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Comparative Assessment of Fish Biodiversity in the Coastal Waters of Eretan, Indramayu Using eDNA and Traditional Methods

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ABSTRACT

Coastal fishery resources are among the most vulnerable components of marine ecosystems due to environmental changes and anthropogenic activities originating from terrestrial areas. One of the key approaches to managing these resources is through the assessment of fish biodiversity. Traditional sampling methods, however, tend to cause habitat disturbances, necessitating more environmentally friendly alternatives such as environmental DNA (eDNA). This study aims to compare fish biodiversity detected using eDNA and traditional methods in the coastal waters of Eretan, as well as analyzing biodiversity indices—including Shannon, Simpson, Pielou, Berger–Parker, and species richness—across both approaches. The research was conducted in October 2024 at three sampling stations along the Eretan coastal area, Indramayu, West Java, Indonesia. Fish were collected using traditional mini trawl fishing and seawater samples for eDNA analysis. Data processing and analysis were carried out using R Studio software, encompassing comparative analysis, relative abundance, and biodiversity index calculations with various statistical packages. The results revealed that the eDNA method detected a total of 43 fish species, whereas the traditional mini trawl method identified 31 species, with 15 species shared between both methods. Biodiversity indices were consistently higher in the eDNA dataset, with Shannon index ranging from 2.00–2.32, Simpson index from 0.75–0.86, and species richness between 27–39 species, compared to 1.79–2.28, 0.75–0.83, and 16–28 species, respectively, for the traditional method. The findings indicate that eDNA metabarcoding provides a broader representation of fish community structure than conventional trawl sampling. Therefore, integrating molecular and traditional approaches enhances the accuracy of biodiversity assessment and offers a reliable foundation for sustainable management of the Eretan coastal ecosystem within the WPPNRI 712 fisheries area.

INTRODUCTION

Coastal ecosystems are among the most productive yet vulnerable environments, providing essential habitats for diverse fish species that support local livelihoods and fisheries. However, increasing anthropogenic activities such as pollution, habitat degradation, and overfishing have significantly altered biodiversity patterns in many Indonesian coastal areas, including the northern coast of Java. The coastal waters of Eretan are part of the Java Sea, located in the northern region of West Java. Rivers that flow into the coastal waters of Eretan include the Cimanuk River and the Cipunagara River. The flow of these rivers serves as an input to the coastal waters, influencing the concentration of organic matter and nutrients in the water **(Wang *et al.*, 2023)**. These inputs can also affect the concentration of chlorophyll-α **(Auricht *et al.*, 2022)**. Nutrient enrichment from river flow can increase the productivity of the aquatic environment; however, if this condition occurs excessively, it may lead to eutrophication **(Ke *et al.*, 2023)**.

Effective management of coastal resources requires accurate and efficient biodiversity assessment methods. Traditional fish surveys, based on netting and visual observation, often underestimate community diversity due to sampling bias and the limited detection of cryptic or rare species. Recently, environmental DNA (eDNA) has emerged as a promising molecular tool for biodiversity monitoring, allowing species detection through DNA traces in water samples without direct observation or capture. The use of the eDNA method in detecting fish diversity effectively reflects the patterns of diversity, seasonality, and relative abundance of fish in coastal waters. Moreover, this method is considered more effective and cost-efficient compared to traditional approaches such as trawling **(Stoeckle *et al.*, 2021)**. This condition highlights the advantages of the eDNA method, which is more environmentally friendly and does not harm or disturb aquatic organisms. In addition, eDNA allows for non-invasive, rapid, and large-scale monitoring of aquatic biodiversity, enabling researchers to detect both resident and transient species that might be missed by conventional sampling **(Deiner *et al.*, 2017; Miya *et al.*, 2020)**. The sensitivity of eDNA also makes it possible to detect rare, endangered, or early invasive species even at low population densities **(Yamamoto *et al.*, 2017; Boussarie *et al.*, 2018)**. Furthermore, eDNA can provide a more comprehensive representation of community composition by integrating DNA signals over time and space, reflecting both current and recent biological presence **(Yang *et al.*, 2021)**. These strengths make eDNA a powerful complementary tool to conventional surveys, particularly for biodiversity monitoring and conservation planning in complex and dynamic environments such as coastal ecosystems.

