TROPICAL BIOINDICATORS UNDER PRESSURE: CALANOID COPEPODS AS INDICATORS OF COASTAL ECOSYSTEM HEALTH IN JAKARTA BAY, INDONESIA

MAQBUL, I.[1*] – ISMAIL, M. R.[1] – ALINA, D. N.[1] – ABDURRAHMAN, U.[1] – SOFYANA, N. T.[1] – ERNAWATI[2]

[1]*Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Jatinangor 40600, Indonesia*

[2]*Department of Fisheries, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Jatinangor 40600, Indonesia*

[*]*Corresponding author*
e-mail: Ismail.maqbul@unpad.ac.id

(Received 22nd May 2025; accepted 26th Jun 2025)

Abstract. This study examines the structure of *Calanoida copepods* communities along a gradient of anthropogenic pressure in Jakarta Bay and the Seribu Islands, Indonesia, utilizing environmental DNA (eDNA) metabarcoding techniques. Water samples were collected from four locations representing high (Tanjung Priok), moderate (Untung Jawa), and low (Pramuka and Harapan Islands) levels of disturbance. The amplification of mitochondrial COI (cytochrome oxidase I) fragments, followed by high-throughput sequencing, revealed significant spatial variation in both *Calanoida* abundance and genus composition. Conservation sites exhibited greater abundance and diversity, with domination of genera such as *Stephos* and *Undinula*. In contrast, urban areas were characterized by low richness and a predominance of tolerant genera such as Paracalanus. Non-parametric statistical tests confirmed significant differences among the sites, while cluster analysis categorized communities based on their level of impact. Although redundancy analysis (RDA) did not yield statistical significance, salinity and dissolved oxygen appeared to influence the patterns within the communities. These findings illustrate the sensitivity of *Calanoida* to environmental gradients and reinforce their role as bioindicators for tropical coastal ecosystems. The study highlights the significance of eDNA metabarcoding as a non-invasive approach for biodiversity monitoring, offering scientific justification for integrating molecular methods into coastal conservation and management strategies.

Keywords: *biodiversity monitoring, Calanoida copepods, community structure, environmental DNA, marine conservation, metabarcoding*

Introduction

Coastal ecosystems, particularly in tropical regions, currently face an escalating threat from intensifying human activities, including pollution, eutrophication, and climate change. The decline in biodiversity not only results in the loss of species but also disrupts essential ecosystem functions, such as nutrient cycling, stability of food webs, and protection of shorelines. Uncontrolled discharges of domestic and industrial waste, combined with poorly managed tourism, exacerbate habitat degradation and facilitate the dominance of invasive species over native communities (Lü et al., 2020; Avram et al., 2021). Furthermore, climate change intensifies these impacts by altering critical marine environmental parameters, such as temperature and salinity, ultimately jeopardizing the long-term resilience of coastal systems (Martínez et al., 2025).

In response to contemporary environmental challenges, effective biodiversity monitoring has emerged as a critical component of conservation planning. Monitoring systems enable the early detection of ecological changes, support assessments of ecosystem health, and support evidence-based decision-making (Kühl et al., 2020; Affinito et al., 2024). Recent advancements in DNA-based technologies—particularly environmental DNA (eDNA) and metabarcoding—have revolutionized the assessment of marine biodiversity. These methodologies enable the identification of species from environmental samples without direct observation, proving particularly beneficial for detecting cryptic or rare taxa that are challenging to identify morphologically (Λεοντίδου et al., 2021; Bella et al., 2024). When integrated with remote sensing and citizen science initiatives, these molecular tools can significantly enhance the spatial and taxonomic resolution of ecological monitoring.

In Indonesia, Jakarta Bay and the Seribu Islands offer contrasting case studies that could significantly impact conservation efforts. Jakarta Bay is a highly disturbed coastal region, receiving waste inputs from over 13 rivers, and is affected by microplastic contamination, eutrophication, and frequent algal blooms, leading to hypoxic conditions (Aziz et al., 2021; Firdaus et al., 2023). In contrast, the Seribu Islands have implemented several conservation measures, including coral reef restoration, sustainable fisheries management, and environmentally conscious tourism development (Damar et al., 2023). Comparative studies suggest that the Seribu Islands maintain more stable environmental conditions, making them a valuable reference site for evaluating the health of coastal ecosystems (Edward and Kusnadi, 2023). Understanding how planktonic organisms respond to these environmental gradients requires a focus on taxa that are both ecologically important and environmentally sensitive.

One such group is the *Calanoida copepod*s, which are dominant herbivorous zooplankton that play a crucial role in transferring energy from phytoplankton to higher trophic levels, while also contributing to carbon cycling and nutrient dynamics in marine systems (Benedetti et al., 2022). Genera such as *Paracalanus, Acartia,* and *Undinula* exhibit distinct ecological preferences and are known to respond sensitively to variations in salinity, temperature, dissolved oxygen, and nutrient load (Ochocka, 2021; Hébert et al., 2022). Due to these sensitivities, *Calanoida* are increasingly recognized as effective bioindicators for assessing marine water quality (Muñoz-Colmenares et al., 2021; Widiastuti and Borumei, 2024).

Despite their potential, eDNA-based studies of *Calanoida* communities in tropical ecosystems remain limited. However, the innovative use of eDNA metabarcoding in this study presents an exciting opportunity to expand our understanding of zooplankton community structure and to detect species that are overlooked by conventional methods (Feng et al., 2022).

This study aims to investigate the composition of *Calanoida copepods* communities across a gradient of anthropogenic disturbance in Jakarta Bay and the Seribu Islands through eDNA metabarcoding. Furthermore, it evaluates their potential as bioindicators of environmental stress in tropical coastal ecosystems.

Materials and methods

Study sites

This research was conducted across four coastal locations in Jakarta Bay: Tanjung Priok Port, Untung Jawa Island, Pramuka Island, and Harapan Island (*Fig. 1*). These locations were selected to represent a range of anthropogenic pressures. Tanjung Priok is exposed to considerable industrial and maritime activities, whereas Untung Jawa Island experiences a moderate level of pressure, positioned in a transitional zone between urban development and tourism. Pramuka and Harapan Islands, which are part of the Seribu Islands National Marine Park, are classified as low-impact zones with conservation status. This spatial framework enabled the investigation of variations in the zooplankton community, specifically within the order *Calanoida*, in response to differing levels of environmental disturbance across urbanized tropical coastal systems.

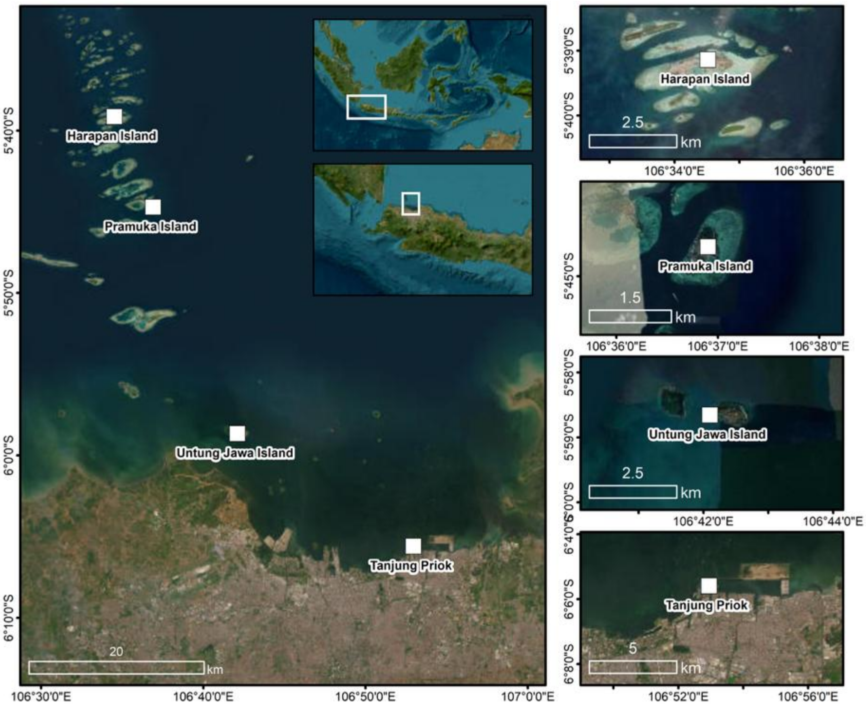

***Figure 1.** Map of sampling sites in the coastal waters of Jakarta Bay and the Seribu Islands, Indonesia. The eDNA sampling points indicate locations subjected to distinct levels of anthropogenic pressure: high (Tanjung Priok), moderate (Untung Jawa), and low (Pramuka and Harapan Islands, designated as conservation zones)*

Sample collection

Four environmental DNA (eDNA) samples, each comprising 4 liters, were collected from each site utilizing sterile sampling bottles. The samples were obtained at depths ranging from 1 to 5 meters below the surface employing SCUBA (Self-Contained Underwater Breathing Apparatus). Immediately upon collection, the water was filtered through sterile 0.45 µm cellulose nitrate membrane filters (47 mm diameter) using a sterile peristaltic pump to capture suspended genetic material. The 0.45 µm pore size was selected based on the recommendations by Stoeckle et al. (2022) for optimal eDNA recovery from seawater.

To mitigate the risk of contamination and DNA degradation, the filters were placed into sterile cryotubes containing 1.5 mL of DNA/RNA Shield (ZymoBIOMICS), stored within a cooling box, and subsequently transferred to a -20°C freezer in the laboratory. All sampling and filtration equipment underwent sterilization with a 10% hypochlorite solution and was rinsed with distilled water following established quality control protocols to ensure the reliability of molecular analyses (Kumar et al., 2021; Truelove et al., 2022; Kuncoro et al., 2023).

