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Using macrobenthic community structure and biotic indices to assess the ecological status of Wulan Estuary, Demak District, Indonesia

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Abstract. *Hartati R, Widianingsih W, Zainuri M, Wardianto Y. 2024. Using macrobenthic community structure and biotic indices to assess the ecological status of Wulan Estuary, Demak District, Indonesia. Biodiversitas 25: 3073-3087.* Estuaries provide critical habitats for species, and they are valued commercially, recreationally, and culturally. They also filter for sediments and pollutants from rivers before entering the ocean, providing cleaner waters for marine biota. This study presents a unique approach to assessing the ecological status of an estuary using microbenthic community structure and biotic indices with a case study of Wulan Estuary, Demak District, Central Java Province, Indonesia. AZTI's Marine Biotic Index (AMBI) community structure and biotic indices were used to assess ecological quality at eleven stations and determine the marine macrobenthic fauna's sensitivity to disturbances in two periods, i.e. August 2021 and 2022. A distinct spatial and temporal variation was evident in the macrobenthic community structure, where higher species richness and density were observed in 2022, with some species being more dominant in 2022. The trophic structure analysis showed the dominance of deposit-feeding bivalves in the fine sediments during those sampling intervals. These trophic groups, however, were dispersed less uniformly in the sandy substratum. Compared to the previous year, the density of herbivorous grazers and suspension feeders was higher in 2022. It is also shown by AMBI values that Wulan Estuary belonged to the good (slightly disturbed) to the high (undisturbed) condition, along with other biotic indices, which showed an enhanced habitat quality for benthic organisms. This result revealed the comparative ecological quality of related estuary ecosystems in Indonesia.

Keywords: AMBI, community structure, estuary, macrobenthic fauna

INTRODUCTION

An estuary is a transitional zone between terrestrial and marine realms in the form of partially enclosed water bodies in coastal areas. It plays significant roles in biogeochemical cycles, such as food webs, energy flows, nutrient cycles, and hydrologic cycles. Estuary delivers various ecosystem services, including nutrient regulation, carbon sequestration, and detoxification of polluted waters (Mehvar et al. 2018). Estuary ecosystems are also well-known as habitats for many benthic communities, nursery grounds, and feeding areas for most coastal and oceanic fish species (Fortune et al. 2023). They are among the most productive and valuable systems with significant resources and socio-economic values that benefit humans. Estuary is also important for various ecological purposes, including preserving biological productivity, protecting the shorelines from abrasion, and reducing storm surges (Stark et al. 2017).

Because estuaries link the system in terrestrial, freshwater, and marine environments, this makes strong spatiotemporal variability of the physicochemical characteristics due to tidal and fluvial forcing (Rehita et al. 2022). Thus, estuaries are susceptible to environmental changes. Such changes might affect many aquatic organisms living in an estuary and adapt to the ecological condition (Arlinghaus et al. 2023). In this regard, habitat quality has a significant influence on the dynamic of biota, including macrobenthic.

The macrobenthic community consists of epifauna (i.e., those that live on the estuarine floor) and infauna (i.e., those that inhabit the bottom sediment). Wan et al. (2018) stated that both types of benthic are essential components of estuarine ecosystems for energy flows and material circulations ecosystems, ecosystem engineering, and function. The sensitivity of macrobenthic to environmental change makes them good indicators of environmental disruption (Shi et al. 2014). Hence, they are widely used as bio-indicators to measure the impact of properties, function change, and the status of estuarine ecosystems (Gjoni et al. 2017). This is because ecosystem function is related to biodiversity, abundance, and biomass of benthic macroinvertebrates (Gjoni et al. 2019; Reizopoulou et al. 2014; Ghezzo et al. 2015; Gjoni and Basset 2018). A study by Xue et al. (2019) in the Yangtze River estuary showed tidal flats altitude, tidal power weakening, and physical and chemical parameters of the water alteration caused by reclamation affect the change in the community structure of benthic macroinvertebrates. In addition, Pierre and Kovalenko (2014) have shown that species diversity and richness decrease when the sedimentary environment is altered. Siltation, as an impact of sediment river runoff, affects the species composition of benthic fauna (Yang et al. 2016).

There are several ways to assess the environment's ecological quality through the community structure of macrobenthic (Gjoni and Basset 2018; Xue et al. 2019), one of which is AZTI's Marine Biotic Index (AMBI). This index has been developed by Borja et al. (2000) to evaluate the health of aquatic environments based on benthic communities. AMBI is an index calculation to determine water status, pressure, and pollution based on biota response, particularly for the diversity of tolerance and intolerance benthos (Borja and Tunberg 2011). AMBI has software to make calculations easier (Borja et al. 2012). The AMBI has been applied successfully in many countries, such as Italy (Munari et al. 2022), Brazil (Netto et al. 2018), Algeria (Belhaouari et al. 2019), India (Feebarani et al. 2016; Dias et al. 2018; Mulik et al. 2020; Nayak et al. 2022). China (Lu et al. 2021; Li et al. 2021; Dong et al. 2021; 2023a; Zhang et al. 2023) and United States (Pruden et al. 2022).

Thus far, only limited studies in Indonesia have employed AMBI to asses marine macrobenthic dynamics in response to environmental disturbances. Some studies successfully used this method, such as in Paiton coastal waters, East Java Province (Mursalin et al. 2014), in Western Java (Wardiatno et al. 2017), and in Tanjung Pasir, Banten, Indonesia (Sahidin et al. 2018). Similar works need to be done in Indonesia's coastal areas since there are increasing pressures caused by human activities, including marine culture, agriculture, domestic sewage, industrial wastewater, and marine transportation, which contribute to the accumulation of organic matter in coastal sediments, affecting the trophic structure and biomass of macrobenthic (Wardiatno et al. 2016).

One important area to asses is Wulan Estuary in Central Java, Indonesia. This estuary faces environmental threats, such as erosion, abrasion, and sedimentation, due to natural and human factors (Muskananfola et al. 2023). Therefore, this study aimed to use community structure and biological indices of AMBI to compare the marine benthic fauna's sensitivity to disturbances and benthic ecological quality in the Wulan Estuary during 2021 and 2022. The results of this study are expected to provide insights and comparative ecological quality of similar estuary ecosystems in Indonesia. It is useful for developing required protective measures in the ecosystems and designing monitoring strategies.

MATERIALS AND METHODS

Study period and area

The study was conducted in August 2021 and August 2022 in Wulan Estuary, Demak District, Central Java Province, Indonesia (Figure 1). Geographically, this estuary is located at the coordinates of 110°32" E to 110°36'0" E and 6°43'30" S and 6°46'30"S. The Wulan River, with an overall catching area of 31.75 km^2, is a component of the Serang Watershed, with a significant water output of 350 m^3.s^{-1} (Fadlillah et al. 2019). The region's lithology primarily comprises fine sand and clay in an alluvial marshy deposit with low permeability. Not only it receives a high sediment load, but this location is also affected by human intervention in the form of aquaculture and settlement in the delta. Fish ponds occupy over 90% of the land, with the remaining 10% comprising settlement, salt marshes, shrubs, irrigated rice fields, and crops (Fadlillah et al. 2018). The coastal community in Wulan Estuary mainly has livelihood as pond farmers and fishermen as the estuary also acts as fishing ground for fish, shrimp, and mussels (Ferdiansyah et al. 2016).

