Preliminary studies on the impact of fish cage culture rainbow trout (*Oncorhynchus mykiss*) on zooplankton structure in the southwestern Caspian Sea

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Abstract

The ecosystem of the Caspian Sea have been strikingly changed by human activities. This area has undergone significant ecological alterations and serious environmental degradation since the beginning of 1990s. The impact of fish cage culture on the zooplankton community was studied in the southwestern Caspian Sea, off Jafrud during January to April 2013. A total of 12 zooplankton belonging to 9 taxa were identified at the study area. Only one species of Cladocera (*Pleopis polyphemoides*), was present in our study. *Acartia tonsa* and *Balanus improvisus* were the most dominant species. Bivalvia larvae, *P. polyphemoides* (Cladocera) and *Synchaeta* sp. (Rotifera) occurred only in January. The average of zooplankton abundance varied between 1,600 and 14,500 ind.m^{-3} in the study. The principal component analysis revealed the spatial variations of zooplankton abundance between the fish cage culture site and the reference site stations. The findings confirmed the impact of fish cage culture on the zooplankton population structure from the prominent abundance of *A. tonas*, *B. improvisus*, *P. polyphemoides*, and Bivalvia larvae at the fish cage site as compared with the reference site.

Keywords: Zooplankton, Impact, Fish cage culture, Caspian Sea

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Introduction

The Caspian Sea is a huge internal water body, with a length of more than 1,200 km measured from north to south, and containing almost 40% of the inland waters on the earth (Rodionov, 1994; Dumont, 1998). Even though it is not connected to any marine system, it is too large to be called a lake (Putans *et al.*, 2010). Over the last thirty years, the Caspian Sea has undergone significant ecological alterations (Rodionov, 1994; Dumont, 1995; Bagheri *et al.*, 2011).

Zooplankton with its significant role in ecosystem is crucially linked in the marine food-webs by its vertical abundance in the sea. The most well-known zooplankton, the copepods, is the most plentiful holoplankton in the Baltic, Black and Caspian Seas (Bagheri *et al.*, 2010; Richardson, 2008). The main significant function of zooplanktons as the most important grazers in marine food-webs, is providing a major corridor for energy transfer from primary producers to consumers at the upper trophic levels (Richardson, 2008; Bagheri, 2012).

Human activities, changes in weather patterns and the introduction of invasive species such as *A. tonsa* Dana, 1849; *Pleopis polyphemoides* Leuckart, 1859, and *Mnemiopsis leidyi* Agassiz,1865 (Dumont, 1995; Finenko *et al.*, 2006; Bagheri *et al.*, 2014; Bagheri *et al.*, 2013) are related to the anomalies in the zooplankton community and fish catches in the Caspian Sea since 2000s (Daskalov and Mamedov, 2007).

The decrease in zooplankton taxa was shown since 1996 and persisted through 2010 in the southern Caspian Sea (Roohi *et al.*, 2008; Bagheri *et al.*, 2012). Only two species of the nine previously recorded Cladocera species were recorded in 2010. Of the five Copepoda recorded in 1996, only one *A. tonsa* was found in 2008 (Roohi *et al.*, 2008; Bagheri *et al.*, 2012; Bagheri *et al.*, 2013).

Recently, countries like China, India, Malaysia, Thailand, Philippines, Japan, Turkey and Australia have established large fish cage culture structures in the sea (Dias *et al.*, 2011). In Iran, the first fish cage culture for rearing the rainbow trout, *O. mykiss,* Walbaun 1792 was launched by Iranian Fisheries Organization (Guilan Province) in the southwestern Caspian Sea in May 2012. The fishes were fed with pelleted feed, which were harvested in October 2012 (Unpublished data by Golshahi). Guo and Li (2003), Dias *et al.* (2011), Abery *et al.* (2005), and Borges *et al*. (2010) found that fish cage culture practices has raised the nutrient levels, caused changes in trophic web, decreased biodiversity, increase in phytoplankton and zooplankton number, introduced alien species and spread diseases in the marine system. Dalsgaard and Krause-Jensen (Dalsgaard *et al.*, 2006) studied the plankton communities at four cage culture sites in the Mediterranean Sea. They concluded, that algae and plankton expansion was maximum at cage sites and elevated within 150 m perimeter of the cages. The zooplankton number inside or near the fish cages was higher than in areas more distant. The ecological stability of the marine ecosystem was in disorder due to the effect of eutrophication in regions where the fish cages were located (Guo and Li, 2003). Additionally, in shore and off shore cage cultures in the sea, elevated organic and nutrient loadings resulted from the feed wastage and faecal excretion which are directly released into the sea (Islam, 2005).

