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The impact assessment of cage culture of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) on benthic communities using biological indicators in the South Caspian Sea, Iran

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Abstract

In this study, the impact of cage culture of rainbow trout (*Oncorhynchus mykiss* Walbaum), on benthic communities using biological indicators located along the coastal areas of the South Caspian Sea (Nashtarud region, Mazandaran Province, Iran) was investigated. Monthly samplings from 4 stations were carried out from January 2016 to May 2016. The physical and chemical characteristics of water were measured on a monthly basis. Sediment samples were taken using Ekman Grab. The ecological indicators such as Shannon–Wiener index of diversity, H, Simpson, S, and Margalef, R, was routinely used. The biomass-dominant faunal groups were Bivalve (146.16 g m^{-2}), Crustacea (24.5 g m^{-2}) and Polychaeta (10.5 g m^{-2}).The greatest variety of benthos was found under the cage and the lowest range was found150-300 m around the cage that was significantly different (p<0.05). Results of current study showed that, the percentage of sand was greater than that of silt and clay throughout the experimental period. Sediment organic matter (OM%) reached extremely high concentrations beneath the control (p<0.05), when compared surrounding area**.** A positive correlation coefficient was found between the Shannon–Wiener diversity index (H'), and biomass of benthic organisms (p=0.001; r=0.684). According to results of species diversity, and amount of organic matter in the cage area, indicated that specific richness levels were in bad ecological statues.

Keywords: Cage culture, Benthic, *Oncorhynchus mykiss*, Caspian Sea, Iran

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Introduction

In recent years, marine fish culture has had remarkable growth and has led to food production that is under the effect of different factors such as hydrodynamics and topographic conditions of the region, texture and composition of sediment, benthic organisms, as well as depth and nutritional techniques (Karakassis *et al.*, 2013; FAO, 2014; Fodelianakis *et al.*, 2014). Additionally, investigated the structure of the native living community in the region before and after the establishment of cages in the sea is of utmost importance (Giles *et al.*, 2009; Châteaua *et al.*, 2015; Santos *et al*., 2015). Information on their status will help identify the changes to the ecosystem brought by fish farms and develop strategies to deal with them (Keeley *et al.*, 2014). Numerous studies have been carried out to assess the health status of marine ecosystems using bio-markers (benthic-organisms), bio-chemical status of sediments and the results of these investigations state that temporal and spatial effects of fish farming on the macro benthic community structure can be used to identify abnormalities in farming management systems (Wilding and Nickell, 2013; Brigolin *et al.*, 2016). Currently, the greatest source of waste in aquaculture used by the benthic population is the OM in form of food and feces that is accumulated in the water column or surface sediments under the farming cages and high abundance of organic waste leads to the reduction of species richness, biomass, dissolved oxygen, creation of anaerobic conditions, increase in hydrogen sulfide, production of toxic wastes and mass mortality of fish (Trichkova *et al.*, 2013; Pulatsü and Kaya, 2016). Macrobenthic invertebrates, maintaining relatively fixed positions in the aquatic environments and sensitive to the environmental turbulences, are widely used as bio indicators by marine ecologists and one of the signs of change in unstable marine environments in the structure of benthic communities and emergence of some of the resistant and opportunist species such as polychaeta that are recently being replaced by Bivalve shellfish in fish farming in cage (Borg *et al*., 2011; Wilding and Nickell, 2013; Tomassetti *et al.*, 2015).

Studies showed that in some regions where farming substrates are not under the impact of pollutants effects non-resistant species are dominant and in the regions where the pressure of pollution exists, the diversity and density of resistant species is higher (Karakassis *et al.*, 2013; Cubillo *et al.*, 2016). It was also reported environmental impacts on benthic organisms decreases gradually with distance from the cages and normal conditions are dominant at 100 to 200 m from the cages and some of the physical-chemical indicators like organic matters and a biotic characteristic such as grain size of sediments reflect the bio-responses from the fish farm (McArthur *et al.*, 2010; Tomassetti *et al.*, 2015)

With the development of marine fish farming in the southern shores of the Caspian Sea (Guilan and Mazandaran

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Provinces, Iran), information on structure and diversity of benthic communities and impact of fish farming in cages on these communities can play an important role in the sustainable development of marine aquaculture. Thus, the continuous monitoring of macrofaunal assemblages and sediment features in marine cage environment can create awareness about the potential risks to food pyramids and sediments close to the substrate. This study carried out with the aim to use various bio-indicators for the assessment of benthic assemblages as well as environmental quality under the cage as a stressful environment and its comparison with the regions of low stress in Nashtarud Region of Mazandaran Province.

