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UTILIZATION OF DOMINANT AND OPORTUNISTIC TAXA OF MACROBENTHIC ASSEMBLAGES INHABITING SEDIMENTS UNDER FISH FARMS FOR THE ENVIRONMENTAL STATUS ASSSESSMENT

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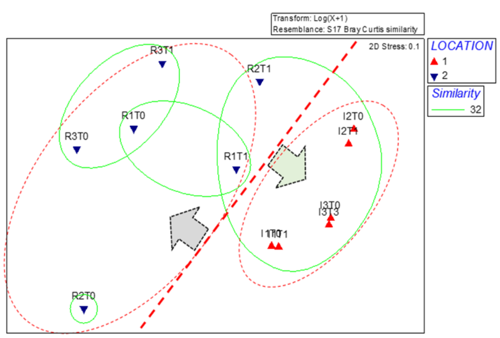
Graphical abstract



Abstract

This study aimed to analyse the environmental quality based on opportunistic taxa and dominant taxa of macrobenthic assemblages, comparing sediment under the IMTA-aquaculture and the reference site, Karimunjawa National Park. Sediments were sampled between May and October 2019, in two sampling times at the IMTA cages in which star pomfret (*Trachinotus blochii*) and tiger grouper (*Epinephelus fuscoguttatus*) were farmed with 3 stations. The reference area was located 1 km away from the fish farm zone. Transformed data of macrobenthic abundance was correlated to abiotic variables using BIO-ENV using Primer V.6.1.5 software. The dominant taxa at IMTA sites were *Diala semistriata*, *Rissoina ambigua*, *Stilifer ovoideus*, *Acteocina fusiformis, Cerithium punctatum, Allorchestes compressa* and *Capitella capitata*, whilst those at the reference sites were dominated by *Barbatia lima, Acteocina candei*, *Cerithium punctatum, Owenia fusiformis*, and *Anaspides spinulae*. Further analyses on the selected dominant and opportunistic taxa of macrobenthic assemblages between the IMTA area (M = 10.18, SE = 2.056) and the reference area (M = 3.18, SE = 0.732) still showed a significant difference ($t_{(42)}$ = 3.207, p = 0.003), indicating a consistency of the results of parametric statistical tests, both using the complete and the selected data set. The use of selected and dominant taxa is effective in determining environmental status and level of disturbance, and speed up the identification process and monitoring time.

Keywords: Macrobenthic assemblages, opportunistic taxa, dominant taxa, environmental status, and biomonitoring

1.0 INTRODUCTION

Menjangan Besar Island is one of the Karimunjawa Islands that has high potential, both in terms of the diversity of its biological resources, as well as coral reef ecosystems that support fisheries production in coastal and ocean areas or in increasing tourist visits [1]. It is suitable for fishery production from aquaculture [2]. Over the last 5 years, the aquaculture industry being developed at farm zone of Indonesia's marine ecosystem is polyculture [3], which farms more than one special in one cage, allowing farmers get more benefits by harvesting more than one farmed commodity. As other anthropogenic activities, fish farming may potentially decrease thewater quality and ecological imbalance in the longrun, one of them is macrobenthic organisms found in sedimentary habitat [4]. The polyculture system that being developed is IMTA (Integrated Multi Trophic Aquaculture) which is a fish farming practice that involves a mutualistic relationship between organisms at low trophic levels to organisms at higher trophic levels. Organisms that may be farmed are fish and prawn as carni-/omnivores, algae as primary producers/soluble organic absorbers, and sea cucumber as deposit feeders and bivalves as filter feeders [5]. One of the advantages of IMTA's practice is that nutrient waste from one species can be a source of nutritional input for other organisms [6, 7], thus it is expected to reduce the potential impact of farming practice, especially organic enrichment at the surrounding.

Macrobenthos are animals that live on the bottom of the sedimentary habitatnd have advantages as bioindicators of environmental change [4], and for sedentary groups are difficult to avoid environmental disturbances that occur around them [8]. As a part of food web, macrobenthic organisms have an important role for life of other organisms because it is the most influential part in the ecosystem of rivers, lakes, coasts, and the sea [9]. Macrobenthic community commonly be used to assess environmental disturbance, especially caused by anthropogenic activities. However, for some reasons, it takes a relatively long time in its implementation, starting from sampling, rinsing, sorting, and identifying specimens to analyzing the complete data set of the macrobenthic structure.

