



Article Seasonal Pattern of Taxonomic Diversity and Functional Groups of Macro-Benthos from a Sub-Tropical Mangrove Estuary

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Abstract: Macro-benthos is commonly considered an indicator for evaluating the health of an aquatic ecosystem. Earlier research from sub-tropical mangrove estuaries, however, has primarily relied on conventional taxonomic methods to determine the pattern of macro-benthos diversity. Therefore, this study aimed to describe the pattern of both taxonomic and functional groups of macro-benthos with respect to ecological variables in three separate seasons (pre-monsoon, monsoon, and postmonsoon) from a mangrove-dominated Pasur River estuary, Bangladesh. The findings revealed significant seasonal variations in the water and sediment parameters (p < 0.05). During the study period, 47 species belonging to 35 families of macro-benthos were identified. The pollution indicator species, Capitella capitata complex was found to be dominant. The highest density of macro-benthos was recorded in post-monsoon (545 \pm 13.76 ind./m²) followed by pre-monsoon (214 \pm 5.57 ind./m²) and monsoon (63 ± 2.27 ind./m²). Diversity indices, Shannon, and evenness also displayed a similar seasonal trend. This pattern may be explained by the more stable bottom and higher food availability during post-monsoon, and on the other side, by erosion and higher turbidity during monsoon. Analysis of similarity (ANOSIM) detected a significant difference in community assemblage among the seasons (R = 0.7222, p = 0.0005), whereas similarity percentage analysis (SIMPER) identified Dendronereis aestuarina as the most contributory species for the overall average dissimilarity. Six functional feeding groups (FFGs) were identified where gathering collectors (GC) had the highest total density (221.83 ind./m²) and relative abundance (26.97%). The community was shown to be shaped by the amount of sedimentary silt and dissolved oxygen in the water main, according to a canonical correspondence analysis (CCA) study, they were positively correlated with the abundance of Pristinella acuminata, Lumbrineris sp., Cossura coasta, C. capitata complex, Neritina violacea, Laccotrephes griseus, Hydrometra butleri, Gomphus sp. and Libellula sp. CCA analysis also revealed a significant positive influence of pH, NO₃-N, PO₄-P, and organic matter, whereas, sand particles of sediments were found to have a negative effect on FFGs. Overall, the study suggests that the estuary is moderately diverse with macro-benthos and their functional feeding groups and influenced by monsoon strongly. The present study on FFGs of macro-benthos in an estuarine river of Bangladesh will provide baseline information for further investigation of other estuaries.

Keywords: macro-benthos; diversity index; functional feeding groups; environmental parameters; Pasur River estuary



Citation: Khatun, B.; Jewel, M.A.S.; Haque, M.A.; Akter, S.; Hossain, M.B.; Albeshr, M.F.; Arai, T. Seasonal Pattern of Taxonomic Diversity and Functional Groups of Macro-Benthos from a Sub-Tropical Mangrove Estuary. J. Mar. Sci. Eng. 2023, 11, 1453. https://doi.org/10.3390/ jmse11071453

Academic Editor: Francesco Tiralongo

Received: 26 March 2023 Revised: 7 April 2023 Accepted: 8 April 2023 Published: 21 July 2023



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1. Introduction

Mangrove-dominated estuaries are regarded as one of the most productive ecosystems. The biological productivity of this type of estuary is influenced by the massive amounts of organic material and nutrients that are released from mangroves [1]. It is well recognized that estuaries support a unique community of macrobenthic organisms that serves as a foundation for the dynamic interplay of biotic and abiotic elements. It offers a crucial base for the aquatic food chain [2]. Primary productivity, pollution detoxification, nutrient cycling, and material translocation are largely dependent on the macro-benthos community structure [3]. The ability of the benthic organisms to mineralize influences how much nutrient is released by the sediments. Furthermore, along with its key role in the mineralization of organic matter, it serves as food for fish and other higher aquatic organisms [4,5]. Therefore, understanding the characteristics and life cycle of macro-benthos living in or near the bottom of a waterbody is required to stimulate the fishery potential of a region [6].

Variations in physicochemical factors (namely temperature, pH, dissolved oxygen, and salinity) and sediment qualities (grain size, organic compounds, and nutrients) of a water body can have a beneficial or detrimental impact on macro-benthic organisms [6]. The macro-benthos can therefore be utilized as a bioindicator for the evaluation of environmental quality because changes in soil properties and an excess of nutrients may alter the composition and abundance of benthic organisms [7,8]. Functional feeding groups (FFGs) illustrate a recent development in the utilization of macro-benthos as bioindicators. The mechanisms of food acquisition serve as the foundation for the classification of functional feeding groups (FFGs). Individuals in FFGs are not specifically categorized according to what they eat, but rather according to how they acquire food and the size of the food particles. It is one of the better convenient techniques for evaluating species assemblage and monitoring water quality currently available [9,10]. Functional group species have a closer relationship to the environment, making them better able to represent the ecological processes impacting aquatic communities and comprehend the aquatic ecosystem and its biodiversity [11].