DNA extraction and amplification

DNA was extracted from the membrane filters utilizing the DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands), following the manufacturer's standard protocol. This kit is widely used in molecular ecology for the extraction of high-quality DNA from various environmental matrices, including seawater, sediment, and soil (Thomson-Laing et al., 2022; Child et al., 2024).

The extracted DNA was subsequently amplified using universal primers mlCOIintF-adpt (5′–GGW ACW GGW TGA ACW GTW TAY CCY CC–3′) and dgHCOI2198-adpt (5′–TAA ACT TCA GGG TGA CCA AAR AAY CA–3′), which specifically target a 313 bp fragment of the mitochondrial cytochrome c oxidase I (COI) gene.

PCR was executed in a total reaction volume of 25 µL, comprising 12.5 µL MyTaq HS Redmix (Bioline), 1.25 µL of each primer, 1 µL of DNA template, and 9 µL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 1 minute, annealing at 48°C for 45 seconds, and extension at 72°C for 30 seconds; followed by a final extension at 72°C for 10 minutes.

Indexed amplification was performed utilizing the Nextera XT Index Kit and KAPA HiFi HotStart ReadyMix for 9 cycles (95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds). The final PCR products were purified through AMPure XP beads and normalized to 4 nM prior to sequencing.

High-throughput sequencing and bioinformatic analysis

Sequencing was performed using the Illumina MiSeq platform with the MiSeq v2 kit, resulting in paired-end reads of 300 base pairs. This platform has been extensively validated for environmental DNA (eDNA) studies in complex marine ecosystems, offering a high depth of read and sequencing precision (Miya et al., 2020; Jackman et al., 2021).

Raw sequencing data were processed through the MBRAVE (Multiplex Barcode Research and Visualization Environment) web-based platform, which provides standardized pipelines for eDNA analysis. The initial steps involved trimming adapters and primers, and implementing length-based filtering (~200 base pairs) to ensure sequence quality and minimize errors (Mathon et al., 2021; Lamperti et al., 2023).

Reads were clustered into Operational Taxonomic Units (OTUs), and taxonomic classification was conducted utilizing two reference databases: BOLD (Barcode of Life Data System) and NCBI GenBank, with a minimum similarity threshold of 98%. Only sequences classified under Kingdom Animalia were preserved for subsequent analysis. Taxonomic classifications were reported across seven hierarchical levels: kingdom, phylum, class, order, family, genus, and species.

The completeness of reference databases is vital for accurate taxonomic identification. Inadequate entries may result in incomplete or ambiguous classifications (Gösser et al., 2022; Jo and Yamanaka, 2022). Consequently, stringent similarity thresholds were enforced to enhance the reliability of identification (Tsuji et al., 2020; Govindarajan et al., 2021).

Community diversity and statistical analysis

The analysis of community diversity was predicated on the number of sequences derived from COI metabarcoding that successfully passed quality filtering and standardization protocols. The unit of analysis was the genus-level taxa within the order *Calanoida*. Abundance at the genus level was quantified as the number of sequences recorded per site and subsequently converted to relative proportions to facilitate comparisons between sites.

Differences in genus distribution across the various sites were examined using the non–parametric Kruskal–Wallis test, as the data did not satisfy the assumptions of normality and homoscedasticity. This analysis was succeeded by a post hoc Dunn test with Benjamini–Hochberg (BH) correction, aimed at identifying statistically significant pairwise differences.

A range of visual representations was generated to illustrate community structure and variations at the site level, which included Bar charts depicting the proportion of *Calanoida* relative to the total taxonomic reads, Stacked bar charts reflecting the relative composition of genera per site, Log- Transformed boxplots to visualize variations in abundance and statistical dispersion, Heatmaps featuring hierarchical clustering to evaluate similarity patterns among the 15 most dominant genera, Heatmaps of Dunn test p- values to emphasize the strength of pairwise differences.

To assess the influence of environmental conditions on the abundance and composition of *Calanoida* genera, a combination of satellite-derived and modelled oceanographic data was utilized. Key ecological parameters—including temperature, salinity, dissolved oxygen (DO), nitrate (NO_3), and phosphate (PO_4)—were retrieved from the *Copernicus Marine Environment Monitoring Service (CMEMS)* database (https://marine.copernicus.eu/). These data represent standardized, high-resolution environmental products commonly used for ecological modeling and marine ecosystem assessments. The datasets were downloaded in NetCDF (.nc) format, a multidimensional data format widely adopted for oceanographic applications. Environmental variables were extracted, subsetted to the sampling coordinates, and averaged over the sampling period using Python (xarray and netCDF4 libraries) and R (ncdf4 and raster packages). This procedure allowed integration of spatially relevant environmental information for correlation analysis and multivariate ordination. All environmental values used in the study represent surface-layer conditions and are spatially matched to the four sampling locations: Tanjung Priok, Untung Jawa, Pramuka Island, and Harapan Island.

To evaluate the relationship between *Calanoida* community structure and environmental parameters, Redundancy Analysis (RDA) was conducted utilizing the abundance of the top five genera and average site-level values for temperature, salinity, dissolved oxygen (DO), pH, nitrate (NO_3), and phosphate (PO_4). To mitigate issues of multicollinearity and overfitting due to the constrained number of sites, only two variables exhibiting low Variance Inflation Factors (VIF) and significant ecological relevance—salinity and DO—were retained for the final model.

All statistical analyses and visualizations were conducted using R (version 2025.05.0) in RStudio (R Core Team, 2024). The `vegan` package was used for multivariate analyses such as redundancy analysis (RDA), Bray–Curtis dissimilarity, and ANOSIM. Non-parametric tests (Kruskal–Wallis and Dunn post hoc) were performed using the `rstatix` and `FSA` packages (Oksanen et al., 2022; Kassambara, 2023). Correlation analyses employed the `Hmisc` package (Harrell, 2024), and heatmaps were generated using `pheatmap`(Kolde, 2019). For barplots, boxplots, and multi-panel visualizations, we used `ggplot2` and `patchwork` (Wickham, 2016). Environmental data in NetCDF format were processed using the `ncdf4` package (Pierce, 2019). Data wrangling and transformation were handled using `dplyr` and `tidyr` (Wickham et al., 2019).

Results and discussion

Results

Abundance and proportion of Calanoida

Preliminary analysis of eDNA sequences revealed clear spatial variation in the abundance and proportion of *Calanoida* among the study sites. The highest relative proportions of *Calanoida* reads were detected at Pramuka Island (20.7%) and Harapan Island (18.9%). At Untung Jawa Island, the proportion decreased markedly to 2.7%, while only three *Calanoida* sequences were identified in Tanjung Priok, resulting in a negligible proportion (<0.01%) (*Fig. 2a,b*).

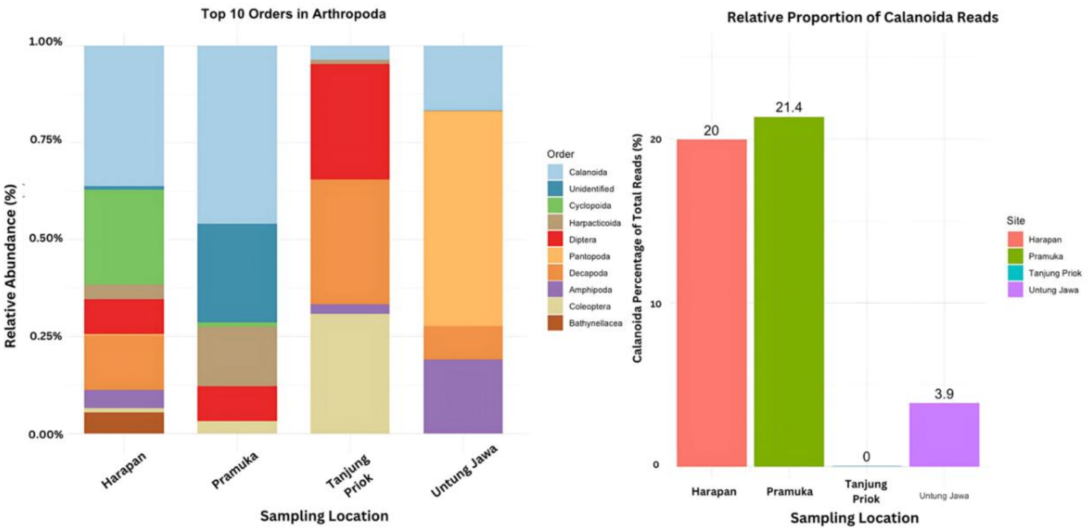

Figure 2. *Presents the taxonomic composition of environmental DNA (eDNA) sequences, which exhibited significant variation across different sites. This variation is illustrated in Figure 2a, which demonstrates the relative abundance of detected taxonomic orders across the study sites, as determined from annotated eDNA sequences. In Figure 2b, the percentage of Calanoida sequences relative to the total annotated eDNA reads at each site is displayed. The highest proportions of Calanoida sequences were observed at Pramuka and Harapan Islands, exceeding 20%, whereas Calanoida sequences were nearly absent at Tanjung Priok*

Absolute sequence counts followed a similar pattern, with the highest *Calanoida* abundance recorded at Pramuka Island (15,007 reads), followed by Harapan Island (8,500 reads), Untung Jawa (1,370 reads), and Tanjung Priok (3 reads). This disparity in abundance reflects a strong environmental pressure gradient, aligned with the ecological characteristics of each site, ranging from marine conservation zones to heavily industrialized urban waters.

Visualization through bar charts emphasized the contrast among sites and underscored the potential of *Calanoida* as a sensitive bioindicator group for detecting variations in coastal water quality. These findings provided a foundational basis for further analysis of community composition at a finer taxonomic resolution.

