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The Relationship between Soft-Bottom Macrobenthic Assemblages and Environmental Variables of Boojagh Marine National Park, Southern Caspian Sea, Iran

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Abstract

Macrobenthic infauna and associated environmental factors influencing the benthic assemblages in the Southern coastal region of Caspian Sea were analyzed in five seasonal surveys from summer 2015 to summer 2016 (18 stations), in order to understand the assemblage structure and the factors influencing the benthic distribution. The results showed that sixteen macrobenthic species in total were collected from the research region. The composition of species was: Polychaeta (4 species); Mollusca (4); Crustacea (6); Clitellata (1) and Thecostraca (1), among which, all of species were common in the sampling of every season. The dominant species varied from season to season; however, the polychaete species *Hypania invalida* and *Clitellata species Stenogammarus carausui* were always present year-round. The results of CLUSTER analysis showed that the similarities of macrobenthic structures between the stations were low; most of the similarities in all seasons were at about 25-30% of similarity value, only two stations were up to 80%-90%. In accordance with the similarity values of the macrobenthic structures, we divided the 18 stations into five groups by the similarity level of 30%. Comparing sand percentages among transects, data based on Levene's test were homogeneous ($P>0.05$). The results of one-way ANOVA showed that the differences between transects were highly significant ($P<0.01$). Results suggested that benthos was controlled by a combination of factors such as sediment structure, salinity, pH, the electrical conductivity, turbidity and temperature, and no single factor could be considered as a main influencing factor. Additionally, significant correlations between species abundances and the tested environmental factors were evident. This study highlights the potential consequences of established non-indigenous species in the Southern Caspian Sea.

Keywords: Benthos; Biodiversity; Assemblage structure; Soft-bottom; Environmental; Caspian Sea; Boojagh marine national park (BMNP)

Introduction

While the Northern basin of Caspian Sea is extremely shallow (depth<25 m), the central and the Southern basins exhibit deep regions where depths reach approximately 800 and 1000 m, respectively [1,2]. Three main sources of inflows are present: river runoff (79%-80% of it coming from the Volga river), rainfall (20%) and groundwater inflow (1%) [3], while outflow is exclusively a result of evaporation. The salinity of the Caspian Sea is around a third of that of the oceans [4-6]. Changes in these factors such as organic matter, grading of sediment, pH, and redox potential affect the abundances of macrobenthos of sediments [7-13] Previous studies have determined a seasonal variation on macrobenthic assemblages with higher densities on spring followed by the lowest on summer [14,15].

Boojagh Marine National Park (BMNP), with an area of 3260 hectares, is a protected area in Astaneh Ashrafieh city of Gilan Province, Iran, being the first "coastal and terrestrial national park" established in the country. BMNP covers approximately 1,600 hectares of coastal area, 160 hectares of wetland meadows, and 1500 hectares of terrestrial areas [16-18]. At BMNP with a variable and diverse environmental conditions a wide range of macrobenthic assemblages might be expected. Due to its biodiversity importance a number of researchers focused on fauna and flora of this National Park [19-22]. Coastal waters of BMNP are to some extent affected by human activities such as pollutants of Sefidrood, port of Kiashahr and fishing activities so benthic study can be an efficient tool to evaluate these impacts [23,24]. However, the Caspian Sea suffers from both natural, e.g., sea level changes and anthropogenic disturbances e.g. pollution, eutrophication and invasive species [2]. The impact of the accidentally introduced ctenophore *Mnemiopsis leidyi* [25] has been tremendous on the Caspian ecosystem causing sharp decreases in macrobenthos densities, pelagic fish stocks and other higher components of the ecosystem [26-28]. Some significant data have been obtained on the recent sea level fluctuations and their chronology, on the development of the Sefidrud River delta and surrounding low-lying plain through the Holocene [2,28-31]. Structure of benthic assemblages are frequently used in pollution effect monitoring programs [32-38]. In this paper we report the results of an analysis of macrobenthos of BMNP, Gilan Province using the data from July 2015 to August 2016. As such, this study attempted to understand the quantitative distribution (abundance) of macrobenthos in the BMNP of the Southern Caspian Sea in relation to seasonal changes in environmental parameters. Ultimately, the comprehensive assessment of factors affecting in this study facilitates a better understanding of the factors influencing in this area. Our aim is evaluate the spatial and temporal variations on macrobenthic total densities, species richness and diversity in the CS and its relation with environmental parameters (Figure 1).

