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Biodiversity and community assemblages of freshwater and marine macrozoobenthos in Gorontalo Waters, Indonesia

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Abstract. Kadim MK, Pasingi N, Alinti ER, Alinti ER, Panigoro C. 2022. Biodiversity and community assemblages of freshwater and marine macrozoobenthos in Gorontalo Waters, Indonesia. *Biodiversitas* 23: 637-647. Macrozoobenthos plays an essential role in the aquatic ecosystem including as primary consumers, food sources, and water quality bioindicators. This study aimed to determine the composition, taxa abundance, community structure of the macrozoobenthos in the lower reach, estuary, and marine areas along Gorontalo waters, also their association with water quality parameters. Biota samples were collected purposively from 33 sampling points. The sampling process was conducted in June 2020 and May 2021. Macrozoobenthos samples were taken using Ekman Grab and prepared with filters (mesh size of 500 μ m). Statistically, a t-test was done to assess the significance of taxa abundance difference among three different ecosystems from both sampling periods. Similarity percentages were displayed through a dendrogram. Furthermore, the biota assemblages and water quality parameters association were interpreted through Eigenvalues of Principal Component Analysis using Minitab ver. 14.0. The results showed that the macrozoobenthic community structure composed 70 taxa belonging to six classes dominated by Gastropoda, followed by Bivalvia, Malacostraca, Clitellata, Polychaeta, and Polyplacophora. The marine area had the highest abundance of macrozoobenthos (469.5 ind. m^{-3} in 2020; 460.9 ind. m^{-3} in 2021), followed by the river station (335.3 ind. m^{-3} in 2020; 2621 ind. m^{-3} in 2021), and estuary (217.3 ind in 2020. m^{-3} ; 848.5 ind. m^{-3} in 2021). The most abundant species in the lower reach area was *Melanooides* sp. (135.5 ind. m^{-3} in 2020; and 1222.5 ind. m^{-3} in 2021), while in the estuary was *Brachidontes* sp. (70.7 ind. m^{-3} in 2020; and 248 ind. m^{-3} in 2021). *Batillaria* sp. (128.3 ind. m^{-3} in 2020) and *Anachis* sp. (1510.7 ind. m^{-3} in 2021) were the species with the highest number in the marine zone. Based on the diversity and evenness index, the sea was in the medium category with no dominant species in the marine area. Biota communities in the lower reach station tend to be more like estuary biota than marine biota.

Keywords: Community structure, Gorontalo Bay, macrozoobenthic, Principal Component Analysis, Tomini Bay

INTRODUCTION

Freshwater and marine areas are habitats for a wide variety of aquatic biota. The confluence of land and seawater is known as coastal areas are an important habitat: estuarine, where the water flows directly to the sea. The quality of rivers as freshwater ecosystems is influenced by the influx of waste originating from surrounding activities (Pasingi et al. 2014a) and by the biological interaction between the living macro-organisms (Rodrigues et al. 2011; Pasingi et al. 2014b; Shaleh et al. 2020; Padja et al. 2021) and micro-organisms.

Bone River that empties into Gorontalo Bay (Kadim et al. 2018) serves as the migration path of amphidromous fish (Olii et al. 2017; Sahami et al. 2019; Pasingi et al. 2020a; Pasingi et al. 2020b; Sahami and Habibie 2020). The bay connected with Tomini Bay is also inhabited by diverse pelagic fishes (Pasingi et al. 2020; Lawadjo et al. 2021; Pasingi et al. 2021; Pasingi et al. 2021) and marine mammals (Mustika et al. 2021). The bay also becomes a center of economic activity in Gorontalo so that the waters potentially receive waste that affects the water quality and its biodiversity (Kadim et al. 2019; Sahami et al. 2020). The interconnection of the ecosystems

contributes to the interaction and survival of living organisms, including macrozoobenthos.

Macrozoobenthos plays an essential role in the aquatic system (Laini et al. 2018; Dewiyanti et al. 2021; Yi et al. 2021), e.g. as a neutralizer of the aquatic environment by breaking down organic matters that enter the bottom of the waters into a food source so that the nutritional conditions of the waters become stable (Alavaisha et al. 2019). Changes strongly influence the macrozoobenthic composition, abundance, diversity, water quality, and the substrate where they live so that macrozoobenthos is also commonly used as biological water quality indicator (Kadim et al. 2013; Laini et al. 2018; Harahap et al. 2018; Herawati et al. 2020; Kefford et al. 2020; Lestari and Rahmanto 2020; Sulaeman et al. 2020; Baryshev 2021; Costa et al. 2021; Ndale et al. 2021; Prajoko et al. 2021; Sueb et al. 2021).

Changes in the structure of macrozoobenthos from estuaries to coastal areas need to be determined, considering the important role of macrozoobenthos in aquatic ecosystems (Ekowicakson et al. 2017). There were no studies that revealed the structure of the macrozoobenthos community in Gorontalo Bay. This study aimed to analyze the composition, taxa abundance,

community structure of the macrozoobenthos in the lower reach, estuary, and marine areas along Gorontalo waters and their correlation with water quality parameters.

