Identification and investigation of species diversity and richness of the Gastropoda in intertidal zone of Bushehr Port coastal area (the Persian Gulf waters)

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Received: March 2017  Accepted: September 2017

Abstract
This study aims to identify and determine species diversity and richness of the Gastropods of the intertidal zone of Bushehr seaport coastal area and assess the ecological status of this area using Welch index. Sampling was carried out at 6 stations in Bushehr seaport in the winter and summer seasons during 2013-2014. Bed sediment sampling was done three times in each station and Gastropod samples were collected using Quadrate (25×25 cm), and were then preserved using 4 % buffered formalin. The preserved samples were transported to the laboratory and were identified and counted by using valid identification keys. Furthermore, physicochemical properties of water including temperature, pH, dissolved oxygen (DO) and salinity were also measured in each station. According to the results obtained from this study, there were totally 14 species identified from 12 families belonging to 7 orders. The most abundantly identified species in the studied stations belonged to Planaxis sulctus, Cerithidea cingulate, and Anachis misera. In this study species diversity was determined by using Shannon-Weiner index, dominant species were investigated using Simpson index and species richness was calculated using Margalef index. The results of Shannon and Margalef indexes showed significant differences between the sampled stations and seasons (p<0.05). Moreover, the results of Simpson index showed no significant differences between the sampled stations and seasons (p>0.05). Results of Welch index showed that based on the mentioned indexes in the sampled stations the status of the water quality was classified as average and rather low/weak pollution.

Keywords: Species diversity, Species richness, Gastropoda, Bushehr seaport, Ecological assessment, Intertidal zone.

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Introduction

Over 70 percent of the earth’s surface is formed of different aquatic ecosystems having relationships with each other in different ways and playing a vital role in the world’s ecosystems (Hamzavi et al., 2012). Bushehr Province is located in south-western Iran and at the edge of the Persian Gulf (Salehi, 2013). The Persian Gulf is a shallow aquatic basin the average depth of which is 35-40 meters and its area is about 232850 square meters. This region is connected to the international waters through the Strait of Hormuz (Annon, 1995; Banat et al., 1998; Saeidi et al., 2008). Water replacement time in this basin is between 3 and 5 years (Saeed et al., 1995). According to the occurrence of different environmental events in this region during recent years including the biggest world oil spill in 1991, ship traffic, transporting and entering of oil contamination and also oil spills, this region has faced a crisis. Generally, it has been specified that about 30 percent of the total world oil transportation is done in the Persian Gulf (Pourang et al., 2005). The Persian Gulf environment undergoes rapid changes, and some of the most principal of these changes result from the coastal region changes (Sheppard et al., 2010).

Coastal regions include about 18% of the earth surface and accommodate about 60% of the world population. 90% of the fishes caught in the worlds come from these regions (Balasubramanian, 1999). Coastal regions are the most important coastal habitats due to their sensitivity, diversity and specific species. These regions have the most complicated and the richest ecosystems that have accommodated a lot of creatures and provide shelters for their reproduction and feeding (Balasubramanian, 1999; Nybakken, 1995; Webber and Thurman, 1995; Nabavi et al., 2011). Intertidal ecosystems are important marine ecosystems due to their vulnerability. These regions are unique marine regions since they are exposed to the air continuously. Thus creatures living in these regions should be able to adapt to their difficulties (Vazirizadeh and Arebi, 2011). These regions reflect the most common species of stuck and dweller creatures which are able to tolerate difficult situations and continue their reproduction (Tait and Desanto, 1972; Solaha and Sohail, 2012).

