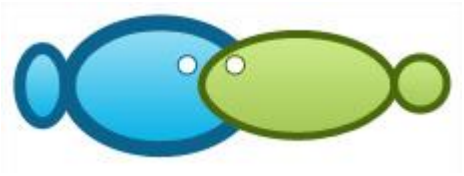

Assessing fish communities post-mass mortality at Serayu Movable Dam through eDNA metabarcoding

Agus Nuryanto, Nuning Setyaningrum, Yuriza Eshanada

Faculty of Biology, Universitas Jenderal Soedirman, Grendeng Purwokerto 53122, Central Java, Indonesia. Corresponding author: A. Nuryanto, agus.nuryanto@unsoed.ac.id

Abstract. Environmental DNA (eDNA) metabarcoding was used to assess freshwater fish community composition in the Serayu Movable Dam, Indonesia, using two mitochondrial 12S rRNA primer sets, MetaFish_1 and Tele02. eDNA was successfully extracted from filtered water samples, and PCR amplification confirmed that both primers efficiently amplified short fragments suitable for degraded environmental DNA. MetaFish_1 produced an amplicon of approximately 170 bp, while Tele02 generated a fragment of approximately 280 bp, consistent with their target regions. Sequencing results demonstrated clear differences in primer performance. MetaFish_1 yielded 41 sequences, of which 53.7% were assigned to fish taxa, whereas Tele02 produced 154 sequences, with 94.8% corresponding to fish, indicating higher specificity and detection efficiency of Tele02. A total of 11 fish species were identified at the species level, with Tele02 detecting more species than MetaFish_1. However, most reads from both primers were assignable only to the class Actinopterygii, reflecting limited species-level resolution likely caused by incomplete regional reference databases. Diversity indices (Shannon, Simpson, and Inverse Simpson) were comparable between primers, indicating moderate but relatively low diversity and dominance by a few taxa, consistent with ecological filtering and potential methodological bias. The detection of several marine fish taxa highlights challenges associated with broad-range primers, environmental DNA transport, and taxonomic misassignment in tropical freshwater systems. Overall, this study demonstrates the utility of eDNA metabarcoding for monitoring fish biodiversity in regulated rivers, while emphasizing that primer selection, sampling design, and reference database completeness critically influence detection accuracy and ecological interpretation.
Key Words: 12S rRNA primers, eDNA metabarcoding, fish community assessment, freshwater fish biodiversity, Serayu River.

Introduction. Freshwater ecosystems are among the most biodiverse yet highly threatened environments globally, providing essential habitats that support fish communities fundamental to ecosystem functioning, fisheries productivity, and human livelihoods. Rivers, lakes, and reservoirs sustain complex biological assemblages, but increasing anthropogenic pressures - including habitat modification, pollution, hydrological alteration, and biological invasions - have led to widespread degradation of freshwater biodiversity. Among these pressures, dams and reservoirs exert particularly strong impacts by altering natural flow regimes, fragmenting longitudinal connectivity, and reshaping physical and chemical habitats, often resulting in shifts in fish community composition and declines in native species richness (Faghihinia et al 2021; Vari et al 2022; Altowairqi & Shafi 2024; Anand et al 2025). Such alterations frequently favor tolerant or generalist taxa, while sensitive species decline, underscoring the importance of accurate biodiversity assessment to support effective conservation and sustainable management (Wegscheider et al 2024; Faro et al 2025).

The Serayu River basin in Central Java, Indonesia, exemplifies these challenges. The construction and operation of the Serayu Movable Dam and the Panglima Besar Soedirman Reservoir have substantially modified local aquatic habitats. In April 2022, a sediment flushing event from the reservoir triggered a catastrophic downstream fish kill, driven by acute deterioration of water quality and elevated suspended solids exceeding physiological tolerance thresholds. A post-event survey conducted in 2025 using

traditional fish sampling methods - netting and visual identification - recorded 27 species and indicated medium to high diversity, suggesting partial recovery of the fish community (Wibowo et al 2025). While these findings provide valuable baseline information for sediment management and conservation planning, conventional survey methods are constrained by logistical limitations, observer bias, and reduced detectability of rare, cryptic, or transient species (Deacon et al 2017; Pinna et al 2023).

