Article

Diversity and distribution of meiofauna Mollusca in the southern Caspian Sea

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Received 27 May 2021; Accepted 2 July 2021; Published 1 September 2021

Abstract
In this study, in order to analyze Mollusca species and determining their relationship with the sediment factors, sediment samples were gathered during spring, summer, autumn and winter 2012 from 12 stations (ranging in depths 5, 10, 20 and 50 meters) in the Southern Caspian Sea from Behshahr to Ramsar (Mazandaran, Iran). The sediment factors (grain size, total organic matter and calcium carbonate concentration) were measured in laboratory. Two classes of Mollusca (Bivalvia and Gastropoda) were identified. Class Bivalvia had 3 species (*Didacna protracta*, *Hypanis caspia*, *Abra ovata*) which belong to 3 genera of 2 families and class Gastropoda had 4 species (*Pergola* sp., *Anisus kolesnikovi*, *Abeskunus sphaerion*, *Ulskia ulskii*) which belong to 4 genera of 3 families. Most dominant species of Bivalvia was *Abra ovata* (46.2 individuals/0.1m^2) and that of Gastropoda was *Abeskunus sphaerion* (58.66 individuals/0.1m^2). The mean of maximum and minimum density of Mollusca was observed in spring (29.67 individuals/0.1m^2) and winter (0 individuals/0.1m^2) respectively. The results of One Way ANOVA showed that density of Mollusca was different significantly with depth and season ($P<0.05$). Results of Pearson correlation showed that between density of Mollusca and salinity, %silt and clay, TOM and depth had been negative positive correlation and had been positive correlation with temperature.

Keywords Mollusca; meiofauna; Caspian Sea; distribution.

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

The biggest inland water body on Earth is the Caspian Sea, with a surface area of 371,000 km^2. It is an anomalohaline lake, whose water level and salinity regimes are determined by a balance of runoff and evaporation (Krijgsman et al., 2019; Sabrina et al., 2019). The Caspian Sea has characteristics common to both seas and lakes. It is often listed as the world's largest lake, although it is not a freshwater lake. The Caspian was once part of the Tethys Ocean but became landlocked about 5.5 million years ago due to plate tectonics. The Volga River (about 80% of the inflow) and the Ural River discharge into the Caspian Sea, but it has no natural outflow other than by evaporation. Thus the Caspian ecosystem is a closed basin, with its own sea level history that is independent of the eustatic level of the world's oceans. The coastlines of the Caspian are shared by Azerbaijan, Iran, Kazakhstan, Russia, and Turkmenistan. The Caspian is divided into three distinct physical regions: The Northern, Middle, and Southern Caspian (Amirahamdai, 2000). Our studied area located in southern of The Caspian Sea (Fig. 1). The Caspian Sea has characteristics common to both seas and lakes. It is often listed as the world's largest lake, although it is not a freshwater lake. The meiofauna specifically Mollusca in size of meiofauna was poorly studied in south of Caspian Sea.

The Mollusca is a very old monophyletic lineage, dating from before the Cambrian (Barker, 2001) and is the second most diverse of all animal phyla (Russell-Hunter, 1983), occurring in many habitat types (Menez et al., 2003). As molluscs are primary consumers in aquatic ecosystems (Brown, 1991), they have been used as environmental indicators. Due to their economical and ecological importance, as well as sedentary life, molluscs have been assumed as an important organism in monitoring contaminants in different ecosystems (Feldstein et al., 2003). They are abundant, sedentary and easy to collect, which makes them ideal organisms in biomonitoring (Bresler et al., 2003) programs. Bivalve and gastropod molluscs are among the most useful organisms for environmental monitoring (Boening, 1999).

However, molluscs are potentially at risk because of the impacts of human activities. Three major ways that fresh/brackish water mollusks have been impacted by human activities are introduction of exotic species, habitat loss or alteration and pollution of water. Sometimes, introduced (alien) species became invasive, causing biodiversity loss and inflicting major economic and/or ecological damage especially, accumulation of silt makes a major impact on the distribution of molluscs. Changing of other physicochemical parameters also directly or indirectly effect on the distribution of aquatic mollusks groups in the water bodies (Lardicci et al., 1997).

Several Ponto-Caspian Molluscan species descended from INEAGES that originated in Lake Pannon, a Miocene–Pliocene enclosed basin that existed in central Europe from 12 to 4 mya. Spectacular adaptive radiation in several Molluscan groups produced nearly 100% endemism in Lake Pannon, which (Geary et al. 2000).