In recent years, few studies have been conducted on zooplankton structure and variations in the southern Caspian Sea (Rowshantabari *et al.*, 2007; Roohi *et al.*, 2008; Hosseini, 2011; Bagheri *et al.*, 2012; Bagheri *et al.*, 2013; Bagheri *et al.*, 2014; Mirzajani *et al.*, 2015). Besides this, there is no study on the impact of fish cage culture in the environment of the Caspian Sea. Hence, the main objective of this study is to investigate how the zooplankton structure is changed within the fish cage culture area due to increase in food availability in the southwestern Caspian Sea, off Jafrud.

Material and methods

Area studied

This study was carried out in the southwestern Caspian Sea, off Jafrud during January to April 2013. Monthly sampling was not possible due to logistic problems and unforeseen natural hindrance in February and March 2013. The fish cage sampling stations (beside the fish cage site) were set at south (S1), northwestern (NW1) and northeastern (NE1) from the cage. Water samples were taken at 25 m depth below the surface (Fig. 1). The fish cage sampling stations were about 5.5 km away from the shore (N 38°89´ 06″; E 41°51´ 06″) and the reference stations were set 1 km away from the fish cage site towards the south (S2), northwestern (NW2) and northeastern (NE2) directions at the same depth (Fig. 1). The sampling of the whole stations grid was performed in one day from 9 am – 2 pm by a speed boat.

Data collection

Zooplankton was sampled using a Juday net (Vinogradov *et al*., 1989); opening diameter: 36 cm, mesh size: 100 μm). At every station, a vertical haul with a Juday net was carried out from bottom to surface using a handle pulley for heaving the net. Zooplankton samples were preserved in neutral 4% formaldehyde and analyzed in the laboratory. Samples were divided into sub-samples using a 1mL Hensen-Stempel pipette and transferred to a Bogorov chamber for identification. At least 100 individuals were counted per sample and identified to species level, and life-cycle stages were determined using an inverted microscope (Harris *et al.*, 2000). Zooplankton taxonomic classification was performed based on Birshtain (1968). Nonparametric analysis (Kruskal-Wallis) followed by the Conover-Inman’s test for zooplankton was used to determine the significant differences of variables among stations.