We propose the use of dominant taxa and opportunistic species of full macrobenthic data set in the routine biomonitoring process, as they may speed up the identification process and monitoring time but keeping the accuracy of determining environmental quality, thereby reducing costs for routine monitoring.

Changes in macrobenthic structure in dominant patterns of abundance and biomass can be used as indicators of aquatic disturbance. The degree of disturbance can be characterized by shifts in the proportions of different phyla and in the relative distribution of abundance and biomass between species with disturbance levels. For example, sediments contaminated by chemical compounds can reduce trophic complexity, where benthic assemblages are dominated by opportunistic species. Ref. [10] noted that the proportion of subsurface feed deposits increased with respect to the contamination gradient, whereas carnivores, filter feeders, and surface deposits decreased in their feed proportions. Opportunistic taxa that exploits disturbed conditions due to environmental pressures by increasing their reproduction can increases their population compared to other organisms that cannot survive [9]. The degree of disturbance can be characterized by shifts in the proportions of different taxa. One of the opportunistic taxa of the macrobenthic community is Polychaeta. The role of Polychaeta animals as the first marine invertebrate organisms to colonize polluted areas has been widely studied [4]. Dominant taxa are groups of animals that dominate in almost all sample stations. Dominant in opportunistic taxa is generally only in disturbed areas, especially by the presence of organic enrichment of the substrate [9].

2.0 METHODOLOGY

The sampling sites were located at Menjangan Besar Island, Kepulauan Karimunjawa, Central Java, Indonesia at the coordinates between 110°25'40' - 110°25'50'E and 5°53'10'-5°53'0'S. Two main sampling locations were assessed with three stations and three replicates for each location, i.e. floating net cage of IMTA and reference locations, which was 1 km away from the farm site. The sediment samples were taken from the two main locations using Eckman Grab. Each sediment sample was put into a 2L plastic jar, containing 10% of formalin and 70% ethanol solutions. The measurement of the physics - chemical water parameters was done three times for each location. The parameters measured *in situ* were pH, temperature (°C), dissolved oxygen (DO), water current, and salinity. Sediment samples were further analysed for each sampling station for sediment composition (coarse sand, fine sand, silt, and clay) and organic contents ($C_{organic}$, N_{total}, and P). Data set of biotic and abiotic parameters were presented in tables, pie diagrams, and histogram with a standard deviation. The samples were taken in two sampling times, i.e. August and November 2018. Macrozoobenthic identification was done at finest taxonomic resolution (species or genus) using identification books as references, followed by enumeration for total abundance, number of species, and tabulation. The relationship between biotic (macrobenthos) and abiotic (physical-chemical) parameters was assessed using a non-parametric multivariate procedure (BIO-ENV). The strength of correlation was expressed by the r value [11]. The diversity of the macrozoobenthic assemblages was analyzed using Shanon-Wiener diversity index (H'), Evenness index (e') to express similarity and dominance index (C) to assess level dominance by certain taxa.

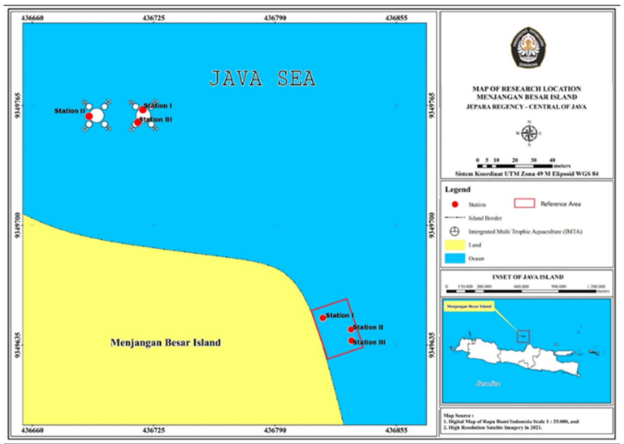


Figure 1 The map of two main sampling sites, i.e. IMTA cage and reference sites at Menjangan Besar, Karimunjawa National Park, Central Java