Although eDNA-based assessments have been increasingly applied worldwide, studies comparing eDNA and conventional methods in Indonesian coastal ecosystems remain scarce. The Eretan coastal waters in Indramayu, West Java, represent an

ecologically significant area with both artisanal fisheries and anthropogenic pressures, making it an ideal site for method comparison. Based on the assessment, the coastal waters of Eretan are generally categorized as moderately polluted, while the trophic level of the area is classified as mesotrophic. The water quality parameters influencing this status include TSS, turbidity, salinity, ammonia, nitrate, and nitrite **(Rosita *et al.*, 2025; Zainalarifin *et al.*, 2025)**. Meanwhile, in terms of fisheries resource utilization, the area is part of Fisheries Management Area of the Republic of Indonesia (WPPNRI) 712, as designated under the Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia No. 19 of 2022. According to this decree, large pelagic fish and demersal fish in WPPNRI 712 are categorized as overfished, indicating that their exploitation levels exceed the sustainable threshold. In contrast, small pelagic fish are considered underfished, suggesting potential for sustainable utilization. Other fishery resources such as reef fish, penaeid shrimp, lobster, crab, swimming crab, and squid are generally classified as being in a moderate exploitation status. These conditions reflect the complex dynamics of fisheries utilization in the region and highlight the urgent need for accurate biodiversity assessments and continuous monitoring to support sustainable fisheries management in the Eretan coastal ecosystem.

Based on the conditions described above, it is important to develop accurate and efficient methods for assessing fish biodiversity in coastal ecosystems such as the Eretan coastal waters. The integration of eDNA and traditional methods is expected to provide a more comprehensive understanding of fish community composition and environmental conditions. Therefore, this study aims to compare the fish biodiversity detected using eDNA and traditional sampling methods in the coastal waters of Eretan, Indramayu. Specifically, it seeks to analyze biodiversity indices, including Shannon, Simpson, Pielou, Berger–Parker, and species richness, at different methods.

MATERIALS AND METHODS

1. Sample collection

The study was conducted in October 2024 in the coastal waters of Eretan, Indramayu, West Java, Indonesia (Fig. 1). eDNA sampling was carried out simultaneously with fish collection using mini trawl fishing gear, which was operated by a 4 GT fishing vessel equipped with a 4-inch mesh size net (Fig. 2). The eDNA samples were obtained by collecting 2 liters of surface seawater along the operational route of the fishing gear. Each 2-liter sample was filtered through a 0.45µm sterile membrane using a water filtration system. After filtration, the membrane filters were placed in 2mL cryotubes prefilled with 1.5mL of DNA Shield (ZymoBIOMICS DNA/RNA Shield). To prevent contamination, all sampling tools were thoroughly sterilized at every stage using a 10% commercial bleach solution. A negative control containing sterilized ddH_2O was included to check for potential contamination during the filtration process. The samples

were subsequently transported to the Oceanogen Laboratory in Bogor, Indonesia, for further analysis.

2. Laboratory analysis

Laboratory analysis of eDNA samples was conducted following the analytical workflow described by **Madduppa *et al.* (2021)**. The eDNA samples and blank DNA controls were extracted using the Qiagen DNeasy Blood and Tissue DNA Extraction Kit according to the manufacturer's instructions. The extracted DNA was eluted in a final volume of 100µL of nuclease-free water. Amplification was performed using the polymerase chain reaction (PCR) method targeting the 12S gene to generate amplicon products. Each sample underwent triplicate PCR runs, accompanied by two negative controls (NC) per run. eDNA samples that failed to amplify during the PCR process were excluded from subsequent sequencing. Successfully amplified (positive) PCR products were then subjected to DNA sequencing using the Illumina sequencing platform.

