

# Multi-class metazoan diversity and community composition in tropical estuaries assessed by eDNA metabarcoding: a baseline dataset from South Sumatra, Indonesia

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# Abstract

The present study employed environmental DNA (eDNA) metabarcoding of the metazoan community composition and diversity in relation to water quality parameters from four major river estuaries of South Sumatra, Indonesia (Sembilang, Banyuasin, Musi, and Air Salek). Surface water sampling was conducted in September 2025; the mitochondrial COI gene was amplified using mICOLintF/jgHCO2198 and sequenced with Oxford Nanopore. In total, 26,300 sequence reads were obtained across eight taxonomic classes: Malacostraca (54.4%), Actinopteri (27.2%), Bivalvia (10.9%), and Thecostraca (7.2%), with contributions from Scyphozoa, Chondrichthyes, Merostomata, and Hydrozoa, all of which either contributed < 1% or failed to be identified as a class-level hit. Banyuasin exhibited the highest read abundance, extreme dominance by the mantis shrimp *Odontodactylus cultrifer*, and very low diversity (Shannon  $H' = 1.35$ ). Conversely, the community of Air Salek was the most balanced ( $H' = 3.18$ ) and had the highest species richness ( $S = 58$ ). PERMANOVA revealed significant differences among stations (pseudo- $F = 3.24$ ,  $p = 0.002$ ), and Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances explained 48.3% and 32.5% of variance, respectively. Simpson dominance was significantly negatively correlated with dissolved oxygen ( $r = -0.99$ ) and salinity ( $r = -0.88$ ), as demonstrated by Pearson correlations. This species-level read abundance matrix is available as Supplementary Table S1. This dataset, which we are revealing for the first time, serves as a reproducible baseline that will facilitate biomonitoring and conservation planning in tropical estuarine ecosystems by leveraging eDNA's unprecedented ability to capture a wide range of biodiversity, extending well beyond fish.

## 1. Introduction

Estuaries are one of the most productive habitats on Earth, as they act as transitional zones between rivers and the ocean, harboring high biodiversity and complex ecological interactions (Cowley et al., 2022; Elliott et al., 2022; Whitfield, 2021). In South Sumatra, Indonesia, the estuaries of four major rivers (Musi, Banyuasin, Komering, and Ogan) are critical to local fisheries (Pramilus et al., 2022; Sudarmo et al., 2025), food security (Syuhada et al., 2020), and biogeochemical cycles (Diansyah et al., 2025). Nevertheless, these ecosystems are subjected to anthropogenic pressures from land-use change, pollution, and climate change (Agussalim et al., 2024; Melki et al., 2018a, 2018b, 2025; Wahyudi et al., 2025), while their long-term sustainability is endangered by the overexploitation of resources (Wulandari et al., 2024), which threatens not only the biodiversity within these estuaries but also the livelihoods of local communities that depend on them.

The physical and chemical variables of estuaries are important drivers for spatiotemporal variation in biodiversity and ecosystem functioning (Chen et al., 2025; Mestdagh et al., 2020). Primary productivity (often measured as chlorophyll *a*) influences relations in food webs and is connected to nutrient loads, turbidity, and hydrodynamic forces, which also influence habitat quality for different taxa (Agussalim et al., 2024; Hu et al., 2021). These environmental baselines are therefore vital for identifying changes in biological communities.

Biodiversity surveys are mostly based on capture methods (e.g., nets, trawls), which suffer inherent drawbacks—they can be selective and labor-intensive, from a taxonomic point of view trivial to complex ones, and cannot easily sample very large or otherwise difficult-to-access areas (Bacheler, 2025; Fediajevaite et al., 2021). Recently, environmental DNA (eDNA) metabarcoding has developed into an advanced, noninvasive technique that shows good taxonomic coverage and high sensitivity for cryptic or rare species through detection of DNA released from organisms in the environment (Çevik & Çevik, 2025; Miya, 2022; Yao et al., 2022). This method has transformed biodiversity monitoring in aquatic ecosystems (Beng & Corlett, 2020; Fonseca et al., 2023; Gold et al., 2021; Peixoto et al., 2021).

Previous studies that have focused on fish communities in estuaries of South Sumatra relied exclusively on conventional fishing gear (gillnets, cast nets, and traps). From 2007 to 2023, the Musi Hilir River experienced a serious loss of fish species. Data from Sudarmo et al. (2025) recorded a sharp decline from 112 to only 23 species, or nearly a fivefold decrease. In the first study conducted in Banyuasin Regency, 31 fish species were recorded (Hernawati et al., 2023), while the second investigated river Sembilang, which had yielded a dominance of 27 marine taxa (78% species) in the estuary region (Sellyndia et al., 2023). 33 fish species collected from the Sugihan River (Setiawan et al., 2019). However, these studies focused on a limited scope of the eukaryotic community (e.g., crustaceans, bivalves, jellyfish, barnacles, and horseshoe crabs) that constitute major components of estuarine food webs and ecosystem functioning. Additionally, traditional methods probably underestimate inconspicuous or uncommon species and are largely ineffective in turbid or deep waters, which can lead to a significant underrepresentation of the true biodiversity present in these ecosystems.

Although estuaries are among the most important ecosystems in South Sumatra, there has been no study using eDNA metabarcoding to comprehensively compare multiple taxonomic classes concurrently (e.g., crustaceans, mollusks, cartilaginous fishes, and other metazoans) across river estuaries before. Although non-fish taxa form an essential part of effective management and conservation plans, the true diversity and community structure of these groups are still largely unknown.

In this study, we conducted the first eDNA-based survey of eukaryotic communities within four prominent estuarine systems in South Sumatra (Sembilang, Banyuasin, Musi, and Air Salek), leading to a comprehensive inventory of their biodiversity. With a single universal marker (COI), we simultaneously recovered eight taxonomic classes: Malacostraca (crustaceans), Actinopteri (ray-finned fishes), Bivalvia, Thecostraca (barnacles), Scyphozoa (true jellyfishes), Chondrichthyes (sharks and rays), Merostomata (horseshoe crabs), and Hydrozoa. In addition, we assess community diversity indices, beta diversity, and the effect of water quality parameters (temperature, salinity, dissolved oxygen, and pH) on the community structure. These results fill a major knowledge gap in tropical estuarine biodiversity and provide a reproducible baseline for future eDNA biomonitoring worldwide, yet particularly in Indonesia.

Here, for this study, we specifically seek to: (1) characterize the species composition and diversity of metazoan communities across four estuaries through eDNA metabarcoding; (2) investigate structural differences in community composition between estuaries; and (3) evaluate how community

characteristics relate to key environmental variables. Ultimately, our goal is to provide a solid scientific foundation for sustainable fisheries management and estuarine conservation.

## 2. Materials and methods

### 2.1. Study area and sampling

The sampling was located in four big river mouths in South Sumatra, Indonesia: Sembilang (1°58'40.57" S, 104°42'30.04" E), Banyuasin (2°12'35.10" S, 104°56'33.93" E), Musi (2°18'39.55" S, 104°56'30.03" E), and Air Salek (2°21'3.17" S, 105°7'30.36" E) (Fig. 1). Sampling was performed in September 2025. A total of three replicate water samples were taken at each estuary and then combined for analysis. Surface water samples (1 L) were aseptically collected from a depth of approximately 30 cm using a sterile bottle.

Briefly, for each water sample, first 1.2 µm mixed cellulose ester (MCE) membrane filter (47 mm diameter, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Ireland) filtration to eliminate large particulate matter was performed before using the vacuum pump for a second MCE filtration at 0.45 µm (47 mm diameter) (Specchia et al., 2023; S. Zhang et al., 2020). These were transferred into tubes containing Longmire's lysis buffer (Edmunds & Burrows, 2020) and kept at – 20°C until DNA extraction.

