

The Macrobenthos Diversity and Dominance in Johor Straits, Malaysia

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ABSTRACT

Johor Strait has received massive anthropogenic traffic in recent years, causing environmental alteration and inevitably harming macrobenthos on the seafloor. A comprehensive assessment was done in several key locations within the strait to identify macrobenthic inhabitants and possible driving factors attributing to differences in macrobenthic assemblages in these areas. Sediments were acquired using Ponar Grab in 13 key locations within the Johor Straits. Seven hundred thirty macrobenthic individuals and 46 known taxa were identified in sediments of 13 locations in the strait. Annelids *Prionospio* ($n=295$), *Minuspio* ($n=95$) and *Mediomastus* ($n=82$) were concentrated in the central zone. Molluscs dominated the Merambong Shoals area (*Arcualuta*, $n=66$), and amphipods dominated waters off Santi River (*Leucothoe*, $n=26$; *Gammarus*, $n=11$; *Cymadusa*, $n=9$). PERMANOVA analyses ($p<0.05$) showed significant differences in benthic taxa composition in all locations overall. BIOENV analyses ($r=0.76$, $p<0.05$) highlighted water acidity, chlorophyll-*a*, silts and total organic carbon as the main influences toward benthic assemblages throughout the study area. PCA graph indicated higher organic carbon and silts in the central area, implying favourable conditions for Sedentarian polychaetes to thrive. The east and west ends of the strait exhibited higher readings of water acidity and chlorophyll-*a*, which may directly contribute to a higher diversity of benthic communities in the areas. Lower oxygen levels in two locations in the central area (J3=2.97 mg/L, J4: 2.63 mg/L) exhibited Sedentaria polychaete-dominated region, but zero benthic organisms

in another part of the central area (J5-J9, 2.97–0.99 mg/L). This study showcased the effectiveness of environmental monitoring using macrobenthos as an indicating subject.

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INTRODUCTION

The Johor (or Tebrau) Straits connect Malaysia, Singapore, and Indonesia, and the Malaccan Straits link to the South China Sea. Due to this, the waterway has served critically as a major economic hub zone as well as a political buffer zone between nations, especially Malaysia and Singapore, for years. On the strait the strait is connected to various rivers and large patches of seagrass at the west and east ends of the strait (Wan-Lotfi et al., 2013; Hossain et al., 2019). At the central part of the strait, a causeway bridges Malaysia and Singapore, which has led to economic growth between the nations for decades since the 1910s (Koh et al., 1991). Due to its strategic location, the waterway was subjected to intensive coastal and urban development between Malaysia and Singapore. It includes the reclamation of Forest City and Pengerang Integrated Complex, in which the projects were intended to accommodate the urban population and increase job employment, respectively (Rahman, 2017; Rezayee et al., 2020).

Unfortunately, concerns have risen over anthropogenic activities' effect on the strait's environmental side, particularly on the lifeforms inhabiting the seafloor. These lifeforms, collectively termed benthic communities, are generally small (~1 m), have limited mobility, and completely rely on the sediment and water surrounding the benthic group (Smith, 1964; Castro & Huber, 2019). Certain groups of benthic organisms (i.e., holothuroids and decapods) are unable to function well or even live in environments that have been altered, such as waters with a lower oxygen level and a higher concentration of heavy metals in the sediment and are, therefore very sensitive to environmental changes in the seafloor ecosystem. Due to these factors, benthic groups, especially macrobenthos, are often used as bioindicators in environmental studies, in which the presence and/or absence of certain groups of benthic taxa are to be associated with the current state of the seafloor ecosystem, ultimately assessing the effect of nearby anthropogenic activities on the environment.

Some studies made in Southeast Asian waters showcased the effectiveness of utilizing macrobenthos as an indicator to assess the extent of environmental damage done in the coastal area. In Port Blair, Andaman Island, records showed the dominance of *Capitella* and *Armandia* polychaetes on the seafloor off the three main ports of Blair. The study highlighted higher organic carbon, hydrocarbons, and heavy metals in sediment as major influences toward lower diversity in the area, regardless of dry and wet seasons. Sediments with such content were identified as favourable toward smaller-sized polychaetes but otherwise to other taxa, leading to the dominance of the former but otherwise of the latter. Malaysia's coastal and port areas showed similar findings to those in Port Blair. Sediments in Port Klang, especially near Southpoint and the Klang River, were inhabited by predominantly Capitellid, Cirratulid, and Spionid polychaetes (Sany et al., 2015; Kasihmuddin & Cob, 2021). In the north of Peninsular Malaysia, the coastal areas of Penang Island experienced major reclamation projects over time (Gholizadeh, 2015). As a result, smaller-sized

polychaetes and gastropods dominate the sediments rather than larger ones. Similar to cases in Blair and Klang, fluxes of reclaimed sediment allowed smaller-sized organisms to thrive there but otherwise for larger ones. All these studies highlighted similar findings, where constant fluxes of organic enrichment in sediment have disallowed larger-sized benthic taxa to thrive. It reduces potential foraging areas due to the smothering of sediments while at the same time providing more opportunities for deposit-feeding and easily reproductive taxa, namely Capitellid, Cirratulid, and Spionid polychaetes, to thrive in the areas (Faulwetter et al., 2014; Kasihmuddin & Cob, 2021).

