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Plankton diversity around Sempu Strait, Indonesia, revealed by eDNA metabarcoding

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Abstract. *Aliviyanti D, Amalia SHN, Dailami M, Prihanto AA, Sari SHJ, Djamaludin H, Wiratno EN. 2025. Plankton diversity around Sempu Strait, Indonesia, revealed by eDNA metabarcoding. Biodiversitas 26: 5848-5856.* Plankton diversity plays a critical role in assessing marine ecosystem health and productivity. Environmental DNA (eDNA) metabarcoding offers a highly informative tool for detecting plankton biodiversity more comprehensively compared to conventional methods. This study assessed plankton diversity and water quality in the Sempu Strait, Indonesia, by analyzing eDNA samples collected from five stations during a single sampling period in August 2024. Environmental parameters (temperature, salinity, pH, dissolved oxygen, nitrate, and phosphate) were measured and found within typical ranges, except for nitrate and phosphate, which exceeded marine quality thresholds. Water samples were filtered and amplified using the 18S V9 region, and sequences were processed using DADA2 and assigned taxonomically against the PR2 database. A total of 361 species across 341 genera were identified from 2.8 million raw reads. The Shannon-Wiener index (H') diversity indices range from 4.61 to 6.43, while Simpson's index (D) ranges from 0.964-0.997, indicating overall high alpha diversity. The most dominant phytoplankton genera were *Leptocylindrus* and *Chaetoceros*, while *Neocalanus* dominated the zooplankton community. Stations near anthropogenic activity showed distinct taxonomic profiles and higher nutrient concentrations. These findings confirm that eDNA metabarcoding is an effective and sensitive approach for monitoring marine plankton communities. This method enhances biodiversity detection and provides valuable reference for future ecological assessments and conservation planning in Indonesian coastal ecosystems.

Keywords: 18S rRNA V9, eDNA metabarcoding, marine biodiversity monitoring, plankton community, tropical coastal waters

INTRODUCTION

Plankton are microscopic organisms that drift within the water column (Ibarbalz et al. 2019). In addition, phytoplankton utilize sunlight as a food source through the process of photosynthesis (Isles et al. 2021). The presence of plankton also indicates primary productivity in waters, so that the presence of phytoplankton greatly affects the lives of other biota in the surrounding area (Bintoro et al. 2023). Plankton communities are fundamental components of marine ecosystems, playing critical roles in food webs, biogeochemical cycles, and as bioindicators of environmental change (Wang et al. 2019). Understanding plankton diversity is essential for monitoring ecosystem health and managing coastal resources effectively.

Monitoring activities of plankton diversity and abundance are generally carried out conventionally through morphological observations using microscopes. However, microscopic identification of plankton often results in subjective interpretations by researchers because this method requires special expertise and relies on extensive taxonomic literature. Currently, the microscopic plankton observation method is considered a conventional method because it can cause variations in the identification process, which can affect the accuracy and consistency of the overall identification results (Gao et al. 2018; Du et al. 2024; Gelis et al. 2024).

Plankton identification is an important key in understanding the dynamics of aquatic ecosystems. However, traditional plankton identification methods often require more time and effort. The use of molecular identification methods, such as environmental DNA metabarcoding, can be an efficient and accurate alternative to overcome the limitations of microscope-based plankton identification (Wang et al. 2019; He et al. 2023). Recent advances in molecular techniques, particularly environmental DNA (eDNA) metabarcoding, have enabled more comprehensive and non-invasive assessments of biodiversity. The 18S rRNA gene, especially the V9 hypervariable region, is commonly used for detecting a wide range of plankton taxa through high-throughput sequencing (HTS) (Ji et al. 2021). Based on this process, it can describe the taxonomic composition of species, as well as provide an economical approach in biodiversity surveys and open up opportunities to overcome the limitations associated with bioassessment that relies on morpho-taxonomy (Yang and Zhang 2020). Studies across tropical and temperate regions have demonstrated the effectiveness of eDNA in revealing patterns of plankton distribution and detecting species that are otherwise overlooked in morphological surveys.

Recent studies highlight the importance of genetic tools, such as DNA barcoding and metabarcoding, for both biodiversity and conservation efforts. For example, Dewi et al. (2025) demonstrated the utility of COX1-based barcoding in assessing stranded dugongs (*Dugong dugon*) in the Java Sea, linking conservation status with habitat conditions such as seagrass meadows. Similarly, Kuncoro et al. (2023) emphasized how molecular approaches improve coral monitoring without destroying habitats and organisms, underscoring its relevance for conservation and management. A previous study of eDNA metabarcoding was conducted at Pondok Dadap fish landing station. This study successfully identified 34 fish species through COI and ITS barcoding, including 13 new ITS entries in GenBank, and revealed 53 species from eDNA metabarcoding around Pondok Dadap, Malang (Andriyono et al. 2019). The findings demonstrate that eDNA metabarcoding provides a more comprehensive and efficient approach than traditional methods, offering valuable baseline data for sustainable fisheries management.

