Fish detection by environmental DNA (eDNA) metabarcoding approach in the Jakarta Bay, Indonesia

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Abstract. Urban areas in tropical countries, particularly coastal and estuarine regions, are highly vulnerable. The dynamic nature of Jakarta Bay has raised concerns about the decreasing diversity of aquatic life. To address this, we have harnessed the power of cutting-edge environmental DNA (eDNA) technology to comprehensively inventory biodiversity potential. This research, which utilizes such advanced technology, aims to evaluate the current status of aquatic biodiversity along Jakarta's coast and identify factors affecting it. The study found sequences from six fish species spanning five genera, five families, and four orders at three sites. At Muara Baru, three species were identified: scalloped perchlet (*Ambassis nalua*), guppy (*Poecilia reticulata*), and sailfin molly (*Poecilia latipinna*). Muara Angke had the Indian mackerel (*Rastrelliger kanagurta*) and the sulphur goatfish (*Upeneus sulphureus*), while Pantai Indah Kapuk (PIK) contained the scalloped perchlet (*Ambassis nalua*) and the pacu (*Piaractus mesopotamicus*). The scalloped perchlet was the most frequently encountered species. The Shannon-Wiener index used to measure species diversity indicates low to moderate diversity across the sites.
Key Words: diversity, eDNA, estuarine, fish.

Introduction. Estuarine areas are complex ecosystems due to the transitional conditions between terrestrial and marine habitats. This area is also a meeting point between two aquatic ecosystems, fresh and salt water, which can produce unique habitats for certain biota (Potter et al 2015). The unique characteristics of estuarine ecosystems also impact the biodiversity potential. The tidal conditions of seawater and river water discharge also make coastal areas have a dynamic aquatic composition that can influence the biota composition within them. Fish, turtles, and migratory birds are a few examples of species commonly found in estuarine ecosystems (Andriyono et al 2021).

Urban areas in tropical countries experience high vulnerability of coastal and estuarine areas. Global climate change can increase seawater temperatures and rainfall which has an impact on high river flows (Filho et al 2022). Dense populations produce excess nutrients and pollutants from agricultural, industrial, and domestic sectors (Zhu et al 2020). These anthropogenic activities leave untreated high concentrations of pollutants in rivers and swept into estuaries. Human intervention and morphological changes in coastal areas such as reclamation, coastal development, and river normalization have a high risk of changing natural conditions and coastal habitats (Bull et al 2021).

Situated in the northern part of Jakarta, Jakarta Bay is a semi-enclosed water system. The bay is a hub for various human activities such as maritime transportation, fishing, residential areas, mangrove zones, beachside tourism, and industrial operations. Furthermore, it is believed that 13 river estuaries play a significant role in transporting organic matter from terrestrial regions. The city of Jakarta saw a population surge of 66,663 individuals in 2023, bringing the total to 10,672,100 (Central Agency of Statistics of Jakarta 2024). This growth signifies a rapid urban expansion, bolstered by industrial and business developments in Jakarta. A larger human population can lead to an increase

in domestic waste discharge, which can accumulate at river mouths through river currents (Aguilar et al 2021). This could potentially lead to a state of nutrient enrichment. If the concentration of these nutrients is high, it can trigger eutrophication, impacting the aquatic environment and leading to a reduction in aquatic biodiversity (Wang et al 2021; Aprilia et al 2024).

Changes in coastal areas have an impact on habitat destruction. Mangrove ecosystem damage has caused decreasing fish habitat and populations. Polluted estuary areas pose a high risk of loss of habitat and aquatic biota. Overexploitation of biodiversity for livelihoods reduces the potential for biodiversity, especially fish. Damaged coastal ecosystems also impact their ability to provide optimal ecosystem services, especially in providing habitat, protecting biodiversity, and space for infrastructure (Hoque et al 2020). Native species in estuarine ecosystems also have a high risk of predation by non-native fish, which can have deleterious impact (Glamuzina et al 2021).

Identification of existing biota in coastal ecosystems has a significant function in seeing the current potential and its benefits for human life. The high diversity of fish can be a source of protein as well as income for local communities (Effendi et al 2022). The potential diversity of fish can be an indicator of the condition of coastal ecosystems (Kjelland et al 2015). Most of the biota that survive in polluted estuarine areas have high adaptability. Both extinct and surviving biota can explain habitat destruction. On the other hand, identifying aquatic biota using conventional methods is unable to produce actual data due to dynamic ecosystem conditions and limited tools (Mishra 2023).

