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Assessing benthic health of hard substratum macrobenthic community using soft bottom indicators and their relationship with environmental condition

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Abstract

This study aimed to assess ecological quality status of hard substratum macroinvertebrates communities of the Caspian Sea with three ecological indices and their relationship with environmental factors. For this purpose, benthic communities of the Caspian Sea basin were studied seasonally during 2014 in 8 sampling sites. Temperature, salinity, dissolved oxygen, pH, nitrate, nitrite, silicate and phosphate were measured as environmental factors. The benthic classification indices AMBI (AZTI Marine Biotic Index), M-AMBI (Multivariate AMBI) and BENTIX (BENthic IndeX) were applied to assess the ecological status of the studied area. Results showed low dissimilarity based on species composition and abundance among seasons, while all seasons discriminated clearly based on environmental factors. In addition, AMBI index was more successful to assess ecological health of hard substratum in the Caspian Sea basin than M-AMBI and BENTIX. Furthermore, AMBI showed high sensitivity to environmental variation. Results indicated that temperature, nitrate, silicate, phosphate and nitrite were the most important factors in the composition and abundance fluctuation of hard substratum macroinvertebrates communities, respectively.

Keywords: Benthic health, AMBI, M-AMBI, BENTIX, Environmental factors, Caspian Sea

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Introduction

Hard substratum benthic communities in coastal areas, especially those areas that located near or at urban and industrial centers, are highly affected by anthropogenic activates. (Fraschetti *et al*., 2001; Zalmon *et al*., 2011; Spaccesi and Rodrigues Capitulo, 2012). Although these shores support a rich biodiversity, but contaminations such as heavy metals and/or bacteria, nutrients from organic or industrial pollution from diverse sources and from sedimentation seriously affected the diversity and functioning of these ecosystems (Terlizzi *et al*., 2002; De Wolf et al., 2004; Piola and Johnston, 2008).

Hard communities are stressed environments; due to waste water discharges that is close to the coastline. These waters cause deterioration in water quality and discourage the settlement of several organisms; that affect these communities thriving in rocky-shores (Arévalo *et al*., 2007). Organic and nutrient enrichment due to domestic wastes is today one of the main reasons explaining the deterioration of marine nearshore ecosystems (Fletcher, 1996). In addition, seasonal oscillations in environmental conditions dramatically influence the macrobenthic communities in coastal waters (Reizopoulou *et al*., 2014). Benthic invertebrates and macroalgae are sedentary long lives, easy sampling organisms that many literatures published on their distribution in specific environments and on their response to different environmental stresses (Zubikarai *et al*., 2014; Abbaspour et al., 2017; Ghorbanzadeh Zaferani et al., 2017). Therefore, these organisms are considered powerful indicators of environmental pollution.

The AZTI Marine Biotic Index (AMBI, also referred to as BC) developed by Borja *et al*. (2000) evaluates ecological health condition in five categories based on the distribution of individual abundances in benthic communities. The species were distributed in those groups according to their sensitivity to an increasing stress gradient (enrichment of organic matter) (Glémarec and Hily, 1981; Hily, 1984). M-AMBI (‘Multivariate AMBI’, Bald *et al*., 2005; Muxika *et al*., 2007) is a multivariate index for assessing the ecological health and quality status of marine and transitional waters. It is based on benthic macroinvertebrates communities and integrates AMBI, a biotic index based on species sensitivity/tolerance, with diversity and richness (Sigovini *et al*., 2013). It aims to integrate the response of species richness, the Shannon diversity index (Shannon and Weaver, 1949) and the biotic index AMBI (Borja *et al*., 2000). Simboura and Zenetos (2002) designed new index based on the AMBI Index (Borja *et al*., 2000) with a re-combination of ecological groups that assigns different weighting coefficients and results to the reduction of macrozoobenthic data in three wider ecological groups (Simboura and Argyrou, 2010).

