

Research Article

An investigation on the effects of fish farming in marine cages on abundance and structure of *Mnemiopsis leidyi* and *Beroe ovata* (Ctenophora: Lobata) in the southwestern Caspian Sea during 2018-2020

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Abstract

The purpose of this study was to investigate changes in abundance of combs and non-biological parameters around fish cage in southwestern Caspian Sea. This study was conducted with 3 stations near the cage fish farming site and 3 reference stations far from the cage during 2018-2020. Total number of *Mnemiopsis leidyi* in the stations near to fish farming site (13200 ind.m⁻³) increased by 42% compared to far stations from the fish farming site (9500 ind.m⁻³) in 2018-2020. *M. leidyi* had the highest monthly abundance at the station near the cage fish farming site with 3011 ind.m⁻³ in February 2019. The lowest abundance of *M. leidyi* was observed at the far station from cage fish farming site almost 4.0 ind.m⁻³ in February 2020. The length group of less than 5 mm dominated *M. leidyi* populations by more than 96% and was the predominant *M. leidyi* population at the cage fish farming site. The findings showed, the abundance of *B. ovata* fluctuated between 27 and 47 ind.m⁻³ at near the stations cage, notably *B. ovate* was not observed at stations far from the cage fish farming site. CCA analysis confirmed that there was a strong relationship between *M. leidyi* abundance and nutrients levels ($r=0.99$). Increasing the amount of nutrients generated from feed and excretion of fish farming in cages is one of the main reasons for increase in the abundance of *M. leidyi* and *B. ovata* at stations near the fish farming site. Therefore due to the closed environment of Caspian Sea, without investigating cage culture effects on native fauna and flora, development of fish farming sites in sea cages is not sustainable.

Keywords: Fish, Cage culture, *M. leidyi*, *B. ovata*, abundance, Nutrients, Caspian Sea

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Introduction

The Caspian Sea is located on the border between Europe and Asia, and is the largest lake in the world with a catchment area of 3.5 million km² (Nazari *et al.*, 2020; Sanaee *et al.*, 2020). The present-day Caspian Sea is part of the Ponto-Caspian Sea which was formed 5 to 7 million years ago. Presence of the shallow northern region with the Volga River Delta and the deep southern region, and water salinity between 0.1 and 13 has created diverse ecological habitats and unique biodiversity (Bagheri *et al.*, 2012; Mertens *et al.*, 2012). Since early 1990s, The Caspian Sea is severely affected by human activities and severe environmental pollution (Dumont, 1998; Bagheri *et al.*, 2013; Mirzajani *et al.*, 2016). Due to increasing use of fertilizers and pesticides, deforestation, concentration of nutrients in rivers is increased and its rate more than doubled in recent years (Dumont, 1998; Nasrollahzadeh *et al.*, 2008; Bagheri *et al.*, 2014a; Mohammadi *et al.*, 2017). The increase in nutrients on southern shores of Caspian Sea has led to an increase in primary producers and an increase in phytoplankton abundance since 2000s (Nasrollahzadeh *et al.*, 2008). Four phytoplankton blooms occurred off the south coast of Caspian Sea in 2005, 2006, 2009 and 2010 (Bagheri *et al.*, 2011; Bagheri and Fallahi, 2014b; Makhloogh *et al.*, 2017). The increase in nutrient contents in Caspian Sea also created the conditions for bloom of non-native species such as *Mnemiopsis leidyi* (Bagheri *et al.*, 2014a). *M. leidyi* is

native to eastern shores of Atlantic Ocean and grow rapidly. The animal entered Black Sea through ship's water balance tank in the mid-1980s and spread throughout Black Sea until the end of the decade, entering the Mediterranean Sea in early 1990s and scattering throughout the ecosystem until 2009 (Mutlu, 2009). It entered the Caspian Sea in late 1990s and spread to all parts of it (Ivanov *et al.*, 2000). It was introduced to North Sea and Baltic Sea in 2006 (Faasse and Bayha, 2006). Diversity and abundance of zooplankton decreased 2 times due to *M. leidyi* feeding, and frequency of phytoplankton increased due to reduction of zooplankton nutrition pressure from phytoplankton, thus increasing the abundance of flagella, protozoa and trophic levels (Shiganova *et al.*, 2001). Entry of *B. ovata* into the Black Sea has increased the abundance of zooplankton and brought fishing back to the 1980s (Kideys *et al.*, 2005). Based on the study for first time, *B. ovata* was reported on southeastern shores of Caspian Sea around a fish cage culture site in 2019 due to increase of nutrient levels by Roohi *et al.* (Unpublished).

