

Ecological status of subtidal zone soft substrate macrozoobenthic community in Amurang Bay, North Sulawesi

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Abstract. This study aims to analyze the ecological quality of the shallow marine environment of Amurang Bay based on bio-indicators of the subtidal zone soft sediment macrozoobenthos community. Sampling used a La Motte grab (modified) (with a cross section of 1110 cm²) at 8 sampling stations. The average abundance of each taxon, Shannon-Wiener diversity index (H'), Pielou evenness index (J'), Margalef richness index (RS), and Berger-Parker dominance index (d) were calculated. The abundance data were analyzed using the multivariate data analysis method with the 'Cluster' Analysis menu options and Correspondence Analysis to assess the ecological condition. The ecological status of macrozoobenthic communities was assessed by AZTI's Marine Biotic Index (AMBI). This study found a total of 685 macrozoobenthos individuals which were sorted and identified from 8 sampling stations, consisting of 101 species (taxonomic groups) and belonging to 15 classes (taxonomic groups) and 7 phylla, namely Annelida, Arthropoda, Mollusca, Echinodermata, Nemertea, Nematoda, and Chordata. The most dominant phyllum was Annelida, especially the Polychaeta class. The lowest individual density was at two stations near the relatively large rivermouth, AB2, with a density of 324 ind m⁻², in front of Nimanga Rivermouth, and AB5 with a density of 342 ind m⁻², located in front of the Ranoyapo River Mouth. The highest density was found at two former land stations, namely AB7 with a density of 1,559 ind m⁻² and AB8 with a density of 1,243 ind m⁻². The lowest number of species, only 9 taxa, occurred at station AB5, and the highest with 34 taxa at station AB1. According to the AMBI criteria, only station AB1 was categorized as 'unpolluted' (BC = 0.810) with a predominance of sensitive species (equilibrium) and few opportunistic species; other sampling stations were categorized as 'lightly polluted'. But when using H', not only station AB1 was categorized as 'high' but also AB3, AB4, AB7 and AB8; stations AB2 and AB6 were categorized as 'good', and station AB5 was categorized as 'moderate'. This study concluded that station AB1 had the best ecological health status, while the worst was station AB5. The stations that have similar characteristics to station AB1, unpolluted, were AB4, AB6, AB2 and AB3, while those that had similar characteristics to station AB5, moderately polluted, were AB7 and AB8. Key Words: AMBI, Amurang Bay, ecological status, macrozoobenthos, multivariate.

Introduction. The ecological footprint shows that human has used natural resources equivalent to two planets due to human dominance over the biosphere known as a new geological era in Earth's history called the Anthropocene (Thrush et al 2021). Increasingly degraded ecosystems, an intensifying climate crisis, and increasing biodiversity loss will threaten the availability of jobs, the economy, the environment, and food security around the world (FAO 2022). In addition to playing an important role in regulating the global climate as the main sink of greenhouse gases, oceans are very important for global food security and stores a huge amount of biodiversity. The loss of marine ecosystem services can have detrimental consequences for human welfare (FAO 2022).

Amurang Bay, North Sulawesi (Indonesia), is increasingly experiencing ecological pressures due to anthropogenic impacts as a consequence of the increasing population and development activities such as agriculture, livestock, fisheries, industry and even

mining in South Minahasa Regency, especially Amurang City and its surroundings. The coastal landslide that occurred in Ranowangko Rivermouth, Amurang Bay, on Wednesday, June 15, 2022, which submerged as many as 33 houses, one bridge, 400 m of roads and their embankments with a total land area of about 2.9 ha, has also caused a significant ecological stress.

In the context of effective marine environmental management, information on biological indicators is needed to estimate the status of the marine environment. Macrozoobenthos are more abundant and more commonly used to monitor and as an indicator of pollution (Gray et al 1988). Macrofauna components in benthic communities are often quantified to determine environmental health because these organisms, despite being relatively sedentary, have a long lifespan and different tolerance to the stress (Dauer 1984). Limitations in movement to avoid unfavorable conditions result in macrozoobenthos often being exposed to contaminants accumulated in sediments and low oxygen solubility in benthic waters so that benthic communities can describe local environmental conditions (Smith et al 2001). Changes in the structure of benthic communities often reflect variations in the physico-chemical factors of the aquatic environment because benthos fauna has properties that are integrated with the surrounding environmental conditions (Le Guellec 1990). Benthic organisms have a wide physiological tolerance, different types of eating patterns and extensive trophic interactions make them sensitive to various environmental stresses (Pearson & Rosenberg 1978; Rhoads et al 1978; Dauer 1984). This study aims to examine the status of benthic ecological quality of Amurang Bay and provide a reference for the management and conservation in the area.

Material and Method. This study was conducted a year after the coastal land slide in Amurang Bay on June 15th, 2022. Sampling started in June 2023 using a modified La Motte grab with a cross section of 1,110 cm² at 8 sampling stations (3 stations in the landslide zone, 5 stations outside the landslide zone in Amurang Bay (Figure 1).

Each station was sampled once. The sediment was directly put into a plastic bag, preserved with 10% formalin and labeled. In the laboratory, the samples were washed with fresh water to remove formalin and then filtered with a mesh sieve size 1 mm to remove the sludge. The filtered sediment was soaked again with Rose Bengal liquid to make the sorting easier. Sorting was carried out under a dissecting microscope. The sorting results were plade into small bottles containing 70% alcohol. Identification was carried out using a binocular microscope $(4-40\times)$ based on available instructions (Day 1967; Abbott 1977; Campbell & Nicholls 1979; Guille et al 1986; Abbott & Dance 1990; Dance 1993) and internet facilities and scientific name adjustments based on WoRMS (2024) instructions.

