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A Baseline of Fish Species Richness Through eDNA Metabarcoding in an Understudied Tropical Mangrove Coast of Java, Indonesia

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ABSTRACT

Mangrove ecosystems support a diverse array of animal species and also provide pivotal ecosystem services, such as coastal protection, food provisioning, and carbon capture. However, these vital habitats are in decline, leading to coastal degradation in many parts of the globe. To address this, a mangrove restoration project in Demak, Java, Indonesia, introduced the use of semi-permeable coastal protective barriers made of bamboo pilings to safeguard the shore zone and hinterlands. The introduction of such hard substrate in the marine environment can attract a range of species, and it is important to be able to monitor changes in biodiversity from a restoration point of view. Here, we assessed whether environmental DNA metabarcoding can be applied to monitor fish biodiversity in an understudied area. Our results show slight but significant differences in species richness and fish community composition within a short timeframe of only 4 months, although we cannot disentangle the effects of seasonal variation from those of the introduction of hard substrate. More importantly, this study demonstrates a useful level of temporal resolution of eDNA metabarcoding and establishes a baseline for fish species richness in an understudied mangrove coastal zone in Demak, Java, Indonesia. Our results are of value for informing future restoration efforts and other (metabarcoding) biodiversity studies in the region.

1 | Introduction

Tropical ecosystems are characterized by being hyper diverse: over 75% of all species on earth are found in the tropics at least part of their life (Barlow et al. 2018). Apart from being biodiversity hotspots, tropical ecosystems also provide a range of ecosystem services such as provision of food, medicine, natural resources, carbon sequestration, and cultural heritage (Barbier et al. 2011; Gajula et al. 2020; Yadav et al. 2022). One key ecosystem of the tropical coastal zone are mangrove ecosystems, which play an important role in

provisioning, regulating, and supporting ecosystem services (Barbier et al. 2011). Healthy mangrove forests provide shelter and feeding grounds and are known to serve as nurseries for many species from different taxa such as reef fish and many crustaceans (Aguaiza et al. 2024; Nagelkerken et al. 2008; Xie et al. 2020). Moreover, mangrove forests are an important coastal barrier: they attenuate wave energy and stabilize the sediment, thereby providing the nearshore areas with pivotal protection (Horstman et al. 2014; Tiggeloven et al. 2022). However, a growing human population has caused high anthropogenic pressure on these vulnerable ecosystems.

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FIGURE 1 | Bamboo poles in support of coastal protection in Demak, Java, Indonesia, as part of the MuMaCo project. The semi-permeable structure functions as a wave barrier and consists of five elements, each 35 m wide, with a 5 m gap in between. Photo credit: Wawan Nugroho.

Pollution, overfishing, and deforestation are just a few of the many stressors that humans exert on coastal mangrove forests (Bhowmik et al. 2022). Not surprisingly, mangrove forests are declining globally: an estimated 4.2% of mangrove forests have been lost between 1996 and 2016 (Worthington and Spalding 2018). Indonesia, the world's largest archipelago with an expansive shoreline, has the largest mangrove cover in the world (Hamilton and Casey 2016). However, it also has one of the highest mangrove deforestation rates: already a decade ago, mangrove cover on the island of Java alone was estimated to be a mere 25% of its cover in the 1800s (Ilman et al. 2016). Mangrove forests are logged and mainly converted to aquacultural ponds for shrimp cultivation (Hamilton and Casey 2016). Conversion of mangrove forests to aquacultural ponds is not only a threat to biodiversity but also to coastal communities. Reduced coastal protection and stability cause regular and extreme floods, which are a serious threat especially in face of climate change and associated sea level rise.

In response to these threats, the MuMaCo project (Mussels as Mangrove facilitators for Coastal defense) was initiated in the coastal region of Demak, Java, Indonesia to restore mangrove forests in the area. Based on previous studies in the same area (e.g., van Bijsterveldt et al. 2020), a configuration of bamboo poles was constructed nearshore with the intention to reduce wave energy and facilitate sediment retention to create a favorable habitat where mangroves can naturally recruit and recover (Figure 1). Simultaneously, the introduction of hard substrate (i.e., bamboo poles) can lead to a local increase in marine biodiversity. Many different species are readily attracted to hard substrate: the surface area can be quickly colonized by pioneer species such as barnacles. Animals from higher trophic levels are attracted that prey on the colonizing epifauna (Toledo et al. 2020), use hard substrate as shelter (Hylkema et al. 2020), and may deposit eggs on it or use it as a spawning site (David da Costa et al. 2022). As such, hard substrate can function as an artificial reef, altering the biological community and supporting local marine biodiversity.

Monitoring the effect of an artificial reef or other restoration effort on local biodiversity is common practice, as succession and the attraction of species are not straightforward processes (Hylkema et al. 2021). Local conditions (e.g., water quality, type of substrate, seasonality) can influence the course of succession (De Mesel et al. 2015; Kingma et al. 2024; Santana et al. 2023). However, visual census monitoring of biodiversity can be time- and cost-intensive. Moreover, these methods are often invasive: an undesirable feature in a nature restoration project. In contrast, Environmental DNA (eDNA) metabarcoding is a noninvasive monitoring method that can overcome these problems, and restoration projects can benefit from its utility. eDNA metabarcoding uses a genomics approach, in which naturally shed DNA in the environment is collected and sequenced to provide an overview of biodiversity in the system (Taberlet et al. 2012). However, one of the most prominent limitations of this technique is its dependence on a DNA reference database (Hestetun et al. 2020; Van Der Loos and Nijland 2020). This has already proven challenging in temperate regions where eDNA metabarcoding is used relatively often and is increasingly being accepted as a monitoring method (Cornelis et al. 2024; Mauffrey et al. 2021). In the tropics, however, this issue is of even greater importance: there are many knowledge gaps in these highly diverse but poorly described biodiversity hotspots (Karlsson et al. 2007; Polanco Fernández et al. 2021). In Southeast Asia, eDNA metabarcoding studies are increasingly being conducted (Clay et al. 2025; Marwayana et al. 2022; Zainal Abidin et al. 2022), but the number of studies lags behind compared to temperate regions (Belle et al. 2019). For example, only a few eDNA metabarcoding studies have been conducted in the Java Sea (Kuncoro et al. 2023; Madduppa et al. 2012, 2021; Sani et al. 2021). Therefore, the main aim of the current study was to see whether eDNA metabarcoding can efficiently capture fish biodiversity on a short timescale in the poorly described Demak Region on the North coast of Java. eDNA shedding and persistence remain an underexplored field and varies greatly between different environmental conditions (Joseph

et al. 2022), but since many studies describe the temporal resolution of eDNA metabarcoding in terms of days to weeks (Lance et al. 2017; McCartin et al. 2022), we hypothesize that differences in fish biodiversity can be detected between time-points in this tropical setting as well. In addition, our work was intended to provide baseline fish diversity data through eDNA metabarcoding that can be used as future reference for other studies in the region.

2 | Materials and Methods

2.1 | Study Site

This research was conducted in the coastal region of the Demak regency, Java, Indonesia (6°53′09.0″S 110°30′23.5″E). This part of the Java Sea is characterized by a North-West monsoon from November to April and has a tidal range of ~1 m (Winterwerp et al. 2020). The coast is lined with many (collapsed) aquaculture ponds, but also some mangrove fringes can be found. As part of the MuMaCo project, a configuration of coastal protective poles was built as semi-permeable wave barriers made up of a double row of bamboo poles parallel to the coast at 25–45 m from the shore at one location in the study area (Figure 1). The rows of bamboo poles consisted of five elements, each 35 m wide, with 5 m gaps in between, of which the most north-eastern element was placed at an angle: the whole structure was 200 m wide. Construction of the semi-permeable bamboo pole wave barriers was completed in October 2021.

2.2 | Sample Collection eDNA

Water samples were collected along six transects: two running perpendicular to the poles (hereafter Pole transects), two transects at control location 1 (~1 km northeast of the Pole transects), and two transects at control location 2 (~500 m northeast of the Pole transects) (Figure 2A). Along each transect, 0.5 L water samples were collected in sterile plastic bottles, in triplicate at three locations: (a) 100 m seaward of the poles (“sea”), (b) in close proximity to the poles in the case of Pole transects and at the approximate location of the poles in the case of control transects (both hereafter called “halfway”), and (c) close to the mangrove edge (“mangrove”) (Figure 2B). Where water depth allowed, the water samples were collected off a boat. If the water was too shallow, sample locations were reached on foot. Water samples were brought to the coast and stored at –20°C. Sampling was performed prior to the placement of poles during the dry season (T0—July 2021) and 1 month after the construction of the poles (T1—November 2021). Tap water control samples were included during the T0 sampling collection.

2.3 | Sample Processing

The frozen water samples were transported to Universitas Diponegoro in Semarang and thawed at room temperature. Once thawed, water was filtered by applying a vacuum and using Cellulose Nitrate filters with a pore size of 1.2 μm. The

filters were stored in 400 μL DNA/RNA shield (Zymo Research) and kept at –20°C until transport to Wageningen University and Research in the Netherlands.

