7 The Roles of Macrobenthic Mollusks as Bioindicator in Response to Environmental Disturbance : Cumulative k-dominance curves and bubble plots ordination approaches

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The Roles of Macrobenthic Mollusks as Bioindicator in Response to Environmental Disturbance : Cumulative *k*-dominance curves and bubble plots ordination approaches

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Abstract. Floating net cage is one of the aquaculture practice operated in Indonesian coastal areas that has been growing rapidly over the last two decades. This study is aimed to assess the roles of macrobenthic mollusks as bioindicator in response to environmental disturbance caused by fish farming activities, and compare the samples within the locations using graphical methods. The research was done at the floating net cage fish farming area in the Awerange Gulf, South Sulawesi, Indonesia at the coordinates between 79º0500'- 79º1500' LS and953º1500'- 953º2000' BT, at the polyculture and reference areas, which was located 1 km away from farming area. Sampling period was conducted between October 2014 to June 2015.The sediment samples were taken from the two locations with two sampling time and three replicates using Van Veen Grab for biotic and abiotic assessment. Mollusks as biotic parameter were fixed using 4% formalin solution and were preserved using 70% ethanol solution after 1mm mesh size. The macrobenthic mollusks were found as many as 15 species consisting of 14 families and 2 classes (gastropods and bivalves). Based on cumulative *k*-dominance analysis projected on each station, the line of station K_3T_1 (reference area; first sampling time) and KJAB P_3T_2 (polyculture area; second sampling time) are located below others curves, indicating the highest evenness and diversity compared to the other stations, whereas station K_2T_1 (reference area; first sampling time) and K_3T_2 (polyculture area, second sampling time) are located on the top, indicate the lowest value of evenness and diversity. Based on the bubble plots NMDS ordination, the four dominant taxa/species did not clearly show involvement in driving/shifting the ordinate position of station on the graph, except *T. agilis*. However, the two species showed involvement in driving/shifting the ordinate position of two stations of the reference areas from the first sampling time by *Rynoclavis sordidula,* and three stations of the polyculture areas from the first sampling time by *Gemulla* sp. away from other stations, thus are considered as bioindicators of environmental disturbance. These results imply that cumulative *k*-dominance curves and bubble plots ordination may be effective method to assess the indication of disturbance of the area.

Keywords : Macrobenthic molluks, polyculture, bubble plots, cumulative *k*-dominance

1. Introduction

Over the last two decades, aquaculture in form of floating net cage has been developed well around the globe. The cage is a pen/impound for fish farming that usually placed in a large flow of water such as lakes, rivers, and sea. The use of floating net cage has proven to facilitate the people for its efficiency of land and food, easier to harvest either selective or total, business units can be determined according to the funding ability by utilizing materials available on farms and easily monitored and do not require water management specifically as in ponds [1]. Floating net cage is one of the aquaculture practice operated in Indonesian coastal areas that has been growing rapidly. The operation of the cage may be done using monoculture and polyculture techniques. Monoculture is growing up a species of farmed biota in one cage over one period of harvest time, while the polyculture is growing up more than one biota in one cage over one period of harvest time. The various types of fish require different types of food so that each type of fish will not compete for food [2]. The use of monocultures system for a long time can cause environmental degradation and can lead to eutrophication due to a buildup of nutrient excretion and inefficient feeding system. Polyculture fish farming system also has the potential to impact on the farm's environment. Intensive mariculture of fish, shrimp, shellfish or the other economic aquatic animals may not only result in an increase of nutrient concentrations in coastal waters, but also lead to changes of the dissolved inorganic nutrient structures and sedim entary environments [3].

Benthic animals are animals that spend some or all of their life in the bottom of waters, either sessile, crawling or digging holes. Benthic animals have an important role in the process of decomposition and mineralization of organic material in the water, as well as occupying several trophic levels in the food chains [4], [5]. Macrobenthos generally cannot move fast, has large body size that make it easy to be identified and spend their lives in the bottom of waters. Their abundance and diversity are highly depended on changes in water quality and substrate tolerance, and also their activity and sensitivity to environmental changes. Tolerance range of macrobenthos in the environment is different one to each other [6]. Macrobenthos provide advantages as biological indicators because of its characteristics, as they are ubiquitous animals, able to provide response spectrum to environmental stress, live a sedentary life in the habitat that may explain the spatial changes and also has longer life cycle that can explain temporal changes. Bioindicators include biological processes, species, or communities and are used to assess the quality of the environment and how it changes over time. Changes in the environment are often attributed to anthropogenic disturbances (e.g., pollution, land use changes) or natural stressors (e.g., drought, late spring freeze), although anthropogenic stressors form the primary focus of bioindicator research [7], [8].

