



OPEN Evaluation of macro and meiobenthic community structure and distribution in the hybrid ocean thermal energy conversion discharge area of Port Dickson

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Over the past two decades, the technology underlying Hybrid Ocean Thermal Energy Conversion (H-OTEC) power plants have progressively matured. These advancements position H-OTEC as a promising alternative energy source with significant potential to replace traditional power plants. The cold discharge from H-OTEC Pilot Plants reduces the temperature of receiving water bodies, thereby directly or indirectly impacting the marine ecological environment. A one-year study was conducted around a pilot-stage 1.0 MW H-OTEC Pilot Plant in Port Dickson to investigate the effects of cold discharge on macro- and meiobenthic communities across different seasons. Apart from the water temperature within a 5-meter range affected by the H-OTEC cold discharge, the impact on other water quality indicators is negligible. A total of 22 macrobenthic species belonging to 4 phyla and meiobenthic organisms belonging to 9 taxa were identified across 15 sampling points. This study demonstrated that cold emissions had a limited impact on the abundance and community structure of benthic organisms across different seasons. The abundance of benthic organisms exhibited a significant increase in Inter-monsoon, followed by a significant decrease in Dry season. Moreover, there was a positive correlation observed between the abundance of benthic organisms and the content of water temperature, conductivity, gavel, sediment pigments and total organic matter. This study identified significant seasonal variations in the structure of both macro- and meio-benthic communities. Specifically, *Umbonium vestiarium* and other gastropoda were the dominant taxa and primary contributors to the observed significant changes in the structure of macro- and meio-benthic communities, respectively. Nevertheless, further study with a higher discharge volume of the outfall is crucial to assist in the outfall pipe placement of the mega-scale OTEC electricity plant. This study provided crucial insights into the ecological impacts of cold emissions from H-OTEC Pilot Plants in tropical coastal areas.

Keywords H-OTEC pilot plant, Macrobenthos, Meiobenthos, Cold discharge

The 12th Malaysia Plan (12MP) sets the ambitious goal for Malaysia to “become a carbon neutral country by 2050 at the earliest”. Considering that fossil fuel power generation currently accounts for 28.2% of all economic activity and is a major contributor to carbon emissions, it is imperative to explore energy-efficient and low-emission alternatives to fossil fuel power generation. Ocean Thermal Energy Conversion (OTEC) power plants offer a significant advantage in this regard, producing one or two orders of magnitude less carbon dioxide emissions per unit of electricity compared to fossil fuel power plants^{1,2}. With technological advancements, the OTEC power plant developed by Makai Ocean Engineering in Hawaii, USA, was connected to the US power grid in 2015, marking OTEC’s shift from experimental development to commercial application. As a novel form of environmentally friendly energy, OTEC power plants hold substantial potential to replace traditional power plants.

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Malaysia's equatorial location presents a promising opportunity for the utilization of OTEC technology, offering the potential to generate an estimated 12 MW of electricity by the year 2025³. This makes the country an ideal candidate for the implementation of OTEC plants along its coast. Currently, collaborative efforts between Malaysian and Japanese universities, spearheaded by Universiti Teknologi Malaysia (UTM) and Saga University of Japan, are underway to develop the first pilot plant, a 1.0 MW H-OTEC facility, situated at the International Institute of Aquaculture and Aquatic Sciences (I-AQUAS) within Universiti Putra Malaysia in Port Dickson, Negeri Sembilan.

H-OTEC is a system that combines the features of closed and open circulation systems⁴. To optimize energy efficiency, it is recommended to discharge the cooled surface water and warmed artificial water closer to the ocean's surface⁵. However, it is crucial to conduct a thorough assessment and evaluation of the potential impact of such a large water discharge on the ocean ecosystem, as it may cause significant disturbances.

According to the environmental assessment conducted by the National Oceanic and Atmospheric Administration (NOAA) on OTEC power plants, the discharge of deep seawater has been recognized as a significant factor impacting the marine environment of OTEC power plants⁶. This is primarily attributed to the discharge of deep-sea water, which contains abundant nutrients and trace metals, into the relatively nutrient-deficient warm surface seawater. As a result, primary productivity in surface water is stimulated^{7,8}. However, the effects of temperature reduction on the marine ecosystem are not well established. Certain studies have indicated that decreasing temperatures can impede the growth and development of eggs and larvae in benthic fish, leading to significant mortality^{9,10}. Conversely, other studies have suggested that cold deep-water discharges from OTEC plants have a negligible impact on phytoplankton production temperatures¹¹. Given the variations in biogeochemical processes and environmental biology unique to different regions, it becomes imperative to undertake a comprehensive investigation into the potential effect of discharging artificially chilled seawater from operational H-OTEC Pilot Plants on the ecological communities within Port Dickson.

The primary components of marine ecosystems, including phytoplankton, fish, zooplankton, and benthic organisms, serve as the principal environmental receptors¹². Among them, benthic organisms play crucial roles in various ecosystem processes, such as nutrient cycling, primary production, and carbon sequestration¹³. Additionally, they provide essential ecosystem services, encompassing their function as a primary food source and their role in creating habitats for other organisms¹⁴. Due to their characteristics such as limited migration ability, extended lifespan in sediments, substantial size, ease of taxonomic identification, and high susceptibility to environmental fluctuations, parameters such as population structure, dominant species, and abundance of benthic organisms are employed as reliable indicators to accurately assess water quality^{15–17}. However, it is important to note that there is currently limited research on the impact of cold emissions from H-OTEC Pilot Plants on benthic organisms. Therefore, a comprehensive assessment and evaluation of the potential ecological impacts of H-OTEC Pilot Plants on marine benthic organisms is imperative.

This study involves in-situ seasonal surveys conducted to assess the environmental conditions and biological communities surrounding the discharge point of a H-OTEC Pilot Plant, where artificially chilled seawater is released. Prior to sampling in this study, seabed water temperature near the cold-water discharge outlet of the H-OTEC Pilot Plant was monitored. A significant temperature drop was observed within a 5-meter radius of the discharge outlet, while beyond this range, the temperature change ($|\Delta T|$) was minimal ($< 0.3^\circ\text{C}$). The study provides a depiction of the biogeochemical attributes of both seawater and sediment. It is followed by an analysis of the spatial distribution patterns of macrobenthic and meiobenthic community structures nearby the discharge point. This study seeks to determine whether cold-water discharge from a H-OTEC Pilot Plant has ecological effects on the surrounding marine benthic ecosystem, with a focus on seasonal variation across four distinct periods.

Materials and methods

Sampling

Sampling site

To investigate the impact of cold discharges from the H-OTEC Pilot Plant on the structural variability of benthic communities across varying spatial scales, a specific sampling design was employed. The discharge outlet of the H-OTEC Pilot Plant was located on the seabed, approximately 50 m from the coastline, at coordinates $2^\circ 27' 57.4''\text{N}$, $101^\circ 50' 54.6''\text{E}$. This study involved a comprehensive examination of 15 distinct sampling sites, each selected to ascertain the temperature gradient. Sampling sites were arranged into four groups, the sampling point d0f as DG group was placed directly at the discharge outlet, and then set up groupings NG, MG, and FG—representing near group, middle group, and far group, respectively, detail information as describe in Fig. 1; Table 1. The map presented in Fig. 1 was generated using Google Maps (<https://www.google.com/maps>).

Sampling time

To investigate the influence of cold emissions originating from H-OTEC Pilot Plants on the seasonal variability of benthic community structure, comprehensive sampling efforts were carried out. Sampling periods were chosen to represent minimum, maximum, and intermediate precipitation conditions^{18,19}. Meiobenthos, macrobenthos, and sediment samples were systematically collected from all 15 designated stations during the months of July (Dry season) 2023, October (Wet season) 2023, April (Inter-monsoon season) 2024, and May (Inter-monsoon season) 2024.

Sampling procedures

Macrobenthic specimens were methodically gathered from the designated sampling stations using a PVC sampling tube with an inner diameter of 15 cm. From each site, four random replicates were collected, and each sample core measured 5 cm in depth²⁰. These macrobenthos samples were rinsed on-site using seawater



Fig. 1. Map of sampling sites in the H-OTEC Pilot Plant (<https://www.google.com/maps>; accessed on June 23, 2024).

