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Assessment of aquatic ecosystem health in Gorgan Bay based on biotic indices

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ABSTRACT
The relationship between spatial patterns of macrobenthic community characteristics and environmental conditions (dissolved oxygen, temperature, salinity, organic matter content and sediment particle size) was studied at 12 stations in Gorgan Bay (South Caspian Sea, Iran), seasonally in 2017. A total of 8803 individuals belonging to 11 families and 11 species were identified. Three species of polychaeta including *Streblospio gynobranchiata*, Tubificidae and *Hediste diversicolor* were the most dominant group in terms of abundance, making up 79.3% of all specimens. The highest density (4733 ind m^{-2}) was observed at St. 1 while the lowest (1166 ind m^{-2}) at St. 9. Biotic indices (i.e. AMBI, BOPA and BENTIX) were used to determine the quality of the bay. Gorgan bay is divided into two distinct zones (eastern and western regions) based on MDS analysis and ecological status. Generally, eastern part of the bay revealed the lowest abundance and biodiversity of macrobenthos. Our results suggest the effect of sediment particle size and TOM as significant parameters on distribution of macrobenthic community in Gorgan bay, South Caspian Sea.

Keywords: Gorgan Bay, Environmental parameters, Biotic indices, Spatial distribution.
Article type: Research Article.

INTRODUCTION
Coastal areas have always undergone significant physical changes due to the operation of natural and anthropogenic factors. One of the major natural factors affecting coastal environments is global warming and climate change, which has become one of the most important human concerns today (Paice & Chambers 2016). Recent assessments of ocean surface, sea level and metrology data over the last century show that ocean levels have risen by an average of 10-20 cm. Earth's mean global surface temperature in 2019 was the second warmest, since modern record-keeping began in 1880. Global temperatures in 2019 were 1.8 degrees Fahrenheit (0.98 ºC) warmer than the 1951 to 1980 mean (GISTEMP Team 2020; Fig. 1). It is predicted that the sea level will rise by about one meter by 2100 and that the geometric distribution of this increase in sea level and oceans will not be the same across all coastal regions (Vermeer & Rahmstorf 2009). Gorgan Bay is economically and ecologically important and plays a significant role in the reproduction of aquatic, bony and cartilaginous fishes and attraction of winter migratory birds (Shahlapour *et al.* 2019). The most recently discussion in the scientific community was on the possibility of drying up in Gorgan Bay due to the rapid decline in the Caspian Sea water level (Ranjbar & Hadjizadeh Zaker 2017; Khoshravan *et al.* 2019). Increasing temperature by 1.5 ºC followed by further evaporation of water and reduced precipitation, over-exploitation of groundwater resources and freshwater (reduced inflow of rivers into Gorgan bay due to dam construction in upstream areas for agriculture activities) and water retreat have drastically reduced the water level of Gorgan Bay (Ranjbad & Hadjizadeh 2017). In addition, rapid urbanization, industrialization and agricultural activities in near coastal zone, has resulted in large quantities of contaminants being discharged into the coastal zone and serious damage to the coastal ecosystem

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(Yang *et al.* 2015). Other intensive anthropogenic activities (e.g., fishing, sewage discharge, aquaculture, and tourism) also have impacts on the environment (Gholizadeh & Patimar 2018). Macrobenthos are the most extensively-used biota for assessing ecological status of water bodies, due to their rapid response to human and natural pressure, and unique community features (e.g., long life span, relatively sedentary, different species composition with different tolerances to pressure, and a significant role in bioturbation; Pearson & Rosenberg 1978; Borja *et al.* 2000). According to the rank of benthic quality assessment indices, in terms of bio-indices, AZTI Marine Biotic Index (AMBI), Benthic Opportunistic Polychaete- Amphipod Index (BO2P) and BENTIX are relatively more responsive to anthropogenic stress (Simboura *et al.* 2007). AMBI is based on a classification of macrobenthos species (ecological groups; EG) regarding to their different tolerances to human pressure, diversity (H′). In addition, AMBI of the macrobenthic assemblage, is a comparison to a reference condition (Borja *et al.* 2000). All indices have been successfully used and validated in many case studies of various coastal areas of the Persian Gulf, Caspian Sea (Iran), Indian Ocean, Yellow Sea and Mediterranean Sea (Simboura & Argyrou 2010; Shokat *et al.* 2010; Sivaraj *et al.* 2014; Li *et al.* 2014; Basatnia *et al.* 2015). Given this introduction, such studies are necessary to identify coastal ecosystem conditions and to assess the health status and knowledge of their benthic fauna as well. Therefore, the present study was carried out with the aim of identification and investigation of spatial changes in macrobenthic community in Gorgan Bay and its relation with environmental stresses by ecological and qualitative evaluation of the bay in the Southern Caspian Sea, Iran using AMBI, BO2P and BENTIX bio-index.