Cage farming is one of the fisheries activities formed in the last eight years on the southern shores of Caspian Sea (Bagheri and Makaremi, 2018) and Persian Gulf (Ziarati *et al.*, 2020). Guo and Li (2003) in their study on water quality, plankton and jellyfish structure near cage fish farming site, showed an increase in phytoplankton and zooplankton abundance due to increased TN, TP concentrations. Furthermore fish

cage culture reduced biodiversity, raised algae blooms, and increased diseases in aquatic ecosystems as well (Borges *et al.*, 2010). The concentration of TN and TP increases from the beginning of feeding the fish in the water, and its rate reaches the peak with the high weight of the fish and feeding; more than 85% of the phosphorus and nitrogen in the environment around the cages are caused by feeding. Cage fish farming increases the amount of nutrients and organic matter, respiration and excretion of wastewater and phytoplankton bloom directly in water due to food waste in the cage fish environment (Islam, 2005).

According to Islam and Tanaka (2004) study, 68 kg nitrogen and 11 kg phosphate per ton of fish are produced and enter the sea under minimal food waste and adequate FCR condition. Studies on the effects of fish farming on Caspian Sea ecosystem are very limited due to the industry's youthfulness in southern shores of Caspian Sea. The first study on the effects of cage fish farming on zooplankton community in Caspian Sea was conducted by Bagheri *et al.* (2016) and followed by Haddadi Moghaddam *et al.* (2020). In this study species diversity and structure of zooplankton populations showed that the population of non-native and opportunistic species near fish farming site increased significantly. In addition, Afraei Bandpei *et al.* (2016) noted that cage fish farming has affected planktonic organisms, and large-scale algae bloom may not be uncommon if cage fish farming sites increase in the

waters of Mazandaran Province in Klar-Abad region.

Also Bagheri and Makaremi (2018) in their study on periphyton at cage fish farming sites showed an increase in the abundance of *Cladophora* sp., *Spirogyra* sp. and *Cordylophora caspia* algae; the highest *Cladophora* algae abundance was reported to be 500,000 ind.m². In addition presence of Nematoda with an abundance of 100 ind.cm⁻¹ in cage fish farming site is an evidence of increased nutrient load and contamination at fish farming site in Jafrud region, southern Caspian Sea (Bagheri and Makaremi, 2018). Parafkandeh *et al.* (2016) studied on distribution, abundance and biomass of macro-benthos in cage location in Klar-Abad in south of Caspian Sea and reported that abundance and biomass of macro-benthos in the cage location declined compared to other areas. The first cage farming in Caspian Sea began in May 2012 in Guilan Province and their numbers increased between 2012 and 2020. Therefore in this study, an attempt was made to investigate some of the effects of fish farming in cages on abundance and structure of *Mnemiopsis leidyi* in southern coast of Caspian, Guilan Province, between 2018 and 2020.

Materials and methods

Study area

Effects of fish farming in cages on structure of *M. leidyi* populations in southern Caspian Sea (special economic zone of Bandar Anzali) were investigated during 2018-2020. The cages were located at 37°31'14' north

latitude and 49°42' 89" east longitude, approximately 7 km from the coastline, at 45m depth (Fig. 1). Since most of the winds in Caspian Sea are from south, northwest and northeast, the study stations (S-n, NE-n, NW-n) were selected in accordance with wind directions in 3 near points of the cage and 3 reference stations (S-f, NE-f, NW-f) in the same directions with a distance of 1000m from near stations of the cage (Bagheri *et al.*, 2016). Sampling was performed on May 2018, September 2018, February 2019, May 2019 and January 2020 between 10 am and 13 pm using a suitable boat.