In Indonesia, which hosts one of the world's most diverse marine environments, applications of eDNA-based biodiversity monitoring are still emerging. The Sempu Strait, located in southern East Java, Indonesia, exhibits a unique regional typology, as it is bordered by Sempu Island, a protected nature reserve, and adjacent a Sendang Biru Beach, which hosts a port area characterized by intensive human activity. Sempu Island is an uninhabited island that supports a fairly high diversity of flora and fauna (Bintoro et al. 2023). Also, the existence of Sempu Island is very important in the Southern Java Region, especially in the South Malang area, because of its role as a wave barrier that directly borders the Indian Ocean and is also the Pondokdadap port area. Despite its ecological importance, the plankton diversity in this area remains poorly characterized, especially at the molecular level.

The purposes of this study are to evaluate plankton diversity and community composition in the Sempu Strait, Indonesia, using eDNA metabarcoding, and to examine the relationship between plankton distribution and key environmental parameters.

MATERIALS AND METHODS

Study area

The research location was determined using an area sampling method by selecting the area of research based on the intensity of human activity around the Sempu Strait, Malang, Indonesia (Table 1). Plankton sampling was carried out along the Sempu Strait in August 2024 (early transition period) at five stations (3 stations were in areas with high human intensity, while the other 2 stations were in the mouth of the strait, where ships pass to the port) with station coordinates recorded using a GPS device (Table 1). The following is a map of plankton sampling and water quality (Figure 1). All measurements were performed in duplicate for each station at depths of 0 and 9 m.

Table 1. Sampling location coordinates of Sempu Strait, Malang, East Java, Indonesia

Stations	Coordinates
1	112°40’94.38"E - 8°25’80.58"S
2	112°40’47.35"E - 8°25’81.67"S
3	112°41’73.89"E - 8°26’29.71"S
4	112°41’86.05"E - 8°26’00.34"S
5	112°41’68.79"E - 8°27’52.23"S

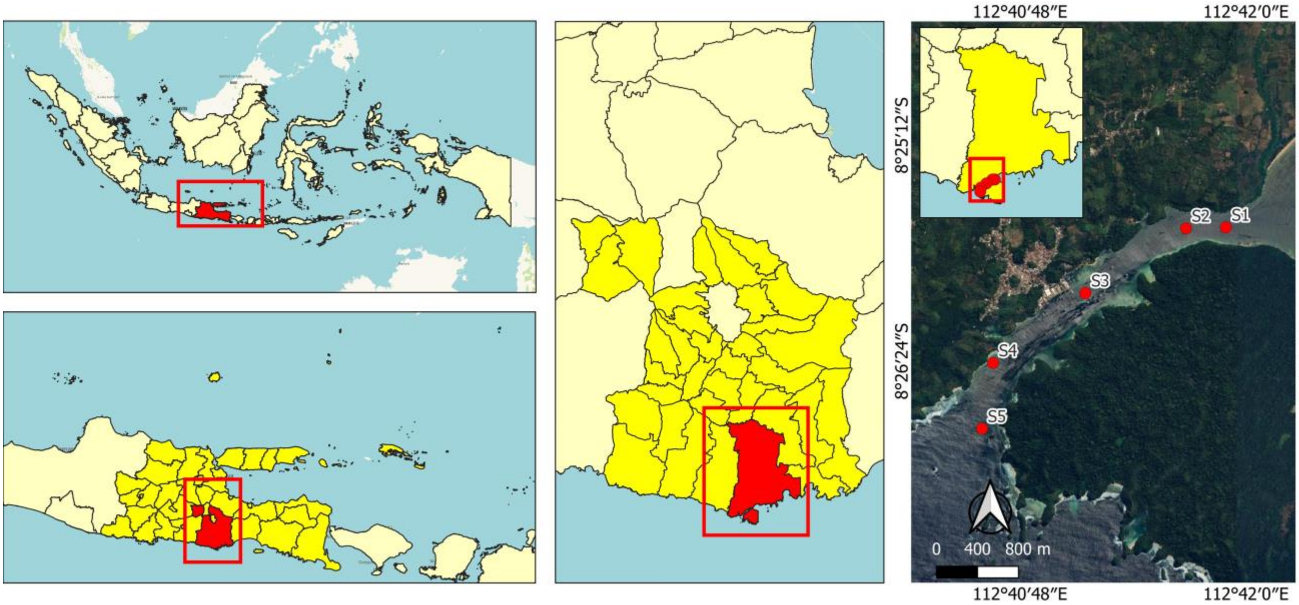

Figure 1. eDNA sampling locations in Sempu Strait, Malang, East Java, Indonesia

Procedures

Measurement of the physicochemical parameter

Physicochemical parameters were measured in situ at each sampling station to assess water quality conditions that may influence plankton distribution. Water sampling for physicochemical analysis was collected in a composite manner at two depths (surface, 0 m; and near-bottom, 9 m) using a water sampler with a total volume of 4.4 L (Gopas 2.2 L; Indonesia). In-situ parameter measurements, including water temperature, salinity, pH, and dissolved oxygen, were carried out directly at the sampling location using a multi-parameter water quality instrument AAQ Rinko (Advance Aquatic Quantifier, Japan).