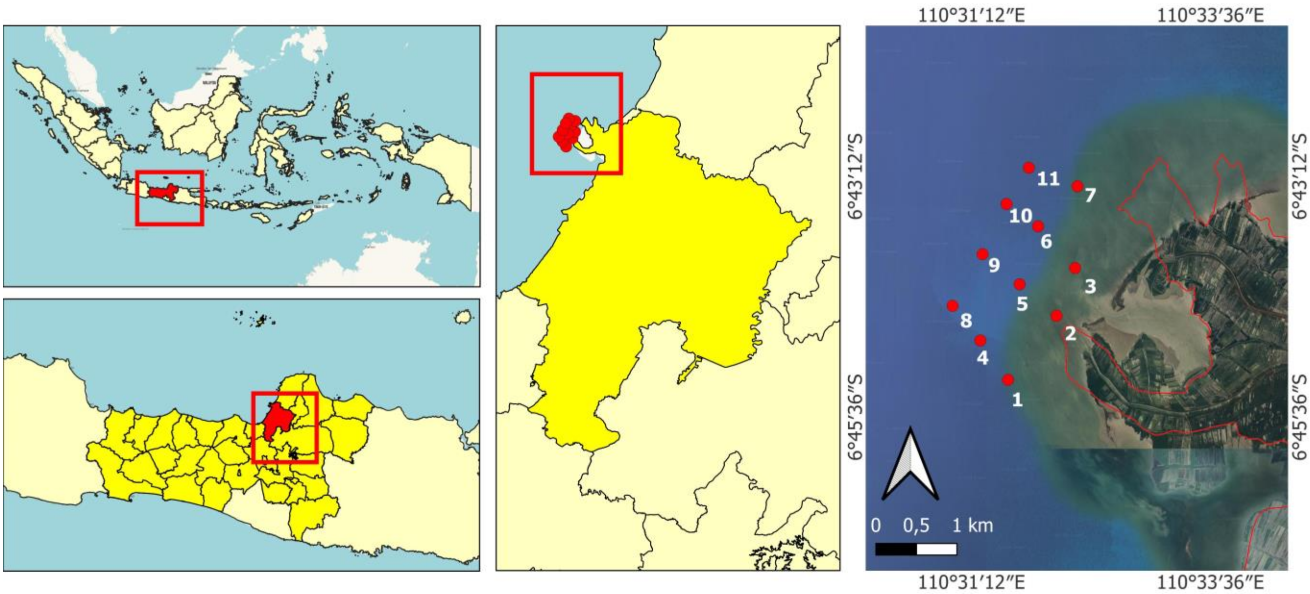

Figure 1. Map of the study area in the Wulan Estuary, Demak District, Indonesia, and the eleven sampling stations

Sampling procedures

The macrobenthic specimens were sampled from the seabed at 11 stations with depths ranging from 2 to 17 meters (Figure 1). The macrobenthic samples were collected using a 1.3-meter dredge, and after being clean washed, they were first put in a 10% formaldehyde solution for 24 hours, then fixed and preserved in 70% denatured ethanol. The observation and recording were carried out. The live color of the specimens was recorded. The collected samples were photographed and sorted into their respective taxa, i.e., Arthropoda (Crustacea), Echinoderm, Mollusk, Annelida (Polychaeta), and Fishes. Twenty two (22) samples (11 samples each for 2021 and 2022) were identified and classified according to their morphological character, following the methods of Short (2014); Gaonkar et al. (2015); Hiebert (2015); Hossain et al. (2015); Xiao et al. (2015); Chanda (2016); Hamid and Wardiatno (2018); Vehof et al. (2018); Hanim et al. (2020; 2023); Redjeki et al. (2020); Setyadi et al. (2021); Mukhopadhyay et al. (2022); Hsu and Chan (2023); Novianingrum et al. (2023) for Arthropoda (Crustacea) Lee (2014); Massin et al. (2014); Michonneau and Paulay (2014); Ong and Wong (2015); Ong et al. (2016); Thomas and MacDonald (2016); Agnello (2017); Filander and Griffiths (2017); Olbers et al. (2019); Cobb et al. (2019); Chagas et al. (2020); Hartati et al. (2021); Purcell et al. (2023); Stara et al. (2023); Winarni et al. (2023), Canessa et al. (2024) for Echinoderm; Reid (2014), Das et al. (2015), Barkalova et al. (2016), Pu et al. (2019), Hakim et al. (2020), Widianingsih et al. (2020); Zhonga et al. (2020), Passos et al. (2022), Dharma (2023), Doustdar et al. (2023), Hartati and Widianingsih (2023), Hsiao and Chuang (2023) for Mollusk; Zhang et al. (2018) and Capa et al. (2022) for Annelida (Polychaeta). Numerical abundance per m^2 was used to estimate the macrobenthic abundance.

Data analysis

Community structure

The diversity of macrobenthic species was measured using the species number (S). Then, the abundance data were used to analyze their functional characteristics using the diversity of the Shannon-Wiener Index (H') (Muzaki et al. 2019), Uniformity Index (E) (Ulfah et al. 2019), and Simpson's Dominance Index (C) (Roswell et al. 2021). The formulations are presented below:

Diversity index

$$H' = -\sum_{i=1}^{s} P_i \log_2 P_i$$ (Muzaki et al. 2019)

Where:
H' : the Shannon-Wiener Diversity Index
Pi : the Ratio ni/N; ni is the number of individuals of species i
N : the Total number of individuals

The resulted Diversity Index values were then categorized for their productivity and the condition of ecosystem based on Sumekar and Widayat (2021) i.e., low diversity and very low productivity, unstable ecosystem (H'<1.0); moderate diversity, moderate productivity, balanced ecosystem (1.0<H'<3.322), and high diversity, high productivity, and stable ecosystem (H >3.322).

Uniformity index

$$E = \frac{H'}{lnS}$$ (Ulfah et al. 2019)

Where:
E : the uniformity index
H' : the Diversity Index
S : the number of species.

The uniformity Index Value were then classified based on Sumekar and Widayat (2021) into low uniformity (E<0.4), moderate uniformity (0.4<E<0.6), and high uniformity (E>0.6).

Dominance index

$$C = \sum (\rho i)^2$$ (Roswell et al. 2021)

Note: C is the Dominance Index, and pi is the number of individuals of the i^{th} species, in which there are no dominant species if 0<C<0.5, and there is a dominant species if 0.5<C<1.

Calculation of AMBI

In the present work, the ecological status of Wulan Estuary was evaluated using the AZTI's Marine Biotic Index (AMBI) based on Borja et al. (2000; 2007). In this method, macro-benthic species were allocated into five ecological groups (EG) (Dias et al. 2018) i.e., EG-I: a species sensitive to organic enrichment and present in unpolluted conditions; EG-II: a species indifferent to enrichment and present at low densities with non-significant variations, EG-III: slightly unbalanced situations stimulate a species tolerant to excess organic enrichment, EG-IV: a second-order opportunistic species with slightly pronounced unbalanced situations, EG-V: a first-order opportunistic species pronounced in unbalanced situations.