One of macrobenthos assessed as a good bioindicator is mollusks group. Mollusks are invertebrates that have the ability as bio-indicators of water pollution, because it has sensitivity to the compound of pollutants in ecosystems. Awerange Gulf is one of the gulfs located in Barru District, South Sulawesi. The gulf has been used as floating net cage over the last 15 years. The water conditions can be changed as a result of farming activities in the bay, and subsequently affect the lives of organisms that live in these waters, one of which is benthos. This study is aimed to assess the roles of macrobenthic mollusks as bioindicator in response to environmental disturbance caused by fish farming activities, and compare the samples within the locations using graphical methods.

2. Research Method

The study was conducted on October 2014 until April 2015 in the Awerange Gulf, South Sulawesi. The research was done at the floating net cage polyculture system in the Awerange Gulf, South Sulawesi, Indonesia at the coordinates between 79º0500'- 79º1500' LS and 953º1500'- 953º2000' BT. The sediment samples were taken from the two locations with two sampling time and three replicates using Van Veen Grab for biotic and abiotic assessment. Samples of mollusks were used as biotic parameters, whilst sediment and water samples were used as abiotic parameters. Mollusks as biotic parameter were fixed using 4% formalin solution and were preserved using 70% ethanol solution after 1mm mesh size. Sediment samples were taken and then sieved using a sieve with a net benthos measuring 1 mm. Siftings then washed under water for subsequent sorting. Results sorting then inserted into a bottle containing 70% ethanol solution and labeled. The next stage is the identification and analysis of data.

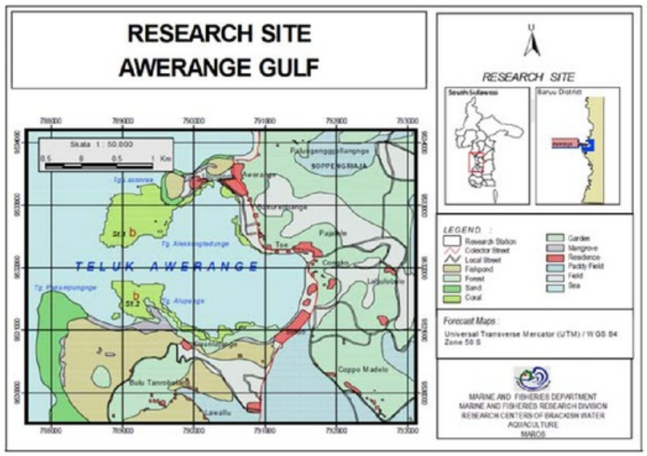

Figure 1. Map of Awerange Gulf, Barru, South Sulawesi as a study site (Source: BPAP Maros, 2014).

The changes in the dominance pattern of macrobenthic mollusk assemblages were assessed using cumulative *k*-dominance curves for each station plotted on the same graph [9]. The extent of disturbance was evaluated by a comparison of the samples between sites and times based on the curves. The dominance (and intrinsically diversity) of the assemblages was generated by plotting cumulatively ranked abundances against log species/taxa rank [10]. Non-metric Multi Dimensional Scaling (NMDS) of Bray-Curtis similarities on log (X + 1) transformed data was used to provide a visual representation of differences between sites over time on the ordination. The analysis incorporates all sites at all times to observe if there was any tendency of separation between polyculture and reference sites. The four dominant taxa were then superimposed on the MDS plots using bubble plots to assess their roles in configuring the ordinations and degree of disturbance. The correlations between physical-chemical factors, substrate granular composition and the carbon and nitrogen content with the abundance of mollusks were analyzed using BIO-ENV. All multivariate and graphical method were analysed using the PRIMER 6.1.5 software packages.

3. Results and Discussion

The macrobenthic mollusks were found as many as 15 species consisting of 14 families and 2 classes (gastropods and bivalves). *Turritella* sp. (Fam. Turritellidae) was the most dominant taxa both at the reference area and the polyculture cage area, followed by *Tellina agilis* (Fam. Tellinidae), *Nassarius castus* (Fam. Nassariidae), and *Anadara* sp. (Fam. Arcidae), as seen in Table 1. Based on sampling location, mollusks abundance at the polyculture area ($\overline{X}$ = 140.67 , SD = 8.71) exhibited significantly different (ρ = 0.001) compared to those at reference area ($\overline{X}$ = 49.2 , SD = 13.9).

