



Zooplankton as indicators of coastal water quality: a comparative study of PlanktonScope imaging and DNA metabarcoding of seawater and mesh-screened samples

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ABSTRACT

Zooplankton serve as a vital link between primary producers and higher consumers and act as key indicators of aquatic environmental health. Traditional zooplankton monitoring methods are often time-consuming and labour-intensive, while eDNA metabarcoding and automated imaging systems offer promising alternatives. However, few studies have compared these methods, particularly regarding their sensitivity to small-scale spatial changes in zooplankton communities. Here, we compared the performance of PlanktonScope—an imaging system enabling in situ monitoring and identification of dominant plankton taxa—with eDNA metabarcoding of water and mesh-screened samples along a 20-km pollution gradient in Hong Kong. All three methods consistently detected a spatial gradient in the relative abundance of the dinoflagellate *Noctiluca scintillans*, jellyfish, and copepods. However, eDNA metabarcoding, especially from net samples, produced very few jellyfish reads, likely due to the fragility of their bodies. Within the eDNA results, *Temora* sp. and *Parvocalanus crassirostris* were dominant in the inner harbour, while *Centropages typicus* and *N. scintillans* prevailed in the outer harbour. Turbidity and orthophosphate phosphorus significantly influenced the abundance of several key taxa. Overall, our findings show that water- and net-based eDNA approaches provide comparable information on zooplankton diversity, capturing a broader range of taxa at finer taxonomic resolution, while PlanktonScope delivers high-resolution spatial and temporal data. Given the considerably lower workload involved, we recommend using automated imaging for the rapid assessment and monitoring of zooplankton taxa, and water-based eDNA metabarcoding for a deeper understanding of coastal zooplankton community dynamics.

1. Introduction

Zooplankton play a vital role in marine ecosystems as secondary consumers, linking primary producers to higher trophic levels (Heneghan et al., 2023; Steinberg and Landry, 2017). Their short life-spans and sensitivity to environmental changes make them effective indicators of anthropogenic impacts on marine ecosystems (Bi et al., 2011, 2022; Richardson, 2008). Thus, monitoring zooplankton diversity and community composition is essential for assessing marine

ecological health.

Traditional monitoring methods rely on plankton net sampling followed by microscopic identification, a process that is both time-consuming and dependent on specialised taxonomic expertise (Holland et al., 2025; von Ammon et al., 2023). DNA metabarcoding has emerged as a high-throughput alternative, often detecting more taxa than microscopy (Chen et al., 2024; Kim et al., 2019; Ratnarajah et al., 2023). However, its accuracy is influenced by primer selection, the completeness of reference databases (Holland et al., 2025; Yang and

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Zhang, 2020), and DNA source—whether from waterborne eDNA or mesh-screened materials (Czeplédi et al., 2021; Du et al., 2024; Kim et al., 2019). Underwater imaging systems offer rapid, in situ observations with fine-scale spatial and temporal resolution. While imaging systems provide moderate taxonomic resolution, they excel at quantifying the distribution and abundance of dominant taxa rapidly and detecting dynamic environmental response, making them a valuable complement to both net and molecular approaches (Bi et al., 2022; Eerola et al., 2024; Song et al., 2020).

Hong Kong, located on China's southern coast along the Pearl River estuary, is bordered by the South China Sea on three sides and by Shenzhen (Guangdong Province) to the north. Covering 1106 square kilometres and home to 7.4 million people, the region faces intense environmental pressures, particularly in its marine ecosystems. Tolo

Harbour, a semi-enclosed coastal body in northeastern Hong Kong, has been heavily impacted by urbanisation and pollution (Chen et al., 2019). Although sewage management improvements in the 1990s enhanced water quality (Xu et al., 2004), the harbour continues to suffer from eutrophication and frequent algal blooms (Deconinck et al., 2025). How environmental conditions have structured the zooplankton communities in this area remains poorly understood.

In this study, we compared three zooplankton monitoring methods—eDNA metabarcoding (waterborne and mesh-screened tissue samples) and PlanktonScope imaging—across four sites along a 20-km spatial scale in Tolo Harbour and Mirs Bay. For the metabarcoding approaches, we selected the 18S rRNA marker due to its outstanding effectiveness in recovering eukaryotic taxa (Amaral-Zettler et al., 2009). We aimed to: (1) evaluate each method's capability in identify of taxa

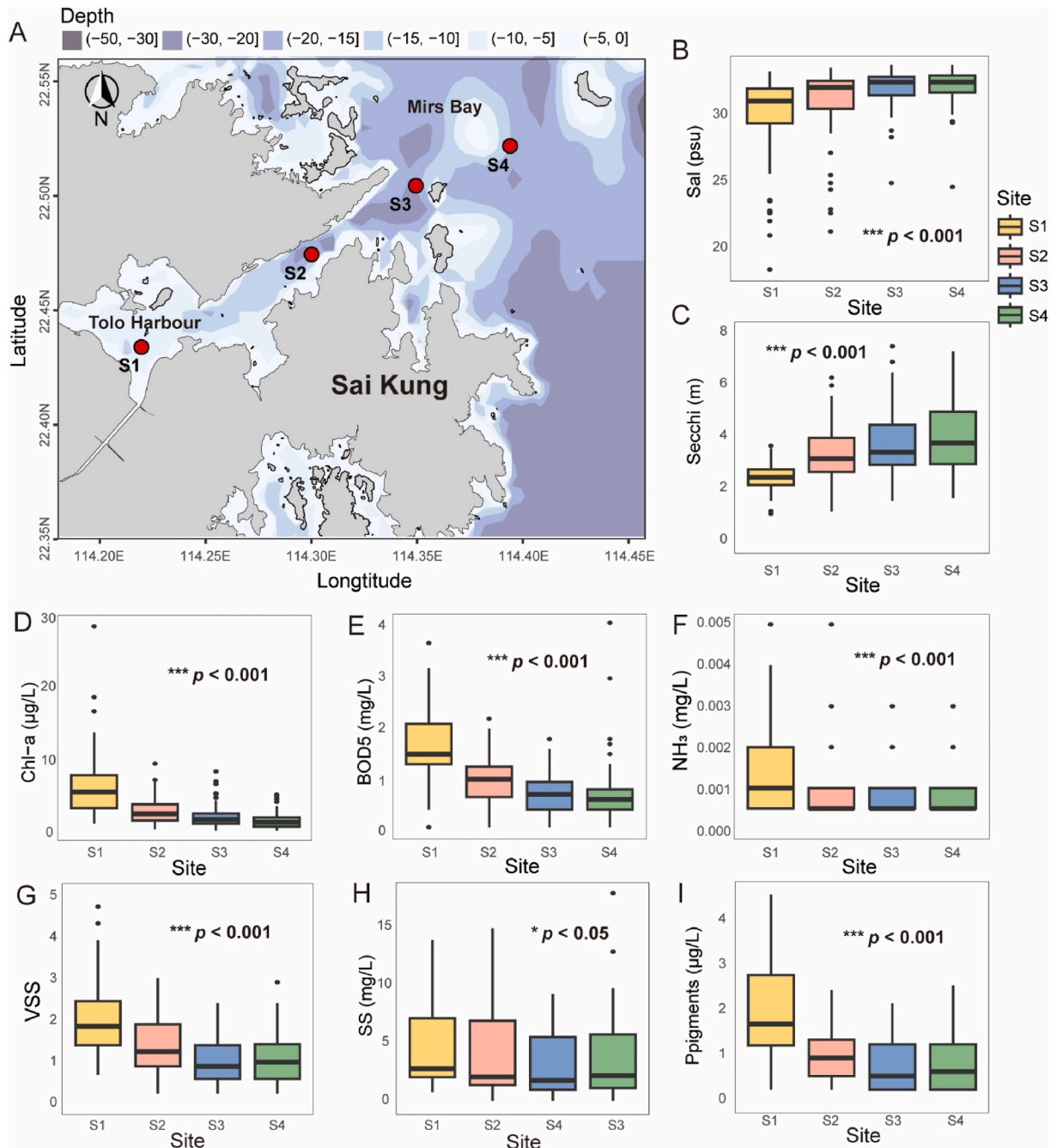


Fig. 1. (A) Sampling sites from Tolo Harbour to Mirs Bay in Hong Kong. (B-I) The changes of environmental variables with significance (* = $p < 0.05$, *** = $p < 0.001$) among four sites, including Sal (B), Secchi (C), Chl-a (D), BOD5 (E), NH₃ (F), VSS (G), SS (H), and Ppigmets (I).

and the resolution of identification; (2) assess the differences between water sampling and net sampling in diversity evaluation by metabarcoding, and (3) the discrepancy of these methods in identifying correlations between dominant species and environmental variables. Our findings will provide critical insights into the strengths and limitations of these approaches for zooplankton monitoring across small spatial scales.