MATERIALS AND METHODS

Sampling area and procedure

Gorgan Bay is located in an east-west direction of Golestan Province, southeast coast of the Caspian Sea, Iran. Its area is around 400 km^2 with the maximum length of 60 km. Most part of the bay has low depth (less than 2 m), the maximum depth is 4.5 m and it increases from west to east.The bay is surrounded by urban areas and agricultural lands. It is the marine part of a larger protected area inclu ding a peninsula called "Miankaleh Wildlife Refuge" and an international wetland (Ramsar Convention on Wetlands 1971). *Roppia maritime* is a seagrass species that dominates the eastern and shallow parts of the bay and in some places becomes so intensive that makes boating impossible. A total of 12 stations were investigated in Gorgan Bay, seasonally (4 times in February, June, August and November) in 2017 to take sediment samples for considering the environmental conditions and biotic index (Fig. 2). Sampling stations are subjected to different sources of disturbance (Table 1). St. 1 was located lose to the jetty of Ashoradeh Island where fishing and tourism boat commuted. The impact of the sturgeon pen culture activities on benthic communities was investigated at three pen culture sites in Ashoradeh Island, Gorgan Bay (Station 3).

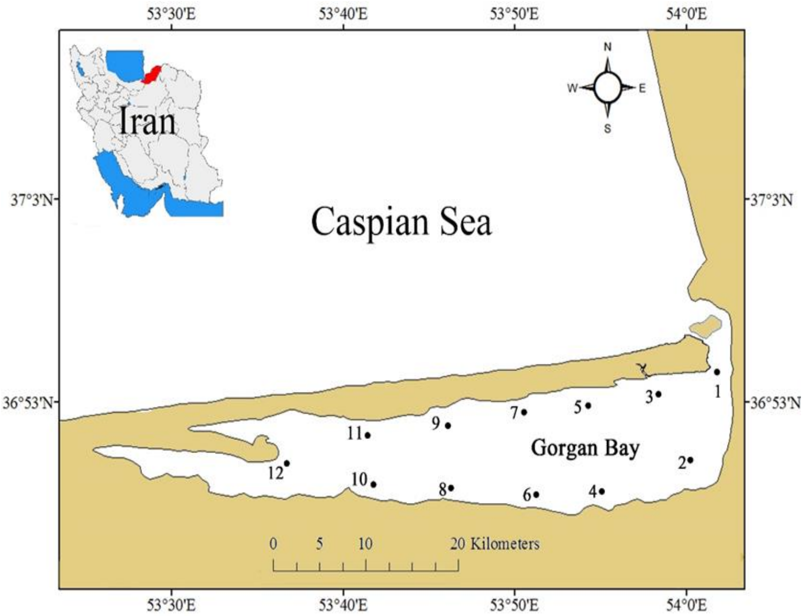

Fig. 1. The sampling stations in Gorgan Bay, southeastern the Caspian Sea, 2017.

Table 1. Stations location and summary of their main disturbance sources.

Studied stations (geographic coordinates)	Depth (m)	Sources of disturbance
Eastern part	2.1	Ashoradeh Island, fishing and tourism
S1 (36°53′32″N/54°01′70″E)		
S2 (36°50′40″N/53°58′43″E)	1.6	Ghareh-Su River
S3 (36°53′05″N/53°58′07″E)	0.9	Sturgeon farming in cages and tourism
S4 (36°49′19″N/53°54′22″E)	1.4	Urban wastewater
S6 (36°48′25″N/53°52′20″E)	1.1	Agricultural activities
Western part		Agricultural activities
S8 (36°48′43″N/53°51′26″E)	0.7	Agricultural activities
S10 (36°48′35″N/53°43′50″E)	0.5	
S11 (36°50′57″N/53°43′38″E)	0.9	livestock activities
S12 (36°49′35″N/53°43′50″E)	0.5	Miankaleh Wildlife Refuge (migratory birds and tourism)

The sediment samples were collected from 3 replicates with a Van Veen grab (0.025 m^2) and screened using a sieve (0.5 mm mesh) then sieved through a 0.5 mm mesh (Gholizadeh *et al.* 2012). The organisms retained on the sieves were preserved in 4% formalin for detailed examination in the laboratory. The organisms found were identified to the lowest taxon possible (Barnes 1987; Milligan & Hulbert 1997; Stock *et al.* 1998; Nikula & Vainola 2003; Bouchet 2014). Sediment samples were also collected to measure the amount of organic matter and determine the sediment grain size. The samples collected at each station were placed in separate containers, along with the details of the sampling stations, and then transferred to the laboratory for examination. The organic matter was measured by burning method (550 °C for 2 h) and metal gradation using a particle analyzer (Tyson 2012).

Environmental factors

The measured chemical variables were temperature, dissolved oxygen (DO) and salinity, which were measured using a Water Quality Checker (Model: HACH sensionTM 156-378 multiparameter meter). Principal components analysis (PCA) was performed to determine different groups of sampling stations based on environmental parameters. Prior to this analysis, all environmental parameters were log transformed and normalized. The sampling station scores on the first two axes were correlated with environmental variables using the Spearman Rank correlation test (Clarke & Warwick 1994).

Statistical analysis

Significant differences in the calculated parameters among different seasons and stations were determined by One-Way ANOVA. LSD test ($P < 0.05$) was performed to measure the significant differences among the study stations (in SPSS Ver. 24). Similarity among sites was analyzed by ordination techniques (nMDS) based on Bray–Curtis similarity matrix. The reliability of nMDS representations of assemblage similarities was assessed by their stress values; stress < 0.2 was considered as acceptable (Clarke *et al.* 2014). The dbRDA classification test was used based on the Distance Based Linear Models (DistLM) to determine how the environmental parameters affecting the presence and distribution of macrobenthos at different study stations. The DistLM analysis was performed using the step-wise method and the determined Akaike's information selection criterion (AIC). Before doing the analysis, the highly correlated variables ($r < 0.9$) were excluded from the set of variables. In addition, environmental variables were first normalized and the Resemblance matrix in the dbRDA plot was based on Bray-Curtis similarity matrix on main data of the benthos abundance without conversion. All the above analyses were implemented in Primer software (version 6) with PERMANOVA+ add-on (Anderson *et al.* 2008).