At each station, in situ measurements of environmental parameters were made. Salinity was evaluated employing a hand-held refractometer (ATAGO Co., Ltd., Tokyo, Japan); pH using a pH meter (SM101, Milwaukee Instruments, Romania); and dissolved oxygen (DO) and temperature were determined by employing a dissolved oxygen meter (HI98193, Hanna Instruments Inc).

### 2.2. eDNA extraction and pcr amplification

DNA extraction was performed using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research Corporation, USA) with the following modifications. Prior to processing, half of each filter paper was finely chopped with a sterile scalpel. A bead-beating step was performed for 5 minutes with incubation on ice after each minute for 30 seconds. The concentrations of DNA were quantified by the Qubit 4.0 Fluorometer (Q33253, Life Technologies) using the Equalbit 1× dsDNA HS Assay Kit from Vazyme (China).

PCR was performed using KOD One™ PCR Master Mix (TOYOBO, Japan) in an Opentrons Flex Thermal Cycler. The reaction mixture was made up of 11.5 µL DNA extract, 12.5 µL master mix, 0.75 µL forward primer, and 0.75 µL reverse primer. Amplification of a 313 bp fragment of the mitochondrial COI gene was performed with the primers mlCOLintF (GGWACWGGWTGAACWGTWTAYCCYCC) and jgHCO2198 (TAIACYTCIGGRTGICCRARAAYCA) (Leray et al., 2013). The PCR program for gene amplification was as follows: initial denaturation at 95°C (10s), touchdown annealing from 62°C (– 1°C per cycle; 30s) to a constant temperature of 46°C (25 cycles), and extension at 72°C for 60s.

For post-PCR DNA concentration, the Qubit 4.0 Fluorometer (Thermo Fisher Scientific) was used again with the same dsDNA HS assay kit. PCR products were purified using VAHTS DNA Clean Beads (Vazyme, China) with a sample-to-beads ratio of 1:2.5 and a final elution volume of 20  $\mu$ L. Purified amplicon sizes were verified by electrophoresis on a 1% (w/v) agarose gel in 1 $\times$ TBE buffer stained with Smobio Nucleic Acid Gel Stain (0.01% v/v) for about 30 minutes at the power of 100 V.

## 2.3. Library preparation and nanopore sequencing

Using approximately 130 ng of DNA amplicon from each sample, sequencing libraries were prepared in parallel on the Oxford Nanopore Technologies (ONT) PromethION platform. Libraries were prepared according to the manufacturer's protocol (Native Barcoding Kit 96 V14; SQK-NBD114-96, ONT, Oxford, UK) with slight modifications for sequencing relying on amplicon ligation. An end-preparation incubation was conducted with the process set to 20°C for 30 minutes, then 65°C for 5 minutes. A native barcode ligation was performed first and incubated at 37°C for 20 minutes, followed by washing the barcode ligation mixture with 80% ethanol. A 30-minute ligation of the adapters was done at room temperature. The adapter-ligated DNA was subsequently eluted by spin-down and incubated at 37°C for 30 minutes on a heat block with gentle tapping every 5 minutes.

Dorado basecall server, see the site for details (v7.9.8) with the model dna\_r10.4.1\_e8.2\_400bps\_sup@v5.0.0 (super-accuracy mode). NanoPlot (De Coster & Rademakers, 2023) was used to produce summary plots and metrics to assess data quality from runs.

## 2.4. Taxonomic classification and diversity analysis

Taxonomic classification and community diversity were carried out on the EPI2ME platform (Oxford Nanopore Technologies, UK). The wf 16S v1.4.0 workflow with the Kraken2 algorithm (Werner et al., 2022) was used against the core\_nt database. This database (updated by the Kraken2 developers on 4 September 2024) is a complete collection of sequences from GenBank, RefSeq, TPA, and PDB. To specifically target the COI amplicons, reads were filtered to keep sequences with a minimum length of 250 bp and a maximum length of 450 bp.

## 2.5. Statistical analysis

The statistical analyses were done with R software version 4.2.2 (R Core Team, 2023), with the vegan package (version 2.6-4) for community ecology calculations (Oksanen et al., 2022). A species-level read abundance matrix, derived from eDNA metabarcoding data (Sembilang, Banyuasin, Musi, and Air Salek; Supplementary Table S.1), was compiled. This approach enabled the computation of several standard biodiversity indices. Species richness (S) was defined as the total number of unique species detected per station. The richness (R) was calculated according to Margalef's richness index as  $(S-1)/\ln(N)$  (Sotomayor et al., 2025), where N is the total number of sequence reads at that station, a non-parametric composite species richness estimate, and therefore an appropriate metric for comparing species diversity across samples of different overlap. The Shannon Wiener diversity index (H') was calculated as  $-\sum(p_i \times \ln(p_i))$  (Sotomayor et al., 2025), where  $p_i$  is the proportion of abundance of the  $i^{\text{th}}$  species ( $n_i/N$ ),

encompassing both richness and evenness. The unweighted abundance data were used to calculate Pielou's evenness index (E) as  $H'/\ln(S)$ , where  $H'$  is the Shannon-Wiener diversity and  $S$  is species richness, in order to assess the degree of equitability among sampled species abundances. Simpson's dominance index (D) was calculated as  $1/\sum(p_i^2)$  (Miyazawa et al., 2020), which gives the probability that two randomly selected reads belong to different species (i.e., the probability of interspecific encounter); higher values indicate greater diversity and lower dominance.

The Importance Value Index (IVI) was used to identify the most ecologically influential species at each station. For a given species at a given station, the IVI was calculated as the sum of its relative read abundance ( $RA = n_i/N \times 100$ ) and its relative frequency across stations ( $RF = f_i/\sum f \times 100$ ), where  $f$  is the number of stations in which the species was present (read count  $\geq 1$ ). Thus, IVI ranges from 0 to 200, reflecting the combined contribution of local abundance and spatial distribution.

Community composition (beta diversity) was analyzed using Jaccard (presence/absence) and Bray-Curtis (relative read abundance) dissimilarity matrices. Both distance matrices were visualized using Principal Coordinates Analysis (PCoA) (Miyazawa et al., 2020). To test for significant differences in community composition among stations, permutational multivariate analysis of variance (PERMANOVA) was performed using the `adonis2` function from the `vegan` package in R, with 9,999 permutations. If the overall PERMANOVA indicated a significant difference, post hoc pairwise comparisons were conducted using Bray-Curtis distances, and  $p$  values were adjusted using the Bonferroni correction to control for the family-wise error rate.

In order to understand how the diversity indices  $H'$ ,  $E$ ,  $D$ , and  $R$  relate to environmental conditions, detailed measures of water quality (temperature, salinity, dissolved oxygen, and pH) were correlated with each component of community structure. As only four sampling stations ( $n = 4$ ) were available, Pearson and Spearman correlation coefficients have been calculated as descriptive measures of association (linear and monotonic, respectively). No statistical significance testing was performed because of the small sample size. The analyses were conducted using R software (version 4.2.2) with the `cor` function from base R and visualized using `corrplot` and `ggplot2` packages. Results are presented as a heatmap (Pearson correlations) and selected scatter plots with linear trend lines. Margalef's  $R$  was constant across all stations (4.0) and therefore excluded from correlation analysis.

## 3. Results

### 3.1. Water quality parameters

To summarize, the four stations differed greatly in terms of water quality parameters (Fig. 2). Air temperature varied from  $28.7 \pm 1.7^\circ\text{C}$  at Sembilang to  $29.6 \pm 1.1^\circ\text{C}$  at Air Salek, with all the stations maintained generally high temperatures throughout the study period. The greatest differences we observed were for salinity; similarly, Air Salek had higher salinity ( $28.9 \pm 5.8$  ppt) than Sembilang ( $27.0 \pm 1.0$  ppt), and both of these values were much greater than those in the other two mouth sites, Banyuasin

( $15.3 \pm 1.5$  ppt) and Musi ( $13.3 \pm 2.9$  ppt). The mean concentration of dissolved oxygen (DO) at Air Salek ( $6.3 \pm 0.5$  mg/L), Banyuasin ( $5.3 \pm 0.5$  mg/L), Sembilang, and Musi was between the two, with relatively high DO levels. Covering information about physical parameters, the pH values were not significantly different across stations, with Musi at  $5.7 \pm 0.6$  and Sembilang at  $6.1 \pm 0.5$ , respectively. These patterns indicate a clear environmental gradient, particularly in salinity and DO, which likely play a key role in structuring the eDNA community (see below).