The highly dynamic condition of Jakarta Bay raises concerns about the decline in the diversity of aquatic biota. If the condition of water eutrophication continues to increase, it will pose a significant risk to the extinction of biota, leading to a weakening of the aquatic ecosystem (Aprilia et al 2023a). On the other hand, the level of biota diversity can be measured through field surveys, but this process can be time-consuming and less comprehensive (Aprilia et al 2023b; Effendi et al 2023). Many fish species on the Jakarta sea coast are known based on fishermen's catches (Pamenan et al 2017). These various species are also caught from the fishing ground, not from estuary areas. Therefore, special studies regarding biota in estuary areas need to be carried out considering that the ecosystem is different from sea and rivers. Environmental DNA (eDNA) technology is used to comprehensively inventory biodiversity potential (Effendi et al 2022; Aprilia et al 2023b; Effendi et al 2024b). By using water samples, all remaining biota materials can be identified through a metabarcoding process. By using this technology, the biota potential in the ecosystem can be determined. This research aims to analyze the current status of the condition of aquatic biodiversity on the coast of Jakarta and identify factors that influence the condition of biodiversity.

Material and Method

Description of the study sites. eDNA samples were collected in February 2024 from three sites along Jakarta Bay (Figure 1). Various sites were selected for sampling, encompassing Muara Baru (site 1), Muara Angke (site 2), and Pantai Indah Kapuk (PIK) (site 3). These three streams flow into Jakarta Bay. These sites were chosen based on a purposive sampling method to account for variations in environmental conditions.

eDNA sampling. eDNA samples were collected directly from the water column and stored in 4 liter water bottles. These samples underwent filtration using 0.45 μm sterilized filter paper facilitated by a peristaltic pump. In cases where the filter was clogged, filtration was paused to prevent flow interruptions. Should the filter become obstructed, the filtration process was temporarily halted to ensure uninterrupted flow. A rigorous procedure was followed to prevent contamination and maintain sterility, including disinfecting all tools between samples and sites with distilled water and a 10% bleach solution. After filtration, the filter paper was meticulously transferred to a 2 mL cryotube containing 1 mL of a DNA/RNA shield to ensure preservation.
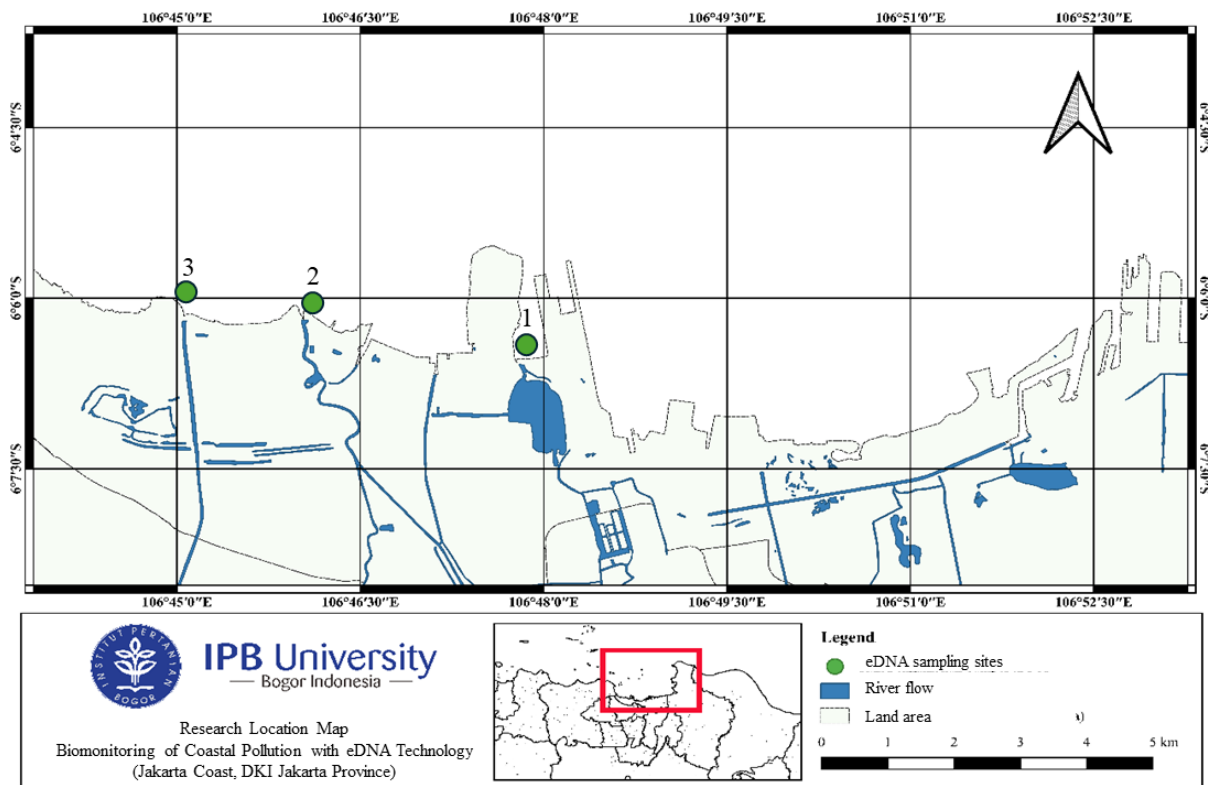
Figure 1. The location of three eDNA sampling points along the Jakarta Bay: site 1 (Muara Baru), site 2 (Muara Angke), and site 3 (Pantai Indah Kapuk).