Advances in molecular ecology have led to the growing application of environmental DNA (eDNA) metabarcoding as a complementary tool for aquatic biodiversity monitoring. eDNA consists of genetic material shed by organisms into their surroundings through skin cells, mucus, feces, gametes, or decaying tissue (Miya 2022). By extracting DNA from water samples and sequencing target genetic markers - commonly mitochondrial 12S rRNA or cytochrome oxidase I (COI) - eDNA metabarcoding enables simultaneous detection of multiple species without direct capture (Miya et al 2015; Shu et al 2021; Cevik & Cevik 2025). This non-invasive approach is rapid, cost-effective, and minimizes disturbance to aquatic organisms, making it particularly suitable for sensitive or recovering ecosystems (Wibowo et al 2022; Flores-Iwasaki et al 2026).

High-throughput sequencing platforms coupled with fish-specific primers such as MiFish have demonstrated high sensitivity for detecting rare, elusive, or early-life stages that are frequently overlooked by traditional methods (Shu et al 2020; Miya 2022; Wang et al 2024). Numerous studies across freshwater systems worldwide report higher estimates of species richness and improved resolution of community composition using eDNA metabarcoding compared with conventional surveys (Lim et al 2016; Deng et al 2024; Liu et al 2024; Lacaruso et al 2026). In Indonesia, eDNA applications in freshwater environments have successfully revealed spatial patterns of fish diversity and identified additional taxa not recorded through capture-based approaches (Wibowo et al 2022; Ambeng et al 2024; Dewi et al 2024).

Beyond species inventories, eDNA metabarcoding offers significant value for early detection of invasive species and monitoring of rare native taxa, both of which are critical in disturbed systems such as reservoirs. Repeated eDNA sampling enables standardized, long-term monitoring of temporal changes in community composition, facilitating adaptive management without the ethical and logistical challenges associated with intensive netting (Lim et al 2016; Huang et al 2025).

This study aimed to evaluate the composition and diversity of fish communities in the Serayu Movable Dam following a mass mortality event using eDNA metabarcoding. The results provide essential insights into post-disturbance ecological conditions and support evidence-based strategies for long-term monitoring, conservation, and sustainable management of freshwater fish communities in the Serayu River system.

Material and Method

Description of the study sites. This study employed a survey approach at the Serayu Movable Dam, Banyumas Regency, Central Java. eDNA samples were obtained from river water collected at three distinct sites within the Serayu Movable Dam area. Sampling points were located in middle-upper part (St 1), edge-middle (St 2), and edge-lower part (St 3) of the dam (Figure 1).

Environmental DNA (eDNA) collection. Water samples were collected during the fieldtrip in June 2024. eDNA was obtained by filtering 5 L of water from each predetermined sampling site through a 0.45 µm Whatman filter paper (Vourka et al 2023; Alenzi 2024). Water samples were collected at a depth of 2 m. The filter paper was subsequently cut into small pieces and placed into 2 mL cryotubes containing 1.5 mL of DNA/RNA Shield solution (Ip et al 2025). The preserved eDNA samples were then packaged and sent to a metabarcoding service provider for eDNA metabarcoding analysis (Yudha et al 2024).

DNA extraction, marker amplification, and sequencing. DNA extraction and marker amplification were carried out at PT. Genetika Science Indonesia Jakarta. This study used

12S rRNA as a molecular marker. The fragment of 12S rRNA was amplified using two MetaFish_1 and Tele02 primer sets (Yang et al 2023).