The spatial variation in water quality parameters corresponded well with the observed community structure. The Banyuasin station, characterized by the lowest salinity (15.3 ppt) and dissolved oxygen (5.3 mg/L), exhibited extreme dominance by a few taxa, particularly the mantis shrimp *Odontodactylus cultrifer* (7,887 reads) and the fish *Trachinocephalus myops* (1,862 reads), as shown in Supplementary Table S1. In contrast, the most saline (28.9 ppt), hot ( $29.6^{\circ}\text{C}$ ), and highest DO in Air Salek sustained a more diverse community, with the highest equitability contributed by bivalves (*Anadara granosa*: 520 reads; *Pilsbryconcha exilis*: 148 reads) and decapod communities (*Portunus pelagicus*: 549 reads). In Musi, which had the lowest pH (5.7) and salinity (13.3 ppt), *Odontodactylus cultrifer* (795 reads) and *Periophtalmodon schlosseri* (124 reads) dominated communities, while in Sembilang, conditions were intermediate, evidenced by representatives of crustaceans with lower salt tolerance like *Harpiosquilla raphidea* (727 reads) and *Lepidotrigla marisiensis* (271 reads). These patterns indicate that salinity and dissolved oxygen were the major environmental drivers structuring the eDNA communities along the study region.

## 3.2. Diversity and composition of eDNA-detected communities

The environmental DNA (eDNA) metabarcoding analysis revealed a total of 26,300 sequence reads across eight taxonomic classes at four sampling stations (Sembilang, Banyuasin, Musi, Air Salek; Table 1). The complete species-level dataset is provided in Supplementary Table S1.

Table 1  
Summary of eDNA sequence abundance per class and study location.

| Class                                                                                                                                          | Sembilang            | Banyuasin                 | Musi                    | Air Salek                | Total                    |
|------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|---------------------------|-------------------------|--------------------------|--------------------------|
| Actinopteri                                                                                                                                    | 1,584                | 4,019                     | 556                     | 998                      | 7,157<br>(27.2%)         |
| Bivalvia                                                                                                                                       | 529                  | 980                       | 124                     | 1,233                    | 2,866<br>(10.9%)         |
| Chondrichthyes                                                                                                                                 | 0                    | 11                        | 0                       | 14                       | 25 (0.11%)               |
| Hydrozoa                                                                                                                                       | 18                   | 0                         | 0                       | 0                        | 18 (0.07%)               |
| Malacostraca                                                                                                                                   | 2,379                | 9,214                     | 1,098                   | 1,613                    | 14,304<br>(54.4%)        |
| Merostomata                                                                                                                                    | 0                    | 11                        | 0                       | 0                        | 11 (0.04%)               |
| Scyphozoa                                                                                                                                      | 0                    | 0                         | 0                       | 22                       | 22 (0.08)                |
| Thecostraca                                                                                                                                    | 976                  | 572                       | 167                     | 182                      | 1,897 (7.2%)             |
| <b>Total</b>                                                                                                                                   | <b>5,486 (20.9%)</b> | <b>14,807<br/>(56.3%)</b> | <b>1,945<br/>(7.4%)</b> | <b>4,062<br/>(15.4%)</b> | <b>26,300<br/>(100%)</b> |
| <i>Note: Numbers are the number of sequences (reads) detected. A complete table per species is available at <b>Supplementary Table S1</b>.</i> |                      |                           |                         |                          |                          |

The class Malacostraca (crustaceans) dominated the total read abundance, contributing 14,304 reads (54.4%), followed by Actinopteri (ray-finned fishes) with 7,157 reads (27.2%), Bivalvia (2,866 reads, 10.9%), and Thecostraca (barnacles and allies; 1,897 reads, 7.2%). The remaining four classes – Scyphozoa (22 reads), Chondrichthyes (25 reads), Merostomata (11 reads), and Hydrozoa (18 reads) – together accounted for less than 0.3% of the total reads.

Banyuasin yielded the highest total reads (14,807, 56.3% of the global total), followed by Sembilang (5,486, 20.9%), Air Salek (4,062, 15.4%), and Musi (1,945, 7.4%). The class-level composition also differed sharply among stations (see Fig. 3 and Table 1 for a visual summary). Banyuasin was overwhelmingly dominated by Malacostraca (9,214 reads, 62.2% of the station total) and Actinopteri (4,019 reads, 27.1%). The mantis shrimp *Odontodactylus cultrifer* alone contributed 7,887 reads at this station, representing 85.6% of all Malacostracan reads in Banyuasin.

Sembilang featured a large fraction of Malacostraca (2,379 reads, 43.4%) and Actinopteri (1,584 reads, 28.9%), but also an important contribution of crustaceans such as Thecostraca (976 reads, 17.8%), more than at other stations. Musi had the lowest total reads (1,945). The station was dominated by Malacostraca (1,098 reads, 56.5%) and Actinopteri (556 reads, 28.6%), with the highest relative contribution of Bivalvia (6.4% of the station total) among all stations.

Air Salek had the most balanced assembly composition with Malacostraca (1,613 reads, 39.7%), Bivalvia (1,233 reads, 30.4%), Actinopteri (998 reads, 24.6%), and Thecostraca (182 reads, 4.5%). The station was unique in having reads of Scyphozoa (22 reads) and Chondrichthyes (14 reads, specifically *Hemiscyllium halmahera*). Additionally, it exhibited the highest bivalve diversity per read count and the greatest species richness.

### 3.3. Diversity indices

Different patterns of diversity indices (Fig. 4) were observed, depending on the station. Air Salek had the maximum species richness ( $S = 58$ ), Margalef index ( $R = 6.90$ ), Shannon-Wiener diversity ( $H' = 3.18$ ), and Pielou's evenness ( $E = 0.78$ ), with the least Simpson dominance ( $D = 0.09$ ), which is indicative of a diverse and reasonably equitable community. In contrast, Banyuasin had low diversity ( $H' = 1.35$ ) and evenness ( $E = 0.37$ ) but high dominance ( $D = 0.54$ ) with the second highest species count  $S (= 40)$ , indicating extreme numerical dominance by only a few taxa.

Sembilang ( $S = 39$ ,  $R = 4.48$ ,  $H' = 2.61$ ,  $E = 0.71$ ,  $D = 0.16$ ) and Musi ( $S = 16$ ,  $R = 2.00$ ,  $H' = 1.58$ ,  $E = 0.57$ ,  $D = 0.37$ ) were intermediate, with Musi being the least species-rich but relatively even. All these indices show a distinct ecological gradient from a strongly dominated, low-diversity community (Banyuasin) to a species-rich, balanced community (Air Salek) and establish a quantitative baseline for future eDNA-based biomonitoring.

The Importance Value Index (IVI) was used to determine the most important species at each station by summing relative read abundance and relative frequency across stations. *Odontodactylus cultrifer* was disproportionately dominant in Banyuasin ( $IVI = 156.2$ ), followed by *Trachinocephalus myops* (75.4) and *Stolephorus commersonii* (42.1). The same mantis shrimp dominated at Musi ( $IVI = 89.4$ ), with *Plotosus canius* (48.2) and *Periophthalmodon schlosseri* (40.3) being the secondary species. The species at Sembilang did not have severe IVI values, and the top three were *O. cultrifer* (48.9), *Lepidotrigla marisiensis* (35.2), and *Harpiosquilla raphidea* (28.7). In Air Salek, the maximum IVI was found for *Parambassis wolffii* (29.6), followed by *Portunus pelagicus* (24.0) and *T. myops* (23.8), which suggests a co-dominant structure where no species had an IVI surpassing 30.

