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ORIGINAL RESEARCH PAPER

Meiofauna and macrofauna community structure in relation with environmental factors at South of Caspian Sea

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ABSTRACT: Biodiversity and distribution of benthic Meiobenthos in the sediments of the Southern Caspian Sea) Mazandaran) was studied in order to introduce and determine their relationship with the environmental factors. From 12 stations (ranging in depths 5, 10, 20 and 50 meters), sediment samples were gathered in four seasons (2012). Environmental factors of water near the bottom including temperature, salinity, dissolved Oxygen and pH were measured during sampling with CTD instrument(conductivity, temperature and Depth) and the grain size and total organic matter percentage and calcium carbonate were measured in the laboratory. From the 4 group animals (Foraminfera, Crustacea, Worms and Mollusca), 40 species were identified belonging to 29 genera of 25 families belonging to meiofauna and 15 species belonging to 15 genera of 13 belonging to macrofauna. Among seven parameters evaluated, Pearson correlation showed that there is a negative correlation between density of meiobenthos, TOM and depth and there is not a correlation between macrofauna and environmental factors. However, according to the results of One Way ANOVA, the density of meiofauna was significantly different from station, season and depth, and macrofaunain was also significantly different from station and season ($P<0.05$). Maximum Shannon–Wiener index was observed in winter.

KEYWORDS: *Benthic community structure; Caspian Sea; Environmental factors; Macrofauna; Meiofauna*

INTRODUCTION

The Caspian Sea is the largest inland body of water in the world and accounts for 40 to 44% of the total lacustrine waters. The coastline of the Caspian Sea is shared by Azerbaijan, Iran, Kazakhstan, Russia, and Turkmenistan. The Caspian is divided into three distinct physical regions: the northern, middle, and

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southern Caspian Sea. The studied area is located in the southern Caspian Sea region (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 Caspian was once part of the Tethys Ocean but became landlocked approximately 5.5 million years ago due to plate tectonics. Both 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 Sea ecosystem is a closed basin, with its own sea- level history that is independent of the eustatic level of the world's oceans (Amirahmadi, 2000). Biodiversity of flora and fauna of the Caspian Sea are unique. Approximate number of plant and animal species native to the Caspian Sea (Simonett, 2006).

There are several groups of benthos commonly distinguished by the body size of organisms: macro-, meio-, microzoo- and microphytobenthos (Balsamo, 2010; Danovaro *et al*., 2004; Higgins and Thiel, 1992). Each of these size groups includes certain taxa and can be considered as a distinctive ecological unit, which has a peculiar set of daptations as well as specific scales of spatio-temporal perception (Burkovsky, 1992; Burkovsky *et al.,* 1994).

The ecology of the main taxa forming these groups has been studied repeatedly, including their spatial distribution, dynamics or feeding modes. Very few attempts have been done, however, to compare the spatiotemporal variability of different size groups. Attention has mostly been paid to the possible between-block trophic interactions, with the main emphasis on such functional characteristics as total abundance, production etc. (Montagna *et al.,* 1995; Buffan-Dubau and Carman, 2000). Much more rarely, the community patterns have been compared for the organisms of different sizes inhabiting the same site. The invertebrate benthic species – meiofauna and macrofauna - provide key linkages between primary producers and higher trophic levels in estuarine food chains (Gee, 1989; Moens and Vincx, 1996).

Meiobenthos and macrobenthos, apart from the difference in size, have a series of distinctive ecological and evolutionary characteristics which suggest different mechanisms for diversity maintenance (Warwick, 1989; Warwick *et al.,* 2006). The dynamics of each component of the benthos may also differ depending on the environmental conditions and trophic state (Danovaro *et al.,* 1995). Surprisingly, data on simultaneous seasonal comparisons between macrofauna and meiofauna in Caspian Sea is not available. It is not clear whether any correspondence exists between the distribution patterns of micro- and meiobenthos. The relative contribution of different spatial and temporal scales to the total variability of their abundance or composition is not well understood (Azovsky *et al.,* 2004).

The aim of this study is to describe and to compare the seasonal variability of the benthic communities – meiofauna, macrofauna – in a coastal water of Caspian Sea.