Anthropogenic disturbances and habitat natural changes are the most important factors in reaction of aquatic organisms (Nouri *et al*., 2008; Saghali *et al*., 2013). Benthic fauna's structure is affected by environmental factors such as temperature, pH, dissolved oxygen and pollution (Sharma and Rawat, 2009; Saghali *et al*., 2013). Variation in these factors cause changes in rate of supply of organic matter and consequently affect the composition and abundance of marine organisms, such as macrobenthic communities (Erftemeijer and Herman, 1994; Bachelet *et al*., 2000).

Assessment of the macroinvertebrates communities' health in soft bottom substratum has progressed in recent years (Borja, 2005; Borja, 2006; Kutser *et al.*, 2006; Pinedo *et al*., 2007; Borja *et al*., 2008), but data on hard substratum is limited. The aim of this study was to examine the hard-substratum benthic community organism in 8 sites of the Caspian Sea rocky seawalls under different environmental conditions and relate it to ecological status indices. Moreover, the study explores the applicability of three of the most commonly used soft bottom benthic indices in assessing the ecological health in these coastlines.

Materials and methods

Sampling was performed in 8 sites form southwest to southeast shores of the Caspian Sea in Iranian waters basin. Sites were located to Astara (S1), Anzali (S2), Chamkhaleh (S3), Ramsar (S4), Sisangan (S5), Babolsar (S6), Amirabad (67) and Khajeh Nafas (S8) (Fig. 1; Table 1). Sampling was done at the midpoint of each season from spring to winter 2014. Temperature, salinity, dissolved oxygen and pH were measured using the portable multi-meters (HACH 51154, USA) with three replicates in each site. Species area curve method applied for sampling from (Browne, 1996). 20×20 cm quadrat (0.04 m^2) was performed on rocky substratum from supralittoral zone to sample from macrobenthic communities with three replicate and samples were preserved in 4% formalin. In the laboratory, the macrofauna were sorted, identified up to species level and counted (Freeman and Bracegirdle, 1971).

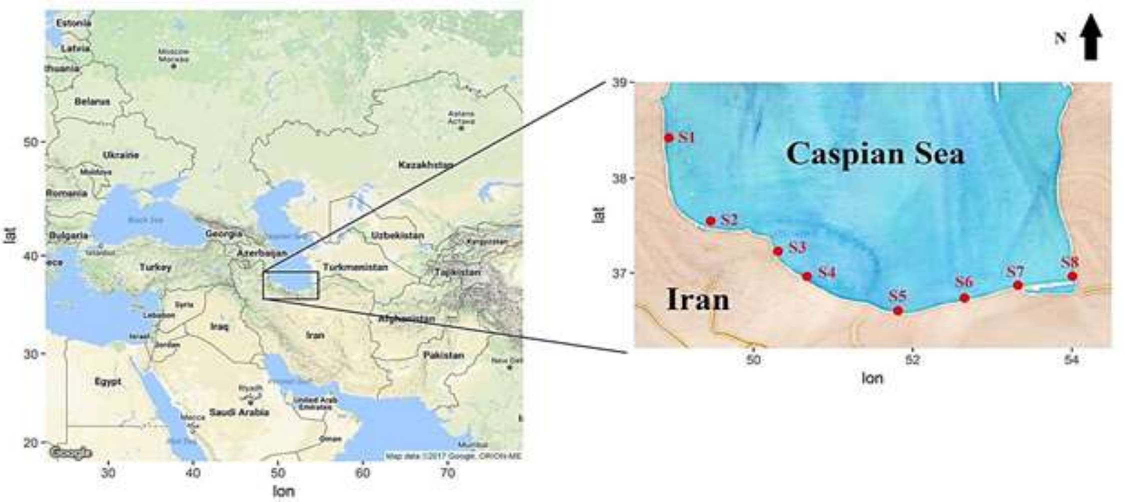

Figure 1: Map showing the sampling locations in the southern Caspian Sea (2014).

