Morphometric study of macrozoobenthos community in Manado Beach, North Sulawesi

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Abstract. This study is aimed at knowing the relationship between morphometric size variables of the macrozoobenthos community in Manado Beach, North Sulawesi. Sample collection was done downstream of Bahu River (station 1), Malalayang District, and Tondano River (station 2), Manado, North Sulawesi using random purposive sampling and quadrat line transect method. Sampling used a corer modified from 3 inch-pipe at 25 cm depth. Sampling site determination used a purposive random sampling method under consideration of topography and sampling site conditions. The morphometric size of the macrozoobenthos community was determined using Image J software, and the relationship between variables using principal component analysis (PCA). Results showed that in station 1, the cumulative eigenvalue of axis 1 (F1) to axis 2 (F2) reached a variance of 1.8229, indicating that data variance could be explained up to axis 2 as much as 83.8095%. The eigenvalue of variance on axis 1 to 2 reached 1.8980, meaning that data variance of the analysis could be explained up to axis 2 (F2) by as much as 84.6361% (Station 2). If the cumulative eigenvalue on both stations up to axis 3 (F3) does not reach 80%, PCA cannot be relied to analyze the issue above. Based on the correlations matrix, it is apparent that one of the morphometric variables, such as Feret Angel (FA) has a relationship with other variables, such as AA, PE, CI, F, FX, FY, AR, and SO in station 1. This condition is similar to that in station 2, except for AA, FY, and MF which could be separated into two groups. The closest relationship in station 1 and station 2 is the correlation matrix that has a correlation coefficient approaching to 100% on other variables that can be explained very high.
Key Words: downstream, eigenvalue, imageJ, taxa, transect.

Introduction. Coastal areas are stretches of land where the terrestrial and marine ecosystems interact and have an impact on one another (Kay & Alder 1999). These are also a boundary where two major environments, terrestrial and marine, change (Sorensen & McCreary 1990). It is a transitional area between the terrestrial and marine environments in which the terrestrial part is still influenced by marine cycles, such as tides, sea wind, and seawater penetration, while the marine part is influenced by terrestrial activities, such as sedimentation and freshwater flow (Ketchum 1972).

Indonesia has 81 km of coastline that holds large natural resources. Manado Beach is also an intertidal zone inhabited by various marine biota, such as corals, coral fishes, and other benthic organisms, such as macrozoobenthos. This area is generally flat and has rocky, sandy, muddy substrates.

Macrozoobenthos (macrofauna) is > 1 mm-sized zoobenthos (Suartini et al 2010). It covers Phylla: Arthropoda (Class: Malacostraca), Annelida (Class: Oligochaeta Polychaeta), Mollusca (Class: Gastropoda), Nematoda and Annelida. According to Pennak (1989), zoobenthos can act as primary, secondary or higher consumers. Macrozoobenthos is also natural food for bottom-feeder fishes.

Macrozoobenthos plays a very important role in the ecological system because it is food for young fish before they grow to be food for other bigger animals. Despite its crucial status, macrozoobenthos has mostly been neglected in conservation studies. Since macrozoobenthos is very important, future studies need to be done on its role in food web and how its population is influenced by pollution and climate change. Macrozoobenthos can also take secondary and tertiary positions in the food chain in aquatic communities and can be used to assess the physical, chemical, and biological environmental equilibrium (Agustinus et al 2013).

Macrozoobenthos possesses an important role as aquatic community building and food source for various fishes. Giere (2009) stated that most macrozoobenthos select the microhabitat in response to food availability to support growth. Its presence can become a bioindicator of ecological status and detect water pollution in coastal environment (Sahidin et al 2018).

The occurrence of macrozoobenthos is dependent upon the availability of nutrients that are the most important materials in the biological function that is able to restrict the productivity in an ecosystem, such as macrozoobenthos is limited by the availability of organic matter. Increased nutrient availability sometimes can reduce the stability of the food chain (Eleftheriou 2013). Since the role of macrozoobenthos is important in the water and there is a lack of information on macrozoobenthos in Manado Beach, this study is focused on morphometric conditions of macrozoobenthos in Manado Beach, North Sulawesi using ImageJ software to know the relationship between morphometric size variables of the macrozoobenthos community.

