



# The Importance of Biodiversity Monitoring in Indonesia's National Priority Lakes: An Environmental DNA in Lake Rawa Pening

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## Abstract

The tropical Lake Rawa Pening, designated by presidential decree as one of Indonesia's national priority lakes, is crucial to the region's biodiversity and ecosystem services. This study analysed the biodiversity of fish species in the lake using Illumina paired-end sequencing, yielding 125,374 reads. The analysis identified 34 taxa across 11 orders and 16 families, with high similarity between sampling sites (0.91–1.00). Order Cypriniformes dominated the fish community, representing 41.80 % of average reads. Conversely, Order Clupeiformes had the lowest total reads count at 0.11 %. Significant presence was also observed for Order Cichliformes (19.90 %) and Order Anabantiformes (19.18 %). At the species level, *Channa micropeltes* was the most dominant species (11.07 %), followed by *Osteochilus vittatus* (10.70 %), *Amphilophus labiatus* (9.63 %), and *Ctenopharyngodon idella* (8.94 %). Native species accounted for 54.7 % of the total reads, while introduced species comprised 43.3 %, including invasive (31.7 %) and non-invasive species (11.6 %). The high presence and dominance of invasive species underscore the need for targeted conservation and management efforts. It is recommended that biodiversity monitoring be promptly initiated in other nationally prioritised lakes, as biodiversity is crucial for ecosystem health and resilience.

**Keywords:** eDNA metabarcoding, fish biodiversity, Indonesia's national priority lakes, invasive species, Lake Rawa Pening

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## Introduction

As an archipelagic nation, Indonesia has numerous ecologically and economically significant lakes. Lake Rawa Pening, located in Central Java, Indonesia, holds substantial ecological, economic, and cultural importance. It is one of the 15 lakes designated as a national priority for conservation by the Indonesian government under Presidential Regulation No. 60 of 2021. The prioritisation of these lakes was based on six key indicators, including the level of lake degradation (e.g., sedimentation, pollution, and eutrophication), the extent of lake utilisation for sectors such as agriculture, fisheries, hydropower, and tourism, as well as the commitment of local governments and communities to lake management. Additional factors include the strategic national importance of the lake, its biodiversity (such as the presence of endemic

species and invasive), and the lake's vulnerability to disasters or climate change impacts. Spanning an area of approximately 2,670 hectares, the Lake Rawa Pening is a critical freshwater resource and a biodiversity hotspot, home to numerous flora and fauna (Mardiatno et al., 2023).

However, the lake is currently under threat due to environmental degradation, driven by rapid urbanisation, sedimentation, agricultural expansion, and the proliferation of invasive species, particularly water hyacinths (*Eichhornia crassipes*) (Irawan & Haryanti, 2020; Prasetyo et al., 2022), and several other invasive species (Kurniawan et al., 2023; Marwoto et al., 2020), which have overwhelmed large portions of the lake. The ecological health of Rawa Pening is inextricably linked to its role in providing essential ecosystem services to the surrounding

communities. The lake is vital for water supply, flood control, and sediment retention, which are indispensable to local agriculture and fisheries (Mardiatno et al., 2023; Piranti et al., 2019; Soeprbowati, 2017). Additionally, the lake supports traditional livelihoods, such as fishing and aquaculture, and plays a central role in tourism, contributing to the local economy (Hakim et al., 2011). However, increasing sedimentation, eutrophication, and pollution from agriculture have significantly reduced the lake's capacity to fulfil these functions, necessitating urgent conservation efforts.

Previous studies on Rawa Pening have shown that the deterioration of the lake's water quality and the decline in biodiversity is closely associated with human activities (Mardiatno et al., 2023; Prasetyo et al., 2022; Soeprbowati et al., 2016). The unchecked spread of invasive plant species, coupled with nutrient pollution from agricultural practices, has altered the lake's ecosystem dynamics, leading to a loss of native species and a decline in fish populations (Gallardo et al., 2016; Hassan & Nawchoo, 2020; Schultz & Dibble, 2012). These changes threaten the lake's biodiversity and the livelihoods of communities that rely on these natural resources (Keller et al., 2018; Mwangi & Swallow, 2008; Shackleton et al., 2019). Despite various restoration initiatives, a comprehensive understanding of the lake's ecosystem and long-term sustainability strategies remains underexplored.

In recent years, environmental DNA (eDNA) has emerged as a powerful tool for monitoring biodiversity in aquatic ecosystems, including lakes. eDNA has proven to be a highly effective tool for detecting species presence in ecosystems and analysing genetic material found in environmental samples (Bohmann et al., 2014; Foote et al., 2012; Ruppert et al., 2019). This method has also been successfully applied in lakes around the world, where it has proven particularly useful in monitoring elusive or rare species, detecting invasive species, ecosystem dynamics, and assessing overall ecosystem health (Bista et al., 2017; Capo et al., 2021; Harrison et al., 2019; Hervé et al., 2022; Priyono et al., 2022; Priyono et al., 2023; Pukk et al., 2021).