Macrobenthos are invertebrate animals which are visible to the naked eye and spend at least a part of their life at the bottom of aquatic resources (Rosenberg et al., 1999; Moghdani et al., 2013) Macrobenthos communities consist of an important part of the aquatic animals in marine environments (Mohammadi Roozbahani, et al., 2010) which play an important role in aquatic ecosystems as primary and secondary consumers (Nassaj et al., 2010). Moreover, they affect some sediments by mixing organic and inorganic matters in the upper sediments through bioturbation (Pavithran et al., 2009). Marine gastropod species of the Persian Gulf are very various and are considered as one important food resource for the upper consumers (Al-Khayat, 2008). Owing to the presence of gastropods at the bottom and their
low capacity to change their place, gastropods can be used as an important index to determine the destructive effects of human activities on coasts (Kohan et al., 2012). The class Gastropoda is the largest class of Mollusca including snails and slugs (Bouchet et al., 2005). This class includes 40000 to 75000 species living in the seas, freshwaters and land. Some of them are herbivorous, some are carnivorous and others are parasites (Pechenik, 2000; Sorensen and Surlyk, 2011). Biological and environmental factors including the Persian Gulf deeply influence the occurrence and dispersion of marine invertebrates generally and gastropods especially (Kohan et al., 2012). Study and investigation of benthic animals especially gastropods is one of the most important issues of ecological science. Since gastropods are considered as one of the most important benthic groups of an aquatic ecosystem, any kind of changes in coastal ecosystems and intertidal zones cause changes in factors dominating the mentioned communities (Andrew et al., 1996). Various studies are published in ecological assessment fields including the studies of Vazirizadeh and Arebi (2011), Moghdani et al. (2013) and Salehi et al. (2015). However there are still many regions where no comprehensive studies are done concerning them or they were done a few years ago. Since Bushehr province coasts have particular importance concerning species abundance and diversity of vertebrates especially gastropods and aquatic animal reproduction and aquaculture ecologically and due to the lack of basic information concerning environmental control in these coasts and also in order to get information concerning gastropoda distribution in this region, this study was carried out to identify and investigate species diversity and richness of gastropods of the intertidal zone of Bushehr seaport coastal area.

Materials and methods
The study area and sampling stations
The study area is the city of Bushehr lying at latitude 28°, 55’, 19.84” north and longitude 50°, 50’, 4.76” east at north-western Iran and at the edge of the Persian Gulf. Fig. 1 specifies the study limits and Table 1 specifies geographical specifications of sampling station. This study was carried out in 6 shores located in the city of Bushehr including Bandargah with a rocky–sandy shore (station A), Marjan Park with a rocky-sandy shore (station B), Jofre Mahini with a rocky-sandy shore (station C), Sheghab park with a rocky-sandy shore (station D), AbShirinKon with a rocky-sandy shore (station E) and Rishehr with a rocky-sandy shore (station F) once per season during both the warm season (the month of September 2013) and the cold season (the month of January 2014).
Sampling sediments and determining physicochemical parameters

Sampling of Gastropods was carried out using Quadrate (25×25 cm) and was based on the methods of Walton (1952), Eleftheriou and McIntyre (2005) and Kohan et al. (2012). Three samples of sediments were taken from each station using Quadrate to study Gastropods for identification, separation and determination of their diversity and density. In order to sample, first sediments were taken from the surface to a depth of 5 cm by using a shovel and were washed through a sieve of 5 mm. Then specifications of each station were recorded on the sampling containers and samples were transported to the laboratory. After transporting samples to the laboratory, samples were washed using water in order to remove the extra materials from them to be completely clean. Then samples were transported to a petri dish and all gastropods present were separated using forceps and using a stereomicroscope. Each gastropod was placed inside a video can containing buffered formalin 4% and relating to particular groups in order to be identified and counted. Then the frequencies of each group of gastropods were calculated during different seasons and important biological indexes concerning them were calculated. In this study it was tried to identify...
gastropods within the limits of the family in the first step and up to the level of genus and species if possible using the present sources and identification keys. In order to identify Gastropoda species, valid identification keys were also used (Taylor and Sohl, 1962; Opresko et al., 1976; Angeletti, 1978; Jones, 1986; Bosch et al., 1995; Abbott et al., 2001; Abbott and Morris, 2001; Bouchet et al., 2005; Tunnell et al., 2010). Moreover, physicochemical parameters of the water such as (pH, salinity, temperature, dissolved oxygen) were also measured with three repetitions in each stage of sampling.