Table 1: Geographical coordinates of the stations.

Station	N	E
Astara	38°25´59.33”	48°52´52.77”
Anzali	37°28´53.00”	49°27´20.55”
Chamkhaleh	37°12´57.68”	50°16´33.22”
Ramsar	36°55´45.75”	50°40´05.19”
Sisangan	36°35´02.49”	51°48´43.41”
Babolsar	36°42´52.43”	52°39´35.40”
Amirabad	36°51´30.55”	53°23´22.17”
Khajeh nafas	36°57´49.43”	54°00´52.51”

Surface water samples were collected simultaneously from rock pools in all the selected sampling sites for analysis the nutrient contents. Water nutrient concentration was measured according to photometric methods (Wood *et al*., 1967; Strickland and Parsons, 1972). Phosphate analyzed by a modified ascorbic acid reduction method and silicate assessed based on calorimeter with formation of molybdic acid (Strickland and Parsons, 1972). Nitrite determined by colorimetric and ion chromatographic methods and nitrate was measured based on cadmium-copper reduction to nitrite (Wood *et al*., 1967).

AMBI, M-AMBI and BENTHIX biotic indices were calculated by following methods: To calculate the AMBI and M-AMBI, the free software (http://www.azti.es v.4) along with the guidelines from the authors (Borja and Muxika, 2005) was used in this study. There are no proposed reference values for M-AMBI in the Caspian Sea; therefore, due to similarity of the Caspian Sea with Mediterranean lagoons, (mean average of temperature: 16 to 23 °C for Mediterranean lagoons and 13 to 31 °C for Caspian Sea in this study; salinity from 0 to 36 ppm for Mediterranean lagoons and 0 to 16 ppm for Caspian Sea in this study; Oxygen from 0 to 14 mL L^{-1} for Mediterranean lagoons and 0 to 11 mL L^{-1} for Caspian Sea in this study; Nitrate from 0.1 to 300 µg L^{-1} for Mediterranean lagoons and 0 to 600 µg L^{-1} for Caspian Sea in this study; pH from 7.5 to 9.8 µg L^{-1} for Mediterranean lagoons and 8.3 to 8.9 µg L^{-1} for Caspian Sea in this study (López and Tomàs, 1989). The reference values for M-AMBI were set as: Diversity=0 to 1.62, S=0 to 7 and AMBI=0.09 to 4, based on AMBI calculation. To calculate the BENTIX (BENthic IndeX) (Add-in v.1.0 version) the software for MS Excel 2007 has been used downloaded free from: http://www.hcmr.gr/en/articlepage.php?id=141. Diversity indices (Margalef, Pielou, Shannon and Simpson) and MDS analyses were carried out using the PRIMER v5 software package, developed in the Plymouth Marine Laboratory. Canonical Correlation Analysis (CCA) and Canonical Discriminant Analysis (CDA) analysis was assessed by R statistical packages (Version 3.13, CCA package). SIMPER analysis preformed to assess dissimilarities between seasons and sites. In addition, Tow-Way-PERMANOVA with 9999 permutations carried out to determine significant difference between environmental factors in different seasons and sites by PERMANOVA 1.6-Anderson.

Results

The differences among the eight sites regarding to the environmental parameters are illustrated in the CDA analysis (Fig. 2). It shows that all seasons discriminated clearly. However, this separation was higher between summer with other seasons. In addition, temperature and pH was the most important factors in discriminant analysis.