Station	Coordinates	Group	Distance (m)	Depth (m)
D0F	2°27'57.4"N 101°50'54.6"E	DG (discharge point)	0	0.53
D0L5	2°27'57.8"N 101°50'54.7"E	NG (near)	5	0.51
D0L10	2°27'58.2"N 101°50'54.8"E	MG (middle)	10	0.51
D0R5	2°27'57.0"N 101°50'54.5"E	NG (near)	5	0.52
D0R10	2°27'56.6"N 101°50'54.4"E	MG (middle)	10	0.50
D5F	2°27'57.4"N 101°50'54.3"E	NG (near)	5	0.65
D5L5	2°27'57.8"N 101°50'54.4"E	MG (middle)	7.1	0.64
D5L10	2°27'58.2"N 101°50'54.5"E	FG (far)	11.2	0.60
D5R5	2°27'57.0"N 101°50'54.2"E	MG (middle)	7.1	0.63
D5R10	2°27'56.6"N 101°50'54.1"E	FG (far)	11.2	0.63
D10F	2°27'57.4"N 101°50'54.0"E	MG (middle)	10	0.78
D10L5	2°27'57.8"N 101°50'54.1"E	FG (far)	11.2	0.75
D10L10	2°27'58.2"N 101°50'54.2"E	FG (far)	14.1	0.80
D10R5	2°27'57.0"N 101°50'53.9"E	FG (far)	11.2	0.76
D10R10	2°27'56.6"N 101°50'53.8"E	FG (far)	14.1	0.78

Table 1. The geographical positions, groupings, and distances from the sampling sites of the H-OTEC discharge of the sampling stations.

and a 500 μ m mesh sieve²¹. To discern living organisms, the contents of the sieve underwent staining with Rose Bengal at a concentration of 1 g/L. Subsequently, the organisms were preserved in a 70% ethanol solution and transported to the laboratory for subsequent macrobenthic analysis.

Meiobenthic specimens were collected from the same sites using a PVC sampling tube with an inner diameter of 5.5 cm. The sampling depth was 10 cm²², and four replicates were randomly taken from each sampling site. These replicates were combined and placed into zip-lock bags for meiobenthos analysis. In the laboratory, the levigation-decantation-sieving method through two stacked sieves of 500 μ m and 42 μ m mesh sizes, respectively, was employed for meiobenthos extraction^{23,24}. To aid in the identification of living organisms, the samples were stained with Rose Bengal at a concentration of 1 g/L and fixed in a 70% ethanol solution. After thorough mixing, the samples were stored at 4 °C.

For sediment analysis, four additional core samples were collected using the same method applied for meiobenthos sampling, and from the same stations. For sedimentary pigment analysis, the uppermost 1 cm layer of undisturbed sediment was carefully extracted using a centrifuge tube. Approximately 5 g of sediment was placed in a centrifuge tube, thoroughly mixed with 10 ml of 90% acetone solution, and prepared for the analysis of chlorophyll-a and pheophytin-a²⁵. The remaining sediment was consolidated and stored in sealed bags, which were rigorously mixed to ensure uniform distribution and the removal of trapped air. These bags were then stored in a dark environment at -20 °C for subsequent analyses of various sediment characteristics, including total organic matter and grain size.

Environmental parameters, comprising water temperature (seabed), conductivity, pH, dissolved oxygen (DO), and salinity, were measured three times at each site employing a multi-parameter device (YSI ProPlus).

Determination of sedimentary quality parameters

For the analysis of sedimentary pigments, the sediment samples were stored in a dark environment at -20 °C until pigment extraction. Fat-soluble pigments were extracted using a 90% acetone solution. The optical density of the acetone extract was measured before and after acidification using a spectrophotometer to determine the concentrations of chlorophyll-a and pheophytin-a²⁵. Organic matter content was analyzed separately using the ignition method²⁵. Prior to this analysis, the sediment samples were dried at 70 °C for 24 h.

For the assessment of sediment grain size, the sediment samples underwent screening using standard sieves (Astem) after the drying process. The grain size of sediments was categorized into seven distinct fractions: > 2 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm, 0.125–0.25 mm, 0.063–0.25 mm and <0.063 mm. Each fraction was meticulously collected, weighed, and subsequently used to calculate the percentage composition of each fraction²⁶.

Determination of benthic community

Meiobenthic specimens were identified and counted utilizing a stereo microscope (Motic Scientific, SMZ-161 Series) operating at a magnification of 50×. Macrobenthos were identified at the species level and meiobenthos at the major taxonomic level based on morphological examination^{27–29}. Ultimately, the macrobenthos species identified were cross-referenced with the known benthic species cataloged in Malaysia³⁰. Following identification, specimens were enumerated, with the number of macrobenthic individuals expressed per square meter (1 m²) and the number of meiobenthic individuals quantified per 10 square centimeters (10 cm²).

Statistical analysis

Biotic and abiotic data underwent normality testing using the Shapiro-Wilk test, where P-values ≥ 0.05 were indicative of normality. These statistical analyses were conducted using SPSS version 17.0. Subsequently, an analysis of similarities (ANOSIM) was performed to determine significant differences in macro- and meiobenthic composition among groups in different seasons. The Kruskal-Wallis test was applied to assess differences in total abundance, diversity indices, and environmental parameters including water temperature, salinity, pH, conductivity, dissolved oxygen (DO), total organic matter (TOM %), chlorophyll-a (Chl-a), pheophytin-a (Pha-a), and sediment grain size among the different sampling groups.

To assess the influence of site and season on meiobenthic communities, the PERMANOVA test was applied. Non-metric multidimensional scaling (nMDS) was employed to visually represent site groups and layer differences based on meiobenthic assemblage structures. Binomial similarity matrices and square-root transformed abundance data were used in constructing the nMDS. Additionally, the SIMPER (Similarity Percentage) test was conducted using R software, Version 4.3.3 (<https://www.r-project.org>) to identify the species primarily responsible for observed differences among the three seasons. There are three commonly used ecological indices for measuring species diversity:

Shannon-Weiner Diversity Index (H'): The Shannon index measures species diversity by taking into account both species richness and evenness³¹.

$$H' = -\sum (P_i \times \ln P_i) \quad (1)$$

Pielou evenness index (J): The Pielou index assesses the evenness or equitability of species distribution within a community³².

$$J = H' / \ln S \quad (2)$$

Margalef richness index (d): reflects a certain spatial range number of species³³.

$$d = (S - 1) / \ln N \quad (3)$$

In the formula: \sum represents the sum of all species in the community; P_i is the ratio of the abundance of the *i*th species at the monitoring station to the abundance of samples; N is the total abundance of all samples; S is the total number of species contained in all monitoring stations.

Similarity between samples was assessed using R 4.3.3 software, with the data subjected to square root transformation to mitigate the influence of dominant species. The Bray Curtis index was employed to calculate similarity across groups during four different seasons. Before redundancy analysis (RDA), the outcomes of detrended correspondence analysis (DCA) revealed that the maximum gradient length of the primary axis for macrobenthic organisms was all below 3. Finally, RDA in CANOCO 5 (Microcomputer Power, Inc., USA) was

employed to discern the sediment environmental variables that impact the community structure of macro- and meio-benthic organisms.

Result
Changes in environmental variables

Changes in water quality variables
Five water quality variables, encompassing water temperature, salinity, pH, dissolved oxygen (DO), and electrical conductivity, have been shown in Table 2. Significant differences in water temperature were observed among the four groups ($\chi^2 = 9.12$, $P < 0.05$ in the dry season; $\chi^2 = 9.02$, $P < 0.05$ in the inter-monsoon season; $\chi^2 = 10.21$, $P < 0.05$ in the wet season). Conductivity differed significantly among groups in the wet season ($\chi^2 = 10.65$, $P < 0.01$), while dissolved oxygen showed significant differences among groups in the inter-monsoon season ($\chi^2 = 11.10$, $P < 0.01$). In contrast, salinity ($P = 0.06 \sim 0.75$) and pH ($P = 0.07 \sim 0.33$) did not differ significantly among groups across all seasons (Fig. 2; Table 2).

Changes in sediment variables
During the inter-monsoon season, Pha-a and TOM significantly varied significantly among sites ($\chi^2 = 8.93$, $P < 0.05$; $\chi^2 = 8.49$, $P < 0.05$). During the dry season, gravel and sand also showed significant variation across sites ($\chi^2 = 11.74$, $P < 0.01$; $\chi^2 = 11.74$, $P < 0.01$). Chl-a ($P = 0.23 \sim 0.79$) and silt and clay ($P = 0.06 \sim 0.66$) did not show significant variation across sites in any season (Fig. 3; Table 3).

Changes in abundance and ecological indices
Macrobenthic abundance and ecological indices

The Kruskal-Wallis's test showed significant differences in the abundance of macrobenthos across all stations ($\chi^2 = 10.16$, $p < 0.05$ in the inter-monsoon season; $\chi^2 = 9.22$, $p < 0.05$ in the dry season; $\chi^2 = 9.78$, $p < 0.05$ in the wet season). During the inter-monsoon season, the highest abundance was recorded in the FG group (951.54 ± 116.72 ind./m²), while the lowest was observed in the DG group (113.23 ± 59.83 ind./m²). In the dry season, peak abundance was found in both the FG and NG groups (667.54 ± 216.72 and 734.73 ± 17.56 ind./m², respectively), with the lowest in the DG group (205.43 ± 119.75 ind./m²). During the wet season, the highest abundance occurred in the FG and NG groups (817.15 ± 209.69 and 800.54 ± 103.95 ind./m², respectively), and the lowest in the DG group (307.88 ± 65.23 ind./m²) (Fig. 4a).