3.0 RESULTS AND DISCUSSION

From the results of research that has been carried out at the two study sites, 4 phyla wereidentified, i.e. Echinoderms, Mollusca, Annelida, Arthropoda, consisting of 6 classes. i.e. ophiuroids 1 fam.), bivalves (6 fam.), gastropods (30 fam.), polychaetes (8 fam.), malacostraca (5 fam.), and branchiopods (1 fam.), as shown at Table 1. The dominant taxa at IMTA sites were *Diala semistriata* (Gastropoda: Dialidae), *Rissoina ambigua (Gastropoda:Rissoinidae), Stilifer ovoideus (Gastropoda: Eulimidae), Acteocina fusiformis (Gastropoda:Tornatinidae), Cerithium punctatum (Gastropoda: Cerithiidae), Allorchestes compressa (Mallacostraca: Dogielinotidae) and Capitella capitata* (Polychaeta: Capitellidae), whilst those at the reference sites were dominated by *Barbatia lima (Bivalvia: Arcidae), Acteocina candei (Gastropoda:Tornatinidae), Cerithium punctatum (Gastropoda: Cerithiidae*), *Owenia fusiformis (Polychaeta: Oweniidae)*, and *Anaspides spinula*. All species recorded from the two study sites consisted of 1 species of ophiuroid, 15 species of bivalves, 47 species of gastropods, 12 species of polychaetes, 5 species of malacostraca, and 1 species of branchiopod. Compared to other groups, gastropod family had the highest number of species and abundance in both sampling locations.

This may be because gastropods can live in various sedimentary structures and prefer to inhabit fine sand substrates. Organic compound in the form of C-organic, total N and P exhibited varied by time and location. It has been reported that sedimentary structure influenced the decomposition of organic content (12). Furthermore, Ref. (13) reported a study on correlation between macrobenthic species and sediment grain size composition and found a strong association between the sediment granulometry and the benthic assemblage composition.

At the two study sites, members of bivalves and polychaetes showed relatively the same abundance and number of species. Although the diversity between the two main sites are considered similar; however, the number of species and abundance at the IMTA location (5168 ind./m2) was 2.8 times higher than the number at the reference location (1836 ind./m2), as shown in Table 1.

In order to compare the two complete data population of macrobenthic assemblages between IMTA site and the reference site, parametric statistic *t*-Student test was used. Before running the test, the transformed log (X+1) data were tested using Kolmogorov-Smirnov test for normal distribution of the data and Levene test for homogeneity of variance. We also run the *t*-Student test from the two set data of selected dominant and opportunistic taxa of macrobenthic assemblages between IMTA site and the reference site to assess the consistency in the result when it is compared to result from the complete data set of the fauna.

Table 1 The composition of macrobenthic structure, comparing the abundance of genus/species between IMTA and Reference sites

No	Phyla	Class	Family	Genus/ Species	Abundance (ind./m²)	
					IMTA	Ref.
1	Echinodermata	Ophiuroidea	Ophiuridae	*Ophiura ophiura*	17	0
2	Mollusca	Bivalvia	Tellinidae	*Tellina lineata*	34	0
			Veneridae	*Lioconcha lorenziana*	17	0
				Circe scripta	17	0
				Timoclea sp.	34	0
			Cardiidae	*Vasticardium orbita*	34	0
				Microfragum festivum	102	68
				Microfragum erugatum	136	85
			Lucinidae	*Anadontia sp.*	51	0
				Notomyrtea scitulum	0	17
				Divalinga quadrisulcata	102	0
				Lucinisca nasulla	34	17
				Lamellolucina	17	0