Methods and materials

Study sites

In this study *O. mykiss* was cultivated in 10 circular floating fiber glass cages with a diameter of 16 m installed at the depth of 25-30 m in Caspian Sea. The sampling stations were located using GPS apparatus and were registered at geographical location of 36°49′40” N 51°53′31 □ /5E).

One station (S2) was located in the center of cage (control station) and 3 stations were located out of it; S1 located 150 m to the west of cages and S3 and S4 stations that located 150 m and 300 m, respectively to the east of the cage (Fig. 1).

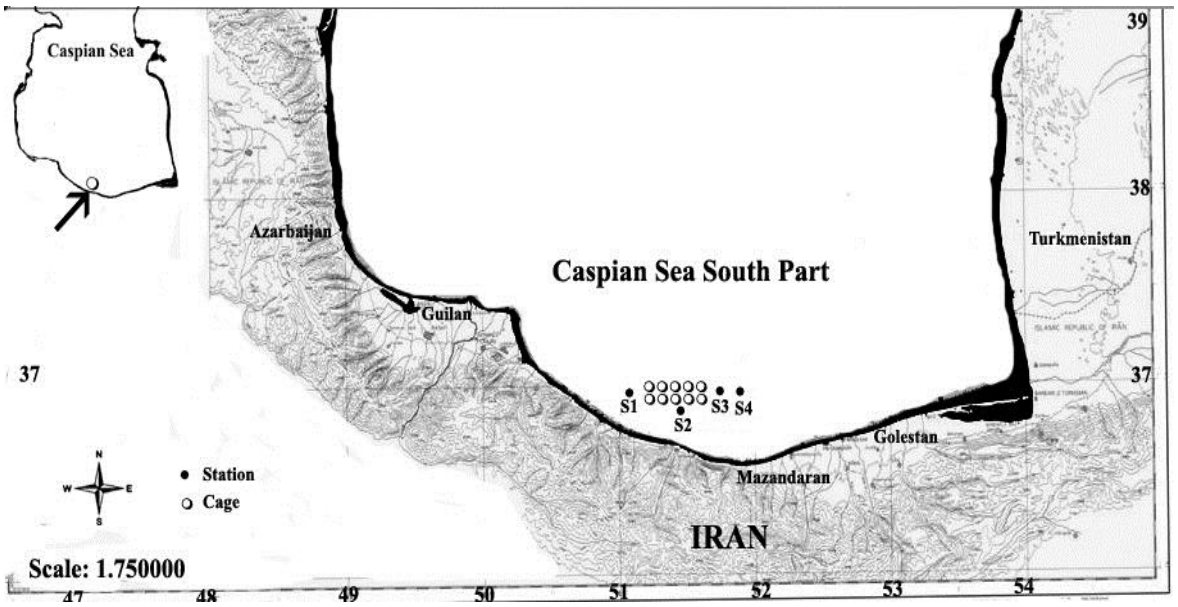

Figure 1: Location of study site and sampling stations in relation to the farm.

Sampling began from January (2016) before the introduction of trout fries to the cages and was carried out in 5 stages and continued for 5 months until May 2016.

Sampling strategy and data analysis

Water temperature (°C), dissolved oxygen (DO, mg L^{-1}) and pH values were measured using a Hach Multi-Parameter liquid analyzer (HQ 40d, HACH-Lange Co, USA). A Refractometer (HR-099 –laser co, UK)

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was used to measure salinity of water, monthly basis. To determine chemical characteristics such as $NO2^{2-}$–N, $NO3^{-}$ N, $PO4^{3-}$–P and total hardness (mg L^{-1}) TSS (mg L^{-1}) water samples were collected from the bottom of each cage by bottles of Niskin bottle (Hydro-Bios Co, UK) sampler Capacity 5 liters in the farming area and control station. All determinations were carried out using specialist kits and spectrophotometer (DR2800, HACH Co, Germany) and according to standard methods (Strickland and Parsons, 1972).

Sediment samples were collected in the farming area and the control station on – monthly basis using the Ekman Grab sampler (box core-0.04 m^2), for the investigation of benthic organisms.

The accumulated sediment from substrate were passed through a sieve (1 mm mesh size) and washed with sea water as well as screened. After separation of organism they were stained and fixed using 0.2 g L^{-1} rose bengal and 95% ethanol (twice the amount of sediment) All samples of benthic invertebrates were transferred to the laboratory. After washing with freshwater and re-passing through the sieve (500 mm mesh size) and weighed using an electronic digital scale to the nearest 0.0001 g, (SZ6045, Olympus Co, Belgium) counted and identified to the species level using stereo microscope (smz89, Nikon Co., Japan) and optical microscopes for statistical analysis. Their biomass was calculated too (Todorova and Konsulova, 2005; Keeley *et al.*, 2012).