For nutrient analysis, 500 mL of surface and deep water were collected in polyethylene bottles and kept in a coolbox with an icepack to maintain the temperature at about 13°C until arrive in the laboratory for further analysis. This temperature helps minimize biological activity and chemical changes prior to laboratory analysis. Nitrate (NO_3^-) and phosphate (PO_4^{3-}) concentrations were determined using spectrophotometric methods with a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) following APHA (2017) standard procedures at the Jasa Tirta Laboratory, Malang, Indonesia.

Sampling plankton

Sampling plankton was conducted at five stations, with sampling times at approximately 07:00-10:00 AM. Samples were collected in a composite manner (Blomqvist 2001) at depths of 0 and 9 m for repetition. Water samples were taken using a water sampler as much as 2.2 L per depth, then filtered using a 0.45 µm Pall Corporation sterilized filter paper (47 mm diameter - Pall, US) using a vacuum pump system. The filtration process was interrupted if the flow stopped due to clogging of the filter. The filter was then stored in a cryogenic tube containing DNA/RNA Shield (Zymo Research, USA) to maintain DNA stability and stored in a liquid nitrogen dewar until DNA extraction. To minimize contamination during fieldwork, all equipment that is used is already pre-sterilized by soaking in a 5% sodium hypochlorite solution (Bayclin®) and rinsed three times with aquades (Georges et al. 2021). During the filtration process, the same protocol was applied to sterilize all equipment used between samples and sampling sites.

Extraction, amplification, and sequencing of DNA

DNA extraction was carried out using the Quick-DNA Magbead Plus Kit (Zymo Research, USA) based on the manufacturer's protocol without any additional pre-treatment steps prior to extraction. Plankton DNA amplification targeting the 18S gene using plankton-specific primer pairs, 1380F 5′-`CCCTGCCHTTTGTACACAC`-3′and 1510R 5′-`CCTTCYGCAGGTTCACCTAC`-3′ (Amaral-Zettler et al. 2009; Stefanni et al. 2018). The 20 µL PCR reaction consisted of 10 µL 2x Phusion™ Plus PCR Master Mix (Provides 1.7 mM $MgCl_2$ at 1X concentration), 0.5 µL forward (0.5 µM) and 0.5 µL reverse (0.5 µM) primers, and 1 µL of DNA extract, 4 µL of 5X Phusion™ GC enhancer, and 4 µL of nuclease-free water. The PCR condition: 1 min at 95°C, followed by 35 cycles of denaturation (95°C for 15s), annealing (46°C for 15s), extension (72°C for 10s), and final extension at 72°C for 3 min (Georges et al. 2021). The PCR products were visualized by gel electrophoresis using agarose gel (1% in TBE buffer), followed by visualization using a UV transilluminator (Dailami et al. 2021). The DNA amplicon was then used for library preparation for DNA sequencing by using Next Generation Sequencing (NGS) technology (Yamindago et al. 2020; Cheng et al. 2023), based on the Illumina platform. Illumina universal adaptor was used in library preparation, and the sequencing was conducted on an Illumina MiSeq using the MiSeq reagent kit V3 600 cycle. All library preparation and sequencing were conducted at the PT Genetika Science Indonesia, Jakarta.

Data analysis

The raw sequencing data were processed using a standardized bioinformatics pipeline to ensure high-quality and reproducible results. Initial quality control was conducted using FastQC to assess read quality. Raw sequence data from the Illumina platform were used for metabarcoding analysis, first by removing adapters and PCR primers from the sequence using Cutadapt V.5.0 (Martin 2011). Then filtered by quality ≥Q30 and removing potential sequence errors and chimera using the DADA2 V.4.4.1 (Callahan et al. 2016). The filtered sequence data, replicated and unique sequence as Amplicon Sequence Variants (ASV), were used for taxonomic analysis by assigning to the PR2 (Protist Ribosomal Reference) database version 4.7.2 (18S rRNA) without applying a minimum identity or coverage threshold to avoid excluding rare taxa although this may increase classification uncertainty; all available sequence data were included in the analysis. Further analysis and visualization were conducted using R v4.4.2 in R Studio (R Core Team 2024), utilizing packages such as Vegan and Ggplot2. Additional visualizations were performed using Krona Tools (https://github.com/marbl/Krona).

RESULTS AND DISCUSSION

Measurement of chemical-physical parameters of the waters

Based on the research conducted in Sempu Strait, there have been obtained on physicochemical parameters covering six environmental parameters, such as temperature, salinity, DO, pH, nitrate, and phosphate (Table 2) have been obtained. The data was observed to describe environmental conditions at the time of the study. Based on Government Regulation No. 22 of 2021, it shows that the parameters of temperature, salinity, DO, and pH are still in accordance with water quality standards, but the dissolved nitrate and phosphate values exceed the threshold of the set quality standards.