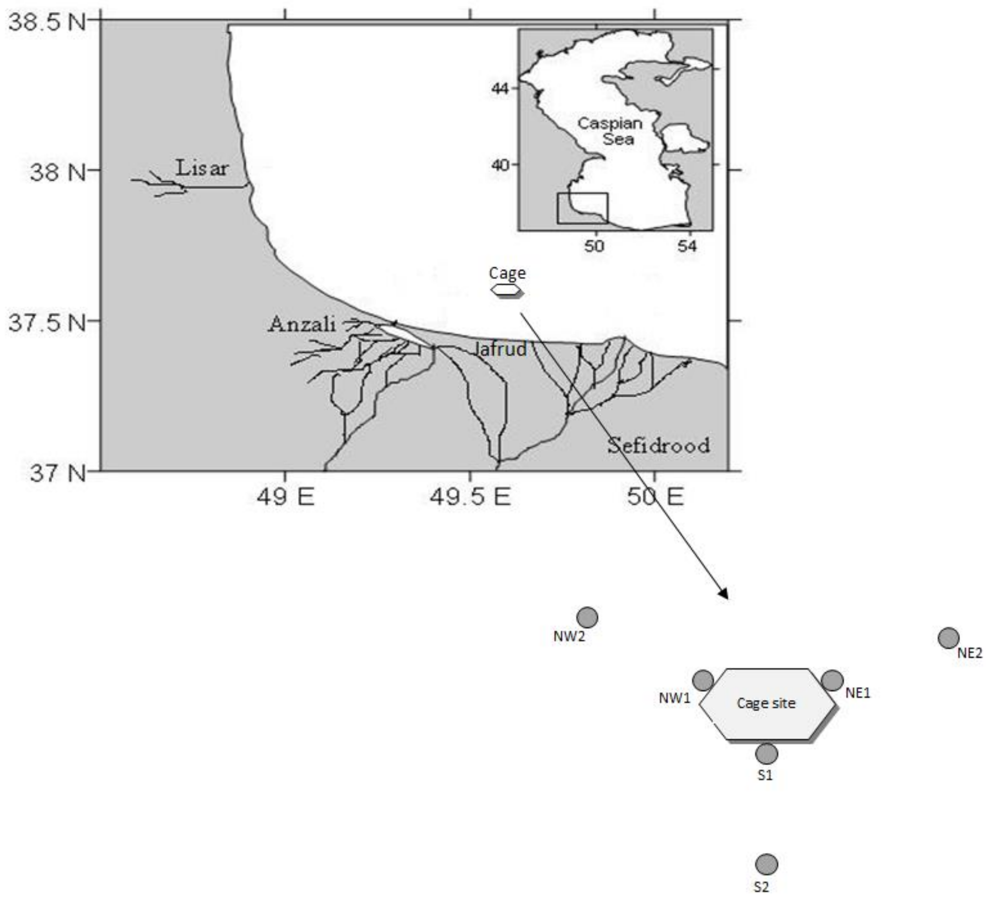

Figure 1: The sampling stations in the southwestern Caspian Sea, off Jafrud in 2013. S_1, NW1, and NE1= near the cage site, and S_2, NW2, and NE2 = 1 km away from the cage site.

The Principal Components Analysis (PCA) was used to determine the dominant taxa and correlation matrix of zooplankton taxa between stations.

Results

Zooplankton taxa

A total of 12 zooplankton items, belonging to 9 taxa (mero–and holoplankton) were found in the area under investigation (Table 1). Fish eggs and larvae were not identified; therefore, it is unclear whether they belong to holoplankton or meroplankton species. The four meroplankton taxa consisted of individuals of water spiders (Arachnida), larvae of Bivalvia, Nematoda, and the Cirripedia *B. improvisus* represented by nauplius and cypris larvae (Table 1).

The four holoplankton taxa belong to Rotifera (*Synchaeta* sp.), Cladocera (*Pleopis polyphemoides*) and Copepoda (*Ectinosoma concinnum, Acartia tonsa*). The three most abundant groups were Cirripedia, Bivalvia and the frequent Copepoda which made up 97% of all the sampled individuals.

Table 1: Average abundances of zooplankton taxa (ind. m^{-3}) per month collected in the southwestern Caspian Sea, off Jafrud in 2013. h: holoplankton, m: meroplankto.

			Freshwater species	January		April			average	
No		Group	Taxa	average	std	average	std	total no	average	std
1	m	Arachnida	Arachnida	0.0	0	0.3	0.8	2	0.166	0.57
2	m	Bivalvia	Bivalvia larvae	0.0	0	2736.8	1889.4	16421	1368	1915
3	m	Cirripedia	*Balanus improvisus* cypris Darwin1854	53.5	22.2	53.2	31.1	640	53.33	25.74
4	m	Cirripedia	*Balanus improvisus* nauplius Darwin1854	983.3	413.8	1472.7	897.8	14736	1228	713.8
5	h	Cladocera	*Pleopis polyphemoides* Leuckart, 1859	0.0	0	290.3	213.9	1742	145.2	209.3
6	h	Copepoda	*Acartia tonsa* nauplius Dana, 1849	3068.5	576.9	968.5	826.0	24222	2019	1290
7	h	Copepoda	*Acartia tonsa* adult Dana, 1849	995.6	230.7	1372.2	827.5	14207	1184	611.6
8	h	Copepoda	*Ectinosoma concinnum* Akatova, 1935	0.0	0	0.2	0.4	1	0.083	0.29
9	m	Nematoda	Nematoda larvae	0.5	1.2	0.0	0.0	3	0.25	0.87
10		Pisces	Pisces larvae	0.0	0	1.8	2.9	11	0.92	2.15
11		Pisces	Pisces ovae	0.0	0	1.3	2.1	8	0.66	1.55
12	h	Rotifera	*Synchaeta* sp. Ehrenberg, 1832	0.0	0	23.3	31.5	140	11.67	24.47
			Total (average)	**5102**	850.2	**6921**	4389	**72133**	6011	3160
			Total (number)	30609		41525				

In each group, only one species was exceedingly abundant: The *A. tonsa* and *B. improvisus* (nauplius and cypris larvae) which were the most dominant species of the whole plankton community in January and April, while the Bivalvia larvae dominated the zooplankton community in April (Table 1).