In grain size analysis of sediment samples, homogenization using the laboratory methods (ASTM, 1990), using the wet and dry sieve method and curve technique in two phases and each one with 3 replications (Martinez *et al.*, 2015). Initial preparation of sediment for determining the amount of organic matter (OM%) was done using the methods suggested by (Carrolla *et al.*, 2003). benthic quality index (BQI) was used for comparison of benthic communities and determination of the eco-classification of the farming environment (Leonardsson *et al.*, 2009) as follows:

Shannon-Wiener diversity index (H')=-$\sum_{i=1}^{s} P_i \ \ln P_i$ (Shannon-Wiener, *et al.*, 2004; Rosenberg *et al.*, 2004; Borja *et al.*, 2009)

Where Pi = n / N

n=one particular species number

N=total number of all individuals in the sample

Σ=the sum of the calculations

In = logarithm to base e

S=number of individuals of one species

between 3-4 indicates that the structure of habitat is stable and in good statues; and values between 2-3 is Moderate statues; and values between 1-2 is Poor statues and the values 1-0 indicates that there are bad and degradation of habitat structure (Türkmen and Kazanci, 2010).

Simpson index $=\frac{1}{\sum_{i=1}^{s}(P_i)^2}$ (Simpson, 1994)

Where Pi = n / N

n= One particular species number

N=Total number of individuals

S=The number of species.

Σ=The sum of the calculations

Simpson index values are between 0 – 1. But while calculating, final result is

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subtracted from1 to correct the inverse proportion.

Margalef index=(S-1)/$\ln_N$ (Gamito, 2010)

Where:

S=is the number of species

N= the total number of individuals in the sample, In =natural logarithm

Have that expressed if the amount of Margalef index is lower than 4, the study area have poor statues.

Statistical data processing

The results were presented as mean ±SD of five stations. Shapiro-Wilk test and the histogram were used to investigate the statistical normal distribution of data in each station .In the case of data normality and to compare the stations statistically (S1, S2, S3, S4) and to determine the difference level between biomass of benthos, one-way ANOVA was used. Statistically significant differences among data (p<0.05) were evaluated by the Duncan's Multiple Range. The correlation amount and coefficient between of benthic and biological indicators close to the substrate grain size and (OM%) was identified using the Pearson correlation coefficient. The statistical analysis of data, plotting of graphs and figures were done using the SPSS and Excel (SPSS vers. 20 for Windows - SPSS, Chicago, IL, USA).

Results

The minimum dissolved oxygen (DO, mg L^{-1}) was 9.3±0.25 and maximum amount was 9.57±0.25 in May. Water temperature (°C) was about 11.1-29.9 in February and 24.3-26.9 in May. Salinity (in ‰) ranged from about 10.11-11.3 ppt and pH value throughout the investigations was above 8 in farming area. No significant difference between the control station and others (p>0.05) has been observed. In general, $NO2^{2-}$–N, $NO3^{-}$N ،$PO4^{3-}$–P and Total hardness ،TSS of water column did not show significant differences between control station and fish farm. The results from the investigation of chemical factors of water such as and Total hardness ،TSS indicate no significant differences between the fish farming areas (p>0.05) (Table 1).

Table 1: Physical and chemical parameters measured in the water column at the fish farm installation at Nashtarud area.

Date	Parameters	Sampling zones			
	Cage-in		Cage-out		
		S2	S1	S3	S4
2016-01	$NO2^{-}$ −N	0.003±0. 03	0.002±0. 01	0.001±0. 01	0.001±0. 01
	$NO3^{-}$ N	0.01±09	0.01±0. 57	0.02±0. 63	0.01±0. 01
	$PO4^{3-}$ −P	0.18±0.01	0.13±0.01	0.12±0. 11	0.11±0. 05
	Total hardness	2200±5.7	2300±4.3	2400±6.3	2500±5.2
	TSS	38.2±0.12	36.2±0.17	35.3±0.17	34.7±0.02
2016-03	$NO2^{-}$ −N	0.005±0. 06	0.005±0. 06	0.006±0. 06	0.004±0. 03
	$NO3^{-}$ −N	0.01±0.05	0.01±0. 011	0.01±0. 08	0.01±0. 05
	PO43- _ p	0.19±0.01	0.18±0.01	0.15±0.05	0.13±0.01

[DOR: 20.1001.1.15622916.2020.19.4.12.0]

Table 1 continued:

	Total hardness	2200±5.7	2320±4.3	2350±6.3	2370±5.2
	TSS	39.8±0.81	23.7±0.12	22.3±0.13	21.7±0.14
2016-05	$NO2^{-}$ −N	0.006±0.03	0.005±0. 03	0.003±0. 03	0.002±0.03
	$NO3^{-}$ −N	0.01±0.02	0.01±0. 02	0.01±0. 02	0.01±0.02
	$PO4^{3-}$ _ P	0.17±0.05	0.16±0.05	0.14±0.02	0.13±0.02
	Total hardness	2200±5.7	2200±5.7	2320±86	2370±6.2
	TSS	57.5±0.12	57.5±0.12	46.3±0.05	37.6±0.02

Values are expressed as (mean±SD)

Effects of fish cage-culture on benthic communities

The total benthic biomass in farm ranged (between 82.66 g m^{-2} and 129.2g m^{-2}). The observed benthic community in the sediments inside and outside the cage were polychaeta (*Nereis diversicolor*), Bivalve (*Abra ovata*, *Cerastoderma lamarki*, *Mytilaster lineatus*) Crustacea (*Balanus improvises*). The highest species diversity was recorded in bivalve (3 species) and the lowest was observed in polychaeta and crustacea (each of them just 1 species). The results showed that at the start of fish culture, the benthic species composition in the farming cages included of crustacean, bivalve and crustacean and cage -out included two species of bivalve and Polychaeta, respectively (Table 2).

Table 2: Species composition of macrobenthos during fish cage culture in the Nashtaroud area.

Species	Beginning (January)		End (May)	
	Cage-in	Cage-out	Cage-in	Cage-out
Bivalvae				
Abra ovata,	+	+	+	+
Cerastoderma lamarki	+	+	+	+
Mytilaster lineatus	+	-	-	-
Polychaeta *Nereis diversicolor*	-	-	+	+
Crustacea *Balanus improvises*	+	-	+	-

The results showed that the maximum variety of benthos under central cage was observed at stations close to the west of the cage during May, March and February and the minimum value was at distances of 150-300 m to east. The dominant species in this research was bivalve shells with a biomass of 49.5% g m^{-2} observed outside the cage in February and the species with the lowest biomass were polychaeta and Crustacean each with a biomass of 1g m^{-2} to the east of the cages in May, April and March.

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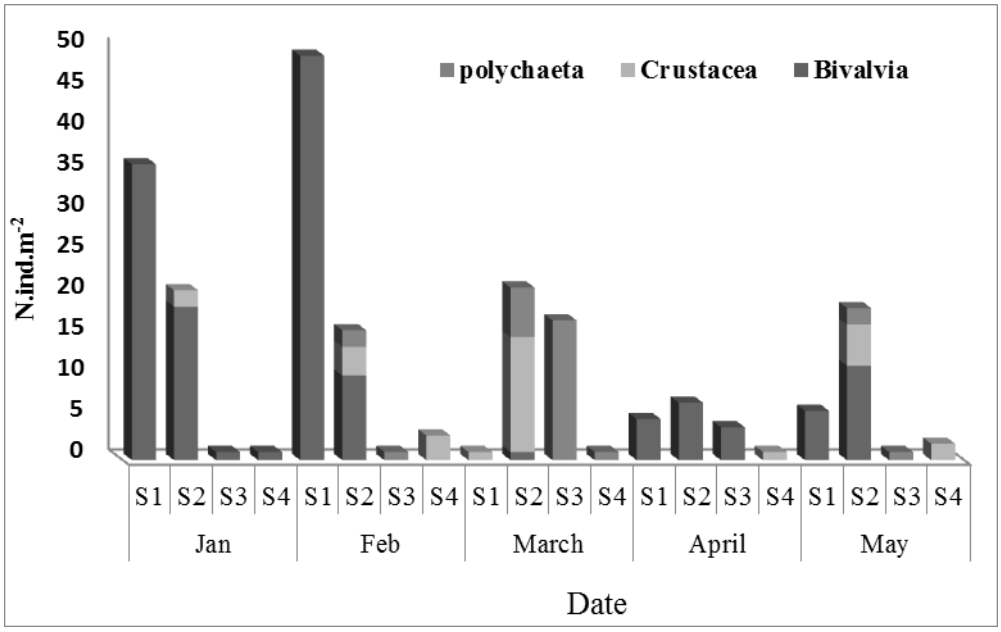

Figure 2: Abundance of macrofauna with the highest number of individuals in the four stations.

The results showed significant difference in macrobenthic communities during the 3rd and 4th phases of sampling (one-way ANOVA, $p<0.05$, Fig. 3).