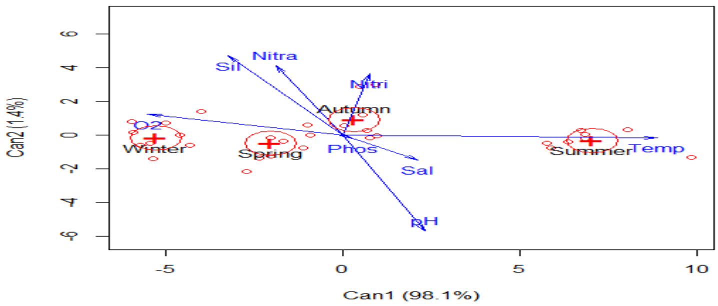

Figure 1: CDA analysis for environmental parameters in all sites (Sal= Salinity, Temp= Temperature, Phos= Phosphate, Sil= Silicate, Nitra= Nitrate, Nitri= Nitrite and O_2= Oxygen).

Tests of dimensionality and standardized for the canonical correlation analysis, as shown in Table 2 and Fig. 2, indicate that one of the four canonical dimensions are statistically significant at the .05 level.

Table 2: CCA analysis between species and nutrients in all sites

Dimension	Correlation	*p* value
1	0.5199	0.01230497
2	0.4258	0.25825982
3	0.2539	0.85435332
4	0.1136	0.94958338
Factors (Species and Nutrient)		Dimension 1
Benthic Macrofauna		
Pontogammarus maeuticus		-0.84062775
Balanus improvisus		0.07808775
Rhithropanopeus harrisii tridentatus		0.06355215
Simulium sp.		-0.70477671
Chironomus albidus		0.79912391
Mytilaster lineatus		0.08378822
Nereis diversicolor		-0.27048504
Tubificoides fraseri		0.26574585
Nutrient		
Nitrate		-1.1022654
Nitrite		0.5875904
Phosphate		-0.6865075
Silicate		0.9421993

Fig. 3 shows CCA plot between environmental factors and macroinvertebrates community. According to the plot, *Chironomus albidus* had strong positive relationship with nitrate. In addition, feebly positive relationship was observed between *Tubificoides fraseri* and nitrate and phosphate.

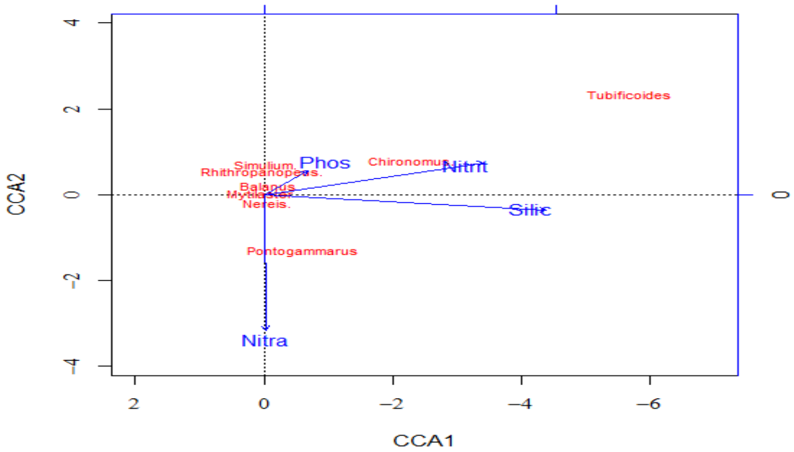

Figure 2: Two dimensional CCA plot for benthic macrofaunal and nutrients variables (Phos=Phosphate, Nitrit=Nitrite, Nitra=Nitrate and Silic=Silicate).

For the Benthic macrofauna variables dimension 1 was most strongly influenced by *Pontogammarus maeuticus*. For the nutrient variables, nitrate was the strongest variable than other parameters.

Fig. 4 shows MDS ordination plot based on environmental (e.g. salinity, temperature etc.), benthic communities and nutrition conditions (e.g. phosphates, nitrates etc.). Results indicate that site 8 and 6 was grouped clearly according to environment, while site 1, 7 and 8 was clearly separated by benthic condition. Furthermore, analyzing based on nutrient condition in sites revealed that site 7, 2, 8, 3 and 6 was clearly grouped and discriminated from other sites.