The aim of the present study was to evaluate the influence of chemical factors (of water and bottom sediments) on the composition, identify and abundance of meiofauna Mollusca in southern of Caspian Sea.

2 Materials and Methods

2.1 Study Area

The study was carried out in spring, summer, autumn and winter 2012, Caspian Sea, Mazandaran province, from Behshar to Ramsar (Fig. 1, Table 1). Sediment samples were collected from 12 stations, ranging in depth from 5 to 50 m.

Fig. 1 Situation of sampling stations in the southern Caspian Sea.

Table 1 Position of sampling stations.

Stations	Longitude (°N)	Latitude (°E)
A1	36° 51' 31"	53° 16' 16. "
A2	36° 53' 10"	53° 16' 12"
A3	36° 56' 48"	53° 16' 09"
A4	37° 00' 52"	53° 16' 16"
B1	36° 43' 18"	52° 39' 33"
B2	36° 43' 58"	52° 39' 36"
B3	36° 45' 55"	52° 39' 28"
B4	36° 48' 41"	52° 39' 29"
C1	36° 40' 32"	51° 27' 43"
C2	36° 41' 04"	51° 27' 44"
C3	36° 41' 47"	51° 27' 42"
C4	36° 43' 47"	51° 27' 41"
D1	36° 56' 47"	50° 39' 20"
D2	36° 57' 18"	50° 39' 21"
D3	36° 58' 29"	50° 39' 26"
D4	37° 03' 17"	50° 39' 16"

2.2 Sampling method

Samples were collected by boat and Stations depths were measured with echo sounder and sampling coordinates were recorded with the Global Positioning System. At each station, a 0.1 m^2 Van-Veen grab sampler was used to collect bottom sediments. Three sets of samples were taken at each station by a 6.60 cm^2 area core sampler with 5cm depth and were stored in plastic boxes. Each sediment for Mollusca studies (33 cm^3 volume) was treated with 1 g/L Rose Bengal solution immediately after its arrival on boat to distinguish living specimens, and then being mixed with 5% concentrated formalin solution (Moghaddasi et al., 2009; MOOPAM,2010; Sadoogh et al., 2013; Zarghami et al., 2019, 2020).

2.3 Mollusca analysis

For determining Mollusca, in the laboratory, wet samples were washed through 63 µm mesh sieve to remove any excess stain and then fixed with alcohol ethanol (70%). Mollusca tests were floated off using the heavy liquid CCl_4 with the upper layer of the liquid consisting of floated foraminiferal tests, which were then filtered by paper and allowed to dry. A stereomicroscope was used to examine and identify tests with reference to and several previous studies (Birshtain et al., 1968; Hayward and Ryland, 1996).

2.4 Environmental factors

The benthic environmental factors including temperature, dissolved oxygen, salinity and pH were measured by CTD during the sampling time. Sediment grain size, Total Organic Matter (TOM) and calcium carbonate concentration ($CaCO_3$) were measured. For the grain-size analysis, 100 g of oven-dried sediment (70°C, 8 h) was mixed with 250 ml of tap water and 10 ml of sodium hexametaphosphate (6.2 g/L) to disaggregate the sediment. The sediment was then stirred mechanically (15 min), allowed to soak (8 h), stirred mechanically (15 min) and dried again (70°C, 24 h). Fifty grams of dried material was then transferred to the uppermost of a stacked series of graded sand sieves with 4, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm mesh. The material that remained on the sieves was removed and weighed. Finally, the percentage of each particle was calculated. (Moghaddasi et al., 2009; MOOPAM, 2010; Zarghami et al., 2020).

TOM in each sample was measured by calculating the loss of weight during combustion. An empty crucible was weighed and then half-filled with wet sediment and dried in an oven (70°C) until a constant weight was reached (about 24 hours). After removal from the oven, the sample was allowed to cool and was reweighed (A). It was then placed in a Muffle furnace (550°C – 8 hours), removed, cooled and reweighed again (B). The TOM content was determined by the loss of weight on ignition at this temperature. [%TOM =100(A-B) / (A-C)] (Moghaddasi et al., 2009; MOOPAM, 2010; Zarghami et al., 2020)

Calcium carbonate concentration was measured based on the reaction with dilute Hydrochloric Acid (HCl). Twenty-five grams (W1) of dried sediment (7 – 8 hrs.) was mixed with HCl (0.1 N) and stirred until no CO_2 bubbles were discernable, and then allowed to soak (24 hrs.). The upper liquid phase was discharged and the remaining sediments were filtered (with filter paper), dried (7 – 8 hrs.) and reweighed again (W2). Calcium carbonate percentage was measured by the following formula: %CaCO3= 100 (W1-W2) / W1 (Moghaddasi et al., 2009).