MATERIALS AND METHODS

Research site

The present study (permitted by Research Institute of Gorontalo State University with number of 248/UN47.DI/PT.01.05/2020 and 186/UN47.DI/PT.01.05/2021) was conducted in June 2020 and May 2021, in Gorontalo Waters, Indonesia (Figure 1). Samples were taken from 33 different points representing three categories of Gorontalo ecosystems: lower reach, estuary, and marine. The sampling areas received inputs from human settlements, agriculture, sand mining, and fishing port activity.

Procedures

Macrozoobenthos sampling and water quality parameters measurement

Biotas from each sampling station were gathered purposively using Ekman Grab with 0.0282 m³ in volume and 150 cm² of sampling area. The samples were collected

by lowering the grab vertically until it hits the bottom of the waters then hauled the trap up once it was fully loaded. The technique followed Overall et al. (2017) and Arfiati et al. (2019). Moreover, the samples were preserved using 70 % alcohol solution. All samples were kept in labeled plastic bags and were taken to a laboratory for the next analysis step. Water temperature, pH, DO, and salinity were measured under the APHA standard (Rice et al. 2012) in situ, along with the biota sampling. Meanwhile, substrate identification was conducted in Hidrobiocology and Biometric Laboratory, Universitas Negeri Gorontalo.

Biota identification

Makrozoobenthos determination was done through preparation (including clearance using a multilevel filter with 35 and 10 mesh sizes), sortation, and biota identification (using a binocular microscope with magnification of 8-32x) steps. In addition, taxa morphology identification was determined according to identification key books by Edington and Hildrew (1981), Hawking and Smith (1997), Quigley (1977), and online reference of WoRMS (World Register of Marine Species). Biota identification down to the genus level was applied whenever possible.

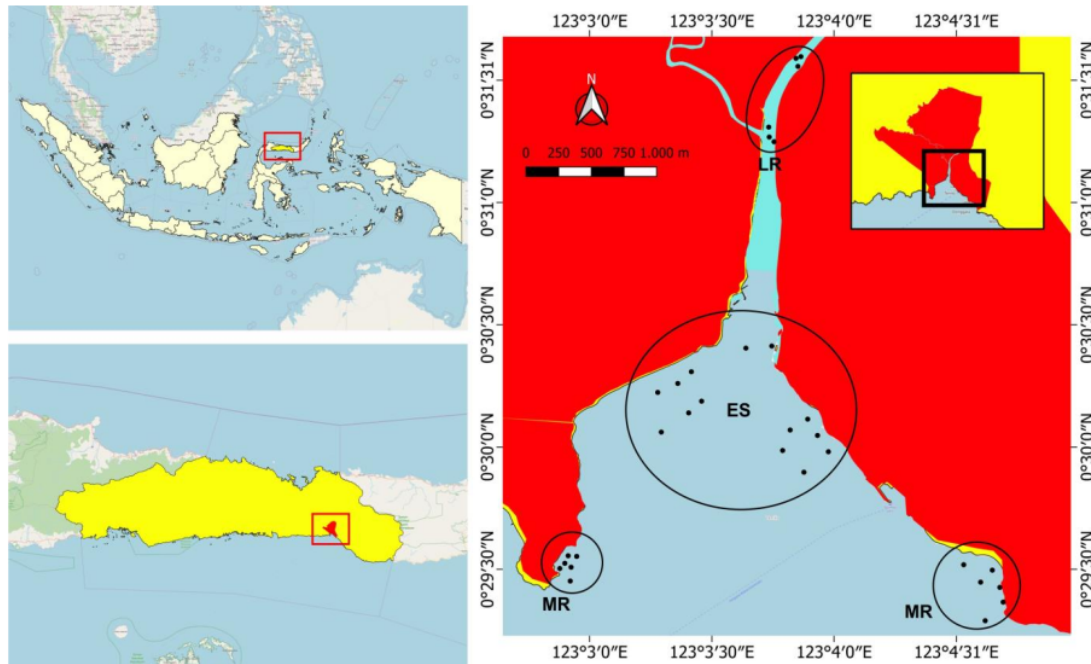


Figure 1. Sampling points in Gorontalo Waters, Indonesia. Note: LR: Lower Reach; ES: Estuary; MR: Marine

Data analysis

Taxa relative abundance, Shannon-Wiener diversity index, evenness index, and dominance index (Shah and Pandit 2013; Strong 2016) were calculated for investigating the macrozoobenthos community structure. Statistically, a t-Test: two-sample assuming equal variances was done to assess the significance of taxa abundance difference between two sampling times in each type of ecosystem. Similarity percentage among the stations was displayed through dendrogram using Minitab ver. 14.0. Furthermore, the biota assemblages and water quality parameters association were interpreted through Eigenvalues using Principal Component Analysis (PCA).