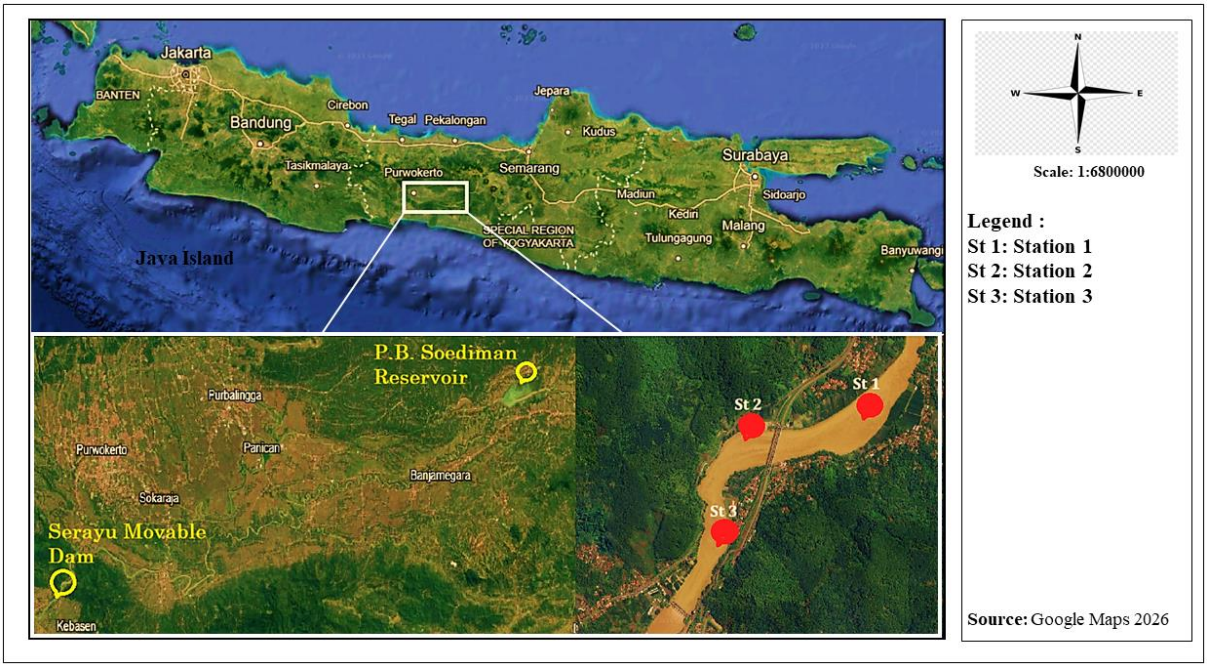

Figure 1. Map of Java Island showing sampling points (St1-St3-red color) at Serayu Movable Dam.

Bioinformatic analysis. Sequence quality control, denoising, and amplicon sequence inference were performed using the Divisive Amplicon Denoising Algorithm 2 (DADA2) pipeline (Callahan et al 2016). Amplicon Sequence Variants (ASVs) generated by DADA2 were subsequently clustered into Operational Taxonomic Units (OTUs) using a de novo approach. Taxonomic assignment was conducted using the MiFish reference database (Miya et al 2015) and the National Center for Biotechnology Information (NCBI) database (Schoch et al 2020).

Alpha diversity was assessed using the Shannon-Wiener, Simpson, ACE, and Chao indices (Kim et al 2017). Visualization of alpha diversity metrics, heatmap generation, and ordination analyses were performed in RStudio using the *phyloseq* package (McMurdie & Holmes 2013). All statistical analyses and data visualizations were conducted in RStudio software (RStudio Team 2020).

Results. eDNA from Serayu Movable Dam water samples was successfully extracted from filter paper. PCR amplification using the MetaFish_1 and Tele02 primers confirmed that both primer sets effectively amplified eDNA from the samples. The successful PCR amplification demonstrates that water serves as an effective medium for DNA preservation and a reliable source of eDNA, with freshwater providing a valuable resource for assessing faunal diversity. The amplification results obtained with the MetaFish_1 primer are shown in Figure 2.

As shown in Figure 2, the amplified fragment is approximately 170 base pairs (bp) in length. This size is consistent with the expected amplicon length, as Yang et al (2023) reported that the MetaFish_1 primer amplifies a ~171 bp fragment of the 12S rRNA gene.

The amplification with the Tele02 primer produced an amplicon of approximately 280 base pairs (bp). This size is slightly larger than the ~170 bp fragment reported by Yang et al (2023) for the Tele02 region of the 12S gene. The difference may reflect the inclusion of flanking sequences, primer extensions, or differences in the reference sequence, but it remains consistent with the expected target region amplified by the Tele02 primer. The amplification of environmental DNA using the MetaFish_1 primer is shown in Figure 3.

