Research Article

Macrobenthos as bioindicator of ecological status in the Yekshabe creek-estuary, Persian Gulf

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Abstract
The present study was conducted with the aim of identification of macrobenthos in the Yekshabe estuary in Persian Gulf. Sampling of macrobenthos was performed from spring 2015 for one year. It was carried out seasonally by the Van Veen Grab (0.04 m²) at four stations, with three replications. The identified macrobenthos belonged to 51 families, 70 genera, 80 species, 4 classes (including Polychaeta, Malacostraca, Bivalvia, Gastropoda) and 5 Phyla. The average total density of macrobenthos was 760±614 per square meter. The most frequently observed classes of macrobenthos belonged to Polychaeta (67.8%), Malacostraca (18.5%), Bivalvia (11.6%) and Gastropoda (1.2%), respectively. According to the results of the Kruskal–Wallis test, there was no significant difference between the macrobenthos classes among the different sampling stations. The highest values of the Shannon-Wiener index of diversity (3.07) and the Margalef's richness index (4.66) were observed in spring, whereas the highest value of the Pielou's evenness index (0.95) was observed in autumn. The values of all indices at station 4 were the highest as compared to those of the others. The results of ANOVA test showed that the mean values of the indices were significantly different (p<0.05) in different seasons, whereas the mean values were not significantly different at stations.

Keywords: Persian Gulf, Yekshabe estuary, Macrobenthos, Biodiversity, Environmental factors

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Introduction
Macrobenthic communities are important component of estuarine ecosystems, playing a main role in the system dynamics (Herman and Heip, 1999). They are special animals in this context limited mobility or most of them are sessile (Olsgard and Gray, 1995; Rosenberg, 2001) and are thus directly depending on environmental conditions, and they show clear reaction to environmental changes depending on toleration of their species specific and sensitivity levels (Ferraro and Cole, 1995; Paiva, 2001; Lancellotti and Stotz, 2004). Moreover, macrobenthic community analysis provides an immediate both snapshot evaluation of current disturbance effects, like chemical and physical analyses, also an united reaction of the disorder effects over the life span of organisms. These assets have applied in benthic community analysis for the assessment of marine habitat quality to become part of international standards (Borja et al., 2003; Rosenberg et al., 2004). Bioindicators involve biological processes, species, or communities which are used to estimate the quality of the environment and how it changes over time. Anthropogenic disturbances or natural stressors are the result of environmental variations. Although, anthropogenic stressors form the main focus of bioindicator research (Mann, 1982; Rosenberg, 2001). There are many researchers that using macrobenthos as a bioindicator index for studying about estuarine or sea ecosystems (Gandomi et al. 2011; Jahani et al., 2012; Reizopoulou et al., 2014; Ejlali et al., 2017, Pazira et al., 2018). Just one study has been conducted about effect of environmental conditions on spatial distribution of macrobenthic community in the Bushehr coasts of the Persian Gulf (Farsi et al., 2013). Studying about macrobenthic species in Obidos lagoon, was identified that, between inorganic and organic contaminants sediment and structure of the benthic community, is a direct relationship (Carvalho et al., 2006). In another study, Ysebaert et al. (2003) tried to contribute macrobenthos reaction to environmental conditions for improvement of statistical predictive model in estuarine ecosystems (Thrush and Dayton, 2002). Hormozgan province has many estuaries and Yekshabe estuary is located in the vast mud lands in eastern part of Bandar Abbas and is connected with flood and seasonal rivers flowing from the plains adjacent to the Persian Gulf (Fatemi et al., 2010). There are no researches about this estuarine macrofauna. The purposes of the present study were to estimate abundance and diversity of the macrobenthic communities, using biodiversity indicators.

Materials and Methods
Study site
To study Yekshabe estuary, four stations with different geographic locations (Table 1) were identified from the beginning of the estuary to its connection to the Persian Gulf (Fig. 1). Station 1 was selected at the estuary
mouth, at the junction of the main part of estuary exposed to the land, Station 2, is located in the central part, Station 3, at the junction of the estuary with the sea, and Station 4, is located at the opposite part of the mouth lying in the Persian Gulf.