RESULTS AND DISCUSSION

Water quality parameters

The key water quality parameters of the three ecosystems are almost identical in the two sampling periods, except the salinity (Table 1). The difference in salinity occurs due to the proportion of fresh water and seawater mixture.

The temperature in each site during the study ranges from 28.6-29.5°C. A suitable water temperature for the growth of macrozoobenthos ranges from 25 to 30°C (Rotvit and Jacobsen 2013). Dissolved Oxygen ranges between 5 and 7.1 mg. L⁻¹, the value is still in normal conditions to support macrozoobenthos life (Wildsmith et al. 2017; Hou et al. 2020). The pH values measured during the study ranged from 6.9 to 7.6. The suitable value for macrozoobenthos natural life is 7 to 7.9. This range is normal and still supports macrozoobenthos survival (Kartikasari et al. 2013). pH affects the survival of biota, as water conditions with strong acid or base will endanger the organism's survival, interfering with metabolic and respiratory processes (Lacoul et al. 2011). The zero salinity in the downstream site continued to increase in the estuary to marine areas from 18 to 32.3‰. The measured salinity range is suitable for macrozoobenthos growth (Roy and Nandi 2012). According to Dudgeon (2006), the abundance of benthos in waters is related to salinity, organic matter, and clay and silt fractions from sediments. A higher salinity value will affect the abundance and diversity of macrozoobenthos (Van Diggelen and Montagna 2016). The types of the substrate at all stations are sandy mud and mud, which are relatively similar.

Composition, abundance, and distribution of macrozoobenthos

Overall, there are 70 taxa found in all stations with a total of 3008 individuals. These taxa represent six classes: Gastropods, Bivalves, Malacostraca, Clitellata, Polychaeta, and Polyplacophora (Table 2). Among the identified macrozoobenthos, Gastropods have the highest number of taxa (48), followed by Bivalvia (7), Malacostraca (10), Clitellata (1), Polychaeta (4), and Polyplacophora (1).

Information on the composition of the macrozoobenthos class is displayed in Figure 2.

Based on the sampling results in June 2020, macrozoobenthos in the river area are composed of two classes. Gastropods are the most dominant class (with a contribution of 99.12% of the total population), and Malacostraca adds only 0.88%. Gastropod and Bivalvia are also the most dominant classes found in the estuary of Donan River (Hakiki et al. 2017) and Lamnyong River (Octavina et al. 2019). As for the estuary, Bivalves contribute 51.35% of the total population, then Gastropods with 48.65%. The marine area is composed of five classes where Gastropods dominate with a contribution of 87.81%, followed by Bivalvia (4.06%), Clitellata (3.75%), Polychaeta (2.81%), and Malacostraca (1.56%).

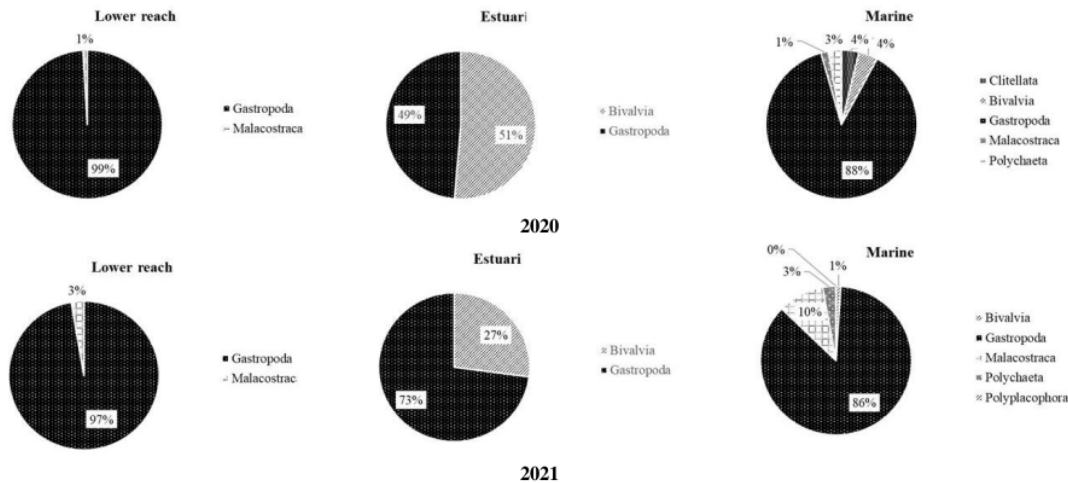
Likewise, in the previous period, macrozoobenthos sampled May 2021 from the downstream only consist of two classes where Gastropods remain the most dominant class with a contribution of 97.3% and Malacostraca by 2.7%. Gastropods dominate 72.9% of the total population in the estuary, followed by Bivalves with 27.1%. The marine area is still composed of five classes, yet the percentage is slightly different where Gastropods still dominate by 6.25%, followed by Malacostraca (9.9%), Polychaeta (2.8%), Bivalvia (1.03%), and Polyplacophora (0.04%). A species from the Gastropods class also dominates Bulaksetra Estuary (Krisnafi et al. 2021). **Chinoderms and Gastropods are the two major classes that make up the macrozoobenthos community in the coastal waters of Marsegu Island (Yunita et al. 2018). In contrast, in Kalibaru Waters of Bengkulu, the dominant macrozoobenthos class is Pelecypoda (Lilisti et al. 2021).**