The AMBI and biotic indices analyses were performed using the AMBI software program v.5.0 (https://ambi.azti.es) according to Borja and Muxika (2005), Borja et al. (2012b), and Yao et al. (2022), in which according to Zhou et al. (2018) prior the analyses, the non-macrofaunal invertebrate species were excluded. The ecological quality status (EQS) of Wulan Estuary was evaluated using biological indicators and divided into five levels, i.e., high, good, moderate, poor, and bad (Table 1) (Lu et al. 2021). A multivariate analysis on AMBI, species richness, diversity, dominance and density to assess eleven stations' reference conditions and benthic ecological status over two consecutive years were conducted according to Muxika et al. (2007).

Table 1. Threshold levels of three indices for benthic ecological quality status assessment (Lu et al. 2021)

Benthic ecological quality	Diversity (H')	AMBI
High (no disturbance)	>4	≤1.2
Good (slight disturbance)	3-4	1.2-3.3
Moderate (moderate disturbance)	2-3	3.3-5.0
Poor (serious disturbances)	1-2	5.0-6
Bad (extremely serious disturbance)	<1	>6

RESULTS AND DISCUSSION

Wulan River, situated in the northern Demak District (Figure 1), experiences high sedimentation caused by the erosion of old, weathered rock and soil and high agricultural use in the upstream area (Marfai et al. 2016). Because of tides and waves, materials flew down from the Wulan River are deposited in the shallow area rather than being transported far from the estuary, causing coastal progradation, and forming the Wulan Delta, which influences the change of land cover, from pond to mangrove (8.81 ha) and mangroves into ponds (29.73 ha) (Ramadhani and Susanti 2020). Wulan water has a mixed microtidal (tidal ranges not more than 2 meters) with the prevailing diurnal tide (Fadlillah et al. 2018). The morphodynamics of the Wulan Delta, tidal, current, wave, and runoff from the Wulan River may affect the organisms inhabiting the Wulan Estuary.

Species composition, abundance, and community structure

Coastal ecosystems, especially estuaries, face many disturbances that affect their ecological quality. Macrofauna has been known to be used to assess ecological environment quality status (Jayachandran et al. 2020; Han et al. 2021; Lu et al. 2021). This is due to the fact that anthropogenic disturbances and shifting environmental conditions primarily alter species composition in macrofaunal assemblages. Marine ecosystems are made up in large part of macrofauna, which is essential to their material cycle and energy flow (Hajializadeh et al. 2020). Macrofauna are a diverse range of biota that mostly reside on the surface as epifauna or in marine sediments as infauna. Examples of these organisms include polychaetes, mollusks, and crustaceans. Furthermore, macrofauna displays many feeding traits and responses to pollution and environmental disruptions (Zhang et al. 2023). As a result, they are frequently employed as ecological quality indicators in pollution assessment and marine environmental monitoring.

This present work has successfully identified 76 species of macrobenthic fauna on August 2021 and 2022. In general, both years at eleven sampling stations were dominated by macrozoobenthic mollusk (53.9%), followed by crustaceans (28.9%), echinoderms (13.2%), polychaeta (annelids) (2.6%), and fish (1.3%) (Figure 2). The richness of macrobenthic fauna species was higher in 2022 than in 2021, in which 5-30 species were found at each station in 2021 and 10-47 species in 2022 (Figure 3). The lowest and highest number of species was found at Stations 1 and 8 in 2021 and Stations 11 and 6 in 2022, respectively. Some species, such as *Acaudina* sp., *A. molpadioides*, *Paracaudina* sp., *P. australis*, and *P. chilensis* were always found in every station during both years. They were soft-bottom-associated sea cucumbers (Hartati et al. 2021). In addition to those species, *Portunus pelagicus*, *Placuna placenta*, and *Paphia undulata* were almost found in all stations in 2022 (Figure 4).

The diversity and abundance of macrobenthic (individuals.m^{-2}) in Wulan Estuary during August 2021 and 2022 were presented in Tables 2 and 3 and summarized in Figure 5. In 2021, the abundance of macrobenthic fauna was in the range of 3.7-71.1 individuals.m^{-2} in Station 11 and 6 and higher abundance (5.6 individuals.m^{-2} in Station 11 and 138.6 individuals.m^{-2} in Station 6) in 2022 (Figure 5).

Macrobenthics are very important organisms that ecologically recycle nutrients by resuspending nutrient-rich sediment into the body of water (Kim et al. 2023). As a major biological element of benthic ecosystems, they are frequently utilized to evaluate the effect of pollution on different stages of their life cycles (Hu et al. 2019). Their responses to environmental pressures can be recognized by analyzing their species' diversity and relative abundance according to their locations. The processes, like benthic primary production and nutrient cycling, can be directly impacted by physical disturbances (Barbier 2015). Hence, studying the macrobenthic community structures can give an understanding of their diversity and distribution in response to environmental changes.

The community structure of the microbenthic fauna in the Wulan Estuary varied among the stations. Diversity expresses the composition of the microbenthic fauna species in the Wulan Estuary. The stations with high diversity were only 27% in 2021 and increased to 72% in 2022; the rest have moderate diversity. This is caused by a higher number of species found in 2022 (10-48 species) than in 2021 (5-35 species), followed by a higher abundance in 2022 (5.6-138.6 individuals.m^{-2}) than in 2021 (1.08-43.3 individuals.m^{-2}).

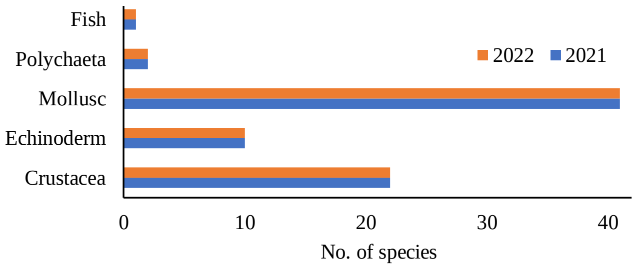

Figure 2. Macrobenthic species composition at 11 sampling Stations in Wulan Estuary, Demak District, Indonesia in 2021 and 2022

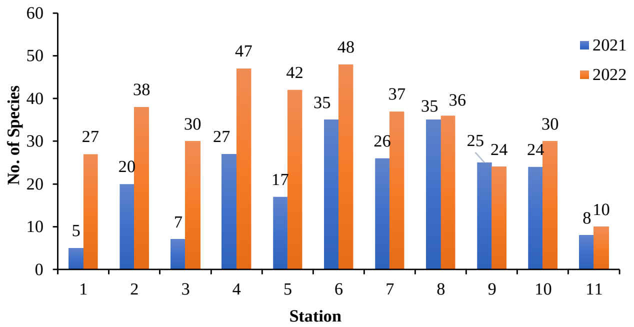

Figure 3. Species richness of macrobenthic fauna in the Wulan Estuary, Demak District, Indonesia in 2021 and 2022