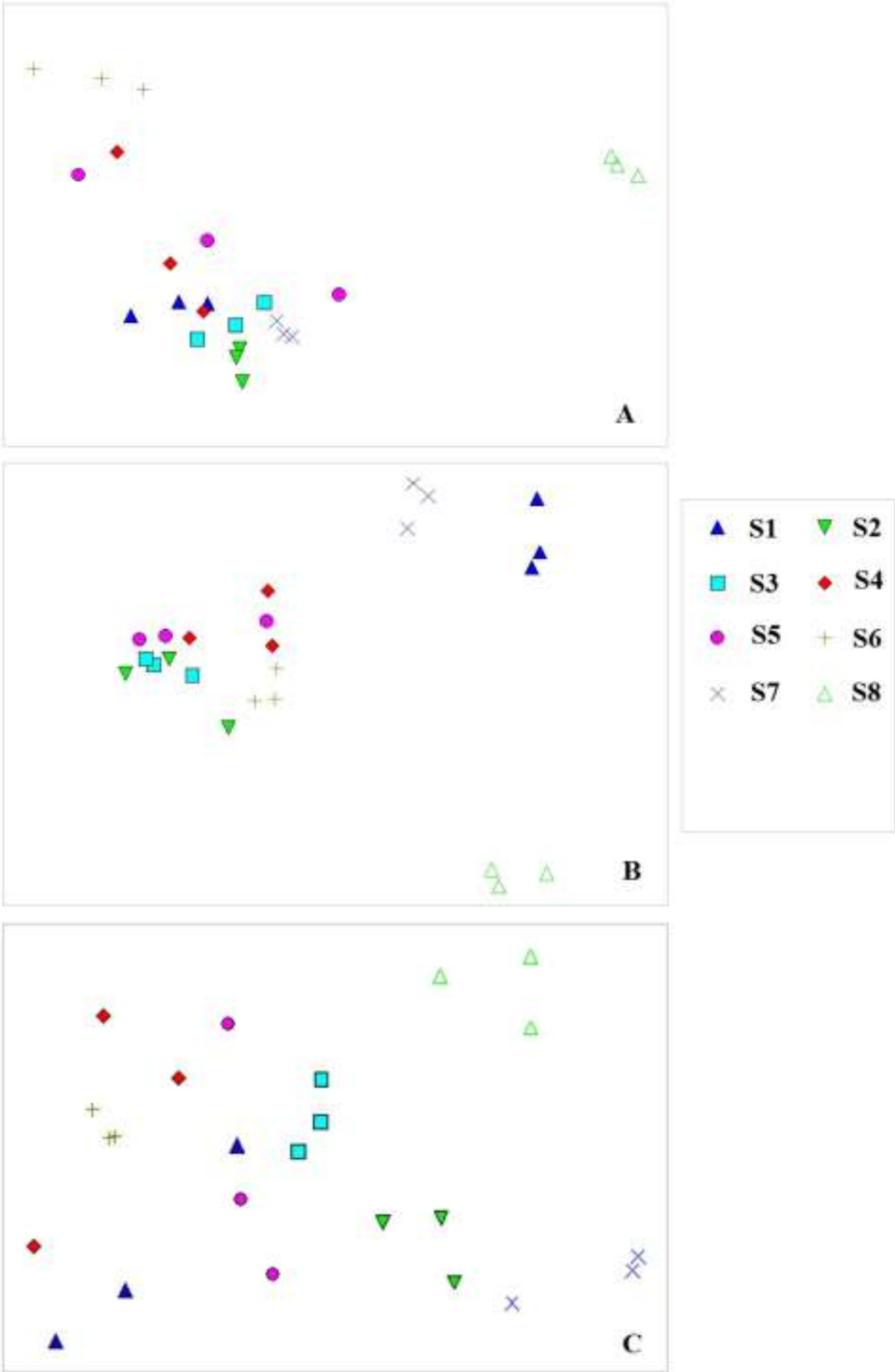

Figure 4: Multidimensional scaling based on: A) environmental condition, B) benthic composition, and C) nutrients conditions in the southern Caspian Sea (2014).