Table 1. The average of abundance of mollusks in the reference and polyculture areas on the first and second sampling time at Awerange Gulf, Barru District, South Sulawesi (per grab).

NO.	CLASS	FAMILY	SPECIES	Polyculture Area		Reference Area	
				Sampling I	Sampling II	Sampling I	Sampling II
1	Gastropod	Turritellidae	*Turritella*sp.	25.67	21.33	126.67	93.00
		Chilodontidae	*Perrinia* sp.	0.00	0.00	0.67	0.00
		Mitridae	*Mitra* sp.	0.33	1.67	0.67	0.67
		Nassariidae	*Nassari uscastus*	5.33	13.33	7.67	16.00
		Cylichnidae	*Cylichnella* sp.	2.33	3.33	5.00	6.67
		Turbinidae	*Guildfordi ayoka*	0.00	0.00	0.33	0.00
		Calliostomatidae	*Photinula caerulescens*	2.00	1.00	0.67	3.67
		Costellaridae	*Vexillum*sp.	0.00	1.67	0.00	0.33
		Epitoniidae	*Epitonium scalare*	0.00	0.33	0.00	0.33
		Cerithiidae	*Rhinoclavis sordidula*	0.00	0.00	0.67	0.00
		Turridae	*Gemmula* sp.	1.00	0.00	0.00	0.00
2	Bivalve	Cardiidae	*Fulvia scalata*	0.67	3..00	3.00	3.67
		Arcidae	*Anadara* sp.	1.00	8.00	1.00	3.33
		Tellinidae	*Tellina agilis*	0.67	5.67	0.67	6.67
Abundance				**39**	**60**	**149**	**134.33**
Total Species				**8**	**10**	**10**	**8**

At the polyculture area, the composition of species abundance in the first and second sampling was dominated by *Turritella* sp. (Gastropoda: Fam. Turritellidae), 70.22 % and 49.19 % respectively, while the lowest abundance was belonged to *Mitra* sp. (Gastropoda: Fam. Mitridae) in the first sampling time, and *Epitonium scalare* (Gastropoda: Fam. Epitoniidae) in the second sampling time, 0.82 % and 0.69 %, respectively. At the reference sites, the composition of gastropods abundance during the study period was dominated by *Turritella* sp. (Fam. Turritellidae), i.e. 88.91% in the first sampling and 61.47% in the second sampling.

Meanwhile, the composition of species abundance of Class Bivalvia was dominated by 63.83% of *Fulvia scalata* Vidal, 1994 (Fam. Acrididae) in the first sampling time and 51.33% of *Tellina agilis* Stimpson, 1857 (Fam. Tellinidae) in the second sampling time, indicating that the species composition of Class Gastropoda is higher than members of Class Bivalvia. *T. agilisis* is a suspension feeder that lives in shallow water with sandy or muddy buttoms [11]. The data of macrobenthic mollusk abundance was highlighted only on the dominant taxa for multivariate analysis using bubble plots of NMDS. They display how the abundance of the four dominant taxa relates to overall assemblage differences between sites. Based on the bubble plots NMDS ordination, the four dominant taxa/species did not clearly show involvement in driving/shifting the ordinate position of station on the graph, except *Tellina agilis* (Fam. Tellinidae), as shown in Figure 2. The differences in relative proportions of *T. agilis* are responsible for the horizontal spread of stations in that the higher proportions of this taxa are plotted to the left on the ordination. However, based on Figure 3, the two species showed involvement in driving/shifting the ordinate position of two stations of the reference areas from the first sampling time by *Rynoclavis sordidula* (Fam. Cerithidae), and three stations of the polyculture areas from the first sampling time by *Gemulla* sp. (Fam. Turridae) away from other stations, thus are considered as bioindicators of environmental disturbance. The differences in relative proportions of *Gemulla* sp. and *R. sordidula* are responsible for the horizontal spread of stations in that the higher proportions of these taxa were plotted to the bottom and the upper right of the configurations, respectively.