### Table 1: Features and numbers of sampling sites (2015-2016)

<table>
<thead>
<tr>
<th>Site</th>
<th>Longitude (E)</th>
<th>Latitude (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St 1</td>
<td>56˚35’</td>
<td>27˚10’</td>
</tr>
<tr>
<td>St 2</td>
<td>56˚35’</td>
<td>27˚09’</td>
</tr>
<tr>
<td>St 3</td>
<td>56˚34’</td>
<td>27˚09’</td>
</tr>
<tr>
<td>St 4</td>
<td>56˚31’</td>
<td>27˚09’</td>
</tr>
</tbody>
</table>

**Sampling**

Sampling of macrobenthos was performed from spring 2015 for one year. Sampling was carried out seasonally by the Van Veen Grab (0.04 m$^2$) with three replications. Sediment samples were transferred to plastic containers, washed and was passed through a sieve with 500-micron mesh, then fixed with alcohol 75% at the sampling site. CTD data recorder at each station performed measurement of environmental parameters including temperature, salinity, dissolved oxygen and pH. In the laboratory, the contents of each container were again passed through a 500-micron mesh size sieve.

Samples were isolated using the stereo microscope and identified by the identification keys (Wolfgang, 1986; Barnes, 1987; Fauchald, 1977; Rouse and Pleijel, 2001). The number of samples per unit area was calculated and therefore the density of each organism was calculated. The sediment was sorted with sieves (Buchannan, 1984) and Total organic matters of the investigated sediments were determined by the loss on ignition method (Holme and McIntyre, 1984).

**Statistical Analysis**

Samples for the study of macrobenthic communities were hand sorted in to
major taxonomic groups, identified to the lowest practical taxonomic level (usually species level) and counted. Macrobenthic community structure was analyzed regarding the univariate variables, abundance (N), number of species (S) and diversity (Shannon Wiener (H') and Margalef's species richness (R) and evenness (Pielou) E indices.

The Margalef's Index of Species Richness (D) is simple ratio between total species (S) and total numbers of individual (N). It can be used to compare one community with another. 

\[ D = \frac{(S - 1)\ln N}{N} \]

Where:

- \( D \) = Margalef's index,
- \( S \) = Number of species in sample,
- \( N \) = Total number of individuals in sample

The Shannon-Wiener index of species diversity (H') (Wilhm and Dorris, 1966) is defined as

\[ H' = -\sum_{i=1}^{S} P_i \ln P_i \]

Where:

- \( S \) = Total number of species in a sample,
- \( P_i = \frac{n_i}{N} \) = Proportion of individuals of the total sample belonging to the i the species, \( n_i \) = Number of individual belonging to the i the species

Evenness index (E) (Pielou, 1969) is defined as

\[ E = \frac{H'}{\ln S} \]

The Kruskal-Wallis test was used to find the significant differences in the abundance of macrobenthos groups among sites. The diversity indices were computed using the PRIMER (Plymouth Routines in Multivariate Ecological Research) Software 6 (Warwick and Clarke, 1993). Differences in the number of individuals and biodiversity index scores between the sites were analyzed using One-Way ANOVA.

**Results**

In the present study, the average total density of macrobenthos in the Yekhabe estuary was 760±614 individuals per square meter and belonged to 5 phyla (Annelida, Arthropoda, Mollusca, Nematoda, Nemertia), 5 classes, 51 families, 70 genera and 80 species (Table 2).

Among the various macrobenthos species, *Prionospio steenstrupi* from the family Spionidae and *Byblis gaimardi* from the Ampeliscidae family had the highest percentage frequency of macrobenthos (Table 2) and were present at all stations. The percentage frequency of polychaeta at Station 2 was higher than the other stations. Polychaeta was the dominant class at Stations 1, 2 and 3. Malacostraca had the highest percentage frequency of macrobenthos at Station 4 (Fig. 2).