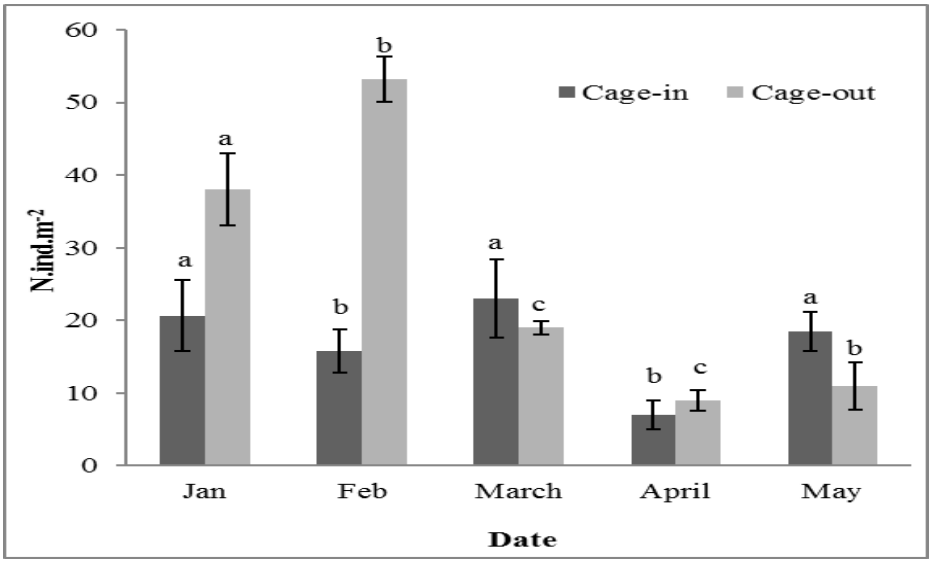

Figure 3: Comparison of benthic macro invertebrates inside the cage and outside during various sampling time.

Sediment physicochemical characteristics

The physiochemical characteristics of sediments including the amount of OM and sediment grain size were studied. The highest amount of organic material (41.49±0.36 %) was measured outside the cage, within a 150 m perimeter of the cage and its lowest level (3.01±0.51 %) was reported in sediments collected within the same distance of the cage before the introduction of fish to the cage. Significant difference (ANOVA-test, $p<0.05$) were reported in OM between the stations in of sampling (Table 3).

[DOR: 20.1001.1.15622916.2020.19.4.12.0]

Table 3: Organic matter content (mean±SD) expressed as dry weight percentage, in sediment.

	Sampling zones			
	Cage-in	Cage-out		
Date	**S2**	**S1**	**S3**	**S4**
2016-01	5.47±0.12[b]	4.05±0.08[c]	3.01±0.51[c]	8.21±0.68[a]
2016-02	21.5±1.33[b]	30.65±0.1[a]	33.19±4.03[a]	36.1±0.26[a]
2016-03	32.75±1.92[a]	34.1±0.57[a]	18.01±1.73[c]	28.74±0.06[b]
2016-04	35.98±2.21[b]	41.49±0.36[a]	37.04±1.23[b]	25.14±0.8[c]
2016-05	10.94±1.18[b]	28.05±1.14[a]	15.29±2.29[b]	3.99±2.14[c]

Means with the same letter in the same row are not significantly different ($p<0.05$).

The percentage of sediment coarse fraction were sandy, clay and silt with a grain size mode between 0.002 and 0.2 mm, and the average size corresponding to very fine grains. The highest and lowest fraction of substrate type in fish cages belonged to sand (64.96%) and clay (10.77%), respectively (Fig. 4).

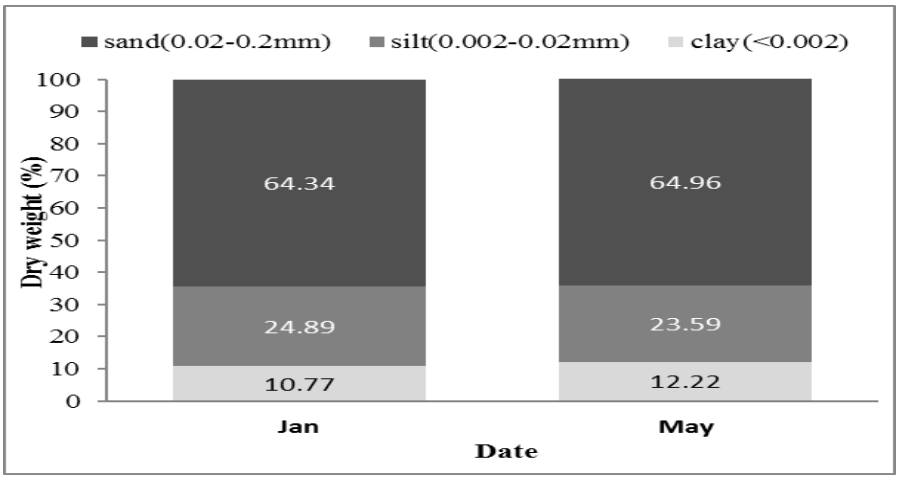

Figure 4: Granulometric structure of sediments expressed as the relative abundance (dry weight percentage) of the different grain-size fractions of samples in the Nashtaroud area.