Table 2. Result of measuring the physical-chemical parameters of Sempu Strait, Indonesia

Parameter	Stations					Average	Quality standard*
	1	2	3	4	5		
Temperature (°C)	23.90	23.17	22.38	22.69	23.17	23.06±0.58	Natural
Salinity (‰)	34.27	34.33	34.38	34.52	34.48	34.40±0.10	33-34
DO (mg/L)	6.90	6.98	7.06	7.04	6.98	6.99±0.06	>5
pH	8.57	8.53	8.52	8.49	8.50	8.52±0.03	7-8.5
Nitrate (mg/L)	**2.156**	**2.268**	**2.750**	**2.997**	**2.865**	2.61±0.3733	0.06
Phosphate (mg/L)	**0.123**	**0.519**	**0.169**	**0.127**	**0.201**	0.23±0.1662	0.015

Note: *Government regulation of Indonesia 2021. Bold indicates the data exceeding the quality standards

The water temperature ranges from 22.38°C to 23.90°C, with an average of 23.06±0.58. The measured salinity also shows stability with values ranging from 34.27 to 34.52‰ with an average of 34.40±0.10. In addition, DO levels at the research location are in the range of 6.90 to 7.06 mg/L with an average of 6.99±0.06, which is still within the optimal limit (>5 mg/L) to support marine life. Meanwhile, the pH parameter shows stability with values ranging from 8.49 to 8.57 with an average of 8.52±0.03, which is still within the safe range (7 to 8.5) for marine ecosystems. The results of the analysis of nutrient content (nitrate and phosphate) showed values that exceeded the water quality standard threshold (PP No. 22 of 2021), with phosphate concentrations ranged from 0.1226-0.5194 mg/L with an average of 0.23±0.1662 mg/L, while nitrate levels also showed a similar pattern with a range of values between 2.156 to 2.997 mg/L and an average of 2.61±0.3733 mg/L which exceeded the water quality standard threshold (0.06 mg/L).

The eDNA metabarcoding

Plankton 18S gene amplification in Sempu Strait was conducted using the 18S V9 primer, followed by visualization on a 1% TBE agarose gel electrophoresis (Figure 2). Bands within the expected 96-134 bp range confirmed successful amplification at all sampling sites, and the samples were subsequently prepared for sequencing. The absence of bands in the negative control (NTC) indicates that no contamination occurred during the PCR process. The consistency and clarity of the bands across all five samples (N850-1 to N850-5) further support the integrity and quality of the amplified DNA, as indicated by the "PASS QC" status in all cases.

Following successful amplification, the samples underwent Next-Generation Sequencing (NGS) to generate high-throughput data. Each station yielded over 100,000 raw reads, indicating a high initial sequencing depth. After quality filtering and denoising (both forward and reverse reads), most of the reads remained, demonstrating minimal data loss during preprocessing. The number of merged reads ranged from 84,895 to 90,052, showing effective read pairing. Final non-chimeric reads, used for downstream analysis, ranged from 67,871 to 77,676, confirming the successful removal of chimeric sequences and ensuring reliable data for taxonomic classification. These results are summarized in Table 3, which presents the sequencing quality and read processing statistics for all five sampling stations.

Discussion

Condition of Sempu Strait

The Sempu Strait is used as an entry and exit route for fishing boats, both from the west and east sides. Based on the results of measurements of physicochemical parameters, the general condition of the waters is similar across sampling sites and still within the range that complies with the seawater quality standards according to Government Regulation No. 22 of 2021. The salinity, DO, and pH parameters are still in accordance with the seawater quality standards. However, the results of monitoring nitrate and phosphate levels showed values higher than the established quality standards, such as 0.06 mg/L for nitrate and 0.015 mg/L for phosphate (Figure 3).

Human activities, such as sewage discharge and agricultural runoff, have disrupted the natural balance of nitrogen and phosphorus in aquatic ecosystems (Oduor et al. 2023). The highest phosphate value was at station 2 (0.519 mg/L), and the highest nitrate value was at station 4 (2.997 mg/L). The three observation points of the sampling location (points 2, 3, and 4) are areas with high human activity, which are the port area and the houseboat. Nitrate and phosphate are essential nutrients that play a crucial role in the growth and development of living organisms, particularly phytoplankton (Aliviyanti et al. 2017; Nindarwi et al. 2021), so changes in their concentration can affect primary productivity and the balance of aquatic ecosystems. Excessive content, which can potentially cause eutrophication when algae growth increases, can disrupt ecosystem stability (Devlin and Brodie 2023).

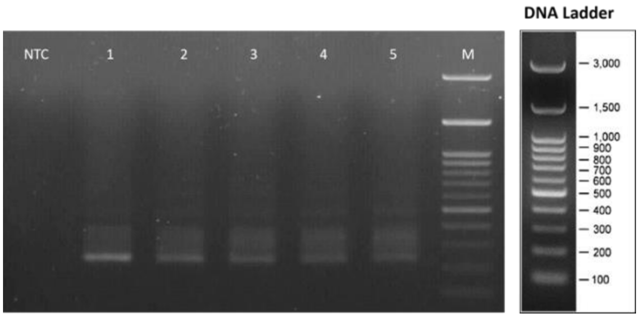

Figure 2. Result of QC by agarose gel electrophoresis of samples from Sempu Strait, Indonesia

Table 3. Sequencing data per station

Sample	Raw reads	Filtered	Denoised-F	Denoised-R	Merged	Nonchim
Station 1	>100,000	95,015	93,557	93,919	90,052	77,676
Station 2	>100,000	94,432	92,824	93,081	87,904	67,897
Station 3	>100,000	94,229	91,892	92,361	85,016	69,422
Station 4	>100,000	94,291	91,868	92,353	85,169	68,408
Station 5	>100,000	94,311	91,631	92,420	84,895	67,871