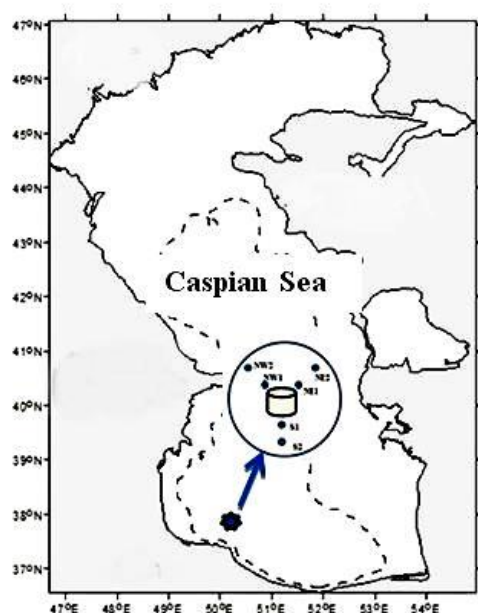


Figure 1: The fish cage farming location and sampling stations in southwestern Caspian Sea, during 2018-2020.

Sampling and laboratory analysis of Ctenophora (M. leidyi and B. ovata)

Sampling of Ctenophora was performed using a METU net sampler with 500 micron mesh, a diameter of 50 cm and a suitable compartment for Ctenophora (Kideys and Moghim *et al.*, 2003). The

sampling method was conducted vertically from 20m depth to the surface water in all stations. After each pull the net was washed with water to collect Ctenophora in the netting compartment. Ctenophora samples were studied immediately after the haul. Net bucket content was emptied into a dish and the Ctenophora were counted by eye. Biomass of each of the measured individuals was calculated using the following equation:

Wet Weight (g) = $0.0013 \times \text{Length}^{2.33}$ (mm); $r^2=0.96$, $n=269$ obtained (Bagheri *et al.*, 2012).

Physicochemical sampling of water

Physico-chemical samples of water were taken from different stations using Nansen bottle device (Hydro-Bios). Water temperature was determined by a return thermometer and water transparency was determined by secchi disk (Clesceri *et al.*, 2005). Total nitrogen and total phosphorus were measured after sample digestion and absorption by spectrophotometer (Valderrama, 1981). Soluble silicate was also determined using molybdenum and silicate (Sapozhnikov *et al.*, 1988) and spectrophotometry. To measure chlorophyll-*a*, a certain volume of water was filtered using 0.45 micron filter paper (GF). Chl-*a* was then extracted from the precipitate obtained on filter paper using 90% acetone. Optical absorption of the sample at certain wavelengths was read by a spectrophotometer and chlorophyll-*a* concentration was calculated with the corresponding formulas (Clesceri *et al.*,

2005). Total suspended solids (TSS) was measured using a vacuum pump (GAST) and a micrometer acetate cellulose filter (MEDAP) and the residual material was weighed with a scale (Bosch, accuracy 0.001, Clesceri *et al.*, 2005).

Statistical analysis

Comparison of frequency changes of *M. leidyi* in stations near the sea cage and reference stations was performed by t-test with version 19 of SPSS software. Correspondence Canonical Analysis was used to determine correlation and relationship between *M. leidyi* abundance and environmental variables (phosphorus, total nitrogen, chlorophyll-

a, silicate, turbidity and water temperature). CCA analysis was performed using MVSP software version 3.13 (Krebs, 1994).

Results

The average abundance of *M. leidyi* in stations near the fish farming site (13,200 ind. m⁻³; C-N) compared to the reference stations (9500 ind. m⁻³; C-F) has increased significantly during the years 2019-20 (Fig. 2). On the other hand statistical analysis of t-test showed a significant difference between the frequency of *M. leidyi*, near stations and far from the cage fish farming site ($p < 0.05$).