Biological data processing

Univariate indices of community parameters including abundance (N, ind), number of species (S), species diversity (Shannon diversity index, (H'), species richness Margalef's (d) and evenness Pielou's (J) were calculated using statistical package of PRIMER Ver. 6. The dominant index (Y) (Chen & Wang 1995) of species was calculated by the following formula:

$Y = (n_i/N) f_i$

where N is the total abundance of all the study stations, n_i is the abundance of the species i of all the study stations, and f_i is the occurrence frequency of the species i of all the study stations. Species i can be defined as the dominant species when $Y > 0.02$.

A species is considered a group indicator if the results are significant to a level of 0.05. The IndVal coefficient was calculated using the PC-ORD v.4.0 program for Windows (McCune & Mefford 1999). AMBI was computed using the AMBI program (version 5.0) freely available online at http://ambi.azti.es, and on the basis of the AMBI guidelines (Borja & Muxika 2005; Table 2). BENTIX only recognized two groups (sensitive and opportunistic species), corresponding to ecological groups 1 and 2; and ecological groups 3-5, respectively, of the AMBI (Table 3). The Benthic Opportunistic Polychaete Amphipod Index (BO2P) is also according to the ecological features of specific taxonomic sets and compares percentage ratios of opportunistic Polychaetes and Amphipods (Dauvin & Ruellet 2007). The environment condition is of good quality, the BO2P is low, but when environmental conditions degenerate, the BO2P is high.

$$\text{BO2P} = Log\ (\frac{fp}{fp+1}+1)$$

where *fp* is opportunistic polychaete abundance, *fa* is the proportion of amphipods (excluding genus *Jassa*) abundance. BO2P index differs between 0 (when *fp* = 0) and 0.30103 (when *fa* = 0).

Table 2. Indices calculated from macrobenthic databases in Gorgan Bay.

Biotic index	Algorithms	References
AMBI	[(0×%GI)+(1.5×%GII)+(3×%GIII)+(4.5×%GIV)+(6×%GV)]/100	Borja *et al.* (2000)
BENTIX	(6×%GS+2×%GT)/100	Simboura and Zenetos (2002)
Shannon index (H')	$-\sum[\left(\frac{ni}{N}\right) log_2 \left(\frac{ni}{N}\right)]$	Pielou (1975)
Margalef (d)	(S-1)/log N	Margalef (1968)
Pielou Evenness (J)	H'/log S	Pielou (1966)

For the AMBI: %GI, relative abundance of disturbance-sensitive species; %GII, relative abundance of disturbance-indifferent species; %GIII, relative abundance of disturbance-tolerant species; %GIV, relative abundance of second-order opportunistic species; %GV, relative abundance of first-order opportunistic species. For the BENTIX: %GS, relative abundance of sensitive species = %GI + %GII; %GT = relative abundance of tolerant species = %GIII + %GIV + %GV. For the Shannon index: *ni*, number of individuals belonging to the i^{th} species; *N*, total number of individuals. For the Margalef index, *S* is the total species richness (as number of taxa) at the sampling station.

Table 3. Ecological quality status (EcoQ) threshold based on biological indicators.

EcoQ status	AMBI	BENTIX	Shannon index (H′)	Benthic community health	Site disturbance category
High	0-0.2 0.2-1.2	6-4	>4	Normal Impoverished	Undisturbed
Good	1.2-3.3	4-3	4-3	Unbalanced	Slightly disturbed
Moderate	3.3-4.3	3.0-2.5	3-2	Transitional to polluted	Moderately disturbed
Poor	4.3-5.5	2.5-2.0	2-1	Polluted- Transitional to heavily polluted	Heavily disturbed
Bad	>5.5	0	<1	heavily polluted	Extremely disturbed
References	Borja *et al.* (2000) Muxika*et al.* (2005)		Simboura & Zenetos (2002)	Labrune *et al.* (2005)	

At each station, two samples were taken:

1- Hydrological sample for the measurement of physico-chemical parameters: temperature, pH, conductivity, salinity, turbidity, dissolved oxygen, nitrites and orthophosphates.

2- Biological sample for the determination of planktonic species.

The planktonic samples were taken using two plankton nets, differentiated by the respective mesh spaces of 100 µm and 500 µm. For each station, vertical lines were conducted to a depth of 10 m and samples were kept in 5% formalin. The identification and counting the planktonic larvae and eggs were made using an inverted microscope and a count of platinum Bogorov 22 mL (80 × 100 mm).

Due to the low abundance of the larvae, the samples were subjected to full counting without splitting. The densities of planktonic eggs and larvae were expressed in individuals m^{-3}.

Statistical processing and data analysis

Hydrological parameters were processed by the principal component analysis (PCA), justified by the different nature of the variables considered and their unit of measurement. This method aimed to evaluate the relative importance of each element in the structure of the whole (Pronier 2000). The relationships between the environmental characteristics and the planktonic densities of different taxa identified were studied using the canonical correspondence analysis (CCA) or correspondence analysis on instrumental variables (AFCVI) (Ter Braak 1986; Lebreton *et al.* 1988), whose objective was to simultaneously process two data tables (environmental and faunal variables) for the same surveys.