2.5 Data Analysis

Principal Component Analysis (PCA) was used to investigate the relationship between seven variables collected during seasonal sampling cruises in 2012 (temperature, pH, dissolved oxygen, salinity, %TOM, %$CaCO_3$ and granulometry). One Way ANOVA was performed to test for possible differences. Shannon-Wiener ($H^{/}$) diversity index has measured for assaying species diversity and ecological assessment in this area (Marques et al., 2009).

3 Results and Discussion

3.1 Environmental factors

The results of environmental factors were showed in Figs 3 to 6 and Tables 2 to 7. The temperature of the water near the bottom was nearly similar in all stations (17.54 to 18.52). The results of measuring dissolved oxygen concentration indicated enough oxygen in water near the bottom and the high average of dissolved oxygen concentration was in Ramsar transect. Salinity was also had low difference between stations (10.94 to 11.28) and increased with the depth increasing. pH was nearly equal in all stations (8.28 to 8.43).

The grain size analysis of the sediments showed that the structure of the sediment samples mostly consisted of; sand, silt and clay and seldom grovel. The grain size decreased with water depth. The silt and clay rate increase with depth in all stations (Fig. 2).

Total Organic Matter (TOM) was higher in station Behshahr (10.59) and increased with depth 50m (14.56%). Calcium carbonate concentration was high in station Behshahr in depth of 20 m.

The grain size analysis of the sediments showed that the structure of the sediment samples mostly consisted of sand; silt and clay that are probably related to nonexistence of rivers follows, which causes reduced the coarse grain-size in this confined. The grain size decreased with water depth. According to Duros et al. (2011), a slight decrease of grain size observed with water depth in the Whittard Canyon area (NE Atlantic). In this area, such as previously study in Thailand golf the grain size of the sediments does not play a significant role on the foraminifer's distribution (Mielis and Violanti, 2006).

TOM was higher in station in stations D4 in spring that is related to increasing silt-clay rate, because according to Moghaddasi et al. (2009) and Sadough et al. (2013) organic compounds increased in silt-clay sediments. The result of Pearson correlation showed the significantly positive correlation with depth and TOM ($P<0.05$).

3.2 Data analysis

The results of PCA showed that granulometry, %TOM and %$CaCO_3$ played an important role (Table 8 and Fig. 7).

The results of One Way ANOVA showed that density of Mollusca was different significantly with depth and season ($P<0.05$). Results of Pearson correlation showed that between density of Mollusca and salinity, %silt and clay, TOM and depth had been negative positive correlation and had been positive correlation with temperature.