Table 2 shows SIMPER analysis among sites and time series. Data analysis indicated there were different guilds of benthic species at each of these eight communities, which are principally responsible for differences between, as well as dissimilarity within benthic assemblages. *Mytilaster lineatus, P. maeuticus* and *Balanus improvises* were contributed to dissimilarity within all compared sites and determined the community structure within benthic communities.

Table 3 lists the average values of the biotic classification indices (AMBI, M-AMBI and BENTIX) and the resulting ecological quality status (EQS) for the eight sites. Based on the average values, results showed that all indices were in a uniform range. In fact, undistributed status in AMBI indices coincided with good status in M-AMBI and high status in BENTIX indices, relatively.

Table 3: Values of the classification metrics and respective EQS assessment in the all sites.

Site	EQS	AMBI				M-AMBI				BENTIX			
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
S1	Score	3.185	4.5	0.409	7.00	0.55	0.16	0.72	-0.05	2.40	2	6	1
	EQS	SD	MD	Un	ED	M	B	M	B	P	P	H	B
	Total S1		SD				G				M		
S2	Score	**0.295**	**0.206**	**0.339**	**0.413**	0.67	0.57	0.83	0.74	5.90	5.9	5.8	5.9
	EQS S2	**Un**	**Un**	**Un**	**Un**	G	M	G	M	H	H	H	H
	Total **S2**		Un				G				H		
S3	Score	0.056	1.283	0.469	0.933	0.63	0.94	0.80	0.93	5.98	4.8	5.8	5.8
	EQS S3	**Un**	**SD**	**Un**	**Un**	G	H	G	G	H	H	H	H
	Total S3		Un				G				H		
S4	Score	0.617	2.855	0.517	1.493	0.65	0.50	0.64	0.94	5.99	3.5	5.7	4.9
	EQS S4	Un	SD	Un	SD	G	P	M	G	H	M	H	H
	Total S4		Un				G				H		
S5	Score	0.305	1.484	1.209	0.589	0.46	0.64	0.95	0.84	6	4.5	5.5	5.5
	EQS S5	Un	SD	SD	Un	P	M	G	G	H	H	H	H
	Total S5		Un				G				H		
S6	Score	1.313	1.700	0.126	0.309	0.93	0.83	0.66	0.78	4.90	4	5.7	5.8
	EQS S6	SD	SD	Un	Un	H	G	M	G	H	G	H	H
	Total S6		Un				H				H		
S7	Score	4.474	3.677	0.136	1.868	0.25	0.55	0.54	0.59	2	3	6	4.3
	EQS S7	**MD**	**MD**	**Un**	**SD**	B	M	P	M	P	M	H	G
	Total S7		SD				G				M		
S8	Score	1.825	7.00	7.00	7.00	0.60	-0.05	-0.05	-0.05	5.5	1	1	1
	EQS S8	SD	ED	ED	ED	M	B	B	B	H	P	P	P
	Total S8		SD				M				H		

SD= Slightly disturbed, Un= Undisturbed, MD= Moderately disturbed, ED= Extremely disturbed, P= Poor, B=Bad, G= Good, H= High and EQS= Ecological quality status.

PERMANOVA test performed on nutrient data and results showed seasonal changes in nutrient content with significant differences between each season ($p<0.05$). According to PERMANOVA test results nitrate significantly varied between seasons in S1, S4 and S8 ($p<0.05$) and Nitrite varied between seasons in S1, S3 and S7 ($p<0.05$). Phosphates and silicates showed significant seasonal oscillation only in site S8 ($p<0.05$). Silicate had significant different amounts in summer in comparison with autumn and winter in all sites ($p<0.05$).