2. Materials and methods

2.1. Environmental variables

To assess water quality in the study area, we obtained surface water data from the Environmental Protection Department (EPD)'s long-term monitoring program (available at <https://cd.epic.epd.gov.hk/>). At four sampling sites aligned with our eDNA and PlanktonScope surveys [i.e., TM4 (S1), TM8 (S2), MM17 (S3), and MM5 (S4), Fig. 1], we extracted the following monthly data for 24 environmental variables from six years (2018–2023): Volatile Suspended Solids (VSS) (mg/L), Unionised Ammonia (NH₃) (mg/L), Turbidity (Turb) (NTU), Total Phosphorus (TP) (mg/L), Total Nitrogen (TN) (mg/L), Total Kjeldahl Nitrogen (TKN) (mg/L), Total Inorganic Nitrogen (TIN) (mg/L), Temperature (Temp) (°C), Suspended Solids (SS) (mg/L), Silica (Si) (mg/L), Secchi Disc Depth (Secchi) (M), Salinity (Sal) (psu), Phaeo-pigments (Pigments) (µg/L), pH (pH), Orthophosphate Phosphorus (OP) (mg/L), Nitrite Nitrogen (NO₂-N) (mg/L), Nitrate Nitrogen (NO₃) (mg/L), Faecal Coliforms (F_{CFU}) (cfu/100 mL), *Escherichia coli* (*E. coli*) (CFU) (cfu/100 mL), Dissolved Oxygen (DO) (mg/L), Dissolved Oxygen (DO%) (%saturation), Chlorophyll-a (Chl-a) (µg/L), Ammonia Nitrogen (NH₄) (mg/L), 5-day Biochemical Oxygen Demand (BOD5) (mg/L). The six-year average data were utilized to depict the mean conditions of each site, as daily parameters may be significantly affected by transient weather events (Table S1). Additionally, PlanktonScope recorded DO (%sat), DO (mg/L), Sal (psu), pH and Temp (°C) while capturing plankton images were also analyzed (Table S2).

2.2. Field sampling

Field sampling was conducted on 17 January 2024 at four EPD monitoring stations along Tolo Harbour and Channel, progressing from the inner harbour (S1) through the middle (S2) and outer channel (S3) to the outer ocean (S4). Prior to sampling, four 2.5-L packs of Milli-Q® water (Milli-Q, MA, USA) were prepared as field controls (FC). At each site, four 2.5-L subsurface water replicates (1 m depth) were collected using sterile Niskin samplers (totalling 10 L per site, 40 L overall) for eDNA analysis, while concurrent net sampling filtered 50 L of surface water per replicate through a 25# plankton net (65 µm mesh) in order to capture both mesozooplankton and microzooplankton. Zooplankton was preserved in absolute ethanol (99%) and kept on ice in a cooler until extraction. All eDNA water samples were filtered the same day using 0.45 µm mixed cellulose ester (MCE) membranes (47 mm diameter, Hawach Scientific Co. Ltd.), selected for their high flow rate and filtration efficiency (Deiner et al., 2018), and stored at –80°C until DNA extraction.

2.3. PlanktonScope sampling and image processing

PlanktonScope is a battery-powered, self-contained shadowgraph imaging system specifically designed for in situ observation of zooplankton larger than 200 µm across a wide range of aquatic environments, including highly turbid coastal waters (Bi et al., 2022; Song et al., 2020). The system utilises red LED illumination in pulsed mode, producing strong strobe lighting to enhance image contrast and clarity. To minimise motion blur caused by boat and water movement, PlanktonScope employs a fast exposure time. It captures images at a frequency of 10 Hz, with each frame representing approximately 240 mL of

sampled water, enabling high-throughput and fine-scale resolution sampling of plankton communities. The system was mounted on the stern and submerged to ~1 m depth for 6–10 min at each site, continuously capturing images. The total estimated sampling volume is 0.25–1.65 m³, including 1.65 m³ for S1, 0.57 m³ for S2, 0.25 m³ for S3, and 0.35 m³ for S4, according to the number of captures. Note that variations in sampling duration were due to in situ conditions and boat traffic.

PlanktonScope images were processed through a multi-step workflow to detect, extract, and classify individual plankton targets (Fig. 2). Raw shadowgraph images were first pre-processed to enhance contrast and suppress background noise (Bi et al., 2015). A Mask R-CNN (Region-based Convolutional Neural Network) model was then applied to segment and identify regions of interest (ROIs) corresponding to potential plankton targets (Bi et al., 2024). This model provided both object boundaries and preliminary taxonomic labels. To improve classification accuracy, particularly for morphologically similar taxa, the segmented ROIs were reclassified using a ResNet-based convolutional neural network trained on a curated plankton image dataset (Cheng et al., 2019). This two-stage approach allowed for robust object detection and refined classification, reducing mislabelling across groups such as copepods, medusae, and larvaceans. Zooplankton densities were reported as individuals per cubic meter (ind./m³).

2.4. DNA extraction, PCR, and sequencing

DNA extraction was performed following our established protocol (How et al., 2024), with membranes containing water filtrates processed using the DNeasy PowerSoil Pro Kit (QIAGEN, Germany), while mesh-screened zooplankton samples were rinsed with water/ethanol through 40 µm meshes to remove salts and residual eDNA. The filtrate was visually examined under a dissecting microscope to ensure no loss of zooplankton due to rinsing. DNA was extracted with the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Laboratory controls (LC), consisting of filtered Milli-Q water, were processed alongside field samples to monitor contamination. DNA concentrations were quantified using Qubit dsDNA High-Sensitivity assays (Invitrogen, Carlsbad, CA, USA). An 8-bp overhanging, sample-specific index was appended to the 5' end of each primer to pool the sequencing libraries for cost efficiency (Kozich et al., 2013). All samples were amplified with indexed V9-18 S rRNA primers, 1389 F (5'-TTGTACACACCGCCC-3') and 1510 R (5'-CCTTCYGCAGGTTACCTAC-3'), targeting the V9 region (Amaral-Zettler et al., 2009). PCR reactions contained 5 µL template DNA (~10 ng), 25 µL KOD 2× premix (Toyobo), 1.5 µL of each 10 µM primer, and 17 µL ddH₂O, using thermal cycling parameters of 98°C for 2 min; 35 cycles of 98°C for 10 s, 55°C for 5 s, and 68°C for 1 s; followed by 68°C for 2 min. Equimolar pools of 10 PCR products (normalized by Qubit quantification) were sequenced on an Illumina NovaSeq PE150 platform (San Diego, CA, USA) at Novogene (Beijing, China).