DNA extraction was performed in a molecular lab in UV-sterilized working cabinets, using the Blood & Tissue kit (Qiagen) following the Tissue protocol. Extractions were performed in six batches, and an extraction blank was taken along for each batch. Concentrations and quality of the DNA were measured on a Nanodrop spectrophotometer.

In general, fish are better represented in the DNA reference database compared to highly diverse invertebrate phyla such as Arthropoda and Annelida (Bucklin et al. 2011). Furthermore, the mitochondrial COI gene has been described to coamplify nontarget eukaryote and prokaryote DNA to such an extent that it reduces the specificity of primers targeting this region (Collins et al. 2019; Deagle et al. 2014). Therefore, we focused on amplification of the mitochondrial 12S rRNA gene of fish DNA using the well-described MiFish U/E primers (Miya et al. 2015) (forward primer: GTYGGTAAAWCTCGTGCCAGC, reverse primer: ATAGTGGGGTATCTAATCCYAGTTTG) with an expected amplicon length of ~170 bp. The primers were extended with a unique, 13 bp long tag (Srivathsan et al. 2021), which enables direct multiplexing of samples after the PCR and omits the need for a barcoding PCR. Samples were amplified in triplicates, where each triplicate reaction was provided the same tag. PCR mixtures contained 2× Phire Tissue Direct PCR Master Mix (ThermoFisher Scientific, USA), 0.2 μL 10 nM F primer, 0.2 μL 10 nM R primer, 0.5 μL sample, and nuclease-free water, to a total volume of 10 μL. Negative PCR controls were taken along for which nuclease-free water was added as a template. The following settings were used for amplification: 3 min of initial denaturation at 98°C, followed by 35 cycles of 98°C for 10 s, 59°C for 10 s, and 72°C for 10 s. Final elongation was set at 72°C for 3 min. PCR products were loaded on a 1.5% agarose gel to determine amplification success.

PCR products were visualized on a 1% agarose gel. Then, the volume to take from each PCR product for equimolarly pooling of the samples was based on the brightness of the target band. Equimolar pooling was done to account for differential amplification between samples and to ensure an even distribution of all tags in the downstream sequencing run. The MiFish primer pair very often also amplifies bacterial DNA, which runs higher on the gel at ~300 bp (Lee et al. 2024). Therefore, we loaded 2×60 μL of the pool on a 1.5% agarose gel again and ran it at 100 V for 1 h. The lower-running target bands of ~170 bp long were cut out of the gel, divided in half, and subjected to gel extraction using the QIAquick Gel Extraction kit following the manufacturer's protocol (Qiagen). After gel extraction, the eluates were pooled again before proceeding with library preparation using the SQK-LSK114 sequencing kit of Oxford Nanopore Technologies (ONT). ONT sequencing was used because of its availability in-house and cost- and time efficiency. Although ONT is often described to have a lower accuracy compared to Illumina sequencing, the latest chemistry and basecalling models in combination with bioinformatics tools (described below) does provide an accuracy of > 99% and have been used in other metabarcoding studies (Doorenspleet,

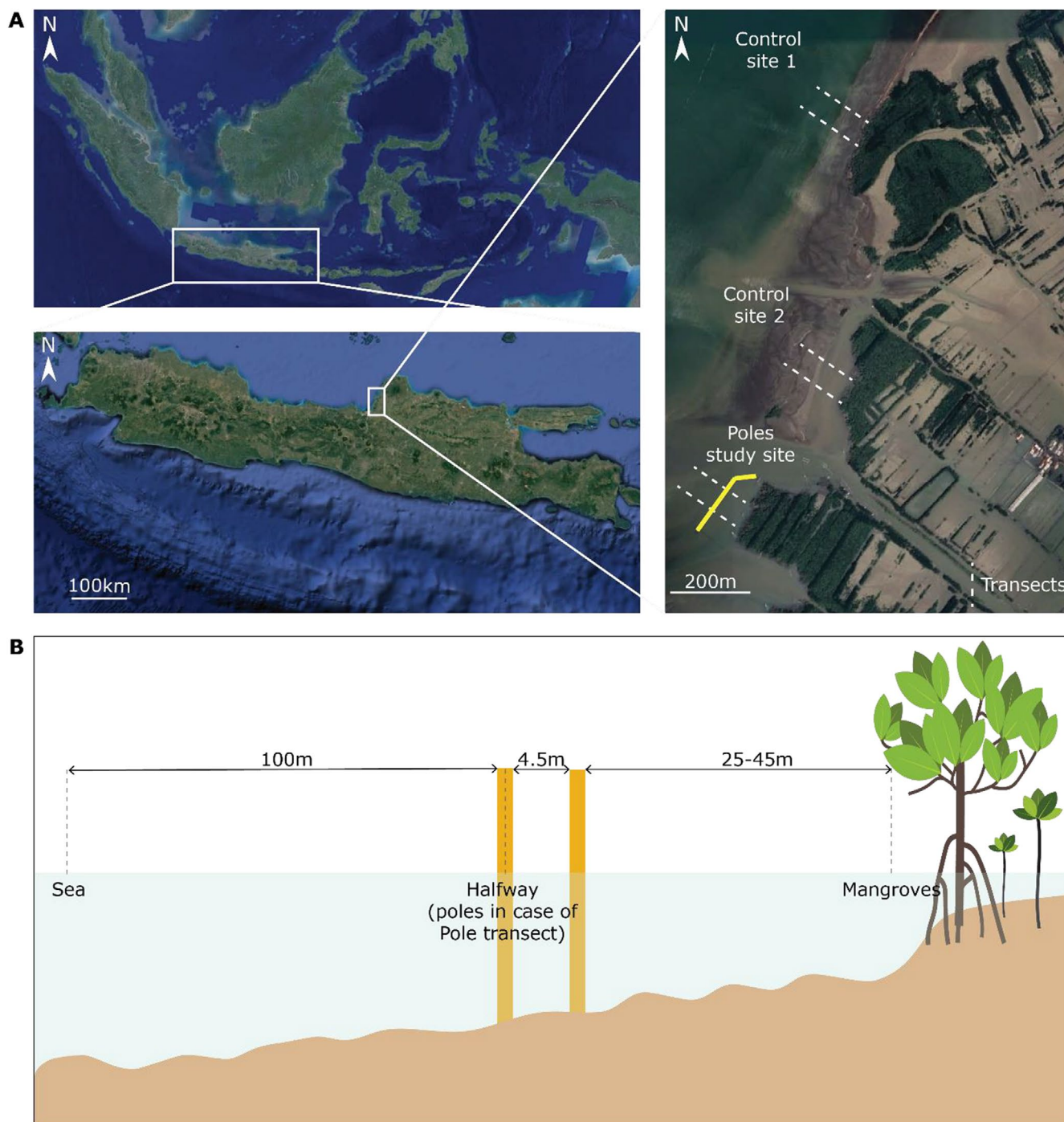


FIGURE 2 | Overview of study site with (A) Aerial photos of Indonesia, Java, and Timbuloko in the Demak region. Transects are indicated with a dashed line, and the location of the bamboo poles with a solid yellow line. (B) Water samples were collected at the sea, halfway (i.e., the location of the poles in case of impacted transects; approximate location of the poles in case of control transects), and at the mangrove forest edge. Aerial images from Google Earth.

Jansen, et al. 2025; Doorenspleet, Mailli, et al. 2025; van der Reis et al. 2023). Since the DNA concentration after gel extraction was very low, we deviated from the ONT protocol and omitted the bead clean-up step after the end-prep and only cleaned the library after adapter ligation. The final DNA concentration was measured on a Qubit 4.0 fluorometer using the dsDNA-HS kit. All DNA (i.e., the pool of 59 tagged amplicon PCRs) was loaded on a primed R10.4.1 flow cell in a Mk1C sequencer and sequenced until an average sequencing depth of

50,000 reads per unique tag was reached, based on real-time data access that ONT provides.