Parameters measured were Shannon-Wiener diversity index (H'), Pielou's evenness index, Margalef's richness index (RS), Berger-Parker's dominance index (d) as follows:

 $H' = -\Sigma((n_i/n)*\log_2(n_i/n))$, where n_i is number of individuals *i* and n is the total number of individuals;

 $J' = H'/log_2S$, where S is the total number of species;

 $RS = (S-1)/log_2n);$

 $d = n_{max}/n$, where n_{max} is the number of individuals of the most abundant species.

The abundance data of ecological groups were entered into a cross table to be then analyzed with the STATGRAPHICS[®] Centurion XIX Program (2023) using the multivariate data analysis method, with the 'Cluster' Analysis menu options (using Ward's Method, City Block distance) and Correspondence Analysis (CA).



Figure 1. Map of sampling stations in Amurang Bay (AB) (top), North Sulawesi (inset); mouth of the Ranowangko River, Amurang Bay (right), condition before the landslide June 15, 2022 (left) (Source: Google Earth).

The ecological status of macrozoobenthic communities was using AZTI's Marine Biotic Index (AMBI) (Borja et al 2000). The use of the AMBI index along with various multivariate statistical tools was essential to assess the health of ecosystems holistically (Sigamani et al 2015). AMBI or biotic coefficient (BC) is a biotic index developed at the Department of Oceanography and Marine Environment, Technological Institute for Fisheries and Food (AZTI), Spain by Borja et al (2000) to determine the quality of marine macrozoobenthos. AMBI is a modification or improvement of the discrete model of Biotic Index based on Ecological Groups developed at the Laboratory of Biological Oceanography, Faculty of Science, University of Bretagne Occidentale, France (Glémarec & Hily 1981; Hily & Glémarec 1990; Grall & Glémarec 1997). Based on the percentage of individual abundance of each ecological group (EG) in each sample, a continuous index or Biotic Coefficient (BC) was obtained = $\{(0\% \text{ EG I})+(1.5\% \text{ EG II})+(3\% \text{ EG III})+(4.5\% \text{ EG})\}$ IV)+(6% EG V)}/100. Borja et al (2000) divided 5 pollution classes, namely unpolluted $(0.0 < BC \le 1.2)$, lightly polluted $(1.2 < BC \le 3.3)$, moderately polluted $(3.3 < BC \le 1.2)$ 5.0), heavily polluted (5.0 < BC \leq 6), very heavily polluted (Azoic). These five AMBI classes are also criteria for estimating the quality of benthic ecology according to Lu et al (2021) when equipped with H' (using log base 2) to become 'High (no disturbance)' (H' \geq 4), 'Good (slight disturbance)'($3 \le H' < 4$), 'Moderate (moderate disturbance)' ($2 \le H' < 4$) 3), 'Poor (serious disturbance)' $(1 \le H' < 2)$, and 'Bad (extremely serious disturbance)' (H' < 1).

Ecological groups are defined based on their sensitivity or tolerance to excess organic matter (Hily & Glémarec 1990; Borja et al 2000). Ecological Group I (EG I) is sensitive taxa that is very dominant under normal conditions, which disappears first and reappears later; this group includes the specialist carnivores, the small Crustacea Peracarida, and some deposit-feeding Polychaeta. EG II, tolerant taxa that are not affected by excess organic matter, is always in small quantities and does not fluctuate markedly in time; this group includes suspension feeders, less selective carnivores such as Glycerides, and "scavengers". EG III, taxa tolerant to excess organic matter, has a wider ecological distribution when there is a disturbance compared to that under normal conditions; for example, substrate surface deposit-feeders such as Spionida "tubicolous" and Bivalvia "ubiquitous". EG IV, second-order opportunistic taxa that reproduces in anoxic conditions, especially subsurface deposit-feeders such as the small Polychaeta Cirratulidae. EG V, the first-order opportunistic taxa, is very abundant in reduced sediments; especially deposit-feeders such as Capitellidae and Oligochaeta.

For granulometry, a sediment sample of 200 g was taken and then dried at 80°C for 24 hours, weighed and washed with fresh water on a 63 µm mesh. The dry residue was sieved on a 2 mm sieve. The percentages of gravel, sand and mud were calculated as fractions of > 2 mm, 63 µm to 2 mm and < 63 µm, respectively, then classified according to the Folk terminology (Thrush et al 2021) based on the comparison of the three sediment fractions. The total organic matter content (% loss on ignition) was obtained by the dry ashing method. The sediment samples were dried at 105°C for 24 hours, then burned at 520°C for 6 hours. The criteria for the percent sedimentary organic matter content were categorized following Reynold (1971): very low (< 3.5%), low (3.5-7%), medium (7-17%), high (17-35%), and very high (> 35%).

Results and Discussion

Location characteristics. The characteristics of the sampling stations are shown in Table 1. The station of Teluk Amurang 1 (AB1) is located about 500 m from Bajo village, near a fish pond (about 10 ha), at a depth of 19 m, and has a muddy sand substrate with a high organic matter content. The station of AB2 is located near the mouth of the Nimanga River, at a depth of 34 m, and has a gravel sand substrate with a high organic matter content. The Nimanga River watershed covers parts of Tomohon City, Minahasa and South Minahasa Regencies, and carries domestic, agricultural and livestock waste. The station of AB3 is located near the Pentu Rivermouth (Lopana Village), at a depth of 15 m, with a sandy gravel substrate and moderate organic matter content. The station AB4 is located near the Port of PT. Cargill Indonesia, at a depth of 30 m, has a gravelly sand substrate with high organic matter content. The station AB5 is located at the Ranoyapo River mouth, at a depth of 36 m, and has a sandy mud substrate with a very high organic matter content. Ranoyapo River is the longest river (54 km) in the Minahasa Peninsula, with a watershed area of about 770.89 km². In addition to transporting domestic waste from settlements in South Minahasa Regency, this river also carries agricultural, livestock and mining wastes. Gold mining carried out both by the community and by PT Sumber Energy Jaya in East Motoling District is suspected to pollute the Ranoyapo River. The stations of AB6, AB7, and AB8 are located at a depth of 26 m, 15 m and 14 m, respectively, near the Ranowangko River mouth with gravelly and muddy sand substrates and the organic matter content is medium, high and very high respectively (Table 1). Before the coastal landslide on June 15, 2022, the stations AB7 and AB8 were a main road, while AB6 was an estuary. The Ranowangko River and the Pentu River which originate from Mount Soputan carry a small amount of domestic and agricultural waste.