However, the Margalef, Shannon, and Pielou indices of macrobenthos showed no significant differences among stations across all seasons ($P > 0.05$) (Fig. 4b, c, d). The highest (0.90 ± 0.08) and lowest (0.45 ± 0.05) values of the Margalef Index were recorded in the wet and dry seasons, respectively. The Shannon Index reached its maximum value (0.97 ± 0.07) during the wet season and its minimum value (0.50 ± 0.03) during the inter-monsoon season. The Pielou Index was highest in the dry season (0.61 ± 0.09) and lowest during the inter-monsoon season (0.29 ± 0.11).

Meiobenthic abundance and ecological indices
The Kruskal-Wallis's test showed a significant difference in meiobenthic abundance among groups in the inter-monsoon season ($\chi^2 = 8.11$, $p < 0.05$), dry season ($\chi^2 = 11.27$, $p < 0.01$), and wet season ($\chi^2 = 9.68$, $p < 0.05$). The maximum abundance of meiobenthos (1206.62 ± 346.53 ind./10 cm²) was observed in FG group, while the minimum abundance (314.42 ± 84.18 ind./10 cm²) occurred in DG group (Fig. 5a).

The Margalef Index, Shannon Index, and Pielou Index of meiobenthos showed no significant variation among different sites ($P > 0.05$) (Fig. 5b, c, and d). The highest (1.01 ± 0.11) and lowest (0.45 ± 0.33) values of the Margalef Index occurred in Dry and Inter-monsoon, respectively. Similarly, the Shannon Index recorded its highest value (0.94 ± 0.19) in Dry and its lowest value (0.47 ± 0.11) in Inter-monsoon. However, the Pielou

Group	Season	Salinity (ppt)	Water Temperature (°C)	pH	Conductivity (ms/cm)	Dissolved Oxygen (mg/L)
DG	Dry	28.63 (28.63–28.64)	28.3 (27.9–28.42)	8.25 (8.24–8.27)	49.85 (49.48–50.2)	3.25 (3.1–3.4)
DG	Inter-monsoon	28.55 (28.55–28.55)	28.4 (28.37–28.52)	8.2 (8.2–8.21)	50.6 (50.4–50.7)	3.7 (3.6–3.8)
DG	Wet	28.41 (28.39–28.42)	28.75 (28.58–28.92)	8.36 (8.36–8.37)	49.3 (49.28–49.32)	5.7 (5.68–5.73)
FG	Dry	28.66 (28.64–28.66)	31.4 (31.37–31.42)	8.25 (8.25–8.27)	49.7 (49.65–49.8)	3.55 (3.35–3.7)
FG	Inter-monsoon	28.55 (28.53–28.56)	31.75 (31.67–31.83)	8.23 (8.23–8.23)	50.9 (50.68–51.1)	4.1 (4–4.3)
FG	Wet	28.13 (27.92–28.27)	30.85 (30.75–31)	8.31 (8.3–8.33)	48.31 (46.38–48.62)	5.5 (5.33–5.75)
MG	Dry	28.64 (28.64–28.64)	31.5 (31.37–31.6)	8.25 (8.25–8.27)	50.05 (49.8–50.32)	3.6 (3.48–3.73)
MG	Inter-monsoon	28.56 (28.49–28.57)	31.75 (31.7–31.8)	8.21 (8.2–8.21)	50.2 (50.13–50.33)	3.45 (3.4–3.55)
MG	Wet	28.3 (28.06–28.35)	30.8 (30.78–30.85)	8.33 (8.32–8.35)	48.7 (48.52–48.85)	5.55 (5.5–5.6)
NG	Dry	28.64 (28.63–28.65)	31.45 (31.27–31.5)	8.26 (8.25–8.28)	50.2 (50.03–50.32)	3.4 (3.2–3.68)
NG	Inter-monsoon	28.55 (28.47–28.56)	31.7 (31.62–31.75)	8.2 (8.2–8.2)	50.3 (49.88–50.78)	3.75 (3.65–3.8)
NG	Wet	28.4 (28.38–28.4)	31 (30.98–31.02)	8.38 (8.37–8.38)	49.3 (49.28–49.31)	5.65 (5.57–5.73)

Table 2. Median (Q1–Q3) of water quality parameters were measured at the sampling sites near the H-OTEC pilot plant within three seasons.

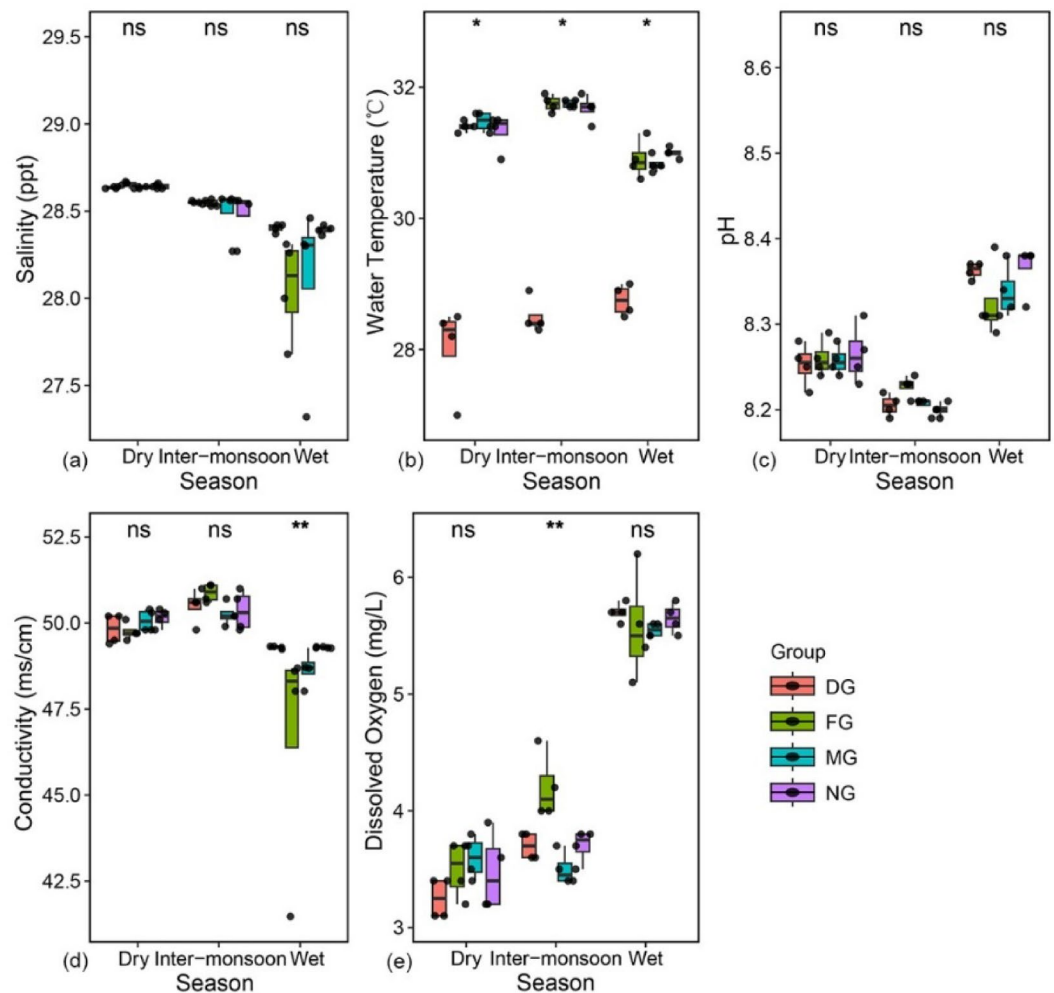


Fig. 2. Salinity, temperature, pH, conductivity, and dissolved oxygen in the study area among sites in three seasons. For the boxplot figures, the box borders represent the interquartile range (IQR), while the horizontal line within the box indicates the median value. The upper and lower whiskers extend to 1.5 times the IQR beyond the upper and lower quartiles, respectively. Statistical significance is indicated by asterisks (Kruskal-Wallis test, p-values: * < 0.05; ** < 0.01; *** < 0.001).

Index reached its highest value (0.62 ± 0.05) in Dry and its lowest value (0.29 ± 0.13) in Inter-monsoon (Fig. 5b, c, and d).

Macrobenthic and meiobenthic composition

Macrobenthic composition

The Bray-Curtis index indicated that there was a significant difference in the macro-benthic community structure among different sites during three different seasons ($P < 0.01$ for the inter-monsoon season; $P < 0.01$ for the dry season; $P < 0.01$ for the wet season) (Fig. 6).

A total of 2089 macrobenthic individuals were identified, belonging to 22 species, 14 families, 10 orders, and 5 classes (gastropod, bivalvia, pilidiophora, malacostraca, and polychaeta) (Fig. 7; Table 4). The dominant macrobenthic species included *Umbonium vestiarium* (95.89%), *Celebratulus lacteus* (1.14%), *Nephtys hombergii* (0.62%), and *Drupella margariticola* (0.53%). The remaining species, including *Peristernia nassatula*, *Pagurus minutus*, *Cerithium trillii*, *Clithon oualaniensis*, and so on, collectively constituted 1.82% of the macrobenthic community.