2.4 | Bioinformatics

Raw sequencing data was processed with Dorado v0.5.3 (<https://github.com/nanoporetech/dorado/tree/release-v0.5.3>). Briefly, the data was basecalled with the super

accurate model (SUP) (./dorado basecaller -r --trim adapters --barcode-sequences --min-qscore> 10) and demultiplexed (./dorado demux --no-classify --no-trim --emit-fastq). Fastq files were then processed using the DECONA (v1.3.1 with adjustments described in Doorenspleet, Jansen, et al. 2025) pipeline, a Linux command line tool specially built for the processing of Nanopore sequencing data from mixed community samples (Doorenspleet, Jansen, et al. 2025; <https://github.com/Saskia-Oosterbroek/decona>). In DECONA, the reads were first trimmed off their unique tags using Cutadapt (Martin 2011), filtered for fragment lengths between 150 and 240 bp, a minimum quality score of 10, and subsequently clustered with a similarity percentage of 96 using CD-hit (Li et al. 2001). A cluster similarity of 96% was chosen based on the study by Doorenspleet, Jansen, et al. (2025). Then they were aligned with Minimap2 (Li 2018), and clusters were assembled using Racon (Vaser et al. 2017). Only clusters with a size of 10 reads or more were retained. The reads were polished with Medaka (<https://github.com/nanoporetech/medaka>), and the consensus sequence reclustered at 99% similarity. Final consensus sequences were generated from a maximum of 500 random reads/cluster. A locally downloaded eukaryote reference database (nt_euk extracted from NCBI April 11th 2024) was used to align all clusters and assign species identifications using the BLAST algorithm (Altschul et al. 1990). DECONA clusters were selected for further processing based on a minimal alignment length of 136 bp (i.e., 80% of the expected amplicon size of ~170 bp) and a percentage identity of 99% or higher to be considered as species level assignments. In one extraction and two PCR controls, we found target species after these filtering steps. We therefore decontaminated our dataset using the R package microDecon with standard settings and the biological replicates set as groups (McKnight et al. 2019). Lastly, we carefully checked the global recording of each species by consulting fishbase.org (FishBase 2025), which sources its data from the widely recognized repositories Global Biodiversity Information Facility (GBIF 2025) and Ocean Biodiversity Information System (OBIS 2025). When a species had never been recorded in South-East Asia, we labeled it contamination and removed it from the dataset before further processing. Species *Scomber japonicus* (Houttuyn, 1782) and *Clupea pallasii* (Valenciennes, 1847) were present in our data, and there have been recordings of these species in South East Asia (although *C. pallasii* not in the Java Sea). However, they only occurred in two and three of our samples, respectively; the total read count was always low (between 10 and 1029 reads), and related North Sea species (*S. scombrus* and *C. harengus*) occurred with a higher number of reads. Since more North Sea species were also detected in our data, and the lab facilities are shared with North Sea experiments, we deemed it more likely that *S. japonicus* and *C. pallasii* assignments were misidentified North Sea species. We therefore also removed those from our dataset.

2.5 | Statistical Analysis

All statistical analyses were performed in R v4.4.1 running in RStudio v2024.4.2. Briefly, read count was converted to presence/absence data for further data analysis. Visualization of data was done using the ggplot2 package v3.5.1 (Wickham 2010)

and further analyzed using the vegan community ecology package v2.6–6.1 (Oksanen et al. 2001). Richness data was checked for normality (shapiro.test) and homogeneity of variance (var.test) before using the Welch's two-sample *T*-tests (t.test, var.equal = FALSE) to determine significance levels for the comparison of species richness between locations along the transects, transects and timepoints separately. The packages VennDiagram v1.7.3 (Chen 2011) and ggplot2 (boxplot) were then used to compare species richness between timepoints. A Jaccard distance matrix (vegdist, method = jaccard) was made to visualize any patterns in beta diversity and tested for significance using a one-way PERMANOVA (adonis2, 999 permutations) with factors Location, Transect type, and Timepoint. Community structures were plotted in a principle coordinate analysis (PCoA, $k = 2$).

3 | Results

3.1 | Data Overview

The sequencing run yielded a total of 3,010,000 raw, unfiltered reads. These are publicly accessible in the European Nucleotide Archive under accession number PRJEB84473 (ENA 2025). A total of 1,531,017 reads (~50% of total raw reads) were left after DECONA processing (average/tag: 29.846 ± 41.748) and a total of 1,062,157 reads (~35% of total raw reads, with an average of 21.243 ± 32.654 reads per tag) after filtering for species level assignments (i.e., > 99% identity and a minimal alignment length of 136 bp). Three control samples were left in the dataset after these filtering steps, corresponding to 5,489 reads in total, with an average read count of 1.830 ± 2.851 and 9 clusters assigned to target species. Target species that were found in the control samples included *Planiliza macrolepis* (Smith, 1846), *Planiliza subviridis* (Valenciennes, 1836), *Anodontostoma chacunda* (Hamilton, 1822), *Carangoides praeustus* (Benett, 1830), *Chanos chanos* (Fabricius, 1775), *Johnius carouna* (Cuvier, 1830), *Lates calcarifer* (Bloch, 1790), *Nibea coibor* (Hamilton, 1822), and *Scatophagus argus* (Linnaeus, 1766). To account for this contamination, we ran microDecon with standard settings. The decontaminated dataset consisted of 1,044,185 reads in total with an average tag distribution of 20.884 ± 32.511 . We then manually removed off-target species such as *Homo sapiens* and North Sea fish, and the final dataset consisted of 882,027 reads in total (average/tag: 17.641 ± 31.639). Table S1 provides a summary of sequencing data processing. An overview of relative read abundance per sample is provided in Figure S1.

3.2 | No Difference in Alpha and Beta Diversity Between Transects and Locations Along Transects

A total of 39 different species of 18 different orders were assigned in our sequencing data. The majority of species (20 in total) belonged to the orders Acanthuriformes, Clupeiformes, Gobiiformes, Mugiliformes, or Perciformes (Table 1). Observed richness per location (Mangroves, Halfway, and Sea) was compared between control and Pole transects for both T0 and T1 using the nonparametric Wilcoxon rank sum test (Figure S2). We found no significant difference between any of the locations between control and Pole transects and therefore pooled the data

TABLE 1 | Overview of the species assignments from eDNA metabarcoding sequencing data. Indicated are which species are detected in what locations, what transect types, and what time points. 1 = detected, 0 = not detected.

Taxonomy	Locations along transect					Transect types (pooled locations)			Timepoints (pooled locations and transect types)			
	Phylum	Order	Family	Genus	Species	Mangrove	Poles	Sea	Pole	Control	July (T0—dry season)	November (T1—early wet season)
Chordata	Acanthuriformes	Sciaenidae	<i>Dendrophysa</i>	<i>Dendrophysa russellii</i>		1	1	1	1	1	1	1
Chordata	Acanthuriformes	Sciaenidae	<i>Johnius</i>	<i>Johnius carouna</i>		1	1	1	1	1	1	1
Chordata	Acanthuriformes	Sciaenidae	<i>Nibea</i>	<i>Nibea coibor</i>		1	1	1	1	1	1	1
Chordata	Carangiformes	Carangidae	<i>Alepes</i>	<i>Alepes djedaba</i>		1	1	1	1	1	1	1
Chordata	Carangiformes	Carangidae	<i>Carangoides</i>	<i>Carangoides praeustus</i>		1	1	1	1	1	1	1
Chordata	Centrarchiformes	Terapontidae	<i>Pelates</i>	<i>Pelates quadrilineatus</i>		0	1	0	0	1	0	1
Chordata	Centrarchiformes	Terapontidae	<i>Terapon</i>	<i>Terapon jarbua</i>		1	0	0	0	1	0	1
Chordata	Chaetodontiformes	Chaetodontidae	<i>Chaetodon</i>	<i>Chaetodon lunula</i>		0	0	1	0	1	1	0
Chordata	Cichliformes	Cichlidae	<i>Oreochromis</i>	<i>Oreochromis aureus</i>		1	1	1	1	1	1	1
Chordata	Clupeiformes	Clupeidae	<i>Anodontostoma</i>	<i>Anodontostoma chacunda</i>		1	1	1	1	1	1	1
Chordata	Clupeiformes	Clupeidae	<i>Escualosa</i>	<i>Escualosa thoracata</i>		1	1	1	1	1	1	1
Chordata	Clupeiformes	Clupeidae	<i>Hilsa</i>	<i>Hilsa kelee</i>		1	1	1	1	1	1	1
Chordata	Clupeiformes	Clupeidae	<i>Sardinella</i>	<i>Sardinella lemuru</i>		0	1	1	1	1	1	1
Chordata	Clupeiformes	Engraulidae	<i>Thryssa</i>	<i>Thryssa baelama</i>		0	1	1	1	1	1	1
Chordata	Cypriniformes	Cyprinidae	<i>Barbonymus</i>	<i>Barbonymus gonionotus</i>		1	1	1	1	1	1	1
Chordata	Gobiiformes	Gobiidae	<i>Acentrogobius</i>	<i>Acentrogobius viridipunctatus</i>		1	1	1	1	1	1	1
Chordata	Gobiiformes	Gobiidae	<i>Drombus</i>	<i>Drombus globiceps</i>		0	1	0	0	1	0	1

(Continues)

TABLE 1 | (Continued)