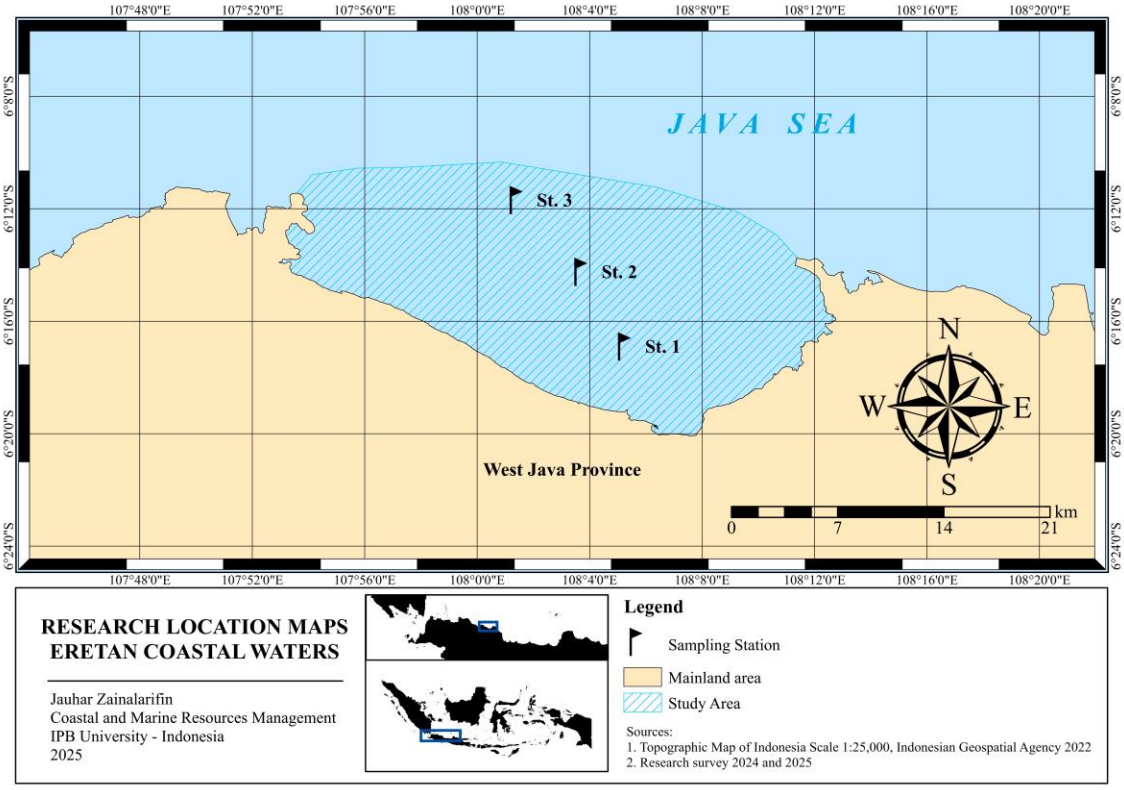

Fig. 1. Research maps location at Eretan coastal waters, Indramayu, West Java

3. Bioinformatics and data analysis

The sequencing data were analyzed using QIIME2 (Quantitative Insights into Microbial Ecology 2; https://qiime2.org/) for quantitative processing **(Leray & Knowlton, 2016)**. The workflow included several main steps: filtering, denoising, non-

chimeric checking, and clustering. Filtering removed primer sequences and reads shorter than ~313 bp (Martin 2011), while denoising corrected low-quality sequences using the DADA2 pipeline **(Callahan *et al.*, 2016)**. The non-chimeric step eliminated mixed sequences from different organisms to ensure accuracy, and clustering grouped similar sequences into Operational Taxonomic Units (OTUs) **(He *et al.*, 2015)**. Taxonomic identification of COI sequences was performed up to the species level using the CRUX database, which provides a well-organized and comprehensive reference collection, facilitating easier data retrieval and management **(Curd *et al.*, 2019)**.

Data analysis in this study was performed using R Studio software. Comparative analysis and visualization of species overlap between eDNA and traditional methods were conducted using the VennDiagram package. Relative abundance analysis was calculated as the percentage of each species from the total number of individuals in a community **(Li *et al.* 2022).** Each DNA read sequence was assumed to represent one individual and was visualized using the ggplot2 and pheatmap packages. Biodiversity indices, including Shannon, Simpson, Pielou, Berger–Parker, and species richness across different methods, were analyzed using the vegan package.

RESULTS

Fish biodiversity

A total of 43 fish species were detected using the eDNA approach, compared to 31 species identified through the traditional method (Fig. 2). Fifteen species were shared between both methods, including chacunda gizzard shad (*Anodontostoma chacunda*), goatee croaker (*Dendrophysa russelii*), fourfinger threadfin (*Eleutheronema tetradactylum*), white sardine (*Escualosa thoracata*), deep-bodied mojarra (*Gerres erythrourus*), false trevally (*Lactarius lactarius*), lunar-tailed puffer (*Lagocephalus lunaris*), common ponyfish (*Leiognathus equula*), moonfish (*Mene maculata*), Indian pellona (*Pellona ditchela*), short mackerel (*Rastrelliger brachysoma*), goldstripe sardinella (*Sardinella gibbosa*), greater lizardfish (*Saurida tumbil*), spotted scat (*Scatophagus argus*), and narrow-barred Spanish mackerel (*Scomberomorus commerson*). These findings reveal that 48% of the species recorded by the traditional method were also captured through eDNA analysis. Additionally, the eDNA technique successfully detected 28 species that were not identified by conventional sampling.