The organic matter in sediments is an important food source for benthic invertebrates (Gray 1974; Pearson & Rosenberg 1978). But if the content of organic matter in the sediment is excessive, it can cause a decrease in oxygen solubility at the bottom of the water due to the decomposition of such an excessive organic matter and can result in the accumulation of toxic byproducts, such as ammonia and sulfides, which can ultimately decrease the species richness, abundance and biomass (Pearson & Rosenberg 1978; Diaz & Rosenberg 1995; Gray et al 2002; Hyland et al 2005; Li et al 2021). Benthic communities are highly sensitive to oxygen solubility, and low oxygen solubility can lead to changes in macrozoobenthic communities from mortality, sub-lethal stress, reduced growth rates and limited habitat availability (Dauer et al 1992; Caswell et al 2019; Li et al 2021).

Table 1

| | | | | Granulometry | , | | Total | Poynold |
|------|-----------------------|--------------|------------------|--------------------|-------------------|-------------------------------|-----------------------|---------------------|
| Site | Geographical position | Depth (m) | Gravel > 2 mm | Sand 0.063-2 mm | Mud < 0.063 mm | Folk (1954) classification | organic matter (%) | (1971) criterion |
| AB1 | 1º16'16.56" N & | 19 | 0.88 | 64.22 | 34.90 | Ms | 30.943 | Н |
| | 124º33'21.34" E | | | | | | | |
| AB2 | 1º15′45.14″ N & | 34 | 14.01 | 79.79 | 6.20 | Gs | 26.866 | Н |
| | 124º35′22.16″ E | | | | | | | |
| AB3 | 1º13′42.01″ N & | 15 | 39.89 | 58.78 | 1.33 | Sg | 14.068 | М |
| | 124º36'17.19″ E | | | | | | | |
| AB4 | 1º11′41.17″ N & | 30 | 26.76 | 69.81 | 3.43 | Gs | 31.857 | Н |
| | 124º33′17.48″ E | | | | | | | |
| AB5 | 1º11′10.39″ N & | 36 | 0.00 | 49.09 | 50.91 | Sm | 58.308 | VH |
| | 124º33'50.86" E | | | | | | | |
| AB6 | 1º11′15.12″ N & | 26 | 24.10 | 65.66 | 10.25 | Mgs | 14.146 | М |
| | 124º34'26.42" E | | | | | | | |
| AB7 | 1º11′13.22″ N & | 15 | 9.85 | 60.45 | 29.70 | Mgs | 28.643 | Н |
| | 124º34'24.14" E | | | | | | | |
| AB8 | 1º11′16.42″ N & | 14 | 7.12 | 54.35 | 38.53 | Mgs | 41.793 | VH |
| | 124º34'30.44" E | | | | | | | |

Geographical position, depth, granulometry, and total organic matter (% LOI) at each sampling station in Amurang Bay (AB)

Ms = muddy sand; Gs = gravel sand; Sg = sandy gravel; Sm = sandy mud; Mgs = muddy gravel sand; M = medium; H = high; VH = very high.

Community structure. A total of 685 macrozoobenthos individuals were sorted and identified from sediment samples in 8 sampling stations in Amurang Bay. They comprised 101 species (taxa) of 15 classes (taxonomic groups) and 7 phyla, namely Annelida, Arthropoda, Mollusca, Echinodermta, Nemertea, Nematoda, and Chordata (Figure 2; Table 2).



Figure 2. Relative abundance of macrozoobenthic phyla.

Annelids are the most abundant phylum in both number of species (species richness) and number of individuals (density) (Grassle & Maciolek 1992; Lumingas et al 2011; Moningkey et al 2017; Lumingas et al 2019). Both in temperate and tropical areas, macrozoobenthic communities are dominated by the phylum Annelida. In Concarneau Bay (France), 88 species (taxa) of macrofauna were found at three sampling stations consisting of Annelid (42 species), Mollusc (24 species), Echinoderms (9 species), Arthropods (7 species), and the rests were Sipuncula, Cnidaria, and Nemertea (Lumingas

1990). In ten sampling stations in the Minahasa Peninsula (Likupang Strait, Totok Bay and Buyat Bay) 114 species consisting of Annelid (52 species), Arthropod (28 species), Mollusc (22 species), and several other phyla were found (Lumingas et al 2011). Also, Moningkey et al (2017) found 78 species consisting of Annelid (44 species), Arthropod (20 species), and Mollusc (6 species), and several other phyla in five sampling stations around lembeh Isand. In Manado Bay, in 6 sampling stations, 102 species were found consisting of Annelid (49 species), Mollusc (24 species), Arthropod (20 species), and several other phyla (Lumingas et al 2019).