This study aims to use environmental DNA (eDNA) monitoring as a cutting-edge method for assessing the biodiversity of Lake Rawa Pening. This approach offers a non-invasive means of cataloguing species, including elusive or rare organisms, which traditional survey methods may overlook. By leveraging the advantages of eDNA metabarcoding, conservationists and policymakers can gain a deeper understanding of the biodiversity within these ecosystems and develop more targeted and efficient management strategies.

## Materials and Methods

### *Ethical approval*

IACUC approval was not required for this study as the methodology relied exclusively on environmental DNA (eDNA) extracted from surface water samples. The methods were entirely non-invasive, and no live animals or fish specimens were captured, handled, or harmed during the research.

### *Water sampling and filtration*

This study was conducted at Lake Rawa Pening, Central Java, Indonesia. (Fig. 1). The eDNA sampling in Lake Rawa Pening was performed only once in April, with two replicates per site and 1 negative control per site, resulting in a total of 8 samples for sequencing. The eDNA sampling conducted in Lake Rawa Pening represents an initial step in understanding the fish diversity in the lake using environmental DNA (eDNA) analysis. While the sampling was limited to a single event, this preliminary study can provide valuable insights into the fish composition in the lake. The chosen locations included two points in the central area of the lake and two sites near the lakeside. The central sampling sites were selected to represent the open water habitat, which is critical for assessing the overall biodiversity of the lake. In contrast, the shoreline points were chosen due to their proximity to fish farming cages and agricultural lands, which are likely to influence the lake's ecological dynamics through nutrients run-off and habitat alteration (Piranti et al., 2019; Sulastri et al., 2016). The Jaccard

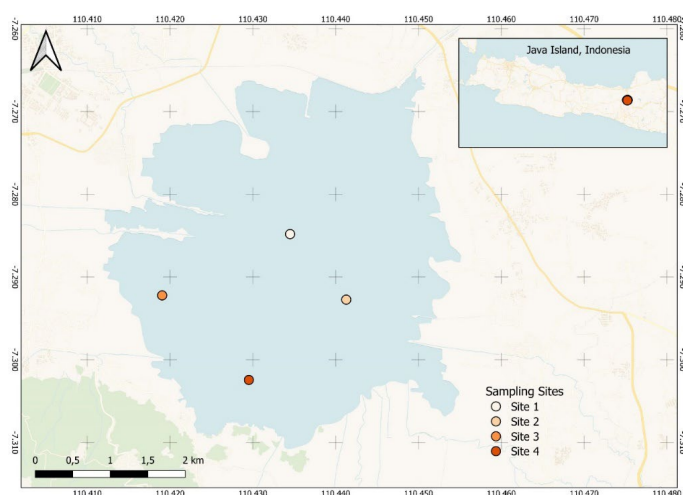


Fig. 1. Map of Lake Rawa Pening showing the four sampling sites selected for fish monitoring using eDNA analysis.

similarity index was used to evaluate the similarity in species composition between the different sampling sites. This index measures the proportion of shared species between two samples, calculated as the ratio of species found in both samples divided by the total number of species in either sample (Fletcher & Islam, 2018). At each sampling location, 1 L of surface water was collected using sterile sampling bottles. Negative controls were included by collecting deionised water at each site to prevent contamination during sampling. These negative controls were handled identically to the water samples by following the same procedures: sterile bottles were used, deionised water was collected alongside environmental water samples, stored in the same conditions, and transported under identical temperature and contamination-prevention protocols. All equipment was sterilised, and gloves were worn throughout the sampling process. Immediately after collection, the water samples were filtered on-site using a 0.45 µm mixed cellulose ester (MCE) membrane filter (Merck Millipore, Germany). After filtration, the MCE membrane filters containing the eDNA were carefully placed into tubes containing Longmire buffer, which is well-known for its effectiveness in preserving eDNA samples (Wegleitner et al., 2015; Williams et al., 2016).

### **DNA extraction, PCR amplification, and sequencing**

DNA was extracted from the samples using the Zymo Quick-DNA™ Miniprep Kit (Zymo Research, USA) following the manufacturer's protocol. DNA was amplified using a two-step PCR method, where barcoded Illumina adapters were incorporated during a second round of amplification. The initial amplification was performed in two replicates for each sample, and the resulting products were pooled to reduce potential PCR bias. All filter and extraction controls were processed alongside the samples during the PCRs. Two-step PCR was used to amplify the DNA, with the second round of amplification (PCR2) incorporating barcoded Illumina adapters. To reduce the possibility of PCR bias, each sample completed four duplicates of the first amplification, and the final results were pooled. When processing the samples for the PCRs, all filter and extraction controls were done concurrently. Using FLUOstar Optima (BMG LABTECH, Germany), 2 µL of product from each reaction was measured. These results were used to calibrate the samples to equal concentrations and clean them using AmPure XP beads (Beckman Coulter, USA). Using the KAPA Library Quantification Kit on a QuantStudio 12K (Applied Biosystems, USA), the Illumina-tagged DNA concentration of each pool was calculated, and a 2100 Bioanalyzer instrument (Agilent, USA) was used to determine the size of each DNA fragment. The resulting pools were quantified using the KAPA Library Quantification Kit and a QUBIT 3.0 with the dsDNA HS test. An Illumina MiSeq platform was used for library sequencing. On a 150 bp Paired-End sequencing run, the MiFish was sequenced MiFish primer, forward 5'-GTCGGTAAACTCGTGCCAGC-3' and reverse (5'-