Species diversity index. Shannon-Wiener diversity index was calculated by equation (1) (Shannon and Weaver, 1963).

\[ H' = - \sum_{i=1}^{R} \rho_i \ln \rho_i \]  

(1)

Where, \( \rho_i \) is the relative frequency, i is in the community and R is the total number of the community.

Species dominancy index. Simpson index was represented by Simpson in 1949 and Krebs has presented its calculation equation in 1972 (Krebs, 1994).

\[ \lambda = - \sum_{i=1}^{R} \rho_i^2 \]  

(2)

where, \( \rho_i \) is relative frequency, i is in the community, and R is the total number of the community.

Species richness index: Margalef formula was used in order to determine species richness:

\[ R = \frac{S-1}{\ln N} \]

In this equation R states species richness, S is the number of species and N is the number of people.

The assessment of pollution status of the area

After determining diversity indexes, Welch index was used to determine pollution status of the area (Table 2) (Welch, 1992).

<table>
<thead>
<tr>
<th>Water quality class</th>
<th>Welch index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low pollution</td>
<td>3-5</td>
</tr>
<tr>
<td>Average pollution</td>
<td>1-3</td>
</tr>
<tr>
<td>High pollution</td>
<td>&lt; 1</td>
</tr>
</tbody>
</table>

Statistical analysis

For statistical investigation of data, first data normality was analyzed by using Kolmogorov-Smirnov test and the difference between stations and seasons were determined using one-way and T-test in the environment of SPSS®15 software. Excel 2013 software was also used in order to draw diagrams.

Results

Physicochemical parameters

According to the investigations carried out, it was specified that there are significant differences between the mean temperature in 6 stations \((p \leq 0.001)\), and also mean temperatures during both the warm and cold seasons have significant differences \((p \leq 0.001)\).
Mean temperature in both warm and cold seasons has been indicated in Fig. 2. Based on the investigation carried out, it was specified that there are significant differences between mean dissolved oxygen in 6 sampling stations ($p \leq 0.001$) and also dissolved oxygen during both warm and cold seasons ($p \leq 0.001$). Mean dissolved oxygen rates during both cold and warm seasons have been shown in Fig. 3. According to the investigations done it was specified that there are significant stations ($p \leq 0.001$) but no significant differences were observed between salinity rate during both summer and winter seasons ($p = 0.147$). Mean salinity rates during warm and cold seasons are shown in Fig. 4. There were significant differences between mean pH in stations ($p \leq 0.001$) and also mean pH in sampling seasons ($p \leq 0.001$). Mean pH rate during both cold and warm seasons are shown in Fig. 5.

**Figure 2:** Water temperature changes during both cold and warm seasons.

**Figure 3:** Dissolved oxygen changes during both cold and warm seasons.

**Figure 4:** Salinity changes during both cold and warm seasons.

**Figure 5:** pH changes during both cold and warm seasons.
Density and dispersion of gastropods

14 species from 12 families belonging to 7 orders of gastropods were totally identified during the sampling (Table 3), and the results of frequencies of benthic groups were represented based on both cold and warm seasons separately. Fig. 6 shows frequency changes of benthic macro-invertebrates counted in different stations. According to the results of one-way ANOVA test, there are significant differences between macrobenthos frequency changes during both cold and warm seasons ($p \leq 0.001$) and also between macrobenthos frequency changes in 6 stations ($p=0.003$).

Figure 6: Macrobenthos frequency changes in sampling stations during cold and warm seasons.