Benthic studies in the Johor Straits focused on the central and both ends of the strait. Studies in the west and east of the straits were more inclined towards seagrass regions, namely the Pulai River, Merambong Shoals, Adang Cape, and Santi River (Wan-Lotfi et al., 2013; Guan, Ghaffar et al., 2014; Guan, Min et al., 2014; Mahadi et al., 2014; Woo et al., 2014; Kassim et al., 2015). Many of these studies solely focus on specific taxa, such as polychaetes (Guan, Ghaffar et al., 2014) and holothuroids (Mahadi et al., 2014). Studies that focus on multiple taxa groups (Guan, Min et al., 2014; Wan-Lotfi et al., 2013) did not fully explain how environmental parameters in the surrounding area influence benthic group distribution overall, as these studies were conducted solely to update the latest distribution benthic data in the region. Moreover, these studies were conducted when coastal development activities had just begun (Rahman, 2017), so records were outdated in this current period. It can be seen in the highly abundant holothuroids and copepods in Merambong and Pulai, respectively (Mahadi et al., 2014; Woo et al., 2014), which may not truly reflect the current state of the area, partly due to the presence of the massive, reclaimed island of Forest City. In the central zone, benthic studies were limited to only one taxon group. Azelee et al. (2014) and Saili and Mohamed (2021) focused on the effect of decreasing oxygen levels in water on green mussels (*Perna viridis*). In Pasir Gudang Industrial Zone, Mahat et al. (2018) and Zawawi (2019) too focused on biomass, weight, and length of green mussels and flower crabs (*Portunus pelagis*) and related their findings to heavy metal concentration in sediment. Eventually, researchers would link these findings with the extent of environmental damage in the central zone due to the immense anthropogenic volume (Koh et al., 1991; Shahbazi et al., 2010; Maznah et al., 2012; Nordin & Ali, 2013; Maznah et al., 2016; Yap et al., 2019; Kong et al., 2020). Though several taxa, such as green mussels and flower crabs, can be considered good bioindicators for environmental monitoring, it would be more effective if other taxa groups were also considered. A lower percentage of a certain taxa group can otherwise indicate a more impactful message about the effect of environmental alteration and hence should be considered altogether within environmental monitoring studies (Huang et al., 2012; Jennings et al., 2012; Equbal et al., 2017).

As more coastal development is on the rise, namely the Forest City, Pengerang Integrated Complex, and tidal barrage in the Johor River, there are concerns that environmental parameters will be altered permanently, leading to the potential destruction of lifeforms in the seafloor ecosystem (Rahman, 2017; Rezayee et al., 2020). As such, it would be pertinent that the latest yet comprehensive benthic assessment be done in several key locations of the Johor Straits, whereby dominance and differences in taxa could help address the underlying environmental disturbance in the region. The assessment must be done with the ever-increasing anthropogenic affluence in the Strait overall. Henceforth, this study aims to assess macrobenthic communities inhabiting the seafloor of the Johor Straits and identify potential environmental factors that influence the differences in taxonomic composition and dominance of certain benthic groups in some areas.

METHODOLOGY

The sampling session was done in April 2021, during the southwest monsoon. Sediment samples were collected in thirteen locations in the Johor Strait (Figure 1), of which two were selected at the west end (J1 and J2), seven at the central zone (J3 to J9, Figure 2), and four at the east end (J10 to J13). Triplicates of sediment were obtained in each sampling location using Ponar Grab with 0.023 m² scoop size and distributed for taxa assessment and sediment quality analysis. Sediments subjected to taxa assessment were first filtered through a 0.5-mm sieve and treated with a 10% formalin solution before packaging, while sediment samples subjected to sediment analysis were directly packaged without filtration or formalin treatment. The multiparameter YSI Pro Plus and fluorometer were lowered to seafloor level in each sampling location to record water quality, particularly water depth, temperature, conductivity, salinity, turbidity, dissolved oxygen, and chlorophyll-*a* (fluorometer only).



Figure 1. Sampling locations

Table 1

Summarized sampling design for benthic assessment in Johor Straits

Sampling Information	Description
Number of sampling locations	13
Duration of sampling	Two days (20–21 April 2021)
Type of sediment	Marine sediment
Sampling tool	Ponar Grab (0.023 m ² scoop size)
Number of replicates	1. Benthic assessment: 3 2. Sediment quality assessment: 1 (divided into three replicates prior to analysis)
Sediment Treatment	1. Benthic assessment: 0.5 mm ² sieve filter, followed by pure formaldehyde solution for preservation 2. Sediment assessment: No treatment

For sediment quality analysis, sediment was dried beforehand before being subjected to organic carbon analysis via the Black-Walkley method (Walkley & Black, 1934) and particle size distribution analysis via the pipette method (Indorante et al., 1990). Sediment and water qualities recorded in all sampling locations were compiled, and the data was transformed before principal component analysis (PCA) could be performed. The PCA test was used to identify which station is attributed to which parameters. Sediments subjected to taxa assessment were preserved with formalin solution and stored in double bags prior to lab analysis. In contrast, sediments subjected to sediment quality analysis were stored immediately without preservation. Water parameters were recorded using YSI multiparameters in each sampling location. In the laboratory, specimens were sorted, and their taxa were identified into genera using appropriate references (Ekman et al., 1945; Smith, 1964; Fauchald, 1977; Cutler, 1994; Rahim & Ross, 2013; Fujita & Irimura, 2015; Baharuddin et al., 2018).

The compiled taxa list for each station was assessed with biotic indices (taxa density, D_n ; taxa number, N ; individual number, n) and ecological indices (diversity, H' ; evenness, J' ; and richness, D_{mn}). A two-way PERMANOVA test was conducted to determine significant differences between two or more benthic assemblages within each sampling location based on polychaete dominance and locality. Lastly, a BIOENV test was performed to link a possible correlation between benthic taxa distribution in all stations with recorded sediment and water quality in Johor Straits. Biotic indices, ecological indices, PCA, and PERMANOVA tests were conducted using PAST 4.03 software, while BIOENV correlation analysis was done using PRIMER E-5.