Table 2

| Na | Tavar | 50 | 70 | AB | AB | AB | AB | AB | AB | AB | AB |
|-----|-----------------------|----------|--------------|----------|----|----|----------|-----|----------|----|----------|
| 10. | iaxon | EG | /G | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | Exotica triradiata | I | Bivalvia | | 10 | | | 2 | | | |
| 2 | Laevicardium crassum | <u> </u> | Bivalvia | 1 | | | | | | | |
| 3 | Latona cuneata | I | Bivalvia | | 1 | | | | | | |
| 4 | Nuculana sp. | I | Bivalvia | 5 | | | | | | | |
| 5 | Echinoidea (juvenile) | I | Echinoidea | 1 | | | | | | | |
| 6 | Echiura | 1 | Echiura | 1 | | | | | 1 | | |
| / | Cerithium columna | 1 | Gastropoda | 1 | | | | | | | |
| 8 | Nassarius haldemani | | Gastropoda | | 1 | | | | | | |
| 9 | Oliva carneola | 1 | Gastropoda | 3 | | | _ | | | | |
| 10 | Asymmetron sp. | I | Leptocardii | 8 | | | 2 | | | | |
| 11 | Branchiostoma sp. | 1 | Leptocardii | | - | 9 | | | | | |
| | Alpneus sp. | NA | Malacostraca | | 1 | | | | | | |
| 13 | Amphipoda | I | Malacostraca | 1 | | 5 | 4 | | | 4 | 1 |
| | Arcania septemspinosa | I | Malacostraca | | | | 1 | | | 2 | |
| 15 | Atnanas sp. | 1 | Malacostraca | - | | | | | | 2 | |
| 16 | Callianassa sp. | <u> </u> | Malacostraca | 1 | | - | | | | 1 | 1 |
| 1/ | Cerapus sp. | <u> </u> | Malacostraca | | | 3 | | - 1 | | | |
| 18 | Hexapus sexpes | | Malacostraca | - 1 | | 1 | | 1 | | | 1 |
| | | | Malacostraca | 2 | | 1 | - 1 | | | | |
| 20 | Processa sp. | I | Malacostraca | 2 | | 1 | 1 | | | | |
| 21 | Ianais sp. | 1 | Malacostraca | 2 | | | 2 | | | | |
| 22 | Nematoa | 111 | Nematoda | | 1 | 2 | 2 | | 2 | 4 | |
| 23 | Olizaabaata | | Olizashasha | 5 | L | 3 | Z | | Z | 4 | 4 |
| 24 | | <u>v</u> | Oligochaeta | | | | | | | 10 | / |
| 25 | Ampinphons squamata | 1 | Ophiuroidea | 5 | | 1 | | | | 1 | 1 |
| 20 | Myodocopido | II T | Optiluioidea | | | 4 | | | | 1 | |
| 27 | Myouocopida | I | Ostracoda | <u> </u> | | | | | | | |
| 20 | Adaphamus dibranchis | I | Dolychooto | 7 | 1 | 2 | | 10 | | 20 | 17 |
| 29 | | I | Polychaeta | / | 4 | Z | | 12 | | 30 | 1 |
| 21 | Ampharata sp. | I | Polychaeta | 1 | | 1 | 2 | | 22 | | 2 |
| 32 | Amplicteis gupperi | | Polychaeta | | | 4 | <u> </u> | | 22 | | <u> </u> |
| 33 | Amphiclena sp | III I | Polychaeta | | | | 1 | | 3 | | 10 |
| 34 | Amphinome rostrata | I | Polychaeta | | | | | | 1 | | 10 |
| 25 | | I | Polychaeta | | | | | | <u> </u> | 2 | 3 |
| 36 | Ancistrosynis sp. | | Polychaeta | | | | 3 | | | 2 | |
| 37 | Aricidea sn | T | Polychaeta | 1 | | | J | | | 2 | |
| 38 | Armandia sp. | T | Polychaeta | 1 | | 1 | | | | | |
| 30 | Chone sp | TT | Polychaeta | | | 2 | | | | | |
| 40 | Cirratulus sn | IV | Polychaeta | | | 2 | | 2 | | 5 | |
| 41 | Cirriformia sp | IV | Polychaeta | | 1 | | | 4 | | 5 | |
| 42 | Cossura coasta | ΝΔ | Polychaeta | | 1 | | 5 | З | | 1 | |
| 43 | Dionatra sp | I I | Polychaeta | | 1 | | 5 | 5 | | 1 | |
| 44 | Dinlocirrus sn | T | Polychaeta | | - | 1 | | | | | |
| 45 | Ditruna arietina | NA | Polychaeta | 4 | | - | | | | | |
| 46 | Dorvillea sp | II | Polychaeta | 7 | | | | | | | |
| 47 | Drilonereis sn | II | Polychaeta | 4 | | | 2 | | | | |
| 48 | Enidionatra sp | T | Polychaeta | | | | 1 | | | | |
| 49 | Fuchone sp | II | Polychaeta | | | | - | | 1 | | |
| 50 | Funice sp | II | Polychaeta | | | 5 | | | - | | |
| 51 | Eurythoe sp. | NA | Polychaeta | | 1 | 5 | | | 1 | | |
| 52 | Glycera longininnis | II | Polychaeta | | - | | | | - | 5 | |