No	Phyla	Class	Family	Genus/ Species	Abundance (ind./m²)	
					IMTA	Ref.
				dentifera		
			Donacidae	*Donax cuneatus*	0	34
			Arcidae	*Barbatia lima*	34	119
				B. fusca	0	34
3		Gastropoda	Pisaniidae	*Engina armillata*	0	17
				E. fusiformis	0	17
			Fissurellidae	*Diadora sp.*	34	17
			Eulimidae	*Stilifer ovoideus*	187	17
				Vitreolina sp.	17	0
			Columbeliidae	*Costoanachis avara*	85	34
				Collumbellia aurantia	17	0
				Anachis semiplicata	17	0
			Naticidae	*Polinices mammila*	0	68
			Trocidae	*Clanculus bicarinatus*	0	17
			Haminoeidae	*Atys angustatus*	17	0
				A. macandrewi	17	0
				Aliculastrum sp.	170	0
			Skeneidae	*Munditiella ammonoceras*	51	0
			Pyramidellidae	*Turbonilla crenata*	153	0
				Herviera gliriella	51	17
			Areneidae	*Arene fricki*	34	0
			Rissoinidae	*Rissoina ambigua*	323	85
				R. crassa	34	0
			Tornatinidae	*Acteocina candei*	136	153
				A. canaliculata	289	136
				A. decorata	68	34
			Cerihipsidae	*Cerithiopsis pulvis*	51	17
			Cerithiidae	*Bittium reticulatum*	17	0
				Cerithium punctatum	323	170
				Ittibitium parcum	0	51
			Triphoridae	*Coriophora cnodax*	51	17
				Mastonia ustulata	17	0
			Dialidae	*Diala semistriata*	816	0
			Colloniidae	*Collonista picta*	17	0
				C.granulosa	51	34
			Vitrinellidae	*Circulus modesta*	136	17
			Neretidae	*Smaragdia tragena*	17	68
				S. rangiana	0	17
				Nerita sp.	0	17
			Nacellidae	*Cellana sp.*	0	17
			Costellariidae	*Vexillum amandum*	0	17
			Eratoidae	*Sulcerato sp.*	0	34
			Marginellidae	*Volvarina paumotensis*	68	0
			Rissoidae	*Alvania mamillata*	34	0
			Epitoniidae	*Epitonium sp.*	34	0
			Mathildidae	*Mathilda retusa*	17	0
4	Annelida	Polychaeta	Spionidae	*Prionospio sp.*	136	0
				Scolelepis squamata	0	17
			Syllidae	*Syllis unzima*	85	17
				S. amicarmillaris	34	17
			Nepthydae	*Nephtys sp.*	0	34
			Capitellidae	*Capitella capitata*	153	0
				Notomastus sp.	17	0
				Heteromastus sp.	51	0
			Nereididae	*Nereis sp.*	0	34
			Oweniidae	*Owenia fusiformis*	34	119
			Cirratulidae	*Aphelochaeta sp.*	51	0
			Dorvileidae	*Ophryotrocha sp.*	68	0
5	Arhtropoda	Malacostra	Leucosiidae	*Ancylodactyla*	34	0
			Dogielinotidae	*Alorchestes*	136	17
			Anaspididae	*Anaspides*	85	119
			Bodotriidae	*Iphinoe trispinosa*	17	0
			Alvatanaidae	*Cretaceous*	102	0
		Branchiopo	Daphniidae	*Daphnia sp.*	17	0
	Total Abundance (N)				5168	1834
	Diversity Index (H')				2.391	2.476
	Pilou Evenness Index (e)				0.801	0.859
	Dominance Index (*D*)				0.232	0.145

Using complete data set of macrobenthic assemblages, result from independent *t*-Student test showed a significant different ($t(160) = 3.184$, $p = 0.002$) between their abundance at IMTA cage sites ($M = 3.73$, $SE = 0.713$) compared to those at the reference sites ($M = 1.32$, $SE = 0.251$). This implies that the activities of fish farming may have influence the sediment properties, especially the composition of silt and clay in which organic materials are sedimented and the fauna rely on it. For the muddy-silty sediments, the absorption of organic matter nutrients because of their tendency to accumulate nutrients from flowing water, where the fine texture and particle size facilitate. Muddy sediments tend to have more nutrients due to fine texture and particle size making it easier for the absorption of organic matter [14]. Further analyses using t-Student test was done using selected dominant and opportunistic taxa of macrobenthic assemblages. Under this circumstance, the results of the analysis their abundance between the IMTA area ($M = 10.18$, $SE = 2.056$) and the reference area ($M = 3.18$, $SE = 0.732$) still showed a significant difference ($t(42) = 3.207$, $p = 0.003$). This indicates the consistency of the results of parametric statistical tests, both using the complete data set of the macrobenthic community and the selected data set of macrobenthic assemblages.