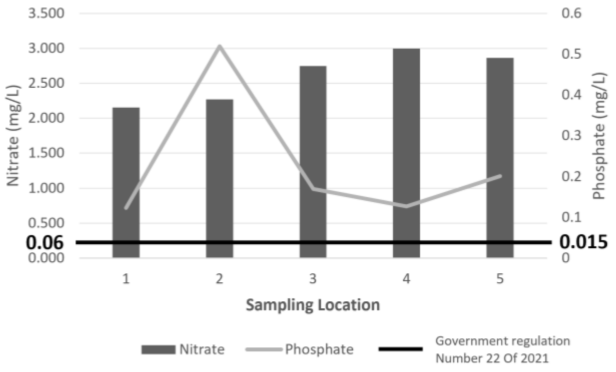

Figure 3. Value of nitrate and phosphate within the sampling location

eDNA metabarcoding analysis

After sequencing and quality filtering, each sampling station yielded between approximately 94,000 and 95,000 high-quality sequence reads (Table 4). Species-level assignments represent PR2 similarity-based classification of ASVs. From these, the number of unique ASVs detected ranged from about 67,000 to 77,000 per station (Table 5). These ASVs were subsequently taxonomically classified, resulting in the identification of 19 phyla, 84 classes, 136 orders, 233 families, 341 genera, and 361 species of plankton, as detailed in Table 4.

The Venn diagram shows the distribution of ASV at each sampling point, with the same or unique number of ASV at each location (Figure 4). The dominance of ASV at each location indicates that the plankton community has a similar structure, although some ASVs were found only at one point. The results of the Venn diagram show that there are 283 (11%) species that are the same at each sampling location. And at each location, there are specific types that are only found at that location, namely at points 1 to 5 are 6; 8; 16; 16; and 17%. The differences at each sampling point may not necessarily correspond to ecological or taxonomic differences due to methodological factors (Bokulich et al. 2013; Callahan et al. 2016). Therefore, further interpretation in this study focuses on species-level trends to support biologically meaningful conclusions. Thus, Venn diagram interpretation should be considered structurally for ASVs only.

Relative abundance of plankton

The results of the relative abundance analysis of 10 main genera at five sampling points showed varying values between each genus in each sample (Figure 5). The eDNA metabarcoding analysis revealed a diverse planktonic community consisting of 361 species across 341 genera from 19 phyla (Table 5). While overall taxonomic composition was dominated by the phyla Bacillariophyta (diatoms) and Arthropoda (copepods), further genus-level resolution uncovered ecological patterns not visible at higher taxonomic levels. The ten most dominant plankton genera identified across all stations included *Chaetoceros*, *Delibus*, *Leptocylindrus*, *Minidiscus*, *Neocalanus*, *Pseudo-nitzschia*, *Paracalanus*, *Prorocentrum*, *Protoperidinium*, and *Thalassiosira*.

Analysis of the plankton community in the Sempu Strait based on relative abundance shows that the plankton composition is dominated by Copepod groups, especially *Neocalanus* and *Paracalanus*, and several diatom genera such as *Leptocylindrus* and *Thalassiosira*, with varying distribution patterns between stations. Diatom is known for their key roles in primary production and carbon cycling in coastal waters (Devlin and Brodie 2023). These genera often dominate during nutrient-rich conditions, as reflected in the high nitrate and phosphate concentrations at stations 2-4 (Retnaningdyah et al. 2025). The proliferation of *Leptocylindrus* and *Chaetoceros* in particular suggests a response to eutrophication, consistent with observations in other tropical estuaries (Akinnawo 2023).

Among the zooplankton, copepods such as *Neocalanus* and *Paracalanus* were most abundant. At station 1, *Neocalanus* is very dominant with a proportion of 0.70, indicating a strong community structure dominated by zooplankton. In contrast, at station 2, the proportion decreased drastically to 0.15 with a significant increase in *Paracalanus* (0.25) and *Delibus* (0.10), indicating a more balanced and diverse community structure. The high zooplankton group is crucial as primary grazers in the pelagic food web and serves as prey for larval fish and other higher trophic levels (Ibarbalz et al. 2019; Chen et al. 2022). Notably, *Neocalanus* was highly dominant at station 1, where lower diversity indices suggest a community under environmental stress, potentially leading to reduced diversity.

In station 3, *Neocalanus* is still dominant (0.42) but followed by *Paracalanus* and *Leptocylindrus* with proportions of 0.12 and 0.09, respectively, indicating strong interactions between zooplankton and phytoplankton. In station 4, the dominance of *Neocalanus* weakens (0.08), and diatoms such as *Leptocylindrus* (0.16) and *Thalassiosira* (0.15) become more prominent. That is likely triggered by increased nutrient availability in this area. Meanwhile, station 5 showed diversity of plankton community with *Leptocylindrus* (0.18) as the most abundant genus, followed by *Thalassiosira* (0.12) and *Neocalanus* (0.13). This pattern indicates that the T5 waters have relatively balanced environmental conditions between the availability of resources for phytoplankton and zooplankton. The differences at each sampling point can be influenced by hydro-oceanographic factors such as currents around the location or the time of sampling (Hwang et al. 2007). Water current patterns can affect the spread of nutrients and plankton distribution, which can have an impact on the level of primary productivity of the waters (Nindarwi et al. 2021; Chen et al. 2022).