Material and Method. The study was conducted from July to October 2021 in Manado Beach, station 1 in Bahu River downstream and station 2 in Tondano River downstream, Manado Municipality, North Sulawesi. Sample identification and analysis were accomplished in the Molecular Biology and Marine Pharmaceutical Laboratory of the Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, North Sulawesi.

Sampling site determination. Sampling site determination used a purposive random sampling method under consideration of topography and sampling site conditions. The sampling site was focused on the littoral zone of Manado Beach by establishing 2 stations, Bahu River downstream (1°27'42" N and 124°49'12" E) (influenced by traditional market activities) and Tondano River downstream (1°29'57" N and 124°50'27" E) (affected by tourism activities (Figure 1). Each station was set with 3 sampling points to represent the site conditions.

Figure 1. Study site map.

Sample collection and handling. Sampling was done in the lowest low tide to ease sample collection and avoid water currents and tidal waves. It was also supported by snorkeling when the tide was rising. Macrozoobenthos was taken with 3 inch-corer as deep as 25 cm and a small scoop on a 1m x 1m quadrat at 100 m x 100 m area as many as 30 sampling points. The macrozoobenthos was hand-sorted, cleaned in the water, and put into labeled sample bottles containing a mixture of rose Bengal, 4% formaldehyde, and 10% alcohol, then put into the coolbox for further analysis. The samples were observed in the laboratory under the microscope to determine the taxa and make the count (Eleftheriou 2013). Large-sized specimens were photographed using a Kodak-merked pocket camera. The identification of macrozoobenthos taxa followed FAO (1998), Hook (2008), WoRMS (World Registers of Marine Species / WoRMS) (2019), and Marine Species Identification Portal (2021).

Data analysis. Morphometric data were analyzed using ImageJ software. The parameters measured were described as follows:

AA = the selected value is in square pixels. An area is a unit that is calibrated, such as square millimeters, square centimeters, and others;
PE = perimeter is the length of the selection's outer boundary;
CI = circ. (circle): 4π * area/perimeter ^ 2. A value of 1.0 indicates a perfect circle. Getting closer to 0.0 indicates an elongated shape. The value may not be valid for very small particles;
F = feret size is based on mean statistics after rotating the object through all possible different angles. Feret diameter is the longest distance between two points along the selected area, also known as the maximum caliper; Feret X and feret Y are the coordinates of the initial feret diameter (on X and Y axes):
FX = feret X is the coordinate of the initial feret diameter (on X axis);
FY = feret Y is the coordinate of the initial feret diameter (on Y axis);
FA = feret angle is the feret value (0-180 degrees), the angle between the feret diameter and the line parallel to the X axis of the image;
MF = MinFeret is the minimum caliper diameter;
AR = AR (aspect ratio): major_axis / minor_axes;
RO = rotation (roundness): 4 * area / (π * major_ axis ^ 2), or the reciprocal of the aspect ratio;
SO = solidity: area / convex area.

To know the correlation between morphometric measurement variables of the macrozoobenthos, principal component analysis (PCA) was applied using Version 2022.1 XLSTAT. The typical data analyzed with PCA are a matrix of n individuals (row) and p-variable (column). Data processing used Excel 2007 and excel add in version 14 Excelstat software.

Results and Discussion

Macrozoobenthos morphometry. Mean FA in station 1 and station 2 has a much higher value than other variables (AA = area; PE = perimeter; CI = circlularity; F = Feret; FX = Feret X; FY = Feret Y; MF = min Feret; AR = aspect ratio; RO = round; SO = solidity (Table 1).

As a whole, the variables in station 1 (Bahu) are higher than those in station 2 (Tondano). This condition could result from that station 1 passing a traditional market without good waste management so that people release various wastes directly into the river. The organic wastes released into the river are available for macrozoobenthos growth. Accumulated organic waste in Labuange sediment impacts the presence of macrozoobenthos (Harahap et al 2018). However, station 2 which is also located near a traditional market has good waste management from the local government so that the wastes are not released into the river, and go through a waste treatment process.