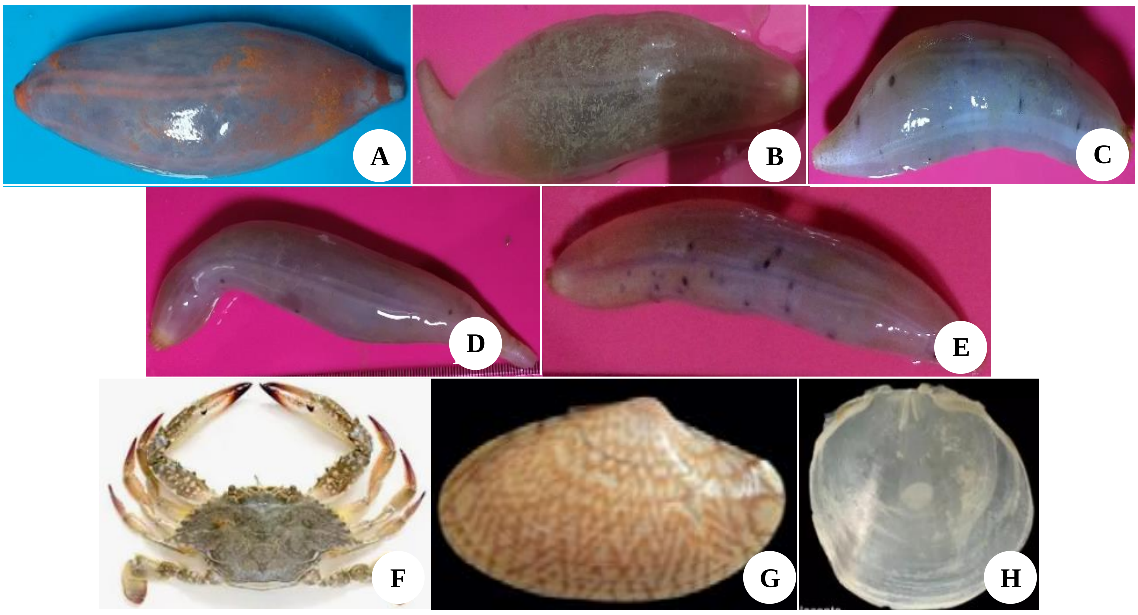

Figure 4. Some dominant species of macrobenthic fauna in Wulan Estuary, Demak District, Indonesia. A. *Acaudina* sp.; B. *Acaudina molpadioides*; C. *Paracaudina* sp.; E. *Paracaudina chilensis*; F. *Paracaudina australis*; G. *Portunus pelagicus*; G. *Phapia undulata*; H. *Placuna placenta*

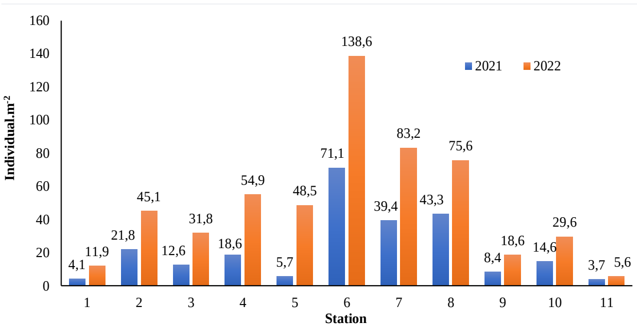

Figure 5. Macrobenthic abundance (individuals.m^{-2}) per station in the Wulan Estuary, Demak District, Indonesia in 2021 and 2022

This may be caused by the reduced anthropogenic stressors. Guerin et al. (2023) stated that anthropogenic stressors are not the only factors driving benthic fauna abundance and composition, particularly in dynamic coastal waters; other factors, such as depth, temperature, and salinity, have well-established relationships with community composition. The natural stressors, such as high sedimentation rates (Robertson et al. 2015) and variable freshwater inputs in estuarine systems (Puente et al. 2022), can also affect infaunal communities but not high hydrodynamic stress in areas with dynamic tidal regimes or intense wave climates (Foulquier et al. 2020). Such dynamic environments can appear relatively unimpacted by anthropogenic influences because contaminated water and sediments are rapidly dispersed, and benthic communities in naturally stressed environments may be more resilient (Callaway et al. 2020). Guerin et al. (2023) showed an increase in benthic species composition and abundance in the absence of acute anthropogenic stressors. In the Wulan Estuary, Dermak District, this could happen with the decrease of fishing activities using dredges and mini trawl done by small-scale fisheries carried out by fishermen around Demak District in 2020-2021 (Wiradana et al. 2021; Wicaksana 2022). This could be understood because since the Indonesian government announced its first SARS-CoV-2 infection case in 2020 (Setiawaty et al. 2020), the implementation of the Large Scale Social Restrictions and Community Activities Restrictions Enforcement from 2020 to 2021 (Andriani 2020; Saraswati et al. 2021) have been directly and indirectly gave a variety of impacts on the marine and fisheries sector in Indonesia (Ferrer et al. 2021), one of which is small-scale fisheries in Demak District. The fishers activity was highly affected by the pandemic due to the lockdown policy, mainly by the restricted fishing access, which gave important and significant setbacks to the fishing operations (Macusi et al. 2022). A study by Edward et al. (2021) showed the possibility of recovery of biodiversity and health of coastal ecosystems when anthropogenic impacts, such as during the COVID-19 lockdown, are reduced. This is very important for biodiversity conservation and sustainable utilization. The decrease of anthropogenic influences in other estuaries, such as Seto Island, Japan (Umehara et al. 2022) and the Avon-Heathcote Estuary in Christchurch, New Zealand (Zeldis et al. 2020) have been associated with improved benthic habitats and increase their diversity and abundance. Monitoring of benthic invertebrates, like done in the present study, can often detect effects resulting from changes in local or regional anthropogenic pressures (Wang et al. 2017; Umehara et al. 2022).

Table 2. Diversity and abundance of macrobenthic (individuals.m^{-2}) in Wulan Estuary, Demak District, Indonesia in August 2021