RESULTS

Both sediment and water quality assessments showed distinct variations. For water quality assessment (Table 2), the highest and lowest depths were recorded on the west side of the

strait, in J1 and J2, respectively. The lowest dissolved oxygen level and turbidity were recorded in J4 to J9 and J1 to J5, respectively. All stations recorded very low chlorophyll-a, except J2, J10, J11, and J12, with J10 having the highest record. For sediment quality assessment (Figure 2), organic carbon analysis indicated that all stations at the central part of the strait recorded organic carbon percentages exceeding 50%, with J9 pertaining to the highest percentage. Stations at the west and east ends of the strait showed a lower percentage of organic carbon except J13, where the station showed a similar percentage as J6 at the central part of the strait. In terms of particle size distribution (Figure 3), the percentage of silt was highest in J1, J3, J6, and J10, while the percentage of sand was highest in J2, J7, J8, J11, J12, and J13. The clay percentage was low throughout the stations. PCA analysis (Figure 4) showed that J2, J10, J11, and J12 were closely attributed to chlorophyll-a, turbidity, and sand particles, while J4, J5, J8, and J9 were closely attributed to temperature and total organic carbon. Lastly, J1 and J10 were attributed to silt particles, depth, water pH, and dissolved oxygen.

Table 2

List of water parameters recorded in every sampling location in Johor Strait. D: Depth (m), T: Temperature (°C), CD: Conductivity; SL: Salinity (ppt); TB: Turbidity (NTU), DO: Dissolved Oxygen (mg/L), pH: Water Acidity and Chl-a: Chlorophyll-a (mg/L)

	D	T	CD	SL	TB	DO	pH	Chl-a
J1	23.60	28.03	55417.00	34.41	14.60	6.71	7.73	0.15
J2	7.40	28.38	55376.00	34.13	119.00	6.30	7.68	3.62
J3	10.00	28.84	52098.00	31.57	168.00	2.97	7.28	0.70
J4	8.00	28.81	50097.00	30.78	107.00	2.63	7.25	0.15
J5	12.00	28.79	51910.00	31.5	11.70	2.85	7.22	0.17
J6	16.00	28.82	52073.00	31.55	11.60	3.05	7.14	0.12
J7	15.00	28.02	52627.00	32.5	104.00	0.99	7.07	0.18
J8	11.00	28.45	52656.00	32.21	104.00	1.20	7.10	0.20
J9	9.00	28.57	52505.00	32.00	13.80	2.19	7.18	0.19
J10	15.00	28.19	53978.00	33.3	120.00	5.61	7.48	7.81
J11	10.50	28.35	54022.00	33.22	121.00	6.11	7.50	2.97
J12	10.00	27.93	54612.00	33.93	140.00	5.90	7.54	3.26
J13	7.70	27.87	54907.00	34.18	113.00	6.35	7.55	0.51

Out of 13 sampling locations, only eight locations recorded the presence of macrobenthic specimens. A total of 730 macrobenthic individuals from 46 known taxa were sorted and identified. The highest specimen count was observed in J4 sediment ($n = 35$), followed by J3 ($n = 150$). Sedentaria polychaetes, particularly from families Capitellidae (*Mediomastus*),

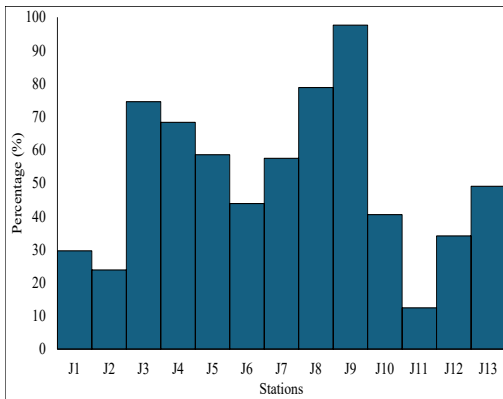


Figure 2. Percentages of organic carbon inside sediments found on the Malaysian side of the Johor Straits

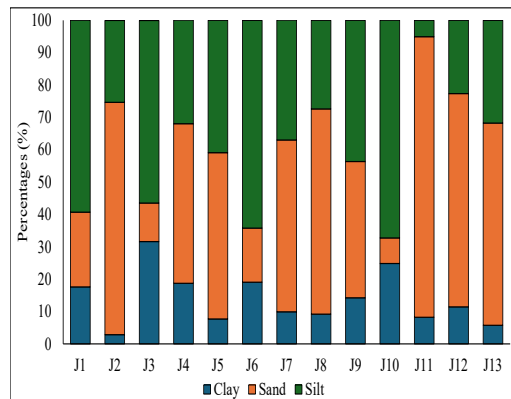


Figure 3. Particle size distribution sediments obtained from all sampling locations in the Johor Straits

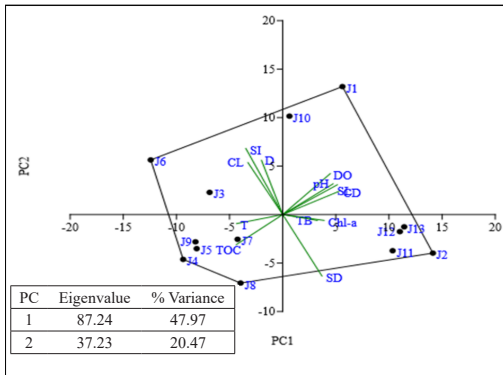


Figure 4. PCA graph on determining the relationship between sampling locations and their related environmental parameters

Cirratulidae (Cirriformia), and Spionidae (*Minuspio* and *Prionospio*), were the highest contributors to the larger abundance in both stations but also observed in other stations such as J1 and J13. Arthropods were highest at the east end of the strait, particularly in J10–J12. Arthropods in this study were mostly peracarids (amphipod, tanaid, mysid). For molluscs, more bivalve individual counts were observed in this study than in gastropods. Gastropods were found only at the westernmost end (J1) and strait’s east (J10 to J13), while a higher

individual count of *Arcualuta* bivalves was found to be extremely abundant in this station. This study’s remaining three phylum groups, brachiopod, echinoderm, and sipunculid, were considered minor phylum groups due to their lower taxa count. For sipunculid, many *Nephasoma* individuals were found in J2 but fewer in J1 and J12, and two individuals of *Themiste* were found only in J1. Echinoderms consisted of one individual of *Holothuria* sea cucumber and six individuals of *Ophiothrix* brittle star, found only in J3, J11, and J12. Lastly, one individual of Brachiopod, namely *Lingula*, was found in sediments from J12.