The Pasur River, one of Bangladesh's major estuaries and a part of the Sundarban mangrove forest (UNESCO world heritage site) is crucial for preserving estuarine fisheries. However, the rapid discharge of industrial effluents and the unplanned construction of numerous industries, including textile, tanneries, TSP and DDT plants, oil refineries, fish processing plants, and factories that release toxic metals and wastes are causing this river to become rapidly polluted. In addition, environmental changes increased fishing pressure, sewage disposal along drains, and dumping of trash by local residents all are contributing to pollution. Recent extensive dredging in the Pasur River estuary to carry coal to the Rampal power station increases turbidity and sedimentation, which has an impact on the recruitment, survival, and abundance of macro-benthos and other inhabitants. The macro-benthic community structure of Bangladesh's rivers and estuaries has been studied by a number of researchers, including Sharif et al. [12], Ullah et al. [13], Matin et al. [14], Haque et al. [15], Sarker et al. [16] and Islam et al. [17]. The majority of the earlier research solely looked at the taxonomic variety of benthos in estuaries while ignoring the functional traits of this major group. Although, compared to the conventional taxonomic indices, measures of functional feeding group classification (FFGs) may be anticipated to be more responsive to various geographical, seasonal, and pollution gradients. Numerous studies have demonstrated that the variety and quantity of macro-benthic organisms in Bangladesh's coastal and marine ecosystems have the potential to act as bioindicators of anthropogenic disturbance [18,19]. However, there is a paucity of understanding of the seasonal variation of taxonomic diversity and FFGs of macro-benthos from the Pasur River estuary. Given this, the present study analyzed and described the overall environmental condition of the ecosystem, and identified the key environmental factors influencing the abundance and seasonal distribution of macro-benthos as well as the variety of their FFGs in the Pasur River estuary, Khulna, Bangladesh.

2. Materials and Methods

2.1. Description of the Study Area

The Pasur River is considered the largest and most vital river in the Sundarban estuarine region that is located in the southwestern (Figure 1) portion of Bangladesh. This river has a length of 142 km with a depth range of 3 to 15 m. It is a tidal river with an approximate tidal area ranging between 1.5–3.0 m [20]. Sampling was conducted at four sites namely Mongla Ferry Ghat (22°27′59.79″ N, 89°35′37.35″ E), Joymoni (22°91'09.63" N, 89°37'20.13" E), Harbaria (22°17'47.91" N, 89°35'54.48" E) and Mazhar Point (22°11′09.10″ N, 89°32′33.30″ E). Mongla is regarded as Bangladesh's second-largest seaport and is surrounded by numerous industries. Rapid industrialization, heavy transport systems, municipal wastewater, domestic sewage, etc. are the major source of pollution at this sampling site. Joymoni is the southwest end of Mongla Upazila (sub-district) and is situated west of Kumarkhali Khal and east of Khulna Reserved Forest. It is approximately 17.5 km away from Mongla. This site is less polluted than Mongla. Salinity concentration is higher and tidal movement is more prevalent. Harbaria is located in the Chadpai range of the Sundarbans beside the Pasur River on the North side of the Bay Bengal. This site is approximately 8 km away from Joymoni and is characterized by a landing zone of numerous large shipping vessels, mechanized river crafts, and fishing boats passing through the Sundarbans. These vessels release waste oil, spillage, ballast water, garbage, and bilge washings. Mazhar Point is approximately 12.4 km away from Harbaria and comparatively less affected by industrial effluents. The salinity of water in Mazhar Point is higher than the other three sites.



Figure 1. Map showing the sampling sites in the Pasur River estuary, Bangladesh.

2.2. Sampling Design, Sample Collection, and Processing

A sampling of water, sediment, and macro-benthos were carried out during the day, between 8:00 am to 11:00 am at each sampling site once in each season (pre-monsoon, monsoon, and post-monsoon) during 2021. The samples were collected from the intertidal zone (close to the bank) during the low tide. During the high tide, the water depth of the sampling sites varied from 30-50 cm. At each sampling date, three replicates of sediment samples containing macro-benthos were collected using an Ekman dredge having a 0.024 m² mouth opening and a penetration depth of 10 cm into the sediment. Therefore, a total of 36 (3 seasons \times 4 sites \times 3 replicates) samples were collected during the study period. Sediment samples were sieved using a net having 0.5 mm mesh. Collected biological materials as well as debris were placed in plastic vials and instantly preserved with a 10% buffered formalin solution. The visibility of the collected organisms was increased by adding a little amount of Rose Bengal diluted with water to the preserved samples. After that, formalin was washed out and biological materials were separated manually from the other residues on a tray under enough light availability. The sorted organisms were then kept in small vials and preserved with a 70% ethanol solution. Seasonal estimate was made for physicochemical parameters, including temperature, pH, DO, transparency, salinity, alkalinity, total dissolved solids (TDS), phosphate-phosphorus (PO₄-P), and nitrate-nitrogen (NO_3-N) from each site.

2.3. Benthos Identification and Functional Feeding Group (FFGs) Classification

Based on morphological features, sorted benthos was identified using stereo and compound microscope as necessary. The major taxonomic group was recognized following the reference of Wilhm and Dorris [21]; Fauchald [22]; Hartman [23]; Pennack [24]; Hossain [25]; Ahmed [26]; Ward and Whipple [27]; Belaluzzaman [28]; Misra [29]; Olomukoro and Egborge [30], Merritt et al. [31], Muir and Hossain [32]. The species was validated using the taxonomic database, WoRMS. The identified macro-benthos was expressed as individual/m² and categorized under six FFGs following the available secondary literature [33–38]. Major functional feeding groups are included Shredders (SH), Scrapers (SC), Filtering collectors (FC), Gathering collectors (GC), Predators (P), and Omnivores (OV) [39].