DOI: http://dx.doi.org/10.15666/aeer/2305_86398660

Genus composition of Calanoida

Genus-level analysis revealed that *Calanoida* communities across the four sites comprised several dominant genera with varying spatial distributions. Based on relative abundance, the five most common genera across all locations were *Paracalanus, Centropages, Undinula, Euchirella, and Stephos*, although each site showed distinct genus dominance (*Fig. 3*).

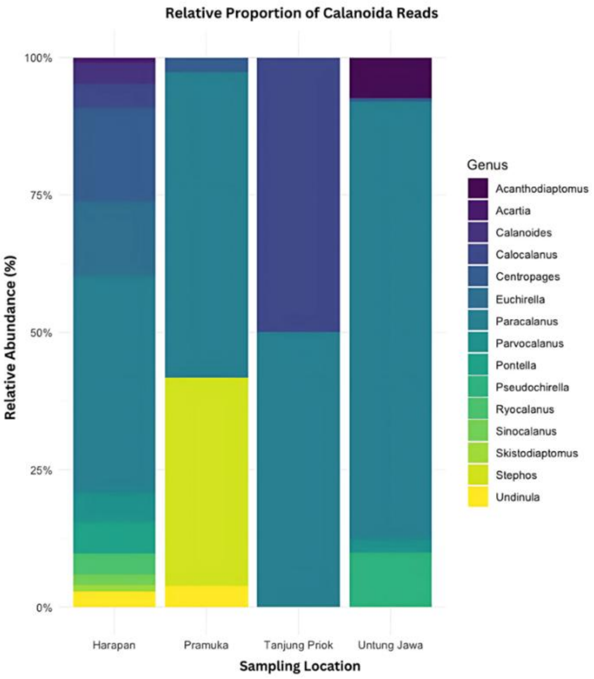

Figure 3. *Relative composition of Calanoida genera based on eDNA sequence proportions at each site. Segment height reflects the contribution of each genus to total Calanoida sequences*

At Pramuka Island, the community was dominated by *Undinula* and *Stephos,* which together accounted for over 60% of the total *Calanoida* reads. In contrast, Harapan Island exhibited a more even distribution of genera, with comparable contributions from *Paracalanus, Centropages,* and *Euchirella*. At Untung Jawa Island, *Paracalanid*s were dominant, while Tanjung Priok yielded too few valid reads for meaningful genus-level visualization and was excluded from the comparative composition analysis.

Stacked bar chart visualizations clearly illustrated the differences in genus composition among sites, suggesting that local environmental factors and anthropogenic pressure have a strong influence on the *Calanoida* community structure. The dominance of particular genera at specific sites further supports their potential as indicator taxa for specific coastal ecosystem conditions.

Genus abundance variation: Statistical testing and visualization

Analysis of *Calanoida* genus abundance revealed statistically significant differences among the study sites. Most genera showed peak abundances at Pramuka and Harapan Islands, with genus-level read counts ranging from very low values (<10) to over 8,000. Conversely, Tanjung Priok showed extremely low abundance across nearly all genera, while Untung Jawa exhibited intermediate values.

A $\log_{10}$-transformed dot plot (*Fig. 4a*) illustrated the genus abundance distribution at each site. The highest variability was observed at Pramuka, which featured several extreme values (outliers), while Tanjung Priok showed a narrow spread, approaching zero.

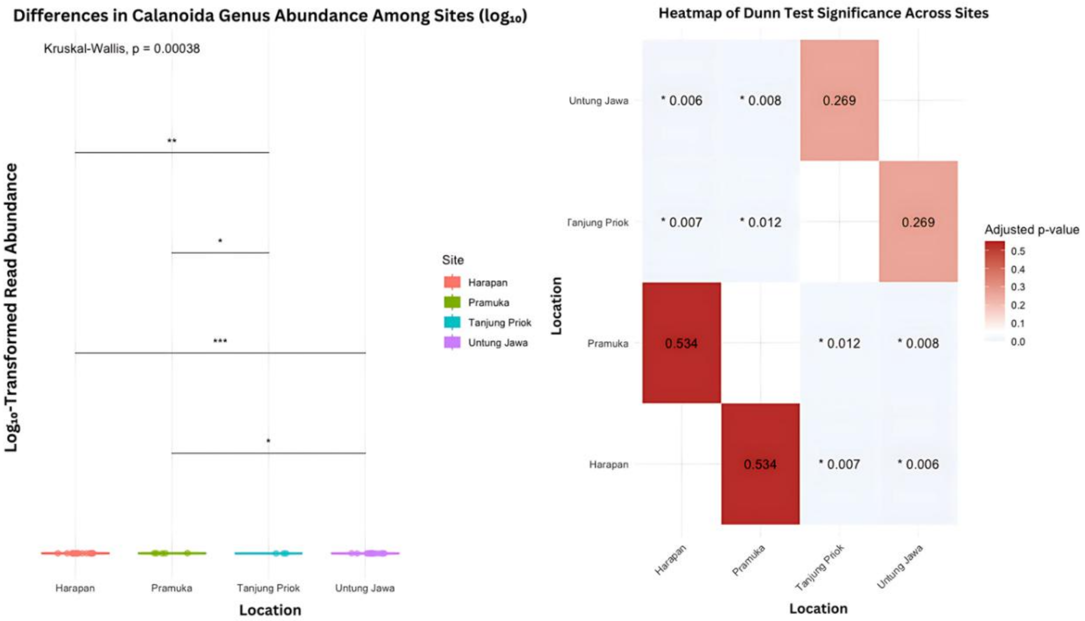

Figure 4. *Statistical comparison of Calanoida genus abundance across sites. (a) dot plot of $\log_{10}$-transformed read counts showing significant differences among locations based on the Kruskal–Wallis test ($\chi^2 = 18.29$; df = 3; $p = 0.00038$). Asterisks indicate pairwise significance levels from Dunn's post hoc test with Benjamini–Hochberg correction: $p < 0.05$ (), $p < 0.01$ (), and $p < 0.001$. (b) Heatmap of adjusted p-values from Dunn's test, highlighting statistically significant differences in Calanoida abundance among sites. Lighter shades indicate higher significance, with the strongest contrasts observed between sites under differing anthropogenic pressure (e.g., Harapan vs. Tanjung Priok)*

The Kruskal–Wallis test confirmed significant differences in genus abundance among the sites ($\chi^2 = 18.29$; df = 3; $p = 0.00038$). Post-hoc Dunn tests with Benjamini–Hochberg correction revealed significant differences ($p < 0.05$) for the following site pairs: Harapan vs. Tanjung Priok ($p = 0.007$), Pramuka vs. Tanjung Priok ($p = 0.012$), Harapan vs. Untung Jawa ($p = 0.006$), and Pramuka vs. Untung Jawa ($p = 0.008$) (*Fig. 4b*). No significant differences were found between Harapan and Pramuka, or between Tanjung Priok and Untung Jawa.

These findings were supported by a p-value heatmap, which indicated the strength of significance across site pairs. The color gradient illustrated the most robust differences, where anthropogenic pressure gradients were most pronounced.

Community pattern based on heatmap and clustering

Community distribution analysis of *Calanoida* genera, based on heatmap visualization and hierarchical clustering, revealed precise spatial segmentation among sites. Z-score transformations of row-scaled data demonstrated that Pramuka and Harapan Islands formed a distinct taxonomic cluster, characterized by the co-occurrence of *Stephos,* *Undinula, Centropages*, and *Euchirella*. These locations exhibited high abundance and relatively even genus diversity, consistent with their status as designated marine conservation zones (*Fig. 5*).

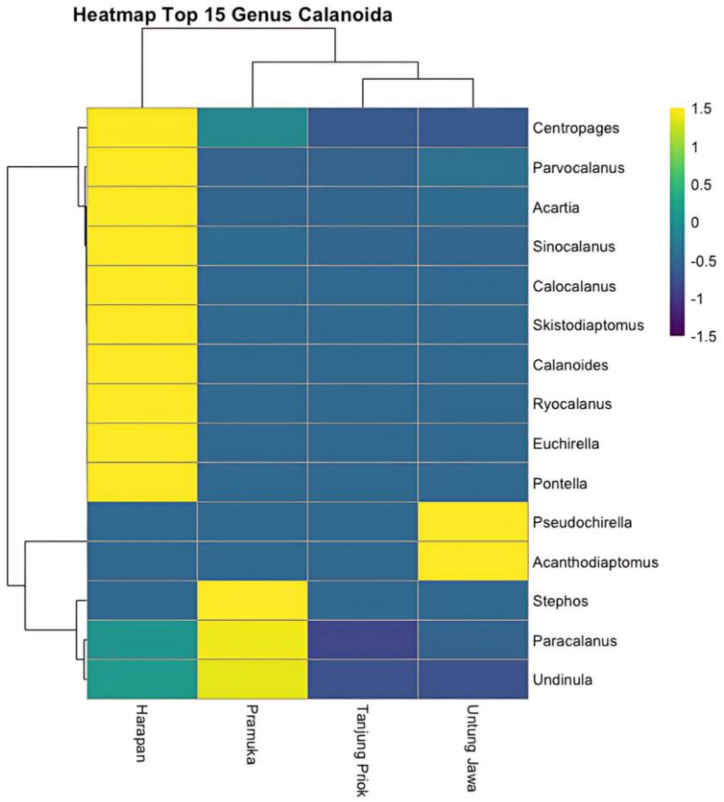

Figure 5. *Heatmap of Calanoida genus distribution based on row-wise z-score transformation of relative abundance, accompanied by hierarchical clustering. The 15 most dominant genera are displayed along the vertical axis, with sampling sites on the horizontal axis. Color intensity reflects relative genus abundance, ranging from low (dark blue) to high (yellow). The Harapan–Pramuka cluster exhibits high community similarity and diversity, in contrast to the Tanjung Priok–Untung Jawa cluster, which is characterized by genus-specific dominance or the absence of key taxa*

In contrast, Tanjung Priok and Untung Jawa formed a separate cluster characterized by lower diversity and limited presence of genera. The *Calanoida* community in Tanjung Priok was almost absent, while Untung Jawa was dominated by *Paracalanus*, a genus known for its tolerance to anthropogenic stress. This clustering pattern reflects a gradient of environmental degradation, ranging from natural to highly disturbed conditions.