eDNA laboratory analysis. Following the manufacturer's guidelines, environmental DNA (eDNA) was extracted from filter papers using the Geneaid gSYNC DNA Extraction Kit. The initial PCR stage targeted a segment of the COI gene, approximately 313 base pairs long, using the mlCOIintF and jgHCO2198 primers for amplification. This segment allows for identifying universal metazoans at the family, genus, and species levels (Leray et al 2013). The PCR amplification process involved a series of steps: starting with a pre-denaturation at 95°C for 5 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 42°C for 30 seconds, extension at 72°C for 30 seconds, and concluding with a final extension at 72°C for 5 minutes, repeated across 35 cycles.

The integrity of the PCR products was assessed through electrophoresis on a 2% agarose gel, with the setup including 100 mL of TAE buffer and 2 g of agarose, run at 50 Volts for an hour. Results were visualized using UV fluorescence through the Alpha Imager Mini Gel Documentation System. PCR products that met quality standards were further processed in a second PCR round for indexing, consisting of an initial denaturation and a series of 9 cycles of temperature variations for denaturation, annealing, and extension, culminating in a final extension. The final step involved DNA sequencing on an Illumina iSeq 100 platform.

Bioinformatics and statistical analysis. The DNA sequencing data underwent analysis using the QIIME2 software (Caporaso et al 2010). A series of quality control procedures were applied to the raw sequence data utilizing the fast toolkit to assess the quality of the sequence reads. Sequences failing to meet quality criteria, such as an average quality score below 20, the presence of ambiguous N bases, stretches of homopolymers, or lengths shorter than 100 base pairs, were excluded. Duplicate sequences were subsequently removed using the VSEARCH tool (Rognes et al 2016). The sequences were sorted and identified based on unique sample tags. The resulting sequencing reads were then organized into operational taxonomic units (OTUs) based on a 97% similarity threshold for nucleotide sequences (Edgar 2010). Taxonomic classification of the OTUs was performed based on the MitoFish database (Sato et al 2018), a component of the mitochondrial genome database, to identify fish species through the BLAST program. The

selection and categorization of OTUs followed established methodologies. A species was considered identified if the OTU sequence exhibited a perfect match and a similarity score exceeding 97% (Yang et al 2023).

The Shannon-Wiener diversity index (H') was analyzed to measure diversity, which considers the number of species present and the relative abundance of each species. As species richness increases, diversity increases (Oksanen et al 2019). The difference in read sequence composition between locations was examined by Non-metric Multidimensional Scaling (NMDS) using the Bray-Curtis distance index to consider species' abundance and presence or absence in each dataset (Jensen et al 2022).