The difference in the morphometric size (Table 1) between both localities could be caused by several factors, such as river currents from the upstream and tides the downstream. This finding is in agreement with Windarto et al (2022) that tides are one of the determining factors for the presence of macrozoobenthos on the coast. Tides also, in general, influence the shell growth of some species of mollusks (Islami 2012). Bartol et al (1999) added that the growth of oyster *Crassostrea virginica* is affected by tide conditions and substrate thickness. The oyster can grow faster at a depth of 90 cm than 25 cm.

Table 1

Summary statistics in station 1 and station 2

Summary statistics in station 1							
Variable	*Observations*	*Obs. with missing data*	*Obs. without missing data*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
AA	8	0	8	0.4909	2.0093	0.8722	0.5295
PE	8	0	8	3.2694	12.4728	5.3297	3.0568
CI	8	0	8	0.2001	0.6469	0.4635	0.1640
F	8	0	8	1.1281	3.6763	1.5738	0.8545
FX	8	0	8	3.5656	17.6033	10.9948	5.9791
FY	8	0	8	2.0718	13.7614	7.7928	3.9914
FA	8	0	8	7.5557	107.7535	85.8117	33.4012
MF	8	0	8	0.6092	1.1793	0.8293	0.2392
AR	8	0	8	1.1653	4.5758	2.1229	1.0832
RO	8	0	8	0.2195	0.8586	0.5670	0.2065
SO	8	0	8	0.7107	0.9696	0.8808	0.0984
Summary statistics in station 2							
Variable	*Observations*	*Obs. with missing data*	*Obs. without missing data*	*Minimum*	*Maximum*	*Mean*	*Std. deviation*
AA	8	0	8	0.1099	1.2339	0.6057	0.4339
PE	8	0	8	1.7586	10.3787	4.1123	2.8416
CI	8	0	8	0.1093	0.7404	0.4969	0.2058
F	8	0	8	0.4557	2.7055	1.2456	0.7427
FX	8	0	8	4.3996	10.7488	8.1025	2.3763
FY	8	0	8	2.7767	13.8478	7.3659	4.3405
FA	8	0	8	2.9697	103.7989	82.5977	32.5572
MF	8	0	8	0.3396	0.9353	0.6219	0.2172
AR	8	0	8	1.3301	4.6444	2.1538	1.1287
RO	8	0	8	0.2222	0.6636	0.5421	0.1652
SO	8	0	8	0.5996	0.9536	0.8648	0.1194

Measurements of macrozoobenthos morphometry used imageJ software, then PCA with Pearson correlation method using XLSTAT 2014.5.03 software, and indicated as Eigenvalue (Table 2).

Table 2

Eigenvalue in station 1 and station 2

Eigenvalue in station 1						
	F1	*F2*	*F3*	*F4*	*F5*	*F6*
Eigenvalue	**7.3961**	**1.8229**	**1.5376**	0.1507	0.0822	0.0104
Variability (%)	67.2373	16.5722	13.9781	1.3702	0.7473	0.0949
Cumulative %	67.2373	**83.8095**	97.7875	99.1577	99.9051	100.0000
Eigenvalue in station 2						
	F1	*F2*	*F3*	*F4*	*F5*	*F6*
Eigenvalue	**7.4109**	**1.8980**	0.8623	0.6658	0.1078	0.0553
Variability (%)	67.3720	17.2541	7.8392	6.0523	0.9800	0.5024
Cumulative (%)	67.3720	**84.6261**	92.4653	98.5176	99.4976	100.0000