The results of SIMPER indicated that within four groups, the most dissimilarity of 76.55% was observed between DG and FG group, with species *Umbonium vestiarium* (Gastropoda: Trochidae), *Celebratulus lacteus* (Pilidiophora: Lineidae), and *Nephtys hombergii* (Polychaeta: Nephtyidae) contributing the most to this dissimilarity, accounting for 96.26% of the cumulative difference (Table 5). The least percentage of dissimilarity (31.56%) was observed between the NG and MG groups, with the same three species contributing 92.91% to the cumulative dissimilarity (Table 5). *Umbonium vestiarium* was the dominant macrobenthos in all sites and seasons, accounting for up to 98.47% of the macrobenthic community in the FG group in the inter-monsoon season (Fig. 7).

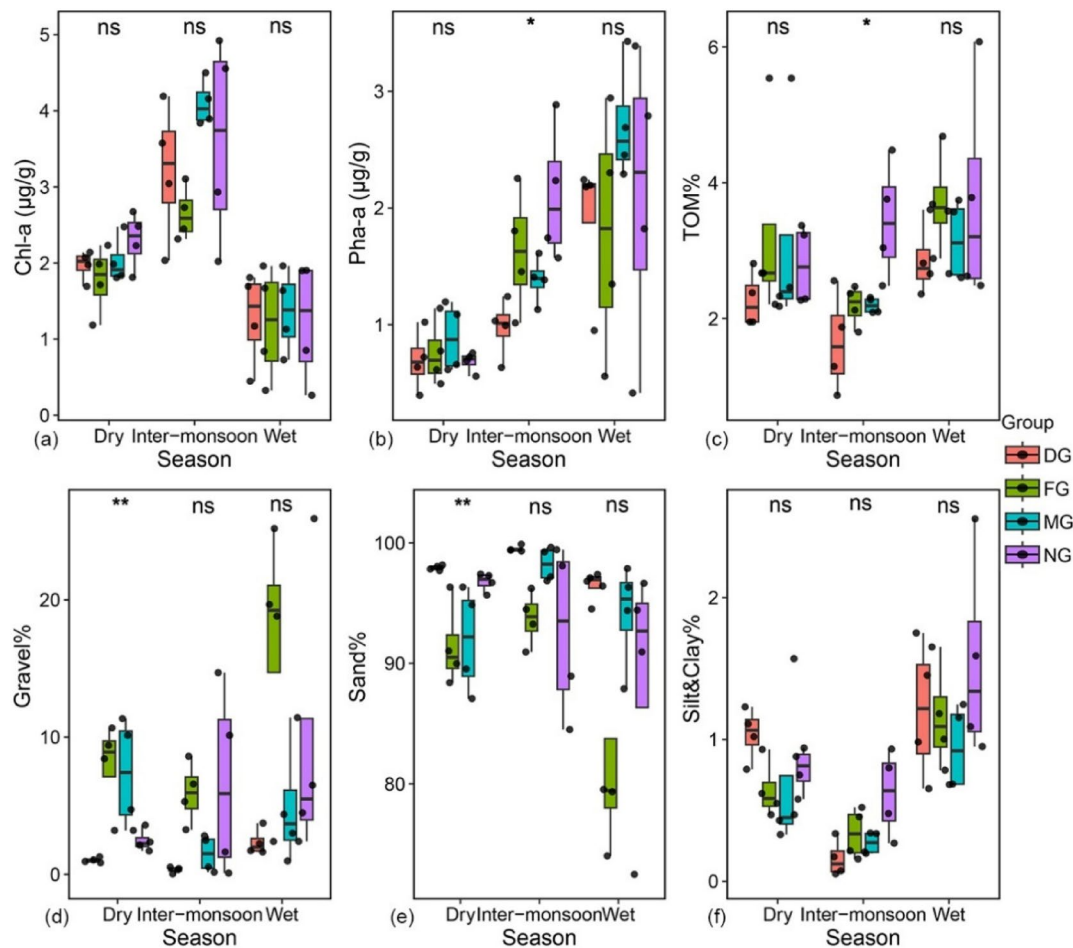


Fig. 3. Chl-a, Pha-a, TOM, gravel, sand, and silt&clay in the study area among sites in three seasons. For a detailed introduction to boxplot figures, please see the caption of Fig. 2.

Group	Season	Chl-a (µg/g)	Pha-a (µg/g)	TOM%	Gravel%	Sand%	Silt&Clay%
DG	Dry	2.02 (1.9–2.09)	0.68 (0.58–0.8)	2.16 (1.95–2.49)	1 (0.92–1.11)	97.94 (97.8–98.07)	1.06 (0.96–1.14)
DG	Inter-monsoon	3.31 (2.79–3.73)	1.01 (0.9–1.08)	1.58 (1.19–2.04)	0.38 (0.26–0.45)	99.41 (99.38–99.53)	0.12 (0.07–0.21)
DG	Wet	1.43 (0.99–1.72)	2.19 (1.87–2.21)	2.74 (2.58–3.01)	1.99 (1.71–2.62)	96.96 (96.24–97.17)	1.22 (0.9–1.53)
FG	Dry	1.85 (1.58–2.05)	0.7 (0.58–0.87)	2.67 (2.55–3.39)	8.91 (7.11–9.72)	90.5 (89.59–92.36)	0.58 (0.53–0.7)
FG	Inter-monsoon	2.59 (2.41–2.82)	1.63 (1.34–1.92)	2.25 (2.04–2.39)	5.94 (4.79–7.09)	93.87 (92.68–94.92)	0.34 (0.2–0.47)
FG	Wet	1.25 (0.71–1.74)	1.82 (1.15–2.46)	3.63 (3.41–3.93)	19.24 (14.71–21.04)	79.44 (78.01–83.76)	1.09 (0.95–1.3)
MG	Dry	1.91 (1.83–2.11)	0.87 (0.65–1.11)	2.4 (2.29–3.23)	7.43 (4.34–10.44)	92.19 (88.92–95.22)	0.45 (0.41–0.74)
MG	Inter-monsoon	4.03 (3.88–4.24)	1.4 (1.32–1.46)	2.19 (2.1–2.29)	1.51 (0.45–2.56)	98.22 (97.11–99.35)	0.27 (0.21–0.34)
MG	Wet	1.38 (1.03–1.72)	2.57 (2.41–2.87)	3.11 (2.65–3.61)	3.69 (2.5–6.13)	95.35 (92.76–96.7)	0.92 (0.69–1.18)
NG	Dry	2.36 (2.12–2.53)	0.71 (0.66–0.73)	2.76 (2.29–3.26)	2.25 (2.03–2.67)	96.99 (96.44–97.31)	0.81 (0.71–0.9)
NG	Inter-monsoon	3.74 (2.7–4.65)	1.99 (1.7–2.4)	3.4 (2.9–3.94)	5.88 (1.25–11.27)	93.52 (87.83–98.43)	0.64 (0.43–0.83)
NG	Wet	1.38 (0.7–1.9)	2.31 (1.47–2.94)	3.2 (2.59–4.36)	5.49 (3.97–11.35)	92.69 (86.34–94.98)	1.34 (1.06–1.83)

Table 3. Median (Q1–Q3) of sediment parameters were measured at the sampling sites near the H-OTEC within three seasons.

Meiobenthic composition

The Bray-Curtis index revealed significant differences in meio-benthic community structure among groups in Dry season ($P < 0.05$), while no significant differences were observed in Inter-monsoon and Wet season ($P > 0.05$) (Fig. 8).