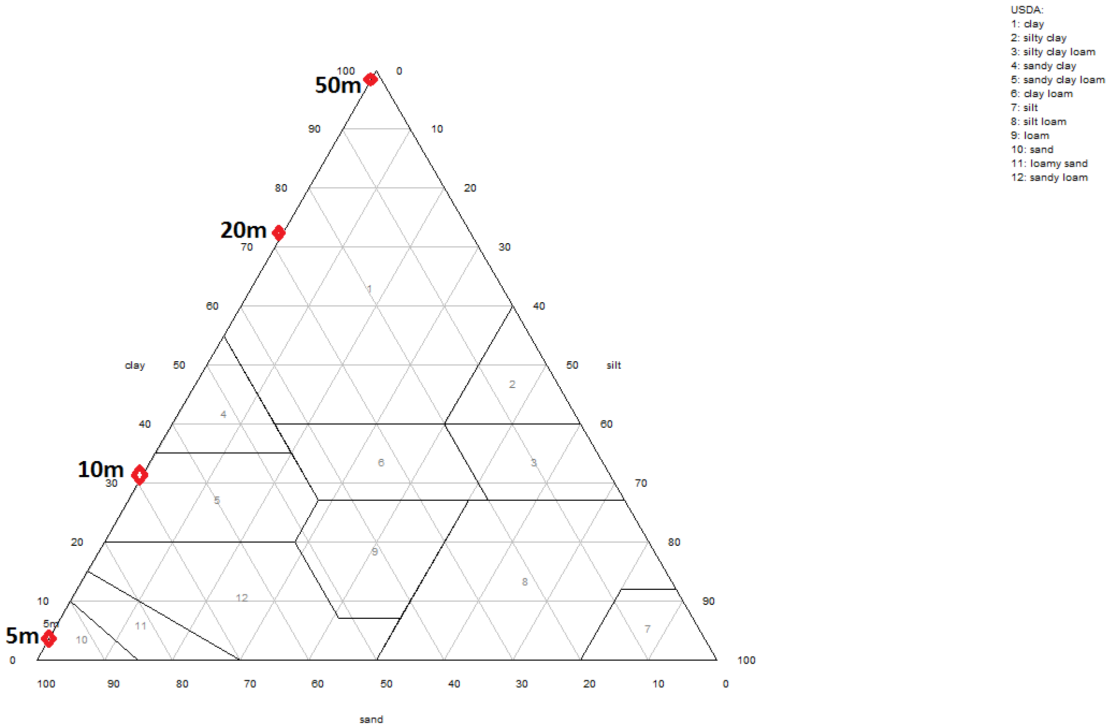

Fig. 2 Percentage of grovel, sand and silt and clay in different depth in the southern Caspian Sea from Behshahr to Ramsar.

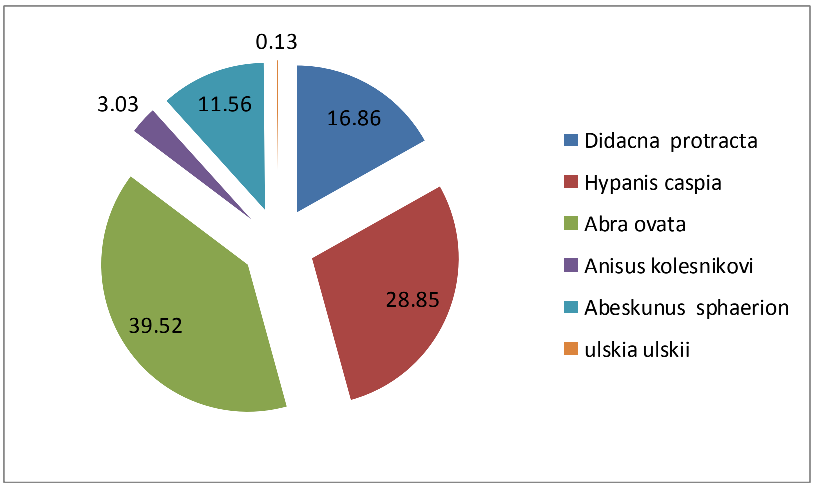

Fig. 3 Percentage of Mollusca density (individuals/0.1m^2) of southern Caspian Sea (Mazndaran).

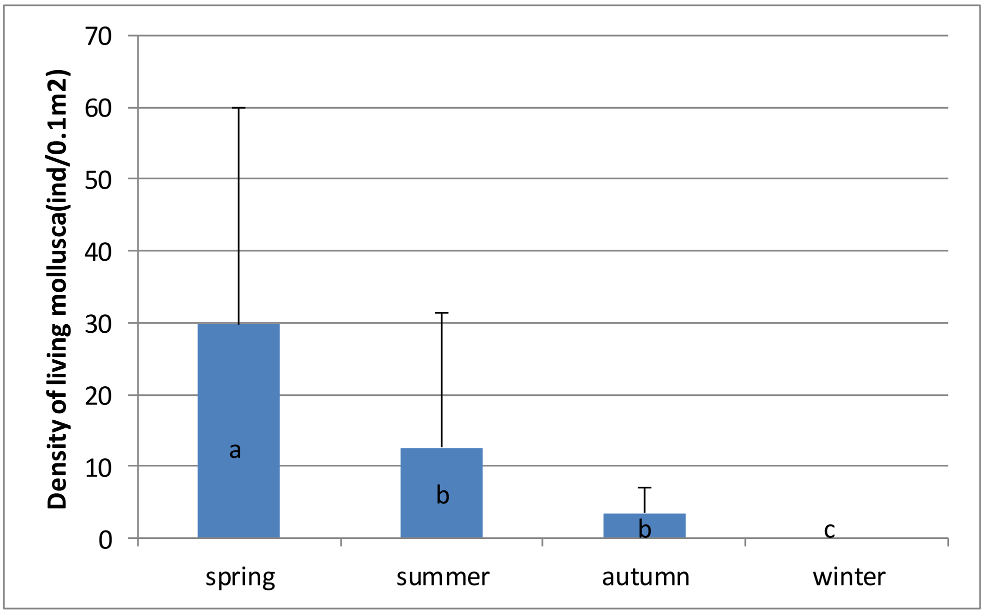

Fig. 4 Density of living Mollusca in different seasons (Mazandran province).

Table 2 The mean of temperature, salinity, DO, pH, Total Organic Matter (TOM) and $CaCO_3$ in different seasons of the southern Caspian Sea from Behshahr to Ramsar (±SD).