RESULTS AND DISCUSSION

Environmental data

The ranges of stations depth were between 0.5 to 2.5 m, which was highest at St. 2 (in the east) and was least at St. 12 (in the west). The maximum water temperature was 28.3 ºC in the summer, while the minimum was 10.1 ºC in winter season (Table 4). The results showed a significant difference between the temperature at various seasons ($p < 0.05$). The recorded average salinity was 11.2 psu in Gorgan Bay, indicating that the water is brackish. The maximum concentration of salinity was 14.7 psu in the summer, while the minimum (8.9 psu) in the winter (Table 4). The results showed a significant difference between the salinity levels at various seasons ($p < 0.05$). The highest amount of dissolved oxygen was 7.9 mg L^{-1} in winter, while its minimum, i.e., 6.2 mg L^{-1} in the summer. The amount of dissolved oxygen showed a significant difference in different seasons ($p < 0.05$). In addition, there was a significant correlation between temperature and salinity ($r = 0.79$; $p = 0.05$) and also dissolved oxygen ($r = 0.69$; $p = 0.05$) during the seasons. The study stations were divided into two different groups: western stations with the lowest depth and higher organic materials, while eastern stations with higher silt and clay. Overall, macrobenthic diversity decreased from the west to the east. The results showed that TOM and sediment texture were the most effective factors on the macrobenthic community characteristics, whereas macrobenthic species were mostly affected by salinity and dissolved oxygen. The macrobenthic communities suffered from anthropogenic activities directly or indirectly, which involved in the interactions across the sediment-water interface (Ahn & Choi 1998; Mirzoev 2021). Human activities, environmental stress and climate change caused the river discharge reduction and elevating salinity. Generally, macrobenthic diversity upraised from the east to the west. Additional results revealed that salinity and sediment texture were the most influencing parameters on the macrobenthic assemblage features, while sediment particle size, salinity and depth affected macrobenthic species. The macrobenthic communities also have shown a decreasing trend in species composition since the 2000s in Gorgan Bay. The mean values of sand, silt, clay, and total organic matter (TOM) measured were 45.76±12, 51.9 ±10, and 2.34 ±1.3 and 6.96 ± 1.3%, respectively. The highest and lowest temporal mean for silt, clay, and TOM were in spring (61.1, 5.9, and 7.1%) and summer (40.1, 0.14, and 6.2%), respectively. The maximum and minimum amounts of sand (23.1-51.9%) were observed in the summer and spring, respectively (Table 4). Generally, sand and silt have been formed over 90% of the bay sediment components. The western sediments of northern sampling stations exhibited coarser composition (mostly composed of sand), where current dynamics prevent the accumulation of fine particles. The textural gradient indicates a shift towards lower sand content which sediments are dominated by silt component toward eastern, mouth and southern part of the bay. The different textural properties of the sediments indicate special hydrodynamic processes and depositional conditions in the two zones of the bay. Based on sediment characteristics in the bay, a clear spatial variability was observed, which was generally dominated by silt-loam sediments. Almost the western stations of the bay were composed of coarser compounds (often sand). These stations have approximately lower depths, so that water currents prevent the accumulation of smaller particles, and towards the eastern portion, the slope of the sedimentary tissue causes a displacement toward smaller- and less sandy- particles. Given the significance of the total organic matter content at different stations and seasons, the trend of organic matter alterations from the west to the east was declining. However, from spring to winter, the elevation can be influenced by the growth of marine plants (especially *R. maritima*), calmness in the western part of the bay in the spring and increase in their growth during summer that has the ability to convert to organic matter. Therefore, spatial and temporal variations of organic matter of sediment can depend on seasonal variations, physical conditions due to the region's current trends and the instability of surface sediments, and on the other hand, it is influenced by the main source of food, soluble and insoluble materials in coastal waters receiving urbane wastes and agricultural fertilizers, which are mainly discharged through rivers (Gholizadeh *et al.* 2012a). Total organic matter as a food item just affected Shannon diversity, while previous studies showed that this factor was correlated with density and distribution of macrobenthos in the South Caspian Sea (Taheri & Foshtomi 2011; Gholizadeh *et al.* 2012b; Ghasemi *et al.* 2016).

Table 4. Environmental variables (mean value of four seasons sampling) measured in the stations.

Station	Sand (%)	Silt (%)	Clay (%)	Tom (%)	DO (mg L^{-1})	Temperature (°C)	Depth (m)	Salinity (ppt)
1	31.7	67.2	1.1	5.17	6.51	20.5	2.1	10.3
2	35.1	60.8	4.1	3.19	7.14	21.6	1.5	10.5
3	35.5	60.8	3.7	7.09	6.57	21.2	2.2	10.1
4	46.8	51.5	1.7	7.3	6.48	20.8	2.7	10.15
5	50.8	47.3	1.9	7.11	6.52	21.1	2.1	10.2
6	41.2	54.48	4.32	7.32	6.47	20.7	1	10.1
7	81.7	17.1	1.2	3.3	6.88	20.75	1.1	10.25
8	52.1	45.4	2.5	6.18	6.46	21.32	1.3	10.52
9	52.1	45.8	2.1	6.37	6.97	21.44	2	10.32
10	59.4	37.8	2.8	7.6	6.65	22.1	0.7	10.7
11	57.6	41.2	1.2	7.37	6.46	21.6	1.2	10.1
12	41.6	56.9	1.5	7.2	6.43	22.18	0.5	10.1