Results

Fish diversity. A total of 6 COI sequences were obtained from six different fish species across five genera, five families, and four orders. At site 1 (Muara Baru), three species were identified based on the sequences with the highest frequency, which are the scalloped perchlet (*Ambassis nalua*), guppy (*Poecilia reticulata*), and sailfin molly (*Poecilia latipinna*). Site 2 (Muara Angke) was home to two species, the Indian mackerel (*Rastrelliger kanagurta*) and the sulphur goatfish (*Upeneus sulphureus*). Meanwhile, PIK harbored two species: the scalloped perchlet (*Ambassis nalua*) and the pacu (*Piaractus mesopotamicus*). The scalloped perchlet emerged as the most prevalent species, with 182 sequences identified (Figure 2).
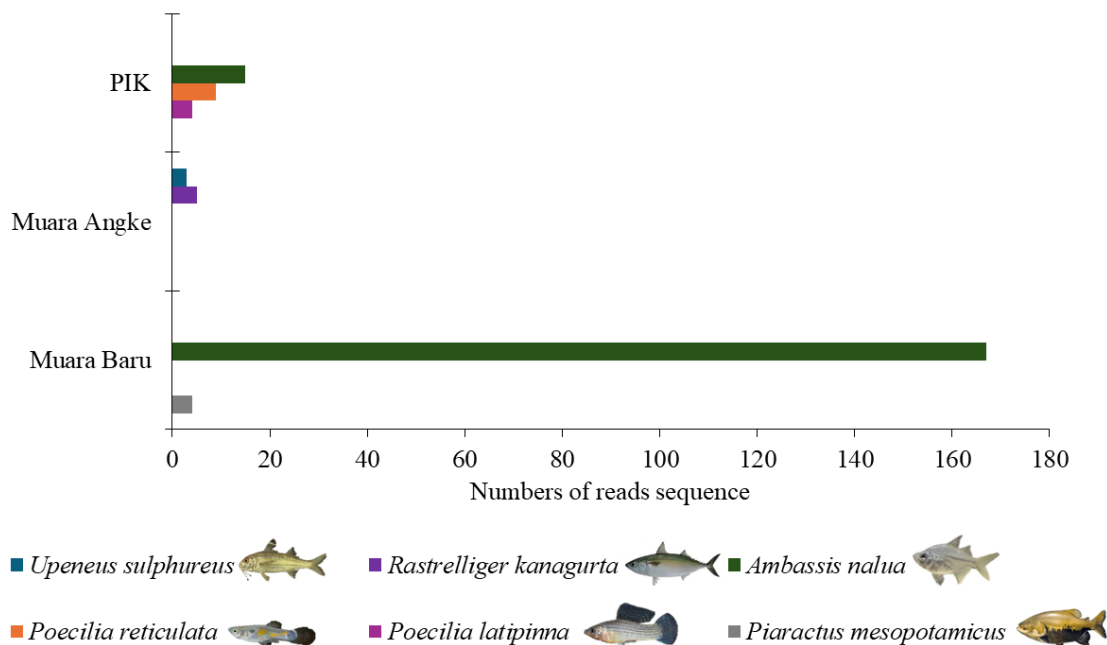

Figure 2. Fish composition in species-level.