A total of 2,563 individuals representing 9 distinct meiobenthic taxa were recorded. The meiobenthic community was primarily dominated by gastropoda (Table 6). The meiobenthic community exhibited the

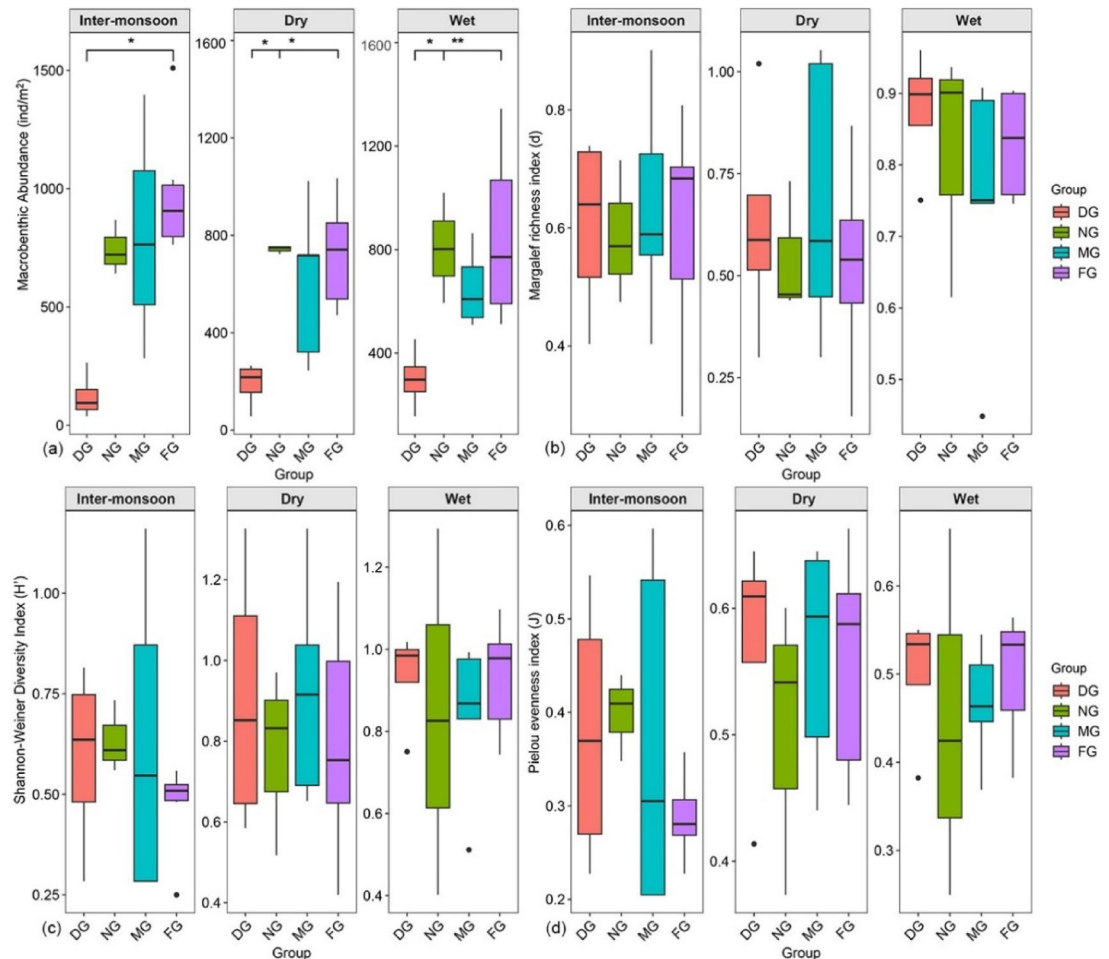


Fig. 4. Comparison of macrobenthic abundance and ecological indices among groups in different seasons adjacent to the H-OTEC Pilot Plant. For a detailed introduction to boxplot figures, please see the caption of Fig. 2.

following distribution in terms of relative abundances: gastropoda (77.02%), nematoda (7.85%), ostracoda (3.97%), bivalvia (3.06%), while foraminifera, polychaeta, copepoda, harpacticoida, and isopoda collectively accounted for 8.10%. Gastropoda was the dominant meiobenthos in all sites and seasons, accounting for up to 86.37% of the meiobenthic community in the FG group in Inter-monsoon season (Fig. 9).

The results of the SIMPER analysis indicated that among the four groups, the greatest dissimilarity (26.90%) was observed between the DG and NG groups, with gastropoda, nematodes, and ostracods contributing the most to this difference, accounting for 73.09% of the cumulative dissimilarity (Table 7). The lowest percentage of dissimilarity (18.48%) was observed between the MG and FG groups, with gastropoda, nematodes, and ostracods contributing 66.70% to the cumulative dissimilarity.

Impact of environmental factors on the benthos in the analysis of RDA

A redundancy analysis (RDA) was used to investigate relationships between macrobenthic species and environmental variables (Fig. 10). The first and second axes explained 39.92% and 21.95% of the variation, respectively (Fig. 10). The first axis was mainly contributed by dissolved oxygen, Pha-a, TOM and gravel, whereas the second axis was influenced by water temperature. The most dominant species, *Umbonium vestiarium* was associated with environments characterized by high water temperature, TOM and gravel concentrations, while it was less prevalent in environments with relatively high silt&clay and sand levels (Fig. 10). *Celebratulus lacteus* was associated with environments exhibiting elevated Chl-a, conductivity, and salinity.

Redundancy analysis (RDA) correlation triplots were used to explore the relationships between meiobenthic taxa and environmental variables (Fig. 11). The first two axes explained 43.97% and 20.03% of the variation, respectively (Fig. 11). The first axis was mainly contributed by Pha-a, gravel and dissolved oxygen, while the second axis was mainly associated with pH, and inversely correlated with conductivity, salinity and Chl-a concentration. Gastropoda were associated with environments characterized by high conductivity, salinity and Chl-a level environment, while nematoda were associated with environments exhibiting relatively high sand content and elevated Chl-a concentrations (Fig. 11).

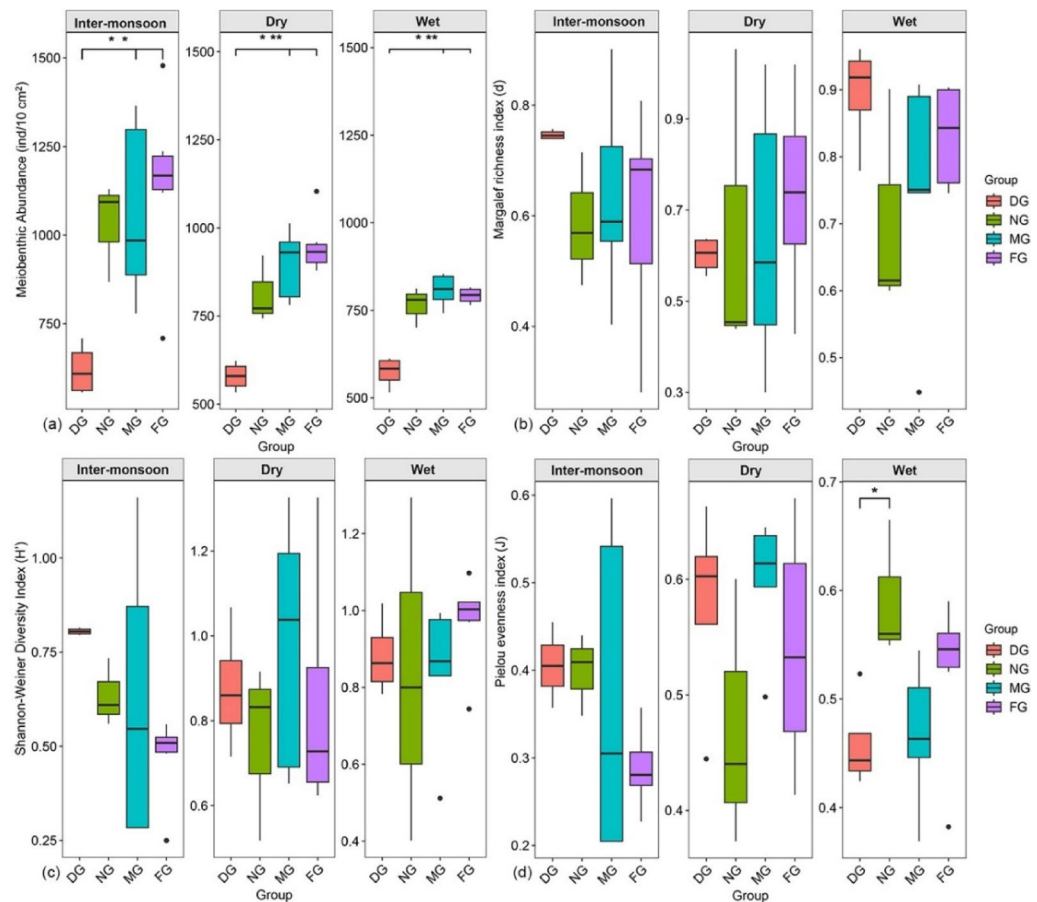


Fig. 5. Comparison of meiobenthic abundance and ecological indices among groups in different seasons adjacent to the H-OTEC Pilot Plant. For a detailed introduction to boxplot figures, please refer to the caption of Fig. 2.

Discussion

Ocean energy production reduces the emission of CO₂ into atmosphere and thus, helps mitigate the effects of climate change on the marine environment^{34,35}. According to Rau and Baird, OTEC systems could be seen having negative CO₂ emissions³⁶. For a tropical marine region like Malaysia, Ocean Thermal Energy Conversion (OTEC) offers advantages such as energy reliability, sustainability, low maintenance costs, and environmental friendliness³. Malaysia is currently constructing a pilot power plant at I-AQUAS UPM in Port Dickson, Negeri Sembilan. Consequently, this study was conducted in the vicinity of the hybrid OTEC facilities to identify the extent and scope of potential environmental changes in the area.