The results showed that during each stage, the percentage of sand was higher than that of silt, and clay although no significant differences were observed (Duncan, test $p\leq0.05$, Table 4).

Table 4: Granulometric structure of sediments in the study zone (inside and outside the cage) expressed as the relative abundance (dry weight percentage).

Date	Parameters (%)	Beginning (January) Cage-in	End (May) Cage-out
2016-01	Silt	23.75±0.14[a]	29.38±0.01[b]
	Clay	18.32±0.17 [a]	8.02±0.73[b]
	Sand	57.93±0.47[a]	62.6±7.78[b]
2016-05	Silt	24.89±0.17 [a]	23.53±2.66[b]
	Clay	10.77±0.47 [a]	12.17±0.93[b]
	Sand	64.34±0.37 [a]	64.3±2.58[b]

Means with the same letter in the same row are not significantly different ($p<0.05$).
Values are expressed as (mean±SD).

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A comparison of frequency rate of the number of benthic organisms and sediments grading showed that with an increase in the rate of clay within the sediments, the biomass increased too (Fig. 5) although the regression between the clay percentage available in the sediments and biomass frequency shows negative correlation (p=0.966; r=-0.018).

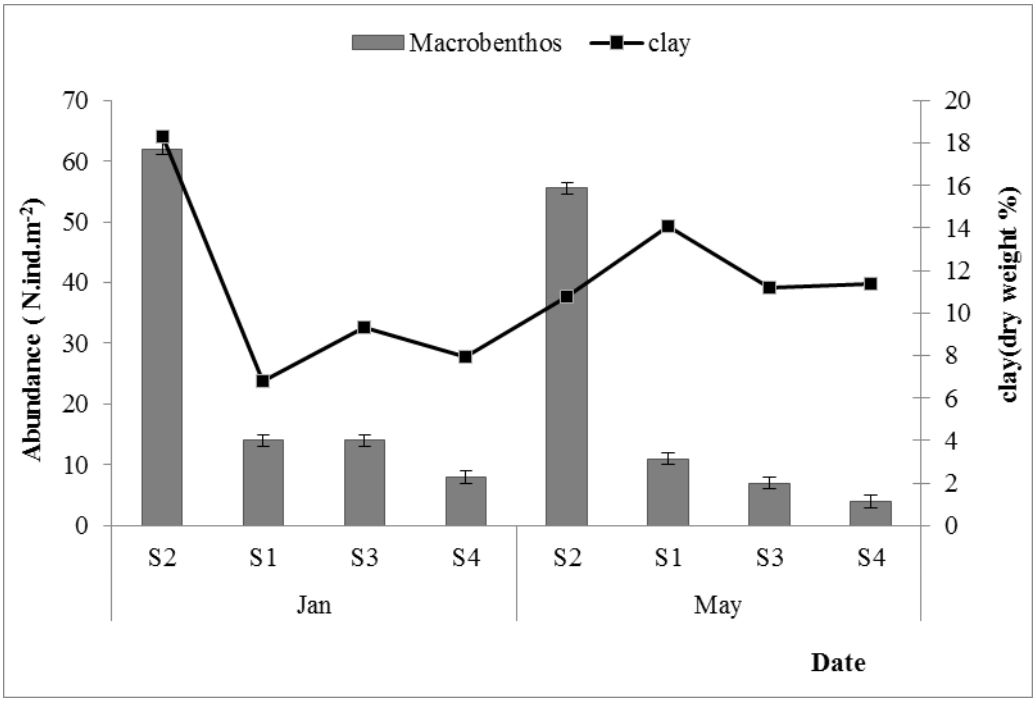

Figure 5: Comparison the abundance of benthic values and clay grain size from Jan-May 2016.

Relationships between indicators and comparison of ecological classifications

Based on the biological quality index (BQI), Shannon's diversity index (H), Margalef's species richness index (S) and Simpson's index (P), the accumulation of OM and ecological status of the study area is classified into five status classes: high, good, moderate, poor and bad. Results showed that in cages with the highest species diversity was (0.73) observed in January and the lowest rate (0.43) was in April. The maximum Simpson's diversity index was 0.65 in January recorded 300 m of the station the cage was installed and the minimum was 0.12, also in the same month and was observed 150 m from the cage. The highest species richness index (1.98) was observed in fish farm in May and the lowest (1.02) was determined in January to the east of the installed cages. The results showed that, fish farming and control station were under stressful and poor status (Table 5).

[DOR: 20.1001.1.15622916.2020.19.4.12.0]

Table 5: Shannon-Wiener(H´), Simpson's dominance (S), Richness(R1) values of the stations in the Nashtarud area and their ecological quality status.