The combined indices show a distinct ecological gradient among the four stations. The environment of Banyuasin is extremely disturbed or strongly filtered, resulting in the dominance of the eDNA signal by one or two species. Musi and Sembilang presented intermediate circumstances with modest dominance and variety. Air Salek is the most even and diversified location, indicating a stable, species-rich community. The gradient provides a quantitative baseline for future eDNA-based biomonitoring in tropical coastal environments.

### 3.4. Beta diversity

Principal Coordinates Analysis (PCoA) of Bray-Curtis distances showed significant patterns of community dissimilarity between the four stations. The first two axes explained 48.3% and 32.5% of the total variation, respectively (Fig. 5). Banyuasin was isolated from the other stations, with coordinates –

0.02 (PCoA1) and 0.01 (PCoA2), showing a community structure strongly dominated by a few abundant taxa (e.g., *Odontodactylus cultrifer* and *Trachinocephalus myops*). In contrast, Sembilang (0.12, 0.14), Musi (0.16, - 0.04), and Air Salek (0.22, 0.25) formed a looser cluster whose community makeup was more similar among these three locations, with Air Salek displaying the largest divergence within the group.

PERMANOVA (adonis2, 9,999 permutations) results indicated substantial differences in community composition between stations for both Bray-Curtis (pseudo-F = 3.24,  $p = 0.002$ ) and Jaccard (pseudo-F = 2.87,  $p = 0.005$ ) distance matrices. These data suggest that both species presence/absence and relative read abundance consistently drive the geographic organization of eDNA communities across the entire research region.

Pairwise comparisons with Bonferroni correction showed that Banyuasin was significantly different from Air Salek ( $p = 0.003$ ) and Sembilang ( $p = 0.009$ ), and Musi was significantly different from Air Salek ( $p = 0.043$ ). No significant difference was seen between Sembilang and Musi ( $p > 0.05$ ). These results are together suggesting a strong ecological gradient, with Banyuasin as a strongly dominated community with low diversity and Air Salek as the most diversified and balanced location.

### 3.5. Relationship between diversity indices and water quality parameters

Pearson correlation coefficients were generated in order to study the relationships between diversity indices (Shannon  $H'$ , evenness  $E$ , and Simpson  $D$ ) and water quality indicators (temperature, salinity, dissolved oxygen, and pH). Margalef's richness index ( $R$ ) was constant at 4.0 across all stations and therefore excluded from the analysis. The correlation heatmap (Fig. 6) showed that Shannon diversity ( $H'$ ) was weakly favorably connected with temperature ( $r = 0.39$ ), significantly negatively correlated with salinity ( $r = -0.43$ ) and pH ( $r = -0.62$ ), and not correlated with dissolved oxygen ( $r = -0.04$ ). Evenness ( $E$ ) was moderately negatively correlated with temperature ( $r = -0.50$ ) and weakly negatively correlated with DO ( $r = -0.38$ ), whereas the relationships with salinity ( $r = -0.04$ ) and pH ( $r = 0.19$ ) were inconsequential. Simpson dominance ( $D$ ) was negatively correlated with salinity ( $r = -0.88$ ), DO ( $r = -0.99$ ), and pH ( $r = -0.66$ ) and positively correlated with temperature ( $r = 0.17$ ). Simpson  $D$ , as expected, showed opposing tendencies to Shannon  $H'$  for most parameters, particularly salinity and pH.

The scatter plots (Fig. 7) further showed two selected relationships: Shannon diversity vs. salinity (left panel) and Simpson dominance vs. dissolved oxygen (right panel). The linear trend lines showed that higher salinity was associated with lower Shannon diversity (Air Salek had the greatest salinity but intermediate  $H'$ ) and lower dissolved oxygen and high Simpson dominance (Banyuasin had very low DO and very high dominance). These descriptive relationships demonstrate that dissolved oxygen and salinity are the two most important environmental determinants of community structure in the research region, corresponding with the separation of stations seen in the PCoA (Fig. 4). Due to the small sample size ( $n = 4$ ), the correlation coefficients are reported solely as exploratory indicators, and no formal significance testing was performed.

## 4. Discussion

The spatial variation of water quality indicators, especially salinity and dissolved oxygen, established distinct environmental gradients among the four sites comparable to those reported for other tropical estuary systems. Air Salek, with the highest salinity (28.9 ppt) and DO (6.3 mg/L), represents a more marine-influenced and well-oxygenated environment. Banyuasin and Musi showed lower salinity (15.3 and 13.3 ppt, respectively) and DO (5.3–5.8 mg/L), which are typical of estuarine or brackish conditions. Similarly, salinity-driven zonation of benthic communities has been demonstrated to be a key driver of species distribution and ecosystem functioning in tropical estuaries (Alves et al., 2020; Dias et al., 2022; Rahman et al., 2022). In estuarine areas, salinity is often the most critical single environmental factor that determines species richness and diversity (Dalkıran & Zünbülğil-Ünsal, 2023; Getz & Eckert, 2023; Koehler et al., 2022; Whitfield, 2021), while freshwater inflow from rivers can sharply reduce salinity and oxygen levels and change nekton community composition in coastal ecosystems (Coffey et al., 2025; Whaley et al., 2024). The temperature across the stations was steady (28.7–29.6°C) and compatible with tropical climates, playing probably a secondary function to salinity, as reported in Mid-Atlantic estuary research (Oleynik et al., 2024). The salinity and DO gradients reported are ecologically significant because they directly affect osmoregulation, respiratory efficiency, species tolerance, and hence the community composition at the study sites.

eDNA metabarcoding caught a wide taxonomic range, demonstrating it is a suitable tool for quick biodiversity evaluation in tropical aquatic habitats (Pansu et al., 2025; Wee et al., 2023; Zainal Abidin et al., 2022; M. Zhang et al., 2023). The most abundant groups were Malacostraca (crustaceans) with a total read abundance of 54.4%, followed by Actinopteri (27.2%) and Bivalvia (10.9%). This assemblage is typical of the benthic and demersal fauna of tropical estuary systems, where decapods, mollusks, and fishes are often co-dominant (Fekri et al., 2024; Rehitha et al., 2022; Whitfield et al., 2024). The mantis shrimp *Odontodactylus cultrifer* is a tropical benthic predator established throughout the Indo-West Pacific at 0–100 m depth (Dias et al., 2022; Duarte et al., 2023; Pratihary et al., 2021). Sembilang has had mantis shrimps described as a high-value economic and ecological group, with commercial species such as *Harpiosquilla raphidea*—also abundant in Sembilang—being exported (Kurniasih et al., 2026). This numerical preponderance, although a product of PCR/sequencing bias, reflects an unbalanced ecosystem that we may speculate is a consequence either of organic nutrient input or intense predation release. By contrast, the balanced community of Air Salek—contributions from Bivalvia (30.4%) and Actinopteri (24.6%) are most significant—suggests a more stable, mature assemblage. These observations are consistent with the broader paradigm that salinity gradients drive species richness and community composition in coastal wetland ecosystems (Dalkıran & Zünbülğil-Ünsal, 2023; González-Sansón et al., 2022; Koehler et al., 2022; Li et al., 2020).