Factors / Season	Temperature (C^0)	Salinity (ppt)	DO (mg/l)	pH	%TOM	%$CaCO_3$
Spring	20.74±0.02	11.01±0.01	10.23±0.04	8.27±0.01	7±1	9±4.47
Summer	23.93±0.008	11.22±0.005	8.17±0.014	8.56±0.005	8.52±1.64	9.61±3.29
Autumn	17.34±0.007	11.14±0.01	8.1±0.007	8.11±0.051	8.08±1.03	9.19±2.22
Winter	9.52±0.009	11.39±0.02	10.53±0.01	8.41±0.01	8.23±1.6	9.72±3.92

Table 3 The mean of temperature, salinity, DO, pH, Total Organic Matter (TOM) and $CaCO_3$ in different seasons of the southern Caspian Sea from Behshahr to Ramsar (±SD).

Factors / Depth	Temperature (C^0)	Salinity (ppt)	DO (mg/l)	pH	%TOM	%$CaCO_3$
5	20.83±0.011	11.08±0.019	8.71±0.034	8.28±0.016	3.41±0.66	3.33±0.653
10	20.71±0.023	11.2±0.008	8.72±0.015	8.29±0.019	6.43±1.14	7.09±1.968
20	18.8±0.019	11.25±0.009	8.6±0.03	8.35±0.03	7.86±0.881	13.4±5.873
50	11.27±0.013	11.21±0.04	9.20±0.007	8.48±0.01	14.56±2.77	12.76±4.572

Table 4 The mean of temperature, salinity, DO, pH, Total Organic Matter (TOM) and $CaCO_3$ in different depths in the southern Caspian Sea from Behshahr to Ramsar (±SD).

Factors / Stations	Temperature (C^0)	Salinity (ppt)	DO (mg/l)	pH	%TOM	%$CaCO_3$
A	18.52±0.34	10.97±0.032	8.69±0.036	8.4±0.024	10.59±1.88	14.73±6.26
B	18.18±0.011	11.24±0.024	8.42±0.036	8.3±0.033	7.78±1.1	7.95±2.59
C	17.37±0.01	11.28±0.014	9.01±0.007	8.43±0.013	7.04±0.95	7.27±1.66
D	17.54±0.017	11.24±0.01	9.1±0.006	8.28±0.005	6.85±1.52	6.63±2.544

Table 5 Density of living Mollusca (individuals/0.1m^2) of southern Caspian Sea (Mazndaran) (Spring, 2012) (±SD).

Stations / Mollusca	A2	A3	A4	B2	B3	C1	C2	C3	C4	D1	D2
Didacna protracta	5.58± 4.32			44.69 ± 33.79	13.96 ± 11.44		2.79 ± 4.32			50.28 ± 77.89	
Hypanis caspia				8.38 ± 7.49		22.34 ± 34.61	2.79 ± 4.32	2.79 ± 4.32		46.09 ± 4.38	41.9± 32.67
Abra ovata	69.44 ± 33.79	11.17 ± 17.3		36.31 ± 2.79		44.69 ± 69.23			2/79± 4.32	27/93 ± 18.86	19/55 ± 18.86
Anisus kolesnikovi			2.79 ± 4.32	2.79 ± 4.32	2.79 ± 4.32				8.38± 12.98		
Abeskunus sphaerion		2.79 ± 4.32	2.79 ± 4.32	2.79 ± 4.32	2.79 ± 4.32			16.76 ± 19.83			
Ulskia ulskii			2.79±4.32								
Total	50.28	13.96	8.38	94.97	19.55	67.04	5.58	19.55	11.17	124.3	61.45