The taxonomic proximity between Harapan and Pramuka communities, as shown in the dendrogram, supports the hypothesis that similar habitat conditions within protected areas exert a homogenizing effect on zooplankton community structure. In contrast, the sharp differentiation from urbanized zones highlights the importance of ecosystem protection in maintaining eDNA-based zooplankton diversity.

Redundancy analysis (RDA)

Redundancy Analysis (RDA) was conducted to evaluate the relationship between *Calanoida* community structure and environmental parameters. The initial dataset included five parameters: salinity, dissolved oxygen (DO), temperature, pH, nitrate (NO_3^-), and phosphate (PO_4^{3-}). Variance Inflation Factor (VIF) analysis revealed strong multicollinearity among pH, NO_3^-, and PO_4^{3-} (VIF > 10), necessitating their exclusion from the final model to prevent overfitting.

The final RDA model retained two ecologically relevant and statistically independent variables: salinity and dissolved oxygen. ANOVA by term revealed that both variables contributed a substantial proportion of the variance in community structure, although neither reached statistical significance at $\alpha = 0.05$ (salinity: $p = 0.333$; DO: $p = 0.125$), likely due to the limited number of sampling sites.

The RDA biplot indicated that the DO vector was positively associated with the communities in Tanjung Priok and Untung Jawa. In contrast, the salinity vector was more closely aligned with Harapan Island. Genera such as *Euchirella, Centropages*, and *Undinula* were positively associated with higher salinity levels, whereas *Stephos* and *Paracalanus* were more prevalent in environments with higher DO and relatively lower salinity (*Fig. 6*).

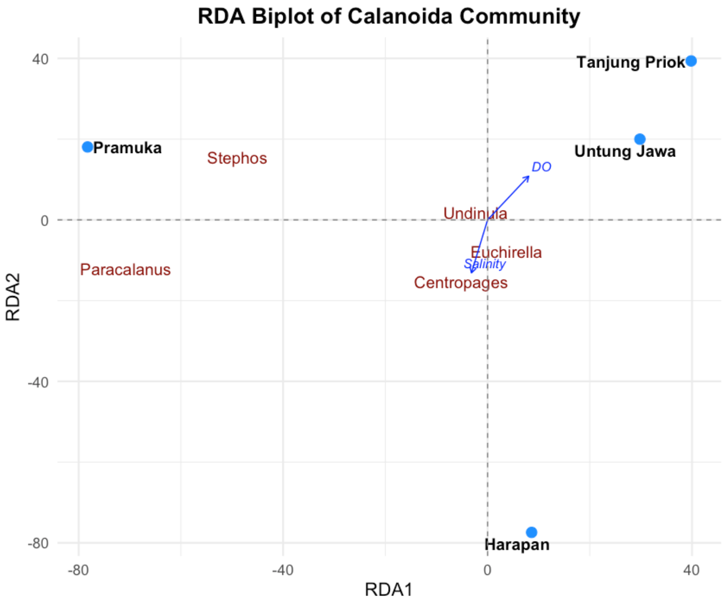

Figure 6. *Redundancy Analysis (RDA) biplot showing the association between Calanoida community composition (five dominant genera), environmental parameters (salinity and dissolved oxygen/DO), and sampling locations. Arrow direction and length represent the relative contribution of each environmental variable to community variation. Centropages, Euchirella, and Undinula were associated with higher salinity conditions, whereas Paracalanus and Stephos were more abundant in sites with elevated dissolved oxygen (DO) levels. The spatial distribution of sites indicates community segregation aligned with an environmental gradient from conservation areas to urbanized coastal zones*

These spatial patterns suggest that *Calanoida* community composition is shaped not only by anthropogenic pressure but also by consistent gradients in physicochemical conditions observed across the tropical coastal and conservation zones.

Discussion

Calanoida abundance variation and ecological implications

The abundance of *Calanoida* displayed pronounced spatial variation across the study sites, aligning closely with the gradient of anthropogenic pressure in Jakarta Bay and the Seribu Islands. The highest proportions of *Calanoida* reads were recorded at Pramuka (20.7%) and Harapan Islands (18.9%), both located within protected marine areas. In contrast, Tanjung Priok—a heavily urbanized site—yielded only three *Calanoida* sequences (<0.01%), and Untung Jawa, representing a transitional zone, showed a moderate proportion (2.7%). These findings demonstrate a clear suppression of *Calanoida* communities in areas with intense urban and industrial activity.

Our results are consistent with those of Isibor et al. (2020), who reported that *Calanoida* abundance was significantly reduced in estuarine regions exposed to high levels of anthropogenic pollution, particularly nutrient enrichment and wastewater discharge. Similarly, Ekpo et al. (2023) found that *Copepods* diversity declined sharply in Nigerian coastal zones adjacent to oil production facilities, emphasizing the vulnerability of pelagic zooplankton to chemical and physical stressors in the water column.

Several dominant genera detected in this study—*Parvocalanus, Undinula*, and *Centropages*—are well-documented for their ecological importance as intermediaries in marine food webs. Specifically, Kortsch et al. (2021) demonstrated that these genera facilitate efficient energy transfer from phytoplankton to higher trophic levels, and Prowe et al. (2022) noted that declines in their abundance can disrupt trophic efficiency and alter nutrient cycling.

Statistical tests confirmed these differences: The Kruskal–Wallis test indicated a significant effect of site ($p = 0.00038$), and Dunn's post hoc analysis identified significant pairwise differences between conservation (Pramuka, Harapan) and impacted sites (Untung Jawa, Tanjung Priok). This pattern reinforces the interpretation that environmental degradation has a direct impact on the stability and composition of *Calanoida* communities.

According to Ochocka (2021), genera such as *Undinula* and *Stephos* tend to inhabit stable, oligotrophic waters, exhibiting strong sensitivity to both nutrient loading and reduced oxygen availability. This aligns with our observation that these genera were dominant in the protected sites but absent or scarce in disturbed areas. Additionally, da Gama Fernandes Vieira Júnior et al. (2020) documented that oligotrophic-adapted *copepods* decline rapidly when exposed to even moderate eutrophication, corroborating the suppressed abundance we recorded in urbanized waters.

Interestingly, *Parvocalanus* was consistently present in Untung Jawa and to a lesser extent in other sites, suggesting its broader ecological tolerance. This supports the findings of Musa et al. (2022), who identified *Parvocalanus* as a genus capable of persisting under fluctuating salinity and nutrient conditions, often occupying transitional zones in tropical estuaries.

In summary, the patterns of *Calanoida* abundance in this study are not merely shaped by natural ecological gradients but serve as concrete evidence of anthropogenic disturbance. The consistency of our findings with previously documented *Copepods* sensitivities (Medellín-Mora et al., 2021; Musa et al., 2022) underscores the reliability of *Calanoida* as a biological indicator for assessing the health of coastal ecosystems under escalating human pressure.

Genus composition as a reflection of environmental quality gradient

The composition of *Calanoida* genera across the study sites revealed a clear ecological response to environmental gradients shaped by anthropogenic disturbance. Three dominant genera—*Stephos, Undinula,* and *Paracalanus*—exhibited spatial distributions aligned with differences in water quality conditions between protected and urbanized areas.

Stephos and *Undinula* were found predominantly on the Pramuka and Harapan Islands, both of which are situated within the Seribu Islands National Marine Park. These genera are known to prefer oligotrophic conditions characterized by high salinity, stability, and elevated dissolved oxygen levels. Ochocka (2021) reported that Undinula tends to dominate in low-nutrient tropical waters, while Zhang et al. (2020) demonstrated that Stephos shows a strong avoidance of eutrophic coastal zones. Additionally, Sadaiappan et al. (2023) identified Stephos as an indicator genus frequently associated with minimally disturbed marine ecosystems, highlighting its ecological sensitivity.

In contrast, *Paracalanus* was dominant at Untung Jawa and occurred in lower proportions at the other sites. This genus is well-documented for its broad ecological tolerance. In a study of semi-degraded eastern Pacific coastal zones, Marín and Tiunov (2023) found that *Paracalanus* often becomes the dominant genus in eutrophic or fluctuating environments, supporting its role as a diagnostic indicator of transitional water quality conditions. The spatial dominance of Paracalanus at Untung Jawa, a transitional zone between conservation and urban influence, reflects this ecological plasticity.

The moderate presence of *Centropages* and *Euchirella* at the conservation sites further supports the pattern of greater community complexity under lower anthropogenic stress. Ochocka (2021) associated *Centropages* with stable open-water conditions, and Zhang et al. (2020) highlighted Euchirella's preference for habitats with higher salinity and minimal disturbance. Their co-occurrence with *Stephos* and *Undinula* reinforces the hypothesis of a more balanced and diverse *Calanoida* assemblage in protected areas.

Although Redundancy Analysis (RDA) did not yield statistically significant results (salinity: $p = 0.333$; DO: $p = 0.125$), likely due to the small number of sampling locations, the ecological associations remain meaningful. The biplot showed that *Centropages, Undinula,* and *Euchirella* were oriented along the salinity gradient, whereas *Paracalanus* and *Stephos* were associated with dissolved oxygen levels. These associations are consistent with the environmental preferences reported in earlier studies (Zhang et al., 2020; Sadaiappan et al., 2023).