Table 3: Species identified during cold and warm seasons.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Species</th>
<th>Warm</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trochoidea</td>
<td>Chilodontidae</td>
<td><em>Euchelus asper</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Trochus radiates</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Cyclonerittimorpha</td>
<td>Trochidae</td>
<td><em>T.erithraeus</em></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neritidae</td>
<td><em>Nerita textile</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaeogastropoda</td>
<td>Acmaeidae</td>
<td><em>Acmaea profunda</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Turbinidae</td>
<td><em>Turbo coronatus</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Caenogastropoda litorinimorpha</td>
<td>Planaxidae</td>
<td><em>Planaxis sulcatus</em></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bursidae</td>
<td><em>Bufonaria echinata</em></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Potamididae</td>
<td><em>Cerithidea cingulata</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Sorbeoconcha</td>
<td>Cerithiidae</td>
<td><em>Clypeomorus sp.</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Muricidae</td>
<td><em>Cronia margaritica</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Nassariidae</td>
<td><em>Nassarius arcularius plicatus</em></td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Neogastropoda</td>
<td>Columbellidae</td>
<td><em>Anachis misera</em></td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>
**Shannon index**

According to the results of ANOVA ($p \leq 0.001$), significant differences were observed between mean Shannon index in sampling stations and also between mean Shannon index during sampling seasons ($p \leq 0.001$).

![Figure 7: Shannon index changes between stations and during cold and warm seasons.](image)

**Simpson index**

Based on the results of ANOVA ($p=0.073$), no significant differences were observed between mean Simpson index in sampling stations and also between mean Simpson index during sampling seasons ($p=0.688$).

![Figure 8: Simpson index changes between stations during cold and warm seasons.](image)
Margalef index
According to the results of ANOVA \((p \leq 0.001)\), significant differences were observed between mean Margalef index in sampling stations and also between mean Margalef index during sampling seasons \((p \leq 0.001)\).

![Margalef index changes between stations during cold and warm seasons.](image)

Determination of pollution status of the area
Welch index was used to determine pollution status. In this index pollution status of each station is determined based on the rate of Shannon diversity index changes. Generally, pollution status in these areas showed an average level (Table 4).

<table>
<thead>
<tr>
<th>Station</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>1.279</td>
<td>1.531</td>
<td>1.386</td>
<td>1.675</td>
<td>1.002</td>
<td>1.016</td>
</tr>
<tr>
<td>Summer</td>
<td>1.254</td>
<td>1.022</td>
<td>1.054</td>
<td>1.005</td>
<td>1.171</td>
<td>1.089</td>
</tr>
</tbody>
</table>

Discussion
Benthic macro-invertebrates form a very important part of the sea bed-fauna which frequently include polychaetes, crustacean and seashells (Tabatabaei and Amiri, 2011). Study of benthic communities of the sea is very important since they are biological indexes and have an important role in food chains, organic matter recycling, removal of the toxicity of the pollutants, dispersion and burial of the secondary production and also they have an effective role in the sea
sediments in the processes of the ecosystem including food circle and pollutant metabolism (Hamzavi et al., 2012; Pourjomeh et al., 2014). Macrobenthic communities of the Persian Gulf are the largest and the most important marine ecosystems (Naser, 2010). The Persian Gulf macrobenthos are described by high level of biodiversity and low species richness and according to the difficult environmental situation and every kind of environmental change affecting these communities (Al-Yamani et al., 2009; Sheppard et al., 2010). Biological diversity and dispersion of the marine macrobenthos are affected by the kind of sediments, temperature, salinity, depth and physical disturbances (Basson et al., 1977; Coles and Maccain, 1990; Pourjomeh et al., 2014). Physical disturbances have the most important effects which can decrease the benthic diversity in the study area (Mohammadi Roozbahani et al., 2010).

The results of the study showed that in the study area Shannon and Margalef indexes showed significant differences between sampling stations and seasons ($p<0.05$) that the highest and the lowest Shannon and Margalef indexes were during the cold and warm seasons, respectively. The obtained results of Simpson index showed no significant differences between sampling stations and seasons ($p<0.05$), and the highest and the lowest values of this index were during warm and cold seasons, respectively. One of useful applications of Shannon species diversity index is ecological assessment in relation to the pollution of the areas (Welch, 2003). The results of Welch index (Welch, 1992) indicate that the study areas have generally an average level of pollution. In this study, mean of Simpson index during the warm season was 0.355 and it was 0.304 during the cold season that indicated that the study shores have good diversity. The obtained results of Shannon-Weiner confirm the results of Simpson index. The highest diversity of macrobenthos was in winter and their lowest diversity was in summer. Based on this index all stations have an average quality during both cold and warm seasons.