CATAGTGGGGTATCTAATCCCAGTTTG-3') (Miya et al., 2015).

### **Bioinformatics analysis**

The fastQ files were then analysed using the QIIME2 pipeline, supported by Python scripts for additional data processing and visualisation (Bolyen et al., 2018). The analysis began with preparing raw sequence data in FASTQ format, where both single-end and paired-end reads were organised according to naming conventions required by QIIME2 (Bolyen et al., 2018). The raw FASTQ files were imported into QIIME2 as demultiplexed sequences and converted into QIIME2 artifacts (\*.qza). Quality control was carried out using DADA2 to denoise the data (Callahan et al., 2016). The pipeline applied a stringent maximum expected error (max-ee) threshold of 2.0 to both forward and reverse reads, thus excluding sequences with potential errors. This filtering, combined with sequence truncation at 130 bp, ideally will effectively remove low-quality bases and reduce noise, though it may have also excluded rare species with lower-quality DNA. Additionally, the cutadapt tool removed non-biological sequences, such as primers and adapters, further ensuring that only high-quality biological data are left (Martin, 2011).

Taxonomic classification of the filtered sequences was performed using the BLAST algorithm against the Mitofish database (Sato et al., 2018), curated explicitly for fish mitochondrial DNA. Filtering was also applied to retain only sequences with sufficiently high BLAST scores, minimising the inclusion of false positives. A custom Python script utilising Seaborn and Matplotlib was used to visualize the distribution of BLAST scores (Bisong & Bisong, 2019), and only sequences meeting the quality threshold were carried forward for taxonomic assignment. Filtering was then applied to retain only sequences with sufficiently high BLAST scores, minimising the inclusion of false positives. Only sequences meeting the quality threshold were carried forward for taxonomic assignment. Following taxonomic classification, the generated feature table summarises the number of reads per operational taxonomic unit (OTU) across all samples. Feature table was created using Pandas understanding of the diversity and abundance of taxa (McKinney & Team, 2015); thus, missing taxonomic assignments were addressed by filling in gaps to ensure all taxa were included. The data were merged with taxonomic classifications and split into hierarchical levels. Afterward, the data was exported from QIIME2 for further analysis. The processed sequences and feature table were converted to BIOM format, ensuring compatibility with downstream bioinformatics tools for subsequent analyses and visualisation using Rstudio (Allaire, 2012). Additionally, the Jaccard index was used to assess the similarity in species composition among the sampling sites.

In determining the native and introduced status of the fish species in Lake Rawa Pening, we consulted the

FishBase (Froese & Pauly, 2010) database for information on species natural distribution. Additionally, we referred to the book “Jenis Ikan Introduksi dan Invasif Asing di Indonesia” (Dewantoro & Rachmatika, 2020) to identify species introduced to Indonesia and those considered invasive. Native species naturally occur and evolve within a specific geographic region or ecosystem over a long period, contributing to its ecological balance. Invasive species, conversely, are non-native organisms introduced to a new environment that spread rapidly and cause harm to the ecosystem, economy, or human health. Introduced species refer to non-native organisms brought into a new area, intentionally or unintentionally. Meanwhile, introduced but not invasive species are those that, despite being introduced to a new environment, do not spread aggressively or disrupt the ecological balance. A list of fish species in Lake Rawa Pening based on a visual census was obtained from a previous study by Weri and Sucahyo (2017). Their research primarily focused on the relationship between fishing gear and captured fish species rather than a biodiversity survey. To compare the species detected in our eDNA study with those identified by Weri and Sucahyo (2017), we created a Venn diagram to illustrate the overlap and differences in species composition between the two methods.