Hierarchical clustering and heatmap visualization revealed a distinct grouping of *Calanoida* communities at Pramuka and Harapan, separate from the urbanized sites of Tanjung Priok and Untung Jawa. Notably, no genera were unique to individual sites, indicating that differences in community composition were primarily driven by shifts in relative abundance rather than species turnover. This finding aligns with observations by Medellín-Mora et al. (2021), who reported that anthropogenic stress often leads to homogenization or simplification of planktonic communities in Caribbean coastal ecosystems.

Overall, the genus-level composition of *Calanoida* serves as a sensitive indicator of environmental quality across the coastal gradient. Incorporating fine-scale taxonomic information into eDNA-based assessments significantly enhances ecological interpretation, particularly in complex tropical systems (Mao et al., 2022; Chen et al., 2023; Mahajan et al., 2024).

Statistical significance in the context of tropical coastal ecosystems

The non-parametric statistical approach employed in this study provides robust evidence for spatial variation in *Calanoida* community structure across coastal sites experiencing differing levels of anthropogenic disturbance. The Kruskal–Wallis test revealed a statistically significant difference in genus-level *Calanoida* abundance among the four sites ($\chi^2 = 18.29$, df = 3, $p = 0.00038$), affirming that these communities are responsive to environmental stress gradients.

This finding aligns with the ecological premise that biological communities in complex and heterogeneous tropical systems rarely conform to parametric assumptions of normality and variance homogeneity. As noted by Orçan (2020), the Kruskal–Wallis test is widely adopted in ecological research for analyzing species abundance data, as it effectively handles non-normal and heteroscedastic distributions. Its suitability is particularly evident in planktonic community assessments where stochastic environmental conditions often lead to skewed or zero-inflated data.

Further insight was gained through Dunn’s post-hoc tests, adjusted using the Benjamini–Hochberg procedure to control the false discovery rate. These pairwise comparisons revealed significant differences ($p < 0.05$) between the conservation sites (Harapan and Pramuka) and the urbanized sites (Tanjung Priok and Untung Jawa), with no significant differences detected between the two conservation zones or between the two urban zones. This pattern reinforces the interpretation that differences in *Calanoida* community composition are primarily driven by the gradient of anthropogenic pressure and conservation status, rather than stochastic variation.

Log-transformed boxplots (*Fig. 4a*) visually confirmed these differences, with conservation areas exhibiting wider abundance distributions and clear outliers, indicative of more dynamic and structured communities. In contrast, the near-zero distributions observed in Tanjung Priok and Untung Jawa reflected severely depauperate communities, likely due to degraded water quality. The corresponding heatmap of adjusted p-values (*Fig. 4b*) provided an intuitive representation of statistical contrasts, highlighting the strongest differentiation between sites with the highest ecological disparity, most notably Harapan vs. Tanjung Priok.

This multifaceted statistical approach—combining non-parametric testing with visual analytics—demonstrates not only the presence of statistically significant community variation but also delivers ecologically meaningful interpretations. This finding aligns with those of Blowes et al. (2020) and Samhouri et al. (2022), who highlighted the importance of protected areas in maintaining more diverse and resilient biological communities compared to those under anthropogenic stress.

In summary, the findings highlight the ecological significance of Marine Protected Areas (MPAs), such as the Seribu Islands, in mitigating biodiversity loss and promoting community stability in tropical marine ecosystems. Furthermore, they emphasize the importance of employing rigorous statistical frameworks that are tailored to the characteristics of ecological data. As shown in the works of Somerfield et al. (2021) and Stoev et al. (2023), non-parametric methods remain indispensable for generating valid ecological conclusions, especially when monitoring biodiversity under shifting environmental baselines.

Environmental parameters and community structure relationships

The structure of *Calanoida* communities in this study exhibited meaningful ecological associations with environmental gradients, particularly salinity and dissolved oxygen (DO), as illustrated through Redundancy Analysis (RDA). Although the relationships were not statistically significant ($p = 0.333$ for salinity; $p = 0.125$ for DO), these patterns remain ecologically relevant. As emphasized by Amer and Mohamed (2022) and Dyson et al. (2023) non-significant p-values in ecological field studies often reflect constraints such as limited spatial replication, site heterogeneity, or inherent ecological variability, rather than the absence of underlying biological interactions.

Salinity is widely recognized as a key abiotic driver shaping the distribution and physiological performance of marine *copepods*. Laboratory experiments and field studies have consistently shown that genera such as *Undinula, Centropages*, and *Euchirella* exhibit distinct salinity preferences that influence their reproductive success and community dominance. For instance, Choi et al. (2021) and Kuismanen et al. (2020) reported that *Undinula* and *Centropages* maintain optimal population densities in meso- to polyhaline conditions, matching our field observations in Harapan and Pramuka Islands, where salinity levels were higher and these genera were more abundant. In contrast, at lower-salinity sites such as Tanjung Priok, *Calanoida* communities were fragmented or largely absent, corroborating the findings of Abo-Taleb et al. (2020) and Xu et al. (2025) regarding salinity-driven habitat filtering in degraded coastal systems.

Dissolved oxygen also emerged as a potentially important factor structuring *Calanoida* assemblages. Studies by Karpowicz et al. (2020) and Wishner et al. (2020) demonstrate that low DO levels can suppress zooplankton diversity by favoring smaller, hypoxia-tolerant taxa. In our study, genera such as Stephos and Paracalanus were more abundant in high-DO environments, indicating their physiological sensitivity to oxygen availability. Similar conclusions were drawn by Obuid-Allah et al. (2020) and Norambuena et al. (2022), who reported reduced reproductive efficiency and survivorship in *Calanoida copepods* under hypoxic conditions.

Despite the RDA model's statistical insignificance, the ordination biplot revealed coherent ecological gradients: *Centropages, Euchirella*, and *Undinula* were strongly aligned with salinity vectors, while Stephos and Paracalanus were more closely associated with DO. These differential associations are consistent with observations by Weinstock et al. (2022) and Ramondenc et al. (2022), who emphasized the interplay of abiotic drivers and anthropogenic stressors in structuring *copepods* communities.

The integration of spatially explicit field data, ecological visualization, and multivariate statistical approaches such as RDA allows for the detection of biologically meaningful signals, even in the absence of formal statistical significance. As noted by Farrar et al. (2022) and Yang et al. (2025), interpreting ecological datasets based on effect sizes and directional patterns is essential in limited-sample studies, where conventional significance thresholds may obscure important trends.

Taken together, the spatial patterns in *Calanoida* genera underscore their varied ecological tolerances, suggesting that these organisms serve as integrative bioindicators. Their distribution reflects the combined effects of physicochemical variation and anthropogenic disturbance, reinforcing their utility for monitoring the ecological status of tropical coastal ecosystems.

Ecological implications and management recommendations

The observed dominance of *Calanoida* in conservation zones—particularly at Pramuka and Harapan Islands—underscores their central role in sustaining trophic balance and ecosystem stability in tropical coastal waters. In our study, *Undinula* and *Stephos* were dominant in these locations, consistent with previous findings that these genera thrive in stable, oligotrophic conditions with high dissolved oxygen and minimal anthropogenic disturbance (Ochocka, 2021; Sadaiappan et al., 2023). Their absence in the highly urbanized waters of Tanjung Priok reflects an ecological shift that may disrupt energy transfer pathways and secondary production, aligning with reports by Isibor et al. (2020) and Ekpo et al. (2023), who documented similar declines in *copepods* populations in polluted tropical estuaries.

Our findings also support the assertion by Biggs et al. (2020) that a loss of sensitive zooplankton taxa can reduce functional redundancy and ecosystem resilience. The dominance of *Paracalanus* in the semi-disturbed site of Untung Jawa—consistent with its known tolerance to eutrophic and variable environments (Marín and Tiunov, 2023)—suggests a transition toward opportunistic community structures, which may persist under stress but fail to support critical ecological functions (Weeks et al., 2025). This shift mirrors patterns documented in other coastal areas undergoing environmental degradation.

Moreover, our findings confirm the role of *Calanoida* in the biological carbon pump, a mechanism critical for oceanic carbon sequestration. Genera such as *Centropages* and *Euchirella*, found in conservation areas, are recognized as significant contributors to vertical carbon flux through diel migration and fecal pellet production (Anderson et al., 2021; Pinti et al., 2023). Their absence or rarity in urbanized sites could imply a diminished capacity for carbon export, echoing concerns raised by Xu et al. (2025) regarding biogeochemical imbalance in polluted coasts.

In the context of management, our study reinforces previous conclusions by Vassallo et al. (2021) and Kim et al. (2020), who demonstrated the effectiveness of Marine Protected Areas (MPAs) in preserving mesozooplankton diversity. The contrasting *Calanoida* assemblages between the Seribu Islands National Park and Tanjung Priok support these findings and highlight the importance of protected areas in maintaining ecological integrity. Conversely, the degraded conditions at Tanjung Priok mirror the ecosystem decline described in Belloulou et al. (2023) for urbanized, unregulated coastal habitats.

Our results also validate the potential of eDNA metabarcoding as an early warning tool, consistent with the claims of Veilleux et al. (2021) and Miya (2022) regarding its efficiency in detecting fine-scale biodiversity changes. Integrating eDNA with ecosystem-based management (EBM), as recommended by Johnston et al. (2022) and Giao et al. (2024), will enhance the capacity of coastal governance frameworks to respond adaptively to multiple concurrent stressors.

In conclusion, this study highlights the utility of *Calanoida* as ecological indicators that reflect environmental quality gradients in tropical marine systems. Their community composition offers insights into ecosystem health and the impacts of anthropogenic stress. By leveraging molecular tools, such as eDNA, and incorporating them into conservation strategies, decision-makers can advance toward more responsive, evidence-based coastal management.