2.4. Determination of Physico-Chemical Parameter

Water temperature (°C) was estimated using a Centigrade thermometer and a Secchi disc (30 cm diameter) was used to measure the transparency level of water. A HACH kit (model FF-2, No. 2430-01; Loveland, CO, USA) was used to assess the alkalinity of the collected samples. Water pH, salinity (ppt), DO (mg/L), and TDS (mg/L) were measured using a pH meter (Adwa AD12 waterproof pH tester); hand-held refractometer (TANAKA, New S-100, Adchi-ku, Japan); DO meter (PDO-519, Taipei, Taiwan) and TDS meter (Adwa AD31 waterproof TDS Testers). Nitrate-nitrogen (NO₃-N) and Phosphate-phosphorus (PO₄-P) were estimated with HACK Kit (DR-2020, Loveland, CO, USA) with high-range chemicals (Nitra Ver. 5 Nitrate Reagent Powder Pillows for 25 mL sample for NO₃-N and Phos. Ver. 3 Phosphate Reagent Powder Pillows for 25 mL sample for PO₄-P analysis).

2.5. Determination of Soil Quality Parameters

In the laboratory, sediment samples for each site were mixed vigorously, air-dried, at room temperature, and sieved with a mesh of 0.5 mm. Samples were then dried in an oven at 105 °C for 24 h and the relative portion of sand, silt, and clay was determined by the method of hydrometer according to Boyd and Tucker [40]. The soil organic matter content was measured with the help of the Walkey and Black wet oxidation process [41].

2.6. Diversity Indices

Shannon-Wiener diversity index and Pielou's evenness index were estimated using the following formula:

$$H = -\sum_{i} \frac{n_i}{N} \ln \frac{n_i}{N}$$

$$e = \frac{H'}{l_n s} [l_n = \text{The natural logarithm}]$$

where, H' = Shannon-wiener's diversity index and S = The number of different species in the sample.

2.7. Statistical Analysis

Seasonal and spatial variation in water quality and sediment parameters were determined by two-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences, version 25.0, IBM Corporation, Armonk, NY, USA). The significant difference was assessed using Duncan multiple range test (DMRT) at a 5% level of significance. The distribution of environmental parameters was analyzed by the principle component analysis (PCA) using Origin (Pro), 2023 (Origin Lab Corporation, Northampton, MA, USA). Multivariate analyses of the macro-benthos community were carried out by the package vegan [42] in R 4.1.3 (R Core Team, Vienna, Austria, 2022). The community assemblage of macro-benthos was determined by one-way Analysis of similarity (ANOSIM) using the function *anosim* and visualized by NMDS ordination on the Bray-Curtis distance measure using the function *metaMDS*. Species contributing most to the seasonal variation in ANOSIM were identified by Similarity percentage analysis (SIMPER) using the function Simper available in the vegan package. PAST 4.10 (Paleontological Statistics) [43] was used to determine the Shannon-Wiener diversity index and Pielou's evenness index, whereas the seasonal variation in total density, number of species, and diversity indices were subjected to ANOVA followed by DMRT test using SPSS (Statistical Package for Social Sciences, version 25.0, IBM Corporation, Armonk, NY, USA) and plotted by Origin (Pro), 2023 (Origin Lab Corporation, MA, USA). The interaction between environmental parameters, macrobenthic community composition, and FFGs was determined by canonical correspondence analysis (CCA) using PAST 4.10 (Paleontological Statistics, Oslo, Norway). Environmental data and abundance data were square-root and $\log(x + 1)$ transformed, respectively before using in the analyses.

3. Results and Discussion

3.1. Environmental Parameters

Water temperature, transparency, salinity, alkalinity, and TDS were significantly higher during the pre-monsoon season, while pH, DO, NO₃-N, and PO₄-P were significantly higher during the post-monsoon season (Table 1). Two-way ANOVA conducted in water and sediment quality parameters showed significant seasonal variation, while did not show significant spatial variation for all the parameters (Table S1). The minimum temperature in post-monsoon might be occurred as a consequence of lower air temperature, whereas the maximum temperature in pre-monsoon might be caused by higher solar radiation. The results of the current study on water temperature were more or less identical to those of Kosari et al. [44] and Shefat [45] as they also recorded the highest temperature in premonsoon and lowest in post-monsoon from Yekshabe creek-estuary, Persian Gulf and Pasur River estuary, respectively. Significantly (p < 0.01) higher transparency was also recorded during the pre-monsoon season which supports the findings of Nabi et al. [46], Akther et al. [47], and Abu Hena et al. [48]. In the present study, maximum salinity was recorded in the pre-monsoon (18.72 ppt) season and might be caused by decreased river discharge from upstream freshwater sources and a higher rate of evaporation, whereas the minimum was recorded in the monsoon (5.18 ppt) season as rainfall and water currents during floods reduce salinity which supports the findings of Rahman et al. [49]. The water pH of the Pasur River estuary was found to fluctuate from 7.12 to 7.98 which indicates the alkaline nature of the water. The elimination of CO_2 by photosynthesis through bicarbonate degradation, a decrease in salinity and temperature, dilution of seawater

by the influx of freshwater, and the decomposition of organic waste may all contribute to higher pH values in post-monsoon seasons. Similar findings were also reported by Chowdhury [50] and Matin et al. [14]. DO was the highest during pre-monsoon and the lowest during post-monsoon season. Higher temperature and the presence of oxygendemanding municipal and industrial effluents might be responsible for the lower DO during the pre-monsoon season and lower water temperature might be responsible for higher DO during post-monsoon. A study conducted by Shefat [45] reported the range of DO was 5.97 to 8.43 mg/L in the Pasur River estuary which is supported by the present findings. Significantly higher alkalinity and total dissolved solids (TDS) were recorded during the pre-monsoon season might be due to the increased ionic concentration of water at higher temperatures which are supported by the findings of Nabi et al. [46], Akther et al. [47] and Abu Hena et al. [48]. Rainfall and upstream water flow were found to regulate the nutrient content of the Pasur River estuary. The monsoon season had a dilution effect on NO₃-N and PO₄-P concentration and therefore, lower level of nutrients was recorded during monsoon season. On the contrary, post-monsoonal deposition of municipal and domestic sewage water, decaying plant debris, and reduced freshwater flow were found to increase the nutrient content of water. The monsoonal effect on the nutrient content of water was also previously reported by Rahaman et al. [49], whereas they recorded lower nutrients from water during monsoon season. The percentage composition of sand was higher in monsoon (Figure 2) following the pre-monsoon and post-monsoon. In contrast to clay and sand, silt particles predominated in the research area. Silt and clay content were higher during the post-monsoon and pre-monsoon seasons, but lower content of these particles were observed during the pre-monsoon and monsoon seasons, respectively. Furthermore, the highest percentage composition of organic matter occurred in post-monsoon and lower in the monsoon season, consequently.