Species	**Station**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Crustacea											
Paradorippe granulata					0.1	0.3	0.2	0.3	0.2		
Portunus pelagicus		0.1		0.4		0.3	0.1	0.3		0.3	0.3
Portunus trituberculatus				0.2	0.1	0.2		0.1		0.3	
Scylla serrata											
Scylla tranquebarica					0.3			0.3			
Thalamita spinimana		0.1		0.1							
Leucosia pubescens		0.1				0.4	0.3		0.1		
Charybdis anisodon				0.3							
Charybdis annulata						0.3					
Charybdis lucifera						0.1					
Matuta victor						0.3	0.2	0.3		0.1	
Matuta planipes										0.1	
Pachygrapsus currogasus				0.1	0.1						
Parapenaeopsis cornuta		0.2	0.02			0.5	0.2	0.2	0.1		
Parapenaeopsis rectacuta				0.1							
Parapenaeopsis coromandelica		0.2		0.1				0.1			
Scylla tranquebarica								0.3			
Diogenes dorotheae										0.2	
Clibanarius longitarsus										0.3	
Balanus amphitrite						8					
Echinoderm											
Asteroidea											
Astropecten sp.								0.2	0.6	0.1	
Holoturia					0.2						
Phyrella sp.				0.6	0.6	0.4	0.1		0.5	0.3	0.2
A. molpadioides	0.6	1	0.1	0.9	0.1	0.8	0.1	0.6	0.3		0.3
Acaudina sp.	0.5	0.6	0.06	0.3	0.9		0.2	0.2	0.2	0.2	0.2
Paracaudina chilensis	0.3	0.9	0.09	1.5	0.6	0.4	0.9	0.4	0.4	0.4	0.5
Paracaudina australis	0.5	1.1	0.11	1	1.5	0.2		0.6	0.2	0.3	0.3
Paracaudina sp.	2.2	6.6	0.66	8		0.6	1.3	1.5	0.8	0.4	0.9
Leusynapta sp								0.5	0.8		
Ophiuroidea											
Amphiodia sp.								0.1			
Echinoidea											
Spatangus sp										1.1	
Mollusk											
Bivalvia					0.3						
Placuna placenta		2.9		1.5	0.1	10.2	4	22.8	1.5	6.5	
Paphia undulata		6.3		1.8		15.7	20.5	4.1	0.4		
Musculista musculista						1.1	0.1				
Anadara unequivalvis					0.1	0.4	0.2		0.1		
Anadara granosa							3.8	6.8	0.8		1
Anadara antiguata				0.1		0.2	0.5	0.1			
Laternula anatica			0	0.1		0.1	0.2	0.2	0.1		
Laternula truncata							0.1				
Tradrycadium subrugrassum		0.2				0.3	1.2	0.5	0.3		
Plagiocardium pseudolatum		0.5				1.7	1.4	0.8	0.1	0.1	
Donax cuneatus								0.1	0.1		
Vepricardium sinense								0.1			
Vepricardium asiaticum						2.1					
Vasticardium flavum						0.5					
Vepricardium subrugosum						1.9		0.1	0.1		
Tapes sulcarius				0.2		0.1		0.1	0.1		
Merica elegans				0.1							
Mipuscrebila mellosus				0.1							
Fimbria fimbriata						12	0.6				
Lopha cristagalli										0.2	
Pharella javanica										0.1	
Amusium pleuronectes										0.1	
Meretrix meretrix										0.2	
Anomia sol										0.1	
Polymesoda erosa										2.7	

Gatropoda					0.1						
Babylonia spirata		0.1		0.2	0.1	0.8		0.4	0.3		
Nassarius dorsatus				0.1	0.2	0.1		0.1			
Turricula nelliae spurius		0.3			0.3	8.3	2.1	0.3	0.1	0.3	
Corralliophila clathrata		0.1		0.2		0.5		0.3			
Murrex concinnus		0.3				1.4	0.4	0.2			
Cerithidea weyersi									0.1		
Eunaticina papilla				0.2							
Beneopsis rectacuta				0.1							
Nactica tigrina						0.5	0.5				
Nactica fasciata										0.1	
Gari truncata							0.1				
Semicassis bisulcata				0.2				0.1			
Gemmula graeffei				0.1							
Bufonaria rana						0.1					
Cephalopoda											
Octopus marginatus		0.1									
Metasepia pfefferi								0.1			
Polychaeta											
Polynoidae (*Stenelais* sp)		0.1						0.1			
Fish						0.3	0.1		0.1	0.1	
No. of Species	5	20	7	27	17	35	26	35	25	25	8
Abundance (Individual.m^{-2})	4.1	21.8	1.04	18.6	5.7	71.1	39.4	43.3	8.4	14.6	3.7

Table 3. Diversity and abundance of macrobenthic (individuals.m^{-2}) in Wulan Estuary, Demak District, Indonesia in August 2022

Species	Station										
	1	2	3	4	5	6	7	8	9	10	11
Crustacea											
Paradorippe granulata		0.3		0.4		0.9	0.4	0.6	0.6	0.2	
Portunus pelagicus	0.3	0.4		0.5	1.5	0.8	0.2	0.6	0.2	0.8	0.7
Portunus trituberculatus	0.5				0.7	0.6		0.6		0.8	
Scylla serrata	0.6	0.7	0.4	1.2	1.2	0.6			0.2		0.2
Scylla tranquebarica		0.3		1				0.9			
Thalamita spinimana	0.1		0.6		0.6		0.2				
Leucosia pubescens		0.8		2.2	1.2	2.3	0.6		0.3		
Charybdis anisodon	0.3										
Charybdis annulata		0.6	0.7	0.2	0.5	2.8	0.4	0.6			
Charybdis lucifera		0.7			0.2	1.7				0.2	0.2
Matuta victor	0.4			0.5	0.4	2.6	0.4	0.9	0.2	0.4	
Matuta planipes	0.4		0.7							0.4	0.2
Pachygrapsus currogasus		0.3			0.1	0.4	0.1				
Parapenaeopsis cornuta	0.3		0.8	0.6	1	2	0.8	0.6			
Parapenaeopsis rectacuta	0.4			0.4	1.1		0.1		0.2		
Parapenaeopsis coromandelica						0.1		0.3		0.2	
Diogenes dorotheae		0.3		0.4			0.1			0.5	
Clibanarius longitarsus		0.3	0.6		1.2	2.3	0.5	0.1		0.6	
Balanus amphitrite	0.3	7.6	3.4	6.7	5.6	12.8	0.1				
Echinoderm											
Asteroidea											
Astropecten sp.		0.6	0.5	0.2		0.3		0.6	1	0.4	
Holoturia											
Phyrella sp.		0.5	0.3	2.3	1.6	1.2	0.3	0.1	1.2	0.4	0.5
Acaudina molpadioides		2.5	0.5	2.9	2.3	2.4	0.3	1.2	0.6	0.2	0.7
Acaudina sp.		2.9	0.3	2.1	0.5	0.2	0.6	1.4	0.8	0.5	0.6
Paracaudina chilensis		2.2	4.3	2.5	1.4	1.4	2.7	1.6	0.9	0.6	0.9
Paracaudina australis		1.9	1.9	1	1.2	0.9	0.3	0.8	0.3	0.6	0.4
Paracaudina sp.		3.4	6.5	6.3	3.4	1.8	3.9	2.3	1.6	0.8	1.2
Leusynapta sp					0.2	0.2		1	1.6		
Ophiuroidea											
Amphiodia sp.		0.3	0.4	0.9	0.1	0.1		0.3			
Echinoidea											
Spatangus sp		0.3	0.4			0.1		0.3		2.2	