The diversity indices measured the ecological gradient more accurately. The highest species richness ( $S = 58$ ), Shannon diversity ( $H' = 3.18$ ), and evenness ( $E = 0.78$ ) were seen in Air Salek, which also showed the lowest Simpson dominance ( $D = 0.09$ ). Such levels are typical of stable, well-oxygenated, high salinity settings where little physiological stress supports the coexistence of many species. In contrast,

Banyuasin demonstrated low diversity ( $H' = 1.35$ ) and evenness ( $E = 0.37$ ), but strong dominance ( $D = 0.54$ ), indicating the extreme numerical domination of a few species. This is a common pattern in organic rich or oxygen stress situations where opportunistic species can outcompete others. A low species pool under intense environmental filtration can also lead to such a dominance pattern. The intermediate positions of Sembilang ( $H' = 2.61$ ) and Musi ( $H' = 1.58$ ) show their transitional nature, which is also consistent with the idea that environmental heterogeneity and salinity gradients generate different alpha and beta diversity patterns in estuarine wetlands (Dalkıran & Zünbülğil-Ünsal, 2023; Fu et al., 2025; Lima et al., 2023). The Importance Value Index (IVI) further explained that at Banyuasin and Musi, *Odontodactylus cultrifer* was ecologically dominant, with the IVI value above 80, while at Air Salek, the highest IVI was only 29.6 which confirmed a co-dominant structure with no species dominating (Kurniasih et al., 2026). A high IVI value reveals ecological dominance of a few species and a low maximum IVI indicates an even distribution of species typical of stable species rich communities (Hou et al., 2025; Shi et al., 2020).

Principal Coordinates Analysis (PCoA) based on Bray-Curtis distances showed a clear community dissimilarity among the four stations. The first two axes accounted for 48.3% and 32.5% of the total variation, respectively. Banyuasin was separated from other stations, which indicates its strongly dominated community structure. In contrast, Sembilang, Musi, and Air Salek formed a less tight cluster, indicating more similar population composition. Both Bray-Curtis (pseudo- $F = 3.24$ ,  $p = 0.002$ ) and Jaccard (pseudo- $F = 2.87$ ,  $p = 0.005$ ) matrices supported significant differences in community composition between stations, suggesting that both species presence/absence and relative read abundance consistently shape the spatial structuring of eDNA communities (Cai et al., 2025; Lamperti et al., 2024). Pairwise comparisons with Bonferroni correction further showed that Banyuasin was significantly different from Air Salek ( $p = 0.003$ ) and Sembilang ( $p = 0.009$ ), while Musi was significantly different from Air Salek ( $p = 0.043$ ). There was no significant difference between Sembilang and Musi ( $p > 0.05$ ). These results demonstrate a clear ecological gradient, from the highly dominated, low-diversity community in Banyuasin to the most diverse and balanced site in Air Salek, similar to the salinity-dissolved oxygen gradient across the study area (Al-Rasady et al., 2021; Mrozińska et al., 2021).

Pearson correlation coefficients showed significant relationships between diversity indices and water quality indicators. Shannon diversity ( $H'$ ) was mildly favorably connected with temperature ( $r = 0.39$ ), significantly negatively correlated with salinity ( $r = -0.43$ ) and pH ( $r = -0.62$ ) and not correlated with DO ( $r = -0.04$ ). Evenness ( $E$ ) was moderately negatively correlated to temperature ( $r = -0.50$ ) and weakly negatively correlated to DO ( $r = -0.38$ ). Correlations between evenness and salinity and pH were negligible. Simpson dominance ( $D$ ) was highly negatively correlated with salinity ( $r = -0.88$ ), DO ( $r = -0.99$ ), and pH ( $r = -0.66$ ) and weakly positively correlated with temperature ( $r = 0.17$ ). These patterns highlight that lower salinity and lower DO are linked to higher species dominance, consistent with the idea that salinity is the most significant environmental factor shaping estuarine biodiversity (Dalkıran & Zünbülğil-Ünsal, 2023; González-Sansón et al., 2022; Harrison & Whitfield, 2024; Mrozińska et al., 2021) and that DO reduction can severely limit sensitive species while promoting tolerant ones (Ali et al., 2022). The very strong negative association between Simpson dominance and DO ( $r = -0.99$ ) implies that

oxygen availability is likely the most important factor causing the excessive dominance at Banyuasin. The limited number of stations ( $n = 4$ ) precludes formal significance testing, but the degree of concordance between correlation patterns and other ordination data provides some support for interpretation that salinity and DO are the key determinants of community organization in this research area.

## 5. Conclusion

This study provides the first comprehensive eDNA-based inventory of metazoan communities across four major river estuaries in South Sumatra, Indonesia, covering eight taxonomic classes. The dataset comprises 26,300 sequence reads, with Malacostraca (54.4%) and Actinopteri (27.2%) as the dominant groups, and reveals a strong ecological gradient from a highly dominated, low-diversity community at Banyuasin (Shannon  $H' = 1.35$ , Simpson  $D = 0.54$ ) to a species-rich, balanced community at Air Salek ( $H' = 3.18$ ,  $S = 58$ ). PERMANOVA and PCoA confirmed significant compositional differences among stations (pseudo- $F = 3.24$ ,  $p = 0.002$ ), mainly driven by salinity and dissolved oxygen gradients, as supported by strong negative correlations (Simpson  $D$  vs. DO:  $r = -0.99$ ; vs. salinity:  $r = -0.88$ ). The complete species-level read abundance matrix (Supplementary Table S1) serves as a reproducible baseline for future biomonitoring, conservation planning, and assessment of anthropogenic impacts in tropical estuarine ecosystems. This work also demonstrates the power of eDNA metabarcoding to capture broad taxonomic diversity beyond traditional fish-focused surveys, filling a critical knowledge gap in the region.

## Declarations

### CRedit authorship contribution statement

**Melki:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Anna Ida Sunaryo Purwiyanto:** Writing – review & editing, Supervision, Methodology. **Wike Ayu Eka Putri:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis. **Andi Agussalim:** Writing – review & editing, Methodology, Investigation. **Freddy Supriadi:** Writing – review & editing, Project administration, Investigation. **Fauziyah:** Writing – review & editing, Supervision, Investigation. **Risnita Tri Utami:** Writing – review & editing,

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

The raw sequencing reads will be made available upon reasonable request. The species abundance matrix is provided as Supplementary Table S1.

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## Figures

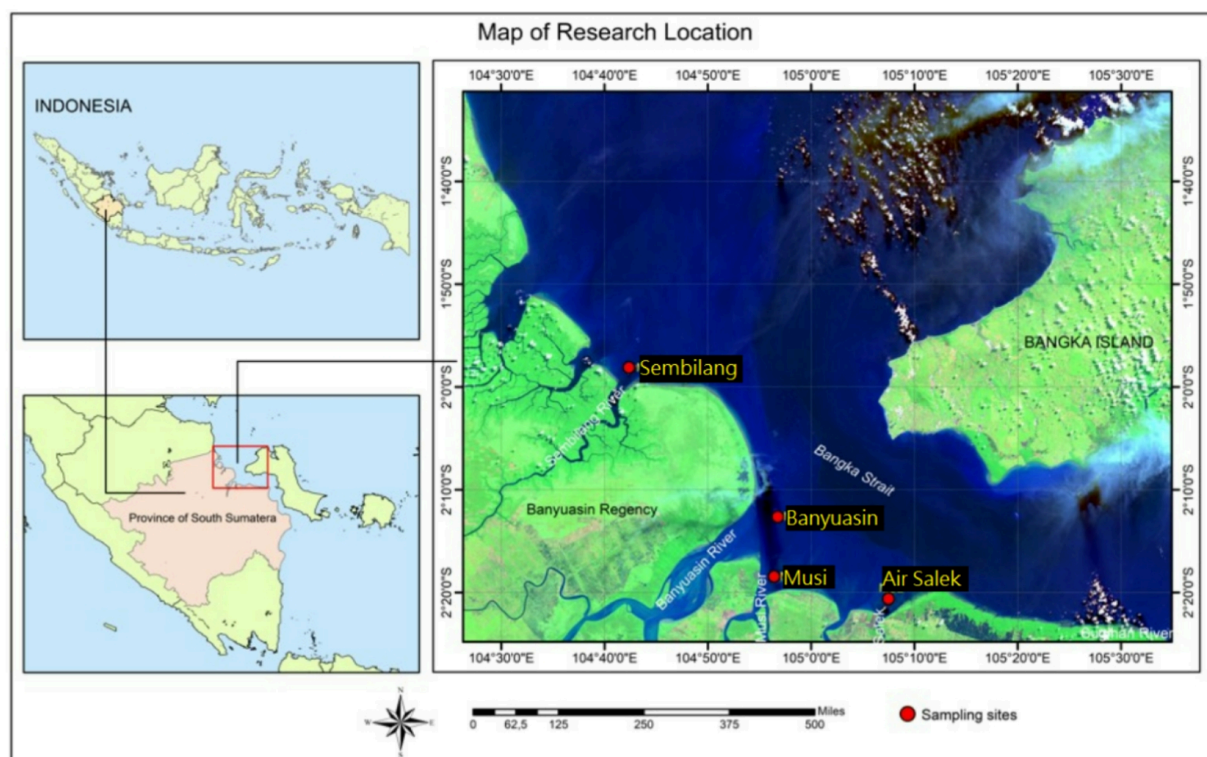


Figure 1

Study area in four principal river estuaries in South Sumatra, Indonesia.