Table 1. Physico-chemica	parameters ((mean and standard	deviation)	of Pasur	River e	estuary.
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Parameters	Pre-Monsoon	Monsoon	Post-Monsoon	F-Value	<i>p</i> -Value
Temperature (°C)	30.01 ± 0.39 $^{\rm a}$	26.06 ± 0.40 ^b	$21.38\pm0.67~^{\rm c}$	880.13	0.000
Transparency (cm)	32.46 ± 1.69 ^a	$14.78\pm1.35~^{\rm c}$	$26.24\pm1.28^{\text{ b}}$	459.47	0.000
Salinity (ppt)	$16.45\pm1.62~^{\rm a}$	$6.70\pm1.11~^{\rm c}$	$10.88 \pm 1.39 \ ^{ m b}$	148.66	0.000
pН	7.49 ± 0.15 ^b	$7.38\pm0.16~^{\rm c}$	7.77 ± 0.14 ^a	21.43	0.000
DO (mg/L)	5.12 ± 0.70 ^c	6.78 ± 0.94 ^b	9.16 ± 0.67 ^a	81.66	0.000
Alkalinity (mg/L)	142.87 ± 21.48 $^{\rm a}$	96.08 \pm 19.26 ^c	134.38 ± 17.82 ^b	19.45	0.000
TDS (mg/L)	169.42 ± 22.59 a	$114.85 \pm 21.83 \ ^{\rm c}$	145.88 ± 24.47 ^b	17.01	0.000
NO3-N (mg/L)	1.08 ± 0.24 ^b	$0.34\pm0.20~^{ m c}$	1.45 ± 0.3 a	59.06	0.000
PO_4 -P (mg/L)	$1.86\pm0.30^{\text{ b}}$	0.68 ± 0.41 ^c	$2.62\pm0.39~^{\text{a}}$	83.15	0.000

Figures in the same row having different superscript letters are significantly (p < 0.01) different. DO = Dissolved oxygen, TDS = Total dissolved solids. Superscript a, b and c indicate the results of multiple comparison test by DMRT.



Figure 2. Properties of sediments collected from the Pasur River estuary.

PCA biplot of the environmental parameters is shown in (Figure 3). In the principle components analysis, PCA 1 and PCA 2 explained 49.31% and 39.97% variability of the data respectively, thus accounting for 89.28% of the total variability. The first principle component axis (PCA 1) discriminated the pre-monsoon samples mainly based on salinity, transparency, and TDS while the second axis (PCA 2) described the post-monsoon samples as per pH, DO, NO₃-N, and PO₄-P. However, a negative correlation of water temperature was observed with a post-monsoon sample. The present findings roughly support the findings of Shabani et al. [37] who reported that temperature, turbidity, pH, conductivity, ammonia-nitrogen, NO₃-N, and total phosphorus influence macro-benthic sample and also observed a positive significant relationship exist between NO₃-N and pH.



Figure 3. Principle component analyses (PCA) on environmental parameters. PrS represents premonsoon samples, MS as monsoon samples, and PoS as post-monsoon samples. WT = Water temperature, Trans = Transparency, DO = Dissolved oxygen, Alka = Alkalinity, TDS = Total dissolved solid.