2.5. eDNA and environmental data analyses

Raw data was demultiplexed using Sabre (<https://github.com/najoshi/sabre>), with no mismatches allowed. Primer removal was performed using Cutadapt 4.8 with a maximum of 2 mismatches allowed for paired reads (Martin, 2011). Meanwhile, a reference dataset was created by using the keywords “18 S” and “invertebrates” to download data from the National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/>). Using CRABS software, we conducted *in silico* PCR and pairwise global alignment to build a primer-specific 18S reference database (Jeunen et al., 2023). QIIME 2 (v.2024.5) was employed for the subsequent processing steps (Caporaso et al., 2010). First, the DADA2 plugin was used to truncate, merge, and filter chimaeras based on their sequencing quality. Subsequently, representative sequences were categorised and annotated using a hybrid mode against the reference dataset. The read counts of the ASVs present in the FCs and LC were

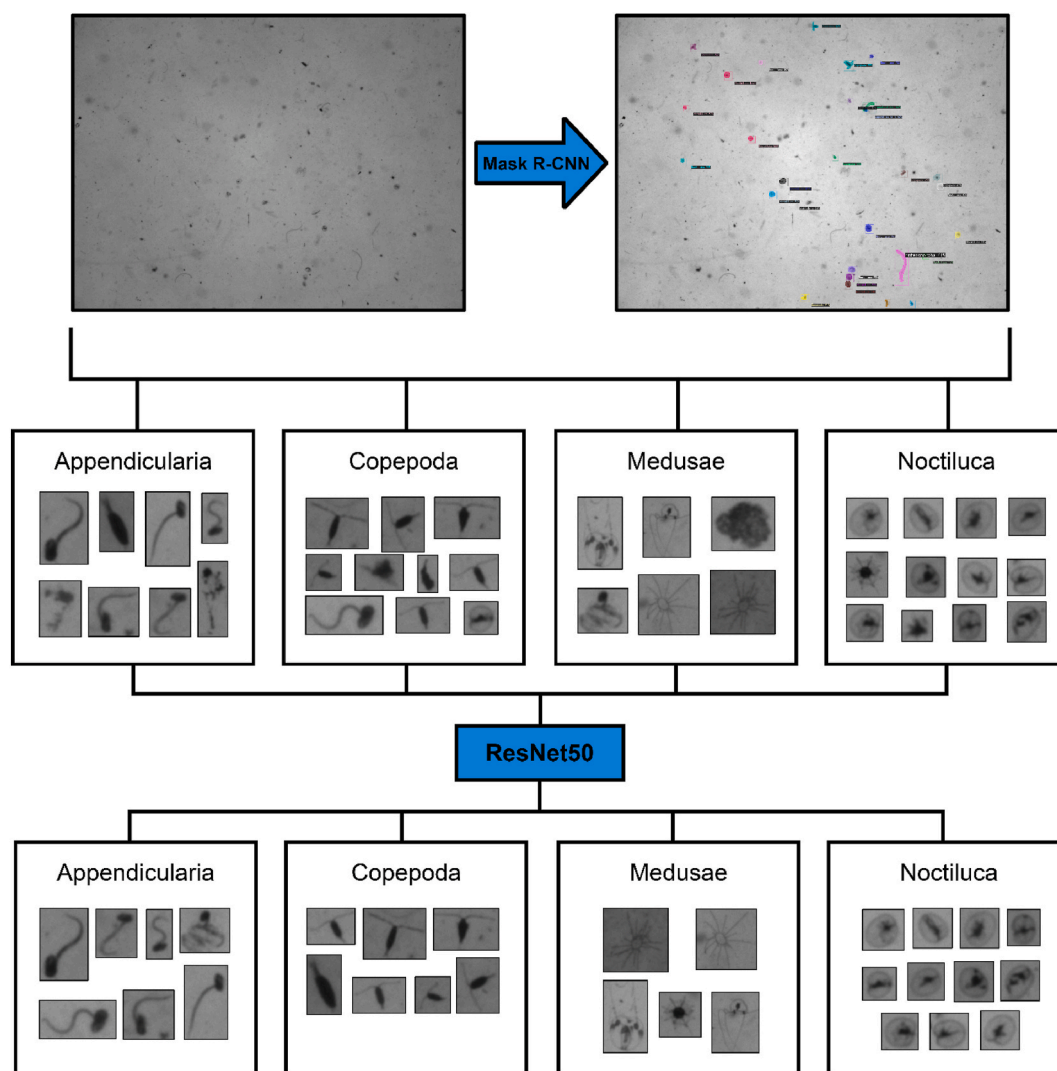


Fig. 2. Image processing workflow and representative plankton imagery collected by PlanktonScope. (A) The upper left panel shows a raw shadowgraph image captured in situ by PlanktonScope at 10 Hz, with each frame representing approximately 240 mL of sampled water. (B) The upper right panel displays predicted targets corresponding to the raw image, as detected by the Mask R-CNN model. (C) The middle panel shows segmented targets along with the initial classification results from the Mask R-CNN procedure; note the presence of misclassifications across different groups. (D) The lower panel presents examples of reclassified targets using a ResNet model, demonstrating improved accuracy in taxonomic assignment.

subtracted from the sample data. To focus on aquatic zooplankton, ASVs below the genus level containing “Insecta”, “vertebrate”, “environmental”, and “uncultured” were also omitted. After filtering, ASVs that appeared in fewer than two samples with a cumulative frequency below 10 were eliminated. Taxa that were only annotated to the genus level and were lacking a species name were referred to as “sp.” in the following analysis. Although *Noctiluca* are dinoflagellates, they were considered zooplankton as they are heterotrophic. Then, we applied WoRMS’s “Taxon match” tool (<https://www.marinespecies.org/index.php>) to exclude terrestrial creatures and cross-reference the taxonomic information. We further refined the species list by confirming that they have been reported from the Asia-Pacific region, based on literature and a local species collection (Zhao et al., 2025).

Multicollinearity analysis was applied to investigate the correlation of the 24 environmental variables. Among those showing high correlations ($VIF = 1/(1 - R^2)$, $VIF > 10$), only one was retained for further investigation. A one-way analysis of variance (ANOVA) was used to determine the environmental variables’ statistically significant differences across the four sites using R Studio (v.2023.9.1.0) (Dixon, 2003). The sampling map was also generated by R Studio. The “iNEXT” package was used to generate rarefaction curves (Hsieh et al., 2016). Data was

rarefied to the lowest total read counts before downstream analysis. Diversity analyses were conducted using the “vegan” package, and the corresponding figures were generated using “ggplot2”. Shannon index and Pielou’s evenness were calculated to evaluate the alpha diversity for samples collected using different methods. Similarity percentage (SIMPER) (Clarke and Warwick, 1994) was adopted to determine the dominant species based on the square root-transformed community data using PRIMER-e software (Auckland, New Zealand). Cluster analysis was calculated from the square roots of the Bray-Curtis distances of community matrices through PRIMER-e software as well, and the significance of each cluster was validated by SIMPROF ($p < 0.01$). BIOENV was chosen to identify the most significant environmental variables, based on Spearman correlation, Bray-Curtis dissimilarity of the community matrices, and Euclidean distances among environmental variables. The associations between dominant and SIMPER-selected taxa and BIOENV-selected variables were determined through Fourth Corner analysis (Dray et al., 2014).

3. Results

3.1. Environmental gradients in the study area

The sampling sites from Tolo Harbour (S1), along the Tolo Channel (S2, S3), and towards Mirs Bay (S4) represented a waterway with changing water quality leading to the ocean (Fig. 1A). The monthly averages of 24 water quality parameters from 2018 to 2023 were calculated (Table S1). One-way ANOVA tests revealed significant differences ($p < 0.05$) in 16 of the 24 variables across the four sampling sites (Table S1). A multi-collinearity test indicated that many variables were strongly correlated ($VIF > 10$), leading to the removal of 10 variables from further analysis. Among the remaining variables with significant site differences, salinity and Secchi disc depth (Secchi) showed an increasing trend from the harbour (S1) to the open ocean (S4) (Fig. 1B and C). In contrast, chlorophyll-a (Chl-a), BOD5, unionised ammonia (NH_3), volatile suspended solids (VSS), suspended solids (SS) and

phaeopigments (Ppigm) exhibited a decreasing gradient from the harbour (S1) to the channel (S2 and S3) and the outer ocean (S4) (Fig. 1D–I). In addition to the data provided by the EPD, the environmental parameters collected by the PlanktonScope while capturing images were also analyzed, which revealed very significant changes: while salinity gradually increased from the inner area to the open sea, DO, pH and Temp gradually decreased from S1 to S3 and then slightly increased at S4 (Fig. S1). Overall, these parameters revealed a clear gradient of water quality in our study area.