Site descriptions

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

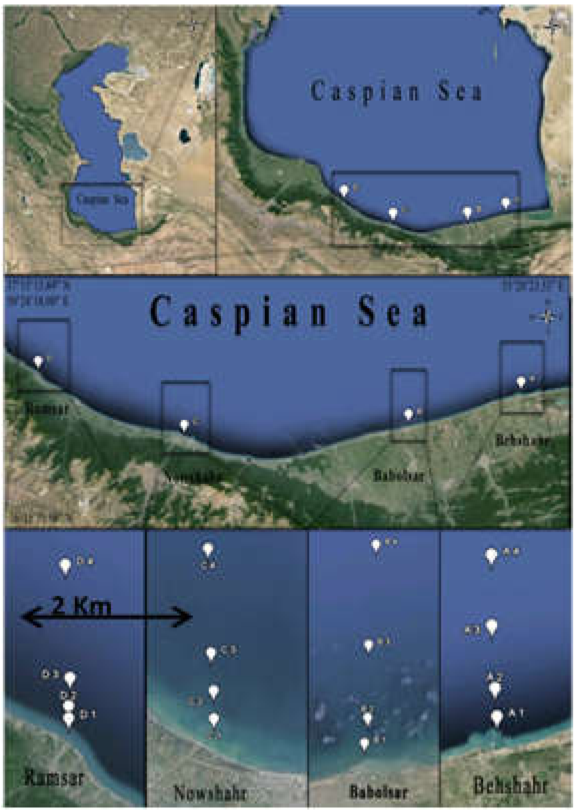

Fig 1: Situation of sampling stations in the Southern Caspian Sea

Table 1: Position of sampling stations

Stations	Depth(m)	Longitude (ºN)	Latitude (ºE)
A1	5	36° 51' 31"	53° 16' 16. "
A2	10	36° 53' 10"	53° 16' 12"
A3	20	36° 56' 48"	53° 16' 09"
A4	50	37° 00' 52"	53° 16' 16"
B1	5	36° 43' 18"	52° 39' 33"
B2	10	36° 43' 58"	52° 39' 36"
B3	20	36° 45' 55"	52° 39' 28"
B4	50	36° 48' 41"	52° 39' 29"
C1	5	36° 40' 32"	51° 27' 43"
C2	10	36° 41' 04"	51° 27' 44"
C3	20	36° 41' 47"	51° 27' 42"
C4	50	36° 43' 47"	51° 27' 41"
D1	5	36° 56' 47"	50° 39' 20"
D2	10	36° 57' 18"	50° 39' 21"
D3	20	36° 58' 29"	50° 39' 26"
D4	50	37° 03' 17"	50° 39' 16"

MATERIALS AND METHODS

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. For benthic studies, each sediment (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; Sadough *et al.,* 2013).

Benthos Analysis

For determining macrobenthic and meiobenthos in order, in the laboratory, wet samples were washed through 500 and 63ìm mesh sieve to remove any excess stain, macrobenthic separated from 500 ìm mesh sieve and fixed with alcohol ethanol (70%) and meiobenthic was then oven dried (75ºC, 8 h) (Schratzberger *et al.,* 2002) Foraminferal, ostracoda and mollusca tests were floated off using the heavy liquid CCl4 with the upper layer of the liquid consisting of floated meiobenthos tests, which were then filtered by paper and allowed to dry. A stereomicroscope was used to examine and identify tests with reference to several previous studies (Birshtain *et al.,* 1968; Murray, 1979; Loeblich and

Tappan, 1988). For determining worms, in the laboratory, wet samples were washed through 500-63 ìm mesh sieve to remove any excess stain and then fixed with alcohol ethanol (70%). Stereomicroscope and microscope were used to examine and identify tests with reference to and several previous studies (Birshtain *et al.,* 1968; Hayward and Ryland, 1996).

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, 8h) 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).

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) 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 discernible, 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).Calciumcarbonate percentage was measured by the following formula [%$CaCO_3$= 100 (W1-W2) / W1] (Moghaddasi *et al.,* 2009).