3.2. Macro-Benthic Community Assemblage

A total of 3286 individuals (Pre-monsoon = 857 ± 45.38 , Monsoon = 251 ± 18.89 , and Post-monsoon = 2178 ± 117.85) were sampled during the study period belongings to 47 species from 35 families, 23 orders, and 6 taxonomic classes (Table 2). Two-way ANOVA showed significant seasonal variation in density and the number of species but insignificant differences among the sites (Table S1). Species accumulation curves are used to estimate the number of species in the present study. To standardize seasonal differences in the sample size, the observed species richness was rarified. The species accumulation curves by rarefaction reflected that post-monsoon was richer with species (overall species richness = 41) than pre-monsoon (34) and Monsoon (27) (Figure 4). In the present study, significantly (F = 114.26, p = 0.000) higher (545 ind./m²) and lower density (63 ind./m²) were recorded in post-monsoon and monsoon season, respectively (Figure 5, Table S1). From the pairwise comparison in density, significant differences were observed between pre-monsoon and monsoon, monsoon and post-monsoon, and pre-monsoon and post-monsoon season. Furthermore, the number of species was significantly maximum (F = 4.641, p = 0.041) in the period of post-monsoon comparing pre-monsoon and monsoon (Figure 5, Table S1). The number of species was found similar to the findings of Noman et al. [51] who also recorded 47 species from the Naf River estuary and widely coherent with the findings of Rahman et al. [52] and Noyel et al. [53] whereas, the findings were fairly incongruous with a former study that recorded only 20 species of macro-benthos from Mouri River, Khulna. Sharif et al. [12] studied macro-benthic diversity in the lower Meghna River estuary and found that species density was minimum (208.1 ind./ m^2) during monsoon at Hatiya and maximum (27,180.0 ind./m²) during post-monsoon at Barisal which was much higher than the present findings. Similar research was also conducted by Kumar and Khan [54], who found that the macro-benthic community along the Indian Pondicherry coast had higher densities throughout the post- and pre-monsoon seasons but lower densities during the southwest monsoon season. Analysis of similarity (ANOSIM) indicated a significant difference in species assemblage among the seasons (R = 0.7222, p = 0.0005) (Table 3). The pair-wise comparison also indicated significant differences between premonsoon—monsoon (R = 0.8021, p = 0.0289), pre-monsoon—post-monsoon (R = 0.7708, p = 0.0293), and monsoon-post-monsoon (R = 0.9896, p = 0.0329). SIMPER analysis (Table 3) detected *Glycera alba* as the most contributory species for the average dissimilarity of pre-monsoon and monsoon samples. Furthermore, D. aestuarina and Lymnaea acuminata were the most contributory species for the dissimilarity of monsoon vs. post-monsoon and pre-monsoon vs. post-monsoon groups. Considering all groups combined, D. aestuarina was the most contributory species for the overall average dissimilarity among the seasons. Seasonal variation in the macro-benthic community assemblage of the Pasur River estuary is visualized more nicely in the NMDS plot. Clear separation of monsoonal samples from the other two seasons is evident in the NMDS plot, while samples collected in the period of pre-monsoon and post-monsoon are quite similar (Figure 6). A distinct pattern of seasonal variation (p < 0.01) was observed in the environmental parameters of the Pasur River estuary and was responsible for the significant variation in macro-benthic diversity and abundance. It is possible that the relatively high density and the number of species shown during the post-monsoon season were the results of stable environmental settings such as high DO and stable salinity, which are crucial for the distribution of fauna. Low temperatures and salinities might cause a decline in benthos during the monsoon season. The changes in ecological parameters brought on by heavy rainfall and the resulting massive freshwater flow during the monsoon period, compared to less or no rainfall during the pre-and post-monsoon, might be the cause of this seasonal variation in the benthic population. The Pasur River estuary is located on the southwest coast of Bangladesh, where the southwest monsoon influences the climatic condition from May through October and the area sees 90% of its annual precipitation. The abundance and variety of the macro-benthos, therefore, changed with the seasons, as did the predominance of opportunistic species. In this research, it was found that the monsoonal rain had an impact on the salinity, DO, OM, and sediment textures, which are important factors in determining the community composition of an estuarine system (Tables 1 and S1). All of the parameters experienced significant fluctuations during the monsoon and were, in comparison, more steady before and after the monsoon. During the monsoon, benthic organisms that could endure a broad range of salinities persisted, and therefore benthic diversity and density were low. Again, the increased flow velocity during the monsoon causes benthic organisms to battle to settle, recruit, and burrow. According to Ullah et al. [13], higher amounts of organic carbon cause oxygen to be depleted, which can reduce species diversity and density. The environmental variables are generally stable during pre- and post-monsoon, which supports the rich biodiversity that was shown in this research. Two major driving forces, such as the incoming tide and the outgoing freshwater flow, regulate the variability in estuarine hydro-geo-chemical variables. The major processes affecting an estuary's biodiversity, such as hydrodynamics (such as water circulation, mixing, and flushing), salinity control, sediment dynamics (such

as sediment delivery, deposition, and erosion), nutrient cycling, and trophic transfer, are all influenced by these two driving forces. During the study period, the most dominant species was *C. capitata* complex with a relative abundance of 10.44% which was possibly due to the high organic matter content of the sediment. The species *C. capitata* complex is commonly used as a pollution indicator organism [55]. Apart from *C. capitata* complex, *Tubifex tubifex*, *Micronephthys oligobranchia*, *Nemalycastis indica*, *C. coasta*, *Filopaludina bengalensis*, *Tegillarca granosa*, *Chironomus* sp., *Cybister* sp. and *Baetis* sp. were also abundant which support the findings of Balachandar et al. [56], Sharma and Chowdhary [57], and Bahuguna and Negi [58].



Figure 4. Species accumulation curve (method = rarefaction) showing differences in the total number of species per individual sample for three seasons. An individual's number is displayed by green, red, and purple lines for pre-monsoon, monsoon, and post-monsoon, respectively.



Figure 5. Density and number of species of macro-benthos of Pasur River estuary.