Abundance of macrozoobenthos

| 53 | <i>Glycera</i> sp. | II | Polychaeta | 6 | 1 | 3 | 6 | | 1 | | 1 |
|-----|-------------------------------|--------|------------|----------|---|----|----------|---|----|-----|----------|
| 54 | <i>Gyptis</i> sp. | II | Polychaeta | | 4 | | | | | | |
| 55 | Hyalinoecia tubicola | NA | Polychaeta | 1 | | | | | | | |
| 56 | Kuwaita heteropoda | II | Polychaeta | | | 7 | | | | | |
| 57 | Laonice cirrata | NA | Polychaeta | | | | 1 | 2 | 1 | | |
| 58 | Lumbrinerides aberrans | II | Polychaeta | 1 | | | | | | | |
| 59 | <i>Lumbrineris</i> sp. | II | Polychaeta | | | | | | | 4 | 2 |
| 60 | <i>Magelona</i> sp. | Ι | Polychaeta | | | | | | | 2 | |
| 61 | <i>Maldanella</i> sp. | Ι | Polychaeta | | | | | | | 3 | 2 |
| 62 | Nereis sp. | III | Polychaeta | 3 | | 1 | | | | | |
| 63 | Nothria conchylega | II | Polychaeta | | | | | | | 1 | |
| 64 | Notomastus latericeus | III | Polychaeta | | | | | 6 | | 38 | |
| 65 | Notomastus sp. | III | Polychaeta | | 7 | | 2 | | 14 | | 9 |
| 66 | Onuphis geophiliformis | II | Polychaeta | | | 12 | | | | | 6 |
| 67 | Onuphis sp. | II | Polychaeta | | | | 1 | | | | |
| 68 | Ophelina longicaudata | Ι | Polychaeta | 6 | | | | | | | |
| 69 | Oriopsis sp. | NA | Polychaeta | | | | | | | 1 | |
| 70 | Oxydromus sp. | NA | Polychaeta | | | | | | | 2 | |
| 71 | Paralacydonia paradoxa | NA | Polychaeta | 1 | | | | | | | |
| 72 | Paramphinome sp. | NA | Polychaeta | 2 | | | | | | | |
| 73 | Paraprionospio pinnata | IV | Polychaeta | | | | | | | 6 | |
| 74 | Pectinaria sp. | I | Polychaeta | | | | | | | | 1 |
| 75 | Phyllochaeptopterus sp. | I | Polychaeta | | | | 1 | | | | |
| 76 | Phyllodoce malmareni | II I | Polychaeta | | | | - | | 1 | | 8 |
| 77 | Phyllodoce sp | II | Polychaeta | | | | 1 | | - | | |
| 78 | Phylo sp | NA | Polychaeta | | | | - | | | 1 | |
| 79 | Pista sp | I | Polychaeta | | | | | | | | 1 |
| 80 | Poecilochaetus serpens | Ī | Polychaeta | 4 | | 1 | 1 | | | | |
| 81 | Polycirrus sp | IV | Polychaeta | <u> </u> | | 1 | 1 | | | | 1 |
| 82 | Prionosnio ehlersi | IV | Polychaeta | 4 | 1 | 4 | 2 | 9 | 5 | 26 | |
| 83 | Prionospio sexoculata | IV | Polychaeta | | - | | 4 | | | _20 | |
| 84 | Prionospio sp | IV | Polychaeta | | | | <u> </u> | | | - | 47 |
| 85 | Protocirriperis chrysoderma | IV | Polychaeta | | | 1 | | | | | - 77 |
| 86 | Scolelenis sn | | Polychaeta | | | - | 2 | | | 1 | |
| 87 | Scolonlos sp | T | Polychaeta | 5 | | | 2 | | | | |
| 88 | Sigambra robusta | TT | Polychaeta | 5 | | | | | 2 | | |
| 80 | Siganibia Tobusta | | Polychaeta | 1 | | | | | 2 | | |
| 90 | Spinerosynis sp. | | Polychaeta | | | 1 | | | | | 1 |
| 01 | Spiophanes sp | 111 | Polychaeta | | | | 1 | | | | <u>-</u> |
| 91 | Sternasnis scutata | | Polychaeta | | | | 1 | | | 1 | |
| 92 | Terebella sp | T | Polychaeta | | | 1 | | | | | |
| 93 | Taraballidas straamii | I | Polychaeta | | | 1 | r | | | | |
| 05 | | | Polychaeta | | | | 2 | | | 2 | |
| 95 | Gadila watsoni | T | Scaphopoda | 3 | | | | | | | |
| 07 | Striocadulus sp | T | Scaphopoda | 1 | | | | | | | |
| 97 | Anioncoma (A.) misakianum | T | Sinuncula | T | | r | 17 | | | 12 | 1 |
| 90 | | T | Sipuncula | | 1 | 2 | 2 | | r | 12 | |
| 100 | Sipunculus sp | T | Sipuncula | | Т | 1 | 3 | | 2 | | |
| 100 | Daratrynauchan microcanhalus | | Teleostoi | | | 1 | | 1 | | | |
| TOT | i aradiypadenen mierocepnalus | IN/A | reieustei | | | Τ. | | Ŧ | | | |

EG = ecological group; TG = taxonomic group; NA = not assigned.

Individual density, number of taxa and macrozoobenthos ecological indices can be seen in Table 3. The lowest individual density was at two stations near the mouth of the relatively large river, namely AB2 with a density of 324 ind m⁻², located in front of the Nimanga Rivermouth, and AB5 with a density of 342 ind m⁻², located in front of the Ranoyapo River mouth. The highest density was found at two former land stations, namely AB7 with a density of 1,559 ind m⁻² and AB8 with a density of 1,243 ind m⁻². The lowest number of species (taxa) was only 9 taxa at station AB5, and the highest with 34 taxa was at station AB1. The low individual density and macrozoobenthos species richness at stations AB5 and AB2 is thought to be due to the influence of freshwater from the river but mainly from anthropogenic stresses from the river transporting domestic, agricultural and livestock wastes, and even the mining wastes at station AB5. In Totok Bay (Southeast Minahasa), Lumingas et al (2011) obtained low individual density (300 ind m⁻²) and macrozoobenthos species richness (9 taxa) near the Ratatotok River mouth which is very turbid due to carrying people's mining waste. But in a station near the Buyat River mouth which is relatively clean, the density reached 1,883 ind m^{-2} and the number of species was 39 taxa. Meanwhile, in the Likupang Strait (North Minahasa), in a station near Bahoi village at a depth of 45 m, they found the individual density of 2,333 ind m^{-2} with a species richness of 39 taxa. In the Gulf of Concarneau (France) (high latitude), a macrozoobenthos density of 1,818 ind m^{-2} with a total of 66 taxa was obtained at the Concarneau station, 2,403 ind m^{-2} with a total of 62 taxa at the Mousterlin station, and 3,933 ind m^{-2} with a total of 57 taxa at Baie de la Foret, respectively (Lumingas 1990). In the Bay of Bengal, on the east coast of India, 73 taxa were obtained at 12 sampling stations consisting of 81.41% Polychaeta and 15.42% Crustacea (Nayak et al 2022). On the northern west coast of the Arabian Gulf, after 12 years of the 1991 oil spill, 392 taxa of macrobenthos were obtained at 58 sampling stations (174 samples) with 71, the density of 3,181 ind m^{-2} , the Shannon-Wiener diversity index (H') of 4.9 (Joydas et al 2017).