Taxonomy	Locations along transect							Transect types (pooled locations)			Timepoints (pooled locations and transect types)	
	Phylum	Order	Family	Genus	Species	Mangrove	Poles	Sea	Pole	Control	July (T0—dry season)	November (T1—early wet season)
Chordata	Gobiiformes	Gobiidae	<i>Drombus</i>	<i>Drombus triangularis</i>	1	0	0	0	1	0	0	1
Chordata	Gobiiformes	Gobiidae	<i>Parapocryptes</i>	<i>Parapocryptes serperaster</i>	1	1	1	1	1	1	1	1
Chordata	Gonorynchiformes	Chanidae	<i>Chanos</i>	<i>Chanos chanos</i>	1	1	1	1	1	1	1	1
Chordata	Istiophoriformes	Sphyraenidae	<i>Sphyraena</i>	<i>Sphyraena jello</i>	1	1	0	1	1	1	0	1
Chordata	Kurtiformes	Apogonidae	<i>Apogon</i>	<i>Apogon hyalosoma</i>	1	0	1	1	1	1	0	1
Chordata	Lutjaniformes	Lutjanidae	<i>Lutjanus</i>	<i>Lutjanus johnii</i>	0	1	0	0	1	1	0	1
Chordata	Lutjaniformes	Lutjanidae	<i>Lutjanus</i>	<i>Lutjanus russellii</i>	1	1	1	1	1	1	0	1
Chordata	Mugiliformes	Mugilidae	<i>Crenimugil</i>	<i>Crenimugil seheli</i>	1	1	1	1	1	1	0	1
Chordata	Mugiliformes	Mugilidae	<i>Ellochelone</i>	<i>Ellochelone vaigiensis</i>	0	1	1	1	1	1	0	1
Chordata	Mugiliformes	Mugilidae	<i>Moolgarda</i>	<i>Moolgarda perusii</i>	1	1	0	1	1	1	1	1
Chordata	Mugiliformes	Mugilidae	<i>Mugil</i>	<i>Mugil cephalus</i>	0	0	1	1	1	1	1	1
Chordata	Mugiliformes	Mugilidae	<i>Planiliza</i>	<i>Planiliza macrolepis</i>	1	1	1	1	1	1	1	1
Chordata	Mugiliformes	Mugilidae	<i>Planiliza</i>	<i>Planiliza subviridis</i>	1	1	1	1	1	1	1	1
Chordata	Perciformes	Centropomidae	<i>Lates</i>	<i>Lates calcarifer</i>	1	1	1	1	1	1	1	1
Chordata	Perciformes	Platycephalidae	<i>Platycephalus</i>	<i>Platycephalus indicus</i>	0	0	1	1	1	0	0	1
Chordata	Perciformes	Scatophagidae	<i>Scatophagus</i>	<i>Scatophagus argus</i>	1	1	1	1	1	1	1	1
Chordata	Perciformes	Serranidae	<i>Epinephelus</i>	<i>Epinephelus coioides</i>	1	1	1	1	1	1	1	1

(Continues)

TABLE 1 | (Continued)

Taxonomy	Locations along transect					Transect types (pooled locations)			Timepoints (pooled locations and transect types)	
	Mangrove	Poles	Sea	Pole	Control	July (T0—dry season)	November (T1—early wet season)			
Chordata	0	1	0	0	1	0	0	1	1	
Chordata	1	1	0	0	1	0	0	1	1	
Chordata	1	1	1	1	1	1	1	1	1	
Chordata	0	0	1	1	0	0	0	1	1	
Chordata	1	0	0	0	1	0	1	1	0	

for the Mangroves, Halfway, and Sea locations of all transects. With all transects pooled, we performed a Kruskal–Wallis test to compare richness between locations but found no significant difference (Figure S2). Per timepoint, similarity between Pole and control transects and between locations was also verified in a Jaccard distance matrix and visualized in a principal coordinate analysis (PCoA) (Figure S3). Again, we found no significant difference between transect type or location along the transect. However, there was a significant interaction effect between transect types and locations for the T0 dataset (PERMANOVA: $R^2=0.211$, $F=2.6$, p value=0.011). This was likely caused by the relatively high richness for pole locations along Pole transects. However, poles were not yet constructed during T0 sample collection, and all other comparisons between control and Pole transects showed high similarity in terms of both alpha and beta diversity. It was therefore decided to pool the data of all transect types and locations along the transects per timepoint, despite the detected interaction effect in T0 samples.

3.3 | Alpha and Beta Diversity Are Different Between Timepoints

Based on the pooled data, there was a significant difference in richness between timepoints. With a mean of 8.7 and 13.6 species, respectively, T0 was found to have a lower mean richness than T1 (Welch's two sample t -test: p -value = 0.029) (Figure 3A). Two species were uniquely detected in T0 samples, 14 in T1 samples, and the timepoints had an overlap of 22 species (Figure 3B). The Jaccard distance matrix also shows a slight but significant difference in community composition between timepoints (PERMANOVA: $R^2=0.068$, $F=3.3$, p value = 0.022) (Figure 3). A complete overview of statistics can be found in Table S2.

4 | Discussion

The aim of this study was to assess whether eDNA metabarcoding could be used as a monitoring method for fish biodiversity in the under-described Java Sea. The research was performed within the MuMaCo project, in which a barrier of semi-permeable bamboo poles was built with the purpose of coastal protection. As such, our data comprised (a) water samples collected along a transect before and 1 month after the construction of the poles, and (b) likewise for control transects where no poles were constructed. A total of thirty-nine different species of eighteen different fish orders were detected, and we verified the presence of these species in consultation with local fishers (pers. comm. Saeri). The actual species richness in the area is possibly higher since 22% of our unfiltered sequencing data had an alignment percentage identity below 99% against the reference database and therefore could not be classified to species level. Our results compare well with the fish diversity study by Debrot et al. (2022) in the same area using traditional monitoring methods, but indeed some species were not found in our data and vice versa. Although it was not the aim of this study to determine the effect of the poles on fish diversity per se, we did not observe a difference in species composition between Pole and control transects, nor between locations along transects (Mangroves, Halfway and Sea) (Figures S2 and S3). Arguably, this is due to the bamboo structures not providing distinct ecological niches

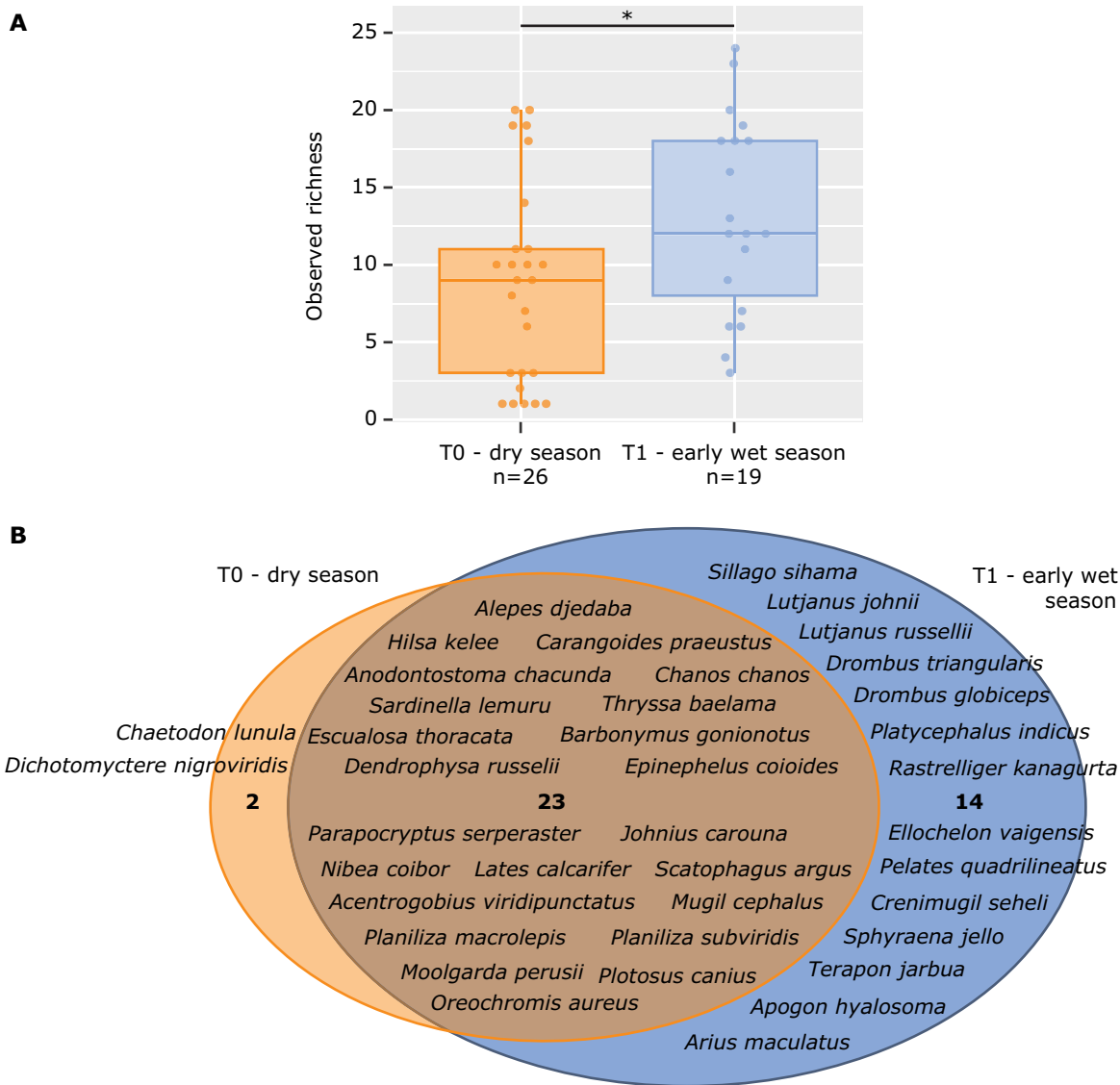


FIGURE 3 | Alpha diversity of timepoints T0 and T1. (A) Mean observed richness is higher in T1 samples than T0 samples (Welch two sample t -test: p value = 0.026 [*]). (B) Venn diagram including species names. A total of 25 species were detected in T0 samples, compared to 37 in T1 samples. 23 species were detected in both timepoints.