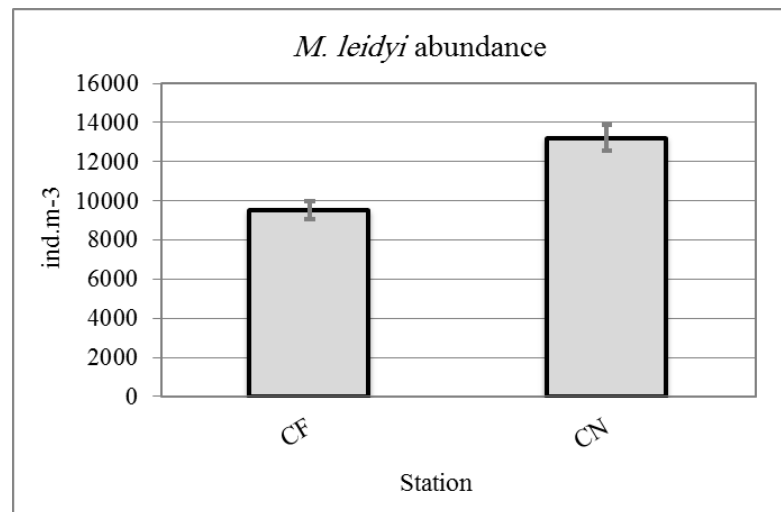


Figure 2: *M. leidyi* abundance on the fish farming site in southwestern Caspian Sea, 2018-2020, CN (near cage) and CF (far from cage).

Comparing the frequency of *M. leidyi* among stations in different months showed that S-f (reference station) with 12 ind. m⁻³ frequency and the station near fish farming site (NW-n) with 90 ind. m⁻³ were significantly different in May 2018.

In addition, the highest frequency of *M. leidyi* was observed near fish farming cage at NE-n station with 1615 ind.m⁻³ and the lowest frequency (643 ind. m⁻³) was away from the fish farming cage (NW-f) in September 2018. The results of *M. leidyi* abundance in February 2019

showed a sharp increase compared to May and September, and the trend of *M. leidyi* abundance changes was increasing from May 2018 to February 2019.

Frequency of *M. leidyi* fluctuated in February 2019 at NW-f and NW-n stations between 1542 and 3011 ind. m⁻³, respectively. Comparing frequency of *M. leidyi* in May 2018 and February 2019 showed that the highest abundance of *M. leidyi* at the near station of fish farming site (NW-n) was 184 ind. m⁻³ in May 2019. The lowest abundance of *M. leidyi* was observed at far-off station of fish farming (S-f) almost 67 ind.m⁻³ in the same month.

The abundance of *M. leidyi* fluctuated between 4 ind. m⁻³ at the far-off station (S-f) of fish farming cage (reference) and 39 ind. m⁻³ at the station NE-n near the fish farming site in February 2020. The t statistical test showed significant differences among frequencies of *M. leidyi* at stations near fish farming cages (S-n, NE-n, NW-n) and reference stations of S-f, NE-f and NW-f ($p<0.05$).

Based on Kruskal-Wallis analysis, significant difference was observed in *M. leidyi* abundance among different months ($p<0.05$). Results showed that abundance of *B. ovata* was fluctuating between 27 and 47 ind. m⁻³ at NW-n and S-n stations, respectively. *B. ovata* was not observed at the reference station S-f far from the fish farming site in the sea (Fig. 3).