Community structure of planktons

Analysis of plankton diversity in Sempu Strait showed significant variation among sampling locations (Table 6). Based on observed ASV, station 5 had the highest richness (1188 ASV), followed by station 4 (1147 ASV) and station 3 (1144 ASV), while station 1 had the lowest richness (643 ASV).

The alpha diversity indices—Shannon (H'), Simpson (D), and Inverse Simpson (1/D)—showed distinct spatial patterns across the five sampling stations in the Sempu Strait (Table 6). Station 5, located in the western part of the strait with minimal anthropogenic activity, exhibited the highest diversity values (H'=6.43; D=0.99; 1/D=337.68). In contrast, station 1, situated near the eastern entrance and closer to active human activities such as port operations and floating houses, recorded the lowest diversity indices (H'=4.61; D=0.96; 1/D=28.08). These gradients suggest that community evenness and richness are highest in less-disturbed areas, consistent with previous studies indicating that environmental pressures, such as nutrient loading and physical disturbance, reduce community complexity (Evita et al. 2021; Devlin and Brodie 2023). The Simpson index at station 1 also reflects this trend, with a stronger dominance of a few taxa—particularly *Neocalanus*—reducing the effective number of species contributing to overall diversity.

Shannon index values above 4.5 in all stations indicate generally high diversity, which is expected in tropical marine systems with complex hydrodynamics (Akinnawo 2023). However, the contrast between station 1 and station 5 (ΔH'=1.82) is ecologically significant, representing changes in richness and relative abundance structure that may be influenced by localized nutrient inflows and water circulation patterns (Oduor et al. 2023). These results support the hypothesis that plankton communities in the Sempu Strait are structured by spatially variable environmental conditions, with diversity declining in response to anthropogenic stressors.

Table 4. ASV Distribution across sampling stations

Sampling station	Number of ASVs
Station 1	77,676
Station 2	67,897
Station 3	69,422
Station 4	68,408
Station 5	67,871

Table 5. Sum of identified plankton taxonomy on-site

Taxonomy level	Total identified	Stations				
		1	2	3	4	5
Phylum	19	15	12	12	15	15
Class	84	61	56	54	54	53
Order	136	87	85	108	106	114
Family	233	118	128	170	169	188
Genus	341	139	167	142	151	138
Species	361	144	178	237	236	246

Table 6. Microbiome alpha diversity metrics from five sampling stations

Obs. point	Observed (ASV)	Shannon	Simpson	InvSimpson
1	643	4.605	0.964	28.084
2	800	5.606	0.991	115.772
3	1144	6.172	0.994	167.608
4	1147	6.371	0.997	315.619
5	1188	6.426	0.997	337.683

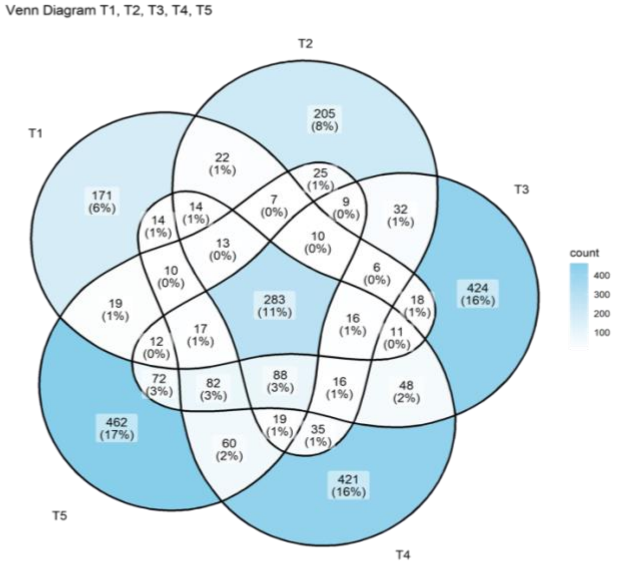

Figure 4. Venn diagram of distribution on plankton species data collection at the ASV level