Effect of cold discharge on benthic abundance and community

The H-OTEC Pilot Plant discharges 30 m³/h of cold water at a temperature of 7–10 °C. A significant temperature drop was observed within a 5-meter radius of the discharge outlet, whereas temperature changes beyond this range were minimal (<0.3 °C). Giraud et al. (2019) conducted a study on the 10 MW OTEC pilot plant located on the Caribbean coast of Martinique. The plant pumped approximately 100,000 m³/h of deep seawater from a depth of 1100 m and discharged it at different discharge depths. Their study found that when the temperature difference threshold was set at 0.3 °C, the cold-water effect was confined to a range of just 1 km—only 2% of the anticipated impact area¹¹. Consistent with the findings of this study, the impact range for temperature changes due to cold emissions was relatively limited.

This study found a significant decrease in the abundance of both macro- and meio- benthic organisms in the DG group, while no significant changes were observed in the NG, MG, and FG groups (Figs. 4 and 6). Water temperature is a critical environmental factor influencing the abundance of benthic organisms in aquatic ecosystems^{37,38}. Reid and Harley (2021) found that low temperatures can cause significant losses to intertidal species such as *Littorina scutulata*. The effect of temperature on the abundance of benthic organisms is primarily due to its role as a key factor regulating the growth and development of these organisms³⁸. Whiteley et al. (1997) found that the protein synthesis rate in the leg and abdominal muscles of *Austropotamobius pallipes* and *Glyptonotus antarcticus* was significantly inhibited as the temperature decreased. Conversely, the transcription rates of several proteins in the muscles, including actin and myosin heavy chain (MHC), increased with rising temperatures³⁹.

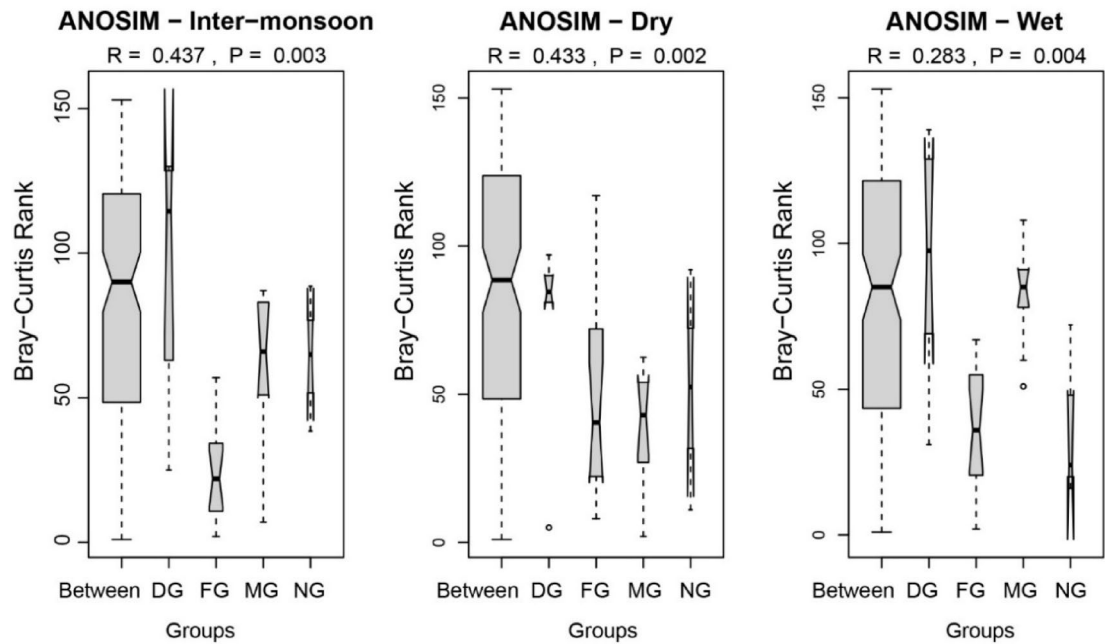


Fig. 6. Comparison of macrobenthic composition within and among groups in different seasons. For ANOSIM figures, the Y-axis represents the dissimilarity rank distribution. The box border represents the interquartile range (IQR), while the horizontal line within the box indicates the median value. The whiskers extend to 1.5 times the IQR beyond the upper and lower quartiles. Differences among groups are reflected in the between. The test statistic R ranges from –1 to 1: $R > 0$ indicates greater similarity within groups, whereas $R < 0$ suggests greater similarity Between groups than within groups. Significance levels are indicated by P values (*, < 0.05 ; **, < 0.01 ; ***, < 0.001).

Phylum	Class	Order	Family	Species	Inter-monsoon	Dry	Wet	Reference
Mollusca	Gastropoda	Trochida	Trochidae	<i>Umbonium vestiarium</i>	737.58 ± 177.69	437.01 ± 89.97	549.71 ± 69.52	Linnaeus, 1758
				<i>Turbo marmoratus</i>	-	1.18 ± 1.18	-	Linnaeus, 1758
		Cycloneritida	Neritidae	<i>Clithon oualaniensis</i>	-	-	1.77 ± 1.21	Lesson, 1831
				<i>Melanoides tuberculata</i>	-	-	1.77 ± 1.21	O. F. Müller, 1774
		Caenogastropoda incertae sedis	Cerithiidae	<i>Clypeomorus bifasciata</i>	-	2.36 ± 1.61	-	G. B. Sowerby II, 1855
				<i>Cerithium trailii</i>	-	2.36 ± 1.61	-	G. B. Sowerby II, 1855
		Neogastropoda	Nassariidae	<i>Nassarius stolatus</i>	1.18 ± 1.18	-	-	Gmelin, 1791
				<i>Peristernia nassatula</i>	-	5.9 ± 2.26	-	Lamarck, 1822
			Muricidae	<i>Drupella margariticola</i>	-	-	11.5 ± 3.47	Broderip, 1833
				<i>Thais clavigera</i>	-	-	0.88 ± 0.88	Küster, 1860
			Columbellidae	<i>Astyrus lunata</i>	-	-	-	Say, 1826
		Littorinimorpha	Naticidae	<i>Tanea lineata</i>	-	-	0.88 ± 0.88	Röding, 1798
		Unionida	Unionidae	<i>Ensidens ingallsianus</i>	-	-	0.88 ± 0.88	I. Lea, 1852
				<i>Hyriopsis biolata</i>	-	-	0.88 ± 0.88	Simpson, 1900
				<i>Pseudodon cumingii</i>	-	-	-	I. Lea, 1851
	Bivalvia	Venerida	Cyrenidae	<i>Polymesoda erosa</i>	-	-	1.77 ± 1.21	Solander, 1786
				<i>Polymesoda expansa</i>	-	-	0.88 ± 0.88	Mousson, 1849
				<i>Polymesoda bengalensis</i>	-	-	1.77 ± 1.21	Lamarck, 1818
				<i>Batissa violacea</i>	1.18 ± 1.18	-	1.77 ± 1.21	Lamarck, 1818
Nemertea	Pilidiophora	Heteronemertea	Lineidae	<i>Cerebratulus lacteus</i>	12.97 ± 5.37	5.9 ± 3.74	-	Leidy, 1851
Arthropoda	Malacostraca	Decapoda	Paguroidea	<i>Pagurus minutus</i>	5.9 ± 2.26	-	-	Benedict, 1892
Annelida	Polychaeta	Phyllodocida	Nereididae	<i>Nephtys hombergii</i>	5.16 ± 2.91	8.26 ± 2.97	-	Savigny in Lamarck, 1818

Table 4. Average (±SE) abundance (ind./m²) of common macrobenthic species adjacent to the H-OTEC pilot Plant.

Location	Group	Cumulative contribution (%)	Average dissimilarity (%)
DG-NG	<i>Umbonium vestiarius</i> ***, <i>Nephtys hombergii</i> , and <i>Celebratulus lacteus</i>	96.03	65.95
DG-MG	<i>Umbonium vestiarius</i> ***, <i>Celebratulus lacteus</i> , and <i>Nephtys hombergii</i>	93.00	64.12
DG-FG	<i>Umbonium vestiarius</i> ***, <i>Celebratulus lacteus</i> , and <i>Nephtys hombergii</i>	96.26	76.55
NG-MG	<i>Umbonium vestiarius</i> , <i>Celebratulus lacteus</i> and <i>Nephtys hombergii</i>	92.91	31.56
NG-FG	<i>Umbonium vestiarius</i> , <i>Celebratulus lacteus</i> and <i>Nephtys hombergii</i>	95.27	33.40
MG-FG	<i>Umbonium vestiarius</i> , <i>Celebratulus lacteus</i> and <i>Drupella margariticola</i>	94.39	39.09

Table 5. SIMPER results illustrate the contribution of the most influential species to the average dissimilarity among the macrobenthic assemblages among the four different locations.