Fluctuation of zooplankton

Spatial fluctuations of the three dominate species; *A. tonsa*, *B. improvisus* and Bivalvia larvae created the spatial variation of the total zooplankton community (Fig. 2b, c, d). The highest abundance of all zooplankton species with more than 14,500 individuals per m^3 occurred in April at the NE1 (the fish cage site station; Fig. 2a) with the lowest 1,600 ind.m^{-3} in January at the NE2 (the reference site station; Fig. 2a). Overall, the total zooplankton abundance was higher at the fish cage site stations (S1, NW1, and NE1) as compared with the reference site stations (S2, NW2, and NE2) during the fish cage culture in the Caspian Sea. Statistical analysis (Kruskal-Wallis) showed, there was significant difference between abundance in different stations during the study period (p<0.05).

The total number of zooplankton in January and April strongly depends on blooming period of the following three taxa (Fig. 2): *A. tonsa* and *B. improvisus* (nauplius and cypris larvae) bloomed in January, April, and Bivalvia larvae bloomed in April.

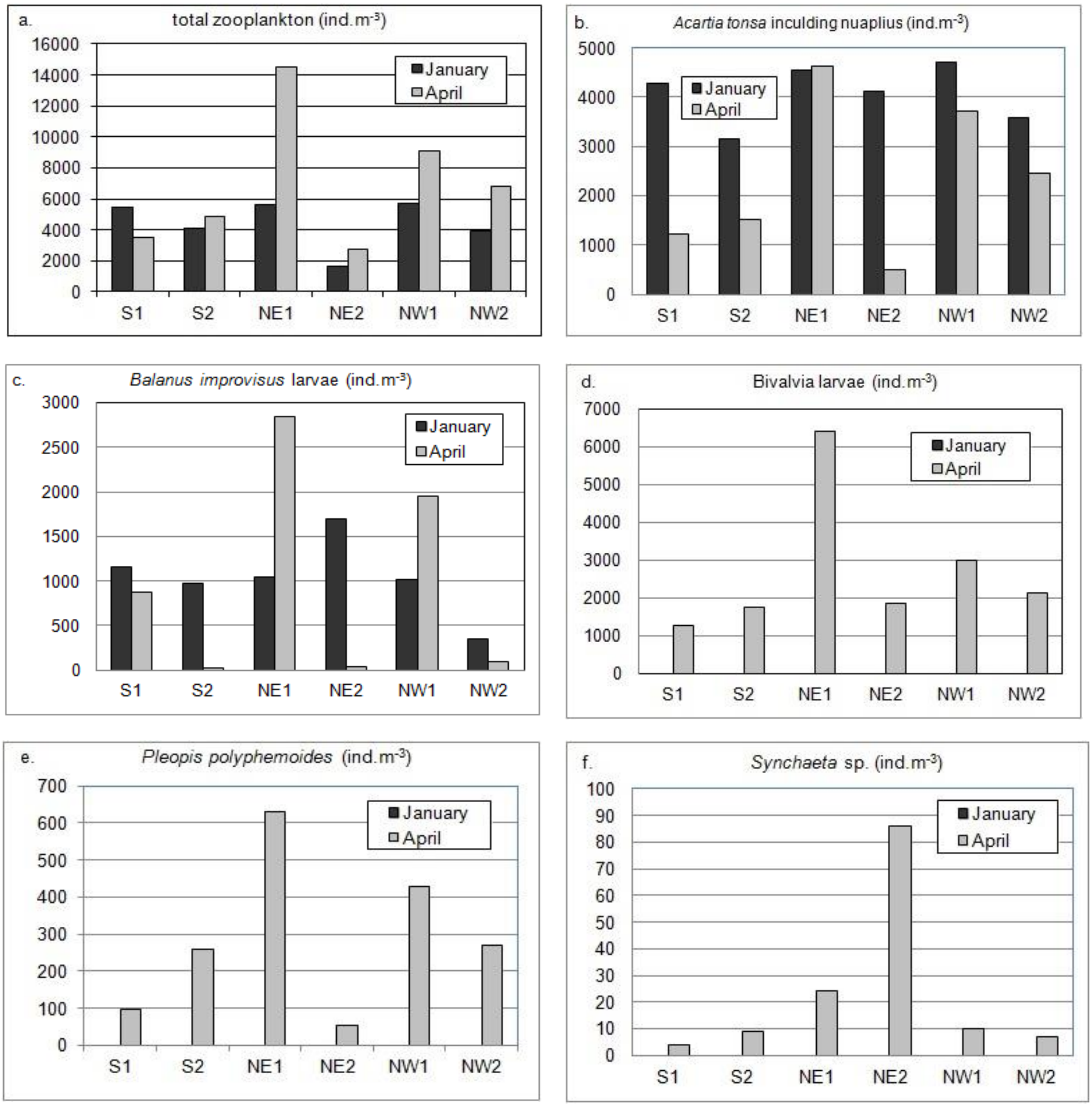

Figure 2: Seasonal fluctuations of dominant zooplankton taxa (average number m^{-3}) in different stations in the southwestern Caspian Sea, off Jafrud in 2013.

The abundance of *A. tonsa* including nauplius increased significantly (3,714 and 4,620 ind.m^{-3}) at the fish cage site stations (NE1 and NW1) in April (Fig. 2b), while there was no significant variation in abundance of *A. tonsa* (700-1,200 ind.m^{-3}) between the fish cage site stations (S1, NW1, and NE1) and the reference site stations (S2, NW2, and NE2) in January (Fig. 2b).

In January, the Cirripedia abundance varied between 350 and 1,700 ind.m^{-3} at the reference site stations (NW2 and NE2), respectively (Fig. 2c). The highest abundance of Cirripedia (*B. improvisus*: nauplius and cypris larvae) recorded 2,800 ind.m^{-3} at the fish cage site stations (NE1) in April , and the lowest Cirripedia abundance recorded only about 30 ind. m^{-3} at the reference site