The highest Shannon-Wiener diversity index was found at station AB1 (H' = 8.815) and the lowest at station AB5 (H' = 2.740). The stations AB1, AB3, AB4, AB7, and AB8 had a benthic ecological quality of 'High' or no disturbance (H' > 4) according to the category of Lu et al (2021). The benthic ecological quality at stations AB2 and AB6 was categorized as 'Good' or mild disturbances ($3 \le H' < 4$), while at station AB5 was categorized as 'Moderate' or moderate disturbances ($2 \le H' < 3$).

The number of species also determines the species richness index. The lowest species richness index was recorded at station AB5 (SR = 1.524), while the highest species richness index was found at station AB1 (SR = 5.011). The highest species evenness index was found at station AB1 (J' = 1.733) and the lowest at the station AB2 (J' = 0.834). The lowest dominance index was found at station AB1 (d = 0.083) and the highest was at the station AB6 (d = 0.386).

Table 3

| Abundance and marces AB 1 AB 2 AB 3 AB 4 AB 5 AB 6 AB 7 AB 8 Number of individuals (n) 96 36 80 67 38 57 173 138 Density (n m ⁻²) 865 324 721 604 342 514 1559 1243 Number of taxa (S) 34 15 26 28 9 14 26 25 Shannon-Wiener diversity index (H') 8.815 3.257 6.859 6.465 2.740 3.359 6.640 6.111 Margalef richness index (SR) 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Pielou evenness index (J') 1.733 0.834 1.459 1.345 0.864 0.882 1.413 1.316 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Abundance and indices | | | | | Station | | | |
|--|-----------------------------------|-------|-------|-------|-------|---------|-------|-------|-------|
| Number of individuals (n) 96 36 80 67 38 57 173 138 Density (n m ⁻²) 865 324 721 604 342 514 1559 1243 Number of taxa (S) 34 15 26 28 9 14 26 25 Shannon-Wiener diversity index (H') 8.815 3.257 6.859 6.465 2.740 3.359 6.640 6.111 Margalef richness index (SR) 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Pielou evenness index (J') 1.733 0.834 1.459 1.345 0.864 0.882 1.413 1.316 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Abundance and mulces | AB 1 | AB 2 | AB 3 | AB 4 | AB 5 | AB 6 | AB 7 | AB 8 |
| Density (n m ⁻²) 865 324 721 604 342 514 1559 1243 Number of taxa (S) 34 15 26 28 9 14 26 25 Shannon-Wiener diversity index (H') 8.815 3.257 6.859 6.465 2.740 3.359 6.640 6.111 Margalef richness index (SR) 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Pielou evenness index (J') 1.733 0.834 1.459 1.345 0.864 0.882 1.413 1.316 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Number of individuals (n) | 96 | 36 | 80 | 67 | 38 | 57 | 173 | 138 |
| Number of taxa (S) 34 15 26 28 9 14 26 25 Shannon-Wiener diversity index (H') 8.815 3.257 6.859 6.465 2.740 3.359 6.640 6.111 Margalef richness index (SR) Pielou evenness index (J') 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Density (n m ⁻²) | 865 | 324 | 721 | 604 | 342 | 514 | 1559 | 1243 |
| Shannon-Wiener diversity index (H') 8.815 3.257 6.859 6.465 2.740 3.359 6.640 6.111 Margalef richness index (SR) Pielou evenness index (J') 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Number of taxa (S) | 34 | 15 | 26 | 28 | 9 | 14 | 26 | 25 |
| index (H') Margalef richness index (SR) 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Pielou evenness index (J') 1.733 0.834 1.459 1.345 0.864 0.882 1.413 1.316 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Shannon-Wiener diversity | 8.815 | 3.257 | 6.859 | 6.465 | 2.740 | 3.359 | 6.640 | 6.111 |
| Margalef richness index (SR) 5.011 2.708 3.954 4.451 1.524 2.229 3.363 3.376 Pielou evenness index (J') 1.733 0.834 1.459 1.345 0.864 0.882 1.413 1.316 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | index (H') | | | | | | | | |
| Pielou evenness index (J') 1.733 0.834 1.459 1.345 0.864 0.882 1.413 1.316 Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Margalef richness index (SR) | 5.011 | 2.708 | 3.954 | 4.451 | 1.524 | 2.229 | 3.363 | 3.376 |
| Berger-Parker dominance index (d) 0.083 0.278 0.150 0.179 0.316 0.386 0.220 0.341 | Pielou evenness index (J') | 1.733 | 0.834 | 1.459 | 1.345 | 0.864 | 0.882 | 1.413 | 1.316 |
| | Berger-Parker dominance index (d) | 0.083 | 0.278 | 0.150 | 0.179 | 0.316 | 0.386 | 0.220 | 0.341 |

Density, number of taxa and macrozoobenthic ecological indices

Prionospio sp. was the most densely populated taxon found at station AB8 with a density of 423 ind m⁻², followed by *Notomastus latericeus* at station AB7, 342 ind m⁻² and *Aglaophamus dibranchis* at the same station, 270 ind m⁻². Several species (taxa) were found in almost all stations. For example, *Prionospio ehlersi* and Nemertea in 7 stations; and *Aglaophamus dibranchis* and *Glycera* sp. in 6 stations.