or structural complexity to attract new species in such a short timeframe (1 month). However, there were significant differences in alpha and beta diversity before and after construction, but these are likely explained by seasonal variation. Samples from the early wet season (November, T1) were found to have a higher observed species richness than samples collected in the middle of the dry season (July, T0) (Figure 3A, p -value = 0.026). Also, beta diversity was found to be slightly but significantly different and hence there were minor differences in species composition between the timepoints (Figures 3B and 4, PERMANOVA: $R^2 = 0.068$, $F = 3.3$, p -value = 0.022). Other fish diversity studies carried out in the Java Sea indeed found the late dry season and transitional monsoon to be characterized by higher species richness than the wet season (Madduppa et al. 2012; Pauly and Martosubroto 1996). The higher richness in our early wet season samples coincided with higher richness in macrobenthos in the area (Muskananfolo and Purnomo 2020) and hence with food availability. The richness found in this time of year may still reflect the build-up of species richness towards the end of the

dry season. Species richness in our dry season samples, however, was lower than expected. Nutrient availability may explain this pattern, as runoff and nutrient levels from rivers are generally low during the relatively calm dry season, possibly leading to lower primary production (Flores-Verdugo et al. 1990). However, many households line the river flowing out in the area under study, making nutrient limitation an unlikely factor for reduced primary production during the dry season.

More likely, though, practical and technical constraints have importantly influenced our results. For example, samples were collected at a remote location with limited resources. Filtration equipment was not available at the field location, making it necessary to store the water samples directly in a -20°C freezer in the local village. Since the village was not easily accessible, water samples were stored as ice for a prolonged period of time (up to 1.5 years). The tropical warmth may have caused the samples to partially thaw during transport to Universitas Diponegoro in Semarang. Once in the local university, samples

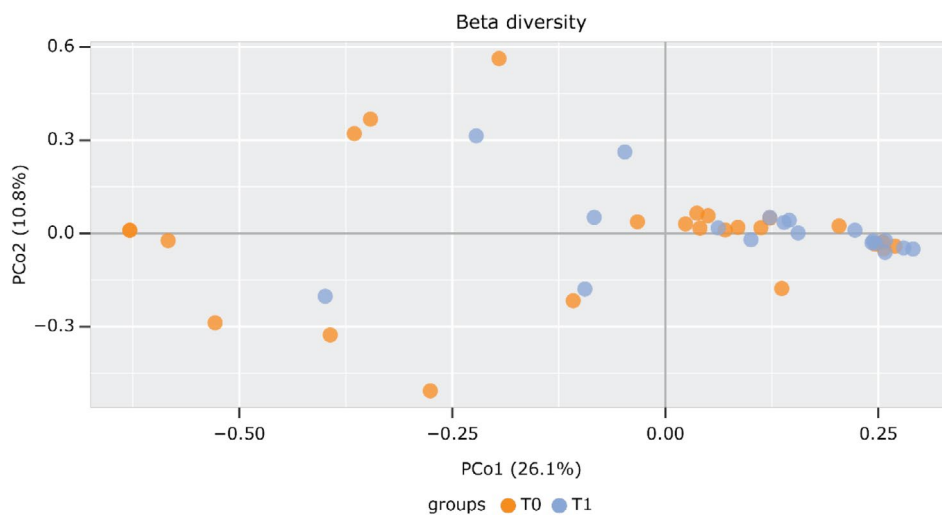


FIGURE 4 | Beta diversity based on the Jaccard distance matrix between timepoints. Community compositions were slightly but significantly different between the two timepoints (PERMANOVA: $R^2=0.068$, p value = 0.022). Orange: T0 samples, blue: T1 samples.

were frozen again until filtration. Freeze–thaw cycles are known to have a detrimental effect on the integrity of DNA (Takahara et al. 2015), and this likely lowered the quality of the DNA. Rare DNA sequences may have degraded and become undetectable in the downstream metabarcoding pipeline due to repeated freeze–thaw cycles. Furthermore, limited resources and several different people helping and handling the samples increase the chance of introducing contamination. The effect of contamination is inflated when the concentration and quality of the DNA is low, since overrepresented DNA sequences may mask the detection of rare sequences (Boessenkool et al. 2012). The loss of and failure to detect rare sequences may have reduced the chances of detecting differences in fish diversity based on the obtained samples. Nevertheless, our approach did detect temporal differences within a 4-month timeframe in our eDNA dataset from a tropical region where coverage of the DNA reference database is low. This shows the robustness and potential of eDNA metabarcoding as a monitoring method when an overview of fish diversity is needed. However, to answer more in-depth ecological research questions through eDNA metabarcoding, it is critical that fieldwork logistics, storage conditions, transport, and handling of samples are optimized. Ideally, water samples need to be filtered immediately after water collection. A handpump or 12V vacuum pump running on a battery pack (as described by Nijland (2020)) would allow for immediate filtration in the absence of electricity, although using pumps this small can be very time-consuming when many samples need to be processed. Also, gravity filtration and passive eDNA samplers are potential alternatives to active filtration in the absence of electricity but are likewise time-consuming due to a long filtration time (up to 1 h per sample) or long emersion time (hours to days), respectively (Chen et al. 2022; Kirtane et al. 2020; Oka et al. 2022). If immediate filtration is logistically impossible, adding a DNA preservative such as Longmire or cationic surfactant directly to the water sample prevents microbial degradation and may help preserve DNA (Williams et al. 2016; Yamanaka et al. 2017). However, filtration as soon as possible remains essential. After filtration, filters should be stored in a DNA preservative and kept at -20°C until further processing (Yamanaka et al. 2016). In our experience, DNA/RNA shield (Zymo) is an easy-to-use

and postprocessing friendly DNA preservative. Cheaper alternatives that can be self-made would be Longmire solution (Longmire et al. 1997) or DESS (Seutin et al. 1991), but these may require DNA extraction methods that are more laborious and hazardous, such as phenol/chloroform extractions. If the samples, either frozen water or filters in DNA preservative, are to be transported from a remote location, the use of a cooler or ice in Styrofoam boxes would help to avoid unnecessary freeze–thaw cycles. Finally, our filtered samples were transported from Indonesia to the Netherlands for further processing. It would be advantageous for the entire pipeline, from sample filtering to sequencing, to be conducted locally, thereby avoiding delays associated with overseas transportation. Apart from logistic considerations, performing molecular biomonitoring locally as much as possible greatly enhances capacity building and local involvement in nature restoration programs in remote areas (Miya et al. 2022; Wee et al. 2023). In the context of mangrove restoration, locally implemented molecular monitoring allows coastal communities and research facilities to track ecological changes over time directly and respond adequately when needed (reviewed by Wee et al. 2023). For example, Zainal Abidin et al. (2022) report eDNA-mediated detection of two invasive species in a mangrove estuary in Malaysia, but eDNA has also been used to monitor the recovery of indicator species after invasive species removal (Lin et al. 2025).

Local lab-processing of eDNA samples would require a well-equipped molecular lab where contamination in any form (human, other organisms or from other experiments) can be avoided. Ideally, eDNA is processed in a completely separate lab, where no other experiments (e.g., DNA extractions from tissue samples) are performed to avoid contamination with highly abundant DNA sources. However, this can often not be achieved logistically. Preasures to work in a shared molecular lab would be to work in UV-cabinets dedicated to eDNA, aliquot all reagents for personal use, use eDNA-only aliquots, strictly separate the pre- and post-PCR workflows and include controls at every step during sample processing to track contamination. Finally, it is absolutely necessary to do a sanity check of the resultant species list. Here, we checked the recordings

of each species in the well-established database of [fishbase.org](https://www.fishbase.org) (FishBase 2025) and verified our results with local fishers. We highly recommend including local ecological knowledge when interpreting biodiversity survey results from remote and poorly-described areas. Although collection of local ecological knowledge data in a structured way can be challenging (e.g., see Valbo-Jørgensen and Poulsen 2000), local knowledge is an invaluable source of biodiversity data and even simple consultation using photos can provide important insights in (metabarcoding) biodiversity studies.