The presence of certain types of taxa is thought to be closely related to the flexibility of each species to the surrounding environments (Gosling 2008; Miltner and McLaughlin 2019). Gastropods are one of the most common groups of mollusks found in various substrates due to their high malleability compared to other classes on rigid and smooth substrates (Turra and Denadai 2006; Baharuddin et al. 2018; Islamy and Hasan 2020). Wildan et al. 2021 report that Gastropod becomes the most abundant taxa in Batang Toru River because of its wide adaptability and tolerance to environmental conditions. However, Gastropods are composed of many species, and their spatial distribution is determined by particular living habitats, certain areas, and certain times (Sahidin et al. 2019).

The taxa recorded during the research are 62, 9, and 7 genus for marine, estuary, and downstream sites, correspondingly; Gastropods are the most dominant class. This number is relatively high compared to Lilisti et al. (2021) and Rimadiyani (2019) research results. Nevertheless, the number is smaller than previous studies outside Indonesia waters. Nebra et al. (2011), in the Ebro Estuary, Mediterranean, observed 214 macrozoobenthos dominated by the Polychaeta group. In addition, in Ria de Aveiro, Western Portugal, 124 macrozoobenthic taxa were discovered (Rodrigues et al. 2011).

Table 1. Water quality parameters (mean \pm standard deviation) of Gorontalo Waters, Indonesia

Parameters	2020			2021		
	Lower reach	Estuary	Marine	Lower reach	Estuary	Marine
Temperature ($^{\circ}$ C)	29.5 \pm 0.72	28 \pm 0.99	29.0 \pm 0.83	29.4 \pm 0.32	29.3 \pm 0.39	28.5 \pm 0.57
DO (mg. L ⁻¹)	6.6 \pm 0.34	6.1 \pm 0.36	7.1 \pm 0.17	5.0 \pm 0.37	6.2 \pm 0.20	5.6 \pm 0.22
pH	7.6 \pm 0.33	7.2 \pm 0.27	7.6 \pm 0.26	6.9 \pm 0.25	7.0 \pm 0.14	6.9 \pm 0.20
Salinity (‰)	0.0 \pm 0.0	18 \pm 0.83	25.5 \pm 0.07	0.0 \pm 0.0	28.2 \pm 0.12	32.3 \pm 0.17
Substrate	sandy mud	mud	sandy mud	sandy mud	mud	sandy mud

**Figure 2.** Mean percentages of different macrozoobenthic groups in 2020 and 2021

In 2020, there were 21 taxa in the marine area; the three main genus were *Batillaria* sp., *Olivella* sp. and *Pleuroploca* sp. from the Gastropoda (33). Moreover, *Phos* sp., *Bulla* sp., *Pugilina* sp., *Polinices* sp., *Conus* sp., *Cypraea* sp., *Iniforis* sp., *Trochus* sp., Tegulidae, and *Siphonaria* sp. were also found. Bivalves classes were represented by *Cyamiocardium* sp., *Brachidontes* sp., and *Vasticardium* sp. Malacostraca classes was denoted by *Panopeus* sp., *Natatolana* sp., and *Emerita* sp. Additionally, *Oligochaeta* and *Lumbrineris* sp. belonged to Clitellata and Polychaeta. In the estuary site, there were six genus, namely *Brachidontes* sp., *Macoma* sp., *Cyamiocardium* sp. from Bivalvia, *Phos* sp., *Thiara* sp., and *Theodoxus* sp. from Gastropods. In the river zone, four taxa were identified. Three genus belonging to Gastropods with the order of genus dominance observed from the highest to the lowest was *Thiara* sp., *Melanoides* sp., *Theodoxus* sp. Meanwhile, *Varuna* sp. is the sole representative of Malacostraca. In Tasi Ana Dead Sea Lake, *Melanoides* were found in all study area sampling (Sombo et al. 2020).

From the 2021 sampling period, the taxa compositions in the year are higher than those in 2020 in all stations. The marine area consisted of 53 taxa. Gastropods are represented by 36 genera, dominated by *Anachis* sp. and *Smaragdia* sp. Malacostraca is represented by six taxa where *Gammarus* sp. dominated. Four genera are from Polychaeta, whereas Polyplacophora is represented by *Acanthopleura* sp. In the estuary area, eight taxa are

discovered. Gastropods are represented by *Phos* sp., *Thiara* sp., and *Theodoxus* sp. Three more genera are *Melanoides* sp., *Clithon* sp., and *Littoraria* sp. Bivalvia was still represented by *Brachidontes* sp. and *Cyamiocardium* sp.