Date	Station	H´	S	R_1	Ecological status
2016-01	**S2**	0.73	0.39	1.95	Poor
	S1	0.5	0.38	1.14	Poor
	S3	0.36	0.12	1.02	Poor
	S4	0.32	0.65	1.36	Poor
2016-02	**S2**	0.64	0.38	1.34	Poor
	S1	0.64	0.52	1.29	Poor
	S3	0.27	0.14	1.65	Poor
	S4	0.22	0.32	1.05	Poor
2016-03	**S2**	0.54	0.35	1.41	Poor
	S1	0.23	0.32	1.63	Poor
	S3	0.5	0.42	1.82	Poor
	S4	0.19	0.33	1.06	Poor
2016-04	**S2**	0.43	0.49	1.35	Poor
	S1	0.41	0.53	1.58	Poor
	S3	0.27	0.18	1.05	Poor
	S4	0.5	0.42	1.96	Poor
2016-05	**S2**	0.49	0.5	1.98	Poor
	S1	0.44	0.52	1.3	Poor
	S3	0.49	0.24	1.88	Poor
	S4	0.25	0.41	1.08	Poor

The results showed that, a positive correlation was observed between the Shannon-Wiener (H´) and benthic biomass during the various phases of sampling (r^2=0.3674, Fig. 6) but this correlation was negative in the biological-indicators of dominance and richness.

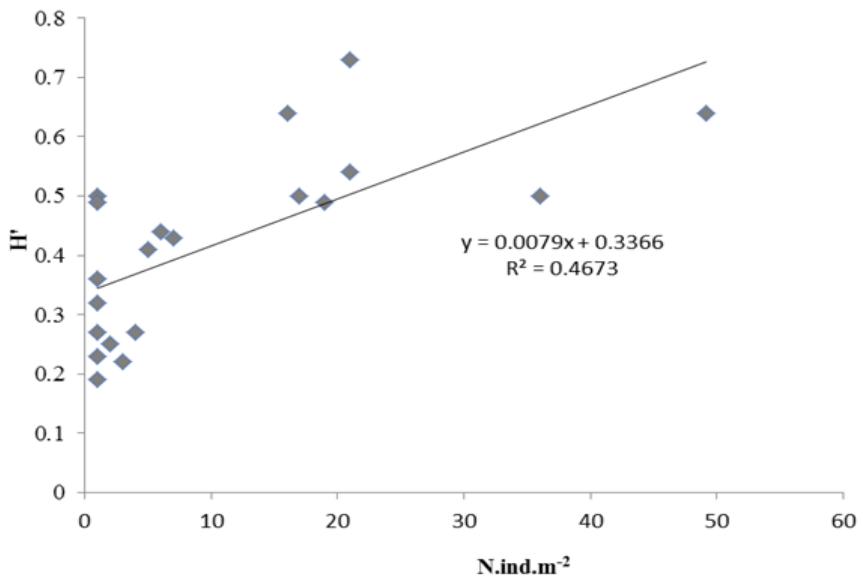

Figure 6: Pearson Correlation between diversity index and benthic biomass in the Nashtaroud area.

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Discussion

The results of the present study clearly illustrated that no significant differences were observed between the physio-chemical parameters of water in fish farm and control station. Similar results also reported in previous studied that thermocline occurred the depths greater than 20 m during the cold seasons of year (Tuzhilkin and Kosarev, 2005a; Zaker *et al.,* 2007). Tuzhilkin *et al*. (2005b) reported that to low salinity in the Caspian Sea, thermal stratification through the water column too low and vertical mixing of Caspian Sea is known as the water gradient. In the present study, create an appropriate stability in temperature and acidity in the farming environment that resulted in adaptation of fish to their environment. In addition, it is through that the low fluctuations in pH values can be a good buffer and on the contrary the freshwaters, has made fish adaptable to their environment (Belle and Nash, 2008).In the present study, chemical parameters in water such as nitrogen and phosphorus remained in the water resources and organic solid particles were deposited in the sediments of fish farm area and control station. Aksu *et al.* (2010) and Tomassetti *et al.* (2015) reported that organic carbon directly causes an increase in soluble oxygen consumption and when demand for oxygen is more than the available oxygen, the sediments become non-aerobic, Moreover lower temperatures, the release and accumulation of the sediments occurs a long period of time. Low water temperatures and high dissolved oxygen concentrations in the sediment surface layers have not provided the opportunity for the accumulation of nutrients.