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, % Caco3 and granulometry). Discriminant Analysis (DA) was used in different depth and stations. One Way ANOVA was performed to test for possible differences. Shannon-Wiener (H/) diversity index and Peilou's Evenness Index have measured assaying species diversity and ecological assessment in this area (Marques *et al*., 2009). Amount of Shannon and Peilous indexes has been showed in Tables 5, 6 and 7:

Table 2: The Mean of Temperature, Salinity, DO, pH, Total Organic Matter (TOM) and Caco3 in different Seasons 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 Caco3 in different Seasons 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 Caco3 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: Shnanon and Peiole index for meiofauna and macrofauna in different seaspns in the southern Caspian Sea from Behshahr to Ramsar

season / index	Spring	Summer	Autumn	Winter
Shannon(meiofauna)	0.5	0.57	0.85	0.9
Shannon(macrofauna)	0.56	0.6	0.6	1.1
Peilou's(meiofauna)	0.31	0.3	0.4	0.46
Peilou's(macrofauna)	0.47	0.63	0.46	0.83

Table 6: Shanon and Peiole index meiofauna and macrofaunain different depths in the southern Caspian Sea from Behshahr to Ramsar

index / Depth(m)	Shannon (meiofauna)	Shannon (macrofauna)	Peilou's (meiofauna)	Peilou's (macrofauna)
5	0.93	0.77	0.52	0.61
10	0.82	0.68	0.39	0.65
20	0.66	0.78	0.31	0.63
50	0.49	0.63	0.39	0.49

Table 7: Shnanon and Peiole index for meiofauna and macrofauna in different stations in the southern Caspian Sea from Behshahr to Ramsar

Index / Depth(m)	Shannon (meiofauna)	Shannon (macrofauna)	Peilou's (meiofauna)	Peilou's (macrofauna)
Behshahr(A)	0.28	0.70	0.13	0.58
Babolsar(B)	0.88	0.75	0.43	0.63
Noshahr (C)	0.78	0.82	0.5	0.67
Ramsar (D)	0.96	0.59	0.55	0.50

Table 8: Density of meiofauna and macrofauna in $10cm^2$ of sediment indifferent depths in the southern Caspian Sea from Behshahr to Ramsar (±SD)

Depth(m) / Density	5	10	20	50
(meiofauna)	606.65±309.46	524.08±325.19	705.93±418.54	279.79±150.46
(macrofauna)	1.76±1.67	1.37±1.76	4.55±2.93[a]	3.67±4.36

Table 9: Density ofmeiofauna and macrofauna in 10cm^2 of sediment indifferent sesons in the southern Caspian Sea from Behshahr to Ramsar(±SD)

Season / Density	Spring	Summer	Autumn	winter
(meiofauna)	362.6±232.81	541.81±347.61	592.01±331.95	820.1±360.04
(macrofauna)	3.74±2.75	2.68±2.14	2.77±3.61	2.16±2.22

Table 10: Density of meiofauna and macrofauna in 10cm^2 of sediment indifferent stations in the southern Caspian Sea from Behshahr to Ramsar(±SD)

Station / Density	Behshar(A)	Babolsar(B)	Noshahr(C)	Ramsar(D)
(meiofauna)	475.6±291.12	654.82±406.75	587.64±270.84	398.39±234.94
(macrofauna)	1.58±1.94	2.54±1.95	2.14±2.23	5.09±4.6