Overall, J1, J3, and J4 showed higher taxa numbers as well as individual counts than other stations (Table 4). While J1 recorded the highest taxa number ($N = 17$), J4 contained the highest individual counts ($n = 325$). The macrobenthic assemblage from the west end to the central part (J1 to J4) showed increasing taxa and individual counts. However, it

Table 3

Taxa composition of macrobenthos found in all eight sampling locations. Stations J5 to J9 were excluded from the list due to a lack of macrobenthic specimens in the sediment

	J1	J2	J3	J4	J10	J11	J12	J13
Phylum Brachiopoda								
Class Lingulata								
Order Lingulida								
Family Lingulidae								
<i>Lingula</i>	-	-	-	-	-	-	1	-
Phylum Annelida								
Class Polychaeta								
Subclass Errantia								
Order Amphinomida								
Family Amphinomidae								
<i>Eurythoe</i>	-	-	-	1	-	-	-	-
Order Eunicida								
Family Lumbrineridae								
<i>Lumbrineris</i>	1	-	-	-	-	-	-	-
Family Onuphidae								
<i>Diopatra</i>	-	-	1	-	-	-	-	-
Order Phyllodocida								
Family Glyceridae								
<i>Glycera</i>	-	-	1	2	-	2	-	-
Family Goniadidae								
<i>Goniada</i>	1	-	7	3	1	-	-	-
Family Hesionidae								
<i>Leocrates</i>	-	-	5	1	-	-	-	-
<i>Syllidia</i>	-	-	-	-	-	-	1	1
Family Nephtyidae								
<i>Micronephthys</i>	3	-	6	5	4	1	1	1
Family Phyllodocidae								
<i>Phyllodoce</i>	-	-	-	-	-	1	-	2
Family Pilargidae								
<i>Sigambra</i>	3	-	-	-	-	-	-	-
Subclass Sedentaria								
Family Chaetopteridae								
<i>Mesochaetopterus</i>	-	-	-	-	-	1	-	1
Order Canalipalpita								
Family Ampharetidae								
<i>Ampharete</i>	-	1	-	-	-	-	-	-

Table 3 (Continue)

	J1	J2	J3	J4	J10	J11	J12	J13
Family Cirratulidae								
<i>Cirriformia</i>	-	-	12	11	-	-	-	-
Family Sternaspidae								
<i>Sternaspis</i>	3	-	-	-	-	-	-	2
Family Spionidae								
<i>Minuspio</i>	3	-	33	59	-	-	-	-
<i>Prionospio</i>	8	-	69	218	-	-	-	-
Family Pectinariidae								
<i>Pectinaria</i>	-	-	-	1	-	-	-	-
Family Poecilochaetidae								
<i>Poecilochaetus</i>	1	-	-	1	-	-	1	1
Order Scolecida								
Family Capitellidae								
<i>Arenicola</i>	-	3	-	-	-	-	-	-
<i>Mediomastus</i>	18	-	35	23	-	2	3	1
Family Maldanidae								
<i>Euchymene</i>	-	-	-	-	-	-	-	1
Family Paraonidae								
<i>Paraonides</i>	1	-	1	-	-	-	-	-
Family Opheliidae								
<i>Ophelia</i>	-	-	-	-	-	1	-	-
Phylum Arthropoda								
Class Malacostraca								
Subclass Eumalacostraca								
Superorder Eucarida								
Order Decapoda								
Family Diogenidae								
<i>Diogenes</i>	1	-	-	-	-	-	-	-
Superorder Peracarida								
Order Amphipoda								
Family Ampeliscidae								
<i>Byblis</i>	-	1	3	-	-	-	-	-
Family Amphitoidae								
<i>Cymadusa</i>	-	-	1	-	-	-	7	2
Family Caprellidae								
<i>Caprella</i>	-	-	2	-	-	-	-	1
Family Gammaridae								
<i>Gammarus</i>	1	-	-	-	1	4	2	4

Table 3 (Continue)

	J1	J2	J3	J4	J10	J11	J12	J13
Family Leuchotoidae								
<i>Leucothoe</i>	-	-	-	-	1	15	5	5
Family Liljeborgiidae								
<i>Listriella</i>	-	1	-	-	-	-	-	-
Family Maeridae								
<i>Ceradocus</i>	-	-	1	-	-	-	-	-
Order Mysida								
Family Mysidae								
<i>Acanthomysis</i>	2	1	-	-	-	-	-	-
Order Tanaidacea								
Family Tanaididae								
<i>Tanais</i>	2	-	-	-	-	-	-	-
Phylum Echinodermata								
Class Holothuroidea								
Order Aspidochirotida								
Family Cucumariidae								
<i>Holothuria</i>	-	-	1	-	-	-	-	-
Kelas Ophiuroidea								
Order Ophiurida								
Family Amphiuridae								
<i>Ophiothrix</i>	-	-	-	-	-	1	5	-
Phylum Mollusca								
Class Bivalvia								
Subclass Autobranchia								
Order Myida								
Family Corbulidae								
<i>Corbula</i>	-	-	-	-	-	-	-	2
Order Mytilida								
Family Mytilidae								
<i>Modiolous</i>	-	66	-	-	-	-	-	-
Class Gastropoda								
Order Caenogastropoda								
Family Cerithiidae								
<i>Cerithium</i>	-	-	-	-	1	-	-	-
<i>Bittium</i>	1	-	-	-	-	-	-	-
<i>Rhinoclavis</i>	-	-	-	-	-	-	1	-
Family Nassariidae								
<i>Nassarius</i>	-	-	-	-	-	-	2	-