The Range of Water Quality Measurement

Water quality parameters were considered in the normal range during the study period The temperature ranged from 29.2 – 33 ^{0}C which indicated a normal range for tropical coastal area. Salinity ranges from 29.1-33.4 ppt, which was in the range of the standard salinity for marine biota is 33-34 ppt [15]. The salinity value is not much different from the salinity value of Indonesian waters, where the average surface of Indonesian waters ranges from 33-34 ‰ [16]. Based on seawater quality standards for marine biota, most of the salinity values at the observation station are still in accordance with the quality standards of seawater for marine biota [15]. The level of salinity of seawater at the location of the fish farm area can be influenced by the presence of deposition of organic material generated from unfed pellets and fish faeces and urine. The pH at the research site is 7.9 – 8.4 which was a normal range for marine organisms and was considered within a normal range according to the quality standard of Ref.[15]. In general, the pH of seawater is relatively more stable and is usually in the range of 7,5 and 8,4, except near the coast. Water conditions that are very basic or very acidic will influence the fitness and growth rate of the farmed fish as it will disrupt the process of metabolism and respiration [17, 15].

Water velocity may carry dissolved and suspended matter, oxygen solubility and can reduce biofouling organisms attached on the surface of the cage facilities. Slow current may reduce the exchange of water in and out of the net of the cage, affecting the availability of oxygen in the maintenance net and the ease of disease [12]. The current water of the study sites was between 3.5 cm.s-1 –10 cm.s-1. This is considered slightly below the current recommended for aquaculture for optimum growth of farmed biotas, which ranges from 5-15 cm.s-1 cm.s-1 [18]. Water currents play an important role in the circulation of water, thus the design of fish cage has to be taken into account in farming practice. Current velocity may affect the position of the net and the anchoring system, by shifting or rafting the facilities. Therefore, cage design, both construction and materials, have to be adjusted to the current speed and bottom waters conditions.

Dissolved oxygen concentrations in the waters of Menjangan Besar Island varied considerably varied, ranging between of 6.1 and 9.2 mg/l. These values are still classified as suitable for marine biota [19]. In general, DO values may indicate the quality of water . The observed values meet seawater quality standards for marine biota life with DO values > 5 mg/l [15]. According to [20], the ideal oxygen content in water is between 3-7 mg/l.

The Concentration of Total Nitrogen, Organic Carbon, and Phosphate: IMTA vs Reference Sites

Sediment composition, especially clay, silt, and fine sand is an important factor in compiling the macrobenthos community. Organic matter as the main food source for marine invertebrates also plays an important role in determining the structure of macrobenthos. Other factors, such as the chemical properties of water, abundance and microbial composition can affect its structure. These factors may vary from time to time, both in quality and quantity, depending on local hydrodynamic patterns [21]. The total nitrogen content in the first sampling was 0,65-1,04mg/l, while the second sampling was 0,107-0.113mg/l. C-organic content in the first sampling was 88,23-98,16mg/l and in the second sampling was 19,7-20,7mg/l. P content in the first sampling 22,97-45,22mg/l, the second sampling 30,63-61,7mg/l. The difference in the results of C-organic, total N and P in the first and second sampling in two locations showed different results may due to different sampling times and may be influenced by fish farming activities which led to accumulation at the sampling location. Another factor that may affects the difference is the season. The first sampling was in May which was the dry season and the second sampling was in December which had entered the rainy season. Furthermore, water velocity may affect the composition of sediment as the habitat of macrozoobenthic community [5].

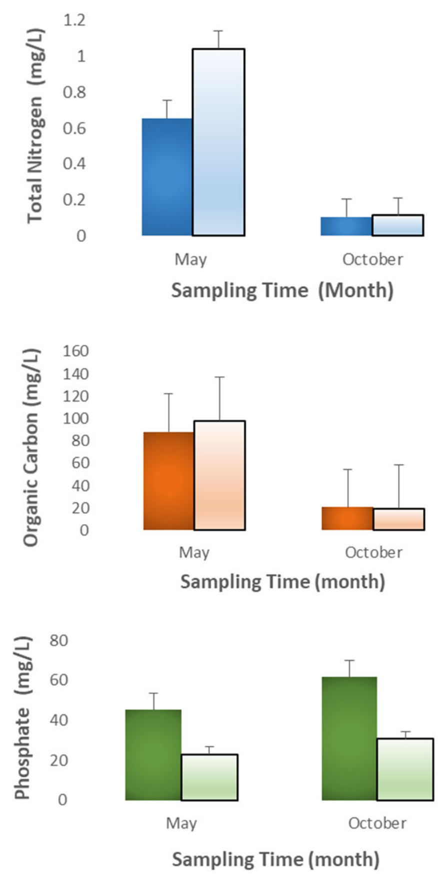


Figure 2 The concentration of total Nitrogen, organic Carbon, and Phosphate at the two main sampling location, comparing values between May and October sampling time (±SD)