3.2. Comparison between PlanktonScope and eDNA approaches for taxa exhibiting abundance gradients

PlanktonScope revealed distinct relative abundance gradients in three taxonomic groups (Medusae, Noctiluca, and Copepoda) across the sampling sites (Figs. 2 and 3). The medusae community consisted primarily of small-sized juveniles (200–400 μm). Taxonomic annotation of

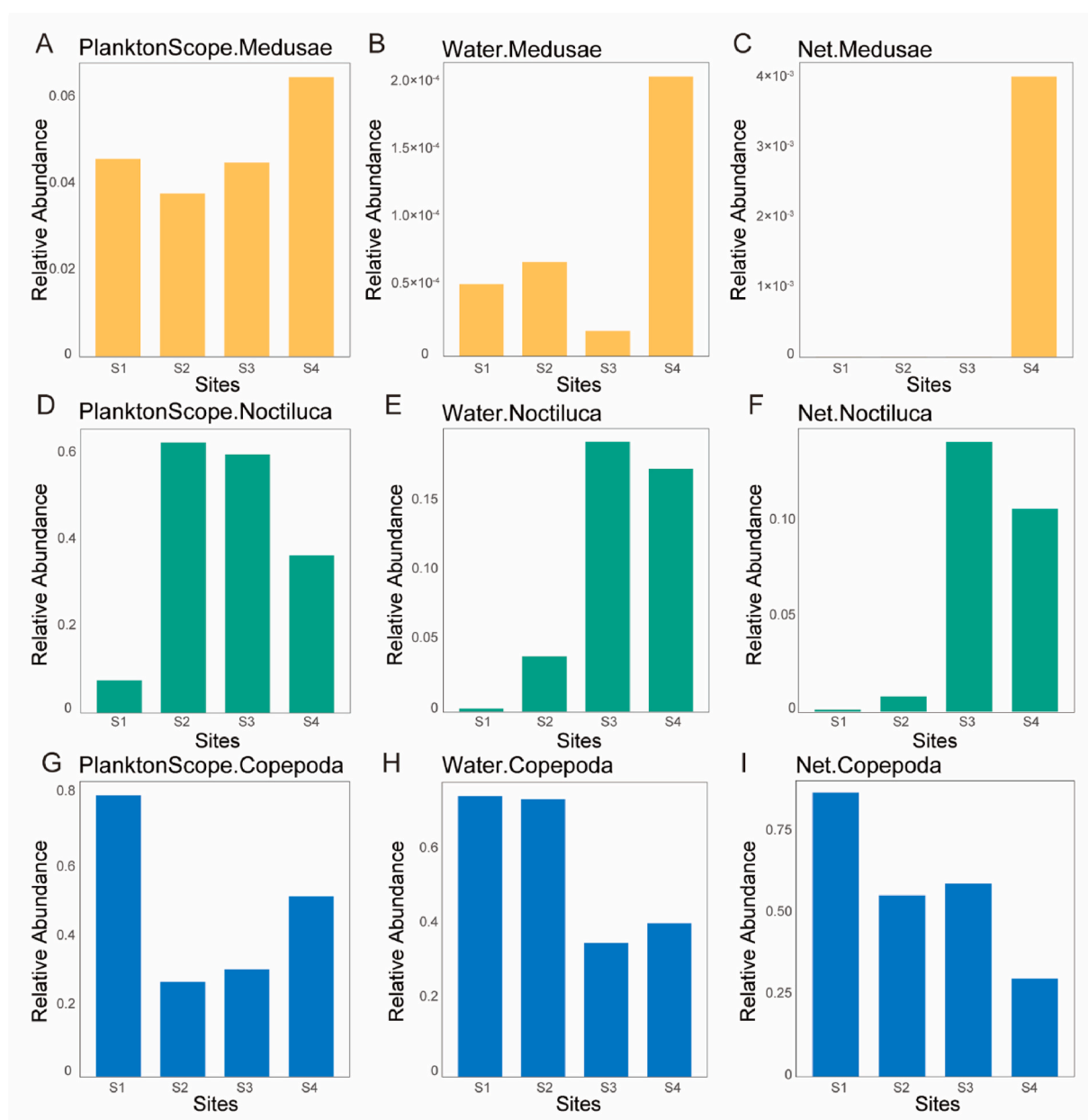


Fig. 3. Comparison of the detection results of Medusae, Noctiluca, and Copepoda using PlanktonScope and metabarcoding of water and net samples. The results of PlanktonScope (A, D, G), water eDNA (B, E, H), and net (C, F, I) samples are shown in relative abundance.

eDNA data identified *Aurelia* sp. and *Liriope tetraphylla*, with *L. tetraphylla* accounting for 99.7% of total medusa reads. PlanktonScope live imaging revealed that the densities of medusae increased progressively from approximately 100 ind./m³ at the inner harbour (S1) to about 3000 ind./m³ in the outer ocean (S4) (Table 1). The three approaches detected similar spatial trends in relative abundance for medusae, though with different proportions at the middle sites, consistently showing the highest proportion at the ocean site (Fig. 3A–C).

For *N. scintillans*, PlanktonScope data demonstrated a spatial distribution pattern similar to that of Medusae, with high densities in the outer channel (S3) and outer ocean (S4) (>15,000 ind./m³), decreasing sharply with only 184 ind./m³ at the innermost station (S1) (Table 1). The relative abundance of *N. scintillans* exhibited a consistent spatial pattern as that identified by the two eDNA-based methods, with significantly higher relative abundances at outer stations compared to S1. However, PlanktonScope detected a high relative abundance in S2, contrary to the two eDNA methods (Fig. 3D–F).

Similarly, three approaches revealed concordant spatial trends, with the relative abundance of copepods decreasing from inner (S1) to outer sites (S4) (Fig. 3G–I). However, PlanktonScope detected the lowest absolute density of copepods in the harbour (S1; ~1935 ind./m³), increasing along the spatial gradient to 25,353 ind./m³ in the outer ocean (S4). Additionally, metabarcoding approaches identified several copepod species, including *Temora* sp., *C. typicus*, *P. crassirostris*, and *B. similis* (Fig. S2). *Temora* sp. was dominant only in the inner harbour (S1 and S2). Its proportion decreased sharply in S3 and S4, from more than half of the total zooplankton abundance to less than 10%. Conversely, *C. typicus* showed the opposite trend, becoming the dominant species in S3 and S4. *B. similis* and *P. crassirostris* varied significantly in relative read abundances across sites, while *Oithona* sp. 1 was only dominant in S3 net samples.

3.3. Comparison of eDNA results between the water and net samples

The sequencing run generated 23,363,687 reads for the water samples and 23,174,636 reads for the net samples. After demultiplexing, denoising, merging reads, and applying quality filtering, we retained 21,912,374 for the water samples and 21,900,041 for the net samples. We excluded the non-zooplankton taxa (Table 1), including the bloom-forming *Phaeocystis* commonly found along China's coastal waters (Song et al., 2023), which were commonly observed in both the PlanktonScope and eDNA surveys and those only annotated to the family level and above. The controls contained an average of 2828 reads—orders of magnitude lower than the samples—so we subtracted these from the sample counts. Then, we removed taxa detected in fewer than two samples or with a total frequency below 10. The rarefaction curves for the sequencing data reached asymptotes, indicating that our sampling and sequencing efforts were adequate (Fig. S3). On average, each water sample contained an average of 106,383 targeted reads, corresponding to 94 zooplankton ASVs; while each net sample contained 422,346 targeted reads, corresponding to 85 zooplankton ASVs (Fig. S4A).

At the class level, both sampling methods detected similar zooplankton compositions, with Copepoda (29.70% to 85.91% of reads) and Polychaeta (3.61% to 30.49% of reads) dominating across sites

(Fig. 4A and B). However, some taxa were represented by far more reads in water samples. For instance, Dinophyceae accounted for 29.62% in S3 and 22.09% in S4—more than double the proportions in net samples (11.46% in S3 and 7.68% in S4). Conversely, Bivalvia and Thecostraca were more prevalent in net samples, particularly in S4, where they made up 18.88% and 11.58% reads, respectively, compared to just 8.38% (Bivalvia) and 0.19% (Thecostraca) in water samples.

At the species level, eDNA metabarcoding detected similar composition in the water and net samples, sharing nine of the top 15 species: *Temora* sp., *Parvocalanus crassirostris*, *Noctiluca scintillans*, *Bestiolina similis*, *C. typicus*, *Prionospio elongata*, *Candacia* sp., *Chaetopterus* sp., and *Pseudebadne tergestina* (Fig. 4C and D). These nine species dominated, representing 86.9% of total reads in water samples and 74.1% in net samples. Copepods—particularly *Temora* sp.—were the most dominant zooplankton group, declining gradually from S1 to S4 in both eDNA datasets (Fig. 4A–D). In contrast, the dominant Dinophyceae species, *Noctiluca scintillans*, increased in relative abundance from the inner harbour to the outer channel before slightly decreasing at the oceanic site.