On the other hand, *Macoma* sp. was not found. However, in the downstream, six taxa are found where four genera represent Gastropods. *Melanoides* sp. and *Thiara* sp. become the dominant genera, followed by *Septarina* sp., and *Neritina* sp. The last two genera mentioned are those that are revealed only at the time of sampling in 2021. Furthermore, Malacostraca was composed of *Varuna* sp. and Palaemonidae.

Biota community structure index

The diversity, evenness, and dominance biota index in each station are presented in Table 3. During the present study, the macrozoobenthic population diversity index ranged from 0.9 (downstream) to 2.3 (marine) in 2020. Meanwhile, in 2021, the value ranges from 1.0 (downstream) to 2.3 (marine), indicating that diversity is in the moderate category except for the lower reach area in 2020. The evenness index can be compared to a diversity index for most macroinvertebrate community analyses (Beisel et al. 2003). The data shows that the diversity level in the low and medium categories signifies that the distribution of individuals for each taxa is increasingly uneven. Pennekamp et al. (2018) state that species decrease and more individuals lead to ecosystem instability.

Table 2. Comparison of Macrozoobenthos Abundance Among Three Different Ecosystems in Gorontalo Waters, Indonesia

	2020						2021					
	Low reach		Estuary		Marine		Lower reach		Estuary		Marine	
	Di	Mean	%	Di	Mean	%	Di	Mean	%	Di	Mean	%
Clitellata	0	0	0	0	0	100	0	0	0	0	0	0
<i>Oligochaeta</i>	0	0	0	0	0	17.7	17.7±61.2	0	0	0	0	0
Bivalvia	0	0	0	0	0	0	0	0	0	0	0	0
<i>Barbatia</i> sp.	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2
<i>Brachidontes</i> sp.	0	0	0	70.7	70.7±122.4	62.3	1.4	1.4±4.9	0	0	11.7	11.7±17.0
<i>Cyamocardium</i> sp.	0	0	0	5.7	5.7±9.8	5.3	16.2	16.2±37.3	0	212.5	212.5±300.5	92.3
<i>Macra</i> s 11	0	0	0	0	0	0	0	0	0	17.5	17.5±24.7	7.7
<i>Macoma</i> sp.	0	0	0	35.3	35.3±61.2	31.6	0	0	0	0	0	0
<i>Tellina</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Vastocardium</i> sp.	0	0	0	0	0	0	1.4	1.4±4.9	0	0	23.6	23.6±74.9
Gastropoda	0	0	0	0	0	0	0	0	0	0	3.9	3.9±16.5
<i>Anachis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Anatillophos</i> sp.	0	0	0	46.7	46.7±20.2	44.4	29.3	29.3±55.5	0	0	1510.7	1510.7±4965.1
<i>Batillaria</i> sp.	0	0	0	0	0	0	128	128.3±149.0	0	70.5	70.5±99.7	11.4
<i>Bulla</i> sp. 9	0	0	0	0	0	0	23.4	23.4±39.4	0	0	0	0
<i>Cerithiopsis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cerithium</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0
<i>Clithon</i> sp.	0	0	0	0	0	0	0	0	0	35	35.0±49.5	5.7
<i>Conus</i> sp.	0	0	0	0	0	0	7.2	7.2±11.6	0	0	0	0
<i>Costapex</i> sp.	0	0	0	0	0	0	0	0	0	0	45.1	45.1±93.1
<i>Cypraea</i> sp.	0	0	0	0	0	0	5.7	5.7±8.4	0	0	15.7	15.7±50.3
<i>Engina</i> sp.	0	0	0	0	0	0	0	0	0	0	9.8	9.8±29.1
<i>Epitonium</i> sp.	0	0	0	0	0	0	0	0	0	0	5.8	5.8±18.0
<i>Gibbula</i> sp.	0	0	0	0	0	0	0	0	0	0	3.9	3.9±16.5
<i>Haminoea</i> sp.	0	0	0	0	0	0	0	0	0	0	11.7	11.7±20.8
<i>Helicacis</i> sp.	0	0	0	0	0	0	0	0	0	0	88.4	88.4±238.6
<i>Iniforis</i> sp.	0	0	0	0	0	0	5.8	5.8±11.3	0	0	3.9	3.9±16.5
<i>Littoraria</i> sp.	0	0	0	0	0	0	0	0	0	17.5	17.5±24.7	2.9
<i>Luria</i> sp.	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2
<i>Mangelia</i> sp.	0	0	0	0	0	0	0	0	0	0	25.4	25.4±53.9
<i>Mamilla</i> sp.	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2
<i>Melanooides</i> sp.	133	132.5±84.4	40	0	0	0	0	0	0	248	248.0±350.7	40
<i>Mitrella</i> sp.	0	0	0	0	0	0	1223	1222.5±1529.5	48	0	0	0
<i>Mitridae</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Murticopsis</i> sp.	0	0	0	0	0	0	0	0	0	0	112.1	122.1±307.3
<i>Nassarius</i> sp.	0	0	0	0	0	0	0	0	0	0	5.9	5.9±25.0
<i>Natica</i> sp.	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2
<i>Neritina</i> sp.	0	0	0	0	0	0	0	0	0	0	35.4	35.4±141.6
	0	0	0	0	0	0	0	0	0	0	41.2	41.2±10.9
	0	0	0	0	0	0	17.5	17.5±24.7	0.7	0	0	0