The Shannon-Wiener index (H') was used to quantify species diversity at each site, with the fish diversity index (H') ranging from 0.11 to 1.01, indicating a low to moderate diversity level, generally leaning towards low diversity (Figure 3). The Non-metric Multidimensional Scaling (NMDS) analysis, a powerful tool in community ecology, showed a tendency for fish species to cluster by site, suggesting significant differences in fish composition among the sites (Figure 4).
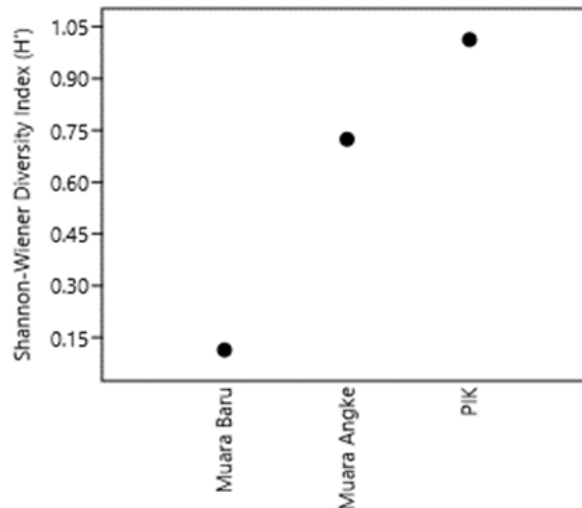
Figure 3. Shannon-Wiener diversity index (H') values for fish identified.
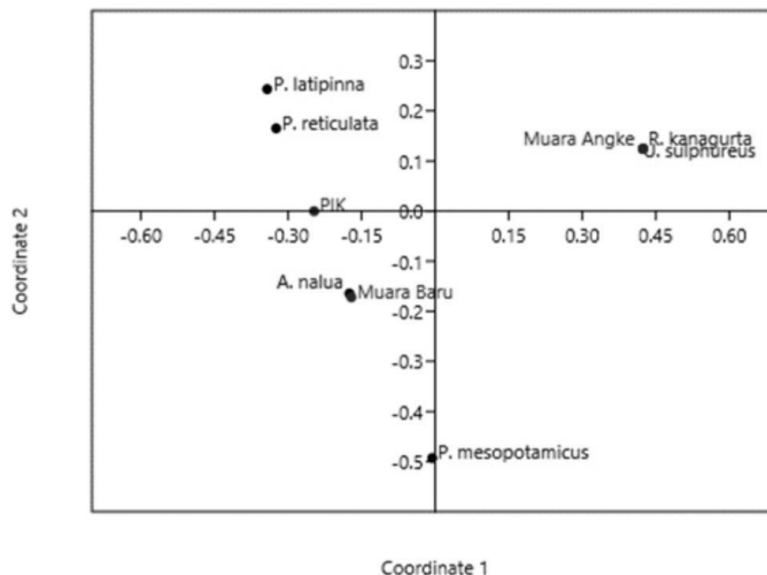

Coordinate 1

Figure 4. NMDS analysis of fish reads sequences based on the Bray-Curtis distance.

Fish conservation status. The comprehensive study under discussion has meticulously categorized the majority of the investigated fish species into two primary conservation status groups: Least Concern (LC) and Not Evaluated, as delineated by authoritative sources such as FishBase (www.fishbase.se) and IUCN Redlist database (www.iucnredlist.org). Among the numerous species scrutinized, only the has been designated as Near Threatened (NT), highlighting its potential vulnerability. Additionally, the study sheds light on the Poeciliidae family, particularly sailfin molly and guppy, pinpointing these species as potential pest threats (Table 1).

Table 1

The IUCN and CITES status of fish species list identified by eDNA

Family	Species	Common name	IUCN	CITES	Threat
Serrasalmidae	*Piaractus mesopotamicus*	Pacu	NT	NE	Harmless
Poeciliidae	*Poecilia latipinna*	Sailfin molly	LC	NE	Potential pest
Poeciliidae	*Poecilia reticulata*	Guppy	LC	NE	Potential pest
Ambassidae	*Ambassis nalua*	Scalloped perchlet	LC	NE	Harmless
Scombridae	*Rastrelliger kanagurta*	Indian mackerel	LC	NE	Harmless
Mullidae	*Upeneus sulphureus*	Sulphur goatfish	LC	NE	Harmless

Discussion. Deiner & Altermatt (2014) states that genetic material from fish and invertebrates can be detected up to 10 km from their original habitats. Rivers serve as conduits that collect and carry genetic signals from various aquatic and terrestrial vertebrates because water can have material from across the catchment area, leading to eDNA accumulation within aquatic systems (Effendi et al 2024b). The diversity of fish species is suspected to be due to the influence of different fertility levels at each location. A study by Damar et al (2020) states that the increase in fertility levels in Jakarta Bay tends to increase each year, measured through nutrient concentration. The measurement results in 2001 showed an annual average value of 22.97 µM dissolved inorganic nitrogen (DIN) and 11.31 µM phosphates, then a significant increase in concentration was observed in 2019 with an annual average value of 88.99 µM DIN and 25.92 µM. This condition causes a high eutrophication status, if reviewed based on the sampling location tends to have a hyper-eutrophic status. The eutrophication that occurred in Jakarta Bay caused a high nutrient enrichment which can cause algae blooming that has an impact on the increase in fish mortality rates (Damar et al 2020; Sidabutar et al 2020). The different water fertility is suspected to be due to different input sources at each location, namely Muara Baru is adjacent to the port and shipyard, Muara Angke is adjacent to the settlement, and PIK is adjacent to the settlement and mangrove area.