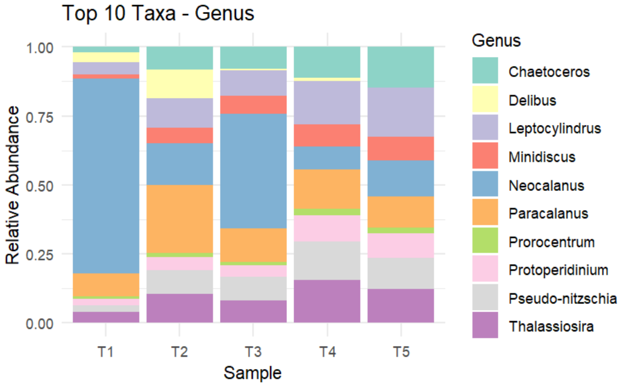

Figure 5. Distribution relative abundance of the top 10 genera

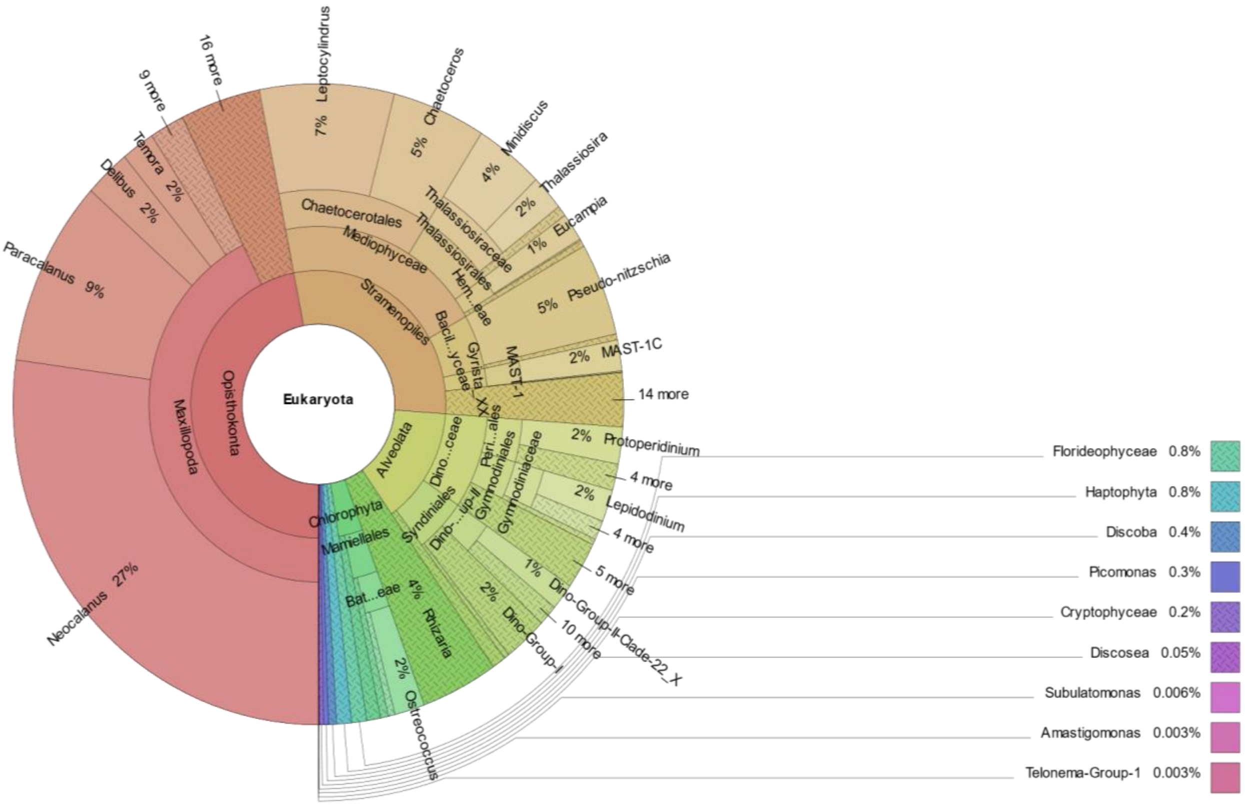

Figure 6. Plankton composition within the sampling location

Compared to previous morphology-based studies in Indonesia that typically identified fewer than 50 plankton species per location (Astriana and Larasati 2021), the eDNA metabarcoding approach applied in this study identified 361 species across 341 genera, reflecting a substantial improvement in taxonomic resolution. This advancement is further supported by diversity indices, where previous research in Demak, Central Java, Indonesia, using morphological identification reported Shannon-Wiener Index (H') values ranging from 1.45 to 2.45 (Evita et al. 2021), while our eDNA-based study revealed higher H' values ranging from 4.60 to 6.43. Additionally, in other tropical waters such as Jakarta Bay (1.2-2.0) and Makassar Strait (1.3-2.57), reported H' values are generally lower, underscoring the higher biodiversity status in the Sempu Strait as detected through eDNA.

In addition to measuring alpha diversity, analysis was also conducted to see the taxonomic distribution of plankton from domain to species level at each sampling location (Figure 6). The plankton community structure revealed in this study highlights both spatial variability and ecological specialization within the Sempu Strait. The analysis showed that the Opisthokonta group, especially the Maxillopoda class, dominated the plankton community with the highest proportion reaching 47%. *Neocalanus* genus is the most abundant Copepod species with a proportion of 27%. In addition, the Stramenopiles group, especially the Bacillariophyta phylum (Diatoms), also has a high abundance. *Leptocylindrus* and *Chaetoceros* genera are the dominant phytoplankton with proportions of 7% and 5% respectively. The Alveolata group, especially Dinophyceae (Dinoflagellates), was also identified with lower abundance. *Protoperidinium* genus was found with a proportion of around 2% of the total plankton detected.