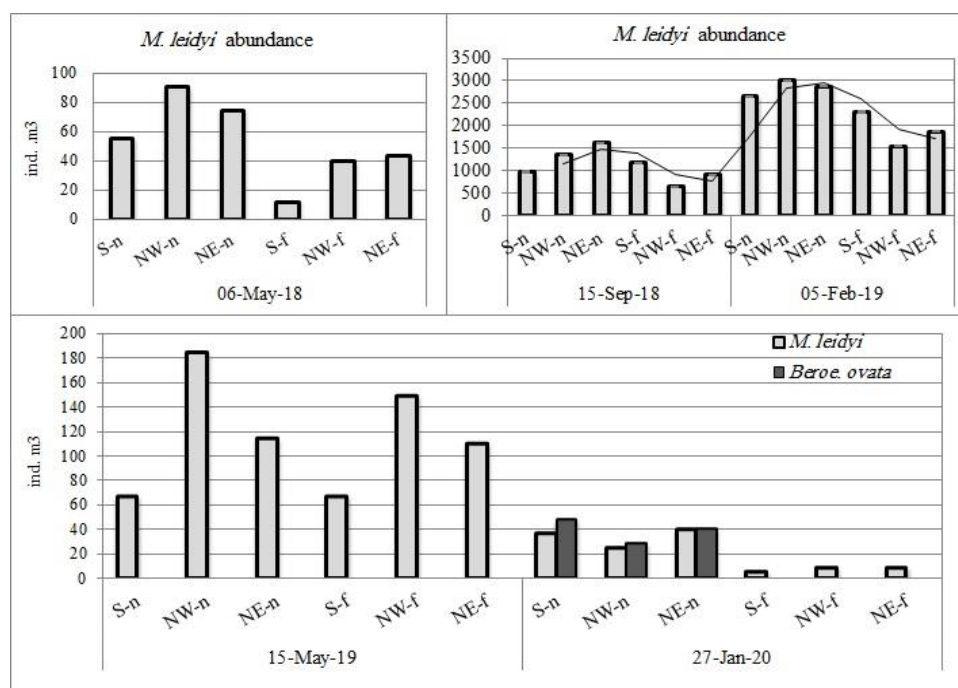


Figure 3: *M. leidyi* and *B. ovata* abundance in different stations of the cage fish farming site in southern Caspian Sea during 2018-2020, near stations (S-n, NE-n, NW-n) and reference stations (S-f, NE-f, NW-f).

Length group

The structure of size groups was determined at stations near the fish farming site (S-n, NE-n, NW-n) and the reference stations (S-f, NE-f, NW-f) (Fig. 4). Size groups of less than 5 mm dominated *M. leidy* population by more than 96%. Size groups of more than 6-10

mm accounted for less than 3% of the population, and more than 11 mm size groups were very small in number. The t-test did not show significant difference among near fish farm stations based on *M. leidy* length structure ($p < 0.05$).

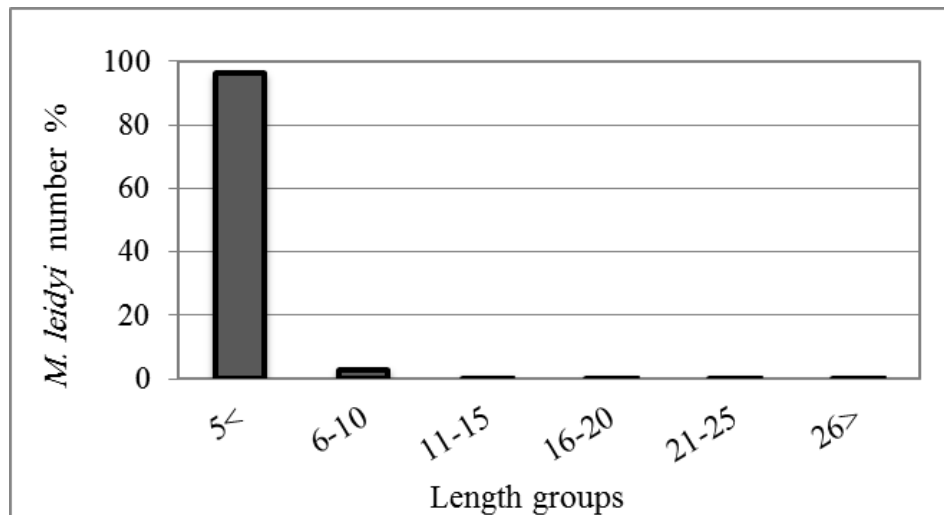


Figure 4: Length groups (mm) of *M. leidy* around the cage fish farming site, 2018-2020.

A comparison of *M. leidy* structure size groups among stations near the fish cage (S-n, NE-n, NW-n) and stations far from the cage (S-f, NE-f, NW-f) in the this study showed that fish cage farming affected the *M. leidy* length structure. The length group of less than 5 mm was predominant *M. leidy* population in different months at stations near the fish cage (S-n, NE-n, NW-n). The highest frequency of the length group of less than 5 mm *M. leidy* was observed in stations near the cage (S-n, NE-n, NW-n) with 8630 ind. m^{-3} in February 2019. The lowest frequency of this length group (>5 mm) was recorded almost 16 ind. m^{-3} in stations far from the cage fish farming (CF) in January 2020. The only

length group observed in Feb 2019 was less than 5 mm. The greatest diversity of *M. leidy* length groups was in September 2018 (from >5 mm to >26 mm length) at far and near stations of fish farming cages. After *M. leidy* with a length of >5 mm, *M. leidy* with a length group of 10-6 mm with 250 ind. m^{-3} had the highest abundance of length groups in September 2018 in stations near the fish farming cage (Fig. 5). The t-test showed significant difference among near fish farming stations in cages based on length structure of *M. leidy* ($p < 0.05$).