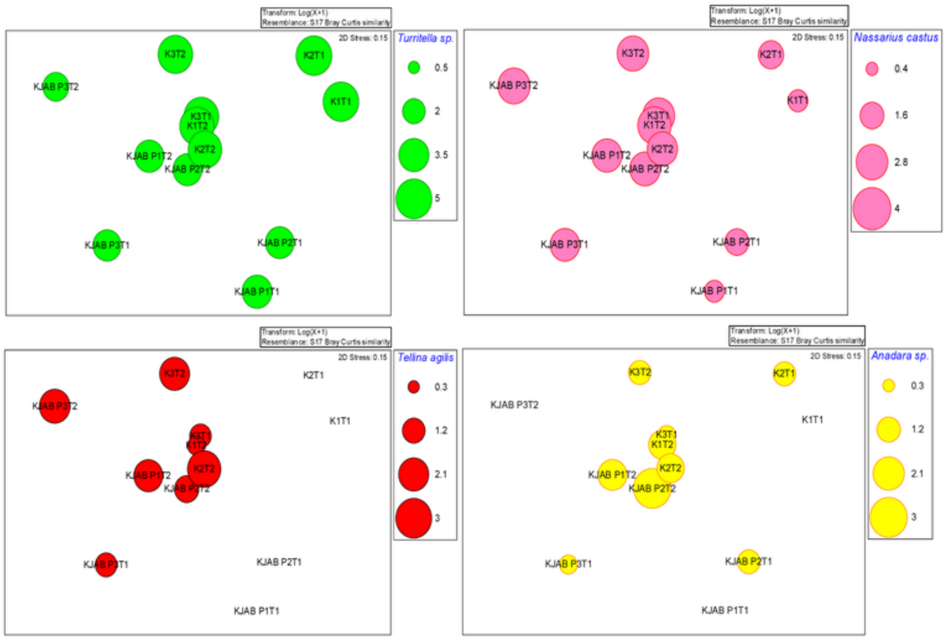

Figure 2. The role of dominant taxa in driving ordinate position on the 2d NMDS superimposed using bubble plots.

Members of Family Cerithidae is a worldwide marine mollusks, most commonly inhabiting shallow waters in tropical and subtropical regions. They are commonly found in a range of habitats such as sand flats and are abundant on trees and roots on the seaward side of mangroves which serve as microalgal or detritus feeders [12], [13], [14]. *R. sordidula* is mostly found in mud at 40 m water depth, few shells in muddy sand and sand between coral patches. In sampled mud station it is frequently colonized by solitary corals. Conservative position of corals on the backside of the shells may suggest that the gastropods were colonized by the coral during lifetime. They can be found in clearer shallow water in coral reefs, preferring sandy to muddy bottoms, feed on deposits in the sediment. *Gemmula* thrive mostly in the deeper waters of the tropics. They can survive in muddy environment. They are carnivores that live by eating polychaetes and seaworms.

The table 2 shows the correlations between physical-chemical factors, substrate granular composition and the carbon and nitrogen content with the abundance of mollusks were analyzed using BIO-ENV on PRIMER V.6.1.5 software. Based on the table, the correlation between the abundance of mollusks with DO, C content and N content have the greatest degree of correlation, which is 0.457. This indicates that the value of DO, the carbon and nitrogen content are the abiotic components that has the most influence in the abundance of mollusks in the Awerange Gulf. The shape of the rank/abundance plot can also provide an indication of dominance or evenness, for example, steep plots signify assemblages with high dominance and shallower slopes indicate higher evenness [15], by then the tendency of environmental disturbance may be assessed [3], [16]. Figure 4 shows cumulative dominance curves between stations at polyculture and reference areas projected based on sampling time.

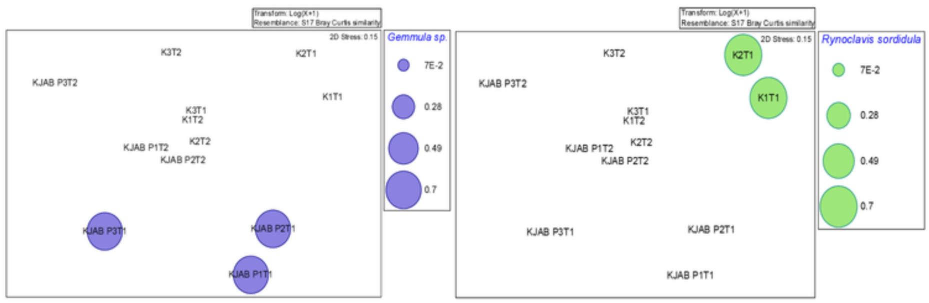

Figure 3. The Tendency of *Gemulla* sp. and *Rynoclavis sordidula* which Present at Certain Location and Absent at Other Location in Driving Ordinate Position on 2D NMDS Bubble Plot.