Table 4: Abundance of species during the study in the southern Caspian Sea (2014)

Species	Ecological Scores		Abundance (number)							
	AMBI	BENTIX	S1	S2	S3	S4	S5	S6	S7	S8
Pontogammarus maeuticus	IV	2	1562	387	1325	4400	2475	450	4286	498
Balanus improvisus	II	1	25	6429	12662	2033	1801	4925	75	8650
Rhithropanopeus harrisii tridentatus	II	1	0	0	75	0	0	25	0	0

Table 4 continued:

***Simulium* sp.**	IV	2	0	37	0	0	0	50	0	0
Chironomus albidus	III	2	1062	262	925	175	0	4000	75	0
Mytilaster lineatus	I	1	575	143137	132250	37050	114587	15736	2475	275
Nereis diversicolor	III	2	0	225	1150	225	175	400	50	200
Tubificoides fraseri	V	2	50	0	0	0	0	0	0	0
Total	--	--	3274	150477	148387	43883	119038	25586	6961	9623

Discussion

Trophic interactions are the most important factor in macrobenthic spatial and temporal variation in coastal environment (Boaventura *et al*., 1999). Koutsoubas *et al*. (2000) declared that frequent fluctuations in environmental parameters (daily, monthly or seasonal basis) would cause changes in the structure and distribution pattern of organisms. Furthermore, natural disturbance often results in the instant destruction of great numbers of individuals (Guelorget and Perthuisot, 1992). These faunal communities' structural changes are principal factors for assessing ecological health by ecological indicators.

Nutrient content is the main factor responsible for fluctuation in benthic macrofaunal assemblages (Aller *et al*., 2001; Kuffner and Pau**l**, 2001; Stief *et al*., 2002; Sivadas *et al*., 2012; Amiri *et al*., 2014). In this study CCA analysis revealed that nutrient group most strongly influenced by nitrate and silicate (Table 2) and Fig. 3 showed that distribution of species was less associated with phosphate than other nutrients. There is plenty of evidence that phosphate is the main limiting nutrient factor in freshwater ecosystems and it was significantly different among seasons. However, Howarth *et al.* (2000) reported that in many coastal marine systems the limiting nutrient is usually nitrogen. In addition, Gao *et al.* (2011) declared that total nitrogen was among the main environmental factors affecting the distribution of macrobenthos, and ammonium nitrogen, nitrate nitrogen, and chlorophyll *a* also had definite effects. In addition, Lamptey and Armah (2008) stated that spatial and seasonal variability in silicate resulted in habitat heterogeneity among the sampling stations and this heterogeneity among the stations because of the environmental variables possibly created conditions that influenced the abundance patterns of the macrobenthic fauna. Overall results showed that seasonal changes of macroinvertebrates communities were highly associated with environmental factors.