We also observed key differences in copepod composition between the two approaches. For example, water samples revealed that *B. similis* thrived in the inner harbour (10.65% in S1) but gradually declined towards the ocean (2.64% in S2 and 3.80% in S3). Net samples, however, showed similar relative abundances of *B. similis* from S1 to S3 (6.74% to 10.51%), with only a modest detection in S4 from both methods. Additionally, *Candacia* sp. was abundant in S3 water samples (12.01%) but constituted only a minor proportion in net samples.

3.4. Comparison of metabarcoding data from water and net samples

We calculated biodiversity indices to assess how sampling methods (net vs. water) influenced eDNA-based community composition (Fig. 5A–E). Net samples exhibited significantly higher Shannon diversity (Fig. 5A) and Pielou's evenness (Fig. 5C) than water samples. However, we detected no significant difference in total ASV counts between the two methods (Fig. 5B). Cluster analysis grouped samples into four distinct clusters, with samples from the same site clustering together for both water and net eDNA (Fig. 5D and E).

We applied SIMPER analysis to identify taxa responsible for clustering patterns (Table 2, Table S3). Between-group comparisons revealed that net and water samples differed significantly (dissimilarity = 0.677; ANOSIM, $R = 0.184$, $p = 0.007$). Copepoda contributed the most to dissimilarity (>20%), followed by Bivalvia, Dinophyceae, Branchiopoda, Thecostraca, and Polychaeta (Table S3). Among the copepods, *Temora* sp., the most abundant species, contributed to 14.77% of dissimilarity between the two methods, while the dinoflagellate *N. scintillans* contributed 6.75%.

Among-group SIMPER analysis highlighted dominant species within each cluster (Table 2 listed taxa contributing to the top 60% of similarity). Half of these dominant species overlapped between methods, including *Temora* sp., *B. similis*, *P. crassirostris*, *Chaetopterus* sp., *N. scintillans*, *Candacia* sp., *C. typicus* and *P. tergestina*.

For Cluster 1, the copepod *Temora* sp. Dominated, contributing 25.83% (water) and 33.11% (net) of within-group similarity, followed

Table 1

Community distribution of six major plankton groups captured by PlanktonScope along the transect from Tolo Harbour to Mirs Bay.

Group	S1		S2		S3		S4	
	Mean (ind/m ³)	Std	Mean (ind/m ³)	Std	Mean (ind/m ³)	Std	Mean (ind/m ³)	Std
Appendicularia	2.4	5.8	155.8	87.7	1096.5	362.1	2378.3	746.7
Chaetognatha	-	-	-	-	8.8	15.2	43.3	48.4
Copepoda	1935.1	1171.0	3660.7	2312.2	8871.3	8162.8	25353.3	10975.5
Medusae	112.7	41.1	521.7	245.9	1330.8	1061.6	3255.0	764.2
Noctiluca	184.2	61.0	8644.8	1463.6	17672.7	4175.9	18296.7	8905.7
Phaeocystis	231.8	69.5	867.5	286.2	655.9	491.4	1028.3	256.6

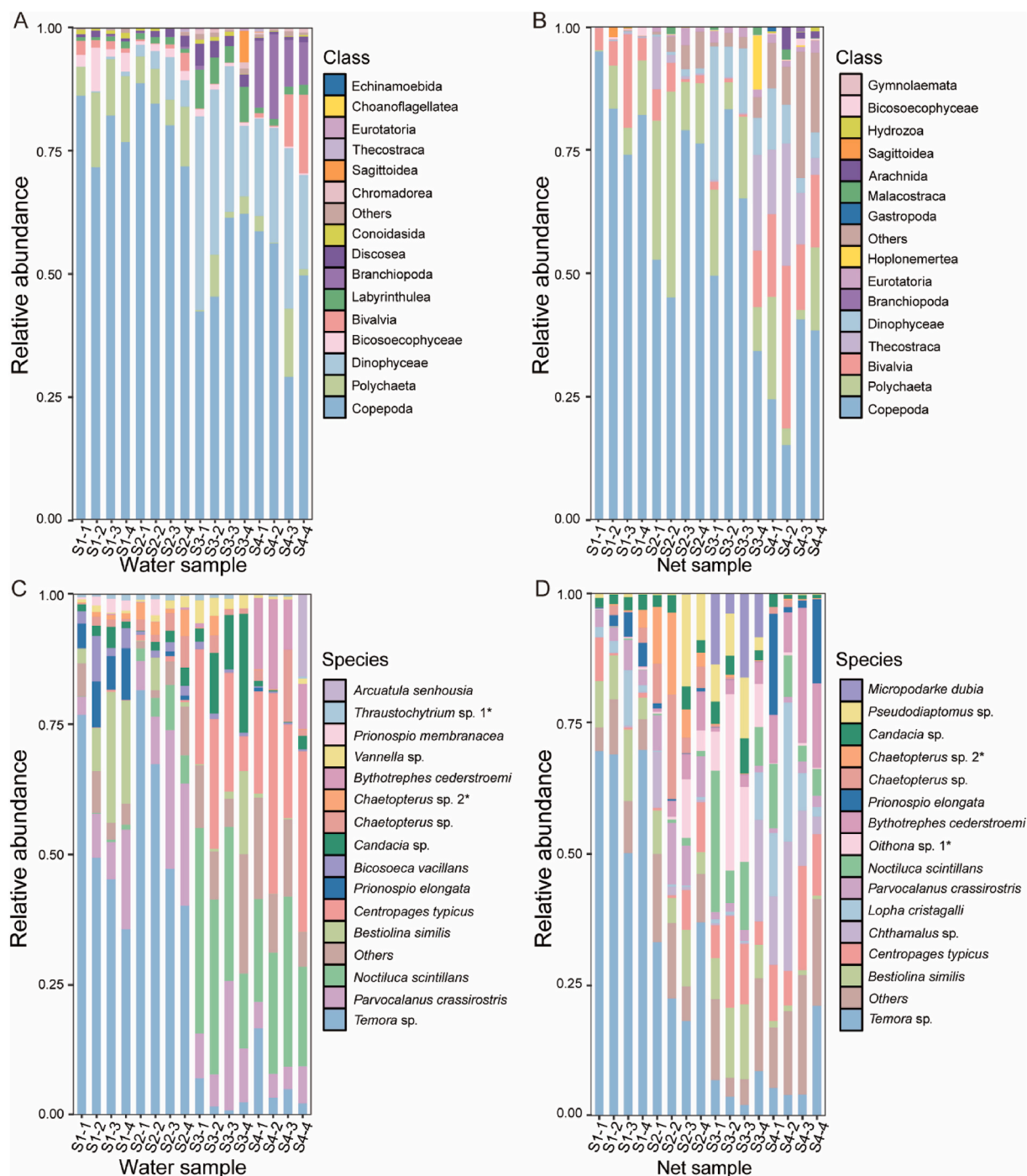


Fig. 4. Comparison of the zooplankton taxa identified by eDNA metabarcoding of water and net samples. Relative abundance of the top 15 zooplankton groups detected by metabarcoding of (A) water and (B) net samples. Relative abundance of top 15 zooplankton species detected by metabarcoding of (C) water and (D) net samples. *Thraustochytrium* sp. 1 means *Thraustochytrium* sp. KL1 identified by annotation, and *Chaetopterus* sp. 2 means *Chaetopterus* sp. 3 J M, 2017. *Oithona* sp. 1 New Caledonia-RJH-2001 is abbreviated as *Oithona* sp. 1.

by the bivalve *B. similis* (10.12% water, 10.58% net) (Table 1). For Cluster 2, *Temora* sp. Again drove grouping (31.57% water, 16.49% net), and *P. crassirostris* was the secondary contributor (13.63% water, 9.32% net). For Cluster 3, water sample similarity was mainly contributed by *N. scintillans* and *C. typicus* (>30% combined within-group similarity), while net samples depended on *Oithona* sp. 1, *B. similis*, and *N. scintillans* (~30% within-group similarity). For Cluster 4, both methods shared key contributors (*C. typicus*, *N. scintillans*, *P. tergestina*, and *Temora* sp.), but their rankings differed.