stations (S2 and NE2) in the same month (Fig. 2c). Bivalvia larvae, *P. polyphemoides* (Cladocera) and *Synchaeta* sp. (Rotifera) did not occur at the reference site and the fish cage site stations in January (Fig. 2d, e, and f). The highest abundance of Bivalvia larvae and *P. polyphemoides* amounted to 6,500 and 630 ind.m^{-3} at the fish cage site station (NE1) in April (Fig. 2d and e). The study revealed that the highest abundance of *Syncheata* sp. was 90 ind.m^{-3} at the reference site station in April (NE2; Fig. 2f).

The PCA based on zooplankton

The principal component analysis (PCA) was performed on the 5 zooplankton taxa (*A. tonsa*, *B. improvisus*, *Synchaeta* sp., *P. polyphemoides*, and Bivalvia larvae) during January and April 2013. According to the PCA, the cumulative values were almost 99% during the study. The co-variances were grouped based on the component loading value along the first two axes (PC1 and PC2) in the PCA bioplot (Fig. 3). Based on the component loading value and the ordinations of the bioplot, *A. tonsa*, *B. improvisus* and Bivalvia larvae were the most dominant taxa with high correlations matrix during the study.

The PCA based on station

The PCA was performed on the 6-stations (S1, S2, NE1, NE2, NW1, and NW2) during the study. According to the PCA, the cumulative values were more than 90 % for zooplankton taxa during the study. The co-variances were grouped based on the component loading value along the first two axes (PC1 and PC2) in the PCA bioplot (Fig. 3). Based on the component loading value and the ordinations of the bioplot, the stations near the fish cage site (S1, NW1, and NE1) were dominated on abundance of zooplankton, while the references site stations (S2, NW2, NE2) were located in separate group with the high variance in the bioplot in the study period (Fig. 3). Furthermore, the component loading value and the ordinations of the bioplot were different between the stations near the fish cage site (S1, NW1, and NE1) and the references site (S2, NW2, NE2) in January and April (Fig. 3).