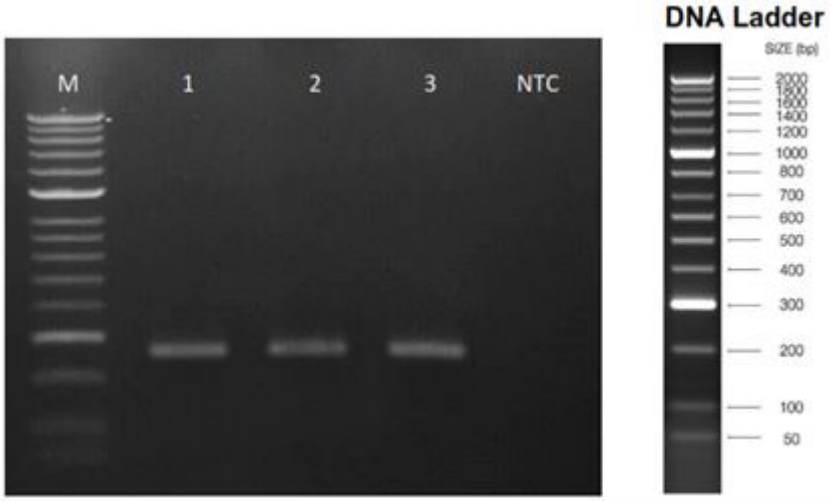

Figure 2. Environmental DNA amplicons amplified using MetaFish_1 primers.

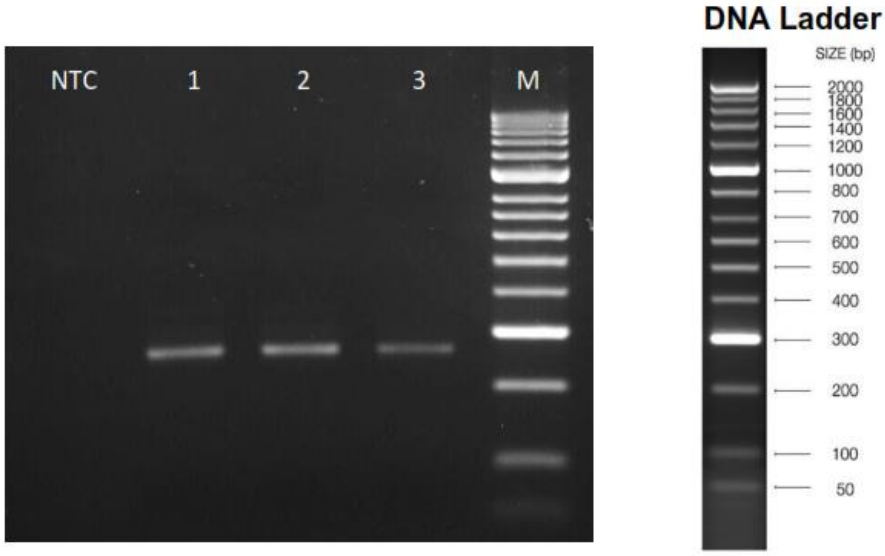

Figure 3. Environmental DNA amplicon amplified using Tele02 primers.

Taxa identification. Sequencing of DNA amplicons from water samples revealed marked differences in primer performance. The MataFis1 primer yielded 41 sequences, of which 22 (53.7%) were fish and 19 (46.3%) non-fish, whereas the Tele02 primer produced 154 sequences, with 146 (94.8%) corresponding to fish taxa and only 8 (5.2%) to non-fish taxa (Figure 4), indicating higher efficiency of Tele02 in detecting fish eDNA.

At the species level, 21 sequences of 22 fish sequences amplified with the Metafish-1 primer were assigned to five species. *Oxyeleotris marmorata* was the most abundant (15 sequences), followed by *Assurger anzac* (3 sequences). *Solegnathus hardwickii*, *Phyllopteryx taeniolatus*, and *Cynoglossus sinicus* were each represented by a single sequence. Of the 146 fish sequences amplified with the Tele02 primer, 21 were assigned to eight species. *O. marmorata* was the most abundant (8 sequences), followed by *Ichthyborus ornatus* (5 sequences), *S. hardwickii* (3 sequences), and *Hemibagrus nemurus* (2 sequences), with the remaining species represented by a single sequence each (Table 1). The majority of sequences obtained with both primers were only assignable to the class level (Actinopterygii; 140 sequences), while six sequences were identified to the family level (Clariidae).