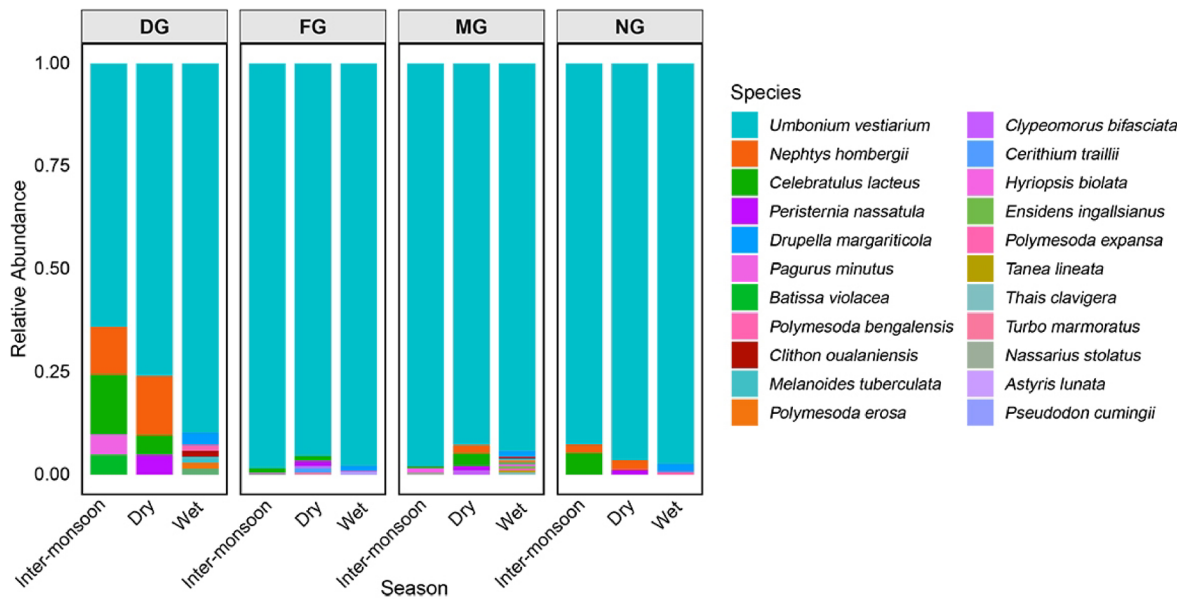


Fig. 7. Comparison of the relative abundance of the 22 macrobenthic species among groups across different seasons.

A marked change in the community structure of macrobenthic organisms in the DG group, with high internal dissimilarity within the group (Fig. 6), whereas the NG, MG, and FG groups exhibited similar community structures (Tables 5 and 7). The cold-water discharge led to reductions in water temperature, gravel content, and TOM in the surrounding area (Fig. 2). *U. vestiarius* exhibited a positive correlation with water temperature, gravel content, and TOM (Fig. 10), which is consistent with its ecological preferences. Specifically, *U. vestiarius* is sensitive to ambient temperature⁴⁰, favors coarser sediments due to its higher mobility and feeding efficiency⁴¹, and is more abundant in environments with high organic matter and rich food resources⁴². As *U. vestiarius* is a dominant species within the macrobenthic community, its decline due to cold discharge likely contributed substantially to the observed shifts in community structure.

Effect of season on benthic abundance and community

Given the extremely limited research on the seasonal variation of benthic organisms in Port Dickson, most studies have focused on the eastern and southern coasts of Peninsular Malaysia. These regions have different climates^{18,19}, which result in distinct environmental indicators compared to those in Port Dickson. As depicted in Figs. 4 and 5, the abundance of macro- and meio-benthic organisms reached its lowest point in Dry and peaks in Inter-monsoon, diverging from findings in previous studies on the seasonality of benthic organisms in Malaysia^{23,43,44}. Additionally, the species composition of benthic organisms varies between these regions, leading to different seasonal trends in benthic abundance and ecological indices. As shown in Figs. 3 and 4, and 5, the Chl-a and conductivity values were highest in Inter-monsoon, corresponding to the highest total abundance of macro- and meio-benthic organisms. In Wet, when Pha-a, TOM and dissolved oxygen were at its highest, the total abundance of macrobenthic organisms was also high. Conversely, in Dry, when the concentrations of Chl-a, dissolved oxygen and Pha-a were the lowest, the total abundance of macro- and meio-benthic organisms reached its minimum. This is consistent with previous studies, which have found that the abundance of benthic organisms is positively correlated with the content of sediment pigments, conductivity, dissolved oxygen and organic matter^{45–48}.

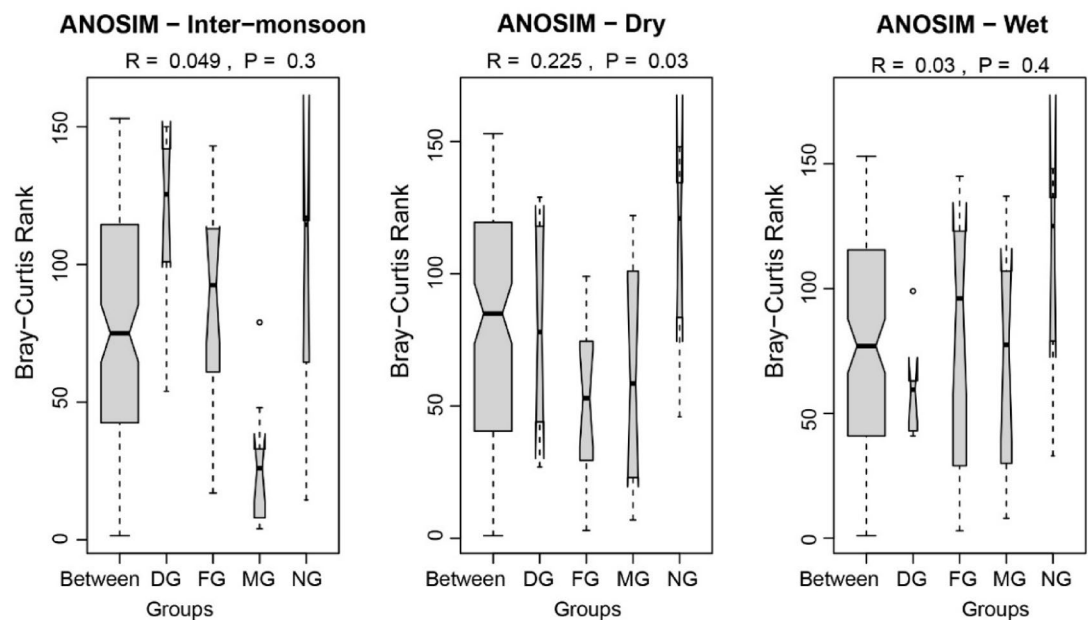


Fig. 8. Comparison of meiobenthic composition within and among groups during different seasons. For a detailed introduction of ANOSIM figures, please refer to the caption of Fig. 6.

Taxa	Inter-monsoon	Dry	Wet
Nematoda	47.48 ± 9.2	97.38 ± 20.88	43.24 ± 6.23
Copepoda	16.15 ± 3.95	7.58 ± 2.59	17.66 ± 3.92
Polychaeta	21.89 ± 6.09	20.86 ± 6.65	6.72 ± 1.76
Ostracoda	20.3 ± 4.7	31.31 ± 8.05	43.62 ± 5.66
Gastropoda	714.99 ± 34.52	591.88 ± 33.29	539.24 ± 26.91
Bivalvia	21.49 ± 3.76	27.74 ± 5.1	24.21 ± 3.5
Harpacticoida	18.05 ± 3.29	8.61 ± 2.62	16.49 ± 3.27
Isopoda	1.95 ± 1.95	9.96 ± 4.18	7.89 ± 3.68
Foraminifera	7.63 ± 3.63	9.72 ± 8.04	22.77 ± 6.88

Table 6. Average (± SE) abundance (ind./10 cm²) of common meiobenthic groups adjacent to the H-OTEC pilot Plant.

This study identified *Umbonium vestiarium*, *Nephtys hombergii* and *Celebratulus lacteus* as the primary species contributing to seasonal changes in the macrobenthic community structure, while gastropod taxa emerged as the main contributors to changes in the meiobenthic community. The abundance of gastropoda was strongly positively correlated with the concentration of Chl-a, salinity, and conductivity^{40,49}. Since *Celebratulus lacteus* preyed on gastropoda⁵⁰, both gastropoda and *C. lacteus* were well-suited to the inter-monsoon season, exhibiting higher relative abundances during this period. Meanwhile, the abundance of *Umbonium vestiarium* was strongly positively correlated with total organic matter (TOM) concentration^{40,42}, contributing to its higher relative abundance during the wet season. Similarly, *Nephtys hombergii* and *Nematoda* were highly resistant to hypoxic conditions^{51,52}, which likely accounts for their increased relative abundance in dry season.

Conclusion

This study evaluated the seasonal impact of H-OTEC Pilot Plant cold discharge on macro- and meio-benthic communities at Port Dickson using in situ monitoring methods over three seasons. Given that the growth and development of benthic organisms are directly influenced by ambient temperature, it is crucial to assess the temperature changes induced by the cold discharge from H-OTEC Pilot Plants. According to the cold discharge water temperature test results, a temperature difference of $|\Delta T| < 0.3$ °C affects the area within 5 m. Additionally, cold discharge does not induce significant changes in the physical and chemical indicators of water quality, apart from water temperature.