The most frequently identified gastropods in the study areas include species of $A. \text{misera}$, $C. \text{cingulate}$, $P. \text{ sulcatus}$. The reason for the frequencies of these groups during both seasons can be due to suitable environmental conditions for these species to live and reproduce. As these species are rather resistant to pollution, it can be stated the study stations have no desirable status qualitatively. $C. \text{cingulate}$, $P. \text{sulcatus}$ are biological indexes of the polluted areas since despite high pollution conditions, they have been able to continue to live with high density. Moreover, these species $C. \text{cingulate}$, $P. \text{sulcatus}$ are able to use decayed food and organic matters and feed and live on the algal covers. Also they are ecologically known as Euryhalin and are able to tolerate sudden environmental changes and a marked oxygen shortage and high levels of hydrogen sulfide (Pearson and Rosenberg, 1978). Hard and harsh environmental situations such as an
increase in temperature may cause diversity to decrease during summer (Simon and Paul, 2002).

According to the results of this study it can be stated that macrobenthos are useful biological indexes in order to assess ecological status of the coastal environments and aquatic resources corresponding with the study results of Borja et al. (2003), Rosenberg et al. (2004), Mistriani and Munari (2008), Anbuchezhian et al. (2009), Vazirizadeh and Arebi (2011), Nourinezhad et al. (2013), Moghdani et al. (2013). The results of the study of Abowei et al. (2012) who studied the effects of water pollution on benthic macrofaunal species in Koluma region in Nigeria showed that diversity of benthic macro-invertebrates in the study stations was generally low that is because of low tolerance of these species to pollution, whereas polychaete species are an index to pollution, and are dominant in brackish waters which can be attributed to their tolerance level to pollution. According to the research of Saunders et al. (2007), an increase in pollution causes a decrease in diversity of frequency of benthic macro-invertebrate species. Also Carvalho et al. (2006) indicated that the pollution increase causes the superiority of polychaete species. In the study of Salehi et al., 2015 it was stated that based on Welch index, the study area is at an average level in terms of pollution. It has been reported that the main reasons or the pollution of these shores are effluents resulting from domestic wastewaters and oil tankers transportation and also the most frequent identified gastropods in the study area include species *P. sulcatus, C. cingulate, A. misera*. In this study the most diversity of macrobenthos was reported during the warm season and their least diversity was reported during the cold season. The study of indicated that diversity decreases due to temperature causing an increase in stress and dryness, i.e. an increase in temperature causes diversity to decrease and dominancy to enhance.

Generally speaking, it can be stated that so far different factors have been reported by the researchers as controlling parameters of frequency in the spread of communities in tropical and subtropical regions including the Persian Gulf (Shappard et al., 1992). Among the mentioned factors, parameters including the size of sediment particles (Basson et al., 1977), water salinity (Coles and McCain, 1990), water flow (Shappard et al., 1992; Basson et al., 1997) and water polluting factors (Coles and McCain, 1990) have the most effects on density of the benthic fauna spread in these regions. Moreover, difficult environment conditions including temperature (Tan and Kastoro, 2004), physical disturbance (like fishing activity) (Muxika et al., 2005) and lack of homogeneous habitats and also an inter-species competition (Simon and Paul, 2002) have great impacts on biodiversity of benthic communities. Therefore all factors should be considered instead of one single factor in the assessment of the effects of environmental factors on frequency, dispersion and diversity of benthic
communities (Kraufvelin and Slovius, 2004; Neubauer et al., 2015).

Thus according to the results of this research, factors causing a change in combination and diversity of macrobenthos species specially gastropods in this region include human activities such as fishing and trade discharge of domestic, industrial and agricultural wastewaters, activities of oil tankers and environmental factors. Finally, coast pollution rates can be investigated based on macrobenthos communities particularly gastropods and they can be used as a key in order to investigate the pollution status of the coasts in future studies.

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