The eDNA method revealed a greater number of species with higher taxonomic diversity compared to the traditional method, as indicated by the larger variety of color segments in each eDNA bar (Fig. 3). This suggests that eDNA captures a broader spectrum of fish biodiversity, including species that might be rare, cryptic, or not easily caught by fishing gear. Across all stations, eDNA consistently detected more species and showed higher evenness in the relative abundance distribution. In contrast, traditional sampling was dominated by a few taxa, as reflected by the larger segments representing

certain species, indicating lower evenness and a more skewed community structure. The variation among stations in eDNA data suggests spatial heterogeneity in fish community composition, while the traditional method showed more uniformity, likely due to selective gear bias.

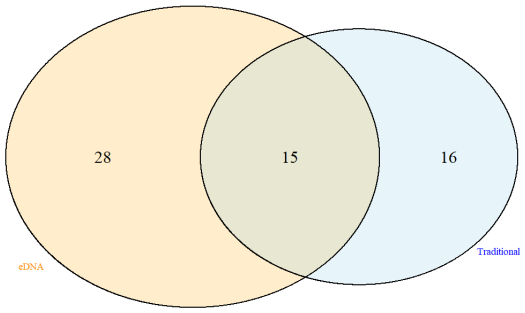

Fig. 2. Venn plot of fish diversity based on eDNA and traditional methods

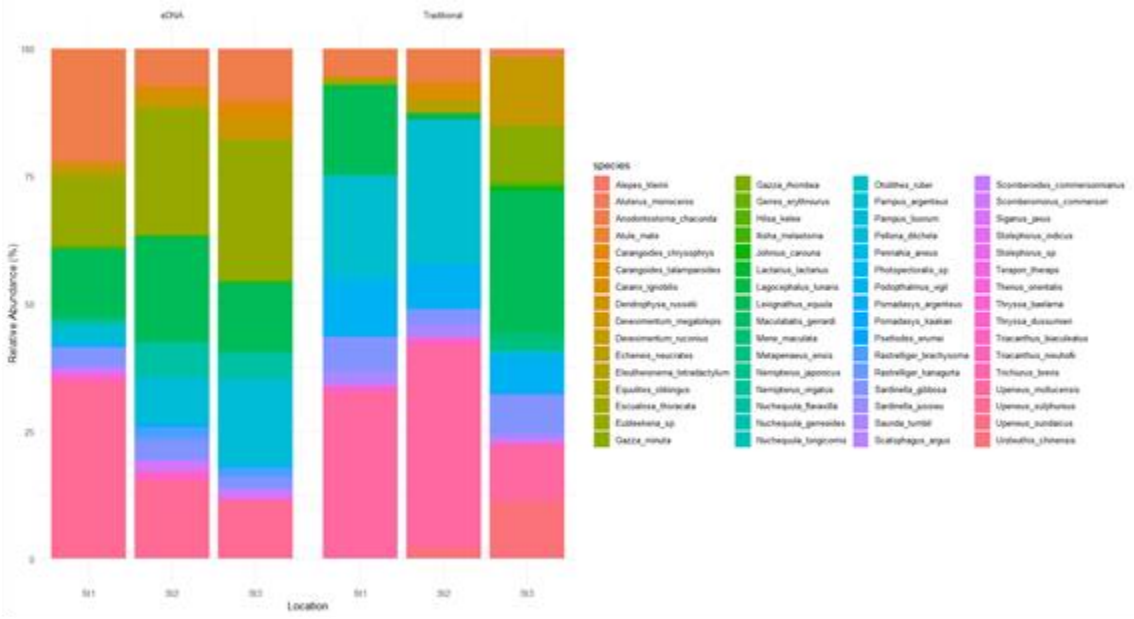

Fig. 3. Relative abundance of fish diversity based on eDNA and traditional methods

Some species such as chacunda gizzard shad (*Anodontostoma chacunda*), fourfinger threadfin (*Eleutheronema tetradactylum*), and white sardine (*Escualosa thoracata*) appeared consistently across both methods, indicating that they are common or dominant species in the study area. However, several species detected through eDNA, such as sunrise goatfish (*Upeneus sulphureus*), toothpony (*Gazza minuta*), and golden threadfin bream (*Nemipterus virgatus*), were absent in the traditional catches, emphasizing the greater sensitivity of eDNA in detecting species present in low abundance or outside the effective range of the mini trawl fishing gear.