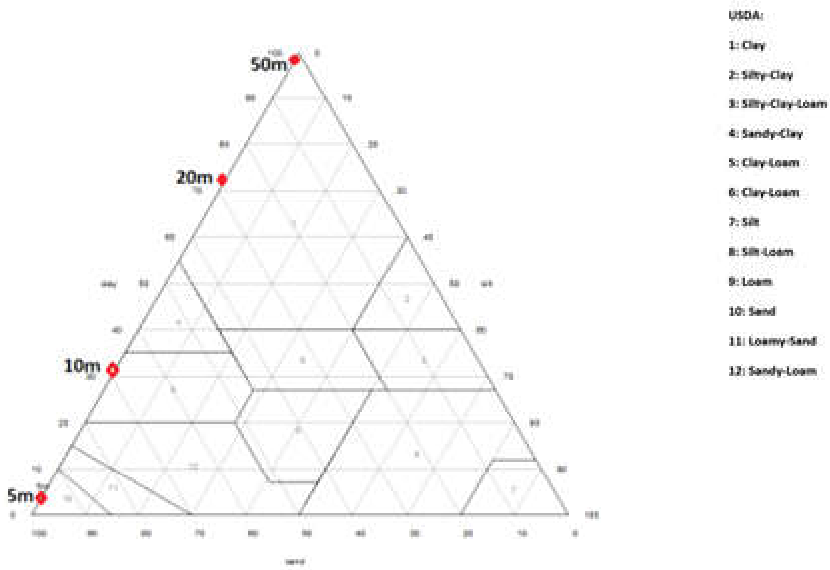

Fig. 2: percentage of grovel, sand and silt and clay in different depth in the southern Caspian Sea from Behshahr to Ramsar

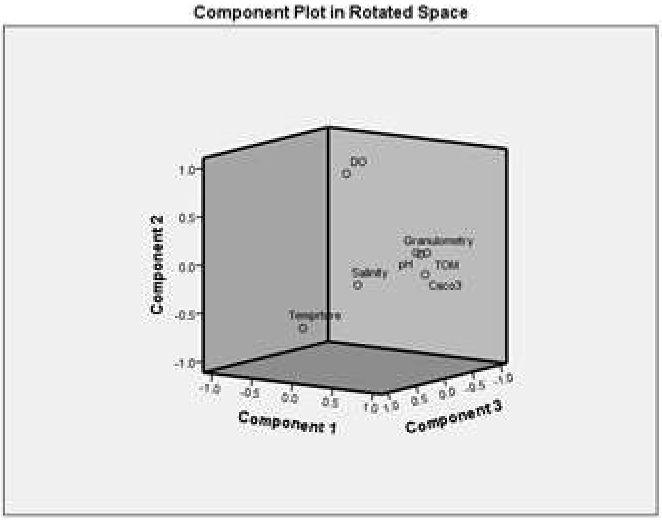

Fig. 3: PCA of environmental factors in southern of Caspian Sea in the sampling period.

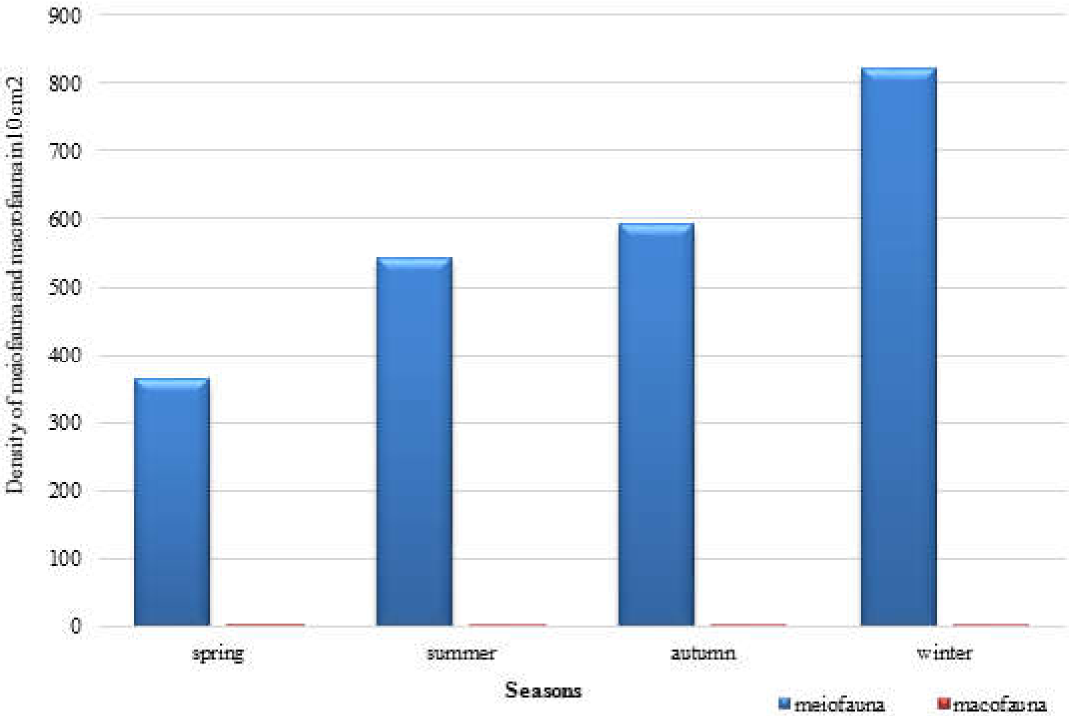

Fig. 4: Density of living benthos in different seasons (in Mazandaran province)