The result of the PCA is revealed in Fig. 3. PCA1 and PCA2 accounted for 32.5% and 23.8% of the total variance, respectively. First axis (PCA1) was related to the percentage of sand (coefficient = 0.86) and silt (coefficient = -0.87). PCA2 summarized variance due to dissolved oxygen (-0.73), clay (-0.66) and TOM (0.58). In summary, western stations with the lower depth and higher clay content separated from eastern deeper stations with higher sand and TOM content. The results showed that silt, clay and depth are the most important factors in stations 1, 2, 3 and 4, while at stations 7 and 10, the sand exhibited the greatest effect.

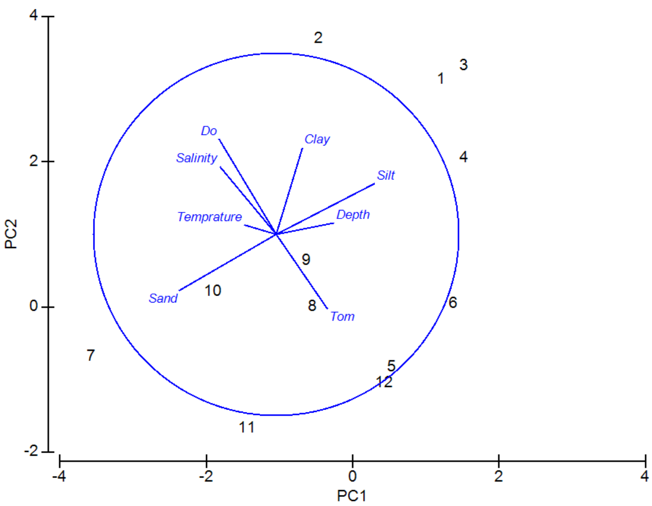

Fig. 3. Principal component analysis (PCA): spatial presentation of the study stations based on environmental data.

Biotic parameters

During the study period, 3 phyla and 11 families of benthos including ringed worms phylum with 79.3% (polychaeta with 2 families and oligochaeta with 1 family), Mollusca phylum with 11.29% (bivalves with 4 families), Arthropoda phylum with 3.03% (crustacean with 2 families) and insects with 1 family (2.69 %) (*Chironomus albidus*) were identified (Fig. 4). The largest distribution of benthos in the western region was related to Arthropoda and Mollusca, while the largest distribution in the eastern region to the ringed worms (Annelida phylum) (Fig. 5). In this study, the total number of collected benthos individual and the average annual density (±SD) were 8803 and 2201 ind m^{-2}, respectively. In addition, the highest number was observed in the spring (3542 ind m^{-2}) while the lowest in the summer (1092 ind m^{-2}). The highest density (5111 ind m^{-2}) was observed at St. 1, while the lowest (600 ind m^{-2}) at St. 9. The maximum abundances were observed along the mouth and eastern stations 1, 2 and 6 (> 6000 ind m^{-2}), while lower abundances in the western zone. The ringed worms were the major group of detected macrobenthos by 95% of samples. Furthermore, Mollusca and Arthropoda were observed in 42% and 33% of the samples. During the current study, *Streblospio gynobranchiata*, Tubificidae and *Hediste diversicolor* were dominant species and observed at all stations (Y > 0.02).

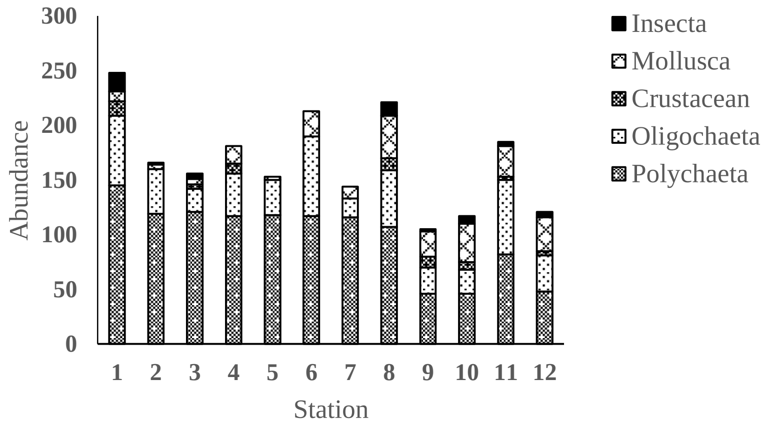

Fig. 4. Macrobenthic abundance of sampling station in Gorgan Bay.