The relationship strength between the variables used is presented in the correlation matrix (Tables 3 and 4). Based on the eigenvalue of the component > 1 (bold black in Table 2), there are 2 factors formed, axes F1 and F2, with percent cumulative variability of 83.81% (station 1) and 84.63% (station 2), and the rest 16.19% (station 1) and 15.37% (station 2) are explained by other axes up to F6. The size variable component in Figure 2 shows that the morphometry which has a direct relationship with one another is PE and F with a very close Euclidean angle and approaches the correlation circle at the most on the positive axis compared to other components (Figures 2 and 3). It could result that several species, such as *Frigidoalvania*, *Hemigrapsus*, and *Oenopota*, are present in the groundwater seepage and not in the seawater seepage. A similar condition is shown in Figures 4 and 5 for PE and F variables. Therefore, PE gives a bigger influence (83.81%) than F (16.19%) on the morphometric size of the macrozoobenthos in station 1 (Figure 2 and Table 2a). In station 2 (Figure 4 and Table 2b), PE also gives a higher effect (84.63%) than F (13.37%) on the morphometric size of the macrozoobenthos. The high percent relationship indicates that the morphometric size gives a good contribution to the macrozoobenthos growth.

Table 3

Correlation matrix (Pearson (n)) in station 1

Variables	*AA*	*PE*	*CI*	*F*	*FX*	*FY*	*FA*	*MF*	*AR*	*RO*	*SO*
AA	**1.00**	**0.92**	-0.50	**0.91**	-0.41	-0.39	**-0.83**	**0.77**	**0.64**	-0.27	**-0.56**
PE	**0.92**	**1.00**	**-0.78**	**0.96**	**-0.67**	**-0.70**	**-0.96**	**0.60**	**0.77**	-0.45	**-0.82**
CI	-0.50	**-0.78**	**1.00**	**-0.63**	**0.95**	**0.95**	**0.80**	-0.32	**-0.57**	0.36	**0.97**
F	**0.91**	**0.96**	**-0.63**	**1.00**	-0.47	**-0.56**	**-0.94**	**0.46**	**0.89**	**-0.63**	**-0.68**
FX	-0.41	**-0.67**	**0.95**	-0.47	**1.00**	**0.87**	**0.66**	-0.38	-0.38	0.15	**0.89**
FY	-0.39	**-0.70**	**0.95**	**-0.56**	**0.87**	**1.00**	**0.78**	-0.24	-0.49	0.32	**0.91**
FA	**-0.83**	**-0.96**	**0.80**	**-0.94**	**0.66**	**0.78**	**1.00**	-0.47	**-0.81**	**0.53**	**0.81**
MF	**0.77**	**0.60**	-0.32	**0.46**	-0.38	-0.24	-0.47	**1.00**	0.02	0.40	-0.34
AR	**0.64**	**0.77**	**-0.57**	**0.89**	-0.38	-0.49	**-0.81**	0.02	**1.00**	**-0.90**	**-0.59**
RO	-0.27	-0.45	0.36	**-0.63**	0.15	0.32	**0.53**	0.40	**-0.90**	**1.00**	0.39
SO	**-0.56**	**-0.82**	**0.97**	**-0.68**	**0.89**	**0.91**	**0.81**	-0.34	**-0.59**	0.39	**1.00**

Note: Bold values indicate correlation > 50% for either positive or negative; AA = area; PE = perimeter; CI = circularity; F = Feret; FX = Feret X; FY = Feret Y; FA = Feret Angel; MF = min Feret; AR = aspect ratio; RO = round; SO = solidity.