Ecology group and AMBI. A total of 88 macrozoobenthos species (taxon) out of 101 species (87%) identified can be determined in their EG. Only 13 species (12.87%) had undetermined NA or EG (Table 2). A total of 44 species are sensitive species included in EG I, 22 species are included in EG II tolerant species, 12 species are included in EG III tolerant species, 9 species are included in 'opportunistic level II' EG IV species, and only 1 species are included in 'opportunistic level I' EG V. The percent of EG (based on the number of individuals) and the BC or AMBI for each station are presented in Figure 3 and Figure 4. In terms of the number of individuals, the station AB1 is dominated by sensitive species (equilibrium), the same as station AB6. The stations AB1, AB2, AB3, AB4 and AB6 had a greater percent of sensitive species individuals (EG I) than those of

opportunistic EG individuals; conversely, the stations AB5, AB7 and AB8 had a greater percent of opportunistic species EG than sensitive species.

Based on the AMBI benthic index (Borja et al 2000), the stations AB1, AB2, AB3, AB4 and AB6 had smaller BC than those at the stations AB5, AB7 and AB8. However, according to the AMBI criteria, only the station AB1 is categorized as 'unpolluted' (BC = 0.810) with the dominance of sensitive species (equilibrium) (EG I = 65.52%) and only a few opportunistic species (EG IV = 4.60%); while other sampling stations are categorized as 'lightly polluted' ($1.2 < BC \le 3.3$).



Figure 3. Percentage of macrozoobenthos ecological groups (based on the number of individuals).



Figure 4. Biotic coefficient (AMBI) of macrozoobenthos.

In this study, there is a difference in results between using the AMBI BC and using the H' criterion according to Lu et al (2021). When using the AMBI criteria, only station AB1 is categorized as 'high' (not disturbed) (BC \leq 1.2), while other stations are categorized as 'good' (lightly disturbed). But when using H', not only station AB1 is categorized as 'high' but also stations AB3, AB4, AB7, and AB8; while the stations AB2 and AB6 are categorized as 'good', and the station AB5 is categorized as 'moderate'. The stations AB7 and AB8, despite classified as 'high' based on their H' values, have more opportunistic species than sensitive species. One year earlier, these two stations were formed due to a coastal landslide, which submerged as many as 33 houses (including septic tanks with organic matter), one bridge, 400 m of the main road and its embankment with a total collapsed land area of about 2.9 ha. The organic matter content is both high and very high in these two stations.

The use of the H' criterion must be done carefully because the value does not consider the characteristics of the taxon as the AMBI index (Lu et al 2021). The conceptual and statistical problems associated with the use of this index in ecology have been discussed that the application of the Shannon-Wiener index results in values that are difficult to interpret and have little or no meaning at all (Barrantes & Sandoval 2009).

Multivariate analysis of ecological groups. The dendogram of sampling station (AB) based on the abundance data of EG macrozoobenthos in a two-way contingency table (5 rows of EG and 8 columns of station AB) is shown in Figure 5. Based on the similarity or distance between the abundance data, the sampling stations can be separated into two different main clusters or very far distances, namely Cluster 1 consisting of AB1, AB4, AB6, AB2, and AB3, and Cluster 2 consisting of AB5, AB8, and AB7. Cluster 1 can also be divided into two sub-clusters, namely Sub-cluster 1A (AB1, AB4, and AB6) and Sub-cluster 1B (AB2 and AB3).

An analysis of correspondence made based on the abundance of individual EG macrozoobenthos is shown in Figure 6. The total inertia for the 4 dimensions is 0.3023 with the contribution of dimension 1 of 0.2217 ($\chi^2 = 144.7389$ (73.32%)), dimension 2 of 0.0483 ($\chi^2 = 31.5470$ (15.98%)), dimension 3 of 0.0222 ($\chi^2 = 14.5153$ (7.35%)), and dimension 4 of 0.0101 ($\chi^2 = 6.6063$ (3.35%)). The first two dimensions explain 89.30% or most of its variability so that the interpretation is carried out only on the two-dimensional plot.

Dendrogram Ward's Method,City-Block



Figure 5. Dendogram of sampling stations based on the abundance of macrozoobenthos ecological groups.



Figure 6. Map of correspondence analysis of sampling stations and macrobenthos ecological groups based on the abundance of ecological groups.

Other important information from correspondence analysis about each row and column category is inertia, quality, absolute contribution and relative contribution. Inertia indicates the proportion of that row or column to the total variability. In this case, the row with the largest inertia (EG I) represents 41.75% of the total variability, while the column with the largest inertia (AB1) represents 25.47%. Quality measures how well the dimensions represent each row and column; categories with higher quality, such as row EG I (quality 0.989) and column AB1 (0.950), are better represented than those with lower quality. Absolute contributions are also quite important, as they represent the contribution of a single row or column to a particular dimension. The column category (station) that is most responsible for the formation of dimension (axis) 1 is AB1 (absolute contribution of 33.0%) followed by AB7 (21.6%); and on the formation of dimension 2 is AB3 (46.4%) followed by AB6 (20.8%). Meanwhile, the row (EG) that is most responsible for the formation of dimension 1 is EG I (55.9%) followed by EG IV (27.0%); and on the formation of dimension 2 is EG II (54.4%) followed by EG III (30.4%). Relative contribution (category-dimension correlation) expresses the share taken by a dimension (factor) in explaining the distribution of a category (element) and describing the row (EG) or column (station) of the exclusive characteristics of a particular dimension (Lebart et al 1982). EG I (relative contribution of 0.982), EG IV (0.834) and EG V (0.650) are ecological groups characteristics exclusive to dimension 1, while ecological groups characteristics exclusive to dimension 2 are EG II (0.789) and EG III (0.599). The

exclusive characteristic stations of dimension 1 are AB1 (relative contribution 0.950), AB8 (0.829), AB4 (0.820), AB7 (0.772), AB6 (0.615), and AB2 (0.602); while the station of the exclusive characteristics for dimension 2 is only AB3 (0.629).