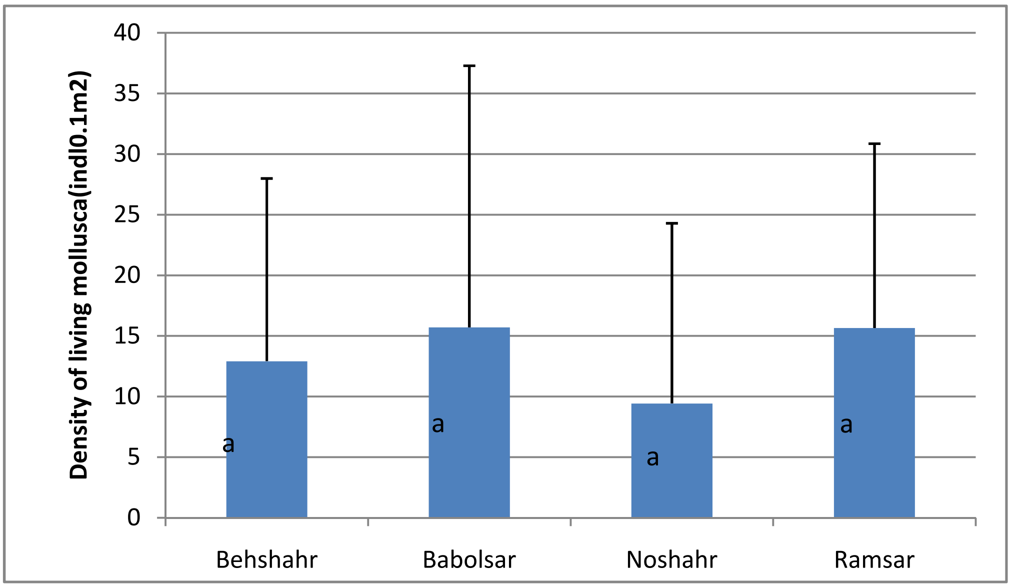

Fig. 5 Density of living Mollusca in different stations (Mazandran province).

Table 6 Density of living Mollusca (individuals/0.1m^2) of southern Caspian Sea (Mazndaran) (Summer, 2012) (±SD).

Stations / Mollusca	A1	A2	A3	B1	B2	B3	C2	C3	D1	D4
Didacna protracta	2.79 ± 4.32		2.79 ± 4.32			5.58 ± 9.67				
Hypanis caspia	2.79 ± 4.32		8.38 ± 12.98		2.79 ± 4.32		2.79 ± 4.32	8.38 ± 12.98	2.79 ± 4.32	
Abra ovata	11.17 ±17.3		8.38 ±0	2.79 ± 4.32	11.17 ±17.3	44.69 ±57.04				
Abeskunus sphaerion	5.58 ± 8.65	2.79 ± 4.32	13.96 ± 11.44			58.66 ± 101.6				2.79 ± 4.32
Total	22.34	2.79	33.52	2.79	13.96	108.94	2.79	8.38	2.79	2.79

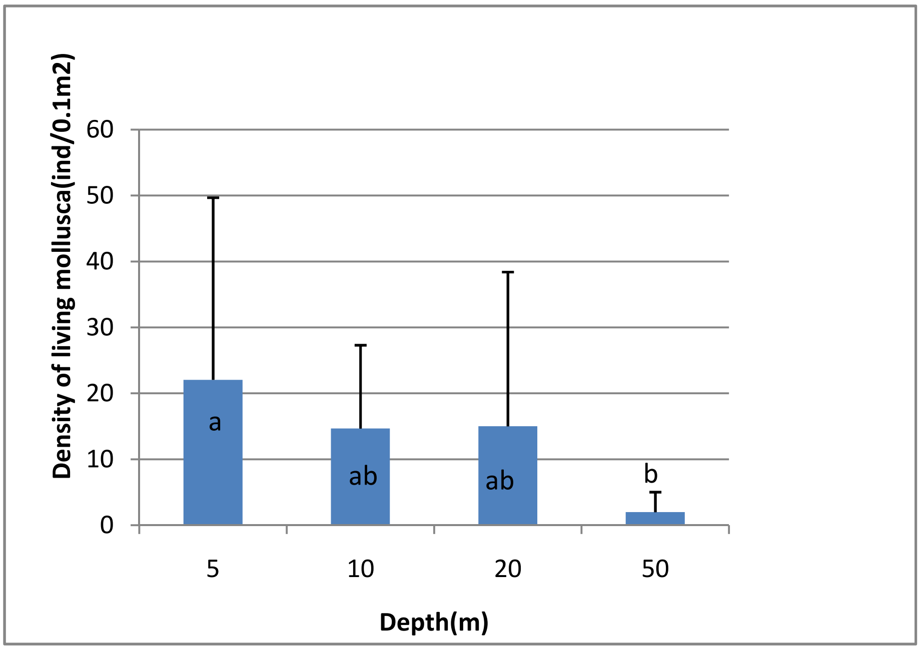

Fig. 6 Density of living Mollusca in different depth (Mazandran province).

Table 7 Density of living Mollusca (individuals/0.1m^2) of southern Caspian Sea (Mazndaran) (Autumn, 2012) (±SD).