The heatmap illustrates the distribution and relative abundance of fish species identified from both eDNA and traditional sampling methods across three stations in the coastal waters of Eretan, Indramayu (Fig. 4). The color gradient from blue to red represents increasing relative abundance, where darker blue indicates lower abundance and warmer colors (yellow to red) indicate higher relative abundance. The eDNA method shows a broader detection spectrum of fish species across all stations, whereas the traditional method reveals fewer dominant species with relatively higher abundance values. This pattern indicates that eDNA captures a wider variety of taxa, including rare and low-abundance species, while traditional sampling tends to reflect species that are physically caught more easily by the mini trawl fishing gear.

Fig. 4. Heatmap of relative abundance based on eDNA and traditional methods

Biodiversity indices

The violin plots illustrate broader data distributions for eDNA (Fig. 5), confirming higher variability and richness in biodiversity indices, while radar charts highlight the overall advantage of eDNA across most metrics (Shannon, Simpson, Richness) (Fig. 6). Conversely, the traditional method shows relatively higher values only for Pielou's evenness and Berger–Parker indices, suggesting dominance by fewer species but with higher individual abundance.

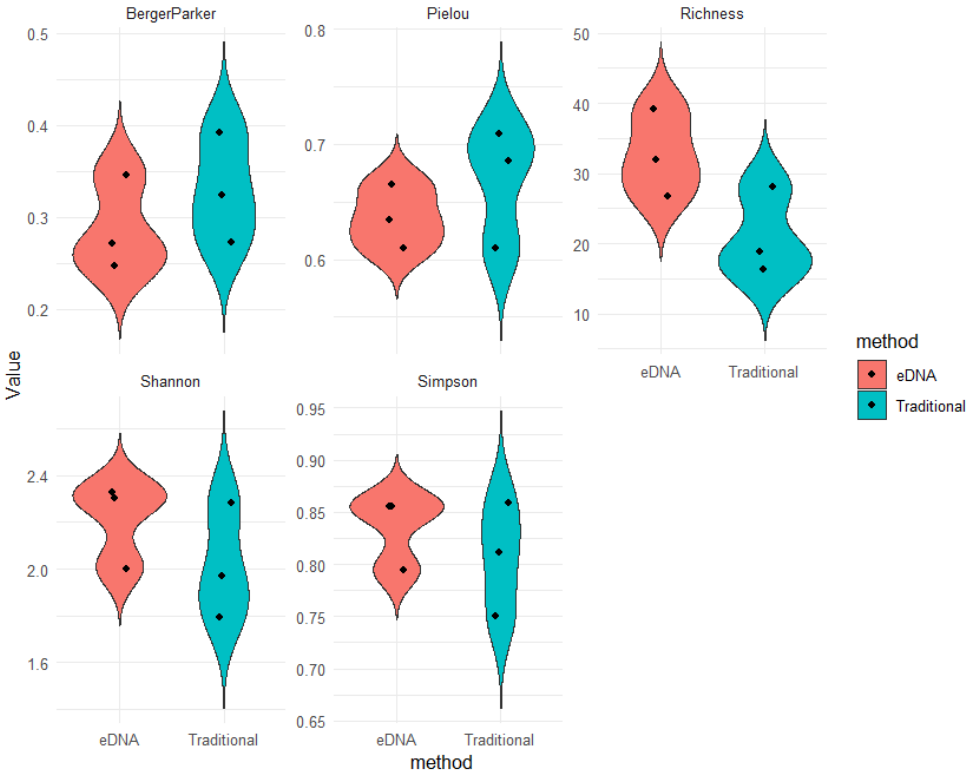

Fig. 5. Violin plot of biodiversity indices

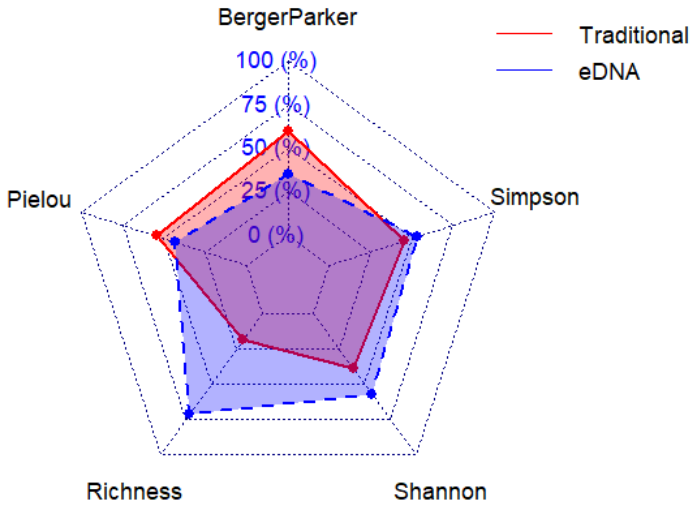

Fig. 6. Radar chart of biodiversity indices

Species richness exhibited the most pronounced difference between the two methods. The eDNA method detected 27–39 species, while the traditional method recorded 16–28 species across stations. This pattern is consistent with the violin and radar plots, where eDNA consistently showed broader and higher richness values.

The Shannon index ranged from 1.79–2.28 for traditional samples and 2.00–2.32 for eDNA samples. Higher Shannon values in eDNA indicate greater diversity and evenness in the fish community detected by molecular methods. Simpson's index values showed similar trends between methods, ranging from 0.75–0.86, but were slightly higher in eDNA-derived data. This pattern indicates that both methods captured communities with moderate to high diversity; however, the eDNA method provided a more balanced detection of species without the dominance of a few taxa, reducing bias caused by gear selectivity.

Pielou's evenness index values ranged from 0.61–0.71 for the traditional method and 0.60–0.66 for eDNA. Although the difference is not substantial, the slightly lower evenness in eDNA may reflect the inclusion of rare or low-abundance species, which tends to reduce evenness but increase richness. This indicates that eDNA captures both common and uncommon species, resulting in a more complete but uneven community representation.