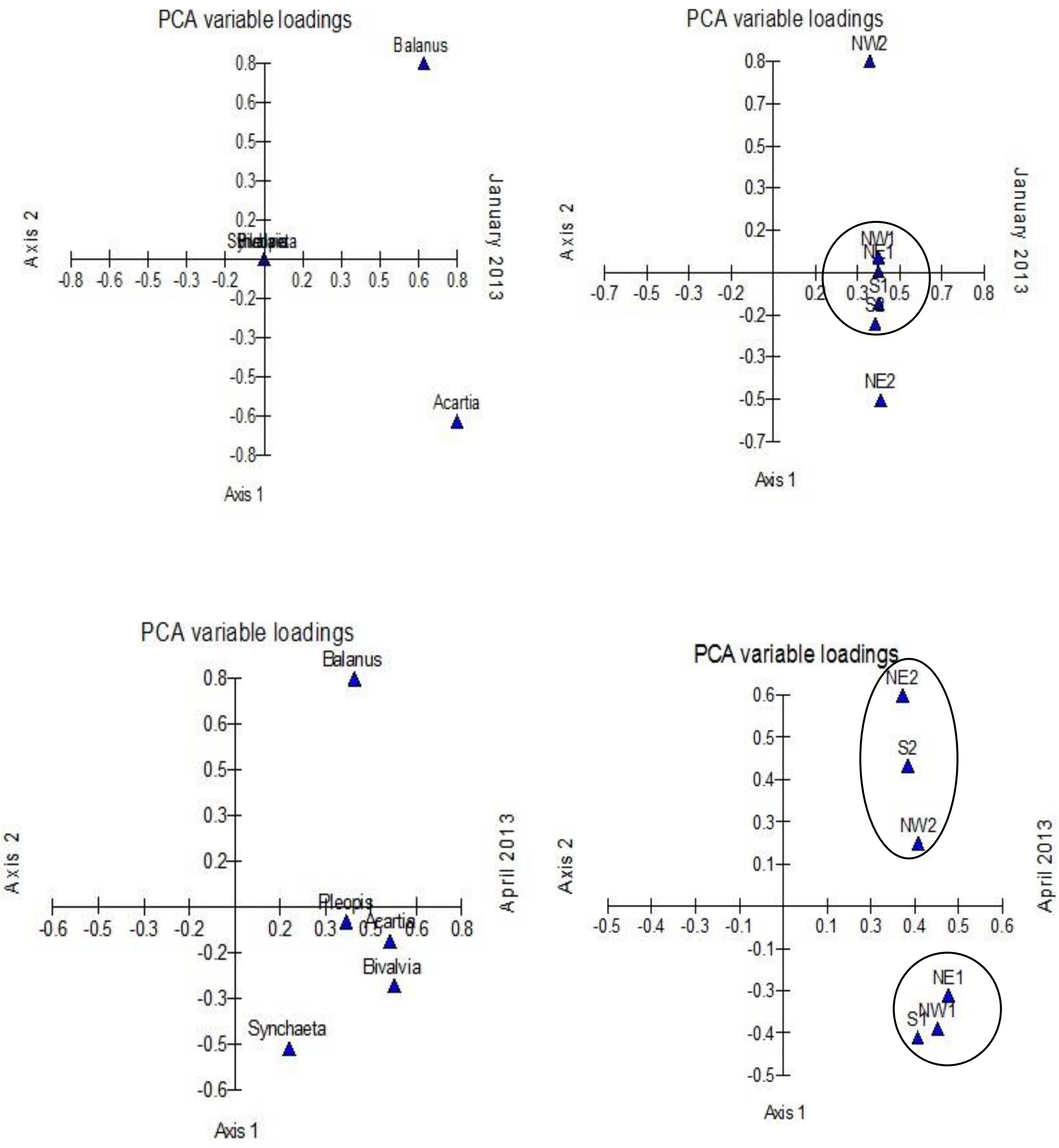

Figure 3: First two axes of principal component analysis (PCA) for zooplankton abundance (ind.m^{-3}) in the southwestern Caspian Sea.