Dimension 1 (horizontal axis) which explains 73.32% of its total variability, separates the EG points from the rightmost (positive part) of the EG I point which is a group of sensitive species to the leftmost (negative part) of the EG V and EG IV points which are opportunistic species. In the center of the horizontal axis (adjacent to the point of origin), are the points of EG II and EG III which are a group of tolerant species. The proximity between the station points means that EG profiles are similar. In this case, the proximity between the station points AB5, AB8, and AB7 reveals that these stations are characterized by opportunistic species of both EG IV and EG V. Likewise, the proximity of the station points AB1, AB3, AB2, AB4, and AB6 shows the similarity of EG profiles especially EG I, but also the influence of EG II and EG III. The station AB1 located in the positive part (right end) of dimension 1 is characterized by the EG I sensitive species group, namely species that are very sensitive to high organic matter content and are predominantly found in substrates with normal or unpolluted conditions.

Various approaches have been used to understand how the functional role of marine macrozoobenthos communities can inform the ecological status of marine waters. When environmental variables are correlated with community structure and ecological groups (Table 4), several conclusions can be drawn. Water depth is negatively correlated very strongly with density, strong with species richness and diversity, but very weakly correlated with ecological groups (AMBI, % sensitive species and % opportunistic species). The content of sludge is positively correlated with the content of organic matter in the sediment, and these two environmental variables are weakly or even very weakly correlated with community structure (density, species richness and species diversity), but on the contrary are moderately and strongly correlated with ecological groups. Species richness is positively correlated very strongly with the species diversity index. The biotic coefficients of AMBI are negatively correlated very strongly with opportunistic species.

The ecological status of macrozoobenthic communities in Amurang Bay shows spatial variations related to anthropogenic stress. According to the AMBI criteria, only the station AB1 is categorized as 'unpolluted' with the dominance of sensitive species (equilibrium) both in terms of individual abundance and in terms of species richness; and there are few opportunistic species; Meanwhile, other sampling stations are categorized as 'lightly polluted'. In Nagapattinam (Southeast India), Selvaraj et al (2024) found maximum AMBI values (4.14-4.17) in locations near fishing ports in estuaries that are high in total organic carbon content and heavy metals as well as low levels of salinity and oxygen solubility; with the dominance of species included in EG III, EG IV and EG V which show somewhat disturbed nature. In contrast, at stations located outside the estuary, the AMBI value ranged from 1.01 to 1.86, with the predominance of EG I (sensitive species) indicating undisturbed nature. The H' index approach needs to be supported by AMBI values to describe the ecological condition of the macrozoobenthic communities in the study area. It is strengthened by multivariate analysis based on the number of individuals of ecological groups, both cluster analysis and correspondence analysis, that yields two different main clusters, namely Cluster 1 consisting of AB1, AB4, AB6, AB2, and AB3, and Cluster 2 consisting of AB5, AB8, and AB7. Cluster 1 can also be divided into two sub-clusters, namely Sub-cluster 1A (AB1, AB4, and AB6) and Subcluster 1B (AB2 and AB3).

Table 4

| | Depth | %mud | %MO | n m ⁻² | S | Η' | AMBI | % sensitive | % opportunistic |
|-------------------|--------|--------|--------|-------------------|--------|--------|--------|-------------|-----------------|
| Depth | 1 | | | | | | | | |
| %mud | -0.05 | 1 | | | | | | | |
| %MO | 0.355 | 0.811 | 1 | | | | | | |
| n m ⁻² | -0.89 | 0.301 | -0.021 | 1 | | | | | |
| S | -0.687 | -0.116 | -0.271 | 0.666 | 1 | | | | |
| Η' | -0.727 | -0.012 | -0.222 | 0.679 | 0.984 | 1 | | | |
| AMBI | -0.118 | 0.605 | 0.599 | 0.398 | -0.308 | -0.273 | 1 | | |
| % sensitive | 0.059 | -0.53 | -0.585 | -0.26 | 0.386 | 0.333 | -0.953 | 1 | |
| % opportunistic | -0.183 | 0.723 | 0.686 | 0.478 | -0.201 | -0.153 | 0.972 | -0.902 | 1 |

Correlation between depth, % sludge, % organic matter, density, number of taxa, Shannon index, AMBI, % of sensitive species, and % opportunistic species

Conclusions. This study concluded that among the eight sampling stations, station AB1 is the best in terms of ecological health status; while the worst is station AB5. Based on cluster analysis and correspondence analysis, the stations that had similar characteristics to the station AB1, namely with the status of "unpolluted" (undisturbed) were AB4, AB6, AB2, and AB3. Meanwhile, the stations whose characteristics are similar to the station AB5, namely "moderate disturbance" (moderately polluted) were AB7 and AB8. It is rare to have a single tool usable to assess the health status of marine benthic ecosystems holistically. Although the AMBI index has been widely used to assess the health status of marine benthic ecosystems, including in tropical areas, the biotic index must also be used in a complementary manner along with the Shannon-Wiener index as well as various multivariate statistical tools, such as cluster analysis and correspondence analysis, to assess the health of marine benthic ecosystems more comprehensively. The study showed that anthropogenic factors could have an impact on granulometry and organic matter content in sediments, which further determine the ecological group of marine macrozoobenthos but less determine the structure of their communities. The benthic environmental monitoring program needs to be implemented in Amurang Bay to protect biodiversity and maintain the health of its benthic ecosystem from increasing anthropogenic impacts.

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