Mollusk											
Bivalvia											
Placuna placenta	0.1	2.1	0.3	1	2.4	23.4	12	33.2	3.4	12.2	
Paphia undulata	0.4	3.7	5.6	2.3	1.6	27.6	22.2	10.2	0.8	0.1	
Musculista musculista				2.2	1	5.6	23.1		1.2		
Anadara unequivalvis	0.4		0.2	0.1		0.9	0.2	0.3	0.3	0.1	
Anadara granosa		2.6			1.4	14.2	0.2	8.6	1.4		
Anadara antiguata			0.2	0.9			0.4	0.4			
Laternula anatica	0.3	0.2		0.5	0.5		0.1	0.4	0.5		
Laternula truncata		0.3	0.1		0.8		0.1		0.2		
Tradrycadium subrugrassum				0.3		0.4	4.5	1	0.6		
Plagiocardium pseudolatum	0.3		0.1	0.3	1.2	4.5	4.3	1.6		0.4	
Donax cuneatus					0.2	0.5		0.3	0.2		
Vepricardium sinense								0.3			
Vepricardium asiaticum					0.2	0.5		0.5			
Vasticardium flavum		0.5	0.1			0.3					
Vepricardium subrugosum		0.4		0.3	0.2	0.5					
Tapes sulcarius		0.6		0.7		0.2			0.3		
Merica elegans				0.5							
Mipuscrebila mellosus	0.2			1.1		0.2					
Fimbria fimbriata	0.3				0.8	12.4	1.3				
Lopha cristagalli		2.3		2.2						0.5	
Pharella javanica					0.9	0.1				0.4	
Amusium pleuronectes		1.2		1.2						0.4	
Meretrix meretrix	2.3			1.2		0.1		0.4		0.5	
Anomia sol		1.2			2.4					0.4	
Polymesoda erosa				0.1						3.4	
Gastropoda											
Babylonia spirata	0.3	1.2		1.4	1.8	1.8	0.2	0.7			
Nassarius dorsatus	0.3		0.5	1.2	1.8	1		0.7			
Turricula nelliae spurius		0.7	0.6		2.3	2.3	0.2	0.8		0.6	
Corralliophila clathrata	0.3			2.2	2.2	0.1		0.5			
Murrex concinnus	0.3					2.3	0.8	0.9			
Cerithidea weyersi				0.1	0.2						
Eunaticina papilla		0.2		0.1							
Beneopsis rectacuta			0.4	0.1	0.2	0.1	0.1				
Nactica tigrina	0.6	0.2					1				
Nactica fasciata	0.7			0.1						0.4	
Gari truncata			0.5		0.2		0.1				
Semicassis bisulcata		0.3		0.6		0.1					
Gemmula graeffei	0.3			0.6			0.1				
Bufonaria rana	0.3										
Cephalopoda											
Octopus marginatus	0.3		0.2		0.2						
Metasepia pfefferi	0.5			0.3			0.3				
Annelida/polychaeta											
Polynoidae (*Stenelais* sp)	0.1	0.4	0.2	0.4		0.1					
Fish		0.1	0.5	0.6		0.9				0.4	
No. of Species	27	38	30	47	42	48	37	36	24	30	10
Abundance (Individual.m^{-2})	11.9	45.1	31.8	54.9	48.5	138,6	83.2	75.6	18.6	29.6	5.6

As one of the most established techniques in benthic ecology, the Shannon-Weiner Index depends on sample size and considers equitability and species richness (Dias et al. 2018). The diversity of macrobenthic fauna in most sampling stations (72%) of 2021 belongs to the moderate category in which they are in moderate productivity and balanced ecosystem (Sumekar and Widayat 2021), and on the contrary, in 2022, in which 72% stations (Stations 1-6, 9 and 10) have high diversity and productivity (24-48 species) and have a more stable ecosystem.

The uniformity and dominance among stations were almost identical in both years, while only one station had one species of dominance in 2021. i.e., *Paracaudina* sp. The diversity, uniformity, and dominance values of macrobenthic at each station in Wulan Estuary in 2021 and 2022 are shown in Table 4. The uniformity of the eleven stations (100%) in 2022 and 81% in 2021 was high, which refers to the high similarity of abundances of each species in the sampling stations and also describes the high commonness of a species among sampling stations in Wulan Estuary (Bowman and Hacker 2020). According to Zhang (2014), the species uniformity of macrobenthic fauna was higher in a deeper site, followed by the coast and estuary. Musale et al. (2015) stated that microbenthic species distribution in the benthic ecosystem and communities is also influenced directly and indirectly by dissolved oxygen, nutrients, and chlorophyll-a.

AMBI

Monitoring biological components is imperative in coastal and transitional waters, such as in estuaries. This includes the diversity and abundance of macrobenthic fauna and the presence of disturbance-sensitive taxa. The macrobenthic fauna sampled in the Wulan Estuary were divided into five ecological groups (EG) according to their response to the stress gradient of organic enrichment (Borja et al. 2012). These groups represent the response to the environment's starting condition and the changes to mild unbalanced conditions and major unbalanced conditions (Li et al. 2022). The result of AMBI analyses (Figure 6) showed that the five EGs of macrobenthic fauna in 2021 and 2022 varied among the stations but mostly belonged to EG-I, i.e., the species that have high sensitivity to organic enhancement and live in unpolluted environments or species that are sensitive to disturbance (Zhou et al. 2018). Only at Station 1 in 2021 did all species belong to EG-II, i.e., the species that is not affected by enrichment, which was found in small populations and had negligible differences, in this case, sea cucumber species (*Acaudina* sp., *A. molpadioides*, *Paracaudina* sp., *P. chilensis*, and *P. australis*). The existence of EG-III at the sampling stations was higher in 2022 than in 2021. The species that is driven by slightly imbalanced conditions-tolerant of excessive organic enrichment (EG-III), and frequently feeds on silt at the surface of sediment (Li et al. 2022) was only found at Stations 6, 9, and 10, namely *Parapenaeopsis cornuta* (shrimp), *Plagiocardium pseudolatum* (bivalve), and *Turricula nelliae spurius* (gastropod), and found in more stations in 2022 (Stations 1-7 and 9-10). As stated by Mehvar et al. (2018), the tidal mudflats of the estuary support the shellfish fisheries, as happened in the Wulan Estuary. In both years, the lowest species diversity belonging to EG-IV was second-order opportunistic species, primarily small-sized polychaetes, that can withstand mild to severe imbalanced environments (which were not found in this research) and subsurface sediment feeders, in 2021 were found at Stations 4-8 (*P. placenta*) and at Stations 1, 3, 4, 6-10 in 2022 (*P. placenta* and *P. undulata*). There was no member of EG-V (i.e., primary opportunistic species, which is more noticeable in imbalanced environments) in both years. This group is mainly deposit feeder organisms that can tolerate the degraded sediments (Li et al. 2022).

The AMBI value 2021 was in the range of 0.231-1.523, indicating that the Wulan Estuary's ecological quality was high or undisturbed to good or slightly disturbed. In 2022, it had a value of 0.370-1.479 in the same EQS/disturbance classification (Table 5). According to AMBI results, some stations (Stations 1, 3, 10) experienced a better status and vice versa at Stations 2, 4, and 7. Lagoons and estuaries are coastal transitional ecosystems that are commonly characterized by low salinity, high saprobity, and strong composite gradients (Ghezzo et al. 2015), both AMBI and species diversity or richness contemplate the natural high pressure (Munari et al. 2022). AMBI may have difficulties showing the effect of pollution other than organic matter, such as physical pressure and high hydrodynamics sites (Sigovini et al. 2013).