DOI: http://dx.doi.org/10.15666/aeer/2305_86398660

Conclusion

This study demonstrates that *Calanoida* communities respond significantly to gradients of anthropogenic pressure along the coastal waters of Jakarta Bay and the Seribu Islands. Both abundance and genus composition differed markedly between conservation and urbanized sites, with sensitive taxa such as *Undinula* and *Stephos* dominating in protected areas. At the same time, degraded locations exhibited limited community structure. Statistical and multivariate analyses suggest that environmental parameters—particularly salinity and dissolved oxygen—play a significant role in shaping community structure; however, the limited number of sampling sites restricts the statistical significance.

These findings underscore the potential of *Calanoida*, as the dominant pelagic zooplankton, to serve as effective ecological bioindicators for monitoring the quality of tropical marine ecosystems. The eDNA metabarcoding approach proved sensitive in detecting spatial community variation and should be integrated into evidence-based conservation monitoring frameworks in coastal regions. Strengthening conservation policies through the use of biological indicators and molecular technologies represents a strategic response to the degradation of marine biodiversity in the era of global environmental change.

Acknowledgements. The authors would like to express their sincere gratitude to all institutions and individuals who provided support throughout the implementation of this research. In particular, the authors acknowledge the Marine Science and Technology Diving School (MSTDS) and the Marine Biodiversity and Biosystematics Laboratory (BIODIVSI) at IPB University for their invaluable technical and logistical assistance during both the fieldwork and laboratory analysis.

REFERENCES

[1] Abo-Taleb, H. A., Ashour, M., El-Shafei, A. A., Alataway, A., Maaty, M. M. (2020): Biodiversity of Calanoida Copepoda in Different Habitats of the North-Western Red Sea (Hurghada Shelf). – Water 12(3): 656. https://doi.org/10.3390/w12030656.

[2] Affinito, F., Butchart, S. H. M., Nicholson, E., Hirsch, T., Williams, J. M., Campbell, J., Ferrari, M. F., Gabay, M., Gorini, L., Stroil, B. K., Kohsaka, R., Painter, B., Pinto, J. C., Scholz, A. H., Straza, T. R. H., Tshidada, N., Vallecillo, S., Widdicombe, S., Gonzalez, A. (2024): Assessing Coverage of the Monitoring Framework of the Kunming-Montreal Global Biodiversity Framework and Opportunities to Fill Gaps. – Nature Ecology and Evaluation 9: 1280-1294. https://doi.org/10.1101/2024.09.25.614896.

[3] Amer, A. S., Mohamed, W. S. E. (2022): Assessment of Ismailia Canal for Irrigation Purposes by Water Quality Indices. – Environmental Monitoring and Assessment 194: 862. https://doi.org/10.1007/s10661-022-10350-y.

[4] Anderson, T. R., Hessen, D. O., Mayor, D. J. (2021): Is the Growth of Marine Copepods Limited by Food Quantity or Quality? – Limnology and Oceanography Letters 6(3): 127-133. https://doi.org/10.1002/lol2.10184.

[5] Avram, S., Onțel, I., Gheorghe, C., Rodino, S., Roşca, S. (2021): Applying a Complex Integrated Method for Mapping and Assessment of the Degraded Ecosystem Hotspots from Romania. – International Journal of Environmental Research and Public Health 18(21): 11416. https://doi.org/10.3390/ijerph182111416.

[6] Aziz, M. Y., Piram, A., Asia, L., Salim, A., Hidayati, N. V., Buchari, B., Doumenq, P., Syakti, A. D. (2021): Organic Pollutants Hazard in Sediments and Green Mussels in Jakarta

Bay, Indonesia. – Soil and Sediment Contamination an International Journal 30(7): 862-885. https://doi.org/10.1080/15320383.2021.1893649.

[7] Bella, A. D., Vecchio, S. D., Fantinato, E., Buffa, G. (2024): Coastal Dune Restoration: A Checklist Approach to Site Selection. – Land 13(2): 135. https://doi.org/10.3390/land13020135.

[8] Belloulou, B., Houma, F., Otmani, H. (2023): Protection and Enhancement of the Coastal Area of the Wilaya of El Tarf (Algeria): Automatic Analysis Using Computer Tools. – Ekológia (Bratislava) 42(3): 279-291. https://doi.org/10.2478/eko-2023-0031.

[9] Benedetti, F., Wydler, J., Vogt, M. (2022): Copepod Functional Traits and Groups Show Divergent Biogeographies in the Global Ocean. – Journal of Biogeography 50(1): 8-22. https://doi.org/10.1111/jbi.14512.

[10] Biggs, C. R., Yeager, L. A., Bolser, D. G., Bonsell, C., Dichiera, A., Hou, Z., Keyser, S. R., Khursigara, A. J., Lu, K., Muth, A. F., Negrete, B., Erisman, B. (2020): Does Functional Redundancy Affect Ecological Stability and Resilience? A Review and Meta-analysis. – Ecosphere 11(7): e03184. https://doi.org/10.1002/ecs2.3184.

[11] Blowes, S. A., Chase, J. M., Franco, A. D., Frid, O., Gotelli, N. J., Guidetti, P., Knight, T. M., May, F., McGlinn, D. J., Micheli, F., Sala, E., Belmaker, J. (2020): Mediterranean Marine Protected Areas Have Higher Biodiversity via Increased Evenness, Not Abundance. – Journal of Applied Ecology 57(3): 578-589. https://doi.org/10.1111/1365-2664.13549.

[12] Chen, J.-Y., Wang, S., Yan, Z., Zhao, X., Feng, M., Wang, J., Zhou, Q. (2023): eDNA of Zooplankton Reveals the Ecological Community Thresholds for Key Environmental Factors in the Baiyangdian Lake Aquatic Ecosystem. – Environmental Sciences Europe 35: 56. https://doi.org/10.1186/s12302-023-00761-0.

[13] Child, H. T., Wierzbicki, L., Joslin, G. R., Tennant, R. (2024): Comparative Evaluation of Soil DNA Extraction Kits for Long Read Metagenomic Sequencing. – Access Microbiology 6(9). https://doi.org/10.1099/acmi.0.000868.v3.

[14] Choi, S. Y., Lee, E. H., Soh, H. Y., Jang, M. (2021): Effects of Temperature and Salinity on Egg Production, Hatching, and Mortality Rates in Acartia Ohtsukai (Copepoda, Calanoida). – Frontiers in Marine Science 8. https://doi.org/10.3389/fmars.2021.704479.

[15] da Gama Fernandes Vieira Júnior, A., de Lima, D. E. P. C., Dos Santos Sales, N., de Freitas Terra, Pessanha, A. 2020): Trade-offs Between Ontogenetic Changes and Food Consumption in Brazilian Silverside *Atherinella brasiliensis* from Two Tropical Estuaries. – Journal of Fish Biology 98(1): 196-207. https://doi.org/10.1111/jfb.14570.

[16] Damar, A., Colijn, F., Hesse, K.-J. (2023): Effects of Different Nutrient Loadings on Planktonic Primary Production in Embayments of Indonesia. – Journal of Tropical Biology & Conservation (JTBC) 11: 63-85. https://doi.org/10.51200/jtbc.v11i.263.

[17] Dyson, K., Dawwas, E., Poulton-Kamakura, R., Alberti, M., Fuentes, T. L. (2023): Say Where You Sample: Increasing Site Selection Transparency in Urban Ecology. – Ecosphere 14(3): e4466. https://doi.org/10.1002/ecs2.4466.

[18] Edward, E., Kusnadi, A. (2023): Review on Monitoring of Water Quality of the Jakarta Bay, Indonesia. – E3s Web of Conferences 454: 02003. https://doi.org/10.1051/e3sconf/202345402003.

[19] Ekpo, P. B., Agu, R. C., Osondu-Anyanwu, C., Nwachukwu, A. A., Nkang, N., Ekpo, I. P. (2023): An Assessment of the Effect of Pollution on Zooplanktons in Calabar Great Kwa River, Nigeria. – Journal of Advances in Biology & Biotechnology 26(3): 11-16. https://doi.org/10.9734/jabb/2023/v26i3623.

[20] Farrar, B., Vernouillet, A., Garcia-Pelegrin, E., Legg, E. W., Brecht, K. F., Lambert, P. J., Elsherif, M. M., Francis, S., O'Neill, L., Clayton, N. S., Ostojić, L. (2022): Reporting and Interpreting Non-Significant Results in Animal Cognition Research. – PeerJ 11: e14963. https://doi.org/10.31234/osf.io/g9ja2.

[21] Feng, Y., Sun, D., Shao, Q., Chen, F., Wang, C. (2022): Mesozooplankton Biodiversity, Vertical Assemblages, and Diel Migration in the Western Tropical Pacific Ocean Revealed

by eDNA Metabarcoding and Morphological Methods. – Frontiers in Marine Science 9. https://doi.org/10.3389/fmars.2022.1004410.

[22] Firdaus, M. R., Thoha, H., Rachman, A., Fitriya, N., Sianturi, O. R., Intan, M. D. B., Prayitno, H. B., Lastrini, S., Bayhaqi, A., Nasution, A. (2023): Study of Plankton in Jakarta Bay and Surrounding Java Sea: A Comparison of Community and Water Quality. – Iop Conference Series Earth and Environmental Science 1271(1): 012040. https://doi.org/10.1088/1755-1315/1271/1/012040.

[23] Giao, N. T., Dien, N. T., Nguyen, H. T., Nhu, T. N., Mi, L., Nhien, H. T. H. (2024): Evaluating Relationship Between Surface Water Quality and Zooplankton Diversity in Coastal Areas of Tien Giang Province, Vietnam. – Jasem 28(3): 841-851. https://doi.org/10.4314/jasem.v28i3.24.

[24] Gösser, F., Schweinsberg, M., Mittelbach, P., Schoenig, E., Tollrian, R. (2022): An Environmental <scp>DNA</Scp> Metabarcoding Approach Versus a Visual Survey for Reefs of Koh Pha-ngan in Thailand. – Environmental DNA 5(2): 297-311. https://doi.org/10.1002/edn3.378.