Stations / Mollusca	A1	B2	B3	B4	C1	C4	D1	D2	D3
Didacna protracta						2.79 ± 4.32		2.79 ± 4.32	2.79 ± 4.32
Hypanis caspia	8.38 ±0	2.79 ± 4.32			5.58 ± 8.65				2.79 ± 4.32
Abra ovata	8.38 ±0			5.58 ± 8.65			8.38 ± 12.98		
Abeskunus sphaerion			2.79 ± 4.32					2.79 ± 4.32	
Total	16.76	2.79	2.79	5.58	5.58	2.79	8.38	5.58	5.58

Table 8 Component Matrix (PCA).

	Component		
	1	2	3
Granulometry	.901	.143	.011
TOM	.834	.148	.023
Caco3	.770	.261	-.180
temperature	-.618	.611	-.072
DO	.222	-.648	.608
Salinity	.309	.554	.332
pH	.245	-.421	-.704

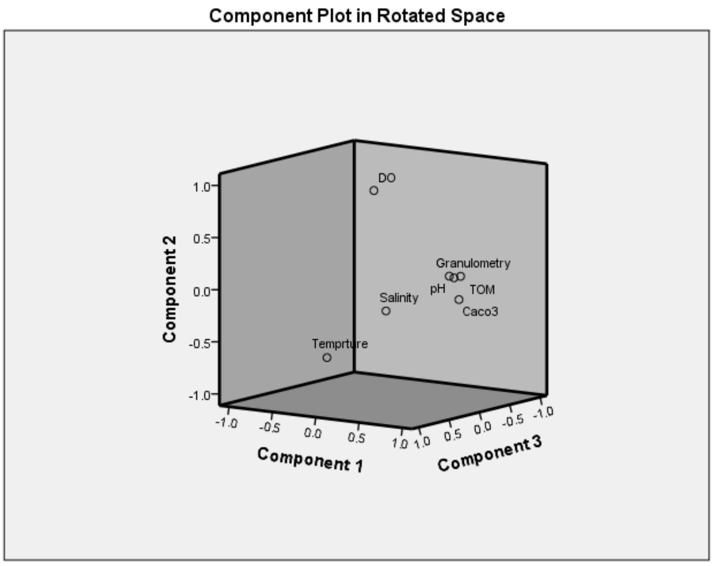

Fig. 7 PCA of environmental factors in southern of Caspian Sea.

4 Discussion

The distribution and dynamics of Molluscan communities in this area is strongly influenced by physicochemical factors. Among seven parameters evaluated, four of them (%silt and clay, salinity, TOM, temperature) had showed considerable variation. Result of Pearson correlation showed that between density of Mollusca and salinity, %silt and clay, TOM and depth had been negative correlation and had been positive correlation with temperature. However, according to the results of One Way ANOVA showed that density of Mollusca has significantly difference with depth and season ($P<0.05$).

The substrate type was varied among the four depths (Fig. 2). The common substrate type consisted with coarse sand, find sand, silt and clay. The highest number of individuals was observed in depth of 5m. In this depth (Fig. 2) substrate structure consisted with fine sand.

Therefore, it can be assumed substrate is one of the major factors that influence on the distribution of meiofauna. The results of PCA showed that granulometry played important role (Table 6 and Fig. 7). In the previous research (Udayantha and Munasinghe, 2009) result showed that distribution pattern of Mollusca which highest abundance of substrate with fine sand, silt and clay.

The observations of Mansingh et al. (2021) in the Bhitarkanika mangrove ecosystem demonstrated that hydrological and soil environmental conditions such as salinity, temperature, pH, electron conductivity and soil biomass carbon may influence the dispersion and distribution of benthic Molluscan faunal assemblages among different mangrove habitats. The results highlighted the need for long-term studies and perspective when evaluating conservation practices in mangrove ecosystems.

Offshore environment with the depth between 10-50 m include sediments with sandy silt, silt clay and clayey marl deposit. Usually substrate composition and environment condition on view of hydrodynamic energy are very suitable for biota habitat (Khoshravan, 2007).

Sediment reworking results from various activities of benthic infauna (i.e. burrowing, feeding and locomotion), the structure and porosity of the sediment matrix (Meysman et al., 2005), the release of nutrients from the sediment to the water column (Biles et al., 2002; Mermillod-Blondin et al. 2005), and the sequestration of pollutants and contaminants (Thompson and Riddle, 2005; Bradshaw et al., 2006).

In this research with the increase TOM distribution was low that (Udayantha and Munasinghe, 2009) result showed the distribution gradually decreases which promote the accumulation of organic matter. Harkantra (1982) made a similar observation in which he stated that low and high value of organic content shows poor fauna and median values show rich fauna that organic matter beyond 6% is noticed to be anoxic.