## Results

All negative controls showed no amplification, confirming the absence of laboratory contamination throughout sample collection, DNA extraction and PCR amplification. Illumina paired-end sequencing yielded 125,374 reads. The dataset encompasses multiple species spanning different orders, families, and species, reflecting a diverse fish community within the lake. After following the pipeline procedure for eDNA metabarcoding, we detected 34 taxa in 11 orders and 16 families. *Channa micropeltes* dominate across all sites, contributing 10.3 % in Site\_1, 10.9 % in Site\_2, 12.1 % in Site\_3, and 10.8 % in Site\_4. *Osteochilus vittatus* emerges prominently in Site\_2 (13.2 %) and Site\_4 (12.1 %), while its abundance is slightly lower in Site\_1 (7.3 %) and Site\_3 (9.8 %). *Rasbora lateristriata* holds significant percentages in Site\_1 (13.0 %) and Site\_2 (9.9 %) but declines in Site\_3 (6.5 %) and Site\_4 (5.9 %). Meanwhile, *Amphilophus labiatus* shows dominance in Site\_3 (12.4 %) and Site\_4 (11.9 %), while being relatively lower in Site\_1 (5.3 %) and Site\_2 (6.9 %). Other notable species include *Barbodes binotatus*, which is particularly abundant in Site\_1 (10.8 %) and Site\_2 (11.3 %), but drops in Site\_3 (5.5 %) and Site\_4 (3.6 %). The results of the Jaccard index analysis show high similarities between sites (0.91–1.00). These values suggest a stable and homogeneous fish community across the lake, which may be attributed to the interconnectedness of habitats that facilitate species movement and mixing (Campbell & Mandrak, 2019; Kurnianto et al., 2021; Taylor, 2004). Such high similarity indices often indicate shared environmental

conditions that support similar species assemblages, as observed in other studies where habitat continuity played a critical role in species distribution (Cardoso et al., 2016; Taylor, 2004; Vitule et al., 2012).

The analysis of eDNA metabarcoding data from Lake Rawa Pening reveals a diverse range of fish orders, families, and species (Fig. 2). Among the orders, Cypriniformes is the most predominant, with a total count of 52,405, which accounts for 41.80 % of the overall reads. This is followed by Cichliformes, with a total of 24,947 reads, representing 19.90 % of the overall count, and Anabantiformes, with a total count of 24,048, making up 19.18 % of the overall reads. On the lower end, Clupeiformes has the lowest total count at just 142, accounting for 0.11 % of the overall reads. At the family level, Cyprinidae dominates with a total count of 36,448, constituting 29.07 % of the overall reads. This is followed by Cichlidae, which has 24,947 reads or 19.90 % of the total, and Channidae, with a total count of 20,219, making up 16.13 % of the overall reads. Conversely, Mastacembelidae is the least represented family with a total count of 111, 0.09 % of the overall reads. Additionally, a small but consistent portion of reads across all sites are categorised as “Unidentified” (2.1 % of the overall reads), pointing to potential limitations in the resolution of the eDNA metabarcoding method.

The bar plot on the left displays the eDNA read counts for each identified fish species (Fig. 3a). The analysis of the average percentage of reads for each species across all sampling sites revealed variations in relative abundance for each species. *Channa micropeltes* emerged as the most dominant species, accounting for an average of 11.07 % of the total reads. Following closely, *Osteochilus vittatus* represented 10.70 % of the total reads, highlighting its strong presence in the lake. Other notable species include *Amphilophus labiatus* and *Ctenopharyngodon idella*, which accounted for 9.63 % and 8.94 % of the total reads, respectively. Conversely, *Systemus rubripinnis* and *Mastacembelus unicolor* were among the least abundant, with average read percentages of 0.08 % and 0.09 %, respectively. The right heatmap shows eDNA reads' intensity for each species across different sampling locations (Fig. 3b). Each cell in the heatmap represents the read intensity for a specific species-location combination, with darker shades indicating higher read counts. From this heatmap, it can be observed that the eDNA read intensity varies among species and locations. For instance, *Channa micropeltes* and *Osteochilus vittatus* exhibit high reads intensity at Site\_3 and Site\_4, indicating that these species are more prevalent at these locations. On the other hand, some species, like *Mystacoleucus obtusirostris* and *Tor tambra*, show lower read intensity, suggesting a more limited distribution or smaller populations in this lake.

The sunburst chart (Fig. 3c) provides a detailed breakdown of the ecological statuses of fish species in Lake Rawa Pening. Figure 3c illustrates species