Table 4. The diversity, uniformity, and dominance values of macrobenthic value at each station in Wulan Estuary, Demak District, Indonesia in 2021 and 2022

Station	Diversity				Uniformity				Dominance			
	2021	Category	2022	Category	2021	Category	2022	Category	2021	Category	2022	Category
1	1.95	Moderate	4.48	High	0.84	High	0.94	High	0.87	D	0.06	ND
2	2.91	Moderate	4.49	High	0.67	High	0.85	High	0.20	ND	0.06	ND
3	1.79	Moderate	3.82	High	0.64	High	0.78	High	0.38	ND	0.11	ND
4	3.17	Moderate	4.84	High	0.67	High	0.87	High	0.21	ND	0.05	ND
5	3.43	High	4.88	High	0.84	High	0.90	High	0.12	ND	0.04	ND
6	3.56	High	4.02	High	0.69	High	0.72	High	0.12	ND	0.10	ND
7	2.74	Moderate	3.18	Moderate	0.58	Moderate	0.61	High	0.29	ND	0.17	ND
8	2.83	Moderate	3.27	Moderate	0.55	Moderate	0.63	High	0.37	ND	0.22	ND
9	4.08	High	4.11	High	0.87	High	0.89	High	0.08	ND	0.07	ND
10	3.01	Moderate	3.53	High	0.65	High	0.71	High	0.24	ND	0.19	ND
11	2.73	Moderate	3.09	Moderate	0.91	High	0.93	High	0.17	ND	0.13	ND

Note: D: Dominant; ND: No Dominant

Table 5. The AMBI values and EQS classes at 11 stations in Wulan Estuary, Demak District, Indonesia in 2021 and 2022

Station	2021		2022	
	AMBI	Disturbance Classification	AMBI	Disturbance Classification
1	1.5	Good (Slightly disturbed)	0.618	High (Undisturbed)
2	0.285	High (Undisturbed)	1.343	Good (Slightly disturbed)
3	1.286	Good (Slightly disturbed)	1.038	High (Undisturbed)
4	0.566	High (Undisturbed)	1.479	Good (Slightly disturbed)
5	0.571	High (Undisturbed)	0.943	High (Undisturbed)
6	1.065	High (Undisturbed)	0.997	High (Undisturbed)
7	0.235	High (Undisturbed)	1.382	Good (Slightly disturbed)
8	0.231	High (Undisturbed)	0.370	High (Undisturbed)
9	0.522	High (Undisturbed)	0.857	High (Undisturbed)
10	1.523	Good (Slightly disturbed)	1.152	High (Undisturbed)
11	0.417	High (Undisturbed)	0.981	High (Undisturbed)

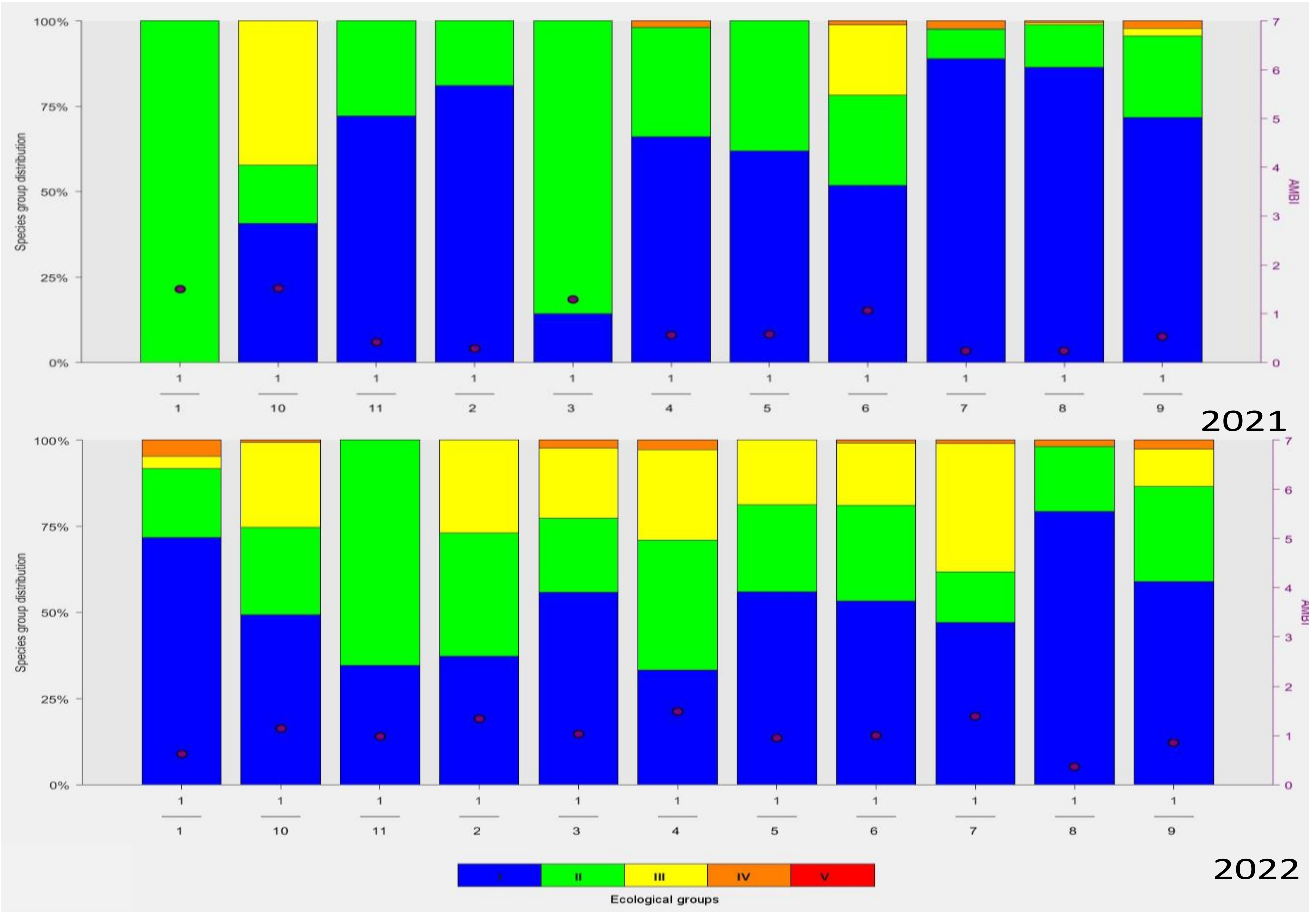

Figure 6. Ecological Group of macrobenthic fauna species based on AMBI analysis in Wulan Estuary, Demak District, Indonesia in 2021 and 2022

Comparing two ecological quality indices (H'/Diversity and AMBI) status among the eleven sampling stations in the same sampling year, as shown in the above two tables, indicated that 54.54 and 63.63% of similar results in 2021 and 2022, respectively. The number of samples may cause the differences among the results of indices. Borja et al. (2008) recommended analyzing more than 50 sampling units to minimize result instability, but it is rather hard. Previous studies showed that the ecological quality of Wulan Estuary had decreased due to very high anthropogenic activities, such as untreated sewage discharge, unenvironmental-friendly catching fish methods, and upstream hydrological changes (Fadlillah 2018, 2019; Ramadhani and Susanti 2020; Muskananfola et al. 2023). However, this study indicates higher species diversity and abundance (Figures 4 and 5); the AMBI and biological indices suggest the AMBI and biological indices, suggesting an improvement in the estuary quality in 2022 compared to the previous year. This may be affected by the COVID lockdown in 2021, which restricted fishing access and operation (Andriani 2020; Saraswati et al. 2021; Wiradana et al. 2021; Wicaksana 2022). A study by Edward et al. (2021) showed that the ecological quality improved in India due to these lockdown activities. In addition, according to Callaway et al. (2020); Foulquier et al. (2020), benthic communities in naturally 'stressed' environments are comparatively resilient.