Table 4

Correlation matrix (Pearson (n)) in station 2

Variables	*AA*	*PE*	*CI*	*F*	*FX*	*FY*	*FA*	*MF*	*AR*	*RO*	*SO*
AA	**1.00**	**0.76**	**-0.51**	**0.82**	-0.01	-0.08	-0.47	**0.96**	**0.51**	-0.64	-0.50
PE	**0.76**	**1.00**	**-0.84**	**0.96**	**-0.51**	-0.47	**-0.84**	**0.75**	**0.89**	**-0.90**	**-0.80**
CI	**-0.51**	**-0.84**	**1.00**	**-0.74**	**0.81**	**0.73**	**0.67**	-0.49	**-0.79**	**0.73**	**0.85**
F	**0.82**	**0.96**	**-0.74**	**1.00**	-0.35	-0.44	**-0.75**	**0.76**	**0.89**	**-0.95**	**-0.66**
FX	-0.01	**-0.51**	**0.81**	-0.35	**1.00**	**0.68**	**0.55**	-0.05	**-0.56**	0.40	**0.69**
FY	-0.08	-0.47	**0.73**	-0.44	**0.68**	**1.00**	0.18	-0.07	**-0.54**	**0.54**	0.31
FA	-0.47	**-0.84**	**0.67**	**-0.75**	**0.55**	0.18	**1.00**	-0.44	**-0.86**	**0.75**	**0.87**
MF	**0.96**	**0.75**	-0.49	**0.76**	-0.05	-0.07	-0.44	**1.00**	0.41	**-0.53**	-0.49
AR	**0.51**	**0.89**	**-0.79**	**0.89**	**-0.56**	**-0.54**	**-0.86**	0.41	**1.00**	**-0.96**	**-0.74**
RO	**-0.64**	**-0.90**	**0.73**	**-0.95**	0.40	**0.54**	**0.75**	**-0.53**	**-0.96**	**1.00**	**0.61**
SO	-0.50	**-0.80**	**0.85**	**-0.66**	**0.69**	0.31	**0.87**	-0.49	**-0.74**	**0.61**	**1.00**

Note: Bold values indicate correlation > 50% for either positive or negative; AA = area; PE = perimeter; CI = circularity; F = Feret; FX = Feret X; FY = Feret Y; FA = Feret Angel; MF = min Feret; AR = aspect ratio; RO = round; SO = solidity.

Based on the correlations matrix, it is apparent that several variables have a relationship with other variables in station 1 and station 2 (Table 3 and 4) that could be separated into two groups. In station 1, the closest relationship is the correlation matrix that has a correlation coefficient approaching 100% on other variables (Table 2) and can be explained in Figure 2 and Figure 3. The positive-positive quadrat (green) is represented by the PE variable due to being the closest to the correlation circle and has a larger value at the horizontal axis than that of AA and MF indicating that the green variable PE has the strongest positive variable compared with other green ones, AA, and MF, while the blue variables in the negative-negative quadrat are represented by SO compared to FY, CI, and FX because SO is the closest to the correlation circle. Red and brown variables that are in the positive-negative quadrat are determined by the closest position to the correlation circle and vertical axis or horizontal axis of the quadrat, and therefore, the brown variable is represented by RO compared to FA, whereas the red variable is represented by AR than F because the distance is closer to the correlation circle. The variable colors above that represent each color are needed to involve in the next analytical model, for instance multiple regression, cluster analysis or other analysis. Figure 2 shows that the most influential morphometric size of macrozoobenthos in quadrat 1 is represented by the PE variable (green) because PE is the closest to the correlation circle and F1 axis compared to that of AA and MF, the quadrat 2 is represented by AR (red), the quadrat 3 is represented by SO (blue), and the quadrat 4 is represented by RO (brown). Figure 3 indicates that the macrozoobenthos influencing the PE variable is *Pagurus* (green), then *Nais* (red), *Melanella* (blue), and *Margarites* (brown). According to Hidayat et al (2004), mollusks are animals common in waters with highly dissolved or suspended organic matter, and these materials are potential food for benthic mollusks, especially filter feeders and deposit feeders. Shalihah et al (2017) also found a positive correlation between mollusks and organic food in downstream of Betahlawang River Kabupaten Demak, Central Java Province, Indonesia.

Station 2 also shows the closest positive correlation to other variables approaching 100% (Table 4). Figure 4 demonstrates that the most influential morphometric size of the macrozoobenthos in quadrat 1 is represented by AA variable (green) due to its position closest to the correlation circle and F1 axis compared to MF, F, and PE, the quadrat 2 is represented by AR (red), the quadrat 3 is represented by RO (blue), and the quadrat 4 is represented by FX (brown). Figure 5 describes that the macrozoobenthos influencing AA are *Melanella* (green), then *Nais* (red), *Pagurus* (blue), and *Margarites* (brown).