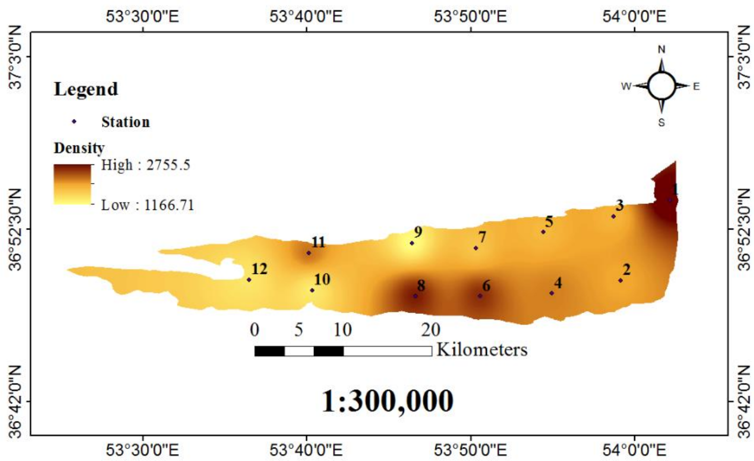

Fig. 5. Spatial distribution of macrobenthic abundance in the sampling stations.

The Shannon-Wiener diversity index (H_0) varied from 1.15 (St. 2) to 1.97 (St. 10), with the average value of 1.59 ± 0.29. The Margalef richness index (D) varied from 0.8 (St. 5) to 2.15 (St. 9), with the average value of 1.37±0.39. The Pielou's evenness index (J) (Pielou, 1966) varied from 0.64 (St. 2) to 0.9 (St. 10), with the average value of 0.77 ± 0.07 (Fig. 6).

The n-MDS analysis showed the similarity between macrobenthic communities in different stations which ranged from 30%-64%. Based on the similarity values, all stations were divided into two groups (west and east regions) at an arbitrary similarity level of 38.84%. Group I (west region) included five stations (8- 12) with a similarity value of 78.05%. Group II (east region) consisted of seven stations (1-7) with a similarity value of 76.45%. Similar results were obtained by n-MDS ordination plots (stress value <0.1; Fig. 7).

Given the definition of sites into groups using west and east regions of Gorgan Bay, the IndVal coefficient revealed fifteen significant indicator species/taxa (Table 5). However, based on IndVal scale proposed, only four species exhibited high indicator values (>70%): The polychaetes *Streblospio gynobranchiata* and *Hypanio lakowalewskii* were belonged to group 1 (east region), while the bivalvia *Abra segmentum* and *Mytilaster lineatus* to group 2 (west region).

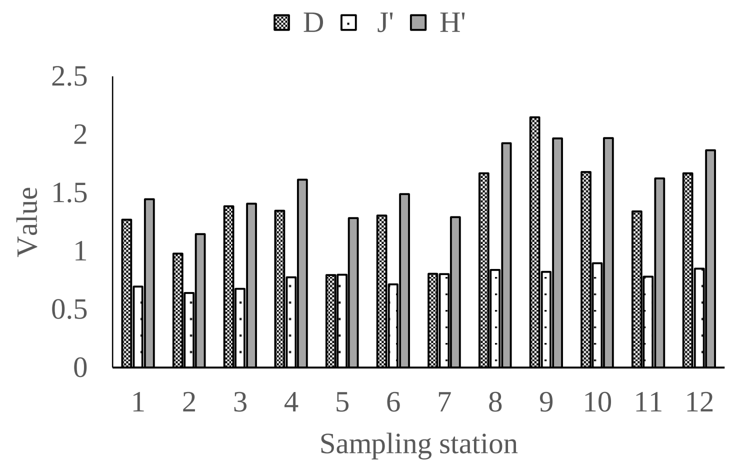

Fig. 6. Margalef richness index (D), Pielou's evenness index (J) and Shannon-Wiener diversity index (H_0) of each sampling station.

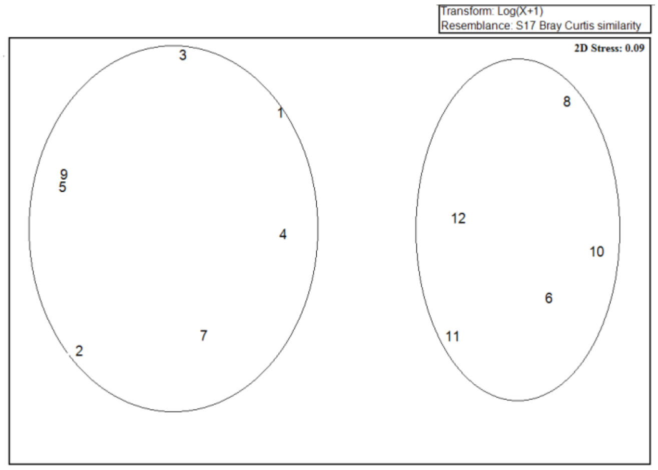

Fig. 7. Non-metric multidimensional (nMDS) plot of macrobenthic communities (based on Bray-Curtis similarity and log (X+1) transformed data on macrobenthic species).

Species–environment relationships

The relationship between macrobenthic community and environmental parameters was analyzed with multiple regression analysis (DISTLM). The results are showed in distance-based redundancy analyses biplots (dbRDA, Fig. 8).

Table 5. Indicator Value (IndVal) coefficient for significant species related to the categorical groups. Groups: (i) west region and (ii) east region.