A multivariate analysis of AMBI, species richness, diversity, dominance, and density were conducted to assess reference conditions of eleven stations and benthic ecological status over two consecutive years following Muxika et al. (2007) (Table 6). This analysis revealed that richness, diversity, and dominance have more impact on benthic ecological quality than AMBI and density (Figure 7). The use of multivariate analysis techniques such as factor analysis (FA) with principal component analysis as an extraction method is an objective tool for assessing benthic ecological quality (Wang et al. 2020; Tampo et al. 2021; Dong et al. 2021, 2023a, b; Lu et al. 2021; Liang et al. 2024). The result of multivariate analyses showed that the ecological quality was influenced mainly by diversity, richness, and density, while the second was AMBI and dominance. Benthic ecosystems have a high species diversity (90%) compared to pelagic ecosystems, and they play important roles through complex trophic relationships in the material cycle and energy flow of benthic (Lu et al. 2021). The diversity of macrobenthos assemblages is also an indispensable indicator for evaluating the benthic ecological quality status (Huang et al. 2021). In this study, the lowest and the highest value of the Shannon-Weiner diversity index was 1.79-4.08 (Stations 3 and 9) in 2021 and 3.18-4.88 (Stations 7 and 5) in 2022. All of these benthic organisms live on the bottom surface or inside of sediment and usually have weak migration capacities, and the different species of macrobenthic differ in their adaptability to environmental conditions, tolerance and sensitivity to adverse conditions such as pollution (Dong et al. 2021), and they have often been used to indicate the quality of benthic environments (Li et al. 2020). As the relationship between macrobenthos communities and the degree of disturbance has been analyzed by previous studies (Yoon et al. 2021), the result of this study is also in line with such studies. Within the same habitat type, soft-bottom, as in Wulan Estuary, benthic community diversity is directly related to the ecosystem quality (Dong et al. 2023a). While diversity is closely related to species richness, in this study, the richness is also the main factor influencing the benthic ecological status of the Wulan Estuary, followed by their density. In 2021, the density of macrobenthic biota was in the range of 3.7-71.1 individuals.m^{-2} and 5.6-138.6 individuals.m^{-2} in 2022 (Tables 2, 3, and Figure 5), with the lowest and the highest, both in the station 11 and 6, respectively.

In the present study, the multivariate analyses showed that the AMBI showed better benthic ecological quality than dominance. The value of AMBI in 2021 ranged from 0.231 (high/undisturbed) at Station 8 to 1.523 (good/slightly disturbed) at Station 10, while in 2022, 0.370 (high/undisturbed) at Station 8 to 1.479 (good/slightly disturbed) at Station 4. The dominance value of all stations in two consecutive years showed almost no dominant species, except for Station 1 in 2021, due to *Paracaudina* sp. as the highest macrobenthic abundance with a high or undisturbed environment. Although estuarine ecosystems are usually under constant threat of multifarious stressors, such as natural and anthropogenic, that lead to the degradation of biodiversity, habitat loss, and shifts within species communities (Garaffo et al. 2018) and can potentially cause these systems to lose their capacity to maintain its original structure and functions (Egres et al. 2019).

Table 6. A multivariate analysis of AMBI, species richness, diversity, dominance, and density of microbenthic fauna in Wulan Estuary, Demak District, Indonesia in 2021-2022

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.695	53.901	53.901	2.695	53.901	53.901	2.583	51.664	51.664
2	1.148	22.970	76.870	1.148	22.970	76.870	1.260	25.207	76.870
3	0.851	17.022	93.892						
4	0.238	4.759	98.652						
5	0.067	1.348	100.000						

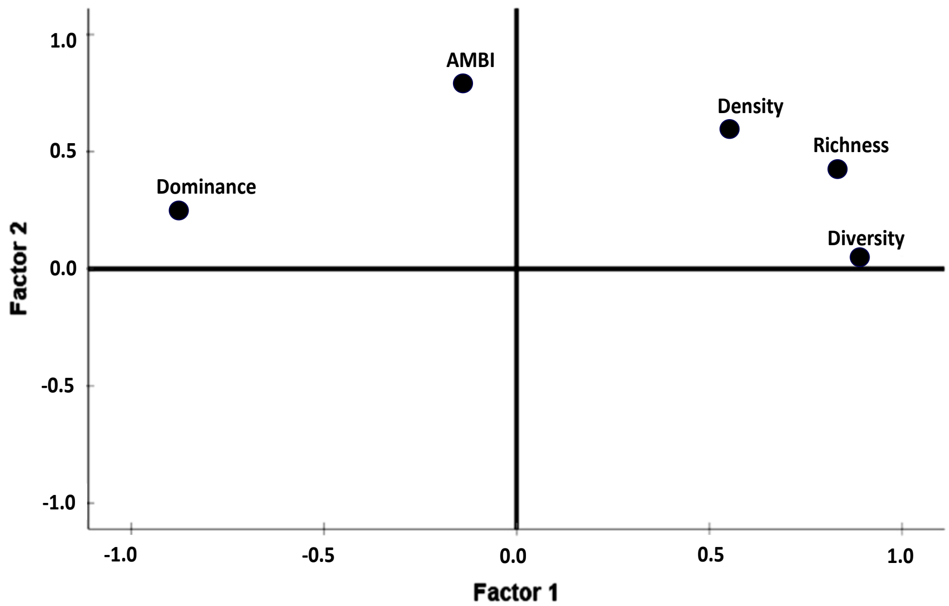

Figure 7. A multivariate analysis on AMBI and other biotic indices assessing the reference conditions and benthic ecological status in the Wulan Estuary, Demak District, Indonesia in 2021-2022

Overall, the ecological quality of Wulan Estuary is slightly disturbed and undisturbed. The higher diversity, species richness, and density of macrobenthic in the Wulan Estuary in 2022 than in 2021 showed a better habitat quality for benthic organisms. The macrobenthic community structure and biotic indices have successfully assessed the ecological status of the Wulan Estuary. It showed that most of the stations have more species richness, higher diversity, and higher density in a better habitat quality in 2022 or pasca COVID lockdown due to reducing fishing operation of small-scale fisheries operated in the Wulan Estuary in the previous year. The multivariate analysis showed that those three indices with AMBI affected the benthic ecological status in the Wulan Estuary. Monitoring of benthic invertebrates is suggested to be carried out regularly to detect the effects of local or regional anthropogenic pressures and the policy to conserve the habitat quality.

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