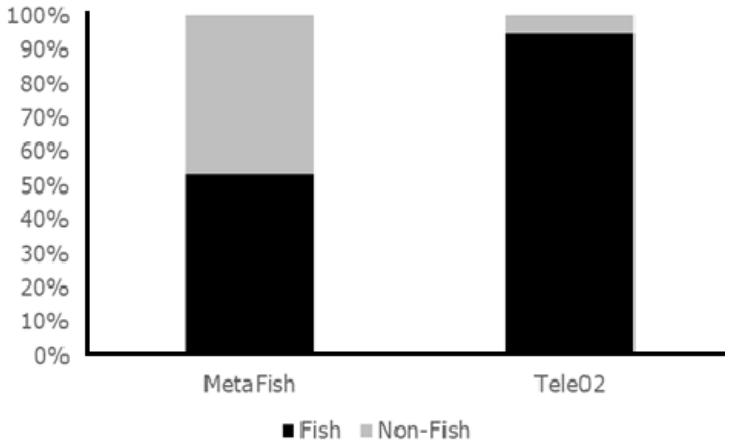

Figure 4. The composition of taxa identified using two primers.

Table 1

Fish species detected in Serayu Movable Dam water using MetaFish1 and Tele02 primers, habitat, and their geographic distribution

Species	*MetaFish1*	*Tele02*	*Habitat*	*Geographic distribution*
Oxyeleotris marmorata	√	√	Freshwater	Asia
Solegnathus hardwickii	√	√	Marine	Western Indian and Western Pacific Ocean
Phyllopteryx taeniolatus	√	-	Marine	Eastern Indian Ocean
Cynoglossus sinicus	√	-	Fresh-brackishwater	Asia
Assurger anzac	√	-	Marine	Atlantic, Indian, Pacific Ocean
Brachyhypopomus sp.	-	√	Freshwater	South America
Ichthyborus ornatus	-	√	Freshwater	Africa
Barbonymus schwanefeldii	-	√	Freshwater	Asia
Hemibagrus nemurus	-	√	Freshwater	Asia
Osteochilus vittatus	-	√	Freshwater	Asia
Poecilia reticulata	-	√	Freshwater	South America

Species-level identifications of all fish detected with the MetaFish1 and Tele02 primers are presented in Table 1. A total of 11 fish species were detected, with several shared between primers and others uniquely identified by either MetaFish1 or Tele02, with Tele02 detecting a greater number of species.

Ecological indices. The MetaFish1 primer yielded Shannon, Simpson and Inverse Simpson indices of 0.719-0.998, 0.357-0.518, and 1.554-2.073, respectively, while Tele02 produced 0.578-1.084, 0.213-0.412, and 1.270-1.701 (Table 2), with overall mean values of 0.892 (Shannon), 0.397 (Simpson), and 1.703 (Inverse Simpson), indicating comparable diversity across primers.

Table 2

Shannon and Simpson indices for fish communities post-mass mortality in Serayu Movable Dam

Indices	*MetaFish1*			*Tele02*			*Average*
	St 1	*St 2*	*St 3*	*St 1*	*St 2*	*St 3*	
Shannon	0.919	0.998	0.719	1.053	1.084	0.578	0.892
Simpson	0.501	0.518	0.357	0.381	0.412	0.213	0.397
Inverse Simpson	2.003	2.073	1.554	1.616	1.701	1.270	1.703

Discussion. eDNA metabarcoding has rapidly emerged as a robust approach for assessing aquatic biodiversity, including fish communities in both lentic and lotic systems, as it allows detection of species presence from water samples without direct capture or observation (Deiner et al 2017). This method is highly dependent on PCR primer selection, which directly influences both the taxonomic breadth and sensitivity of detection, particularly in environments where DNA may be degraded, such as dam spillways or rivers with high sediment loads (Chen et al 2025).

MetaFish_1 primer is among the most widely used universal fish eDNA primers, targeting a ~163-185 bp hypervariable region of the mitochondrial 12S rRNA gene conserved across diverse teleosts (Miya et al 2015). They were designed based on alignments of over 880 fish mitogenomes to maximize taxonomic coverage across marine and freshwater taxa (Shu et al 2022). Tele02 primers, another commonly applied 12S rRNA marker, have been optimized for freshwater systems and have demonstrated strong detection efficiency for local fish taxa with relatively low false positives when combined with rigorous bioinformatic pipelines (Xu et al 2024).