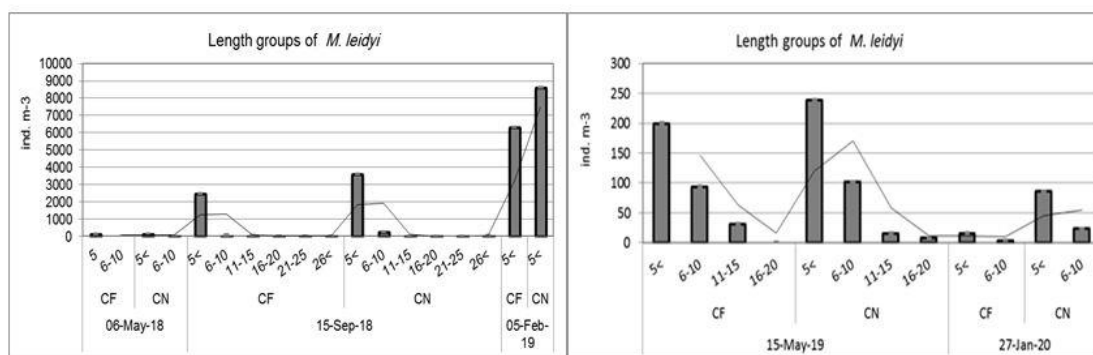


Figure 5: *M. leidy* length groups at near stations (CN) and far from cage fish farming site (CF), 2018-2020.

Hydrochemical

The findings of water transparency showed significant changes (Fig. 6). The results showed a decrease in transparency at stations near the fish farming site. The highest level of transparency was observed at the c-f station (away from the cage, September 2018) with 6.5m and the lowest level was observed at the c-n station (near the cage, February 2018) with a transparency of 2.5m. Based on statistical analysis, significant differences were observed at study stations ($p<0.05$). Total nitrogen concentrations ranged from 0.7 to 1.4 mg L⁻¹ at the c-f stations (away from cage, September 2018) and c-n (near cage, Feb 2018), respectively. The highest average total phosphorus concentration was observed in the station near the cage in Sep 2018 (c-n) with a rate of more than 0.2 mg L⁻¹ and the lowest in May 2019 with a rate of 0.03 mg L⁻¹ in the station away from the cage (c-f).

Statistical tests showed that the concentration of total nitrogen and total phosphorus in different stations were

significantly different ($p<0.05$). Findings showed that chlorophyll *a* ranged between 1.33 and 0.28 µg. L⁻¹ in near cage (September 2018) and away from cage (January 2020), respectively. The highest average turbidity of seawater was recorded in May 2018 as 4.7 mg L⁻¹ and the lowest with an average of 1.3 mg. L⁻¹ in January 2020. The t-test showed that the amount of chlorophyll *a* and turbidity in far and near stations of the sea cage had a significant difference ($p<0.05$). The last parameter studied was water silicate, with the highest silica content of 0.7 mg L⁻¹ in May 2019 and the lowest concentration near station at 0.09 mg L⁻¹ in May 2018.

Canonical correspondence analysis (CCA) among 7 environmental factors of water: transparency (Secchi); total nitrogen (TN); total phosphorus (TP); silica (Sio₂); chlorophyll (Chl-*a*); turbidity and abundance (Ml. n⁻³), and biomass (Mlg⁻³) of *M. leidy* were performed. This analysis showed that Eigen value for the first axis (CCA1) was 0.004 and for the second axis (CCA2) was the same, almost 46.3% of

variance was calculated for the first axis (CCA1) and 83.4% of variance for the second axis (CCA2). Based on CCA analysis for first and second axes

(CCA1, CCA2), strong correlation ($r=0.99$) was observed among abundance and biomass *M. leidyi* and 7 environmental variables (Table 1).