Table 2. Analysis of the correlation between biotic and abiotic component in reference area and polyculture cage area

No.	Correlation (r)	Variable	Variable amount
1	0.457	4,7,8	3
2	0.447	1,7,8	3
3	0.437	1,4,7,8	4
4	0.416	2,4,7,8	4
5	0.41	1,3,4,7,8	5
6	0.401	1,2,4,7,8	5
7	0.394	1,2,7,8	4
8	0.391	3,4,7,8	4
9	0.388	2-4,7,8	5
10	0.388	1-4,7,8	6

Note: 1) pH, 2) Temperature, 3) Salinity, 4) DO, 5) Sand, 6) Silt, 7) C Content, 8) N Content

Station KJAB P1T1 (polyculture area, first sampling time), KJAB P2T1 (polyculture area, first sampling time), and KJAB P1T2 (polyculture area, second sampling time) represent curves with steep plots, indicate the dominance value of the stations are high. On the other hand, curve with shallower slope as shown for K1T1 (reference area, first sampling time) indicates higher evenness.

In a stressful condition, macrobenthos community are dominated by organisms with *k*-selection strategy in their lives, called opportunistic species, with characteristics such as relatively small body, short lifetime, dominance in species quantity with low biomass, has high potential reproductive level and early maturity [17]. Based on cumulative *k*-dominance analysis projected on each station by sampling time, station K3T1 (reference area; first sampling time) and KJAB P3T2 (polyculture area; second sampling time) located below others curves, indicate the highest evenness and diversity rather than the other stations and station K2T1 (reference area; first sampling time) andK3T2 (polyculture area, second sampling time) located on the top, indicate the lowest value of evenness and diversity [18].

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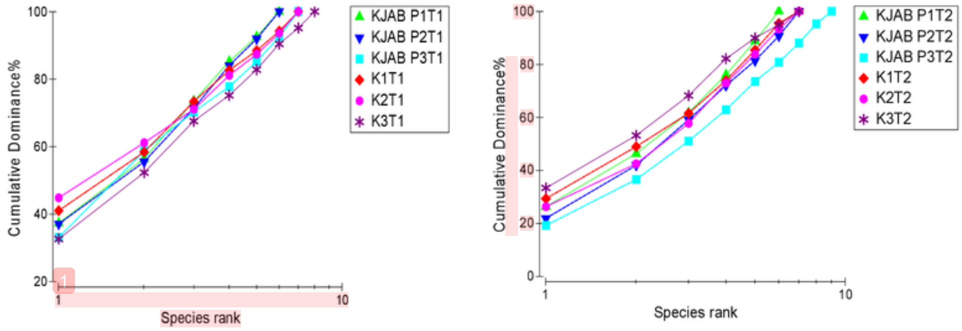

Figure 4. Cumulative dominance curves between stations at polyculture and reference areas projected based on sampling time: (A) First sampling time (October 2014); and (B) Second sampling time (April 2015).

4. Conclusions

Based on the bubble plots NMDS ordination, the four dominant taxa/species did not clearly show involvement in driving/shifting the ordinate position of station on the graph, except *T. agilis*. Furthermore, the two species of *Rynoclavis sordidula* (Fam. Cerithidae) and *Gemulla* sp. (Fam. Turridae) showed involvement in driving/shifting the ordinate position of the stations. The higher proportion of *Gemulla* sp. was plotted to the bottom, whilst the higher proportions was plotted to the upper right of the configurations. Based on abiotic-biotic correlation analysed using BIO-ENV, the value of DO, the carbon and nitrogen content are the abiotic components that has the most influence in the macrobenthic mollusk abundance in the Awerange Gulf. Based on cumulative *k*-dominance analysis projected on each station by sampling time, station K3T1 (reference area; first sampling time) and KJAB P3T2 (polyculture area; second sampling time) located below others curves, indicate the highest evenness and diversity rather than the other stations and station K2T1 (reference area; first sampling time) and K3T2 (polyculture area, second sampling time) located on the top, indicate the lowest value of evenness and diversity. These results imply that the graphical methods using cumulative *k*-dominance curves and bubble plots ordination on the macrobenthic mollusk abundance are effective and sensitive method to assess the indication of disturbance of the area.

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