Regarding the results of the statistical analysis, it was determined that the data on the density of identified macrobenthos had an abnormal distribution. According to the results of the Kruskal–Wallis test, there was no significant difference between the macrobenthos classes among the different sampling stations. Except for the density of gastropoda, which had a significant difference in different seasons (\( p<0.05 \)), the density of other classes in the four seasons was not significantly different (\( p<0.05 \)).
Table 2: Some dominant identified macrobenthos genera and species in the Yekshabe estuary (2015-2016)

<table>
<thead>
<tr>
<th>Polychaeta</th>
<th>Malacostraca</th>
<th>Bivalvia</th>
<th>Gastropoda</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prionospiosteensrump</strong></td>
<td>Byblisgaimardi</td>
<td>Tellinavernalis</td>
<td>Tornatina sp.</td>
</tr>
<tr>
<td><strong>Nepthys sp.</strong></td>
<td>Amphipod sp.</td>
<td>Pitar sp.</td>
<td>Hypermastic sp.</td>
</tr>
<tr>
<td><strong>Glyceria p.</strong></td>
<td>Corophium sp.</td>
<td>Lioconcha sp.</td>
<td>Tricoliaios</td>
</tr>
<tr>
<td><strong>Lamberineris sp.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Boccardia sp.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to Fig. 3, the highest percentage frequency of polychaeta was observed in summer, which gradually decreased in cold seasons. This pattern is exactly the opposite of the percentage frequency of Malacostraca and Bivalves in different seasons of the year.

According to the normal distribution of the data of the studied indices, the results of ANOVA test showed that the mean values of the Shannon-Wiener index of diversity, the Margalef's richness index and the Pielou's evenness...
index were significantly different ($p<0.05$) in different seasons (Fig. 4), where as the mean values of the Shannon-Wiener index of diversity, the Pielou’s evenness index and the Margalef’s richness index ($d$) were not significantly different between stations (Fig. 4).

![Figure 4](image.png)

**Figure 4:** Comparison of the mean values of the Shannon-Wiener index of diversity ($H'$), the Pielou’s evenness index ($J'$) and the Margalef’s richness index ($d$) at different study stations.

![Figure 5](image.png)

**Figure 5:** H) Seasonal changes in the average values of the Shannon-Wiener index ($S$), d) Margalef’s richness index ($d$) and J) Pielou’s evenness index ($J'$).

The organic matter of sediments does not show much variation in different seasons (Fig. 6) and the granulometric result show that the sediments are composed of sand, silt and clay (Fig. 6). Based on the correlation matrix of macrobenthos density with TOM and granulometric composition (Table 3), the total density of benthos organisms showed a positive correlation with silt ($p<0.05$), ($r=0.513$). Among different classes, a negative correlation was observed between Malacostraca and salinity ($p<0.05$), ($r=-0.612$).
The distance based Redundancy Analysis explained the effective environmental factors and distribution pattern of dominant species.

**Discussion**

The results of the present study showed that the most frequently observed group of macrobenthos during one year of sampling belonged to polychaeta with the frequency of 67.8% and the family Spionidae was the dominant family. In the coastal waters of Qeshm Island (Nassaj *et al.*, 2010) also, estuaries of Hormozgan province (Sharifinia *et al.*, 2018) it was found that the polychaeta were most frequent groups. In this investigation, the maximum amount of the total density of macrobenthos per unit area was observed in summer (1770 individuals per square meter) whereas the lowest amount of the total density of macrobenthos was observed in fall (181 individuals per square meter). The study of macrobenthos density in the Gabric Estuary in Jask city showed that the macrobenthos density in winter was less than summer (Soleimanirad *et al.*, 2011). It should be noted that in the present study, this

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**Table 3: Pearson’s correlation between macrobenthos density and A) TOM and granulometric composition, B) Abiotic variables.**