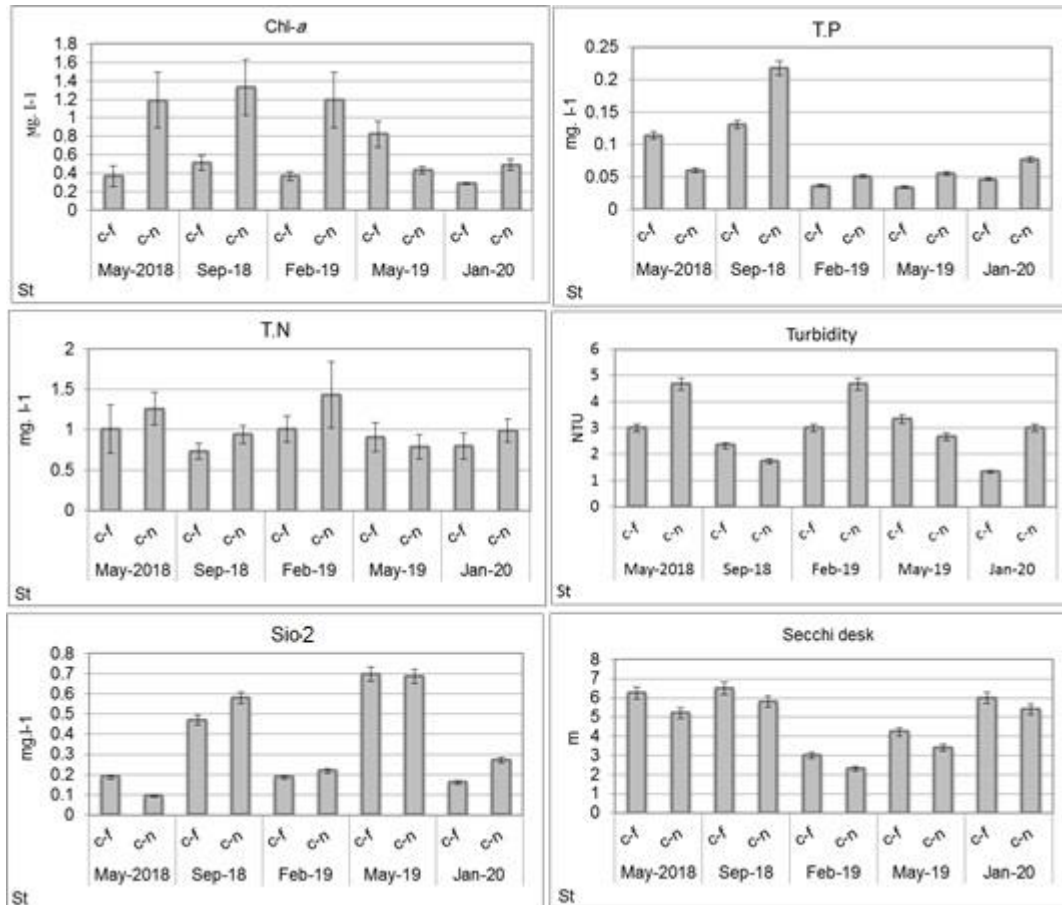


Figure 6: The Average (\pm sd) secchi disk and nutrient concentration (total phosphorus and nitrogen; silicate), chlorophyll *a*, turbidity at cage fish farming site in southern Caspian Sea (2018-2020), Cage near (c-n), cage far (c-f).

Table 1: The CCA analysis among environmental factors and *M. leidyi* abundance in the fish cage farming site in southern Caspian Sea, 2018-2020.

Environmental factors	CCA1	CCA2
Eigenvalues	0.004	0.004
Percentage	46.3	37.14
Cum. Percentage	46.3	83.441
Cum. Constr. Percentage	55.489	100
Spec.-env. correlations	0.992	0.838

The CCA analysis showed that nutrients including, total nitrogen, total phosphorus, chlorophyll, silica and water temperature were the most important environmental factors that had

the greatest impact on fluctuations of abundance and biomass of *M. leidyi* (Ml.n^{-3} and M.g^{-3}). The biplot showed that *M. leidyi* abundance is located on left side of the diagram indicating that

there is strong relationship between total phosphorus (TP), chlorophyll, silica (SiO_2) and water temperature, and *M. leidyi*. While

turbidity (Turb) and transparency (Secchi) are located on the opposite side of the biplot, which indicates lack of correlation between *M. leidyi* abundance and turbidity and transparency (Fig. 7).