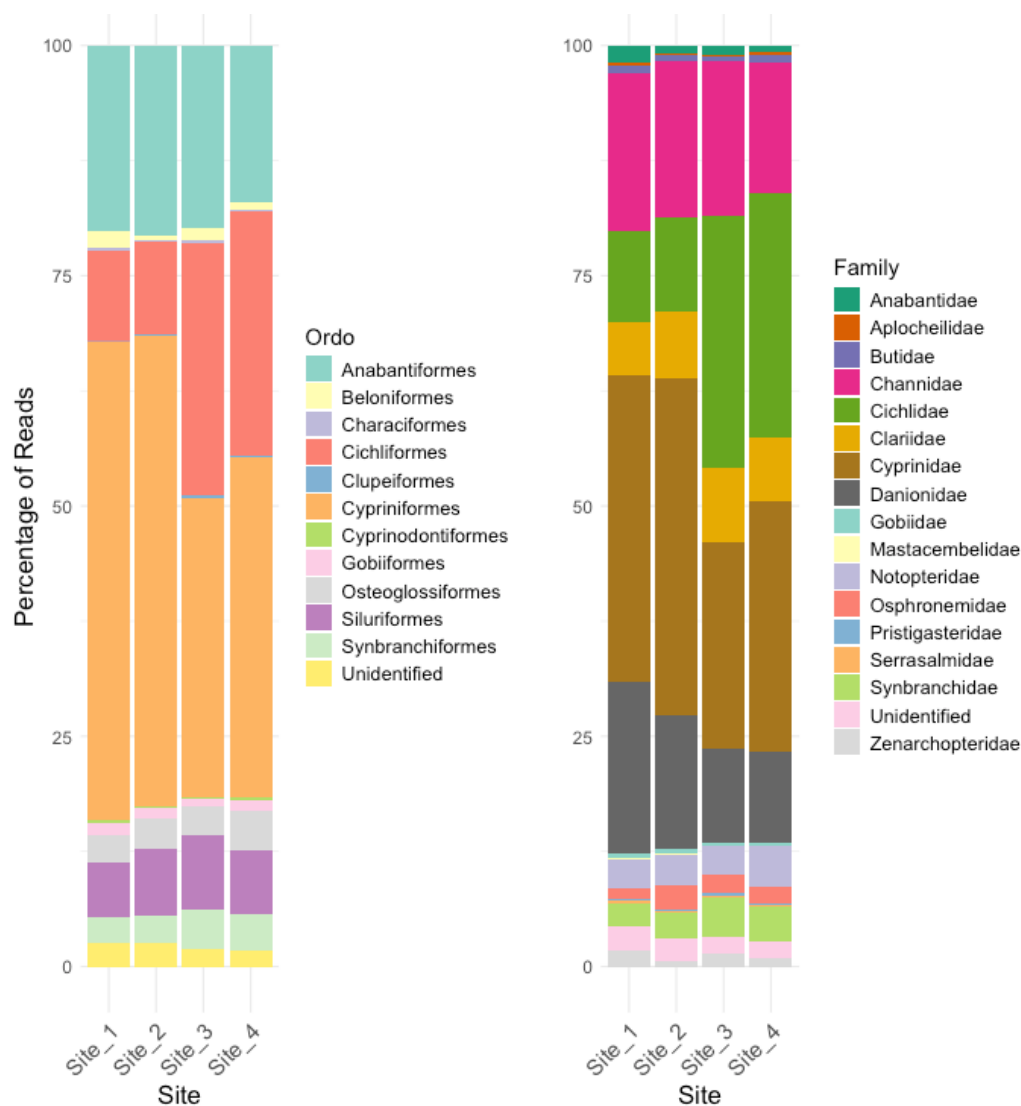


Fig. 2. Stacked bar plot of average reads percentages by taxonomic order and family across sampling sites.

classification using a sunburst chart. The inner layer shows the primary categories: native (54.7 %) and introduced (43.3 %). The outer layer further divides the introduced species into invasive (31.7 %) and non-invasive (11.6 %). Additionally, 2.1 % of the species remain unidentified.

A comparison of fish species detected through eDNA (this study) and visual surveys (Weri & Sucahyo, 2017) in Lake Rawa Pening revealed both overlap and unique species in each dataset. Thirty-four fish species were identified through eDNA, while the visual survey recorded 17 species (Fig. 4). Seven species were common to both studies, including *Oreochromis niloticus*, *Ctenopharyngodon idella*, *Channa striata*, *Amphilophus labiatus*, *Trichogaster trichopterus*, *Trichogaster pectoralis* and *Clarias batrachus*. Notably, eDNA identified 27 species not recorded in the visual survey, namely *Channa micropeltes*, *Barbichthys laevis*, *Anabas testudineus*, *Dermogenys pusilla*, *Colossoma macropomum*, *Oreochromis mossambicus*, *Opisthopterus tardoore*, *Barbodes binotatus*, *Barbonymus balleroides*, *Barbonymus gonionotus*, *Cyclocheilichthys apogon*, *Hampala macrolepidota*,

*Mystacoleucus obtusirostris*, *Neolissochilus soro*, *Osteochilus vittatus*, *Systemus rubripinnis*, *Tor tambra*, *Tor tambroides*, *Rasbora argyroteenia*, *Rasbora lateristriata*, *Aplocheilus armatus*, *Oxyeleotris marmorata*, *Glossogobius aureus*, *Chaenogobius annularis*, *Notopterus notopterus*, *Mastacembelus unicolor*, and *Monopterus albus*. In contrast, the visual survey detected ten species not found in the eDNA analysis, including *Osteochilus hasselti*, *Glyptosternum platypogon*, *Chela oxygastroides*, *Amphilophus trimaculatus*, *Chitala lopis*, *Anguilla rostrata*, and *Amyda cartilaginea*.