Table 3 (Continue)

	J1	J2	J3	J4	J10	J11	J12	J13
Order Vetigastropoda								
Family Trochidae								
<i>Gibbula</i>	-	-	-	-	-	-	-	1
Kelas Cephalopoda								
Order Octopoda								
Family Octopodidae								
<i>Histoctopus</i>	-	1	-	-	-	-	-	-
Phylum Sipuncula								
Class Sipunculidea								
Order Golfingiida								
Family Golfingiidae								
<i>Nephasoma</i>	1	-	10	-	-	-	1	-
<i>Themiste</i>	2	-	-	-	-	-	-	-



Figure 5. Compilation of taxa specimens found in sampling stations throughout Johor Straits. **Annelid:** (a) *Minuspio*, (b) *Mediomastus*, (c) *Pilargis*; **Mollusk:** (d) *Arcualuta*; (e) *Bittium*; **Brachopoda:** (f) *Lingula*; **Echinoderm:** (g) *Ophiothrix*, (h) *Holothuria*; **Arthropod:** (i) *Diogenes*, (j) *Acanthomysis*, (k) *Gammarus*; **Sipuncula:** (l) *Nephasoma*

abruptly reduced to zero when reaching J5 until J9, located at the central part. On the other hand, macrobenthic assemblages at the east end (J11 to J13) have almost similar taxa and individual counts compared to the east end and central part of the strait. J10 has the fewest taxa and individual numbers of macrobenthos compared to others. In terms of ecological indices, a higher diversity and richness index was seen in macrobenthic assemblages in J1, J12, and J13; a higher evenness index was seen in J10, J11, and J12. Oppositely, J2 showed the lowest diversity index, while J4 showcased the lowest value in the evenness and richness index.

Table 4

Compiled list of biotic and ecological indices in macrobenthic communities throughout the straits. St: Sampling locations; N: Taxa number; n: individual number; De: Taxa density; H': Shannon-Wiener diversity index; J': Pielou's evenness index; D_{mn}: Menhinick's richness index

St	N	n	De	H'	J'	D _{mn}
J1	7.67 ± 4.51	17.33 ± 10.41	753.62 ± 452.54	1.66 ± 0.79	0.81 ± 0.14	1.90 ± 0.98
J2	3.67 ± 2.08	24.67 ± 24.01	1072.46 ± 1043.78	0.73 ± 0.88	0.67 ± 0.28	1.07 ± 1.04
J3	10.33 ± 1.53	62.67 ± 18.15	2724.64 ± 789.02	1.77 ± 0.06	0.58 ± 0.11	1.31 ± 0.02
J4	7.00 ± 2.00	108.33 ± 53.00	4170.14 ± 2304.48	1.03 ± 0.29	0.41 ± 0.02	0.72 ± 0.30
J10	2.00 ± 1.00	2.67 ± 0.58	115.94 ± 25.10	0.60 ± 0.56	1.00 ± 0.00	1.24 ± 0.60
J11	4.00 ± 1.73	9.33 ± 5.51	405.80 ± 239.46	1.10 ± 0.17	0.81 ± 0.18	1.35 ± 0.30
J12	5.67 ± 0.58	10.00 ± 4.58	192.31 ± 88.13	1.59 ± 0.04	0.87 ± 0.07	1.86 ± 0.27
J13	6.00 ± 3.00	8.33 ± 6.81	160.26 ± 130.90	1.63 ± 0.48	0.94 ± 0.10	2.14 ± 0.37

The two-way PERMANOVA test for polychaete dominance indicated a significant difference between sampling locations but not for localities ($p < 0.05$). However, when paired together (polychaete dominance and locality), a two-way PERMANOVA showed a significant difference between sampling locations ($p < 0.05$). Lastly, BIOENV analysis (Table 4) indicated water pH, chlorophyll-a (Chl-a), silt (Si), and total organic carbon (TOC) parameters as the main environmental parameters that influence macrobenthic taxa distribution throughout sampling locations in Johor Straits ($r = 0.76, p < 0.05$).

Table 5

List of parameters with strongest correlation towards macrobenthic assemblages in Johor Straits ($p < 0.05$)

Number of Variables	Correlation value (r)	Parameter
4	0.76	pH, Chl-a, Si, TOC
3	0.76	pH, Chl-a, Si
4	0.71	DO, pH, Chl-a, Si
4	0.71	TB, pH, Chl-a, Si, TOC
5	0.71	CD, pH, Chl-a, Si, TOC

DISCUSSION

Relationships Between Environmental Parameters and Macrobenthic Assemblage in the Strait

Results from the PERMANOVA analysis showed significant differences between sampling stations in terms of locality and polychaete dominance. This analysis indicated that such differences occurred due to differences in environmental parameters throughout the strait. BIOENV identified pH level, chlorophyll-*a*, silt, and organic carbon as the main driving factors for macrobenthic distribution across the straits. Organic carbon and silt contents in sediment influence polychaetes, particularly those from Subclass Sedentaria (Cho et al., 2013; Faulwetter et al., 2014). Sedentaria polychaetes are generally smaller-sized and thrive more efficiently within sediment with higher percentages of smaller-sized sediment (Dai et al., 2015; Bolam et al., 2016; Rosli et al., 2018). This group is often associated with bioaccumulation activities (Dai et al., 2015), which heightens the sedimentation rate in the area (Kristensen et al., 2012; Abessa et al., 2019). These were seen in J3 and J4, where both stations showed a higher abundance of Sedentaria polychaetes and consequently recorded a higher percentage of organic carbon and silt in the sediments.