The results indicate significant seasonal variations in macro- and meio-benthic communities along the Port Dickson coast. The abundance of macro- and meio-benthic organisms reaches its lowest point in Dry and peaks in Inter-monsoon. At the same time, this study found significant differences in the structure of macro- and

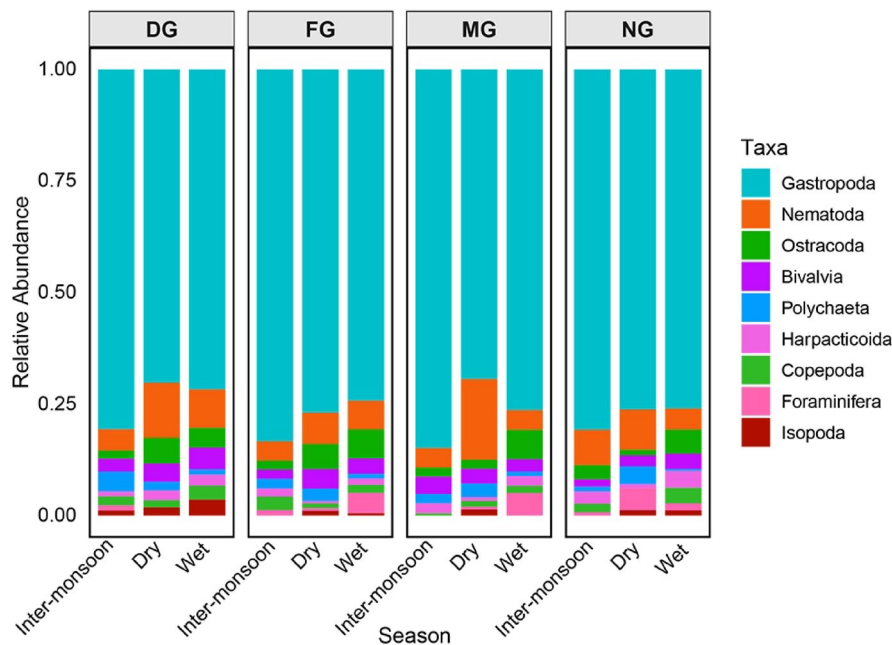


Fig. 9. Comparison of the relative abundance of various meiobenthos taxa among groups across different seasons.

Location	Group	Cumulative contribution (%)	Average dissimilarity (%)
DG-NG	gastropoda**, nematoda, and ostracoda	73.09	26.90
DG-MG	gastropoda**, nematoda, and ostracoda	75.48	26.75
DG-FG	gastropoda***, nematoda, and ostracoda	73.27	26.43
NG-MG	gastropoda, nematoda and foraminifera	67.81	18.99
NG-FG	gastropoda, nematoda, and ostracoda	66.28	19.96
MG-FG	gastropoda, nematoda, and ostracoda	67.70	18.48

Table 7. SIMPER results illustrate the contribution of the most influential taxa to the average dissimilarity among the meiobenthic assemblages among the four different locations.

meio-benthic communities across different groups in different season and station, respectively. The abundance of gastropoda in both macro- and meio-benthic communities may be influenced by one or more factors, such as water temperature, conductivity, gravel, Chl-a, and TOM.

This study provides preliminary insights into the ecological response of benthic communities to cold-water discharge from the 1.0 MW H-OTEC Pilot Plant. At this pilot stage, with a relatively low discharge volume, the cold discharge significantly altered the abundance and community structure of macro- and meio-benthic organisms within 5-meter. These findings serve as a valuable baseline for assessing potential ecological changes associated with larger-scale OTEC operations. However, due to the limited scale and early stage of the plant's operation, further studies involving higher discharge volumes and long-term ecological monitoring of benthic communities are essential. Such research will be critical for supporting future environmental assessments and guiding the sustainable development of full-scale H-OTEC facilities.

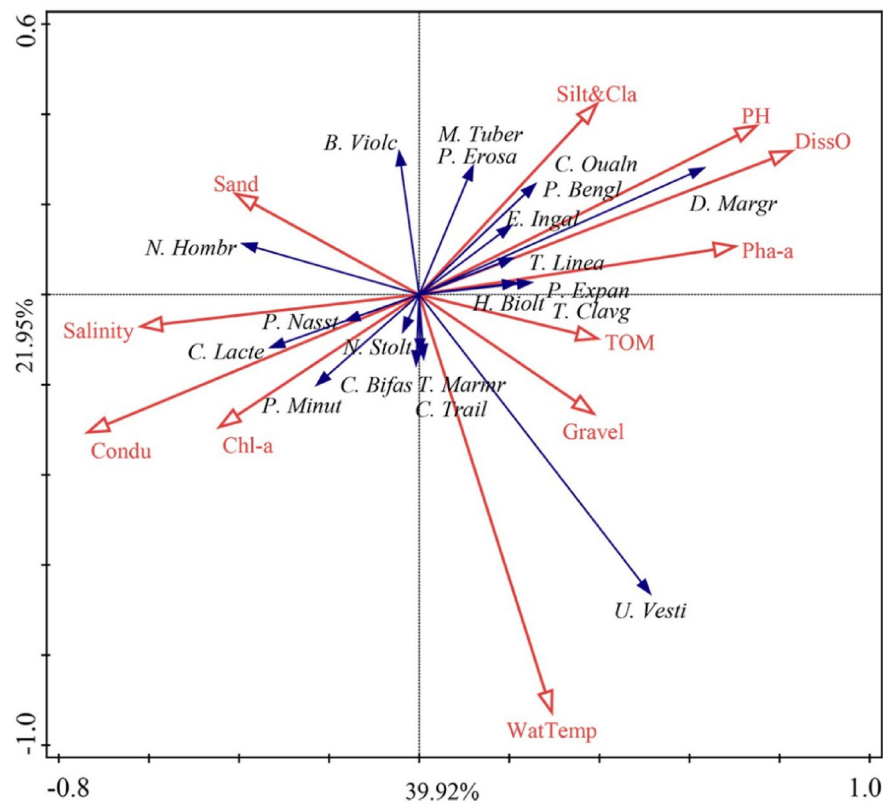


Fig. 10. Redundancy Analysis (RDA) correlation triplots depict the distribution of macrobenthic species (blue line) in relation to sediment environmental variables (red line).

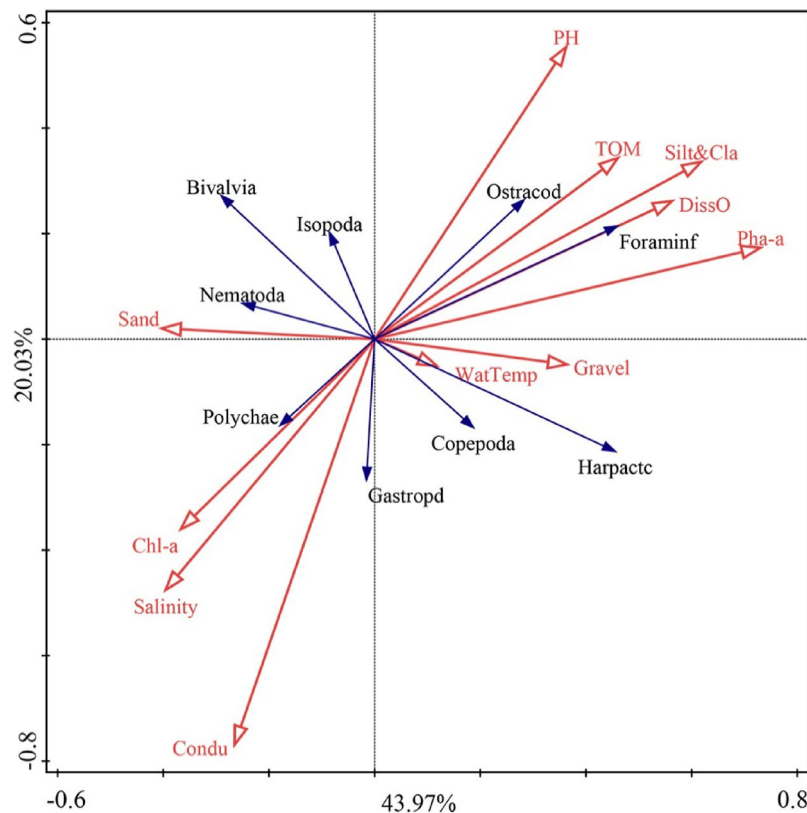


Fig. 11. Redundancy Analysis (RDA) correlation triplots depict the distribution of meiobenthic species (blue line) in relation to environmental variables (red line).

Data availability

All data generated or analysed during this study are included in this published article.

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FMY: Conceptualization, Supervision, Funding acquisition, Writing-Review and Editing
 QXL: Methodology, Writing - Original Draft, Writing- Reviewing and Editing
 KNM: Methodology, Writing-Reviewing and Editing
 NSZ: Visualization, Investigation, Validation
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Declarations

Competing interests

The authors declare no competing interests.

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