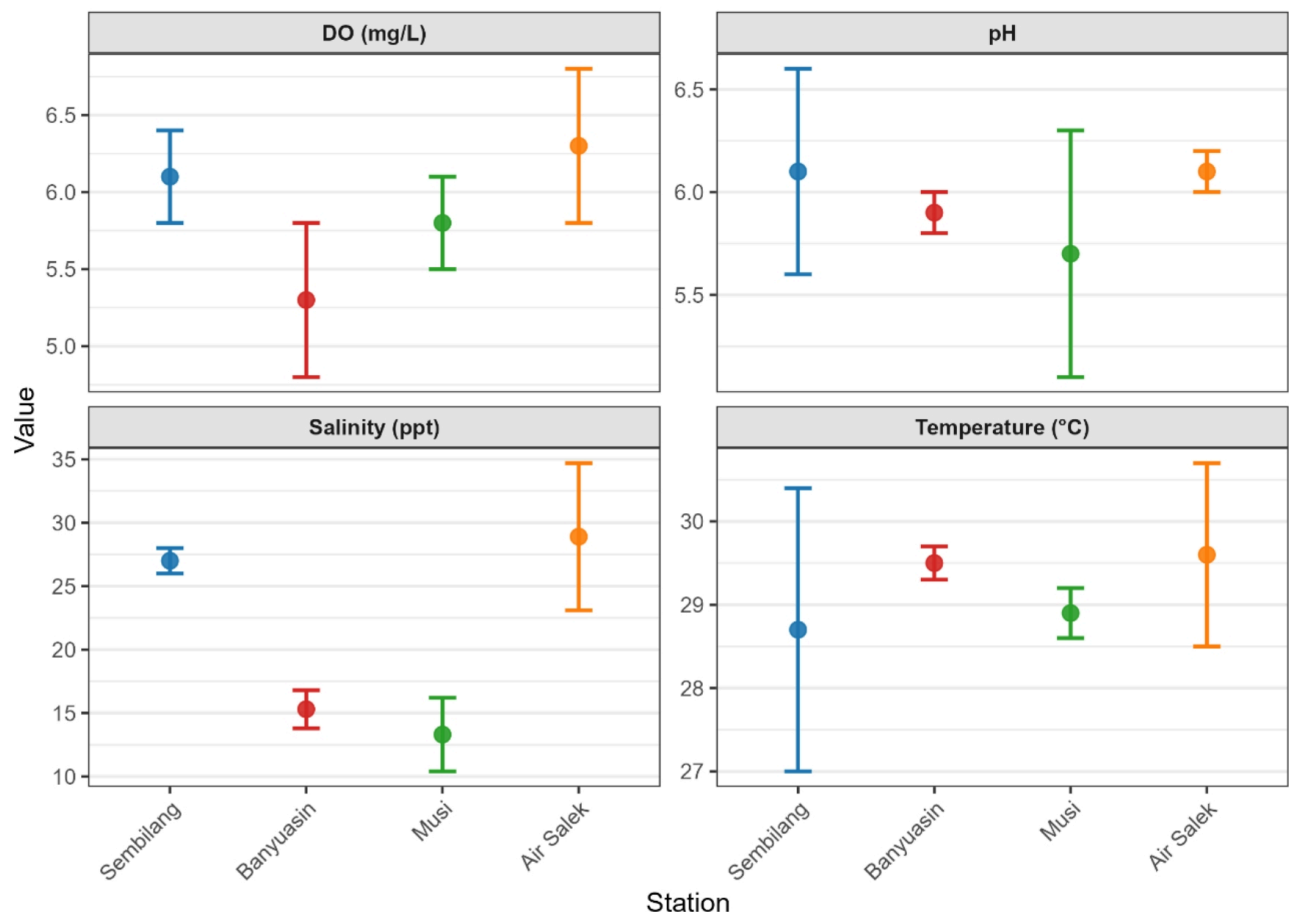


Figure 2

Water quality parameters (mean ± SD) at the four sampling stations.

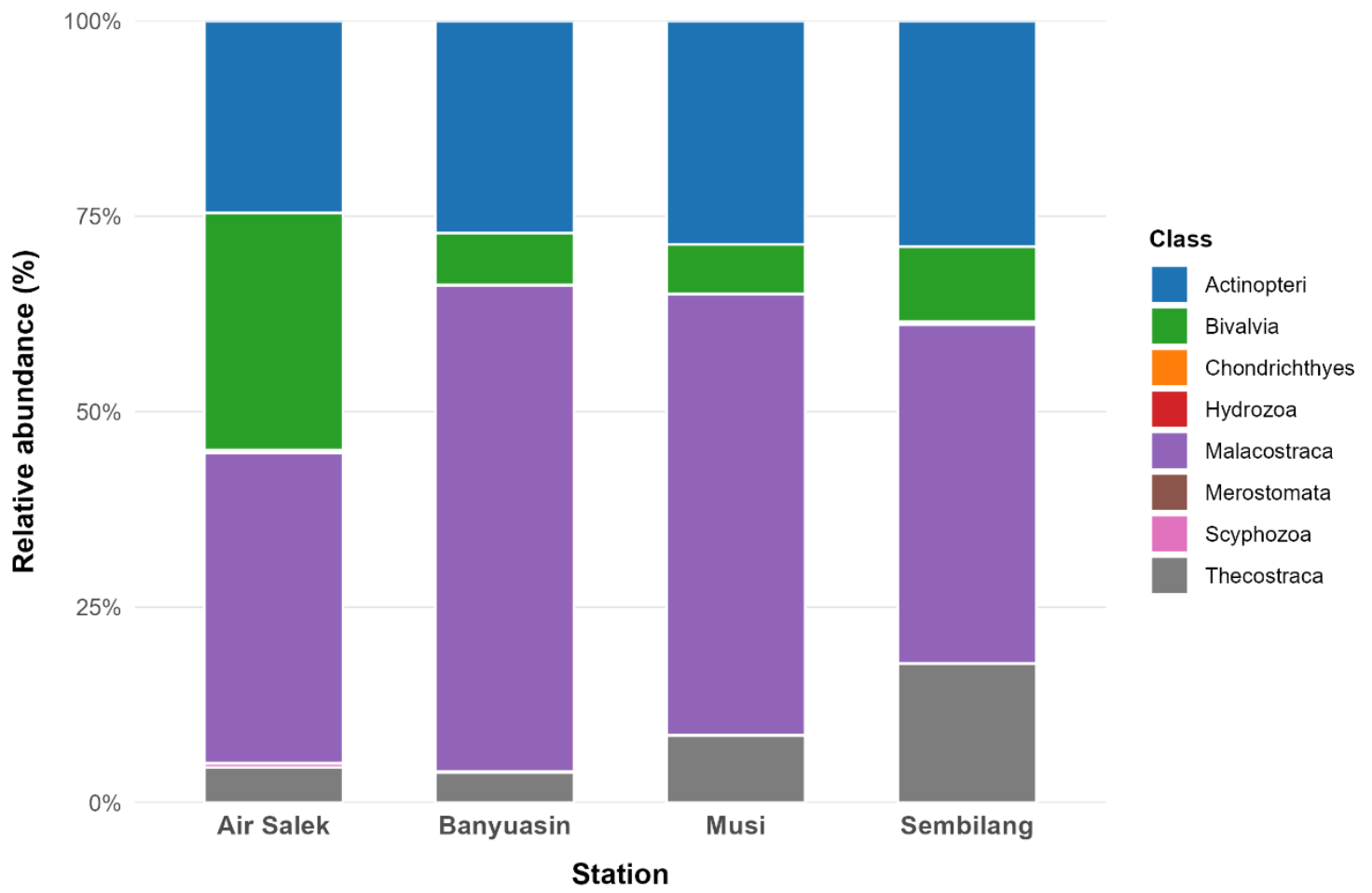


**Figure 3**

Composition of eDNA reads by taxonomic class at each station.