Indicator species	EG AZTI list	BENTIX EG	Indicator value (IndVal)	Significance level (p)
Hediste diversicolor	III	GS	50.4	0.98^{n}
Streblospio gynobranchiata	IV	GT	**70**	0.002
Hypaniola kowalewskii	III	GT	**85.5**	0.002
Tubificoides fraseri	V	GT	50.7	0.93^{n}
Pontogammarus rubustoides	IV	GS	64.9	0.08
Stenocuma gracilis	II	GS	14.7	0.72^{n}
Cerastoderma glaucum	III	GT	65.2	0.03
Abra segmentum	III	GT	**82.6**	0.01
dreissena polymorpha	IV	GS	55.3	0.13^{n}
Mytilaster lineatus	I	GS	**80.2**	0.02
Chironomus albidus	III	GT	63.6	0.13^{n}

According to Marginal tests within the DistLM procedure, the most environmental parameters, including sand, silt, clay and depth, displayed an important relationship with the macrobenthic community structure when measured alone (Table 6). There was no correlation between TOM ($p = 0.57$), temperature ($p = 0.88$), salinity (p = 0.61), dissolved oxygen (p = 0.54) and macrobenthic abundances. In marginal tests, sand and silt contributed alone with 98%, clay with 25% and depth with 21% to the model (Table 6). Although, the dbRDA revealed that the highest correlation ($R^2 = 0.63$) with the macrobenthic community structure using all 8 environmental parameters, the *F*-values revealed that sand and silt were the most effective at environmental parameters based on their biota (Table 6). dbRDA revealed that sand ($R^2 = 0.97$), silt ($R^2 = - 0.96$) and temperature (R2 = 0.58) were positively correlated with dbRDA axis 1, whereas clay (R2 = 0.5) was correlated with dbRDA axis 2 (Fig. 7). In the case of the R^2 criterion, according to the stepwise selection, the sequential tests showed a combination of four factors, namely sand, TOM, clay and depth which significantly elucidated 32.3% of the variation in macrobenthic assemblage structure (Table 6). Therefore, sand, TOM and clay are the best parameters to account for variation in the macrobenthic assemblage structure in this study.

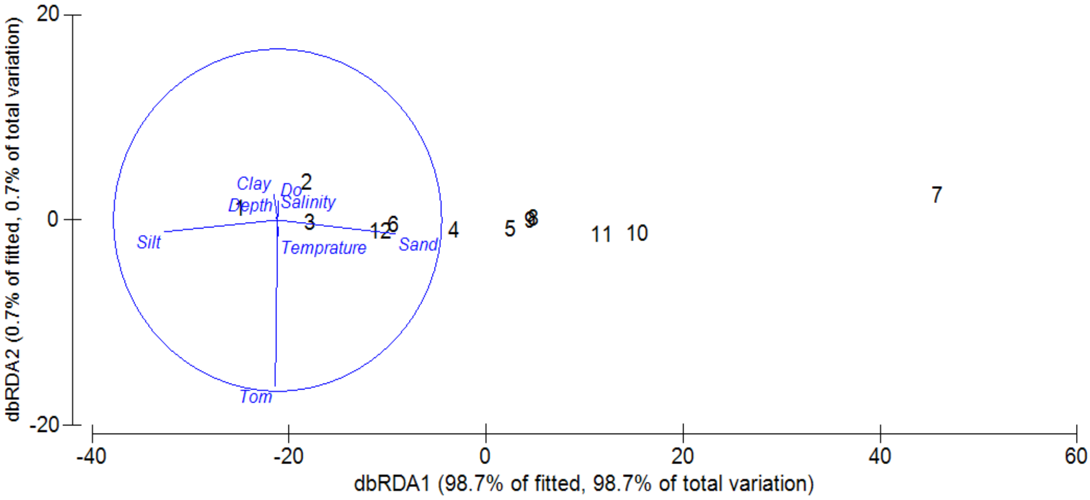

Fig. 8. A distance-based redundancy analysis (dbRDA) ordination of study samples by the macrobenthic assemblage structure.

Among all the macrobenthos identified (11 taxa), over 20% were not listed in any ecological group (EG) in the AZTI database. The highest value (4.73) of AMBI index was observed at St. 2, while the lowest value (3.6) at St. 10. The trend of AMBI index changes from west to east of the bay was increasing. According to AMBI index, 75% of the stations exhibited a moderate status, while 25% (stations 1, 2 and 8) were in poor condition.

Table 6. DistLM marginal and sequential tests, displaying the influence of environmental parameters on the macrobenthic assemblage.

Marginal tests				
Variable	SS (trace)	Pseudo-F	P- value	Proportion %
Sand	3998.5	676.71	0.001	0.98
Silt	3997	659.65	0.001	0.98
Clay	604.36	3.75	0.04	0.25
Tom	139.58	0.35	0.57	0.03
Temperature	14.554	0.04	0.88	0.04
Salinity	111.69	0.28	0.61	0.03
Do	153.16	0.39	0.54	0.04
Depth	446.85	3.24	0.04	0.21
Sequential tests				
Variable	SS (trace)	Pseudo-F	P- value	Proportion %
Sand	3998.5	676.71	0.001	0.98
Tom	26.908	7.5254	0.002	0.006
Clay	24.426	25.199	0.001	0.006
Depth	5.2586	14.749	0.001	0.03

Total average of BO2P index in Gorgan Bay was 0.04. The range of BO2P index was changed based on depth (0.02-0.07). The maximum value of BO2P index (0.07) was observed at high depth (2-3.5 m), while the minimum value (0.02) at low depth (0-1 m). Therefore, the trend of changes from shallow to deep was increasing. The range of BO2P index alterations was based on station (0.009- 0.073). The maximum BO2P index was observed at St. 2