With increasing depth distribution was decreased and we observed maximum density in depth of 5m.in previous study by Michel et al. (2007). Although a reduction in macrofaunal diversity in deeper waters is a general trend. Because with increasing depth percent of silt and caly and TOM is increased that distribution was decreased.

Whereas most Molluscan classes are restricted to marine habitats, bivalves have repeatedly colonised freshwater ecosystems. They often represent a major part of the benthic biomass, as they do in certain soft-bottom marine ecosystems such as muddy grounds. There are many independent gastropod lines that have invaded brackish and freshwater as well as terrestrial ecosystems. Indeed, gastropods are found in practically all ecosystems on Earth and are certainly one of the most successful colonisers in the animal kingdom (Haszprunar, 2020).

Seasonal changes in environmental parameters can be significant in temperate areas.in this research maximum density observed in spring that showed significant difference with other seasons. Temperature may also act indirectly since it is one of the major environmental factors interfering in the reproduction activity of benthic invertebrates (Kinne, 1963).

In the study of Mollusca in Mersin Bay result showed that the species abundance showed seasonal and depth-wise variations. The highest species richness (6-16 species) was encountered between depths of 10 and 100 m in winter and spring, whereas the Molluscan population increased in warm seasons due to the high abundance. Bivalves and gastropods were abundantly found at depths of 10-200 m, 10-25 m, and 75-100 m (Mutlu and Ergev, 2012).

Two classes of Mollusca (Bivalvia and Gastropoda) were identified. Class bivalvia had 3 species (*Didacna protracta, Hypanis caspia, Abra ovata)* that belong to 3 genera of 2 families and class Gastropoda had 4 species (*Pyrgula* sp., *Anisus kolesnikovi, Abeskunus sphaerion, Ulskia ulskii)* that belong to 4 genera of 3 families. Most dominant species of Bivalvia was *Abra ovata (*39.52%) and that of Gastropoda was *Abeskunus sphaerion* (28.85%) the rare species was *Ulskia ulskii* (Fig. 3).

Abra ovata was dominant species in this research. Available data show that populations of *A. ovata* are characterized by a long settlement period with differences being closely related to the prevailing water temperature and sufficient food resources, salinity and oxygen concentration (Nicolaidou and Kostaki-Apostolopoulou, 1988; Kevrekidis and Koukouras, 1992). Specimens of *A. ovata* used during winter experiments were thus in a resting reproductive stage. Differences in sediment reworking rates between summer and winter therefore probably resulted from a direct effect of temperature on feeding activity (Maire et al., 2007). We worked in meiofauna size than after the reproduction season the number of them will be increased. The size -frequency revealed that recruitment takes place mainly in autumn. Few recruits were also recorded in winter, a fact indicating a rather prolonged reproductive activity (i.e. from spring to early summer) (Gontikaki et al., 2003).

The studied Holocene Turali fauna represents a snapshot of a pre-20th Century crisis middle Caspian open bay to shore face mollusc community. Comparisons with present-day data are qualitative, but indicate the loss of many endemic Caspian species and a very large turnover in such communities (Sabrina et al., 2019)

We studied meiofauna Mollusca thus our study consisted immature Mollusca. Despite of macrofauna, meiofauna is poorly studied in Caspian Sea that should be done more study on them. In the area, the Environmental Factors of the sediments play a significant role on the Mollusca distribution. We recommended that research should be done in other area in south of Caspian Sea, for example in Guilan and Golestan provinces and in deep water (more than 50 m) and to do phylogenetic experiments on Mollusca species.

5 Conclusion

Distribution and diversity of meiofauna Mollusca in southern of Caspian Sea (Mazandaran - Iran) and two classes of Mollusca (Bivalvia and Gastropoda) were identified. Class bivalvia had 3 species (*Didacna protracta*, *Hypanis caspia*, *Abra ovata*) that belong to 3 genera of 2 families and class Gastropoda had 4 species (*Pyrgula* sp., *Anisus kolesnikovi*, *Abeskunus sphaerion*, *Ulskia ulskii*) that belong to 4 genera of 3 families. Most dominant species of Bivalvia was *Abra ovata* (46.2 ind/0.1m^2) and that of Gastropoda was *Abeskunus sphaerion* (58.66 ind/0.1m^2). The mean of maximum and minimum density of Mollusca was observed in spring (29.67 ind/0.1m^2) and winter (0 ind/0.1m^2) respectively. The results of One Way ANOVA showed that density of Mollusca was different significantly with depth and season ($P<0.05$). Results of Pearson correlation showed that between density of Mollusca and salinity, %silt and clay, TOM and depth had been negative positive correlation and had been positive correlation with temperature.

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