C-organic content in the first sampling was 88,23-98,16mg/l and in the second sampling was 19,7-20,7mg/l. The difference value between the two sampling time may be due to difference in the season, where the second sampling was carried out in the beginning of rainy season. Ref.[22] stated that during the rainy season, a lot of organic matter content could be dissolved and carried away by the water current. The higher concentration of carbon may be caused by the accumulation of waste or residual organic matter, as well as the enrichment of nutrients in the process of decomposition of waste in the waters [23]. Organic matter on the substrate is needed as a food source for biota, which will then affect the number of biota populations. Organic matter in the form of C-organic is the main constituent of organic matter in all marine organisms and thus become energy source for all organisms. However, the excessive of this matter may lead to reduce their diversity and increase their dominance of certain taxa of macrobenthic structure [5, 12]

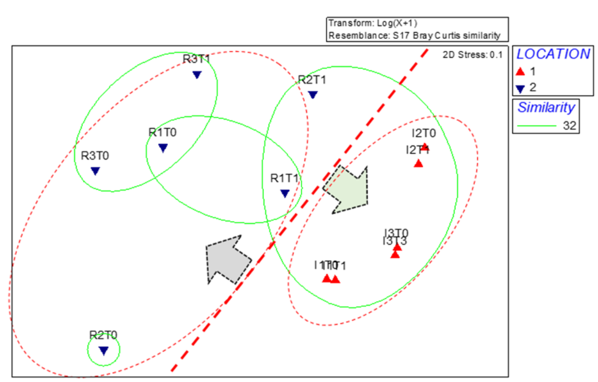
Total nitrogen content in the first sampling was 0,65-1,04mg/l, while the second sampling was 0,107-0.113mg/l. Based on Ref.[15], concerning on the quality standards, the nitrate concentration of 0.008 mg/l is suitable for marine life. This shows that the total nitrogen in the first and second sampling both exceeds the threshold. High and low nitrogen concentration is also influenced by temperature, which may affects the metabolic rate of organisms. In warm waters, it causes an increase in the decomposition of organic matter and an increase in oxygen consumption [24]. Furthermore, sediment texture also affects the concentrations of total Nitrogen, as the smaller the particle size of the sediment, the greater the organic matter content [25].

P content in the first sampling 22,97-45,22 mg/l, the second sampling 30,63-61,7mg/l. Based on Ref.[15] concerning on quality standards, the proper phosphate concentration for marine life is 0.015 mg/l. The P content in the first and second sampling was above the threshold value of P concentration for marine biota. The high value of P concentration can be vulnerable for the life of marine biota as it may cause eutrophication [12]. According to Ref.[26] that the maximum recommended phosphate level for rivers and waters that have been reported is 0,1 mg/l.

Macrobenthic Structure: Multivariate and Ordination

Based on 2D-NMDS differentiated by macrobenthic abundance of all stations at the two main sampling locations, ordination using a complete data set is able to show a tendency for clustering between main locations (Figure 3[A]). This shows that the differences in macrobenthos structure between main locations can be described clearly so that the stations representing the Reference location tend to group on the left side, while the stations representing the IMTA location tend to group to the right side of the ordination. However, this does not occur if the ordination is carried out using dominant and opportunistic taxa selected from the macrobenthic assemblage. In Figure 3[B] it can be seen that the stations representing the Reference locations and IMTA farms tend not to group together. This means that the use of the selected data has not been able to reflect the differences in macrobenthic structure between the main locations. This was possible due to the insufficient samples at the two main locations to reflect the difference on the ordination. Another factor is that the n umber of dominant and opportunistic taxa is not sufficient to describe the difference in grouping when plotted in the NMDS ordinance, although the results of the t-Student parametric test revealed a significant difference ($t(42) = 3.207$, $p = 0.003$). As has been reported by several authors [5, 27], the presence of dominant and opportunistic taxa in the macrobenthos community can describe the level of environmental disturbance, especially the presence of organic substrate enrichment.