The original intent of this study was to also assess whether the construction of the semi-permeable bamboo pole barriers in support of coastal protection (i.e., introduction of hard substrate) could measurably affect local fish diversity across a timeframe of 1 year in the coastal region of Demak, Java, Indonesia. As such, we aimed for year-round sampling of all locations. However, research and fieldwork in a tropical ecosystem are challenging, and several setbacks caused us to deviate from our original aim, whereby our comparison only assessed short-term changes occurring over 4 months. Importantly, bad weather conditions during sample collection of T2 (monsoon period) led to the collection of a limited number of samples. Our research was performed during the COVID-19 pandemic, and traveling to Indonesia was not an option. We were lucky to have the help of talented locals, but with personnel and equipment being only available for a short period of time, we were not able to postpone sample collection and had to do with the limited number of samples collected during T2. Future research may benefit from incorporating back-up windows for sample collection, especially during the unpredictable monsoon period. The collection of our last timepoint (T3) was successful, but the samples likely suffered from the technical constraints in storage and repeated freeze–thaw cycles mentioned above. Despite numerous attempts, we could hardly amplify any DNA from T3 samples. Hence, we ultimately were unable to sequence half of our envisioned samples.

Biodiversity research in tropical regions is underrepresented and much needed for optimal decision-making in conservation. We hope that by sharing the challenges we faced in this study, others may be able to avoid the same limitations. Even so, we were able to compile baseline fish diversity and show eDNA metabarcoding can be applied as a monitoring method in a poorly documented tropical region.

5 | Conclusion

This study uses eDNA metabarcoding to show that slight but significant differences in species richness and composition occur and can be detected in a timeframe of 4 months in the coastal region in Demak, Java, Indonesia. Bamboo poles were introduced in one of the studied transects and our second timepoint was collected 1 month after their construction. Although we cannot disentangle seasonal effects from those stemming from the introduction of hard substrate in the area, our results do demonstrate the ability to detect temporal changes through eDNA metabarcoding in an understudied area. The results support the potential of this method in remote areas. Our data also provide baseline fish diversity data of the Demak

region and can be used as a reference for future research in Southeast Asia.

Author Contributions

The study design was conceptualized by L.J., A.O.D., and R.N.; samples were acquired and prepared for transport by R.W.A., L.L.W., and S.R.; and processed and sequenced by L.J. and E.L. Data analysis was performed by L.J. and data interpretation by L.J. and R.N. The manuscript was written by L.J., and all coauthors provided feedback.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequencing data will be archived in the European Nucleotide Archive (ENA 2025) under project accession number PRJEB84473.

References

- Aguaiza, C., P. Mumby, E. Rastoin-Laplaine, O. Aburto-Oropeza, and P. Salinas-De-León. 2024. “Mangroves as Nursery Habitats for Galapagos Reef Fishes.” *Marine Ecology Progress Series* 747: 99–115. <https://doi.org/10.3354/meps14702>.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. “Basic Local Alignment Search Tool.” *Journal of Molecular Biology* 215, no. 3: 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Barbier, E. B., S. D. Hacker, C. Kennedy, E. W. Koch, A. C. Stier, and B. R. Silliman. 2011. “The Value of Estuarine and Coastal Ecosystem Services.” *Ecological Monographs* 81, no. 2: 169–193. <https://doi.org/10.1890/10-1510.1>.
- Barlow, J., F. França, T. A. Gardner, et al. 2018. “The Future of Hyperdiverse Tropical Ecosystems.” *Nature* 559, no. 7715: 517–526. <https://doi.org/10.1038/s41586-018-0301-1>.
- Belle, C. C., B. C. Stoeckle, and J. Geist. 2019. “Taxonomic and Geographical Representation of Freshwater Environmental DNA Research in Aquatic Conservation.” *Aquatic Conservation: Marine and Freshwater Ecosystems* 29, no. 11: 1996–2009. <https://doi.org/10.1002/aqc.3208>.
- Bhowmik, A. K., R. Padmanaban, P. Cabral, and M. M. Romeiras. 2022. “Global Mangrove Deforestation and Its Interacting Social-Ecological

- Drivers: A Systematic Review and Synthesis." *Sustainability* 14, no. 8: 4433. <https://doi.org/10.3390/su14084433>.
- Boessenkool, S., L. S. Epp, J. Haile, et al. 2012. "Blocking Human Contaminant DNA During PCR Allows Amplification of Rare Mammal Species From Sedimentary Ancient DNA." *Molecular Ecology* 21, no. 8: 1806–1815. <https://doi.org/10.1111/j.1365-294X.2011.05306.x>.
- Bucklin, A., D. Steinke, and L. Blanco-Bercial. 2011. "DNA Barcoding of Marine Metazoa." *Annual Review of Marine Science* 3: 471–508. <https://doi.org/10.1146/annurev-marine-120308-080950>.
- Chen, H. 2011. "VennDiagram: Generate High-Resolution Venn and Euler Plots." <https://cran.r-project.org/package=VennDiagram>.
- Chen, X., Y. Kong, S. Zhang, J. Zhao, S. Li, and M. Yao. 2022. "Comparative Evaluation of Common Materials as Passive Samplers of Environmental DNA." *Environmental Science & Technology* 56, no. 15: 10798–10807. <https://doi.org/10.1021/acs.est.2c02506>.
- Clay, C. G., F. Gösser, M. Glue, et al. 2025. "Exploring Species and Functional Diversity of Fishes in Cambodian Coastal Habitats Using eDNA Metabarcoding." *Coral Reefs* 44, no. 1: 221–241. <https://doi.org/10.1007/s00338-024-02599-1>.
- Collins, R. A., J. Bakker, O. S. Wangensteen, et al. 2019. "Non-Specific Amplification Compromises Environmental DNA Metabarcoding With COI." *Methods in Ecology and Evolution* 10, no. 11: 1985–2001. <https://doi.org/10.1111/2041-210X.13276>.
- Cornelis, I., A. De Backer, S. Maes, et al. 2024. "Environmental DNA for Monitoring the Impact of Offshore Wind Farms on Fish and Invertebrate Community Structures." *Environmental DNA* 6, no. 4: e575. <https://doi.org/10.1002/edn3.575>.
- da Costa, I. D., J. L. da Silva Santos, L. L. Costa, J. S. Lima, and I. R. Zalmon. 2022. "Reproductive Potential and Production Role of Artificial Reefs—Southeastern Brazil." *Estuarine, Coastal and Shelf Science* 265: 107710. <https://doi.org/10.1016/j.ecss.2021.107710>.
- De Mesel, I., F. Kerckhof, A. Norro, B. Rumes, and S. Degraer. 2015. "Succession and Seasonal Dynamics of the Epifauna Community on Offshore Wind Farm Foundations and Their Role as Stepping Stones for Non-Indigenous Species." *Hydrobiologia* 756, no. 1: 37–50. <https://doi.org/10.1007/s10750-014-2157-1>.
- Deagle, B. E., S. N. Jarman, E. Coissac, F. Pompanon, and P. Taberlet. 2014. "DNA Metabarcoding and the Cytochrome c Oxidase Subunit I Marker: Not a Perfect Match." *Biology Letters* 10, no. 9: 20140562. <https://doi.org/10.1098/rsbl.2014.0562>.
- Debrot, A. O., A. Plas, H. Boesono, et al. 2022. "Early Increases in Artisanal Shore-Based Fisheries in a Nature-Based Solutions Mangrove Rehabilitation Project on the North Coast of Java." *Estuarine, Coastal and Shelf Science* 267: 107761. <https://doi.org/10.1016/j.ecss.2022.107761>.
- Doorenspleet, K., L. Jansen, S. Oosterbroek, et al. 2025. "The Long and the Short of It: Nanopore-Based eDNA Metabarcoding of Marine Vertebrates Works; Sensitivity and Species-Level Assignment Depend on Amplicon Lengths." *Molecular Ecology Resources* 25, no. 4: e14079. <https://doi.org/10.1111/1755-0998.14079>.
- Doorenspleet, K., A. A. Mailli, B. B. van der Hoorn, et al. 2025. "Advancing Molecular Macroinvertebrate Biodiversity Monitoring: A Comparison Between Oxford Nanopore and Illumina Based Metabarcoding and Metagenomics." *PeerJ* 13: e19158. <https://doi.org/10.7717/peerj.19158>.
- ENA. 2025. "ENA Browser Accession Number PRJEB84473." <https://www.ebi.ac.uk/ena/browser/view/PRJEB84473>.
- FishBase. 2025. "FishBasever.04/2025." <https://www.fishbase.org/>.
- Flores-Verdugo, F., F. González-Farías, O. Ramírez-Flores, et al. 1990. "Mangrove Ecology, Aquatic Primary Production, and Fish Community Dynamics in the Teacapán-Agua Brava Lagoon-Esuarine System (Mexican Pacific)." *Estuaries* 13, no. 2: 219–230.
- Gajula, H., K. Vadlapudi, P. D. Vijendra, J. Rajashekar, T. Sannabommaji, and G. Basappa. 2020. *Secondary Metabolites From Mangrove Plants and Their Biological Activities*. INC.
- GBIF. 2025. "Free and Open Access to Biodiversity Data." <https://www.gbif.org/>.
- Hamilton, S. E., and D. Casey. 2016. "Creation of a High Spatio-Temporal Resolution Global Database of Continuous Mangrove Forest Cover for the 21st Century (CGMFC-21)." *Global Ecology and Biogeography* 25, no. 6: 729–738. <https://doi.org/10.1111/geb.12449>.
- Hestetun, J. T., E. Bye-Ingebrigtsen, R. H. Nilsson, A. G. Glover, P.-O. Johansen, and T. G. Dahlgren. 2020. "Significant Taxon Sampling Gaps in DNA Databases Limit the Operational Use of Marine Macrofauna Metabarcoding." *Marine Biodiversity* 50, no. 5: 70. <https://doi.org/10.1007/s12526-020-01093-5>.
- Horstman, E. M., C. M. Dohmen-Janssen, P. M. F. Narra, N. J. F. van den Berg, M. Siemerink, and S. J. M. H. Hulscher. 2014. "Wave Attenuation in Mangroves: A Quantitative Approach to Field Observations." *Coastal Engineering* 94: 47–62. <https://doi.org/10.1016/j.coastaleng.2014.08.005>.
- Hylkema, A., A. O. Debrot, R. Osinga, et al. 2020. "Fish Assemblages of Three Common Artificial Reef Designs During Early Colonization." *Ecological Engineering* 157: 105994. <https://doi.org/10.1016/j.ecoeng.2020.105994>.
- Hylkema, A., Q. C. A. Hakkaart, C. B. Reid, R. Osinga, A. J. Murk, and A. O. Debrot. 2021. "Artificial Reefs in the Caribbean: A Need for Comprehensive Monitoring and Integration Into Marine Management Plans." *Ocean and Coastal Management* 209: 105672. <https://doi.org/10.1016/j.ocecoaman.2021.105672>.
- Ilman, M., P. Dargusch, and P. Dart. 2016. "A Historical Analysis of the Drivers of Loss and Degradation of Indonesia's Mangroves." *Land Use Policy* 54: 448–459. <https://doi.org/10.1016/J.LANDUSEPOL.2016.03.010>.
- Joseph, C., M. E. Faiq, Z. Li, and G. Chen. 2022. "Persistence and Degradation Dynamics of eDNA Affected by Environmental Factors in Aquatic Ecosystems." *Hydrobiologia* 849, no. 19: 4119–4133. <https://doi.org/10.1007/s10750-022-04959-w>.
- Karlsson, S., T. Srebotnjak, and P. Gonzales. 2007. "Understanding the North–South Knowledge Divide and Its Implications for Policy: A Quantitative Analysis of the Generation of Scientific Knowledge in the Environmental Sciences." *Environmental Science & Policy* 10, no. 7: 668–684. <https://doi.org/10.1016/j.envsci.2007.04.001>.
- Kingma, E. M., R. ter Hofstede, E. Kardinaal, et al. 2024. "Guardians of the Seabed: Nature-Inclusive Design of Scour Protection in Offshore Wind Farms Enhances Benthic Diversity." *Journal of Sea Research* 199: 102502. <https://doi.org/10.1016/j.seares.2024.102502>.
- Kirtane, A., J. D. Atkinson, and L. Sassoubre. 2020. "Design and Validation of Passive Environmental DNA Samplers Using Granular Activated Carbon and Montmorillonite Clay." *Environmental Science & Technology* 54, no. 19: 11961–11970. <https://doi.org/10.1021/acs.est.0c01863>.
- Kuncoro, I., N. P. Zamani, B. Subhan, and N. K. D. Cahyani. 2023. "eDNA Assessment of Scleractinian Diversity and Distribution in Lemukutan Island, Indonesia." *Biodiversitas Journal of Biological Diversity* 24, no. 7: 4185–4191. <https://doi.org/10.13057/biodiv/d240758>.
- Lance, R., K. Klymus, C. Richter, et al. 2017. "Experimental Observations on the Decay of Environmental DNA From Bighead and Silver Carps." *Management of Biological Invasions* 8, no. 3: 343–359. <https://doi.org/10.3391/mbi.2017.8.3.08>.
- Lee, B.-Y., G. A. Milne, C. Freedman, J. Miksis-Olds, and B. L. Brown. 2024. "eDNA Metabarcoding Analysis of Bony Fish Identification in Coastal Water of Gulf of Maine Using Nested Polymerase Chain Reaction of 12S rRNA Universal Primers." *Environmental DNA* 6, no. 6: e70033. <https://doi.org/10.1002/edn3.70033>.