In highly managed systems such as the Serayu Moveable Dam, environmental factors such as flow dynamics, sediment load, and water quality can influence eDNA transport and degradation. Using both MetaFish_1 and Tele02 primers in combination can be complementary: MetaFish_1 offers broad teleost detection, while Tele02 can enhance detection of local freshwater species. This dual-primer approach reduces the likelihood of missing taxa that amplify poorly with a single primer and can improve species richness estimates from metabarcoding data (Shu et al 2022).

However, eDNA recovery is also influenced by primer-target mismatches and environmental DNA characteristics. Short amplicons, such as those targeted by MetaFish_1 and Tele02, are more likely to amplify successfully from degraded DNA than longer fragments. Nonetheless, universal primers can exhibit taxonomic bias, preferentially amplifying some lineages while underrepresenting others, potentially skewing relative abundance estimates and species richness assessments (Min et al 2023). Consequently, eDNA results should be interpreted alongside primer characteristics, environmental context, and, where possible, validated with complementary methods such as qPCR or conventional surveys.

Reference database completeness is another critical factor in accurate taxonomic assignment. In tropical regions like Indonesia, incomplete barcode libraries can limit species-level resolution, even when amplification is successful. Expanding regional reference databases and integrating multiple primer sets, including alternative mitochondrial markers such as 16S, can improve assignment accuracy and survey reliability (Kumar et al 2022; Chen et al 2025).

In this study, both MetaFish_1 and Tele02 primers predominantly amplified sequences assigned to Actinopterygii, with a smaller proportion of reads representing mammals and other non-target vertebrates. This outcome reflects the primers' design, targeting conserved 12S rRNA regions across ray-finned fishes, while allowing occasional amplification of closely related non-fish taxa when present in environmental samples (Polanco et al 2021; Li et al 2024). The dominance of Actinopterygii reads highlights a trade-off in universal primer design: primers must balance specificity and universality, but broad teleost primers may also amplify vertebrate DNA under conditions of high non-target template abundance or degraded eDNA (Valsecchi et al 2020).

Primer bias and PCR amplification dynamics further influence observed community composition. Variations in primer-template binding efficiency, template concentration, and fragment length can disproportionately favor Actinopterygii reads relative to other taxa, potentially underestimating species that are less abundant or have mismatched priming sites (Min et al 2023). Strategies such as blocking primers, multiple primer sets, or enhanced reference databases can help mitigate these biases and improve taxonomic resolution (Li et al 2024).

Taxa identification. Despite these limitations, eDNA metabarcoding using MetaFish_1 and Tele02 detected 11 fish species at the species level in the Serayu Moveable Dam, fewer than the 27 species recorded through conventional sampling (Wibowo et al 2025).

Similar phenomenon was also reported by previous study (Bylemans et al 2019). The discrepancy is likely influenced by primer-specific amplification efficiency, the shallow water sampling depth (2 m below the surface), and limited reference coverage. Environmental DNA is unevenly distributed vertically and horizontally, and single-depth sampling may miss taxa inhabiting deeper or benthic zones (Dan et al 2024; Ahmed et al 2025). Combined with primer bias, these factors can lead to underestimation of species richness compared with direct surveys that physically sample organisms across habitats and depth strata (Schenekar 2023).

eDNA metabarcoding remains highly effective in detecting elusive, low-abundance, or cryptic taxa that conventional methods may overlook, including nocturnal, benthic, or rare fishes (Shen et al 2022; Deng et al 2024). The approach also facilitates detection of non-native or invasive species, providing critical information for management and conservation that may not be captured through morphological surveys alone (Deng et al 2024).

Unexpected detection of marine fish species in this freshwater dam highlights additional methodological and ecological considerations. Broadly designed primers like MetaFish_1 and Tele02 can amplify marine taxa due to conserved priming sites. Moreover, DNA may be transported into the dam through anthropogenic vectors such as ballast water, fishery effluent, or contaminated equipment, leading to detections of non-resident species (Macher et al 2023). Reference database limitations can further contribute to misassignments if marine taxa are overrepresented relative to local freshwater congeners (Roesma et al 2023).

These findings underscore the strengths and caveats of eDNA metabarcoding: high sensitivity and broad taxonomic reach allow detection of taxa that may be missed by conventional surveys, but results must be interpreted carefully in the context of primer choice, sampling strategy, environmental DNA transport, and reference database completeness (Shu et al 2022; Xu et al 2024; Xu et al 2025). Integrating eDNA with traditional monitoring provides a more comprehensive understanding of fish community composition, supporting improved conservation and management in fragmented river systems (Deng et al 2024) like the Serayu Moveable Dam (Wibowo et al 2025).