The higher the level of disturbance, the greater the number of these taxa. This also resulted in a decrease in the diversity index and an increase in the dominance index. The higher number of taxa and abundance of opportunistic polychaetes at the IMTA cage sites compared to the reference site implies that the farmed sites might have been ecologically disturbed by farming activity. The presence of *Capitella* sp. at IMTA cage sites is related to its feeding type as a sub-surface deposit feeder. They are well known to respond to changes in sediment organic content and are therefore considered an opportunistic taxon . Opportunistic species refers to one of the life strategies of macrobenthic species (*r*-life strategy), especially polychaetes, which have the ability to adapt to a disturbed environment and are able to take advantage of situations that are unfavourable to other organisms by increasing their reproduction rate, but reduce their biomass. [5, 28].



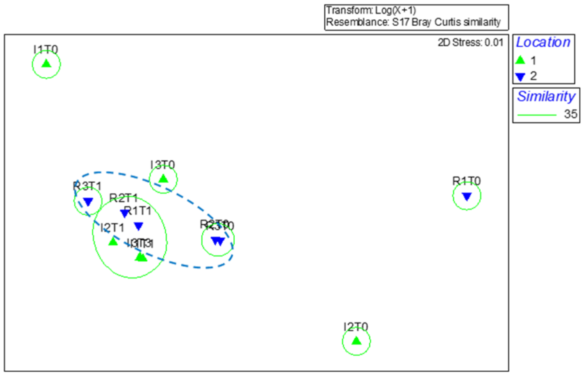


Figure 3 The comparison of ordination 2D-NMDS based on full set data of macrobenthic assemblages [A] and selected dominant and opportunistic taxa of macrobenthic assemblages [B]

Furthermore, *Nereis* sp. are omnivores and detritivores and feed on the surface of muddy and sandy sediments. *Nereis* sp. may also function as a suspension feeder [29, 30].

It has been reported that a high abundance of some opportunistic taxa and low biomass values were observed to shift from a highly polluted phase to a transitional phase at shallow, coastal soft-bottom sediment ecosystems. Furthermore, the main environmental factors affecting the distribution and structure of macrobenthic animals reported by most authors are food availability, particularly organic matter, salinity and sediment characteristics, especially mud or clay content, and hydrodynamic water ecosystem as significant factors influencing macrobenthic spatial and temporal patterns [31, 32, 33].

Degree of Environmental Disturbance: EWS-3SWJ for Environmental Status

Further analysis using the EWS-3SWJ software showed a light to moderate level of disturbance, as shown in Table 2. This software combines abiotic (water and sediment quality) and biotic data (macrobenthos structure and opportunistic taxa) to assess the level of disturbance, and it has been effectively described the level of disturbance at tropical coastal regions [34, 35, 12]. The reference locations both on the first and second sides indicate a slight level of environmental disturbance, while the IMTA farms location indicates minor disturbances on the first side, but moderate disturbances in the second sampling.

4.0 CONCLUSION

Gastropod family had the highest number of species and abundance, whilst bivalves and polychaetes showed relatively the same abundance and number of species at both sampling locations. Analyses using *t*-Student test on the selected dominant and opportunistic taxa of macrobenthic assemblages between the IMTA area and the reference area still showed a significant difference, indicating a consistency of the results of parametric statistical tests, both using the complete and the selected data set. However, the graph form 2D-NMDS carried out using selected dominant and opportunistic taxa did not group the IMTA and reference sites. Further analysis using software EWS-3SWJ categorised all sampling sites as slightly disturbed area, except only for IMTA sites at second sampling time as moderately disturbed area. The use of selected and dominant taxa is effective in determining environmental status and level of disturbance, and speed up the identification process and monitoring time, thereby reducing costs for routine biomonitoring. Yet, further assessment may need to be considered in a larger spatial and temporal scale.

Table 2 The environmental status of IMTA cage and Reference sites based on EWS-3SWJ software

No	Location & Sampling Time	Environmental Status
1	IMTA cage sites, First Sampling	Lightly disturbed area
2	Reference sites, First Sampling	Lightly disturbed area
3	IMTA cage sites, Second Sampling	Moderately disturbed area
4	Reference sites, Second Sampling	Lightly disturbed area

*Notes: the data of each location and sampling time was taken from 3 stations and 3 replicates.

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writing of the manuscript, or in the decision to publish the results.

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