The Berger–Parker index ranged from 0.27–0.39, with higher values in traditional catches, particularly at St2 (0.39), indicating stronger dominance of a few species. In contrast, eDNA showed lower dominance (0.25–0.34), suggesting a more evenly distributed community. This supports the view that traditional gear tends to overrepresent dominant or larger-bodied species that are more easily captured, while eDNA detects a more proportionate array of taxa.

DISCUSSION

Comparasion between methods

The findings confirm that eDNA-based monitoring detects higher species diversity than traditional mini trawl sampling in the coastal waters of Eretan, Indramayu. This aligns with previous research by **Yamamoto *et al.* (2017)** and **Boussarie *et al.* (2018)**, indicating that eDNA can capture species that are elusive or present at low abundance. eDNA metabarcoding has been particularly effective in detecting cryptobenthic, pelagic, and rare fishes that are often overlooked by classical visual surveys **(Boulanger *et al.*, 2021)**. The detection of 28 unique species by eDNA, including *Pampus liuorum*, a cryptic species of silver pomfret **(Wei *et al.*, 2021)**, and small benthopelagic species such as various ponyfishes (Leiognathidae), as well as large pelagic species such as talang queenfish (*Scomberoides commersonnianus*), demonstrates the method's superior sensitivity for comprehensive biodiversity assessment. This finding is also consistent with studies in other coastal regions by **Stoeckle *et al.* (2021)**, which have reported similar advantages of eDNA in capturing a broader spectrum of fish diversity compared to conventional methods.

The traditional trawling method, although detecting fewer species overall, provided valuable information on dominant and commercially important species such as goldstripe sardinella (*Sardinella gibbosa*) and short mackerel (*Rastrelliger brachysoma*). The higher Berger–Parker index values in traditional samples reflect the gear's efficiency in capturing abundant, school-forming species that dominate the catch. This methodological complementarity underscores the importance of employing both approaches to obtain a comprehensive understanding of fish community structure, as each method contributes unique and valuable insights into different aspects of the ecosystem.

Ecological implications

Moderate evenness and relatively high dominance indicate a potential community imbalance, possibly driven by environmental stressors. The fish community structure revealed in this study provides important insights into the ecological status of Eretan coastal waters. The moderate biodiversity indices and the dominance of a few species suggest that the community may be experiencing environmental pressure, consistent with previous findings that characterize these waters as mesotrophic and moderately polluted due to riverine inputs from the Cimanuk and Cipunagara rivers **(Rosita *et al.*, 2025; Zainalarifin *et al.*, 2025)**. The correlation between biodiversity and water quality parameters further underscores the importance of maintaining coastal ecological integrity through effective pollution control and habitat protection.