3.5. Influence of environmental variables on community structure

We used BIOENV analysis to identify environmental variables affecting zooplankton community structures (Table 3), finding that turbidity and orthophosphate phosphorus (OP) produced the highest correlation coefficients for both water (0.845) and net samples (0.797), with no improvement from adding more variables. Incorporating these two variables into fourth-corner analysis revealed that community responses differed substantially between the two methods (Fig. 6). While some taxa like *Chthamalus* sp., *Vannella* sp., *Micropodarkie dubia*, and

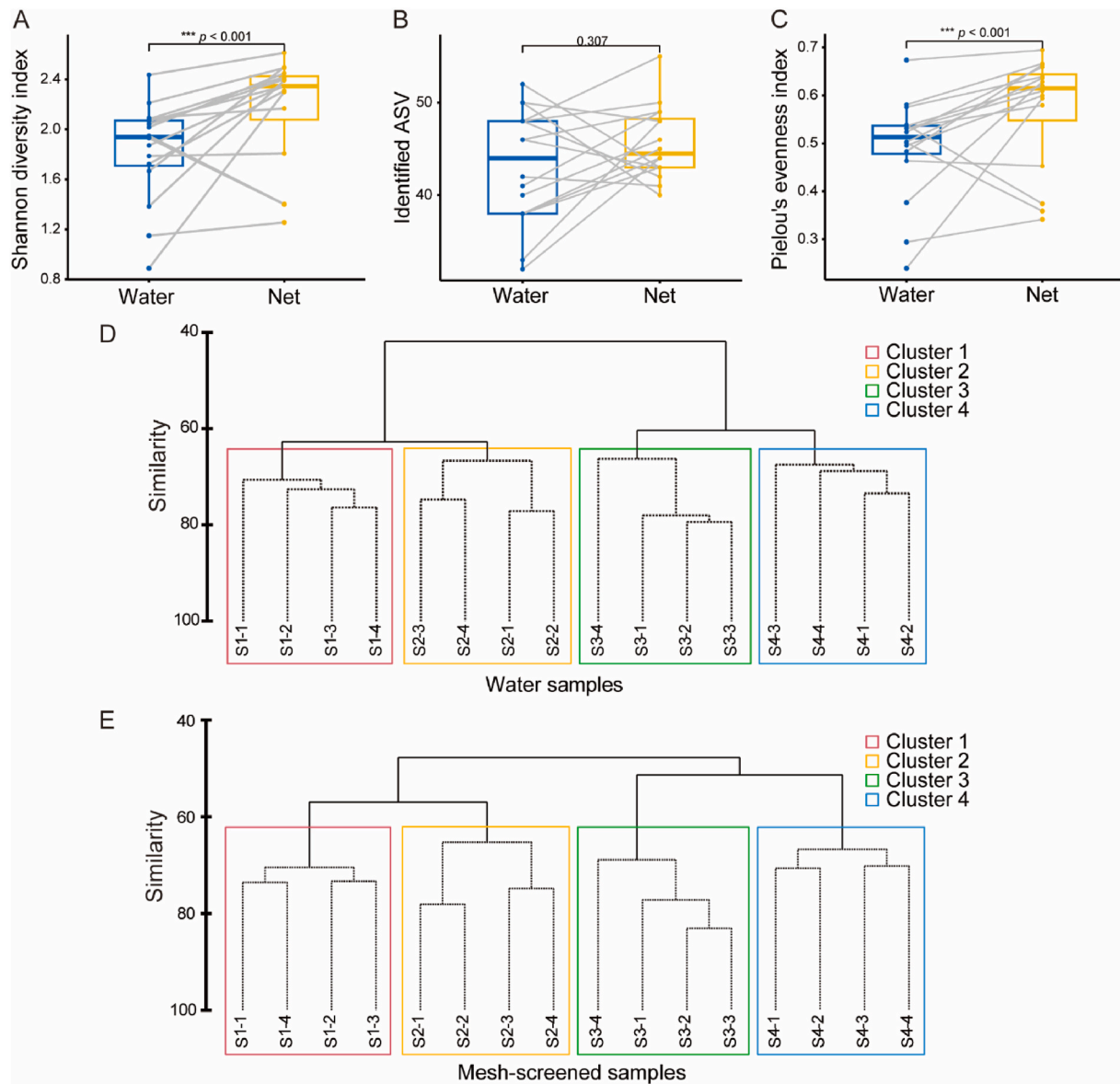


Fig. 5. Pairwise comparison of α diversity indices, including (A) Shannon diversity index, (B) identified ASV numbers and (C) Pielou's evenness index. The significance was assessed using the Wilcoxon U test ($*** = p < 0.001$). CLUSTER analysis results based on zooplankton species abundance data identified by eDNA metabarcoding of water (D) and net (E) samples. The significance of each clade in the cluster diagram was determined using the SIMPROF test. The solid lines indicate a significant difference ($p < 0.01$) between the two branches, whereas the dashed lines indicate no significant difference. Samples in the red box correspond to Cluster 1, the yellow box to Cluster 2, the green box to Cluster 3, and the blue box to Cluster 4.

Prionospio membranacea showed opposite correlations between water and net samples, other species, including *N. scintillans*, *Bicosoeca vacillans*, *L. cristagalli*, *P. elongata*, and *Chaetopterus* sp., displayed highly similar responses to turbidity and OP changes. Notably, the polychaete *P. elongata* exhibited a strong positive correlation with both variables, whereas the copepods *Oithona* sp. 1 and *Pseudodiaptomus* sp. exhibited strong negative correlations. In contrast, *Temora* sp. maintained consistently positive correlations with turbidity and OP across both methods. Similarly, *P. elongata* and *Temora* sp. were positively correlated with PlanktonScope-recorded environmental data except DO, but other taxa showed limited correlation (Fig. S6). Overall, these results indicate that different groups of zooplankton respond differently to the water quality gradient, represented by turbidity and OP, in our study area.

4. Discussion

This study highlights the complementary strengths of imaging and

eDNA approaches. While the imaging system excels at capturing fine-scale spatial distributions, it provides limited taxonomic resolution. In contrast, eDNA methods offer greater insight into species diversity, but the relative read abundance may be influenced by sample collection methods, including water sampling techniques, net type, and the depth from which samples were taken.

4.1. Cross-validation between monitoring strategies reveals congruent spatial trends in three common zooplankton groups

DNA-based metabarcoding and automatic imaging systems each offer unique advantages and disadvantages for zooplankton monitoring. Metabarcoding provides superior species resolution and detects a broad range of species via molecular identification, although its effectiveness is contingent on comprehensive reference databases and appropriateness of the primers and PCR protocols (Holland et al., 2025). In contrast, imaging systems such as PlanktonScope deliver absolute abundance and

Table 2

Results of SIMPER analysis showing species with >60% cumulative contribution to station group similarities detected by DNA metabarcoding of water and mesh-screened samples. Clusters were determined by Cluster analysis. Clusters 1 to 4 correspond to samples collected from S1 to S4, respectively. *Thraustochytrium* sp. 1 means *Thraustochytrium* sp. KL1, and *Chaetopterus* sp. 2 means *Chaetopterus* sp. 3 J M, 2017. *Oithona* sp. 1 New Caledonia-RJH-2001 is abbreviated as *Oithona* sp. 1.