Higher chlorophyll-*a* levels in the column water suggested a larger presence of phytoplankton in the surroundings (Deininger et al., 2017; Asha et al., 2020). Phytoplankton is an important element within the benthic ecosystem as part of the food web (Braeckman et al., 2015; Leoni, 2016). A higher presence of arthropods, particularly peracarids, is common in ecosystems with a higher phytoplankton count (Mercer et al., 2016; Zhao et al., 2019). In this study, chl-*a* record was found higher in sampling locations near Merambong Shoals (J2), Kim River (J10), Belungkor River (J11), and Santi River (J12). These locations were all seagrass biotopes, which such biotopes are commonly known to contain higher nutrient affluent due to intensive photosynthesis and nitrogen cycling activities taking place within seagrass areas (Duarte, 1995; Setiabudi et al., 2016; Asha et al., 2020).

This study also identified water acidity as the main parameter affecting macrobenthic distribution. The optimum pH in a normal benthic ecosystem is 7.50 and above (Hinga, 2002). pH levels above 7.50 are ideal for phytoplankton to perform photosynthesis (Egerton et al., 2014; Scholz, 2014). Conversely, a lower pH value could potentially harm benthic organisms, especially arthropods and molluscs (Rosenberg et al., 1991; Tripole et al., 2008). These negative effects included exoskeleton corrosion within arthropods and molluscs as well as denaturation of certain enzymes inside these macro-benthos, causing these macrobenthos to be unable to perform their life processes normally (Hale et al., 2011). Lower pH in seafloor biotopes strongly relates to the dissolved oxygen parameter. Although dissolved oxygen was not listed as a main parameter in BIOENV analysis, there was a noticeable observation of a higher number of Sedentaria polychaetes, particularly from the families Capitellidae, Cirratulidae, and Spionidae, but otherwise for other benthic

groups inside sediment with a lower oxygen level (Huang et al., 2012; Martinez-Garcia et al., 2013; Bolam et al., 2016). It was seen in J3 and J4, where the macrobenthic community in J3 has a limited number of taxa other than *Sedentaria* polychaetes, while J4 is fully dominated by polychaetes only.

The dissolved oxygen levels in J3 and J4 were lower than other stations and hence identified as hypoxic zones. Meanwhile, sediments from stations J5 to J9 did not contain any macrobenthic taxa in other parts of the central zone. Dissolved oxygen in these stations was even lower than in J3 and J4, with dissolved oxygen in J7 considered the lowest and approaching anoxic level. The absence of even these three polychaete families (Capitellidae, Cirratulidae, and Spionidae) in these locations suggested that even though these groups can live in sediments with lower oxygen levels, there is still a range of acceptable oxygen levels in the sediment required for these groups to live normally. Therefore, this parameter should also be regarded as the most important parameter differentiating sampling locations with macrobenthic communities dominated by polychaetes or otherwise and sampling locations without macrobenthos, as stated in the PERMANOVA analysis result.

Overview of Macrobenthic Assemblage Within Johor Strait

Starting on the west end, J1 and J2 had low organic carbon percentages. Both stations were located within seagrass biotopes, spanning from Piai Cape to Merambong Island (Kassim et al., 2015; Hossain et al., 2019). Macrobenthic communities in J1 were more varied than in J2, comprising more polychaetes than other taxa groups. Taxa found in this study were mostly common on the seafloor with higher depth and turbidity and further off the coast, such as annelids *Lumbrineris* and *Sigambra*, arthropods *Diogenes* and *Tanais* and molluscs *Bittium* (Dong et al., 2018; Shafie et al., 2021; Sivadas et al., 2021). Smaller-sized annelids were found in greater abundance in this region. *Goniada*, *Micronephthys*, *Minuspio*, *Prionospio* and *Mediomastus* polychaetes were commonly known to inhabit sediments with higher percentages of silt and clay in which smaller particles of sediment enabled these taxa to propagate easily (Kristensen et al., 2012; Faulwetter et al., 2014) just as reflected in BIOENV analysis. J2 was surrounded by multiple seagrass biotopes, namely Pulai Estuary, Merambong Shoals and Adang Cape, where seagrass patches are in higher volume.

However, the seagrass patches might be lower than previously recorded by Woo et al. (2014) and Hossain et al. (2019), largely caused by dredged sediments from still ongoing Forest City projects (Rahman, 2017). Molluscs dominated the macrobenthic community in J2. *Arcualuta* mussels were highly abundant there and have previously been recorded in similar abundance at the Pulai River estuary, Merambong Shoals and Adang Cape (Guan, Min et al., 2014; Kassim et al., 2015; Mukhtar et al., 2019; Wong & Sigwart, 2019). This taxon is very common in seagrass meadows, where water depths are lower enough for

seagrass to absorb sunlight in a better position. The mussel's dominance is highly attributed to lower silt and clay particles, higher chlorophyll-*a*, dissolved oxygen and pH in water, as stated in the PCA graph toward Station J2 and BIOENV correlative analysis. The mussels prefer larger sediment particles to safely attach to sediments without sinking into smaller-sized silts and clays (Khongpuang, 2011). The genus is a filter feeder, foraging food particles consisting of marine litter from upwelling to survive. The mussels are generally smaller, lack predators, are able to spawn as many as hundreds of larvae into the waters at once and have a faster growth rate from larva to adult (Otero et al., 2013; Watson et al., 2021).