Phylum	Class	Order	Family	Species	RA (%)	FFG
				Tubifex tubifex	4.23	GC
				Limnodrilus hoffmeisteri	2.76	GC
		Tubificida		Branchiura sowerbyi	2.00	GC
	Clitellata		Naididae	Pristinella acuminata	0.81	GC
				Nais simplex	1.48	GC
				Aulodrilus pigueti	0.75	GC
			Nephtyidae	Micronephthys oligobranchia	8.12	SH
Annelida		-	Nereididae	Nemalycastis indica	5.85	SH
		Phyllodocida		Dendronereis aestuarina	5.02	PR
				Perinereis nuntia	3.23	OV
	Polychaeta			Tylonereis bogoyawlenskyi	2.80	OV
			Glyceridae	Glycera alba	4.24	PR
PhylumClassOrderFamilyAnnelidaClitellataTubificidaNaididae $\begin{bmatrix} Lit \\ h \\ p \\ p$	Eunicida	Lumbrineridae	Lumbrineris sp.	1.30	PR	
	Cossura coasta	7.05	GC			
		Aciculata	UrderFamilySpeciesKA (v_0)Tubifer tubiferLA (v_0)Tubificit laTubifer tubifer2.76Branchiura socuerbuj2.00Prisinella acuminata0.81NaididaeMephtyidaeMeinorphilhys oligobranchiaNephtyidaeMeinorphilhys oligobranchia8.12NereididaeNereididaeColscar alba4.24EunicidaLumcindaeClayceridaeClayceridaeClayceridaeCapitellidaCossuridaeCossuridaeCossura coasta7.05AciculataNeritidaePila globosa0.57typophilaLymnaeidaeLymnaeidaeLymnae acuminataOldoneritidaNeritidaePila globosa0.57typophilaLymnaeidaeLymnaeidaeLymnaeidaeLymnaeidaePila globosa0.57typophilaLymnaeidaePila globosa0.57typophilaLymnaeidaePila globosa0.57typophilaLymnaeidae <td< td=""><td>SC</td></td<>	SC		
		Cuelonoritido	NT	Vittina smithii	0.71	SC
		Cyclohernida	Neritidae	Neripteron violaceum	0.46	SC
		Architaenioglossa	Ampullariidae	Pila globosa	0.57	SC
	Gastropoda	Hygrophila	Lymnaeidae	Lymnaea acuminata	0.66	SC
		Littorinimorpha	Littorinidae	Littoraria melanostoma	0.40	SC
		Architaenioglossa	Viviparidae	Filopaludina bengalensis	2.03	SC
Mollusca		Caenogastropoda	Cerithiidae	Cerithium tenellum	0.44	SC
	Bivalvia	Venerida	Veneridae	Meretrix meretrix	2.98	FC
		Cardiida	Donacidae	Donax carinatus	1.46	FC
		Ostreida	Ostreidae	Magallana gigas	1.18	FC
		Myida	Pholadidae	Pholas sp.	1.05	FC
		Arcida	Arcidae	Tegillarca granosa	1.89	FC
		Diptera	Chironomidae	Chironomus sp.	4.52	GC
			NT	Ranatra digitata	0.82	PR
		Hemiptera	Nepidae	Laccotrephes griseus	0.69	PR
			Belostomatidae	Lethocerus indicus	0.88	PR
A He		Hydrometrinae	Hydrometra butleri	1.00	PR	
			Gomphidae	Gomphus sp.	0.96	PR
		Odonata	Libellulidae	Libellula sp.	0.56	PR
	Hexapoda		Destingidae	<i>Cybister</i> sp.	2.42	PR
		Coleoptera	Dyfiscidae	Dytiscus sp.	0.87	PR
Arthropoda		concopieru	TT 1	Hydrobius sp.	1.42	PR
			Hydrophilidae	Hydrophilus piceus	2.03	PR
			D	<i>Baetis</i> sp.	2.54	GC
		Ephemeroptera	Baetidae	Platybaetis sp.	0.63	SC
		-	Heptageniidae	Heptagenia sp.	1.18	SC
		Discoutour	Nemouridae	Amphinemura sp.	0.94	SH
		Plecoptera	Perlidae	Tetropina sp.	1.30	PR
			Palaemonidae	Leptocarpus potamiscus	0.83	GC
	Malaassiass	Decanada	Penaeidae	Metapenaeus monoceros	0.76	OV
	Maiacostraca	Decapoua	Ocypodidae	Ocypode macrocera	1.32	PR
			Sesarmidae	Episesarma mederi	0.45	SH
		at 11 arr a				

Table 2. Taxonomic list of macro-benthos species of Pasur River estuary with their functional feedinggroups (FFGs) classification.

Note: Shredders = SH, Scrapers = SC, Filtering collectors = FC, Gathering collectors = GC, Omnivores = OV, Predators = PR.

Crouns	ANOSIM		Dissimila	% Contribution		
Groups	R P		Ave. Diss. (%)	Typical Species		
				Glycera alba	4.88	
		0.0289	55.55	Branchiura sowerbyi	4.82	
Pre-monsoon vs. monsoon	0.8021			Baetis sp.	4.77	
				Tegillarca granosa	4.63	
				Dytiscus sp.	3.74	
				Dendronereis aestuarina	4.36	
		0.0293	65.22	Namalycastis indica	4.30	
Monsoon vs. post-monsoon	0.7708			Glycera alba	3.67	
				Cossura coasta	3.60	
				Hydrobius sp.	2.88	
				Lymnaea acuminata	4.52	
	0.9896	0.0329	30.89	Cossura coasta	4.37	
Pre-monsoon vs. post-monsoon				Hydrometra butleri	4.22	
				Tylonereis bogoyawlenskyi	3.44	
				Micronephthys oligobranchia	3.23	
				Dendronereis aestuarina	3.93	
			50.55	Namalycastis indica	3.65	
Overall or pool of all groups	0.7222	0.0005		Glycera alba	3.61	
				Branchiura sowerbyi	3.02	
				Tegillarca granosa	2.75	

Table 3. Results of ANOSIM and SIMPER analysis of macro-benthic assemblage in Pasur River estuary.



Figure 6. Non-metric multidimensional scaling of sampling seasons.

Moreover, the presence of freshwater species namely *Amphinemura* sp. and *Baetis* sp. indicated freshwater flow and low saline zone in the present study. The amount, quality, and timing of flows pose a threat to an ecosystem's ability to sustain and maintain a diverse and hardy population of organisms, according to Andreasen et al. [59]. Again, upstream freshwater carries organic matter and transports nutrient-rich water to the estuary, which increases productivity and biodiversity. Attrill and Rundle [60] found that there was a continuum of assemblages along the salinity gradient, with fauna occupying the midestuary being either freshwater or marine species at the limit of their range, rather than 'true estuarine organisms'. So, along the estuary gradient, some opportunistic species and euryhaline species can be found. According to Williams and Williams [61], aquatic insects

made up 32% of the entire invertebrate population. They also noticed that during periods of low flow, freshwater chironomids and mayflies moved down towards the river.