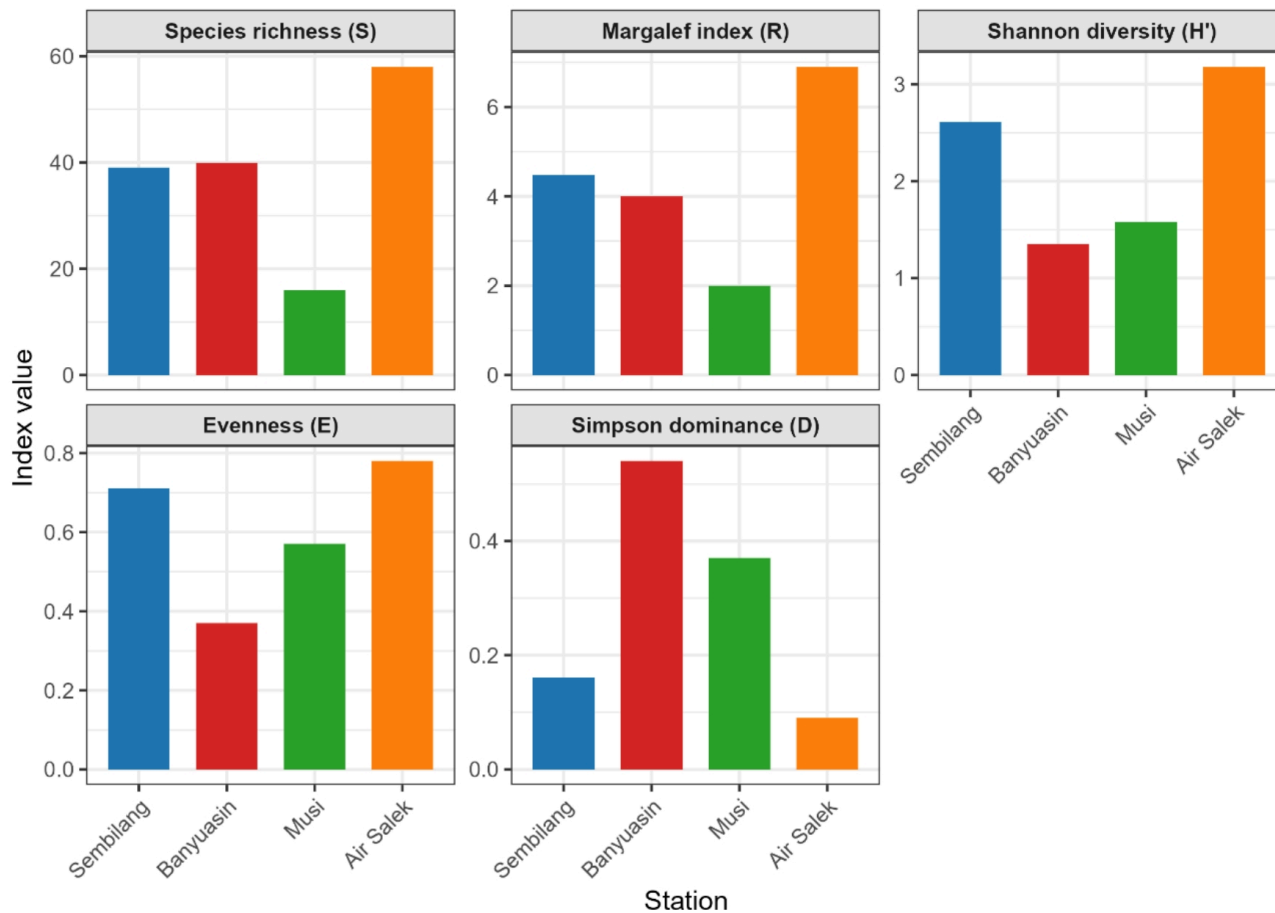


**Figure 4**

Diversity indices per station: Species richness (S), Margalef richness (R), Shannon diversity (H'), Evenness (E), and Simpson dominance (D).