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Materials and Methods

Sampling

Eighteen sampling stations were established in the coastal water of BMNP, Southern coast of the Caspian Sea, within the area of 37°24'45.37"N, 50°2'17.90"E. We sampled in the region every season, from July 2015 to August 2016: including summer 2015, autumn 2015, winter 2016, spring 2016 and summer 2016. Sediment samples were taken to study the macrobenthos by Van Veen grab with a sampling surface area of 0.22 m^2. Each replicate was placed in a separate container and tagged with transect and sampling station specifications (Table 1). Then a 73 g/l solution of Magnesium Chloride was used to relax the species [39-43]. In the laboratory of Science and Research Branch of Islamic Azad University samples were fixed with 10% formalin. In order to identify macrobenthic invertebrates, samples were washed though a 0.5 mm mesh sieve. Specimens of the macrobenthos were sorted and identified with the help of microscope. Identified taxa were kept in 80% ethanol for further reference. Species were identified to the lowest possible level of taxonomy using Birshtain et al. as identification main reference with up to date corrections from World Register of Marine Species (WoRMS) reference website. To evaluate the significance of differences a one-way ANOVA was performed using SPSS 16 software and a post hock test of LSD was used to detect differences among sites. Also, Microsoft Excel was used for the calculation of location and dispersion of gathering environmental parameters [44].

Environmental factors

The environmental factors were obtained in situ, including water depth, temperature, salinity, pH, turbidity, dissolved oxygen (hereafter referred to as DO). Among these, water depth, temperature and salinity

Figure 1: Study area and sites of sampling, BMNP (6 transect; 18 sampling stations).

Transect season	TRANSECT									
Season	Transect	SAND (%)	SILT (%)	TOM (g/l)	Turbidity (m)	pH	Conductivity (µz)	Salinity (ppt)	O2 (g/l)	Temperature ©
	1	87.52	2.03	0.09	2	8.2	13.13	17	5.3	31
	2	83.4	4.86	0.09	1.8	8.2	13.12	18	5.3	31
	3	85.89	1.46	0.09	1.8	8.2	13.13	17	5.4	31
Autumn 2015	4	95.5	0.29	0.07	2.3	8.3	13.08	5	5.4	31
	5	84.85	1.81	0.07	1.8	8.2	13.06	18	5.2	31
	6	93.55	1.24	0.07	1.8	8.3	13.07	18	5.4	31
	1	89.13	9.84	0.08	0.8	8.3	12.49	11	6.7	15.6
	2	69.41	15.44	0.09	1	8.4	12.71	12	6.3	15.6
	3	76.09	11.01	0.09	0.9	8.5	12.67	10	6.7	15.6
Winter 2015	4	87.51	1.4	0.09	0.4	8.7	12.56	6	6.3	15.7
	5	65.37	29.45	0.18	1.3	8.4	12.76	10	7.7	15.6
	6	88.7	11.28	0.1	1.2	8.4	12.76	11	7.6	15.6
	1	98.78	0.3	0.1	1.1	8.6	12.17	16	7.3	11.5
	2	96.39	1.45	0.07	1	8.6	12.04	16	7.3	11.5
	3	92.95	1.82	0.09	1	8.5	12.2	16	7.5	11.5
Spring 2016	4	95.36	1.21	0.09	0.4	8.5	12.76	8	7.7	11.5
	5	93.22	4.75	0.22	0.8	8.5	13	17	6.6	11.3
	6	96.47	2.35	0.1	1.1	8.5	13.01	17	7.8	11.5
	1	92.05	4.72	0.1	1.8	8.4	12.11	18	6.2	19.4
	2	90.39	5.18	0.09	1.5	8.4	12.72	19	6.1	19.4
Summer 2016	3	93.16	2.35	0.09	1.4	8.4	12.95	17	6.3	19.3
	4	82.4	5.2	0.11	1.8	8.4	12.67	9	6.1	19.3
	5	91.86	4.8	0.1	1.7	8.4	12.47	18	6.1	19.3
	6	93.98	4.78	0.1	1.8	8.4	12.5	18	6.1	19.3
	1	93.9	1.04	0.1	2.6	8.6	13.15	16	6.1	30
	2	82.38	6.12	0.08	2.6	8.7	13.59	16	5.8	30
	3	95.34	2.65	0.09	2.4	8.7	12.81	16	6.1	29.6
Autumn 2016	4	97.49	1.45	0.07	2.3	8.6	13.6	8	6	29.6
	5	95.27	2.76	0.1	1.9	8.5	13.35	16	6	29.6
	6	88.71	2.96	0.09	2.2	8.6	13.31	16	6.2	30

Table1: Localities of the sampling transect of BMNP with the sediment type and concentrations of organic matter (%).