3.3. Species Diversity and Evenness Index

The Shannon diversity index is an estimation of diversity that integrates species richness and their relative abundances whereas species evenness is defined as how species are evenly distributed in an ecosystem. Species diversity and evenness were significantly higher in the period of the post-monsoon and lowest in the monsoon. A significant difference was visualized in the Shannon (F = 10.22, p = 0.004) and Evenness index (F = 9.915, p = 0.005) among the seasons (Figure 7 and Table S1). Tukey's multiple comparison tests indicated insignificant differences occurred in diversity between pre-monsoon and monsoon, while significant variation appeared between pre-monsoon with post-monsoon and monsoon with post-monsoon. Significant differences in evenness index were observed between pre-monsoon vs. monsoon and monsoon vs. post-monsoon. However, the evenness index was insignificant between pre-monsoon vs. post-monsoon. The Shannon diversity (F = 10.22, p = 0.004) was significantly higher in the post-monsoon (3.51) compared to the pre-monsoon (3.25) and monsoon (2.83) period subsequently, which was more or less similar to the value (1.69–3.09) reported by Kumar and Vyas [62] from the selected reach of River Narmada, India. However, lower diversity of macro-benthos was reported by Matin et al. [14] in the Feni estuary and Sharif et al. [12] in the lower Meghna estuary. On the other hand, higher macro-benthic diversity was recorded in the different mangrove ecosystems of Tamil Nadu Coast, India by Thilagavathi et al. [63]. Magurran [64] stated that the value of H' need to remain in the range of 2.5 to 3.5 for a healthy environment, which supports the present findings. In addition, the evenness index (F = 9.915, p = 0.005) increased with the increasing $H'_{,}$ and a significantly higher value was observed in the period of post-monsoon (0.92) compared to pre-monsoon (0.75) and monsoon (0.50) season respectively. Sarkar et al. [16] observed the evenness value ranged from 0.61–0.85 at Meghna and Bakkhali River estuary, which supports the present findings.



Figure 7. Shannon and evenness index of macro-benthos of Pasur River estuary.

3.4. Interaction between Environmental Parameters and Macro-Benthic Community

The interaction between environmental parameters and the macro-benthic community composition of the Pasur River estuary is shown in canonical correspondence (CCA) analysis (Figure 8). Axis-1 has a positive influence on water temperature, salinity, pH, transparency, alkalinity, TDS, NO₃-N, PO₄-P, clay, and organic matter therefore, these parameters are positively influenced the abundance of *Branchiura sowerbyi*, *Nais simplex*, *Tylonereis bogoyawlenskyi*, *G. alba*, *Vittina smithii*, *Pila globosa*, *F. bengalensis*, *Cerithium tenellum*, *Donax carinatus*, *Magallana gigas*, *Pholas* sp., *T. granosa*, *Ranatra digitata*, *Lethocerus indicus*,

Dytiscus sp., *Hydrobius* sp., *Baetis* sp., *Platybaetis* sp., *Heptagenia* sp., *Amphinemura* sp. and *Episesarma mederi*. However, Axis-2 is found to have a positive influence on salinity, pH, DO, transparency, alkalinity, TDS, NO₃-N, PO₄-P, silt, clay, and organic matter. The abundance of *T. tubifex*, *L. hoffmeisteri*, *Aulodrilus pigueti*, *M. oligobranchia*, *N. indica*, *D. aestuarina*, *Perinereis nuntia*, *L. acuminata*, *L. melanostoma*, *Meretrix meretrix*, *Chironomus* sp., *Cybister* sp., *Hydrophilus piceus*, *Tetropina* sp., *Leptocarpus potamiscus*, *Metapenaeus monoceros* and *Ocypode macrocera* were positively influenced by water temperature and sand. Furthermore, the higher assemblage of several species, namely *P. acuminata*, *Lumbrineris* sp., *C. coasta*, *C. capitata* complex, *N. violaceum*, *L. griseus*, *H. butleri*, *Gomphus* sp. and *Libellula* sp. were observed with increasing DO and percentage of silt concentration in the bottom as they are positively correlated with these species.



Figure 8. Canonical correspondence analyses between environmental parameters and macrobenthic community.

3.5. Macro-Benthic Functional Feeding Groups

Macro-benthic species sampled during the present study are grouped into six FFGs such as SH, SC, FC, GC, OV, and PR (Table 4). Functional feeding groups (FFGs) are a classification approach mainly based on the type of food resource that a species utilizes in an aquatic ecosystem. Forty-seven macro-benthic species identified in the present study were categorized into 4 Shredders (SH), 10 Scrappers (SC), 5 Filtering collectors (FC), 10 Gathering collectors (GC), 3 Omnivore (OV), and 15 Predator (PR). The present findings were widely coherent with the findings of Shabani et al., [37] who conducted a study on the Sanjiang plain wetland and reported 57 macro-benthos were classified as predators (19), gathering-collectors (15), scrapers (7), filtering collectors (6), omnivores (5) and shredders (5). During pre-monsoon and monsoon periods, GC was the most dominant FFG with a group density of 71.17 and 19.33 ind./m², respectively, while, PR was the most dominant with a group density of 144.08 ind./m² during post-monsoon season. However, the least contribution was made by OV in the period of pre-monsoon and monsoon, while by FC during the post-monsoon period. Furthermore, GC represents the highest total density $(221.83 \text{ ind.}/\text{m}^2)$ and relative abundance (26.97%) among the other FFGs. During the study period, the least dominant group was OV with (55.83 ind./ m^2) a total density and

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(6.79%) relative abundance which included only three species, namely *Perinereis nuntia*, *T. bogoyawlenskyi*, and *M. monoceros*.