<i>Odostomia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.5	23.5±47.0	0.59
<i>Oliva</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.7	9.7±16.1	0.25
<i>Pleuroploca</i> sp.	0	0	0	0	0	0	0	91.1	91.1±96.8	22.1	0	0	0	0	0	0	0	0	0	0	0
<i>Pugilina</i> sp.	0	0	0	0	0	0	0	73.6	73.6±81.2	17.8	0	0	0	0	0	0	0	0	0	0	0
<i>Rissoina</i> sp.	0	0	0	0	0	0	0	22.1	22.1±66.2	5.3	0	0	0	0	0	0	0	0	0	0	0
<i>Septarina</i> sp.	0	0	0	0	0	0	0	0	0	0	106	106.0±149.9	4.2	0	0	0	0	0	198.7	198.7±414.4	5
<i>Siphonaria</i> sp.	0	0	0	0	0	0	0	1.4	1.4±4.9	0.4	0	0	0	0	0	0	0	0	0	0	0
<i>Smaragdia</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	931.7	931.7±3542.8	23.42
<i>Strombus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.7	9.7±20.1	0.25
<i>Tegulidae</i>	0	0	0	0	0	0	0	2.8	2.8±6.6	0.7	0	0	0	0	0	0	0	0	0	0	0
<i>Terebra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.8	5.8±18.0	0.15
<i>Theodoxus</i> sp.	23.3	23.3±34.6	7.1	17.7	17.7±30.6	16.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thiara</i> sp.	177	176.7±316.2	53	41.3	41.3±71.6	38.9	0	0	0	0	1205	1205.0±1404.3	47	194.5	194.5±75.7	31.4	0	0	0	0	0
<i>Tricolta</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.9	3.9±11.3	0.05
<i>Trivirostra</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.9	3.9±16.5	0.1
<i>Trochus</i> sp.	0	0	0	0	0	0	0	4.3	4.3±7.7	1.1	0	0	0	0	0	0	0	0	0	0	0
<i>Turbonilla</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	411.6	411.6±1295.6	0.1
<i>Turridae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17.7	17.7±47.4	10.53
<i>Vexillum</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2	0.45
Malacostraca																					
<i>Emerita</i> sp.	0	0	0	0	0	0	0	1.4	1.4±4.9	20	0	0	0	0	0	0	0	0	0	0	0
<i>Gammarus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	413.4	413.4±1421.9	90.52
<i>Grapsus</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.8	5.8±18.0	1.29
<i>Grapsidae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.8	11.8±34.3	2.59
<i>Leucosviidae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2	0.43
<i>Natatolana</i> sp.	0	0	0	0	0	0	0	1.4	1.4±4.9	20	0	0	0	0	0	0	0	0	0	0	0
<i>Palaemonidae</i>	0	0	0	0	0	0	0	0	0	0	52.5	52.5±24.7	75	0	0	0	0	0	0	0	0
<i>Panopeus</i> sp.	0	0	0	0	0	0	0	4.3	4.3±10.8	60	0	0	0	0	0	0	0	0	15.7	15.7±44.1	3.45
<i>Penaeidae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.8	7.8±19.2	1.72
<i>Varuna</i> sp.	2.8	2.8±6.9	100	0	0	0	0	0	0	0	17.5	17.5±24.7	25	0	0	0	0	0	0	0	0
Polychaeta																					
<i>Glyceridae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45.1	45.1±73.6	35.38
<i>Lumbrineris</i> sp.	0	0	0	0	0	0	0	13.3	13.3±36.3	100	0	0	0	0	0	0	0	0	49.2	49.2±183.3	38.46
<i>Nereis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.8	9.8±26.5	7.69
<i>Sigalionidae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	23.4	23.4±40.0	18.84
Polyplacophora																					
<i>Acanthopleura</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.9	1.9±8.2	100

Table 3. Diversity, Evenness, and Dominance Index

Index	2020			2021		
	Lower reach	Estuary	Marine	Lower reach	Estuary	Marine
Diversity (H')	0.9	1.6	2.3	1.0	1.7	2.3
Evenness (E)	0.7	0.9	0.7	0.6	0.8	0.6
Dominance (D)	0.4	0.2	0.2	0.4	0.2	0.2

The evenness index value both in 2020 and 2021 for all stations, which is close to 1, reveals that the individual distribution of each genus at each station has moderate uniformity and no genus dominates. According to Odum (1998), a uniformity index value close to 1 indicates a stable ecosystem. It illustrates that no genus dominates; therefore, the taxa distribution of each genus is very uniform and equitable. Cai et al (2016) reported that the index of feeding group macrozoobenthos as number as 0.2 indicated the poor of ecological quality in most Bohai Bay research area.