<table>
<thead>
<tr>
<th></th>
<th>Total Benthos</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>TOM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Total Benthos</td>
<td>1</td>
<td>-.482</td>
<td>.513**</td>
<td>-.243</td>
</tr>
<tr>
<td></td>
<td>Sand</td>
<td>1</td>
<td></td>
<td>-.988***</td>
<td>.024</td>
</tr>
<tr>
<td></td>
<td>Silt</td>
<td>1</td>
<td></td>
<td>-.180</td>
<td>.715**</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>1</td>
<td></td>
<td>-.192</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOM</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| B | Total Benthos |       | .175    | .130    | .422   | -.417  |
|   | Temp          | 1       |         | .191    | -.019  | -.554* |
|   | Sal           | 1       |         | .359    | -.096  |
|   | pH            | 1       |         | -.219   |        |
|   | O2            | 1       |         |         |        |

* = p<0.05  ** = p<0.01
increase was due to the high density of polychaetes in summer (Fig. 3), even the Malacostraca had the highest density, whereas the densities of other classes declined from spring to summer. According to the study conducted in the mangrove forests in India, the high density and diversity of macrofauna in winter was due to low temperature and stability of environmental parameters such as salinity. It was also concluded that low species diversity in summer was due to reduction of gametogenesis and reproduction, reduction of dissolved oxygen and increase of hydrogen sulfide in sediments (Sarvankumar et al., 2008). The temporal variation of macrobenthos diversity in the Yekshabe Estuary (Fig. 5) was consistent with this pattern. The disturbance of sediments due to hydrodynamic forces, such as waves and storms, cause erosion of the benthic fauna and affects its seasonal and spatial patterns (Olafsson et al., 1994). Correlation analysis (Table 4) showed that pH had a positive effect on the density and distribution of Polychaeta (p<0.05), (r=0.530), whereas pH had a negative effect on the population of Gastropoda (p<0.05), (r=-0.528). Among the sediment parameters, there was a significant correlation between silt and density of Gastropoda (p<0.05), (r=0.532). In the Yekshabe Estuary, a strong and positive correlation of silt and negative correlation of sand (Table 4) were observed with Bivalvia (p<0.01), (r=0.663) and total density of macrobenthos (p<0.05), (r=0.513). In the study of the density of Gastropoda in the coastal waters of the Oman Sea, it was found that stations with silt and loam grains have high densities of Gastropoda (Asghari et al., 2011). Studies by researchers on muddy beds indicated that densities of macrobenthos were high in these sediments (Beukema and Cadee, 1997) and living organisms such as worms were accumulated. The sediment grain property is an important factor affecting other environmental factors and plays an important role in the distribution of benthos (Gray, 1981; Dauvin et al., 2004).

Table 4: Pearson’s correlation between group of macrobenthos and TOM, granulometric composition and Abiotic variables

<table>
<thead>
<tr>
<th></th>
<th>Bivalvia</th>
<th>Gastropoda</th>
<th>Polychaeta</th>
<th>Malacostraca</th>
<th>Nematoda</th>
<th>Nemertea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>-0.633**</td>
<td>-0.522*</td>
<td>-0.406</td>
<td>-0.191</td>
<td>0.118</td>
<td>0.021</td>
</tr>
<tr>
<td>Silt</td>
<td>0.663**</td>
<td>0.523*</td>
<td>0.448</td>
<td>0.123</td>
<td>-0.11</td>
<td>-0.015</td>
</tr>
<tr>
<td>Clay</td>
<td>-0.258</td>
<td>-0.065</td>
<td>-0.307</td>
<td>0.417</td>
<td>-0.04</td>
<td>-0.037</td>
</tr>
<tr>
<td>TOM</td>
<td>0.355</td>
<td>0.26</td>
<td>0.321</td>
<td>0</td>
<td>-0.06</td>
<td>0.023</td>
</tr>
<tr>
<td>Temp</td>
<td>-0.062</td>
<td>0.02</td>
<td>0.223</td>
<td>-0.194</td>
<td>-0.156</td>
<td>0.218</td>
</tr>
<tr>
<td>Sal</td>
<td>0.314</td>
<td>-0.3</td>
<td>0.224</td>
<td>-0.612*</td>
<td>0.202</td>
<td>-0.049</td>
</tr>
<tr>
<td>pH</td>
<td>0.153</td>
<td>-0.528*</td>
<td>0.530*</td>
<td>-0.489</td>
<td>0.173</td>
<td>-0.27</td>
</tr>
<tr>
<td>O2</td>
<td>-0.334</td>
<td>-0.363</td>
<td>-0.414</td>
<td>0.067</td>
<td>-0.044</td>
<td>-0.059</td>
</tr>
</tbody>
</table>