Favourable sediment and water conditions and suitable physiological aspects enable the mussels to thrive easily in the seagrass area. *Ampharete* and *Arenicola* polychaete *Listriella* amphipod, *Acanthomysis* mysid and *Histoctopus* octopus are common in mangrove and seagrass biotopes and usually higher in ecosystems with lesser anthropogenic influence (Jennings et al., 2012; Reise, 2012; Egerton et al., 2014; Samper-Villareal et al., 2016; Equbal et al., 2017; Bang et al., 2018; Jombodin et al., 2021; Asha et al., 2020; Sordo & Lana, 2020). Lesser polychaetes were noticed in this region, which can be attributed to a lower percentage of organic carbon in the sediment (Cho et al., 2013). Sediments in seagrass biotopes often contain a lower percentage of organic carbon due to continuous absorption by seagrasses in the region (Samper-Villareal et al., 2016).

Annelids are a major phyla group in the central region, particularly in J3 and J4. However, annelids' full domination was seen only in J4. Taxa composition in J3 was similar to that in J1, in which the station recorded the presence of arthropods and sipunculids, albeit in a smaller percentage than annelids. Arthropods (mainly amphipods) and sipunculids are common in estuaries, even with heavy anthropogenic interference, such as the Skudai River. Taxa composition in this area was almost similar to those in the Garaguacu estuary in Brazil (Gusmao et al., 2016), Swarnamukhi in India (Pandey et al., 2021) and the closest one which was Langat estuary in the state of Selangor (Azrina et al., 2006). *Minuspio* and *Prionospio* polychaetes were the most abundant annelids found in this study.

Most taxa individuals were in these two stations, with J4 retaining the highest individual count. While J3 was located near the estuary of the Skudai River, J4 was closer to a massive dredging area. J4's ecosystem was marked with distinct variations of particle size distribution and an even higher organic carbon percentage than other stations, even J3. *Minuspio* and *Prionospio* are both capable of not only surviving in environments with deprived oxygen levels but can even reproduce faster in sediments with higher organic matter (Huang et al., 2012; Cho et al., 2013; Bolam et al., 2016; Dauvin, 2018). These genera are known to be very small in size (<1 mm) and capable of reproducing at a very fast rate (Faulwetter et al., 2014). Spionid polychaetes are deposit feeders and traverse easily between smaller silt particles for both foraging and protection from larger-sized predators such as Errantian polychaetes and decapods (Cho et al., 2013).

Greater availability of silt particles enables the polychaetes to thrive better, along with increasing organic enrichment in sediment due to dredging areas nearby (Dauvin et al., 2017). Decreasing oxygen levels in water renders most benthic taxa unable to inhabit the region, hence allowing these taxa to grow numerically even faster and unhindered. The dominance of Spionid polychaetes in heavily enriched sediments but poorer oxygen levels was highly documented, as seen in Algerian, Andaman and Klang ports (Dauvin et al., 2017; Equbal et al., 2017; Kasihmuddin & Cob, 2021). The three ports' records highlighted very small percentages of Errantian polychaetes and molluscs but higher individual counts of Spionidae polychaetes. Due to the positive growth shown by Spionidae polychaetes toward increasing bioaccumulation and lowering oxygen levels in sediments, these taxa are well-suited to be utilized as bioindicators to assess the extent of organic pollution occurring in seafloor biotopes (Borja & Tunberg, 2011; Kristensen et al., 2012; Cho et al., 2013; Marin et al., 2015; Dauvin, 2018; Jorissen et al., 2018).

Sampling locations from J5 to J9 are all considered dead zones. Similar to J4, both sediments of J5 and J6 originated near the same dredging zone, recorded a higher percentage of organic carbon and lower oxygen levels, but did not contain any macrobenthos. The dredging zone encompassed from J4 to J6 and has previously recorded higher concentrations of harmful algal bloom and heavy metals (Koh et al., 1991; Egerton et al., 2014; Syaizwan et al., 2015; Curren et al., 2019; Yap et al., 2020; Hii et al., 2021). J4 was furthest from the dredging zone but closest to the Skudai River estuary. Due to its proximity to the estuary, water mixing is still possible, and the macrobenthic community could still receive nutrients and oxygen from rivers (Egerton et al., 2014). Hence, J4 can still be considered a safer zone for macrobenthos to thrive due to the area's connection to the river, albeit lower oxygen levels and higher organic carbon may restrict distribution to all but Sedentaria polychaetes in the area (Selck et al., 2011; Villnäs et al., 2012; Faulwetter et al., 2014; Ganesh et al., 2014; Dauvin, 2018), as observed in taxa composition for J4. Stations J5 and J6 were closest to the Causeway, where water current and mixing rate were noticeably lower.

The effect of these intrusions was more obvious in J7, J8 and J9, which were situated on the eastern side of the Causeway. Oxygen levels in this area were very low, with J7 almost reaching an anoxic level (0 mg/L). According to a report by Koh et al. (1991), water quality in the inner Johor Strait has declined ever since the Causeway was opened to the public in 1911. In addition to the Causeway itself, increasing dredging, settlement building, factories, and mariculture activities nearby the Causeway have led to a decrease in water pH and oxygen levels in sediment. Sediment and water quality in this area worsened, as records of faecal matters (Nordin & Ali, 2013), heavy metals (Yap et al., 2019) and hydrocarbon (Shahbazi et al., 2010) were found to be even higher than previously recorded in older environmental reports prepared by Koh et al. (1991) in the 90's. All these inevitably led to an uninhabitable seafloor ecosystem, unsuitable to even Sedentaria polychaetes themselves,

which are supposedly able to adapt even in seafloor ecosystems with extremely lower percentages of oxygen levels (Dauvin et al., 2017).