Another condition that is suspected to influence the level of diversity of fish species is the occurrence of hypoxia due to the high process of organic decomposition that generally occurs in estuarine areas. Damar et al (2021) stated that the hypoxic condition that occurs in Jakarta Bay is quasi-persistent hypoxic, caused by low transport of surface to bottom water, resulting in isolation of low oxygen of the near-bottom water. This case is caused by large oxygen demand, weak tidal mixing, high river discharge, and lack of continuous convection, causing benthic mass mortalities (Ladwig et al 2016; Hayami et al 2020).

The consequence of this event is the limited ability of fish species to live, in this case, scalloped perchlet is suspected to have a high survival ability because it is found quite high at the Muara Baru station and is also found in the PIK area. Various environmental factors, encompassing physical, chemical, and biological elements, can significantly influence the distribution of eDNA particles across different locations. Variations in eDNA abundance and diversity can be attributed to the topographical conditions affecting water transport. Factors such as water temperature, currents, pH levels, dissolved oxygen, organic content, UV exposure, and the specific amount and nature of the material used in sampling can all impact the quality and preservation of eDNA (Aprilia et al 2023b). Additionally, eDNA analysis depends significantly on the choice of primers used. Primers are short sequences of nucleotides that provide a starting point for DNA synthesis during the polymerase chain reaction (PCR). The selection of appropriate primers is crucial because they determine the specificity and efficiency of the PCR, directly influencing the accuracy and reliability of the eDNA analysis. Different primers target different DNA regions, which can affect the detection and identification of particular species or groups of organisms within a given environmental sample (Effendi et al 2024a).

Pacu is a ray-finned fish from South America, initially found in the Paraguay-Paraná River basin. However, due to aquaculture practices, its presence has expanded to a broader region. The pacu is listed as Near Threatened mainly due to habitat loss, overfishing, pollution, and climate change (Delcorso et al 2020). In Jakarta Bay, these issues are particularly pressing: rapid urbanization and industrial activities have degraded natural habitats, while overfishing depletes pacu populations. Pollution from factories and households harm water quality, and climate change affects water temperatures and river flows (Aprilia et al 2022; Aprilia et al 2023a). To help the pacu, protecting and restoring habitats, enforcing sustainable fishing practices, reducing pollution, and raising public awareness about conservation efforts are crucial.

The Poeciliidae family, part of the Cyprinodontiformes order, also known as tooth-carps, encompasses popular live-bearing aquarium fish species. This family was originally found in the Southeastern America down north of Argentina's Río de la Plata and across Africa, including Madagascar. This research focuses on the Poeciliidae family, particularly

on species such as sailfin molly and guppy, highlighting their potential as pest threats. Due to their high adaptability and quick reproduction rates, these species can flourish in various environments. Upon introduction to new habitats, they pose a risk by competing with native fish species for resources, disturbing the balance of local ecosystems and leading to a decline in biodiversity. Their invasive characteristics and rapid dissemination underscore the need for vigilant monitoring and management strategies to protect native aquatic species from their impact (Gomes-Silva et al 2020).

Conclusions. A study obtained sequences from six fish species across five genera, five families, and four orders from three sites. At Muara Baru, three species were identified: scalloped perchlet (*A. nalua*), guppy (*P. reticulata*), and sailfin molly (*P. latipinna*). Muara Angke hosted the Indian mackerel (*R. kanagurta*) and the sulphur goatfish (*U. sulphureus*), while Pantai Indah Kapuk had the scalloped perchlet (*A. nalua*) and the pacu (*P. mesopotamicus*). The scalloped perchlet was the most common species found. Species diversity, measured by the Shannon-Wiener index, indicates low to moderate diversity across the sites. The future of eDNA analysis in estuarine areas is promising, offering a powerful tool for monitoring and managing these complex ecosystems. eDNA can provide detailed insights into biodiversity, enabling the detection of a wide range of species, including those that are rare or elusive. It also serves as an effective method for assessing ecosystem health by identifying bioindicator species and tracking pollution levels. The early detection of invasive species through eDNA can help prevent ecological disruptions. Additionally, eDNA is valuable for studying the impacts of climate change on species distribution and phenology in estuaries. As methodologies improve and become more standardized, eDNA analysis will play a crucial role in restoration efforts and conservation planning, ensuring the protection of these vital ecosystems.

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

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