The findings of *Pampus liuorum*, a cryptic species of silver pomfret using eDNA method, vs *P. argenteus* only from traditional method using conventional visual fish identification may show eDNA method urgency. This is because highly similar external

morphologies between *Pampus* genus then may cause the species misidentification **(Wei *et al.*, 2021)**. This may also show thas species stock for silver pomfret from Eretan waters is actually for *Pampus liuorum*, not *Pampus argenteus*.

The detection of species such as lunar-tailed puffer (*Lagocephalus lunaris*), which is often associated with disturbed habitats, along with the general pattern of moderate diversity indices, highlights the need for continued monitoring and adaptive management of these coastal ecosystems. Moreover, the presence of both estuarine-dependent species such as fourfinger threadfin (*Eleutheronema tetradactylum*) and marine species such as the narrow-barred Spanish mackerel (*Scomberomorus commerson*) emphasizes the ecological importance of Eretan waters as a transitional zone linking freshwater and marine environments.

Methodological considerations

While eDNA offers superior detection efficiency for biodiversity assessment, several methodological considerations must be acknowledged. The success of eDNA metabarcoding depends on various factors including DNA degradation rates, water movement patterns, and the completeness of reference databases **(Deiner *et al.*, 2017; Mashar *et al.*, 2025)**. In this study, some taxa could only be identified to genus level (e.g., *Eubleekeria* sp., *Photopectoralis* sp.), highlighting the need for continued development of comprehensive DNA reference libraries for Indonesian marine fishes.

The traditional trawling method, while subject to gear selectivity bias, provides essential biological data including size distribution, maturity stages, and biomass estimates that are crucial for fisheries management. While eDNA metabarcoding allows the detection of a wider range of species compared to conventional methods, it still falls short of providing reliable abundance estimations **(Yao *et al.*, 2022)**. The higher evenness values (Pielou's index) in traditional samples likely reflect the gear's tendency to quantitatively sample dominant species while missing rare taxa. Hence, integrative monitoring combining eDNA and capture-based approaches is recommended for robust biodiversity assessment.

Management relevance

The findings of this study have significant implications for fisheries management within Fisheries Management Area (WPPNRI) 712, where several key stocks, including large pelagic and demersal fishes, have been categorized as overfished. The ability of the eDNA method to rapidly and comprehensively assess biodiversity makes it a valuable tool for routine stock assessment and the implementation of ecosystem-based fisheries management. For effective management of Eretan coastal waters, an integrated monitoring approach that combines eDNA metabarcoding with traditional surveys is recommended.

The use of eDNA enables rapid assessment of biodiversity patterns and facilitates the detection of rare or endangered species, while traditional methods remain essential for collecting biological data and validating fishery resource information **(Aprilia *et al.*, 2025).** Establishing long-term eDNA monitoring stations would further allow the tracking of temporal changes in biodiversity and community composition. The detection of both overfished species, such as the narrow-barred Spanish mackerel (*Scomberomorus commerson*), and underutilized species, including various small pelagics, through both methods demonstrates the potential for developing more targeted and adaptive management strategies based on comprehensive biodiversity data. Overall, this study underscores the potential of eDNA as a rapid, non-invasive, and cost-effective biomonitoring tool for coastal fisheries management, particularly in data-deficient regions such as northern Java. Routine implementation of eDNA-based surveys could enable early detection of biodiversity loss and support timely adaptive management interventions to ensure the sustainability of coastal fishery resources.

CONCLUSION

This study concludes that eDNA methods detected a significantly higher number of fish species (43 species) compared to the traditional sampling (31 species) in the Eretan, Indramayu coastal waters. Among them, 15 species were shared between both methods, while 28 species were uniquely identified through eDNA analysis. Biodiversity indices (Shannon, Simpson, and richness) were consistently higher in eDNA datasets, with Shannon index values ranging from 2.00–2.32, Simpson index from 0.75–0.86, and species richness between 27–39 species, compared to 1.79–2.28, 0.75–0.83, and 16–28 species for the traditional method. The integration of molecular and conventional methods enhances the accuracy of biodiversity monitoring and provides a reliable framework for coastal ecosystem management.

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