*=p<0.05  **=p<0.01
Benthic organisms always have a tendency to choose a sediment bed that is easier to penetrate (Hossein Khezri, 2000) and sand-silt bed has a high density of benthos (Mohammed, 1995). It should be noted that the dominance of the sand substrate will decrease the content of organic matter in the sediments, thereby decreasing the presence of macrozoobenthic biota in both abundance and type and will ultimately affect ecological status (Zetler et al., 2009). The present study also showed that there was a significant negative correlation between TOM and sand ($p<0.01$), ($r=-0.696$) (Table 3). The incidence of high species diversity and total abundance of polychaetes might be attributed to lose texture of sediment character due to high content of sand (Musale and Desai, 2011) and (Ansari, 1977) reported high density of polychaetes are associated with sandy substrate.

The distance based RDA demonstrated that a 75.5% of multi variate distinction of dominant species were explicated by two original RDA axis, further proved the positive grouping influence of temperature, salinity and sand besides a negative influence of TOM and silt on spatial and temporal differentiation of *Urothoe* sp, whereas the test showed a reverse combination effect of mentioned parameters on distribution pattern of *Glycera* sp, *Paphias* sp., *Nephthys* sp., *Lumberineris* sp. and Amphipoda (Fig. 7). It should be noted that as estuaries are complex diverse ecosystems, benthic communities are controlled by a combination of factors, such as salinity, pH, tidal fluctuation, dissolved oxygen, sediment composition, and organic matter, and no single factor could be considered as an ecological ‘master’ factor (Hernandez-Alcantara and Solis-Weiss, 1991; Gray and Elliott, 2009). pH (especially its low and high values) is one of the important factors that influence the specific water chemistry regime (Spyra, 2017).
Based on water quality directive of Shannon-Wiener Index, the relationship between the indices and ecological level explained as follows: high status: >4, good status: 3-4, moderate status: 2-3, poor status: 1-2 and bad status: 0-1 (Kalyoncu and Zebek, 2011). In the present study, the values of the Shannon-Wiener index of diversity ranged from the minimum value of 0.27 to the maximum value of 3.07. According to the categorization presented by Kalyoncu and Zebek (2011), the biodiversity in the Yekshabe estuary was estimated from the second to the last level of ecological levels in the annual cycle. The highest amount of biodiversity was registered at station 4 in spring, and in this regard, the Margalef’s richness index and the Shannon-Wiener index of diversity confirmed each other (Figs. 4 and 5). The highest number of taxa was observed this season (Fig. 5). The Shannon-Wiener index of diversity was less than two in summer. Considering the high number of species in the spring and at all stations, the cause of the low levels of all indices in some stations and seasons, especially in summer, can be due to the dominance of some species in natural stress caused by the seasonal fluctuations of parameters after intensive evaporation and reduction of water volume in Yekshabe estuary as well as the climatic conditions of the
Persian Gulf. Hard and harsh environmental situations such as an increase in salinity and temperature may cause diversity to decrease during summer (Thrush and Dayton, 2002).

As a final point, Yekshabe estuary is a marine ecosystem in the Persian Gulf, far from residential areas as well as vessels traffic (Iran and Rohanian, 1985). Considering the results of community composition, comprising the identification of 80 macrobenthic species, high diversity (H’) and richness (d) reaching to 3.075 and 4.66, it was evaluated as a diverse and healthy ecosystem.

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