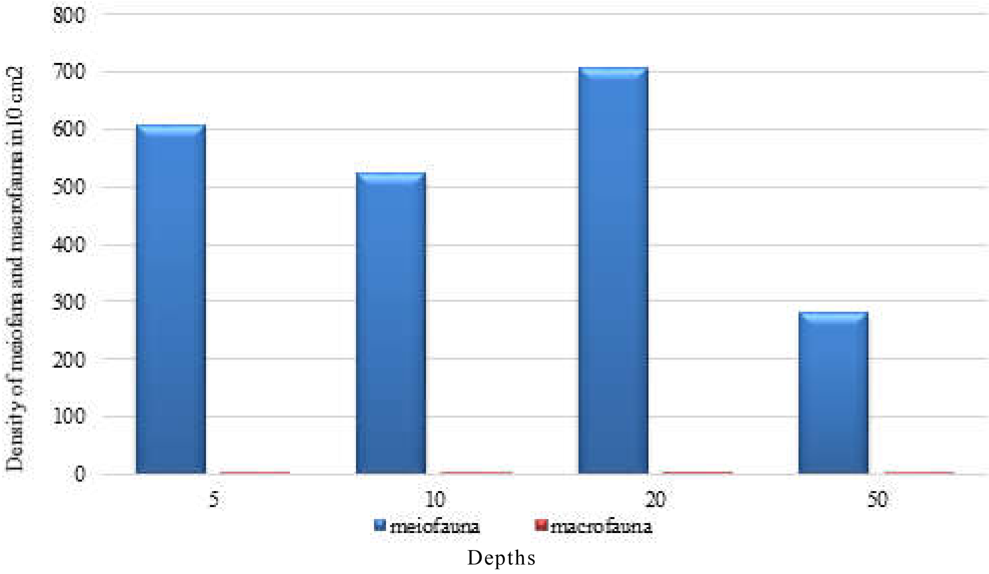

Fig. 5: Density of living meiobenthos in different depths (m) (in Mazandaran province)

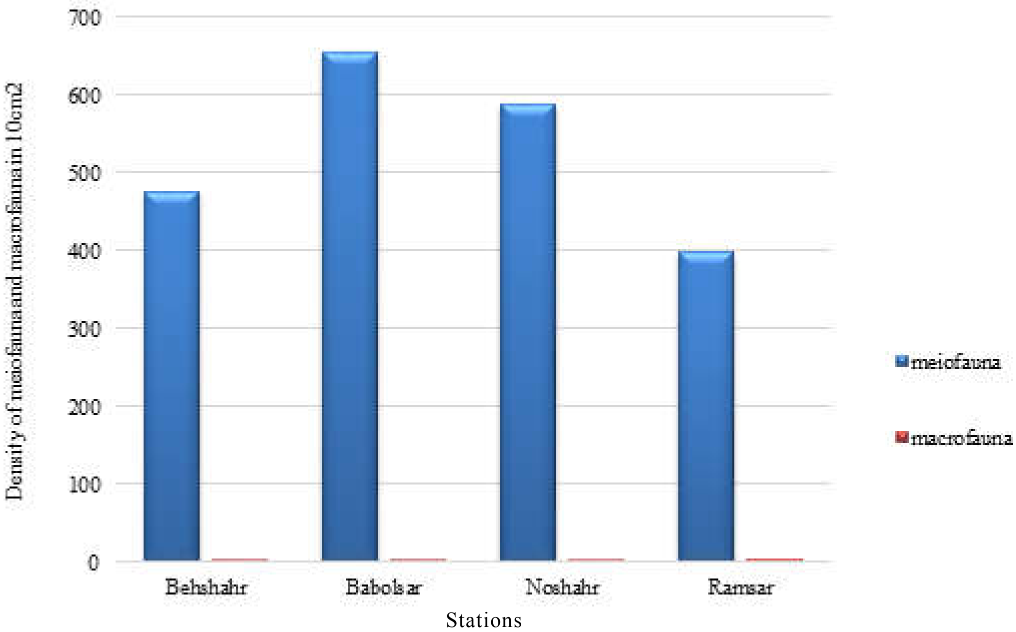

Fig. 6: Density of living meiobenthos in different stations (in Mazandaran province)

Table 11: Living identified species of meiofauna in the southern Caspian Sea from Behshahr to Ramsar in different seasonss.