The dominance index values for all stations less than 0.5 are classified as a low category (Odum 1998). The low dominance index is related to the evenness index (Yusuf and Kadim 2019; Pritchard and Martel 2020). The low dominance index in the results showed that the macrozoobenthos were in good habitat circumstances. Vijapure and Sukumaran (2019) stated that the greater the evenness index value, the greater the type of taxa uniformity. The density of each type of individual is relatively the same and tends not to be dominated by certain types and vice versa.

Correlation between ecosystem characters, water quality, and taxa distribution

During the two sampling periods, marine areas had the highest total abundance of macrozoobenthos (in 2020 it was 469.5 ind. m⁻³ in 2020; 605.9 ind. m⁻³ in 2021) followed by river areas (335.3 ind. m⁻³ in 2020; 26201 ind. m⁻³ in 2021) and estuary areas (217.3 ind. m⁻³ in 2020; 848.5 ind. m⁻³ in 2021). Yusuf and Kadim (2019) point out that different substrate conditions, organic matter, or food can cause an ecosystem's high and low population. Substrate texture serves as an environmental factor contributing to the intertidal macrozoobenthos community structure (Darmarini et al. 2021), although it is further determined by the physical and chemical contents of the substrate type (Sahidin et al. 2019). Rodrigues et al. (2018) state that the spatial distribution of benthic fauna is strongly influenced by hydrodynamics and salinity gradients. Based on the variation in the abundance of taxa found at all sampling points, the similarity of the presence of taxa in the lower reach and estuary stations arrives at more than 80%; meanwhile, the similarity value is only 46.75% (Figure 3).

Ecological conditions in estuaries often undergo transitions that affect the success of benthic fauna. Fewer genera are found in estuaries than organisms living in both freshwater and marine (Noh et al. 2019). The small number

of individuals living in the estuary waters is due to large environmental fluctuations, especially salinity (Whitfield et al. 2012; Van Diggelen and Montagna 2016) and temperature during high tides (Basset et al. 2013). Macrozoobenthos abundance is higher in 2021 compared to 2020. There is a significant difference between the sampling period and the abundance in the marine area, and there is no significant difference for the lower reach and estuary areas (Figure 4).

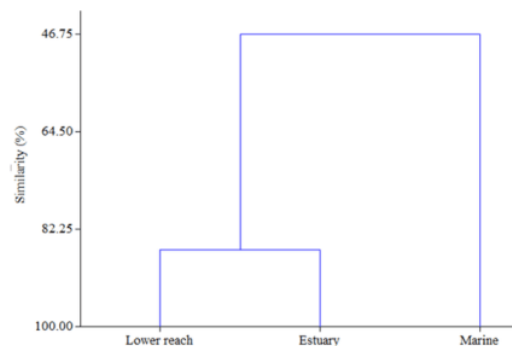


Figure 3. Similarity dendrogram of the three ecosystem characteristics based on macrozoobenthos taxa abundance

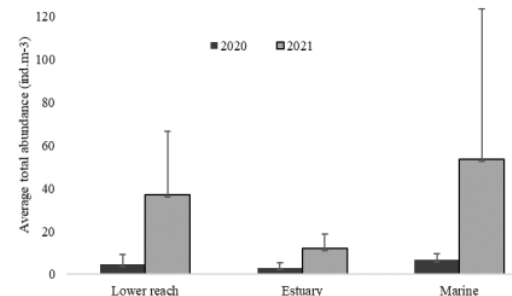


Figure 4. Comparison of macrozoobenthos average total abundance (mean ± standard error) between two different sampling periods from each ecosystem category

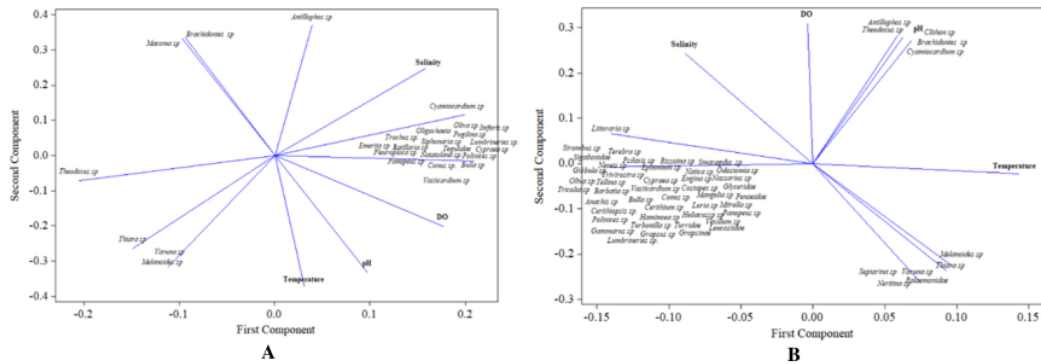


Figure 5. Biplot of macrozoobenthos distribution and water parameters based on the abundance of each existing taxa during the sampling time in the years 2020 (A) and 2021 (B)

Based on the principal component analysis in Figure 5, the taxa found in the two different sampling times are scattered in different ways. The cumulative eigenvalues from both years are relatively representative to be used as a basis for concluding the analysis of the main components of water quality variables and the macrozoobenthos community structure. They imply that all taxa and the four water quality variables (temperature, pH, DO, and salinity) are reduced to two variables, resulting in two new components explaining more than 95% of all variables in 2020 and 2021. There are no water quality parameters that characterize the abundance of certain taxa, except for pH in 2021, which shows its association with *Antillophos* sp., *Theodoxus* sp., *Clithon* sp., *Brachidontes* sp., and *Cyamioecardium* sp.