Species	Class	Mean abundance	%contribution	Cumulative %contribution
Cluster 1				
Water samples				
<i>Temora</i> sp.	Copepoda	118.75	25.83	25.83
<i>Bestiolina similis</i>	Bivalvia	58.06	10.12	35.95
<i>Prionospio elongata</i>	Polychaeta	44.99	9.77	45.72
<i>Parvocalanus crassirostris</i>	Copepoda	49.66	9.43	55.15
<i>Bicosoeca vacillans</i>	Bicosoecophyceae	31.99	5.86	61.00
Net samples				
<i>Temora</i> sp.	Copepoda	134.11	33.11	33.11
<i>Bestiolina similis</i>	Bivalvia	48.23	10.58	43.70
<i>Parvocalanus crassirostris</i>	Copepoda	31.37	7.19	50.89
<i>Candacia</i> sp.	Copepoda	25.40	5.89	56.79
<i>Lopha cristagalli</i>	Bivalvia	30.51	5.53	62.31
Cluster 2				
Water samples				
<i>Temora</i> sp.	Copepoda	126.65	31.57	31.57
<i>Parvocalanus crassirostris</i>	Copepoda	64.39	13.63	45.20
<i>Noctiluca scintillans</i>	Dinophyceae	36.34	8.12	53.32
<i>Chaetopterus</i> sp.	Polychaeta	28.73	6.33	59.65
<i>Chaetopterus</i> sp. 2*	Polychaeta	27.28	5.86	65.52
Net samples				
<i>Temora</i> sp.	Copepoda	87.16	16.49	16.49
<i>Parvocalanus crassirostris</i>	Copepoda	47.66	9.32	25.81
<i>Bestiolina similis</i>	Bivalvia	43.21	7.82	33.63
<i>Chaetopterus</i> sp. 2*	Polychaeta	46.77	7.56	41.18
<i>Chaetopterus</i> sp.	Polychaeta	45.62	6.96	48.14
<i>Candacia</i> sp.	Copepoda	29.05	5.56	53.71
<i>Pseudevadne tergestina</i>	Branchiopoda	31.03	4.71	58.42
<i>Centropages typicus</i>	Copepoda	30.06	3.66	62.08
Cluster 3				
Water sample				
<i>Noctiluca scintillans</i>	Dinophyceae	88.94	17.32	17.32
<i>Centropages typicus</i>	Copepoda	71.04	13.54	30.86
<i>Parvocalanus crassirostris</i>	Copepoda	55.95	9.83	40.69
<i>Candacia</i> sp.	Copepoda	53.95	8.93	49.62
<i>Oblongichytrium</i> sp.	Labyrinthulea	36.90	6.97	56.59
<i>Vannella</i> sp.	Amoebozoa	29.27	5.65	62.24
Net samples				
<i>Oithona</i> sp. 1*	Copepoda	63.53	10.18	10.18
<i>Bestiolina similis</i>	Bivalvia	53.02	9.59	19.77
<i>Noctiluca scintillans</i>	Dinophyceae	58.29	9.38	29.14
<i>Micropodarke dubia</i>	Polychaeta	52.18	8.72	37.86
<i>Centropages typicus</i>	Copepoda	51.00	8.48	46.34
<i>Pseudodiaptomus</i> sp.	Copepoda	43.86	7.42	53.75
<i>Temora</i> sp.	Copepoda	37.30	6.15	59.91
<i>Candacia</i> sp.	Copepoda	33.02	5.75	65.65
Cluster 4				
Water samples				
<i>Centropages typicus</i>	Copepoda	86.04	18.69	18.69
<i>Noctiluca scintillans</i>	Dinophyceae	80.85	18.47	37.15
<i>Pseudevadne tergestina</i>	Branchiopoda	57.71	12.76	49.91
<i>Parvocalanus crassirostris</i>	Copepoda	38.18	8.74	58.65
<i>Temora</i> sp.	Copepoda	39.76	6.96	65.61
Net samples				
<i>Pseudevadne tergestina</i>	Branchiopoda	61.80	10.83	10.83
<i>Centropages typicus</i>	Copepoda	57.33	10.49	21.32
<i>Chthamalus</i> sp.	Thecostraca	57.00	9.37	30.69

(continued on next page)

Table 2 (continued)

Species	Class	Mean abundance	%contribution	Cumulative %contribution
<i>Lopha cristagalli</i>	Bivalvia	52.82	7.64	38.33
<i>Noctiluca scintillans</i>	Dinophyceae	43.02	7.49	45.81
<i>Temora</i> sp.	Copepoda	45.34	7.24	53.05
<i>Prionospio elongata</i>	Polychaeta	44.24	5.59	58.65
<i>Musculus</i> sp.	Bivalvia	29.66	5.32	63.97

Table 3

Results of BIOENV procedures showing the best matches between the biotic and environmental similarity matrices, as determined by the spearman rank correlation (Corr.). 14 environmental factors included: 1, VSS; 2, NH₃; 3, Turb; 4, OP; 5, Si; 6, Secchi; 7, Sal; 8, Ppigmets; 9, pH; 10, NO₂; 11, Chl-a; 12, BOD5; 13, TP; 14, SS.

No.	Water samples			Net samples		
	No. Vars	Corr.	Selections	No. Vars	Corr.	Selections
1	2	0.845	3, 4	2	0.797	3, 4
2	3	0.845	3, 7, 10	3	0.797	3, 7, 10
3	4	0.845	1, 3, 9, 10	4	0.797	1, 3, 9, 10
4	5	0.8381	1, 2, 4, 5, 9	5	0.765	1, 3, 4, 9, 10
5	6	0.8381	1, 2, 4, 5, 7, 9	6	0.7469	1, 2, 4, 5, 7, 9
6	7	0.8381	1, 2, 4, 6, 7, 9, 12	7	0.7469	1, 2, 4, 6, 7, 9, 12
7	8	0.8381	1-4, 6, 9-11	8	0.7469	1-4, 6, 9-11
8	9	0.8381	1-4, 6, 7, 9-11	9	0.7469	1-4, 6, 7, 9-11
9	10	0.8381	1, 3, 4, 6-12	10	0.7469	1, 3, 4, 6-12

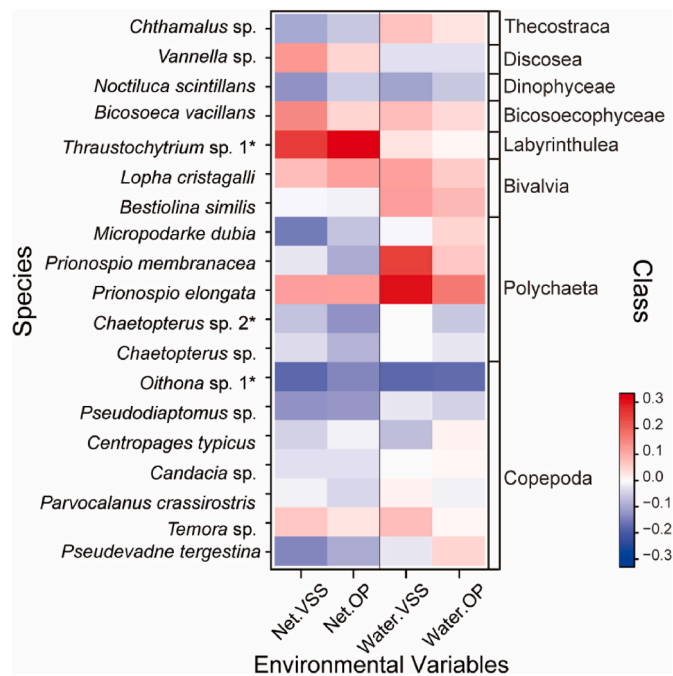


Fig. 6. Results of fourth-corner tests. Positive associations are shown by red, and negative associations are represented by blue. “water.” and “net.” corresponds to water and net samples for eDNA analyses, respectively. The left is annotated with species names, corresponding to the taxonomic group on the right panel. *Thraustochytrium* sp. 1 represents *Thraustochytrium* sp. KL1, and *Chaetopterus* sp. 2 means *Chaetopterus* sp. 3 J M, 2017. *Oithona* sp. 1 New Caledonia-RJH-2001 was abbreviated as *Oithona* sp. 1.

allow real-time capture of morphological features, including shape, size, and developmental stages—characteristics that molecular methods cannot discern (Ciranni et al., 2024). Our comparison of the relative abundance of three common taxonomic groups (Noctiluca, Medusae,

and Copepoda) revealed generally congruent distribution patterns across the three methods. Nonetheless, notable differences were observed. For instance, while the relative abundance pattern of Medusae was similar across all approaches, PlanktonScope identified them as a common group of zooplankton, with abundance increasing from approximately 100 ind./m³ at the inner harbour (S1) to about 3000 ind./m³ in the outer ocean (S4). In contrast, Medusae represented only a minor taxon in the two eDNA datasets, with read counts ranging from 0 to 4112 only. The underrepresentation of Medusae in the metabarcoding data is likely attributable to their tissue fragility and high water content, resulting in poor retention of DNA during filtration, particularly for net samples, as observed in previous studies (Minamoto et al., 2017; Remsen et al., 2004). Jellyfish blooms have been reported in coastal region, causing negative effects on marine ecological health (Lee et al., 2023; Sun et al., 2020). The exceptionally high medusae abundance recorded by PlanktonScope (up to 3000 ind./m³) is notable because they act as both top-down predators and contributors to bottom-up nutrient regeneration in the marine food web (Wang et al., 2026). Such elevated densities may indicate a jellyfish bloom in the harbour. However, the exact causes remain unknown. Further investigation combining time-series sampling and environmental characterization is warranted to clarify the ecological implications of such high medusae densities.