## Discussion

The eDNA metabarcoding analysis from Lake Rawa Pening reveals a diverse fish community. One of the dominant fish species in Lake Rawa Pening is *Channa micropeltes*. This species has also been reported as dominant in several other lakes in Indonesia, such as Lake Sermo (Hedianto, 2023; Suryandari et al., 2021) and Lake Sentani (Ohee et al., 2018, 2020). This indicates that *C. micropeltes* is a widely distributed and dominant species in various lake ecosystems across

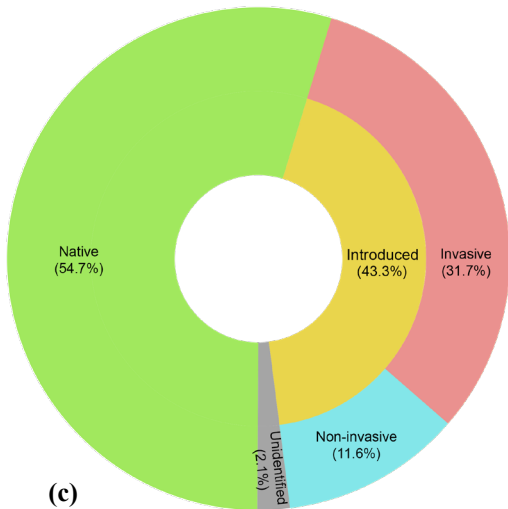
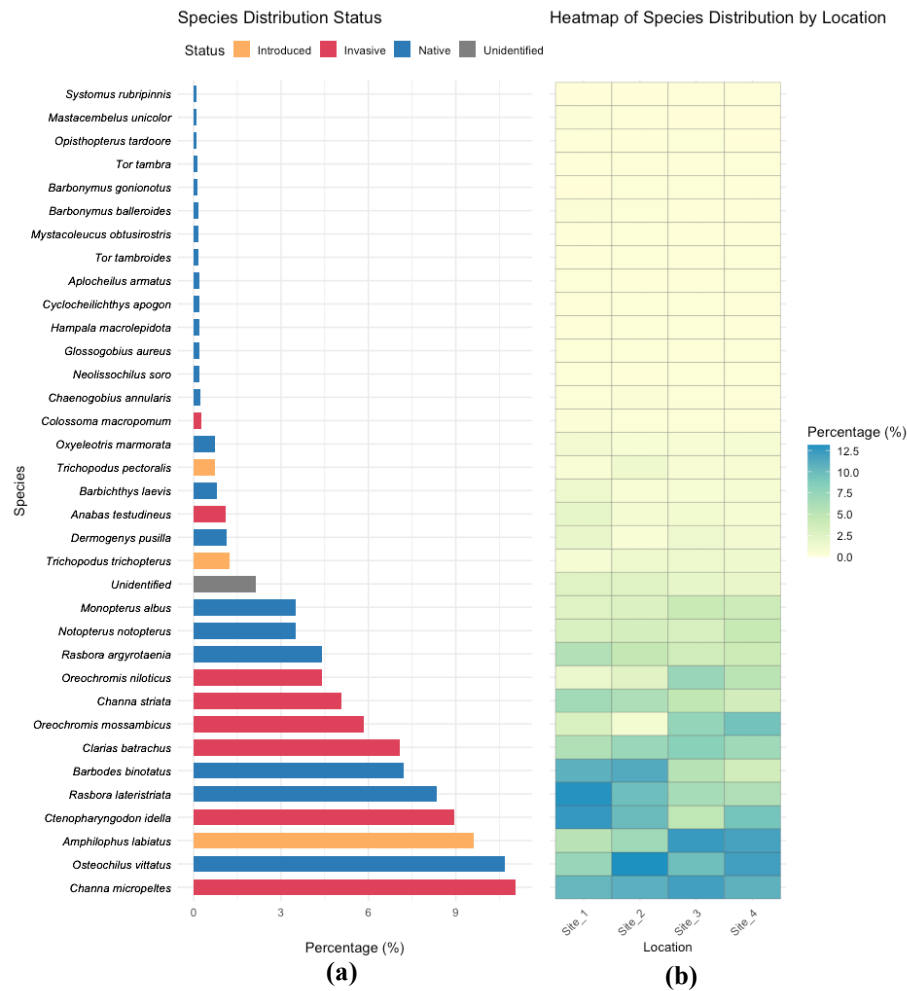


Fig. 3. Overview of species composition and distribution based on eDNA metabarcoding of four sampling sites in Lake Rawa Pening. (a) Percentage of sequencing reads per species, categorised by status (native, introduced, and invasive), (b) Species distribution across the four sampling sites, represented by read counts in a heatmap, (c) Proportional representation of native, introduced, and invasive species in the lake, visualised in a circular plot.