Table 4. Distribution of functional feeding groups (FFGs) of the macro-benthic assemblage of Pasur

 River estuary.

	Pre-Monsoon		Monsoon		Post-Monsoon			DA (0/)
FFGs	Density (ind./m ²)	RA (%)	Density (ind./m ²)	RA (%)	Density (ind./m ²)	RA (%)	Iotal Density (ind./m ²)	KA (%)
SH	28.17	13.08	7.83	12.50	90.42	16.60	126.42	15.37
SC	41.08	19.07	11.33	18.08	91.50	16.80	143.92	17.49
FC	23.92	11.10	5.42	8.64	41.08	7.54	70.42	8.56
GC	71.17	33.04	19.33	30.85	131.33	24.12	221.83	26.97
OV	5.67	2.63	4.00	6.38	46.17	8.48	55.83	6.79
PR	45.42	21.08	14.75	23.54	144.08	26.46	204.25	24.83

Note: Shredders = SH, Scrapers = SC, Filtering collectors = FC, Gathering collectors = GC, Omnivores = OV, Predators = PR.

Gholizadeh and Heydarzadeh [65] found gathering collectors (52%) were major FFGs of the Zarin-Gol River, Iran. Furthermore, Addo-Bediako [66]; Linares et al. [67]; Subramanian & Sivaramakrishnan [68], and Callisto et al. [69] also found gathering-collectors as a dominant group in their studies which may occur due to the higher proportion of organic matter in water [70]. Distribution of gathering collectors including *T. tubifex, L. hoffmeisteri, Aulodrilus pigueti,* and *Leptocarpus potamiscus* were positively influenced by water temperature and sand. Furthermore, the distribution of scrapers (*P. globosa, F. bengalensis, C. tenellum*) and filtering collectors (*M. gigas* and *Pholas* sp.) were positively influenced by NO₃-N, PO₄-P, and organic matter. A similar study was carried out by Sharmin et al. [71] where they found that several environmental parameters namely temperature, depth, pH, soil organic matter, and soil organic carbon reflected a close relationship with Mollusca and Annelida therefore, less association were observed with Arthropods.

3.6. Interaction of Environmental Parameters and FFGs of Macro-Benthos

The interaction between environmental parameters and FFGs of macro-benthos is shown in Figure 9. The first two CCA axes represent 90.71% eigenvalue for describing the interaction. Water temperature, salinity, DO, silt, and clay were found as the most important parameters describing the structuring of macro-benthos FFGs. Axis 1 has a positive influence on SH, OV, and PR, and a negative influence on SC, FC, and GC. Furthermore, Axis 2 has a positive influence on GC and OV, and a negative influence on SH, SC, FC, and PR. CCA analysis revealed a general trend of seasonal variation rather than spatial variation.



Figure 9. Canonical correspondence analyses between environmental parameters and macro-benthos functional feeding groups (FFGs).

4. Conclusions

The overall goal of this study was to uncover the taxonomic and functional groups of macro-benthos in the Pasur river estuary in connection to the seasons and ecological variables. A total of 47 species comprising six FFGs were identified with fewer than 35 taxonomic families. With a relative abundance of 10.44%, the pollution indicator species C. capitata complex was the most prevalent one. Gathering collectors (GC) had the highest overall density (221.83 ind./m²) and relative abundance (26.97%) of all the FFGs, indicating organic enrichment of the area. Values for diversity ranging from 3.51 to 2.83 indicated a moderate level of diversity in the estuary. The density, Shannon diversity, and Pielou's evenness index were significantly higher during post-monsoon and lower during monsoon. This pattern may be explained by high DO, food availability, and stable salinity during the post-monsoon and by high rainfall and low salinity during the monsoon. The species, D. aestuarina contributed most to the overall average discrepancy (50.55%) between the seasons. C. coasta, C. capitata complex, N. violaceum, H. butleri, Gomphus sp., and Libellula sp. were found to be positively influenced by the water's DO and the silt particles of the bottom sediment. The study will provide baseline information for characterizing macro-benthic community structure and FFGs in similar estuarine habitats.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11071453/s1, Table S1. Two-way ANOVA of water and sediment quality parameters of Pasur River estuary by sites and seasons. Significance is highlighted in bold.

Author Contributions: Conceptualization, M.A.S.J., B.K. and M.A.H.; methodology, M.A.S.J. and B.K.; software, M.A.H.; validation, M.B.H. and M.A.H.; formal analysis, M.A.H., M.A.S.J., B.K. and S.A.; investigation, B.K.; resources, M.B.H.; data curation, B.K.; writing—original draft preparation, M.A.H., B.K. and M.A.S.J.; writing—review and editing, M.B.H., M.F.A., T.A. and S.A.; supervision, M.A.S.J.; funding acquisition, B.K., M.A.S.J., M.F.A. and T.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was also funded by the Researchers Supporting Project Number (RSP2023R436), King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are provided in the article.

Acknowledgments: This study was partially funded by Universiti Brunei Darussalam under the Faculty/Institute/Center Research Grant (No. UBD/RSCH/1.4/FICBF(b)/2020/029), (No. UBD/RSCH/ 1.4/FICBF(b)/2021/037) and the FOS Allied Fund (UBD/RSCH/1.4/FICBF(a)/2023). The authors would like to acknowledge the support provided by the Researchers Supporting Project Number (RSP2023R436), King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

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