At the east end, the last four stations (J10-J13) showed varied sediment and water quality records and consequently varied macrobenthic composition. J10 has the lowest depth but retained the highest chlorophyll-*a* and organic carbon percentage, yet retained the lowest macrobenthic specimen yield in this study. This station's sediment was obtained near Kim River's estuary, where the notoriously abundant number of factories around it had been associated with water pollution cases that led to local populaces' toxic poisoning (Kamaruzzaman et al., 2010; Yap et al., 2019; Kong et al., 2020). The constant release of toxic materials from inland may cause the area unsuitable for macrobenthos to thrive despite higher chlorophyll-*a* and organic carbon in water and sediment, respectively. J11 and J12 were located near the Belungkor and Santi River estuaries. Seagrass meadow coverage was present in both locations but lower (Amri et al., 2005; Wan-Lotfi et al., 2013; Hossain et al., 2019).

Amphipods, namely *Cymadusa*, *Gammarus*, and *Leucothoe*, were found highly abundant here, similar to previous findings by Wan-Lotfi et al. (2013) in regions with higher seagrass patches. However, the study was done only in the Santi River estuary. Amphipods are common in river estuaries, especially with higher chlorophyll-*a* and small-sized sediment particles (White, 2015; Podlensinska & Drabowska, 2019; Shafie et al., 2021). Lower organic carbon percentages in both stations made these locations suitable for echinoderms (*Ophiothrix*) and brachiopods (*Lingula*). Lesser anthropogenic activities, coupled with higher chlorophyll-*a* in water and lower organic carbon in sediment, allow many phyla groups to thrive in these areas more easily than in the central region. J13's sediment was obtained off Pengelih Cape, Johor. It was also located close to a reclamation site off Pengerang Cape. The reclamation is still underway, intending to construct a petroleum processing plant (Brelsford, 2016). Sediment samples from this location retained a higher percentage of organic carbon, similar to those in J3 and J4, reflecting the extent of dredging activities in the area.

However, varied macrobenthic phyla taxa can still be found inside sediment samples. Amphipods were found highly abundant in this station, but so were Sedentaria polychaetes, such as *Euchymene* and *Poecilochaetus*, which are more common in less disturbed seafloor ecosystems (Idris & Arshad, 2013; Magalhães & Bailey-Brock, 2014). Dissolved oxygen was still high in this location despite the higher percentage of organic carbon and silt particles. The presence of amphipods and Sedentaria polychaetes other than Capitellidae, Cirratulidae, and Spionidae indicated that J13 are still habitable to macrobenthos despite increasing reclamation activities that led to increasing organic carbon and smaller-sized sediment particles in the area. However, as the reclamation project is still ongoing, there is a possibility that the accumulation of organic carbon and silt in the sediment may cause

a lowering of water pH and oxygen level, and this could cause the area to end up with the same fate as in J4, where the area becomes unsuitable for most macrobenthic groups but Sedentaria polychaetes to thrive, or even worse, the oxygen level went so low that not even Sedentaria polychaetes can live in the sediment. Therefore, long-term surveillance must be done in the area to assess the extent of damage wrought by Pengerang development towards the macrobenthic community on the seafloor.

CONCLUSION

Unlike previous studies, this study covers the larger scope of the area, focusing on the east, central and west sides of the strait, taking into account localities as major driving forces besides environmental factors behind the distribution of macrobenthos in Johor Straits overall. Macrobenthos was found in most locations in the strait and considerably varied thanks to spatial variations of environmental parameters. PCA and BIOENV analyses both revealed higher water acidity, oxygen level, and chlorophyll-*a* were driving factors for myriads of macrobenthos, especially amphipods, bivalves (particularly Asian Date Mussels), and Errantian polychaetes, in the west and east side of the strait, where anthropogenic traffic was noticeably lower compared to the central region. In the central region, however, major urban areas and the Causeway, Capitellid, Cirratulid, and Spionid polychaetes dominated the seafloor habitat, especially in areas close to dredging zones. Higher levels of organic carbon and silt sediment particles but lower oxygen levels at the central region render the ecosystem unsuitable for all but these three taxa. These polychaete's dominance, however, does not guarantee continuous survival in the region should the oxygen level in water decrease further, as they are too unable to thrive in extremely low oxygen levels in waters, as observed in sediments from other parts of the central region. These polychaetes' resilience in ecologically stressed ecosystems showcased their suitability as bioindicator species to assess the extent of environmental disturbance in any given marine ecosystem, especially in coastal areas and ports, where the level of anthropogenic activities is higher.

This study reached two concerning conclusions about the marine ecosystems in Johor Straits. The dominance of three Sedentarian polychaetes in the Central Zone implies that the anthropogenic activities in the region have gone too far. Increasing land reclamation, uncontrolled domestic release to the sea, and the effectiveness of the Causeway as a geographical barrier that permanently altered the water current since the 1990s may have led to total environmental destruction in the area, hence making the seafloor inhabitable to most lifeforms except Capitellid, Cirratulid, and Spionid polychaetes. The diversity of macrobenthic communities on the west and east ends may not last long due to multiple land projects underway nearby. The enlargement of Forest City, the construction of a tidal barrage off the Johor River, the enlargement of ports off Belungkor Cape, and the

development of the Pengerang Integrated Complex could potentially transform the current seafloor ecosystem at the west and east ends into those of the central region. Thus, it is imperative that proper planning be made to prevent the destruction of marine habitats or at least mitigate the extent of degradation in the area. This study showed the effectiveness of utilizing macrobenthos to address key environmental issues in marine environments. It could be a useful reference for future benthic studies in the Johor Straits or Malaysian waters.

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