The correlation between variables with the main axis can be seen in the correlation circle, namely the variable coordinate or the quality of the variable representation at the main axis indicated with the variable distance to the axis F1 (Figures 2, 3, 4, and 5). The closer the variable is to the axis, the stronger the correlation will be (positive or negative). The interpretation of variables affecting the macrozoobenthos morphometry is shown in the correlation circle of axis 1 and axis 2 (F1-F2) in station 1 (Figures 2 and 3) and station 2 (Figures 4 and 5).

Figures 2 and 3 show the taxa in station 1, with the high morphometric role (blue), are represented by *Frigidoalvania*, *Melanella*, *Cylichna*, and *Oenopota*, due to having similar positive vector direction to the morphometric size of FX, FY, SO, and CI that approach to F1 axis and positive correlation circle, whereas the morphometry of CI tends to be reduced due to being closer to F2 axis and far from the correlation circle. *Margarites* (brown) is the only macrobenthos taxa representing the morphometric variables RO and FA whose position is close to the F2 axis and far from the correlation circle. It means that *Margarites* is less influential to describe the morphometric sizes. *Hemigrapsus* and *Pagurus* (green) describe the MF, AA, and PE variables. The F variable is only represented by *Nais*. The crucial roles influencing the macrozoobenthos morphometric size are the variables on the F1 axis and approach the correlation circle since PE is the closest to F1 and the correlation circle indicating that PE has the strength to describe the extent of the effect on the morphometric size compared to AA and MF (Figure 2).

Figures 4 and 5 show that in station 2, the taxa possessing high morphometric role (blue taxa) are represented by *Pagurus Oenopota*, *Hemigrapsus*, and *Frigidoalvania* due to having a similar positive vector direction to the morphometric size of RO that approaches the F1 and negative correlation circle. Only *Nais* represents the size of AR (red) and *Melanella* (green) significantly affects PE, F, MF, and AA. The PE variable is the most influential variable because it exists on F1 axis and approaches the correlation circle, even though AA is closer to the correlation circle but near the F2 axis.

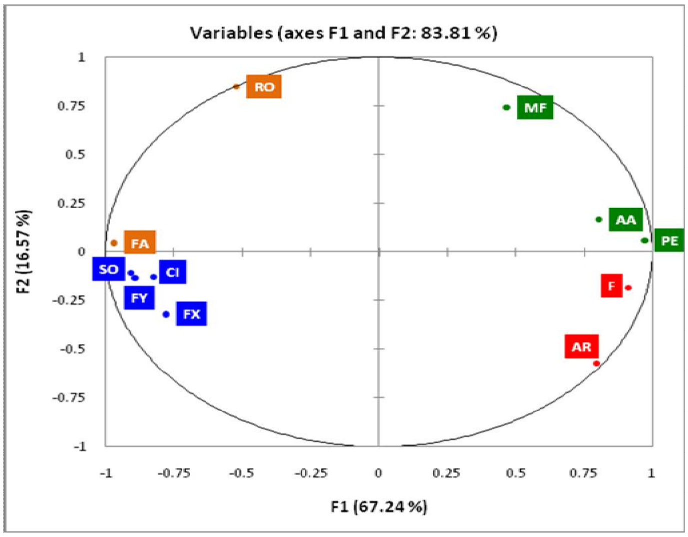

Figure 2. Correlation circle between variables at the main axis (F1 & F2) in station 1.

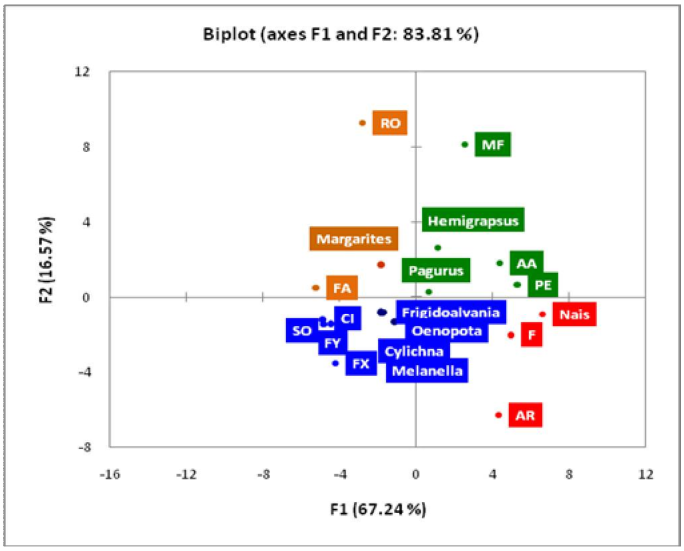

Figure 3. Taxa distribution at biplot at the main axis (F1 & F2) in station 1.