In the present study there is no variation on the numbers, types, and frequencies from those found of the central cage at a distance from the farm and other unused station sand the low diversity in benthic communities to the unstable conditions of sediments and physico-chemical parameters of water result from climatic change and turbulent flows in the water (Forchino *et al.,* 2011; Mirto *et al.,* 2012). Adriatic Sea results showing a trends of these changes are related to daily feeding management of fish (manually or automatic), close to installed of cages, input size of sediments to grain size, reduction of phytoplankton abundance and width and flow of water has reported (Mantzavrakos *et al.,* 2007; Borja *et al.,* 2009; Forchino *et al.,* 2011; Tomassetti *et al.,* 2015).

The present research, there was no significant differences observed between the sediment grain size but a positive correlation was reported between biomass and the amount of clay in the sediments at the beginning of the study period. Particles size of sediments is one of the factors which are important in the investigation of the development and accumulation of benthic assemblages. Trichkova *et al.* (2013) reported that there is a direct relationship between the benthic populations and particles size by filled up water as with an existing or escreates an appropriate environment for benthic accumulation between the

[DOR: 20.1001.1.15622916.2020.19.4.12.0]

soil particles and sediments, so that the larger particles create more space for macrofauna within the sediment.

Li *et al.* (2013) also reported that organic matter available in the sediments consisted of fine particles or microscopic organisms and counted as a source of nutrition for benthic organisms.

Increase in organic materials in the sediments alone cannot cause the concentration and accumulation of benthic species in these types of sediments and other factors are effective as well (Teixeira *et al.,* 2010; Kristensen *et al.,* 2012). Generally, it can be concluded that penetration of oxygen to the deep layers in the sediments with sandy substrates and low temperature provides favorable conditions for suspended food from upper layers of water that causes the growth of benthic communities and their diversity increase in central cage and the role of hydrodynamic conditions of the Caspian Sea cannot be ignored (Karakassis *et al.,* 2013). In the present study, significant differences were observed in OM which increased around 150 m to the west of the installed farm. Significant differences had found in the performance of benthic assemblages and richness of OM in the regions close to strong oceanic currents (Papageorgiou *et al.,* 2010; Martinez *et al.,* 2013). These findings are supported by other studies as well Papageorgiou *et al.* (2010) and Jyväsjärvi *et al.* (2014) reported the sedimentation amount of OM under the cages is 500 times higher and feces and food wastes within sediments accumulated under the cage can increase organic enrichment of sediments load and results increased in opportunistic species as well as that of other species. The local flows of Caspian Sea can be one of the factors which by its movement in a clockwise direction cause this accumulation (Moscow, 2013; Naderi Beni *et al.,* 2013). In this study, it was observed that a suitable condition of oxygen under the substrate and in the water and unsuitable conditions in the substrate for settling the polychaeta, bivalve had a greater share in the increase of diversity and biomass of benthic macrofauna and has coin habited with Polychaeta of worms. Further studies are required to better understand these results also we observed, a positive correlation between the Shannon–Wiener diversity with biomass of benthic organisms (Fig. 6). The effects of pollution and natural perturbations caused by farming or environmental factors using the benthic quality index and in form of variation in diversity, majority and richness in the benthic population supported by reported of (Karakassis *et al.,* 2013; Tomassetti *et al*., 2015). Fish farms located in the Mediterranean, Greece and Italy shores showed that effected with a reduce at 25 m that reported by Mantzavrakos *et al.* (2007) and Forchino *et al.* (2011). However, Tavoli *et al.* (2016) and Ghasemi *et al.* (2016) have been reported at 5m depths of the Gorgan Bay and at 20m depths of Chalus shores, diversity and dominance of species, and the ecological were at an average level. The marine flows have negative impact on the bio-indicators

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and macrofaunal accumulations in the cages located on the eastern shores of the Mediterranean Sea (Simpson, 1994; Gamito, 2010; Simboura and Argyrou, 2010; Türkmen and Kazanci, 2010). The results of the present study showed decrease in bio-indicators. The low diversity in opportunistic species of polychaeta and larger grain size of sediments in the region caused the dominance of large benthic species within those stations supported by the report of research (Villnas *et al.*, 2011) was also observed in this study.

Regarding to the accumulation of benthic populations, it could be due to the hydrodynamic state of the Caspian Sea (as the natural stress) that causes instability of macrofaunal communities in the study region (Table 5). The result of Wan Hussin *et al.* (2012) stated that to better understand the ecological status of region other variables such as geochemical terms of sediments and relationship between benthic organisms and marine flows should be investigated. It can be therefore concluded that in general that marine fish farms has minimal effects on the benthic communities and some characteristics of the sediment.

An effective management plan for the cage enterprises built after all appropriate research may give rise to a structure that can provide environmental, social and economic sustainability. Besides, ecology of the area, wild fish and human factors should be monitored regularly, for sustainable aquaculture farming.

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