For copepods, body size and swimming ability can influence eDNA recovery rates (Ershova et al., 2021), yet relative abundance patterns were consistent among the different methods (Fig. 4, S2; Table 1). This agreement was apparent when the PlanktonScope counts were expressed as relative abundance [e.g., the copepods’ average density (1935.1 ind./m³) divided by the total density of detected zooplankton (2466.2 ind./m³)]. Intriguingly, when PlanktonScope counts were presented as individuals per cubic metre, the spatial trend of zooplankton was negatively correlated with relative eDNA read abundances. This result suggests that relative abundance, rather than absolute density in imaging data, should be used when correlating with eDNA read abundance. Compared to imaging methods, eDNA analysis offers higher taxonomic resolution, enabling more precise characterisation of local biodiversity, elucidation of biological relationships, and exploration of associations between species and environmental or geographical parameters (Di Capua et al., 2023; Schroeder et al., 2020). In our study, metabarcoding enabled species-level resolution of copepod trends, revealing opposing spatial patterns for *Temora* sp. (decreasing towards the open ocean) and *C. typicus* (increasing towards the ocean) (Fig. S2). These contrasting spatial distributions align with previous studies, suggesting habitat partitioning and environmental preferences (Mazzocchi and Di Capua, 2004). Conversely, differences in DNA composition and sample quantity may introduce PCR amplification biases, potentially contributing to the observed spatial gradient. Zooplankton size is a crucial indicator in marine ecosystems, reflecting demographic structure, species diversity and trophic status (Ratnarajah et al., 2023), yet this factor remains underexplored in eDNA assessments. PlanktonScope, as an auto-imaging system, could capture metrics such as individual length, or size-class distribution, supplementing molecular methods with more detailed species information (Zhang et al., 2024). By incorporating metrics in the future, researchers can better account for sampling biases and establish a more robust framework for interpreting method-specific strengths and limitations. Overall, this comparison underscores the complementary strengths of each approach: PlanktonScope provides

robust quantitative imaging data, while metabarcoding offers superior taxonomic resolution. Despite these methodological differences, our cross-validation demonstrates that metabarcoding using either water eDNA or mesh-screened tissues effectively captures major abundance trends in zooplankton communities.

4.2. DNA source matters: water versus net samples

Recent studies have extensively compared eDNA and traditional microscopic identification methods for biodiversity assessment (Czeglédi et al., 2021; Du et al., 2024; Ershova et al., 2021; Koda et al., 2023). While eDNA metabarcoding excels at detecting more species, including rare and microscopic species across large spatial scales (Fediajevaite et al., 2021; Suarez-Bregua et al., 2022), including zooplankton (Feng et al., 2022; Wong et al., 2022; Yang and Zhang, 2020), few studies have evaluated water eDNA versus mesh-screened tissues for zooplankton metabarcoding (Du et al., 2024).

In this study, we compared water eDNA versus mesh-screened tissues for zooplankton metabarcoding. Water eDNA detected significantly more taxa than net collections across (Fig. S4), with pronounced methodological biases: water samples better captured small species like Labyrinthulea, while nets preferentially collected larger taxa, including Hoplonemertea and Malacostraca. This size selectivity arises because net collection may exclude organisms smaller than the mesh, as well as fragile or rare organisms, whereas water samples filtered through fine membrane filters retain DNA from the entire water volume (Brandt et al., 2021). The case of Medusae exemplifies this bias: their high water content (Minamoto et al., 2017), combined with fragile body and net avoidance, likely explains their absence in S1-S3 net samples (Fig. 4). Moreover, the net used in this study was relatively small and primarily intended for collecting large phytoplankton, micro-zooplankton, and rotifers, potentially underestimating meso-zooplankton taxa such as copepods and jellyfish (Pitois et al., 2016).

The contrasting detection of *Vanella* sp. (water only) and *Lopho cristagalli* (net dominant) further illustrates methodological biases. *Vanella* sp. can pass through nets, while *L. cristagalli* larvae benefit from the large water volume filtered during net collection. These findings emphasise the critical influence of mesh size in plankton studies (Ratnarajah et al., 2023; Zhang et al., 2024). Despite the rapid degradation of eDNA in flowing systems (Thomsen et al., 2012), the stagnant conditions in Tolo Harbour minimised transport effects (Harrison et al., 2019), resulting in broadly comparable community patterns across methods. Similar conclusions have been drawn from riverine studies (Du et al., 2024), confirming the efficiency and practicality of water sampling for large-scale surveys (2.5 L for water samples vs. 50 L for net samples per replicate). However, limitations of metabarcoding remain, including incomplete reference databases, primer biases, and amplification artefacts (Krehenwinkel et al., 2017; Marinchel et al., 2023). While PCR-free approaches may reduce biases (Manu and Umaphathy, 2023), improved reference databases remain essential for both molecular and imaging methods (Bucklin et al., 2021).

4.3. Influence of sampling methods on identifying influential environmental variables

Although BIOENV analysis identified the same key variables (turbidity and orthophosphate phosphorus) as drivers of zooplankton community structure for both sampling methods (Table 2), the fourth-corner analysis revealed method-dependent species responses (Fig. 6). Notably, the bloom-forming species *Noctiluca scintillans* showed negative correlations with both variables, consistent with its known bloom dynamics involving phosphate-mediated feedbacks (Choi et al., 2024; Li et al., 2024; Liu et al., 2025). Three polychaete species (*P. elongata*, *Chaetopterus* sp., and *M. dubia*) demonstrated potential as pollution indicators, with *P. elongata* showing particularly strong positive correlations with turbidity and phosphorus (Fig. 6). Given that several species

spionid polychaetes, including *P. elongata*, are established indicators of organic pollution (Elías et al., 2005), these results suggest that water eDNA offers superior sensitivity for detecting environmental relationships. The consistent harbour-associated distributions of these taxa across methods (Table 1) reinforce their utility as bioindicators, supporting the integration of eDNA monitoring with water quality assessments to understand anthropogenic impacts on coastal ecosystems.

5. Conclusion

In this study, we compared two metabarcoding approaches (water and net-collection of eDNA) with automatic imaging (PlanktonScope) for zooplankton biodiversity assessment in Tolo Harbour, and linked the biodiversity pattern to water quality. Our results demonstrate that both eDNA metabarcoding approaches provided higher taxonomic resolution than imaging while achieving similar performance in biodiversity assessment, spatial distribution analysis, dominant species detection, and crucial environmental variable identification. Given these results, we propose a complementary monitoring strategy: continuous auto-imaging for real-time zooplankton monitoring alongside periodic eDNA metabarcoding of water samples for detailed biodiversity analysis, thereby capitalising on their respective strengths in temporal and taxonomic resolution. Future studies should include body-size data to enable a more systematic evaluation of method efficacy. To fully realise the potential of eDNA metabarcoding, future work should expand reference databases using diverse marker genes, develop balanced primer sets to optimise both taxonomic breadth and specificity, investigate the effects of total sample DNA abundance on PCR amplification of specific taxa, and minimise sequencing errors to enhance accuracy for zooplankton monitoring programmes.

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CRediT authorship contribution statement

Yingbei Peng: Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Chun Ming How:** Data curation, Investigation, Visualization, Writing – review & editing. **Dai Liu:** Data curation, Investigation, Visualization, Writing – review & editing. **Dumas Deconinck:** Investigation, Writing – review & editing. **Mei-Hong Zhao:** Investigation, Writing – review & editing. **Jack Chi-Ho Ip:** Writing – review & editing. **Leo Lai Chan:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Hongsheng Bi:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Jian-Wen Qiu:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2026.108238>.

Data availability

The raw sequences related to this study are available at 10.5281/zenodo.16659582 and 10.5281/zenodo.16665148.

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