rare, or migratory species, leading to gaps in biodiversity data critical for conservation planning (Thomsen et al., 2012; Simmons et al., 2020; Baudry et al., 2023).

The pilot study conducted Mangde Chhu River Basin Pilot Study demonstrated eDNA's potential to detect diverse fish species, including cryptic and endangered ones, by analyzing DNA fragments shed into the water (DoFPS, 2024). This is particularly significant for Bhutan, where many fish species are endemic, elusive, and face growing pressures from hydropower development, habitat fragmentation, and invasive species.

The adoption of eDNA-based monitoring in Bhutan addresses several critical conservation needs:

1.Species such as the Golden Mahseer and Snow Trout, which are highly vulnerable to habitat fragmentation caused by hydropower dams and other developmental activities, can be effectively monitored using eDNA (Karmacharya et al., 2023; DoFPS, 2024).

2.The introduction of invasive species such as Brown Trout has already caused significant declines in native fish populations in Bhutan's rivers (Dorji and Gurung, 2017). eDNA enables the early detection of invasive species, even at low abundances, allowing for rapid management interventions to mitigate their spread (DoFPS, 2024).

3.eDNA provides a scalable and reliable approach to monitoring community composition and tracking changes in biodiversity over time. eDNA can capture baseline data and assess shifts in aquatic communities, which are crucial for understanding ecosystem responses to hydropower development, climate change, and pollution (DoFPS, 2024; Gardham et al., 2014).

4.Bhutan's rugged topography and high-altitude freshwater systems often hinder comprehensive biodiversity assessments using traditional methods. eDNA's reliance on water samples, which are easier to collect and transport, makes it ideal for surveying species in remote and otherwise inaccessible habitats (DoFPS, 2024). This broadens Bhutan's capacity to document biodiversity across its freshwater ecosystems, filling critical data gaps.

Table 2: Comparison of eDNA Metabarcoding and Traditional Methods

Method	Traditional Techniques	eDNA Metabarcoding
Sensitivity	Low (common species detected)	High (detects cryptic and rare species)
Invasiveness	High (requires physical capture of species)	Non-invasive (water sampling only)
Time and Cost	Time-intensive and costly	More efficient and cost-effective
Species Detection	Biased towards common and easily captured species	Comprehensive (multiple species in one sample)
Taxonomic Expertise	Requires experts for identification	Less reliant on in-field expertise (DNA analysis)

Challenges and future directions

Challenges of implementing eDNA in Bhutan

While eDNA holds immense potential for revolutionizing biodiversity monitoring in Bhutan, there are several key challenges to its implementation. These challenges are not unique to Bhutan but are common to many regions with limited resources and infrastructure.

1.One of the primary challenges in implementing eDNA in Bhutan is the lack of necessary laboratory infrastructure and sequencing technology. eDNA analysis relies on sophisticated techniques such as PCR amplification and high-throughput sequencing, which require specialized equipment and clean fab facilities (Valentini et al., 2016). The College of Natural Resources and other few agencies have spearheaded the addition of lab facilities and they are at early stage currently. However, Bhutan currently lacks the laboratory capabilities to carry out large-scale eDNA metabarcoding in-country, necessitating the outsourcing of DNA sequencing to laboratories in other countries.

2.The successful implementation of eDNA also requires trained personnel with expertise in molecular biology, genetics, and bioinformatics. Bhutan has a limited pool of researchers with the necessary skills to conduct eDNA analysis, particularly in the areas of DNA extraction, sequencing, and data interpretation (Dorji, 2021). To overcome this challenge, there is a need for capacity-building programs that train local researchers, university students, and conservationists in the technical aspects of eDNA monitoring. Collaborations with international research institutions can also help to address this gap by providing training and knowledge exchange.

3.Implementing eDNA-based monitoring in Bhutan requires significant investment in infrastructure, training, and equipment. While eDNA has the potential to be more cost -effective in the long run, the initial setup costs can be prohibitive for a country like Bhutan, which has limited financial resources allocated for research and biodiversity monitoring. Securing funding from international conservation organizations, governmental bodies, and donor agencies will be critical to establishing eDNA capacity in Bhutan (Simmons et al., 2020).

Collecting water samples for eDNA analysis requires careful planning and coordination, especially when field sites are located in areas that are difficult to reach by vehicle or on foot (Gurung & Thoni, 2015). Bhutan's rugged terrain and remoteness pose significant logistical challenges for fieldwork, particularly in terms of accessing high-altitude rivers and streams. Additionally, the cold temperatures in Bhutan's alpine regions may affect DNA preservation, requiring the use of specialized equipment for sample storage and transport to ensure the integrity of the samples (Karmacharya et al., 2023).