In this study, eDNA metabarcoding using MetaFish_1 and Tele02 primers unexpectedly detected marine fish species in the Serayu Moveable Dam, a freshwater system in Indonesia. Several methodological and ecological factors likely contributed to these observations.

The choice of primers in eDNA metabarcoding directly influences both taxonomic breadth and detection sensitivity (Fang et al 2025). Primers such as Tele02 and MetaFish/MiFish target short fragments of the mitochondrial 12S rRNA gene and are designed to amplify a wide range of teleost fishes, encompassing both marine and freshwater lineages due to conserved priming sites (Macher et al 2023). Comparative studies have shown that these primers perform well in amplifying diverse fish DNA sequences, yet their broad taxonomic coverage can lead to amplification of marine species even when applied in freshwater contexts (Macher et al 2023).

eDNA may originate from sources beyond the immediate local community, particularly in lotic systems or hydrologically complex environments. Marine fish DNA could be introduced into inland dam waters through anthropogenic pathways such as ballast water discharge, transport of marine products into the watershed, release of fishery effluents, or contamination of sampling equipment. Once released, DNA fragments can persist in the environment and be transported downstream, resulting in detections of taxa not established in the local ecosystem (Abidin et al 2022; Xu et al 2024).

Accurate taxonomic assignment in metabarcoding depends on comprehensive reference databases. In regions with high biodiversity but limited barcode coverage, such as Indonesia, incomplete reference sequences for local freshwater congeners may lead to misassignments to marine relatives that are better represented in global databases. Consequently, apparent detections of non-indigenous marine species may reflect database biases rather than true presence (Roesma et al 2023).

Ecological indices. Shannon's diversity index (H') quantifies biodiversity by integrating both species (or taxon) richness and evenness in relative abundance. Conceptually rooted in information theory, Shannon's H' represents the uncertainty in predicting the identity of an individual randomly drawn from a community: the more taxa present and the more evenly distributed they are, the greater the uncertainty and the higher the H' value. This index is widely used in ecological studies to characterize community complexity because it increases with both richness and evenness. Typical empirical ranges of H' in ecological applications are often between ~0 and ~4, with many natural communities falling between ~1.5 and ~3.5; values closer to zero indicate very low diversity (dominated by one or few taxa), whereas higher values reflect richer and more even assemblages. Because absolute H' values depend on taxonomic scope and sampling design, interpretation is most meaningful when comparing communities of similar context rather than against universal thresholds (Kunakh et al 2023).

Simpson's diversity index emphasizes dominance structure and evenness by estimating the probability that two randomly selected individuals belong to different taxa. When expressed as $1 - \Sigma p^2$ (the Gini–Simpson form) or as an inverse (1/D), higher values indicate greater diversity and evenness, whereas lower values indicate that a few taxa dominate the community. Simpson indices are mathematically more sensitive to the relative abundance of common taxa than to rare taxa, so they provide a measure of how dominance shapes the community's structure. Because different formulations exist (e.g., complement vs. reciprocal), it is important to specify which form is used, but all share the property of increasing with diversity in intuitive contexts (Morris et al 2014).

Using Shannon and Simpson indices together helps disentangle contributions of richness and evenness to community structure. Shannon's H' responds to changes across all taxa, including rare ones, whereas Simpson's index places stronger weight on abundant taxa. When both metrics are relatively low to moderate, it suggests communities with limited numbers of abundant taxa and comparatively low representation of rarer taxa. In eDNA studies, such patterns frequently arise because sequencing depth, primer bias, and taxonomic assignment limitations can underestimate rare taxa relative to abundant ones, yielding diversity estimates that reflect both ecological patterns and methodological constraints (Drummond et al 2021).

The average observed Shannon H' of 0.892 suggests moderate but comparatively low diversity, consistent with communities where richness is limited and/or relative abundances are uneven. Similarly, average Simpson values (0.397) and corresponding average Inverse Simpson values (1.703) imply that the effective number of abundant taxa is low, meaning that a small set of taxa dominates the detected eDNA signal, while rare taxa contribute less to diversity estimates. This combination supports the interpretation that evenness is modest and that dominant taxa strongly influence overall α-diversity patterns (Morris et al 2014).