[25] Govindarajan, A. F., Francolini, R., Jech, J. M., Lavery, A. C., Llopiz, J. K., Wiebe, P. H., Zhang, W. G. (2021): Exploring the Use of Environmental DNA (eDNA) to Detect Animal Taxa in the Mesopelagic Zone. – Frontiers in Ecology and Evolution 9. https://doi.org/10.3389/fevo.2021.574877.

[26] Harrell, F. E. (2024): Hmisc: Harrell Miscellaneous. – R package version 5.1-1. https://CRAN.R-project.org/package=Hmisc.

[27] Hébert, M., Symons, C. C., Cañedo-Argüelles, M., Arnott, S. E., Derry, A. M., Fugère, V., Hintz, W. D., Melles, S. J., Astorg, L., Baker, H. K., Brentrup, J. A., Downing, A. L., Ersoy, Z., Espinosa, C., Franceschini, J. M., Giorgio, A. T., Göbeler, N., Gray, D. K., Greco, D., … Beisner, B. E. (2022): Lake Salinization Drives Consistent Losses of Zooplankton Abundance and Diversity Across Coordinated Mesocosm Experiments. – Limnology and Oceanography Letters 8(1): 19-29. https://doi.org/10.1002/lol2.10239.

[28] Isibor, P. O., Imoobe, T. O., Dedeke, G. A., Adagunodo, T. A., Taiwo, O. S. (2020): Health Risk Indices and Zooplankton-Based Assessment of a Tropical Rainforest River Contaminated with Iron, Lead, Cadmium, and Chromium. – Scientific Reports 10: 16896. https://doi.org/10.1038/s41598-020-72526-1.

[29] Jackman, J. M., Benvenuto, C., Coscia, I., Carvalho, C. O., Ready, J. S., Boubli, J. P., Magnusson, W. E., McDevitt, A. D., Sales, N. G. (2021): eDNA in a Bottleneck: Obstacles to Fish Metabarcoding Studies in Megadiverse Freshwater Systems. – Environmental DNA 3(4): 837-849. https://doi.org/10.1002/edn3.191.

[30] Jo, T., Yamanaka, H. (2022): Meta-analyses of Environmental DNA Downstream Transport and Deposition in Relation to Hydrogeography in Riverine Environments. – Freshwater Biology 67(8): 1333-1343. https://doi.org/10.1111/fwb.13920.

[31] Johnston, N. M., Murphy, E. J., Atkinson, A., Constable, A. J., Cotté, C., Cox, M. J., Daly, K. L., Driscoll, R., Flores, H., Halfter, S., Henschke, N., Hill, S. L., Höfer, J., Hunt, B. P. V, Kawaguchi, S., Lindsay, D. J., Liszka, C. M., Loeb, V. J., Manno, C., … Yang, G. (2022): Status, Change, and Futures of Zooplankton in the Southern Ocean. – Frontiers in Ecology and Evolution 9. https://doi.org/10.3389/fevo.2021.624692.

[32] Karpowicz, M., Ejsmont-Karabin, J., Kozłowska, J., Feniova, I. Y., Dzialowski, A. R. (2020): Zooplankton Community Responses to Oxygen Stress. – Water 12(3): 706. https://doi.org/10.3390/w12030706.

[33] Kassambara, A. (2023): rstatix: Pipe-Friendly Framework for Basic Statistical Tests. – https://cran.r-project.org/package=rstatix.

[34] Kim, H., Lee, C.-R., Lee, S., Oh, S., Kim, W. (2020): Biodiversity and Community Structure of Mesozooplankton in the Marine and Coastal National Park Areas of Korea. – Diversity 12(6): 233. https://doi.org/10.3390/d12060233.

[35] Kolde, R. (2019): pheatmap: Pretty Heatmaps. – R package version 1.0.12. https://CRAN.R-project.org/package=pheatmap.

[36] Kortsch, S., Frelat, R., Pécuchet, L., Olivier, P., Putnis, I., Bonsdorff, E., Ojaveer, H., Jurgensone, I., Strāķe, S., Rubene, G., Kruze, E., Nordström, M. C. (2021): Disentangling Temporal Food Web Dynamics Facilitates Understanding of Ecosystem Functioning. – Journal of Animal Ecology 90(5): 1205-1216. https://doi.org/10.1111/1365-2656.13447.

[37] Kühl, H. S., Bowler, D. E., Bösch, L., Bruelheide, H., Dauber, J., Eichenberg, D., Eisenhauer, N., Fernández, N., Guerra, C. A., Henle, K., Herbinger, I., Isaac, N. J. B., Jansen, F., König-Ries, B., Kühn, I., Nilsen, E. B., Pe'er, G., Richter, A., Schulte, R., … Bonn, A. (2020): Effective Biodiversity Monitoring Needs a Culture of Integration. – One Earth 3(4): 462-474. https://doi.org/10.1016/j.oneear.2020.09.010.

[38] Kuismanen, L., Forsblom, L., Engström-Öst, J., Båmstedt, U., Glippa, O. (2020): Salinity Effects on Egg Production, Hatching, and Survival of Eurytemora Affinis (Copepoda, Calanoida). – Crustaceana 93(3-5): 429-445. https://doi.org/10.1163/15685403-00003988.

[39] Kumar, G., Farrell, E., Reaume, A. M., Eble, J. A., Gaither, M. R. (2021): One Size Does Not Fit All: Tuning eDNA Protocols for High- and Low-turbidity Water Sampling. – Environmental DNA 4(1): 167-180. https://doi.org/10.1002/edn3.235.

[40] Kuncoro, I., Zamani, N. P., Subhan, B., Cahyani, N. K. D. (2023): eDNA Assessment of Scleractinian Diversity and Distribution in Lemukutan Island, Indonesia. – Biodiversitas Journal of Biological Diversity 24(7). https://doi.org/10.13057/biodiv/d240758.

[41] Lamperti, L., Sanchez, T., Moussi, S. S., Mouillot, D., Albouy, C., Flück, B., Bruno, M., Valentini, A., Pellissier, L., Manel, S. (2023): New Deep Learning-based Methods for Visualizing Ecosystem Properties Using Environmental DNA Metabarcoding Data. – Molecular Ecology Resources 23(8): 1946-1958. https://doi.org/10.1111/1755-0998.13861.

[42] Λεοντίδου, K., Vokou, D., Sandionigi, A., Bruno, A., Lazarina, M., Groeve, J. D., Li, M., Varotto, C., Girardi, M., Casiraghi, M., Cristofori, A. (2021): Plant Biodiversity Assessment Through Pollen DNA Metabarcoding in Natura 2000 Habitats (Italian Alps). – Scientific Reports 11: 18226. https://doi.org/10.1038/s41598-021-97619-3.

[43] Lu, Y. L., Yuan, J. J., Lu, X., Su, C., Zhang, Y., Quan, Y., Xie, W. (2020): Major Threats of Pollution and Climate Change to Global Coastal Ecosystems and Enhanced Management for Sustainability. – EGU General Assembly 2020. https://doi.org/10.5194/egusphere-egu2020-22316.

[44] Mahajan, D., Thakur, K., Kumar, S., Kumar, R. (2024): Ecological Perspectives on Water Quality and Zooplankton Diversity in the Ravi River. – World Water Policy 10(3): 891-908. https://doi.org/10.1002/wwp2.12216.

[45] Mao, Z., Cao, Y., Gu, X., Zeng, Q., Chen, H., Jeppesen, E. (2022): Response of Zooplankton to Nutrient Reduction and Enhanced Fish Predation in a Shallow Eutrophic Lake. – Ecological Applications 33(1): e2750. https://doi.org/10.1002/eap.2750.

[46] Marín, I., Tiunov, A. V. (2023): Terrestrial Crustaceans (Arthropoda, Crustacea): Taxonomic Diversity, Terrestrial Adaptations, and Ecological Functions. – Zookeys 1169: 95-162. https://doi.org/10.3897/zookeys.1169.97812.

[47] Martínez, M. L., Silva, R., Chávez, V., López-Portillo, J., Salgado, K., Marin-Coria, E. J., Pérez-Maqueo, O., Maximiliano-Cordova, C., Landgrave, R., de la Cruz, V. (2025): The Challenges of Climate Change and Human Impacts Faced by Mexican Coasts: A Comprehensive Evaluation. – Plos One 20(4): e0320087. https://doi.org/10.1371/journal.pone.0320087.

[48] Mathon, L., Valentini, A., Guérin, P., Normandeau, É., Noël, C., Lionnet, C., Boulanger, E., Thuiller, W., Bernatchez, L., Mouillot, D., Déjean, T., Manel, S. (2021): Benchmarking Bioinformatic Tools for Fast and Accurate eDNA Metabarcoding Species Identification. – Molecular Ecology Resources 21(7): 2565-2579. https://doi.org/10.1111/1755-0998.13430.

[49] Medellín-Mora, J., Escribano, R., Corredor-Acosta, A., Hidalgo, P., Schneider, W. (2021): Uncovering the Composition and Diversity of Pelagic Copepods in the Oligotrophic Blue

Water of the South Pacific Subtropical Gyre. – Frontiers in Marine Science 8. https://doi.org/10.3389/fmars.2021.625842.

[50] Miya, M., Gotoh, R. O., Sadõ, T. (2020): MiFish Metabarcoding: A High-Throughput Approach for Simultaneous Detection of Multiple Fish Species from Environmental DNA and Other Samples. – Fisheries Science 86(6): 939-970. https://doi.org/10.1007/s12562-020-01461-x.

[51] Miya, M. (2022): Environmental DNA Metabarcoding: A Novel Method for Biodiversity Monitoring of Marine Fish Communities. – Annual Review of Marine Science 14(1): 161-185. https://doi.org/10.1146/annurev-marine-041421-082251.