- Li, H. 2018. "Minimap2: Pairwise Alignment for Nucleotide Sequences." *Bioinformatics* 34, no. 18: 3094–3100. <https://doi.org/10.1093/bioinformatics/bty191>.
- Li, W., L. Jaroszewski, and A. Godzik. 2001. "Clustering of Highly Homologous Sequences to Reduce the Size of Large Protein Databases." *Bioinformatics* 17, no. 3: 282–283. <https://doi.org/10.1093/bioinformatics/17.3.282>.
- Lin, L., Y. Guo, G. Wang, et al. 2025. "Changes in Macrobenthos Communities During the Invasive *Spartina Alterniflora* Removal and Mangrove Restoration." *Frontiers in Marine Science* 12: 1581442. <https://doi.org/10.3389/fmars.2025.1581442>.
- Longmire, J. L., M. Maltbie, R. J. Baker, and Texas Tech University. 1997. *Use of 'Lysis Buffer' in DNA Isolation and Its Implication for Museum Collections, Occasional Papers*. Museum of Texas Tech University.
- Madduppa, H., N. K. D. Cahyani, A. W. Anggoro, et al. 2021. "eDNA Metabarcoding Illuminates Species Diversity and Composition of Three Phyla (Chordata, Mollusca and Echinodermata) Across Indonesian Coral Reefs." *Biodiversity and Conservation* 30, no. 11: 3087–3114. <https://doi.org/10.1007/s10531-021-02237-0>.
- Madduppa, H. H., S. C. A. Ferse, U. Aktani, and H. W. Palm. 2012. "Seasonal Trends and Fish-Habitat Associations Around Pari Island, Indonesia: Setting a Baseline for Environmental Monitoring." *Environmental Biology of Fishes* 95, no. 3: 383–398. <https://doi.org/10.1007/s10641-012-0012-7>.
- Martin, M. 2011. "Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads." *EMBnet. Journal* 17, no. 1: 10–12. <https://doi.org/10.14806/ej.17.1.200>.
- Marwayana, O. N., Z. Gold, C. P. Meyer, and P. H. Barber. 2022. "Environmental DNA in a Global Biodiversity Hotspot: Lessons From Coral Reef Fish Diversity Across the Indonesian Archipelago." *Environmental DNA* 4, no. 1: 222–238. <https://doi.org/10.1002/edn3.257>.
- Mauffrey, F., T. Cordier, L. Apothéloz-Perret-Gentil, et al. 2021. "Benthic Monitoring of Oil and Gas Offshore Platforms in the North Sea Using Environmental DNA Metabarcoding." *Molecular Ecology* 30, no. 13: 3007–3022. <https://doi.org/10.1111/mec.15698>.
- McCartin, L. J., S. A. Vohsen, S. W. Ambrose, et al. 2022. "Temperature Controls eDNA Persistence Across Physicochemical Conditions in Seawater." *Environmental Science & Technology* 56, no. 12: 8629–8639. <https://doi.org/10.1021/acs.est.2c01672>.
- McKnight, D. T., R. Huerlimann, D. S. Bower, L. Schwarzkopf, R. A. Alford, and K. R. Zenger. 2019. "microDecon: A Highly Accurate Read-Subtraction Tool for the Post-Sequencing Removal of Contamination in Metabarcoding Studies." *Environmental DNA* 1, no. 1: 14–25. <https://doi.org/10.1002/edn3.11>.
- Miya, M., T. Sado, S.-i. Oka, and T. Fukuchi. 2022. "The Use of Citizen Science in Fish eDNA Metabarcoding for Evaluating Regional Biodiversity in a Coastal Marine Region: A Pilot Study." *Metabarcoding and Metagenomics* 6: e80444. <https://doi.org/10.3897/mbmg.6.80444>.
- Miya, M., Y. Sato, T. Fukunaga, et al. 2015. "MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA From Fishes: Detection of More Than 230 Subtropical Marine Species." *Royal Society Open Science* 2, no. 7: 150088. <https://doi.org/10.1098/RSOS.150088>.
- Muskananfolo, M. R., and P. W. Purnomo. 2020. "Impact of Environmental Factors on Macrobenthos Distribution and Abundance in Mangrove Ecosystems on the Northern Coast of Java." *AACL Bioflux* 13, no. 5: 2745–2756.
- Nagelkerken, I., S. J. M. Blaber, S. Bouillon, et al. 2008. "The Habitat Function of Mangroves for Terrestrial and Marine Fauna: A Review." *Aquatic Botany* 89, no. 2: 155–185. <https://doi.org/10.1016/j.aquabot.2007.12.007>.
- Nijland, R. 2020. "Collecting eDNA From Marine Water Samples in the Field." <https://protocols.io/view/collecting-edna-from-marine-water-samples-in-the-f-6yfhftn>.
- OBIS. 2025. "Vision and Mission." <https://obis.org/about/>.
- Oka, S.-i., M. Miya, and T. Sado. 2022. "Gravity Filtration of Environmental DNA: A Simple, Fast, and Power-Free Method." *MethodsX* 9: 101838. <https://doi.org/10.1016/j.mex.2022.101838>.
- Oksanen, J., G. L. Simpson, F. Guillaume Blanchet, et al. 2001. "Vegan: Community Ecology Package." <https://doi.org/10.32614/CRAN.packages.vegan>.
- Pauly, D., and P. Martosubroto. 1996. *Baseline Studies of Biodiversity: The Fish Resources of Western Indonesia*. WorldFish.
- Polanco Fernández, A., V. Marques, F. Fopp, et al. 2021. "Comparing Environmental DNA Metabarcoding and Underwater Visual Census to Monitor Tropical Reef Fishes." *Environmental DNA* 3, no. 1: 142–156. <https://doi.org/10.1002/edn3.140>.
- Sani, L. M. I., A. K. Husna, B. Subhan, and H. Madduppa. 2021. "Environmental DNA (eDNA) Reveals Endangered Narrow Sawfish Across Indonesian Reefs." *IOP Conference Series: Earth and Environmental Science* 944, no. 1: e012020. <https://doi.org/10.1088/1755-1315/944/1/012020>.
- Santana, E. F. C., M. Mies, G. O. Longo, et al. 2023. "Turbidity Shapes Shallow Southwestern Atlantic Benthic Reef Communities." *Marine Environmental Research* 183: 105807. <https://doi.org/10.1016/j.marenvres.2022.105807>.
- Seutin, G., B. N. White, and P. T. Boag. 1991. "Preservation of Avian Blood and Tissue Samples for DNA Analyses." *Canadian Journal of Zoology* 69, no. 1: 82–90. <https://doi.org/10.1139/z91-013>.
- Srivathsan, A., L. Lee, K. Katoh, et al. 2021. "ONTbarcoder and MinION Barcodes Aid Biodiversity Discovery and Identification by Everyone, for Everyone." *BMC Biology* 19, no. 1: 217. <https://doi.org/10.1186/s12915-021-01141-x>.
- Taberlet, P., E. Coissac, F. Pompanon, C. Brochmann, and E. Willerslev. 2012. "Towards Next-Generation Biodiversity Assessment Using DNA Metabarcoding." *Molecular Ecology* 21, no. 8: 2045–2050. <https://doi.org/10.1111/J.1365-294X.2012.05470.X>.
- Takahara, T., T. Minamoto, and H. Doi. 2015. "Effects of Sample Processing on the Detection Rate of Environmental DNA From the Common Carp (*Cyprinus Carpio*)." *Biological Conservation* 183: 64–69. <https://doi.org/10.1016/j.biocon.2014.11.014>.
- Tiggeloven, T., H. de Moel, V. T. van Zelst, et al. 2022. "The Benefits of Coastal Adaptation Through Conservation of Foreshore Vegetation." *Journal of Flood Risk Management* 15, no. 3: e12790. <https://doi.org/10.1111/jfr3.12790>.
- Toledo, M.-I., P. Torres, C. Díaz, V. Zamora, J. López, and G. Olivares. 2020. "Ecological Succession of Benthic Organisms on Niche-Type Artificial Reefs." *Ecological Processes* 9, no. 1: 38. <https://doi.org/10.1186/s13717-020-00242-9>.
- Valbo-Jørgensen, J., and A. F. Poulsen. 2000. "Using Local Knowledge as a Research Tool in the Study of River Fish Biology: Experiences From the Mekong." *Environment, Development and Sustainability* 2, no. 3: 253–376. <https://doi.org/10.1023/A:1011418225338>.
- van Bijsterveldt, C. E., B. K. van Wesenbeeck, D. van der Wal, et al. 2020. "How to Restore Mangroves for Greenbelt Creation Along Eroding Coasts With Abandoned Aquaculture Ponds." *Estuarine, Coastal and Shelf Science* 235: 106576. <https://doi.org/10.1016/j.ecss.2019.106576>.
- Van Der Loos, L. M., and R. Nijland. 2020. "Biases in Bulk: DNA Metabarcoding of Marine Communities and the Methodology Involved." *Molecular Ecology* 30, no. 13: 3270–3288. <https://doi.org/10.1111/mec.15592>.