The dominance of Diatoms such as *Leptocylindrus*, *Chaetoceros*, and *Skeletonema* at stations 2, 3, and 4 coincides with elevated nitrate and phosphate concentrations, suggesting eutrophication-driven Diatom proliferation (Retnaningdyah et al. 2025). These taxa are well-documented as fast-growing r-strategists that respond rapidly to nutrient pulses, playing a central role in primary production and carbon export in coastal food webs (Devlin and Brodie 2023). *Leptocylindrus*, in particular, has been recognized as a bioindicator of high-nutrient and mesotrophic conditions, especially in semi-enclosed tropical systems. Diatom *Leptocylindrus* is one of six important diatom genera that form a stable relationship with marine epiphytic bacteria. These epiphytic bacteria play a role in the remineralization of organic matter by degrading dead organisms or body parts and producing inorganic elements such as carbon, nitrogen, and phosphorus. The success of this interaction shows the important role of *Leptocylindrus* in the marine ecosystem, which works together with bacteria to support the biogeochemical cycle in marine waters (Younas 2024).

The abundance of copepods, especially the genus *Neocalanus*, shows that zooplanktons have an important role in the aquatic ecosystem of the Sempu Strait. Copepods are a group of small crustaceans that are widely distributed in various aquatic environments. In aquatic ecosystems, copepods can be found in both pelagic and benthic habitats. In the pelagic environment, they are the most dominant zooplankton in terms of number and act as the main link in the food chain (Zamora-Terol et al. 2020). In addition, copepods have an important role in the biogeochemical cycle, especially in the global carbon cycle. Their daily vertical migration activities contribute to the transfer of carbon to the deep sea. In the benthic environment, copepods are included in the main group of meiofauna that live in sediments (Kwok et al. 2015). Although *Neocalanus* appeared highly abundant at station 1, this pattern should be interpreted with caution. *Neocalanus* is a calanoid copepod typically associated with high-latitude or deep-water upwelling zones, but its presence in tropical coastal waters may reflect hydrodynamic mixing or advection from deeper layers (Yang and Zhang 2020; Hou et al. 2022). Its detection could also result from eDNA transport from deeper layers, or amplification bias rather than true ecological dominance. Therefore, its high relative abundance does not necessarily indicate environmental stress or reduced community resilience.

The patterns observed in Sempu Strait are consistent with those found in other tropical ecosystems. For example, in Jakarta Bay, Indonesia, nutrient enrichment led to recurrent blooms of *Skeletonema* and *Thalassiosira*, while copepod communities became less diverse (Susanti et al. 2022). Similar trends have been reported in Vietnam's coastal estuaries, where diatom-dominated regimes emerge under high anthropogenic nutrient loads (Nguyen et al. 2022). Compared to these systems, Sempu Strait still retains relatively high overall diversity, particularly at station 5 (H'=6.43), suggesting that certain areas remain ecologically intact and should be prioritized for conservation.

Overall, these results indicate that the plankton community at the research site is dominated by zooplankton from the Opisthokonta (Maxillopoda) group and phytoplankton from the Stramenopiles (Bacillariophyta/Diatom) group. The presence of other groups, such as Dinoflagellates, also indicates variations in the plankton community that can affect the dynamics of aquatic ecosystems. These findings emphasize the functional implications of taxon-level shifts, where changes in plankton composition influence nutrient cycling, trophic dynamics, and carbon fluxes. The eDNA-based identification of both autotrophic (phytoplankton) and heterotrophic (zooplankton) indicators enables a more nuanced understanding of ecosystem status and pressures. Such insights are crucial for supporting long-term monitoring, early detection of ecological shifts, and the formulation of evidence-based management policies in Indonesian coastal waters.

In conclusion, eDNA metabarcoding is a highly informative tool for assessing marine plankton diversity with high accuracy and wide coverage area in the Sempu Strait. By targeting the 18S V9 region, eDNA metabarcoding analysis successfully identified 361 species across 341 genera from five stations, revealing clear spatial variations in community composition. The highest plankton diversity was observed at station 5, a less disturbed area, while lower diversity and higher dominance of specific taxa (e.g., *Neocalanus*) occurred near sites with anthropogenic pressure. Physicochemical parameters such as nitrate and phosphate concentrations were found to be associated with the abundance of nutrient-responsive Diatom genera, suggesting that eutrophication influences community structure. Alpha diversity indices (Shannon, Simpson, and Inverse Simpson) further confirmed the ecological gradient across stations, highlighting the spatial structuring of plankton communities within a narrow coastal zone.

Despite providing valuable insights into plankton diversity in the Sempu Strait, this study has several limitations. Sampling during a single season may not capture temporal changes driven by monsoonal or tidal cycles. Spatial coverage, while representative of key habitats, was limited relative to the strait's hydrographic complexity. The exclusive use of the 18S rRNA marker may bias detection toward well-represented taxa, while eDNA degradation, amplification variability, and transport dynamics could affect abundance estimates. Moreover, the absence of comparison with morphology-based identification limits validation of taxonomic assignments. Future studies incorporating multi-seasonal sampling, additional genetic markers, and cross-method validation will strengthen the reliability of eDNA metabarcoding for marine biodiversity monitoring in Indonesia. Overall, this work establishes a molecular biodiversity baseline for the Sempu Strait, Malang, Indonesia, and underscores the importance of integrating eDNA-based approaches into long-term marine monitoring programs to support effective conservation and management of Indonesia's coastal ecosystems.

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