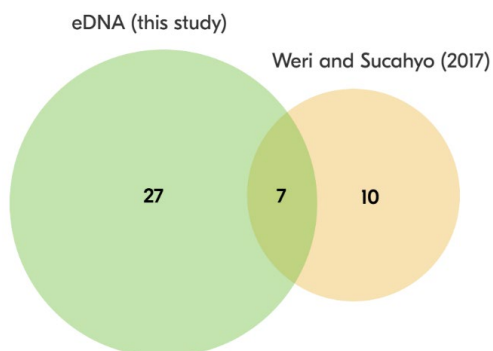


Fig. 4. Venn diagram comparing fish species detected in Lake Rawa Pening using eDNA (this study) and the visual survey conducted by Weri and Sucahyo (2017).

Indonesia. *Osteochilus vittatus*, also known as the bony lip barb, is a dominant fish species in various lakes and reservoirs in Indonesia. Furthermore, Sathyananta (2023) specifically examined the food composition of *O. vittatus* in Tamblingan Lake, Bali, and found that it was an omnivorous fish, consuming a variety of phytoplankton, zooplankton, and aquatic plants. This suggests that *O. vittatus* is well-adapted to thrive in diverse lake ecosystems in Indonesia. Additional studies have also reported the dominance of *O. vittatus* in other water bodies, such as Benanga Reservoir in East Kalimantan (Jusmaldi et al., 2020a, 2020b) and the Lebak Kalong Floodplain in South Sumatra (Irawan, 2023). These findings indicate that *O. vittatus* is a widely distributed and ecologically important species in Indonesian freshwater habitats.

The high Jaccard index values between sites suggest a stable and homogeneous fish community across the lake. This high similarity could be due to the interconnectedness of habitats within the lake, facilitating the movement and mixing of species across different areas. The dominance of Cyprinidae suggests these groups are key components of the lake's ecosystem. Similar dominance patterns by Cypriniformes have been observed in other Indonesia's lake ecosystems, indicating their critical role in such habitats (Gustiano et al., 2023; Haryani & Samir, 2020; Syandri & Yunus, 2014). Cyprinidae are known for their adaptability to various environmental conditions and prolific breeding capabilities, which could explain their prevalence in Indonesian lakes. Order Cichliformes also showed substantial presence. The high relative abundance of Cichliformes can be attributed to their diverse feeding strategies, evolution, and adaptability to various ecological niches, which allow them to thrive in different environments (Albertson et al., 2005; Liem, 1973; Pease et al., 2018). The strong presence of these taxonomic groups, especially generalist species, suggests that Rawa Pening may be experiencing similar ecological pressures that drive species composition in a particular direction, favouring species with broader ecological tolerances.

The diversity of fish orders and families detected through eDNA highlights the lake's complex and dynamic ecosystem role. However, the presence of both native and non-native species suggests potential disruptions to the natural balance. Non-native species, particularly invasive ones, can significantly impact the native biodiversity by outcompeting local species for resources, altering habitat structure, or introducing new diseases (Colin et al., 2018; Cucherousset & Olden, 2011; Gozlan et al., 2010; Manchester & Bullock, 2000). In Rawa Pening, the high proportion of invasive species raises concerns about long-term ecological sustainability. As noted in other studies, invasive species have the potential to alter entire aquatic food webs, which can result in declines in native populations and even local extinctions (Carey & Wahl, 2010; David et al., 2017; Goren et al., 2016). The ecological impact of invasive species in Rawa Pening is

particularly concerning, given the lake's status as a national priority lake. In this context, species like *Channa micropellets* and *Ctenopharyngodon idella*, may contribute to shifts in the lake's fish community structure. Other notable species include *Amphilophus labiatus*, the red devil cichlid, for its aggressive nature and adaptability (Kumar et al., 2021; Ohee et al., 2018; Robin et al., 2023). Their high abundance across various sampling sites indicates that they have established themselves successfully, possibly at the expense of native species.

The high detection of non-native species indicates potential vulnerability in the lake's native biodiversity. Native species play a crucial role in maintaining the natural balance of ecosystems by contributing to the stability of food webs and nutrient cycling (Carey & Wahl, 2010; da Silva et al., 2024; Eby et al., 2006; Martin et al., 2010; Schindler et al., 2001). However, the dominance of invasive species could indicate ongoing ecological shifts where native species are either outcompeted or displaced. This finding is consistent with other freshwater systems impacted by invasive species, where native species often decline in the presence of more aggressive or adaptable non-native species (Crowl et al., 2008; Cucherousset & Olden, 2011; Hermoso et al., 2011).