Discussion

The number of Cladocera species was reduced in 2013 as compared to the findings of Hosseini (2011), and Bagheri *et al*. (2014): They listed 24 and 13 Cladocera species which were known in the southern Caspian Sea in 1996-1997, including the dominant species *Polyphemus exiguous* Sars, 1897. Only one of them (*P. polyphemoides*), could be found in 2013 during the study (Table 1). Seven Copepoda species were present in 1996-1997 (Roohi *et al.*, 2008), of them six were not found in 2013 (Bagheri *et al.*, 2013) (Table 1). Although, the comparison of our results with those of earlier studies was not easy since the region of study and the lists of species were dissimilar by all contributing authors.

The *Pleopis polyphemoides*, *Synchaeta* sp. and Bivalvia larvae did not occur in January (Fig. 2d, e, f). Based on Bagheri (2012) these taxa occurred in higher abundance in April-May after 2000, they were absent during summer in all years till 2010 due to low water temperature (Kasimov, 1994; Bagheri *et al.*, 2012; Bagheri *et al.*, 2013; Bagheri *et al.*, 2014; Abery *et al.*, 2005).

The findings showed the abundance of *A. tonsa* adult including nauplius varied between 2,340 and 4,064 ind.m^{-3} in January and April 2013, respectively (Table 1). The average annual abundance of *A. tonsa* presented by Rowshantabari *et al*. (2007), Hosseini (2011), and Bagheri *et al*. (2012) in the period of 1996, 2001-2006 and 2009-2010, respectively revealed a decrease of *A. tonsa* abundance. The decrease of *A. tonsa* abundance during this study could be related to month and time of sampling, because increase in the abundance of *A. tonsa* occurred in summer and continued until autumn in the southwestern Caspian Sea, as reported by Bagheri (2012).

The total zooplankton abundance was high at the fish cage site stations (S1, NW1, & NE1) as compared with the reference site stations (S2, NW2, and NE2) during the study. These findings revealed the impact of the fish cage culture on the zooplankton structure (Fig 2a).

According to Bagheri *et al.* (2013), the zooplankton abundance such as *A. tonsa, B. improvisus, P. polyphemoides* were strongly affected by high levels of nutrients. In this study the increasing of zooplankton species at the fish cage site stations (Figs. 2) could be related to high nutrient levels due to the rise of bacteria, small food particles similar to what has been noted by Matsumura and Tundisi (2005) in a Brazil lake. Likewise, Dias *et al*. (2011) reported a high spatial zooplankton abundance near the fish cage culture and low abundance at the reference site in a Turkish lake. They observed high nitrate, phosphate concentrations and the phytoplankton number next to the fish cage culture. Furthermore, Guo and Li (2003) reported similar results in a Chinese lake, with zooplankton taxa (e.g. Cladocera and Copepoda) occurred with high abundance near the fish cage culture due to increase of nutrient levels at the cage. Similar findings were reported by **Sanitos** *et al*. (2009). Unfortunately no nutrients parameters and small food measurements were carried out in this study.

However, the PCA confirmed the spatial variations of zooplankton abundance, and separated the stations near to the fish cage site (S1, NW1, and NE1) from the reference site stations during the study period (1 km away from the fish cage site; S2, NW2, and NE2; Fig. 3). Based on the PCA, there is temporal variation of zooplankton abundance between January and April (Fig. 3). It might be related to the increase of zooplankton abundance and biodiversity as Bivalvia larvae (2737 ind.m^{-3}; Table 1) and *P. polyphemoides* (290 ind.m^{-3}; Table 1) in April as compared with January. Richardson (2008) and Bagheri *et al*. (2014) believed that in the beginning of spring (March-April), the increase of water temperature facilitated Bivalvia larvae and Cladocera to increase abundance in the Baltic and Caspian Sea.

It can be concluded that the hypothesis was accepted because the dominant zooplankton species were abundant at the stations near the fish cage culture except S1 (south of the fish cage culture) which could be related to the wind direction in January and April 2013, which transferred the food availability from northwestern to northeastern of the fish cage site in the sea. The findings confirmed the impact of the fish cage culture on the zooplankton population due to striking increase of *A. tonsa*, *B. improvisus*, *P. polyphemoides*, and Bivalvia larvae abundance at the fish cage site.

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