Environmental gradients or stressors often correlate with changes in these diversity metrics: habitats with more stable or less disturbed conditions tend to exhibit higher Shannon and Simpson diversity, whereas stressed or filtered systems tend to show lower values reflecting reduced richness or increased dominance by tolerant taxa. Because diversity index values are relative to sampling design, taxonomic resolution, and ecological scale, comparisons of H' or Simpson values should be made within consistent methodological frameworks to avoid confounding effects of differing protocols or community compositions.

Implications for monitoring and management. The detection of non-indigenous marine taxa illustrates both the strengths and limitations of eDNA metabarcoding for inland biodiversity monitoring. While such detections demonstrate high analytical sensitivity and broad taxonomic coverage, they also highlight the need for cautious interpretation when results conflict with known biogeographic patterns. Marine fish eDNA in freshwater systems does not necessarily indicate the presence of viable populations and may reflect DNA transport, incomplete reference libraries, or primer bias. Consequently, eDNA results should be evaluated within a broader ecological and hydrological context (Shu et al 2022).

Integrating eDNA data with hydrological information, local species inventories, and confirmatory methods - such as conventional sampling or species-specific qPCR - can reduce false positives and increase confidence in management decisions (Shu et al 2022). Primer choice and reference database completeness remain critical determinants of taxonomic accuracy. Employing multiple, complementary primer sets improves inventory completeness and reduces false negatives associated with primer bias (Evans et al 2016; Deiner et al 2017; Stat et al 2017). Similarly, investment in region-specific reference libraries, supported by targeted barcoding of local fauna, is essential for improving taxonomic resolution and minimizing assignment errors (Curry et al 2018; Schenekar et al 2020).

Hydrodynamic modeling combined with spatial patterns of eDNA detection can further help distinguish between local species presence and transported DNA signals (Harrison et al 2019). Integrated monitoring frameworks that combine eDNA metabarcoding with traditional survey methods provide more robust and multidimensional data, supporting both early detection and, where necessary, demographic assessments (McClenaghan et al 2020a). Diversity metrics derived from eDNA should be interpreted cautiously and ideally calibrated against known abundance data or analyzed using occupancy modeling to separate ecological signals from methodological artifacts (McClenaghan et al 2020a, b; van der Heyde et al 2022).

From a management perspective, detections of unexpected or high-risk taxa should prompt targeted validation through species-specific assays and focused field surveys. Such tiered monitoring approaches enhance the reliability of eDNA-based assessments and support rapid response strategies aimed at preventing the establishment and spread of invasive species.

Conclusions. Environmental DNA (eDNA) metabarcoding effectively characterized fish diversity in the Serayu Movable Dam, with Tele02 primers showing higher specificity for freshwater taxa compared with MetaFish_1. A total of 11 fish species were detected, including several unique to each primer, highlighting the complementary value of multi-primer strategies. Diversity indices (Shannon H' = 0.892; Simpson = 0.397; Inverse Simpson = 1.703) indicate a moderately diverse but uneven community dominated by a few taxa, notably *Oxyeleotris marmorata*. Occasional detection of marine species reflects the combined effects of broad primer specificity, environmental DNA transport, and incomplete reference databases. These results demonstrate the utility of eDNA as a rapid, non-invasive tool for aquatic biodiversity assessment while underscoring the need for careful interpretation. Future research should enhance regional reference libraries, apply multiple genetic markers, and conduct repeated spatial and vertical sampling to improve detection of rare or underrepresented species and refine community-level diversity estimates.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:
Agus Nuryanto, Faculty of Biology, Universitas Jenderal Soedirman, dr. Soeparno Street no. 63 Grendeng Purwokerto 53122, Central Java, Indonesia, e-mail: agus.nuryanto@unsoed.ac.id
Nuning Setyaningrum, Faculty of Biology, Universitas Jenderal Soedirman, dr. Soeparno Street no. 63 Grendeng Purwokerto 53122, Central Java, Indonesia, e-mail: nuning.setyaningrum@unsoed.ac.id
Yuriza Eshananda, Faculty of Biology, Universitas Jenderal Soedirman, dr. Soeparno Street no. 63 Grendeng Purwokerto 53122, Central Java, Indonesia, e-mail: yuriza.eshananda@unsoed.ac.id

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