Group of meiofauna	season / species	spring	summer	autumn	winter
Foraminfera	Ammonia beccarii	*	*	*	*
	Ammonia tepida	*	*	*	*
	Ammonia parkinsoniana	*	*	*	*
	Elphidium littorale	*	*	*	*
	Criboelphidium sp.	*	*	*	*
	Elphidium excavatum	*	*	*	*
	Ammobaculites agglutinans	*	*		*
	Ammotiumsp.	*	*		
	Miliammina fusca	*			
	Miliammina sp.	*	*	*	*
	cornuspira sp.		*		
Crustacea	Amnicythere longa				
	Amnicythere bacuana	*			
	Amnicythere reticulata				
	Amnicythere striatocostata	*	*		
	Loxoconcha lepida		*	*	*
	Loxoconcha rhomboidea				*
	Xestoleberis depressa	*			*
	cyprideis littoralis	*	*		*
	Darwinula stevensoni	*	*	*	*
	Polyphimidae				*
	Copepoda		*		*
	Mysidae	*	*	*	*
Mollusca	Didacna protracta	*	*		*
	Hypanis caspia	*	*		*
	Abra ovata	*	*		*
	Anisus kolesnikovi				
	Abeskunus sphaerion	*	*		*
	ulskia ulskii				
Worms	Paranais litoralis	*		*	*
	S. gynobranchiata	*	*	*	*
	Nereis diversicolor				
	Annulovortex sp.	*		*	*
	nematoda	*	*	*	*
Total		25	24	22	14

Table 12: Living identified species of macrofauna in the southern Caspian Sea from Behshahr to Ramsar in different seasonss.

Group of macrofauna	species \ season	spring	summer	autumn	winter
Mollusca	*Dreissena* sp.	*		*	
	Hypanis caspia	*	*	*	
	Abra ovata	*	*	*	
	Anisus kolesnikovi	*	*	*	*
	Abeskunus sphaerion	*	*	*	*
	ulskia ulskii	*	*	*	*
Crustacea	*pontogammarus maeoticus*	*	*		*
	cumacea				*
	mysidacea	*			
	ostracoda	*		*	*
	barnacle		*		
Worms	*Streblospio gynobranchiata*	*	*	*	*
	nereis diversicolor	*	*	*	*
	paranais littoralis	*	*	*	*
	nematoda			*	
Total		12	10	11	9

RESULTS AND DISCUSSION

From the four group animals (Foraminfera, Crustacea, Worms and Mollusca), 40 species were identified belonging to 29 genera of 25 families belonging to meiofauna and from three group macrofauna (Crustacea, Worms and Mollusca) and 15 species belonged to 15 genera of 13 families.

The distribution and dynamics of benthos communities in ecosystems are strongly influenced by fluctuations of the physicochemical factors. Among seven parameters evaluated, Result of Pearson correlation showed that there had been a negative correlation between density of meiobenthos, TOM and depth and there was no correlation between macrofauna and environmental factors. However, according to the results of One Way ANOVA, the density of meiofauna was significantly different from station, season and depth also macrofauna was significantly different from station and season ($P<0.05$). The substrate type varied among the four depths (Fig. 2). The common substrate type consisted of coarse sand, find sand, silt and clay. The highest diversity was observed in depth of 5m. In this depth (Fig. 2) substrate structure consisted of fine sand.

Therefore, it can be assumed substrate is one of the major factors that influence the distribution of benthos. The result of PCA showed that granulometry had had an important role (Table 6 and Fig. 7).

Offshore environment with the depth between 10-50 m includes 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).

In this research TOM density of animals was low with the increase 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.

When increasing depth density was decreased, we observed maximum density of meiofauna and macrofauna in depth of 20m. In the previous study by Michel *et al.* (2007), however, a reduction in macrofaunal diversity in deeper waters is a general trend. Because the depth percent of silt and clay and TOM is increased, that distribution was decreased.