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were measured, turbidity was measured with a secchi disk, and for gathering sample water in different depth by Niskin bottle sampler.

Statistical analysis

Softwares of Plymouth Routines in Multivariate Ecological Research (PRIMER 6.0), SPSS 16.0 and Excel with office 2010 were used for the statistical analysis. The biological properties include abundance (A), the number of species (S), Shannon-Wiener diversity index (H_), Margalef richness index (d), and dominant index (Y). The dominant index of species was calculated by the following formulae: $Y=(n_i/N) \times fi$. (1) Where, N is the total abundance of all the stations; n_i is the abundance of the species i of all the stations; fi is the occurring frequency of the species i of all the stations. In order to reduce the disturbance of the opportunistic species in the analysis of the biological properties of the macrobenthos, those species with their abundance proportions less than 1% in the whole abundance from the study region were deleted from the species of analysis, unless the species had more than 3% abundance proportion at any station of all the 18 stations.

Results

Analysis of environmental factors

The first and most important factor for the distribution of macrobenthoses in some cases is salinity. The total minimum average of salinity was in the spring 2016 than in other seasons. In the case of pH, the review made, the pH somewhat more were reported at lower depths. The transparency of water at depths of 10 meters was much less than the other depths. Furthermore, the maximum depth of transparency was measured in summer and lowest depth of transparency in winter 2015. The amount of dissolved oxygen is somewhat similar assessment at different depths, but on closer examination at a depth of ten meters deep was found in the winter than in other seasons. In the case of the temperature also decrease at different depths somewhat similarly measured, but in different seasons was as follows: In the summer 2015, 31°C; in the autumn 2015, 15.6°C; in winter 2016, 11.5°C; in the spring 2016, 19.4°C and in summer 2016, 30°C.

Species composition

In this study according to Table 2, sixteen species were identified.

Gastropoda represented the best taxon with 4 species (occupying 47.26% of all the species), followed by Crustacea with 6 species (19.60%), Polychaeta with 4 (13.22%) and Clitellata with 1 (9.09%) and other groups Barnacle with (10.8%). The total species number of each of the five sampling was different: As well as generally in summer 2015, Bivalves; in autumn 2015, Bivalves and Clitellata; in winter 2016, Bivalves and Polychaeta; the spring 2016 Bivalves and Cumacea and in summer 2016, Bivalves and Polychaeta were the largest and most of Macrobenthos of all five sampling, with one sampling in every season, sixteen species were identified as dominant species in the study area in accordance with their dominant values (P>0.05). Most of the species macrobenthos at a depth of ten feet and then five and one meter deep, respectively, were observed.

A total of 5 chapters sampling, Gastropoda ranks highest with an average density of 588 per square meter, class malacostraca with an average of 225 per square meter, class polychaeta with an average of 175 per square meter, category lepadiform with an average of 109 per square meter finally clitellata class with an average density of 98 per square meter was the lowest.

The results of analysis of variance benthic it should be noted that compared data on species *Cerastoderma glaucum* in the autumn 2015 of transect data on Levene's test with homogeneity of variance were significantly (P>0.05). The Shapiro-Wilk normality test results showed that normal data were significantly (P>0.05). Based on the high number of tests it was found that there is a possibility of doing parametric analysis (Table 2). As a result, one-way ANOVA was performed and the results showed that the difference between transect there was very significant (P<0.01) (Table 3).

LSD test results in the Fall 2015, according to the density of this species was divided into two groups in transect. Station 3 in transect 3, 4 and 5 had very significant difference with the rest of stations. It seems that this species tends to other deeper stations of eastern. This is probably due to Sefidrood river output is approaching nutrients such as transect 4 on the mouth of the river and around the transect 3 and 5.