(0.073), while minimum at St. 12 (0.009). The trend of alterations in BO2P index from west to east of the bay was increasing. The average of BENTIX index during the sample period at different stations was 1.6 ± 0.1. Its maximum value was 2.09 at St. 10, while its lowest (1.21) in St. 10. The process of changing BENTIX index based on the station from the west to east of Gorgan Bay was decreasing. Based on this index, all stations exhibited poor ecological status (Table 7). Due to spatial variations in the number and frequency of macrobenthic population in Gorgan Bay, Shannon diversity index in the eastern part displayed lower ecological quality than the western part with poor condition which may be due to the inflow of rivers and surface water channels around the bay, especially in the eastern margin, with greater depth and stress in this area, consequently leading to the specific population distribution in the bay and affecting its quality. Generally, according to the average of all calculated indices, Shannon and Benthic indices showed a poor ecological status of Gorgan Bay; AMBI index showed a moderate ecological situation in the bay. The results of different indices showed various degrees of ecological situations (from excellent to bad) at different stations. Almost most of the lower quality stations were located in east of the bay, which can be due to the hydrodynamic conditions of the area and more unfavorable conditions for different species of benthos because of its proximity to Qarasu River and other discharges from around the bay. Gholizadeh & Patimar (2018) reported that there was a negative correlation between all of the measured biological indices and the metals concentrations in sediments, exhibiting a negative effect of metal concentrations on macrobenthic communities. Therefore, environmental variables and pollutions have relative importance in the structure of ringed worm (Annelid) communities in the interstitial waters of the bay. Due to changes in BENTIX index values at different stations, the trend of this index increased from east (to greater depth) to west (to lower depth) of the bay, indicating better ecological status of the western part than the eastern. The ecological quality of BENTIX index was the same at all stations and the quality was poor. Therefore, this index cannot separate the eastern and western parts.

Table 7. Values of the classification metrics and respective EQS assessment in the all stations.

Station	%GS	%GT	Shannon index (*H'*) (ECoQ)	BO2P (ECoQ)	AMBI (ECoQ)	BENTIX (ECoQ)
1	3.3	96.7	1.96 (poor)	0.063 (good)	4.4 (poor)	2 (poor)
2	2.1	97.9	1.67 (poor)	0.07 (good)	4.73 (poor)	2 (poor)
3	2.5	97.5	2.03 (moderate)	0.041 (good)	4.17 (moderate)	2.01 (poor)
4	6.7	93.3	2.33 (moderate)	0.062 (good)	4.08 (moderate)	2 (poor)
5	0	100	1.85 (poor)	0.047 (good)	4.29 (moderate)	2.04 (poor)
6	10	90	2.7 (moderate)	0.032 (good)	3.72 (moderate)	2 (poor)
7	11.9	88.2	2.71 (moderate)	0.044 (good)	3.7 (moderate)	2.03 (poor)
8	1.4	98.6	2.09 (moderate)	0.05 (good)	4.57 (poor)	2 (poor)
9	0	100	1.86 (poor)	0.046 (good)	4.02 (moderate)	2.05 (poor)
10	14.7	85.3	2.72 (moderate)	0.031 (good)	3.59 (moderate)	2.09 (poor)
11	6.5	93.4	2.34 (moderate)	0.022 (good)	4.3 (moderate)	2.01 (poor)
12	5.8	94.2	2.69 (moderate)	0.009 (high)	4.05 (moderate)	2 (poor)

The range of the BO2P index alterations was based on station (0.009-0.073). The highest and lowest values were observed at St. 2 (0.073) and St. 12 (0.009), respectively. The increasing trend of BO2P index from west to east of the bay may indicate a difference in the sediment quality of the bay at some stations. But according to the BO2P index, the entire Gorgan bay bed is in acceptable condition (good and excellent), which is in contrast to the results of other indices, including Shannon and BENTIX. However, BO2P index like BENTIX index, cannot distinguish the eastern and western parts of the bay well. Using common benthic indices (AMBI, BO2P, BENTIX, H') to assess the ecological status of semi-closed coastal ecosystems and transitional aquatic ecosystems showed that the results of these indices are not in agreement with each other and different results of ecological situation of Gorgan Bay is based on the limitations of indicators. Particularly, the dependence of most of these indicators and the classification of their ecological status is attributed to the habitat characteristics, especially the natural level of silt-clay particles and the location of stations in tidal zone (Blanchet *et al.* 2008).

CONCLUSION

This study investigated macrobenthic communities and their relationship with environmental parameters in surface sediments of Gorgan Bay. The results showed that three species of ringed worms (*S. gynobranchiata*, *H. versicolor,* and *T. Fraseri*) are dominant species of the bay, which were present in different seasons and stations. *S. gynobranchiata* by 51% of the worm's abundance was dominant species. The distribution of 3 species of ringed worms was constant in different habitats throughout Gorgan Bay. In this study, AMBI index was a more reliable and strong indicator for assessing the bay and identified two main zones in the bay. This zoning verifies the classification of multivariate analysis (MDS), cluster and environmental parameters, and also soil gradation. The results showed that the western part of the bay has a better ecological status than its eastern part where has more depth and is located close to the mouth of Qarasu River. Therefore, human activities (urban, agriculture and tourism) in sediments of Gorgan bay exhibited a low ecological risk to biological species. Finally, future studies should include more sites and time for sampling, as well as toxicological aspects of macrobenthic community in this bay.

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