Table 3 showed that all sites had acceptable ecological conditions based on AMBI index. Overall sites, 3 slightly disturbed and 5 undistributed status occasions were observed. According to PERMANOVA results temperature, oxygen, phosphate, and nitrite were the main factors that varied significantly between seasons in whole study area. In addition, all nutrient factors varied significantly between sites. MDS plot based on the benthic communities, demonstrated discrimination between S1, S7 and S8 with other sites (Fig. 4B). AMBI clearly separated these sites from the rest in the analysis, M-AMBI could not distinguish these sites and BENTIX was successful in separating S1 and S7 from other sites (Table 3). However, M-AMBI was successful to distinguish S1, S7 and S8 in seasonal analysis from other sites similarly to other indices. Table 4 shows that S1, S7 and S8 had fewer total species than other sites. *P. maeuticus* and *B. improvisus* were dominant in S1 and S7, and S8, respectively. High dominance of *P. maeuticus* with IV score in AMBI index, resulted changes in ecological status for these sites. The resulted ecological status by M-AMBI diverge from the results of AMBI due to the diversity components included in the method. Species scores are equal in AMBI and M-AMBI formula; and changes in reference value for AMBI, Diversity and Richness; caused changes in calculation of M-AMBI in comparison of AMBI. In addition, *B. improvises* with score 1 in BENTIX index, was dominant in S8 and resulted to high status classification for this site, while *P. maeuticus* with score 2 was dominant in S1 and S7 (Table 4). Furthermore, analysis of nutrient content and environmental condition showed that S7 and S8 were clearly separated from other sites (Figs. 4 A, 4C), while S1 overlapped. These changes in ecological health assessment based on macroinvertebrates were also observed in Mediterranean basin. Reizopoulou *et al*. (2014) reported that BENTIX and M-AMBI underestimated and AMBI overestimated the ecological status of Mediterranean coastal lagoons. Borja *et al*. (2008) stated that the greatest number of disagreements when comparing AMBI or M-AMBI with other indices is found in low salinity locations. The Caspian Sea is an enclosed inland body of water with average salinity of 13 ppm (Karbassi *et al*., 2008) and is very low in contrast of estuarine and marine salinity. The problems in assessing the benthic ecological status in low salinity or highly changing salinity habitats have been discussed under the 'Estuarine Quality Paradox' (Dauvin *et al*., 2007; Elliott and Quintino, 2007). In addition, Fig. 2 showed that all seasons were discriminated based on environmental condition and the Caspian Sea basins have highly variable ecosystems. Zubikarai *et al*. (2014) described two reasons for variation in species richness between rocky substratum s. They declared that reasons for these differences can be (i) lower discharge or (ii) much higher wave energy between sites. However, equitable distribution of trophic groups in environment and sampling area, indicate a healthier ecosystem functioning and, as such, an improvement in the quality of the environment (Bremner *et al*., 2006).

Although many benthic indices were successfully validated during the last decade, most indices and assessment scales were developed for local geographic regions, and often only for specific habitats within the region (Borja and Tunberg, 2011). In fact, species composition and reference conditions change naturally with ecoregion and habitat (Borja *et al*., 2009). Therefore, many studies have been conducted to establish different reference conditions for different estuarine habitats before benthic condition assessment (Weisberg *et al*., 1997; Borja *et al*., 2008; Teixeira *et al*., 2008). However, authors set reference value for M-AMBI based on AMBI calculation (by default. Furthermore, these three indices were established to apply for soft bottom communities and employed for hard substratum communities in this study. Nevertheless, some of them have been also successfully applied also for hard bottom communities as BENTIX index in Bhosphorus Strait communities (Kalkan *et al*., 2007) and was successful to separate control and discharge communities.

Results of this study revealed the weakness of the biotic indices to reflect and discriminate among the anthropogenic and natural stress in the hard substratum ecosystems as well. In addition, species sensitivity, richness and diversity as benthic community traits do not seem to function well in assessing the ecological quality status in these ecosystems. However, AMBI index was more successful to assess ecological health of hard substratum in the southern Caspian Sea basin compared to M-AMBI and BENTIX. In addition, AMBI showed high sensitivity to environmental variation. Indeed, successful classification of these indices is highly relevant to regional ecological conditions. For instance, Simboura and Reizopoulou (2008) studied the rocky deep and sedimentary shallow water body type three lagoonal sites located in Greece (Eastern Mediterranean) and stated that In the studied the rocky deep areas the BENTIX index seems to give a more biologically relevant and consistent with the environmental conditions classification, compared to the AMBI assessment, while BENTIX was more successful in this study. Furthermore, results indicated that temperature, nitrate, silicate, phosphate and nitrite were the most important factors in spatial and temporal variations of hard substratum macroinvertebrates communities, respectively.

Further studies should be conducted to determine reference value and boundary limits for hard substratum region especially in the southern Caspian Sea. It should be noted that dominant species, food webs, habitat structure, life span and cycle, reproductive rate and dispersal potential are important factors that affect ecological quality status of ecosystems and should be considered in analysis.

Acknowledgments

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