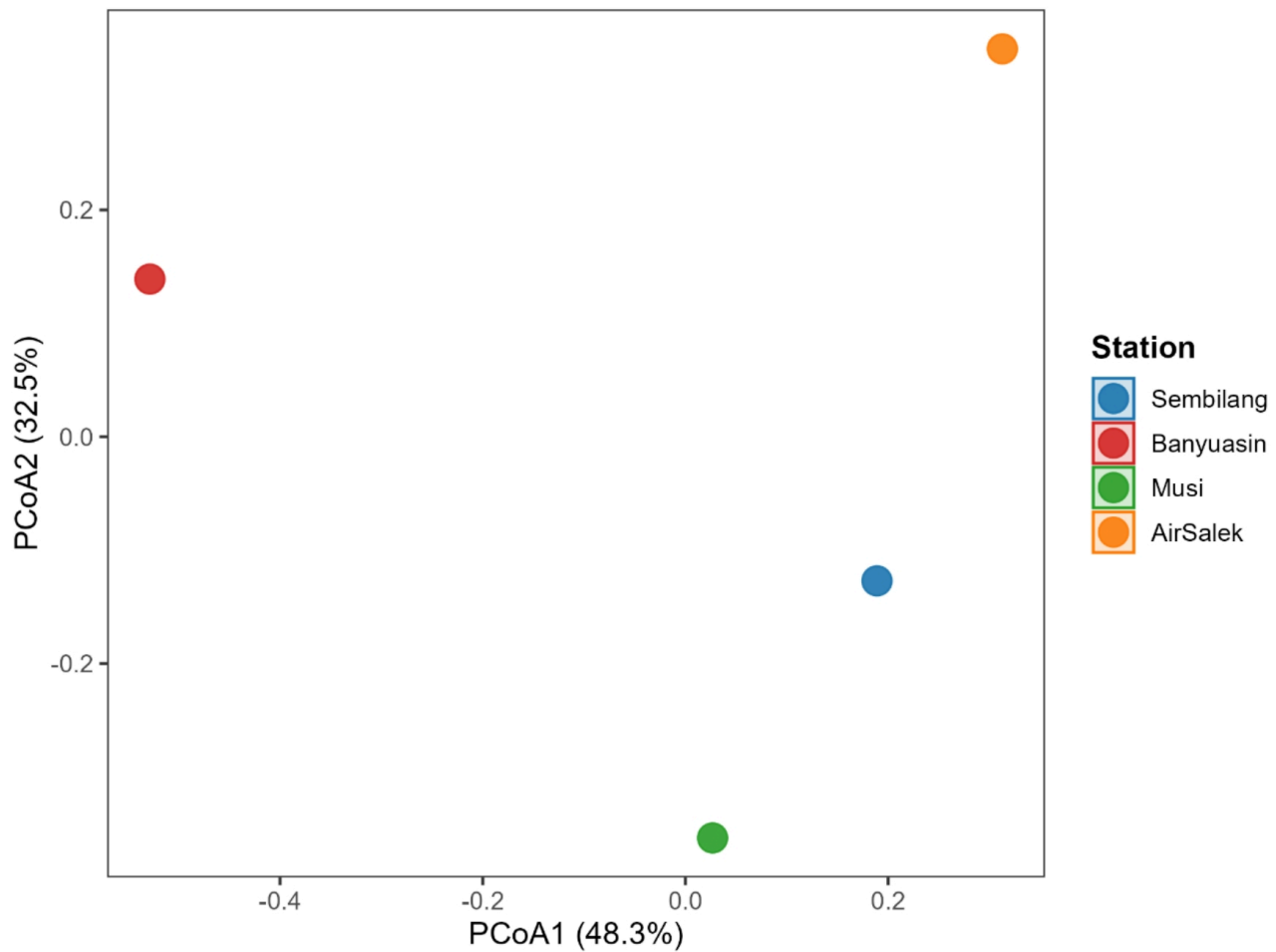


**Figure 5**

Principal Coordinates Analysis (PCoA) ordination of eDNA communities based on Bray-Curtis distances, showing the dissimilarity among four stations (Sembilang, Banyuasin, Musi, Air Salek). Ellipses represent 95% confidence intervals. PERMANOVA confirmed significant differences among stations (pseudo-F = 3.24,  $p = 0.002$ ). Pairwise comparisons with Bonferroni correction: Banyuasin vs. Air Salek ( $p = 0.003$ ), Banyuasin vs. Sembilang ( $p = 0.009$ ), Musi vs. Air Salek ( $p = 0.043$ ); other pairs not significant ( $p > 0.05$ ).

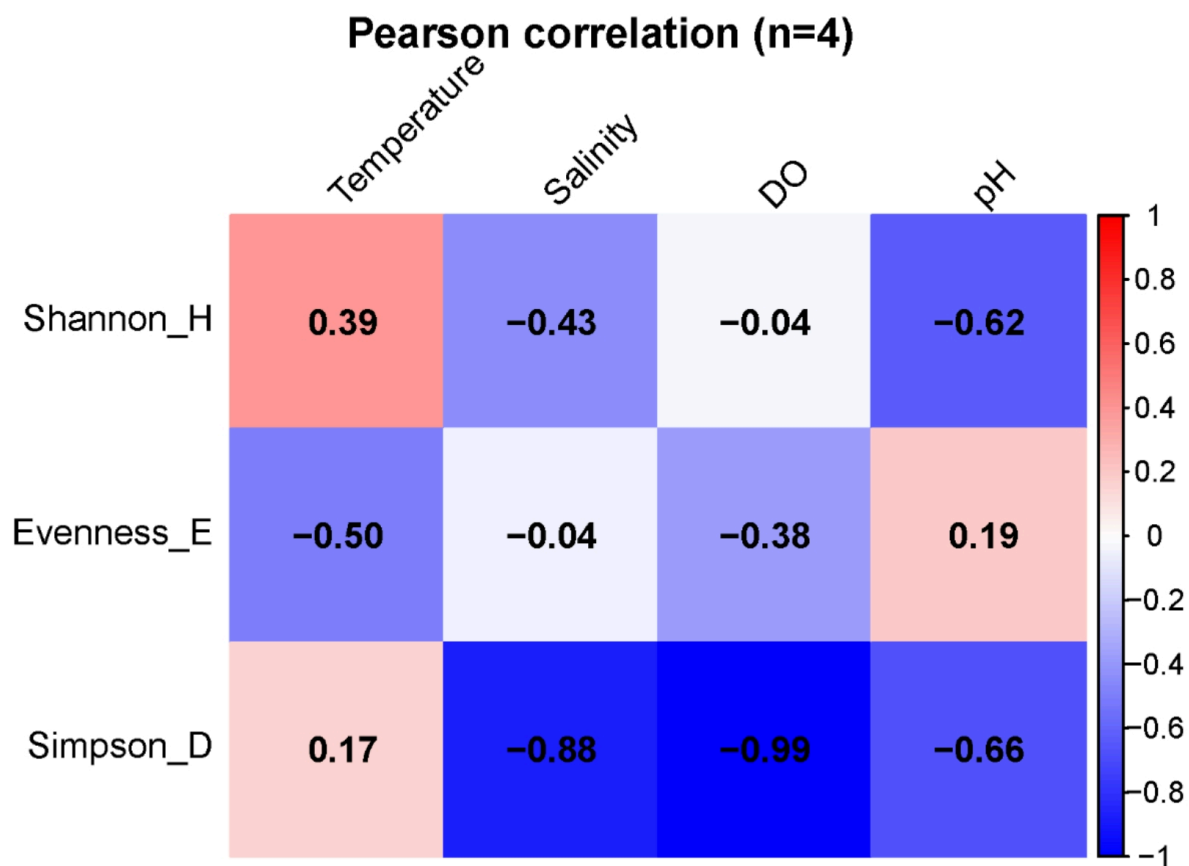


**Figure 6**

Heatmap of Pearson correlation coefficients between diversity indices and water quality parameters.

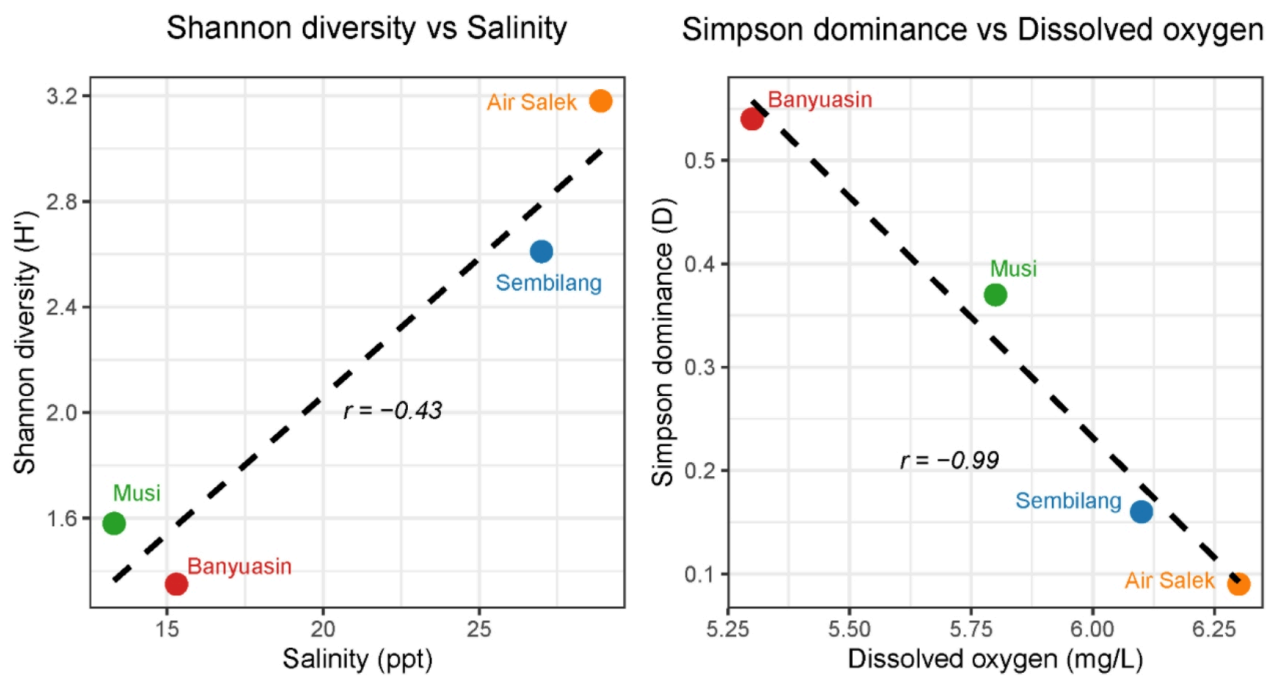


**Figure 7**

Scatter plots showing relationships between (left) Shannon diversity (H') and salinity and (right) Simpson dominance (D) and dissolved oxygen, with linear trend lines and station labels.