- van der Reis, A. L., L. E. Beckley, M. P. Olivar, and A. G. Jeffs. 2023. "Nanopore Short-Read Sequencing: A Quick, Cost-Effective and Accurate Method for DNA Metabarcoding." *Environmental DNA* 5, no. 2: 282–296. <https://doi.org/10.1002/edn3.374>.
- Vaser, R., I. Sović, N. Nagarajan, and M. Šikić. 2017. "Fast and Accurate De Novo Genome Assembly From Long Uncorrected Reads." *Genome Research* 27, no. 5: 737–746. <https://doi.org/10.1101/gr.214270.116>.
- Wee, A. K., S. G. Salmo Iii, K. Sivakumar, et al. 2023. "Prospects and Challenges of Environmental DNA (eDNA) Metabarcoding in Mangrove Restoration in Southeast Asia." *Frontiers in Marine Science* 10: 1033258. <https://doi.org/10.3389/fmars.2023.1033258>.
- Wickham, H. 2010. "A Layered Grammar of Graphics." *Journal of Computational and Graphical Statistics* 19, no. 1: 3–28. <https://doi.org/10.1198/jcgs.2009.07098>.
- Williams, K. E., K. P. Huyvaert, and A. J. Piaggio. 2016. "No Filters, no Fridges: A Method for Preservation of Water Samples for eDNA Analysis." *BMC Research Notes* 9, no. 1: 1–5. <https://doi.org/10.1186/S13104-016-2104-5/FIGURES/2>.
- Winterwerp, J. C., T. Albers, E. J. Anthony, et al. 2020. "Managing Erosion of Mangrove-Mud Coasts With Permeable Dams—Lessons Learned." *Ecological Engineering* 158: 106078. <https://doi.org/10.1016/j.ecoleng.2020.106078>.
- Worthington, T., and M. Spalding. 2018. *Mangrove Restoration Potential. A Global Map Highlighting a Critical Opportunity*, 1–36. Apollo—University of Cambridge Repository.
- Xie, X., Z. Wu, C.-C. Wang, et al. 2020. "Nursery Habitat for Asian Horseshoe Crabs Along the Northern Beibu Gulf, China: Implications for Conservation Management Under Baseline Gaps." *Aquatic Conservation: Marine and Freshwater Ecosystems* 30, no. 2: 260–272. <https://doi.org/10.1002/aqc.3259>.
- Yadav, V. S., S. S. Yadav, S. R. Gupta, et al. 2022. "Carbon Sequestration Potential and CO₂ Fluxes in a Tropical Forest Ecosystem." *Ecological Engineering* 176: 106541. <https://doi.org/10.1016/j.ecoleng.2022.106541>.
- Yamanaka, H., T. Minamoto, J. Matsuura, et al. 2017. "A Simple Method for Preserving Environmental DNA in Water Samples at Ambient Temperature by Addition of Cationic Surfactant." *Limnology* 18, no. 2: 233–241. <https://doi.org/10.1007/S10201-016-0508-5/FIGURES/4>.
- Yamanaka, H., H. Motozawa, S. Tsuji, R. C. Miyazawa, T. Takahara, and T. Minamoto. 2016. "On-Site Filtration of Water Samples for Environmental DNA Analysis to Avoid DNA Degradation During Transportation." *Ecological Research* 31, no. 6: 963–967. <https://doi.org/10.1007/s11284-016-1400-9>.
- Zainal Abidin, D. H., S. A. Mohd. Nor, S. Lavoué, M. A. Rahim, and N. A. Mohammed Akib. 2022. "Assessing a Megadiverse but Poorly Known Community of Fishes in a Tropical Mangrove Estuary Through Environmental DNA (eDNA) Metabarcoding." *Scientific Reports* 12, no. 1: 16346. <https://doi.org/10.1038/s41598-022-19954-3>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** edn370201-sup-0001-FigureS1.pdf. **Figure S2:** edn370201-sup-0002-FigureS2.pdf. **Figure S3:** edn370201-sup-0003-FigureS3.pdf. **Table S1:** edn370201-sup-0004-TableS1.xlsx. **Table S2:** edn370201-sup-0005-TableS2.xlsx.