Overcoming challenges

A well equipped eDNA laboratory was successfully established in December 2024 at the College of Natural Resources, Royal University of Bhutan, with support from ETH Zurich, Switzerland. The laboratory is currently capable of performing DNA extraction and PCR analyses. This initiative is part of a broader effort to enhance biodiversity monitoring in Bhutan, as highlighted by a recent pilot study, emphasizing the need for an expanded species reference database to maximize the potential of eDNA methods in ecological research. Several regions with limited resources have successfully implemented eDNA by building the necessary infrastructure and expertise, providing valuable lessons for Bhutan. For example, in Nepal, which shares similar geographical and environmental challenges with Bhutan, researchers have successfully used eDNA to assess fish biodiversity in remote Himalayan rivers (Manandhar et al., 2023). By partnering with international institutions and securing funding from conservation organizations, Nepal has been able to establish local capacity for eDNA analysis.

Similarly, countries in Southeast Asia have made significant progress in building eDNA databases for their aquatic biodiversity, despite limited resources. By developing region-specific reference databases of fish species, these countries have improved the accuracy of eDNA species identification, enabling more reliable biodiversity assessments (Valentini et al., 2016). Bhutan can take inspiration from these success stories by prioritizing the development of a national eDNA database for its freshwater species. Building a comprehensive database of fish DNA sequences will enhance the ability to identify species accurately and will support future biodiversity monitoring initiatives.

The path forward for eDNA in Bhutan

To fully realize the potential of eDNA in Bhutan, several steps must be taken. Establishing eDNA laboratories and acquiring the necessary equipment will be critical to enabling Bhutan to conduct in-country eDNA analysis. This will reduce dependence on external laboratories and lower the costs associated with sample processing. Currently clean lab is established in CNR, but limited trained personal is the main issue. Further, training programmess for local researchers and conservationists need to be strengthen to ensure that Bhutan has the skilled personnel needed to carry out eDNA sampling, analysis, and data interpretation. Collaboration with international research institutions can facilitate the transfer of knowledge and technical expertise. Building a reference library of fish DNA sequences specific to Bhutan will improve the accuracy of species identification and contribute to global biodiversity research. Partnering with international databases such as NCBI GenBank can also enhance Bhutan's ability to integrate its data into global conservation efforts. Bhutan will need to secure financial support from international conservation organizations, governmental bodies, and donor agencies to cover the initial costs of establishing eDNA capacity. By demonstrating the long-term benefits of eDNA for biodiversity conservation, Bhutan can attract the necessary funding to support this important initiative.

Conclusion

eDNA has emerged as a transformative tool for fish biodiversity monitoring, offering a non-invasive, cost-effective, and highly efficient alternative to traditional methods. In the context of Bhutan's freshwater ecosystems, where traditional approaches like net sampling and electrofishing have been limited by logistical challenges, species detection biases, and resource constraints, eDNA provides a promising solution. The ability of eDNA to detect cryptic, rare, and migratory species that might otherwise evade detection through conventional methods makes it particularly valuable for Bhutan, where many fish species are endemic, threatened, or occupy remote habitats.

The non-invasive nature of eDNA is especially advantageous in a country like Bhutan, which places a high value on environmental conservation and the preservation of biodiversity. By simply collecting water samples, eDNA minimizes the disturbance to aquatic ecosystems, allowing researchers to monitor fish populations without physically capturing or harming species. This method is not only more species-sensitive but also more resource-efficient, reducing the time, labor, and costs associated with traditional fieldwork (Valentini et al., 2016). Moreover, eDNA enables comprehensive ecosystem monitoring, as it can detect multiple species from a single sample, providing a broader understanding of community dynamics and species interactions.

To fully capitalize on the potential of eDNA for aquatic biodiversity management in Bhutan, several steps should be prioritized. First, investment in infrastructure—including the establishment of eDNA laboratories and the acquisition of high-throughput sequencing technology—will enable Bhutan to conduct in-country analyses and reduce reliance on external facilities. Second, capacity building through training programs will ensure that local researchers have the necessary expertise in molecular biology, genetics, and bioinformatics to implement eDNA-based monitoring.

Third, the development of a national eDNA database for Bhutan's freshwater species will enhance species identification accuracy and support long-term biodiversity monitoring efforts. Finally, securing financial support from international conservation organizations and government bodies will be essential to cover the initial costs of implementing eDNA technologies.

By integrating eDNA into its biodiversity conservation strategies, Bhutan can position itself at the forefront of cutting-edge environmental monitoring, ensuring the protection and sustainable management of its unique aquatic ecosystems. As Bhutan faces growing pressures from hydropower development, urbanization, and climate change, eDNA offers a crucial tool for adaptive management and informed decision-making. The continued application of eDNA will not only strengthen Bhutan's conservation efforts but also contribute valuable data to global biodiversity initiatives, reinforcing Bhutan's role as a leader in environmental stewardship.

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