Class	Order	Family	Species names
Bivalvia	Cardiida	Cardiidae	*Cerastoderma glaucum*
	Mytilida	Mytilidae	*Mytilaster lineatus*
Gastropoda	Littorinimorpha	Hydrobiidae	*Pyrgula grimmi*
	Hygrophila	Planorbidae	*Anisus kolesnikovi*
Malacostraca	Amphipoda	Pontogammaridae	*Stenogammarus carausui*
			Paraniphargoides motasi
		Uristidae	*Onisimus caspius*
	Cumacea	Pseudocumatidae	*Pterocuma pectinatum*
			Pterocuma sowinskyi
			Pseudocuma (Stenocuma) gracile
Hexanauplia	Sessilia	Balanidae	*Amphibalanus improvisus*
Polychaeta	Terebellida	Ampharetidae	*Hypania invalida*
	Sabellida	Fabriciidae	*Manayunkia caspica*
	Spionida	Spionidae	*Streblospio gynobranchiata*
	Phyllodocida	Nereididae	*Hediste diversicolor*
Clitellata	Haplotaxida	Tubificidae	*Nais sp.*

Table 2: Identified species in the 5 seasons from summer 2015 until summer 2016.

ANOVA	Sum of squares	Degrees of freedom	Mean square	F	P
Between groups	1111.200	17	65.365	3.860	0.000
Within groups	1219.200	72	16.933	-	-
Total	2330.400	89	-	-	-

Table 3: One-way ANOVA, samples *Cerastoderma glaucum* species in BMNP, Caspian Sea.

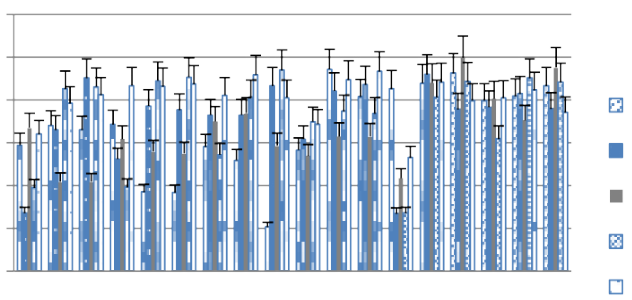

Figure 2: Shannon biodiversity index at various stations in total sampling period (5 chapters) from summer 2015 to summer 2016 BNMP (Error bars show standard deviation).

Biodiversity

According to Shannon index, most stations were good in most seasons biodiversity, including stations with good biological diversity can be noted in the following stations: Figures 2 and 3 show the two biodiversity indices of the macrobenthos at all stations in five seasons. All stations that were located at a depth of 10 meters (S3). The depth of 5 meters stations was also somewhat appropriate that this well is visible on the graph.

Discussion

Tajalipour [45] examined the Iranian coast of the Caspian Sea and he divided coastal zone into eleven sections, each station at depths of 5-200 m with three replications because ampharetids and *S. gynobranchiata* inhabit similar habitats and are both considered surface deposit feeders [46], it appears that *S. gynobranchiata* is able to outcompete native ampharetids in shallow waters and displace them to greater depths in the Southern Caspian Sea. Likewise, because *H. diversicolor* occupy overlapping habitat, they are also potential competitors [47]. Because *H. diversicolor* preys on larger organisms [48], *H. diversicolor* becomes suppressed increases in abundance [49]. Hence, *H. diversicolor* is usually confined to fresher waters, greater depths, and high-sulfidic sediment areas where it is able to tolerate extreme conditions, albeit at the expense of competitive ability [50,51]. For example, the introduction of macrobenthos into aquaculture pens resulted in increased species richness, enhanced the productivity and biomass of benthic fauna, and improved environmental conditions for fish production by reducing the organic matter content in sediments [52]. Also, in comparing data related to oligochaete, *Nais sp.* To solve the problem of data transfer with reflow was used. After transferring the homogeneity of variance Levene test results significant ($P>0.05$) and Shapiro-Wilk normality test results significantly ($P>0.05$). Based on the high number of tests it was found that the possibility of doing parametric analysis after transferring there. LSD test results in the Fall 2015, according to the density of this species was divided into two groups of in transect. Stations 2 and 3 in transect 5 had significant difference from the rest of the stations in all transect, of course transect and station 3 was very significant difference to all stations even two stations have the same transect. It seems the station and partly station 2 in this transect environment is appropriate to increase the density of these oligochaet.

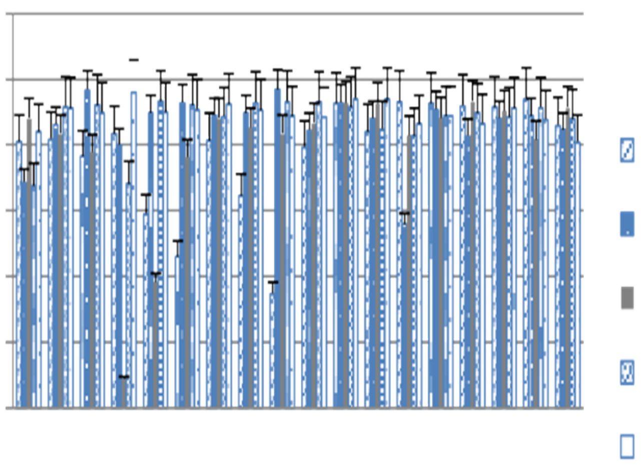

Figure 3: Simpson biodiversity index at various stations in total sampling period (5 chapters) from summer 2015 to summer 2016 BNMP (Error bars show standard deviation).