Seasonal changes in environmental parameters can be significant in temperate areas. In this research, maximum density of meiofauna and macrofauna as observed in winter and spring showed a 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).

According to table (8, 9 and 10), the result showed that density of meiofauna is very high compared with macrofauna. Maximum and minimum density of meiofauna was observed in winter and spring respectively. In about macrofauna maximum and minimum density was observed in spring and winter. We observed highest Shannon–Wiener index for meiofauna and macrofauna in winter. We also measured high Pielou index in winter thus Shannon–Wiener index was high in winter rather other seasons despite we had maximum richness in spring (Table 5).

The studied benthic fauna components of the Laguna Estuarine System showed a clear seasonal variation, though with an opposite pattern of variation. Whilst the number of species and abundance of the macrofauna were significantly higher in the spring and summer, for the meiofauna, both the number of taxa and abundances were significantly higher during the winter and autumn. Moreover, values of correlations between benthic fauna and the environmental variables (meiofauna and nematodes ositively correlated with salinity and macrofauna positively correlated with temperature). At studied site, the increase of reproductive activity of macrofaunal species during spring and summer, as showed by the highest densities of temporary meiofauna, coincided with the lower peak of the meiofauna densities. Moreover, the highest peak of the meiofauna, during autumn and winter months, corresponded to the decrease of the macrobenthos recruits. Indeed, Danovaro *et al.* (1995) showed that selective predation operated by meiofauna on the dominant polychaete families of the temporary meiofauna may structure macrofaunal communities both altering density and acting selectively on a few families of macrofaunal juveniles. Therefore, these results probably indicated that the divergent seasonal variations of the meiofauna and macrofauna may be linked to their different life strategies, and that possible biological interactions between meiofauna and macrofauna may also play a significant role in structuring these associations (Meurer and Netto, 2007).

It is already known that meiofauna and macrofauna have different mechanisms for diversity maintenance (Warwick, 1984). Although scanty, the studies that simultaneously compared seasonal variability between estuarine meiofauna and macrofauna did show different trends in variation (Fonseca and Netto, 2006). In addition to be conservatively separated on the basis of size, meiofauna and macrofauna each have a series of distinctive biological traits resulted from evolutionary adaptations to the spatial and temporal structure of the marine environment, rather than ecological constraints imposed by the physical nature of particular habitats (*Warwick et al*., 2006).

Reproduction, growth and feeding strategies differ between meiofauna and macrofauna. Moreover, the response of the meiobenthos to the constant and unpredictable disturbances of which shallow sedimentary bottoms are subjected to is not always the same as that those exhibited by the macrobenthos (Austen and Widdcombe, 2006; Gallucci and Netto, 2004).

Therefore, these results probably indicated that the divergent seasonal variations of the meiofauna and macrofauna may be linked to their different life strategies, and that possible biological interactions between meiofauna and macrofauna may also play a significant role in structuring these associations. Aside from the different life strategies, biological interactions between meiofauna and macrofauna could possible contribute to the observed opposite seasonal variation showed by the benthic faunal components. Warwick (1989) suggested that the reason why it should have been necessary for larger animals (macrofauna) to have evolved a planktonic larva was to avoid competition with and predation by the permanent meiobenthos, which constitute a highly efficient consumer unit.

CONCLUSION

In summary exploring the biodiversity and distribution of benthic Meiobenthos in the sediments of the Southern Caspian Sea (Mazandaran) showed that Foraminfera, Crustacea, Worms and Mollusca were dominant. The First major finding was that, the meiofauna group had a higher diversity (40 species belonging to 29 genera of 25 families compared with 5 species belonging to 15 genera of 13 belonging to macrofauna). Pearson correlation also revealed a negative correlation between density of meiobenthos, TOM and depth. However, there was not a correlation between macrofauna and environmental factors.

The second major finding was that the density of meiofauna was significantly different from station, season and depth, and macrofaunain was also significantly different from station and season. Further similar investigation into other parts of the Southern Caspian Sea coastline is strongly recommended.

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CONFLICT OF INTEREST

The authors declares that there is no conflicts of interest regarding the publication of this manuscript.

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