The proliferation of invasive species in Lake Rawa Pening can be closely linked to human activities, particularly aquaculture and land use practices. The expansion of fish farming, such as using fish farming cages, has introduced non-native species into the lake ecosystem (Hakim et al., 2011; Soeprbowati, 2017; Sulastri et al., 2016). These species, such as *Ctenopharyngodon idella* (grass carp) and *Channa micropeltes* (peacock bass), often outcompete local species for resources, disrupt food webs, and alter ecological dynamics. As noted by Geraldi et al. (2019), introducing non-native species through aquaculture can lead to significant shifts in biodiversity, with invasive species frequently dominating ecosystems. Furthermore, agricultural practices around the lake contribute to nutrient run-off, leading to eutrophication and creating favourable conditions for establishing and spreading invasive species. The use of fertilisers and pesticides not only deteriorates water quality but also encourages the growth of invasive aquatic plants, such as water hyacinths (*Eichhornia crassipes*), which can smother native flora and fauna (Beaury, 2023). This nutrient overload, coupled with land-use changes, has been shown to promote the dominance of non-native species and alter ecosystem services. Research indicates that areas with high human activity often experience increased propagule pressure from non-native species, further facilitating their establishment and spread (Barbosa et al., 2022; da Silva et al., 2024; Gustiano et al., 2023). This phenomenon is evident in Lake Rawa Pening, where the combined impacts of aquaculture and agricultural run-off likely contribute to the dominance of non-native species. The presence of species such as

*Channa micropeltes* and *Ctenopharyngodon idella* not only threatens native biodiversity but also disrupts habitat structures and nutrient cycling, further endangering the ecological integrity of the lake (Capps & Flecker, 2013; da Silva et al., 2024; Lafage et al., 2019).

Another important finding is the identification of “Unidentified” taxa, which points to potential limitations in the eDNA metabarcoding methodology. The incompleteness of reference databases for species identification is a known challenge in eDNA studies (Blackman et al., 2023; Priyono et al., 2022; Schenekar et al., 2020; Westfall et al., 2023). In the case of Rawa Pening, some species remain unresolved at the taxonomic level due to gaps in the genetic reference libraries. This highlights the need for continued efforts to expand these databases, particularly for tropical and understudied ecosystems, to improve the precision of species identification and enhance our understanding of local biodiversity. Despite these limitations, eDNA metabarcoding has proven an effective tool for assessing biodiversity in aquatic environments. Compared to traditional methods like netting or electrofishing, eDNA offers a non-invasive approach that can capture a broader snapshot of species diversity, including those that are elusive or occur in low abundances (Taberlet et al., 2018). The method’s ability to detect a wide range of species across multiple sampling sites in Rawa Pening demonstrates its value in ecological monitoring and conservation planning. As environmental pressures such as habitat degradation and species introductions continue to shape the lake’s ecosystem, eDNA will be crucial for tracking these changes.

The comparison of fish species detected through environmental DNA (eDNA) analysis and visual surveys in Lake Rawa Pening reveals insights into the biodiversity of this aquatic ecosystem. However, the eDNA analysis revealed 27 species not recorded in the visual survey, suggesting that visual methods may underestimate biodiversity, particularly for species with low abundance or those inhabiting complex environments (Muha et al., 2019; Yamanaka and Minamoto, 2016). This discrepancy is consistent with findings from other studies, which indicate that eDNA can capture a broader range of species compared to traditional survey methods (Muha et al., 2019; Nakagawa et al., 2018; Seymour et al., 2020; Valdivia-Carrillo et al., 2021). These findings emphasise the importance of employing multiple methodologies for comprehensive biodiversity assessments in aquatic ecosystems. Integrating eDNA with traditional methods can enhance understanding of fish community dynamics and inform effective conservation strategies (Matern et al., 2019; Sales et al., 2021). In summary, while eDNA metabarcoding provides a powerful, non-invasive tool for biodiversity monitoring, it is constrained by limitations such as incomplete reference databases, primer bias, lack of quantitative resolution for abundance, potential false

positives from DNA persistence or transport, and risks of contamination. These considerations highlight the importance of careful interpretation and the integration of eDNA with conventional survey methods for robust ecological assessments.

From a conservation perspective, the findings underscore the urgent need for active management and restoration efforts in Rawa Pening. The high proportion of invasive species significantly threatens the lake’s ecological integrity, requiring targeted interventions to mitigate their impact. Conservation strategies should include measures to control invasive species populations, such as biological control programs, barriers, or habitat restoration projects that enhance the resilience of native species (Arthington et al., 2016; Green & Grosholz, 2021; Jones et al., 2021; Rahel et al., 2008). Community engagement and awareness campaigns can also play a critical role in preventing the introduction of non-native species through aquaculture or other human activities (Atchison et al., 2017; Shackleton et al., 2019). Additionally, regular eDNA monitoring should be implemented to track the effectiveness of these interventions and detect early signs of new species introductions in Rawa Pening or other national priority lakes.

## Conclusion

This study represents a significant advancement in Indonesian freshwater biology by successfully utilising environmental DNA (eDNA) metabarcoding for the first time to assess fish biodiversity in Lake Rawa Pening, a national priority ecosystem. By identifying a diverse range of both native and non-native fish taxa, the research demonstrates that eDNA metabarcoding is a highly effective, non-invasive alternative to traditional sampling methods in tropical freshwater environments. The findings particularly highlight the ecological threat posed by invasive species, providing crucial, actionable insights for conservation management. This study underscores the urgent need to integrate regular eDNA biomonitoring into national conservation strategies to safeguard Indonesia’s vital aquatic biodiversity.

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