The effect of stress based on their population structure parameters are detected [53]. Several studies also prove that anthropogenic activities and environmental factors would influence the spatial and temporal distributions of macrofaunal abundance and biomass [54-56]. Furthermore, significant correlations between macrobenthos abundance and percentages of silt–clay and various sand types were also observed in the current study. These observations suggest that sediment grain size and %TOM may be influential contributors to macrobenthic assemblage composition; similar suggestions have been made by others [57,58]. McIntyre [59] suggested that, coupled with the direct operation of temperature and salinity, grain size operates indirectly to control the distribution of benthic organisms. In brackish waters, salinity is an important factor influencing the spatial distribution of animals [60] and is considered the most important abiotic factor in the Caspian Sea [61]. Historically, Caspian endemic species adapted to inhabit fresher waters of the northern Caspian Sea [62]. As such, lower diversity in the Southern Caspian Sea could be related to higher salinities [62]. Species introductions can have both positive and negative impacts on their invaded such as different ecosystems. Ecological impacts of invasions are often inferred by assuming that impacts become more severe as non-indigenous species (NIS) become more abundant and widespread. However, studies where distribution and abundance of NIS can be evaluated relative to native species are typically conducted at restricted spatio-temporal scales in areas known to be infested, potentially overestimating the importance of the NIS over broader scales [63]. The geographical range of several species has been increased by human activities, both intentionally (e.g. aquaculture) and unintentionally (e.g. interconnection of water basins through canals; shipping activities) [64]. As such, many invasive species have arrived in the Caspian Sea. For example, the oligochaete *Tubificoides fraseri* and polychaete *S. gynobranchiata* have been unintentionally introduced into the Caspian Sea [65], most likely through the Volga Don canal, which opened in 1954. Additionally, *H. diversicolor* and have been intentionally introduced to the Caspian Sea to increase food resources for commercially exploited fish [65]. Consequently, in the Caspian Sea, these worms represent a significant food resource for commercially valuable fish and could potentially facilitate the recovery exploited fish stocks. Because these invasive macribenthos are usually dominant taxa in benthic assemblages of the Caspian Sea, they could be utilized as indicator species of environmental conditions [66]. In addition, errantia worms, could potentially enhance the oxygenation, organic matter mineralization decomposition, nutrient accessibility and bacterial activity of sediments by means of bioturbation [67,68]. Ellingsen [69] studied macrobenthic infauna in relation to environmental variability in Norway and found that the best correlative variable combination included depth, median grain size and silt–clay content. Nevertheless, physical disturbance and chemical contamination in sediment may have higher effects on macrobenthic infauna than sediment characteristics in coastal waters [70]. Han et al. [71] studied the macrobenthic assemblage structure in the eastern and central Bohai Sea and reported that "water depth and nitrate concentration in the bottom water, followed by microfauna abundance, had the closest relationships with the macrobenthic assemblage". Jayaraj et al. [72] studied the macrobenthos and associated environmental factors in the northwest Indian shelf, and thought that "benthos were controlled by a combination of factors such as temperature, salinity, dissolved oxygen, sand and organic matter and no single factor could be considered as an ecological master factor" [73,74]. Although the establishment of these some nonindigenous macrobenthos as an additional food source could facilitate the rehabilitation of commercially exploited fish stocks, the consequences for native benthic assemblages remain unclear. As such, benthic assemblages are at risk of being subjected to unforeseen negative impacts. Ultimately, NIS have the potential to provide many favorable ecosystem services and enhance the overall health of an ecosystem; however, further studies are required to monitor the potential impacts of these non-indigenous macrobenthos on the benthic assemblages of the Caspian Sea.

Conclusion

Our recent study showed that the salinity, temperature and sand in the research region were the most important factors impacting the assemblage structure of macrobenthos and without "one master factor" controlling the macrobenthos assemblage. The combination of factors of concentrations of sand and temperature would impact mainly the assemblage structure of macrobenthos. Our results are different from that of the above mentioned, which may be due to the different environmental factors in the water regions.

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