[52] Muñoz-Colmenares, M. E., Sendra, M. D., Sòria-Perpinyà, X., Soria, J., Vicente, E. (2021): The Use of Zooplankton Metrics to Determine the Trophic Status and Ecological Potential: An Approach in a Large Mediterranean Watershed. – Water 13(17): 2382. https://doi.org/10.3390/w13172382.

[53] Musa, S., Aura, C. M., Tómasson, T., Sigurgeirsson, Ó., Thorarensen, H. (2022): Impacts of Nile Tilapia Cage Culture on Water and Bottom Sediment Quality: The Ability of a Eutrophic Lake to Absorb and Dilute Perturbations. – Lakes & Reservoirs Research & Management 27(4): e12413. https://doi.org/10.1111/lre.12413.

[54] Norambuena, J.-A., Poblete-Grant, P., Beltrán, J. F., De Los Ríos-Escalante, P., Farías, J. G. (2022): Evidence of the Anthropic Impact on a Crustacean Zooplankton Community in Two North Patagonian Lakes. – Sustainability 14(10): 6052. https://doi.org/10.3390/su14106052.

[55] Obuid-Allah, A., Moustafa, A. S., Hussien, E. H. M., Gaber, A. M., Mohammad, W. A. (2020): Distribution of Zooplankton Community in the River Nile at Esna Barrages, Egypt in Relation to Some Ecological Factors. – Egyptian Journal of Aquatic Biology and Fisheries 24(1): 131-143. https://doi.org/10.21608/ejabf.2020.68904.

[56] Ochocka, A. (2021): ZIPLAS: Zooplankton Index for Polish Lakes' Assessment: A New Method to Assess the Ecological Status of Stratified Lakes. – Environmental Monitoring and Assessment 193: 664. https://doi.org/10.1007/s10661-021-09390-7.

[57] Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D. (2022): vegan: Community Ecology Package (al.). – https://CRAN.R-project.org/package=vegan.

[58] Orçan, F. (2020): Parametric or Non-Parametric: Skewness to Test Normality for Mean Comparison. – International Journal of Assessment Tools in Education 7(2): 255-265. https://doi.org/10.21449/ijate.656077.

[59] Pierce, D. (2019): ncdf4: Interface to Unidata netCDF (version 4) format data files. – R package version 1.17. https://CRAN.R-project.org/package=ncdf4.

[60] Pinti, J., Jónasdóttir, S. H., Record, N. R., Visser, A. W. (2023): The Global Contribution of Seasonally Migrating Copepods to the Biological Carbon Pump. – Limnology and Oceanography 68(5): 1147-1160. https://doi.org/10.1002/lno.12335.

[61] Prowe, A. E. F., Su, B., Nejstgaard, J. C., Schartau, M. (2022): Food Web Structure and Intraguild Predation Affect Ecosystem Functioning in an Established Plankton Model. – Limnology and Oceanography 67(4): 843-855. https://doi.org/10.1002/lno.12039.

[62] R Core Team (2024): R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing. https://www.r-project.org/.

[63] Ramondenc, S., Nöthig, E., Hufnagel, L., Bauerfeind, E., Busch, K., Knüppel, N., Kraft, A., Schröter, F., Seifert, M., Iversen, M. H. (2022): Effects of Atlantification and Changing Sea-ice Dynamics on Zooplankton Community Structure and Carbon Flux Between 2000 and 2016 in the Eastern Fram Strait. – Limnology and Oceanography 68(S1): S39-S53. https://doi.org/10.1002/lno.12192.

[64] Sadaiappan, B., Balakrishnan, P., Vishal, C. R., Vijayan, N. T., Subramanian, M., Gauns, M. (2023): Applications of Machine Learning in Chemical and Biological Oceanography. – Acs Omega 8(18): 15831-15853. https://doi.org/10.1021/acsomega.2c06441.

[65] Samhouri, J. F., Shelton, A. O., Williams, G. D., Feist, B. E., Hennessey, S. M., Bartz, K. K., Kelly, R. P., O'Donnell, J. L., Sheer, M. B., Stier, A. C., Levin, P. S. (2022): How Much

City Is Too Much City? Biodiversity and Ecosystem Functioning Along an Urban Gradient at the Interface of Land and Sea. – Frontiers in Marine Science 9. https://doi.org/10.3389/fmars.2022.931319.

[66] Somerfield, P. J., Clarke, K. R., Gorley, R. N. (2021): A Generalised Analysis of Similarities (ANOSIM) Statistic for Designs with Ordered Factors. – Austral Ecology 46(6): 901-910. https://doi.org/10.1111/aec.13043.

[67] Stoeckle, M. Y., Adolf, J. E., Ausubel, J. H., Charlop–Powers, Z., Dunton, K. J., Hinks, G. (2022): Current Laboratory Protocols for Detecting Fish Species with Environmental DNA Optimize Sensitivity and Reproducibility, Especially for More Abundant Populations. – ICES Journal of Marine Science 79(2): 403-412. https://doi.org/10.1093/icesjms/fsab273.

[68] Stoev, Y., Uzunov, T., Stoyanova, N. S., Grozdanova-Uzunova, R. G., Kosturkov, D., Taneva, I. (2023): Mechanical Properties of Materials for 3D Printed Orthodontic Retainers. – Folia Medica 65(6): 986-992. https://doi.org/10.3897/folmed.65.e107299.

[69] Thomson-Laing, G., Howarth, J., Vandergoes, M. J., Wood, S. A. (2022): Optimised Protocol for the Extraction of Fish <scp>DNA</Scp> from Freshwater Sediments. – Freshwater Biology 67(9): 1584-1603. https://doi.org/10.1111/fwb.13962.

[70] Truelove, N. K., Patin, N., Min, M. A., Pitz, K. J., Preston, C. M., Yamahara, K. M., Zhang, Y., Raanan, B. Y., Kieft, B., Hobson, B., Thompson, L., Goodwin, K. D., Chávez, F. P. (2022): Expanding the Temporal and Spatial Scales of Environmental DNA Research with Autonomous Sampling. – Environmental DNA 4(4): 972-984. https://doi.org/10.1002/edn3.299.

[71] Tsuji, S., Maruyama, A., Miya, M., Ushio, M., Sato, H., Minamoto, T., Yamanaka, H. (2020): Environmental DNA Analysis Shows High Potential as a Tool for Estimating Intraspecific Genetic Diversity in a Wild Fish Population. – Molecular Ecology Resources 20(5): 1248-1258. https://doi.org/10.1111/1755-0998.13165.

[72] Vassallo, P., Bellardini, D., Castellano, M., Dapueto, G., Povero, P. (2021): Structure and Functionality of the Mesozooplankton Community in a Coastal Marine Environment: Portofino Marine Protected Area (Liguria). – Diversity 14(1): 19. https://doi.org/10.3390/d14010019.

[73] Veilleux, H. D., Misutka, M. D., Glover, C. N. (2021): Environmental DNA and Environmental RNA: Current and Prospective Applications for Biological Monitoring. – The Science of the Total Environment 782: 146891. https://doi.org/10.1016/j.scitotenv.2021.146891.

[74] Weeks, T. L., Walkden, P. A., Edwards, D. P., Lees, A. C., Pigot, A. L., Purvis, A., Tobias, J. A. (2025): Land-Use Change Undermines the Stability of Avian Functional Diversity. – Preprint. https://doi.org/10.1101/2025.01.15.633202.

[75] Weinstock, J., Vargas, L. G., Collin, R. (2022): Zooplankton Abundance Reflects Oxygen Concentration and Dissolved Organic Matter in a Seasonally Hypoxic Estuary. – Journal of Marine Science and Engineering 10(3): 427. https://doi.org/10.3390/jmse10030427.

[76] Wickham, H. (2016): ggplot2: Elegant Graphics for Data Analysis. – Springer-Verlag. https://ggplot2.tidyverse.org/.

[77] Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., … Yutani, H. (2019): Tidyverse: Easily Install and Load the Tidyverse. – R package version 1.3.1. https://CRAN.R-project.org/package=tidyverse.

[78] Widiastuti, W., Borumei, D. (2024): Indication of Feeding Ground Inside the Manta Ray's Cleaning Station by Investigation of the Zooplankton Community Composition in Raja Ampat Islands, Indonesia. – Biodiversitas Journal of Biological Diversity 25(3). https://doi.org/10.13057/biodiv/d250340.

[79] Wishner, K. F., Seibel, B. A., Outram, D. M. (2020): Ocean Deoxygenation and Copepods: Coping with Oxygen Minimum Zone Variability. – Biogeosciences 17(8): 2315-2339. https://doi.org/10.5194/bg-17-2315-2020.

[80] Xu, Y., Cheng, F., Ma, X., Mao, M., Zhu, Y., Jiang, Z., Zheng, Z., Zeng, J., Shou, L., Zhou, F., Du, P. (2025): The Impact of Summer Yangtze River Runoff Fluctuations on Estuarine Fronts Dynamics and Zooplankton Communities from 2016 to 2023. – Frontiers in Marine Science 12. https://doi.org/10.3389/fmars.2025.1556561.

[81] Yang, Y., Noble, D. W. A., Senior, A. M., Lagisz, M., Nakagawa, S. (2025): Interpreting Prediction Intervals and Distributions for Decoding Biological Generality in Meta-Analyses. – Preprint. https://doi.org/10.7554/elife.103339.

[82] Zhang, X., Liao, J., Xu, S., Liu, P., Huang, Q., Dumont, H. J., Han, B. (2020): A Set of New Primers for COI (Cytochrome Oxidase Subunit 1 Mitochondrial Gene) Amplification in *Phyllodiaptomus tunguidus* (Copepoda, Calanoida, Diaptomidae). – Mitochondrial DNA Part B 5(2): 1622-1624. https://doi.org/10.1080/23802359.2020.1745100.