In 2020, the output analysis shows that the first and second components' eigenvalues were 22.888 and 7.112, respectively. Furthermore, the cumulative eigenvalue proportion indicated that the two components represented 76.3% and 19% of the total variability. All water quality parameters positively correlated with the first principal component (PC1) and negatively correlated with the second principal component (PC2), except salinity that still had a positive correlation with PC2. Additionally, most taxa positively correlated with PC1, excluding *Melanoides* sp., *Theodoxus* sp., *Thiara* sp., and *Varuna* sp. that negatively correlated with PC1 and PC2.

Unlike the previous sampling time, in 2021, the investigation shows that the eigenvalue is 48.535 and 10.465 for the first component (PC1) and the second component (PC2), respectively. The cumulative eigenvalue proportion of 100% signifies that the two principal components represent 82.3% and 17.7% of the total variability. In addition, water quality parameters that positively correlate with PC1 are temperature and pH; in contrast, DO and Salinity negatively correlate with PC1. Next, all taxa negatively correlate with both PC1 and PC2, except the *Brachidontes* sp., *Cyamioecardium* sp., *Antillophos* sp., *Clithon* sp., *Melanoides* sp., *Theodoxus* sp.; they correlate positively with both principal components of PC1 and PC2.

Like other freshwater ecosystems, *Thiara* sp. was the most dominant in the downstream area with a zero salinity in 2020 (average abundance of 176.7 ind. m⁻³) and *Melanoides* sp. in 2021 (average abundance of 1222.5 ind. m⁻³). The same dominance in freshwater ecosystems was reported by Ekowicaksono et al. (2017). In 2021, *Melanoides* sp. is the most abundant (average abundance 248 ind. m⁻³), while in the coastal area of *And* sp. is found to be the most abundant with an average abundance 1510.7 ind. m⁻³. *Brachidontes* sp. in estuaries (mean abundance of 70.7 ind. m⁻³) and *Batillaria* sp. (average abundance of 128.3 ind. m⁻³) at marine stations were dominant in 2020. *Anachis* sp. was also reported to be found in the waters of the Gulf of Thailand by Sanpanich and Duangdee (2013) and Wells et al. (2021). In Indonesia, *Brachidontes* sp. is also discovered in the waters of Badur Beach, Madura, by Tan et al. (2021).

The results suggest that there is a mixed population between freshwater and estuary, and between estuary and marine. *Theodoxus* sp., *Thiara* sp., and *Melanoides* sp. are macrozoobenthic types downstream to the estuary with a relatively similar abundance. *Phos* sp., *Brachidontes* sp., *Littoraria* sp., and *Cyamioecardium* sp. are distributed in the estuary to marine areas, but with an abundance that tends to decrease in coastal areas. Substrate and organic contents in sediments are essential factors in benthic community structures. The type of substrate in all stations in this study is relatively identical, and the adaptability of the six taxa to substrate conditions is predicted to be one of the causes of the mixed population. The type of substrate affects the ability of mollusks, especially gastropods, to attach and survive. Mud substrate can bind organic matter in the sediments and become a food source for Gastropoda and Bivalvia. Due to flushing and mixing between freshwater and marine in estuaries, many species/taxa from downstream and marine zones must be adapted to the daily and seasonal fluctuating environmental conditions (Nursuhayati et al. 2013; Van Diggelen and Montagna 2016). *Melanoides* sp. and *Thiara* sp. are two groups of Gastropods native in rivers and invasive and can live in fresh and brackish water (Facon et al. 2003; Ng et al.

2016). Genus of *Thiara* sp. can tolerate moderate salinity levels to still live in the mouths of rivers bordering the sea. This taxa is often found in abundant populations (Kumar et al. 2017; Sinambela et al. 2019). *Phos* sp. belongs to the Buccinidae family.

In the connection area of marine and freshwater, generally, salinity level alteration is a vital environmental factor determining the benthic macrovertebrate community structure and distribution (El Asri et al. 2021; Anchenko et al. 2017). Boutoumit et al. (2021) reveal that key factors determining the species composition patterns of macrozoobenthos assemblages are the hydrographic regime (marine and terrestrial freshwater), sediment distribution and characteristics, and the sort of habitat (vegetated area).

Based on the composition and abundance data, the macrozoobenthos community structure in Gorontalo Waters shows that only marine genus dominate the marine station, which is also valid for downstream areas. In this study, the lower reach composition is similar to the estuary than the marine area.

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