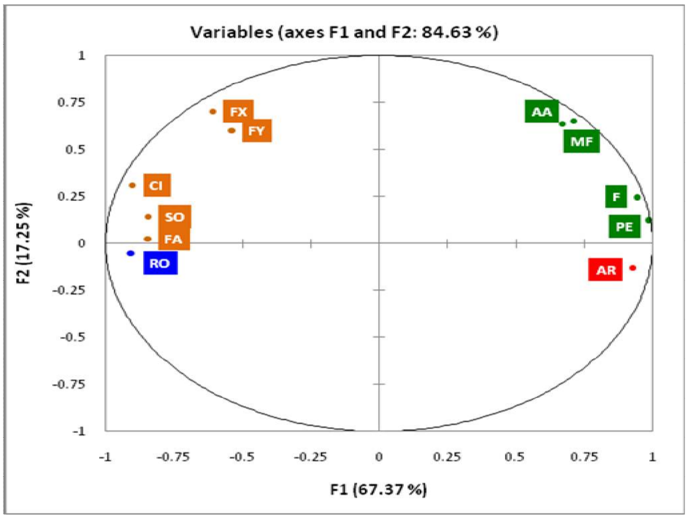

Figure 4. Correlation circle between variables at the main axis (F1 & F2) in station 2.

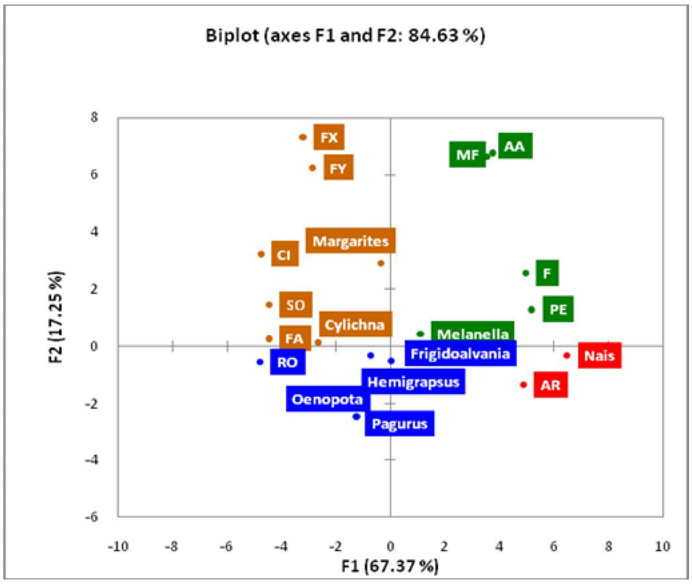

Figure 5. Taxa distribution at Biplot of the main axis (F1 & F2) in station 2.

Conclusions. The highest role of the morphological size of macrozoobenthos in station 1 was represented by *Pagurus* for the morphology of AA and PE, *Nais* for F and AR, *Melanella* for CI, FY, SO, and FX, and *Margarites* for FA, respectively, since all those taxa had the largest role at the horizontal axis and correlation circle. In station 2, the highest role of the morphological size of the macrozoobenthos was represented by *Melanella* for PE and F, *Nais* for AR, *Pagurus* for RO, and *Cylichna* for CI, SO, and FA, because those taxa had the largest role at the vertical axis and correlation circle. This information could help study the ecological importance of macrozoobenthos occurrence and could be used for environmental management along the coastal regions.

Conflict of interest. The authors declare that there is no conflict of interest.

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