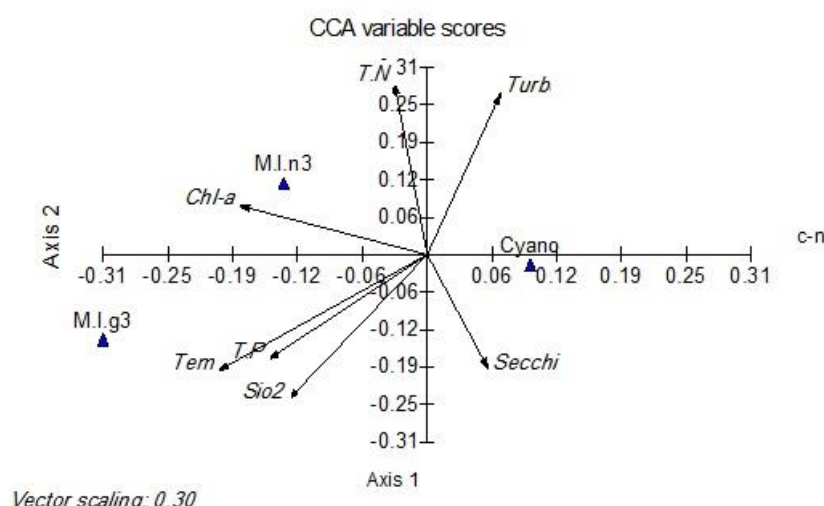


Figure 7: The two CCA axes for comb abundance and environmental parameters at the cage fish farming site in southern Caspian Sea, 2018-2020.

Discussion

Fish farming in marine cages introduces undigested food and waste products such as urea and ammonia organic matter into the aquatic environment (Islam and Tanaka, 2004). Quality of fish food in cages, management of fish farming site, and the ratio of feed weight to fish weight (FCR) play an important role in nutrient levels (Islam, 2005). Increased *M. leidyi* abundance at near the cage fish stations (S-n, NE-n, NW-n) with an abundance of more than 13,000 ind. m^{-3} compared to far from cage stations (S-f, NE-f, NW-f) with an abundance of 9000 ind. m^{-3} (Figs. 2 and 3) indicates an increase in the amount of nutrients in the environment around fish farming cages, because non-biological parameters such as total phosphorus, total nitrogen, chl-*a*

and decreasing transparency and turbidity have increased in the environment around fish farming cage (Fig. 6). The CCA Analysis showed that nutrients including, total phosphorus, chl-*a*, silicate and water temperature were the most important environmental factors that had the greatest impact on fluctuating abundance and biomass of *M. leidyi*, so that strong correlation showed between *M. leidyi* abundance and total phosphorus, chl-*a* and water temperature (Fig. 7). According to Purcell *et al.* (2007), Richardson (2008), and Bagheri *et al.* (2012), *M. leidyi* have a strong positive relationship with increasing water temperature and nutrients concentration. They believe that increasing *M. leidyi* abundance is due to human activity increase due to

organic matter loads. Studies by Dias *et al.* (2011) and Bagheri and Makaremi (2018) also showed an increase in nutrients, decreased water transparency and rise of phytoplankton abundance in the area of fish farms on shores of Mediterranean Sea and freshwater lakes and south-western Caspian Sea.

Bloom of ctenophores depends on the concentration of nutrients (Bagheri *et al.*, 2014a) and organic matter due to fish excretion and undigested fish in marine ecosystems (Dias *et al.*, 2011). Findings of Islam (2005) showed that for production of one tonne of fish in the sea, 70 kg of nitrogen and 11 kg of phosphorus are imported directly into the sea, so increasing nitrogen and phosphorus as a result of aquaculture in the sea increases the abundance of micro-plankton such as protozoa, dinoflagellata and tiny diatoms. Similar effects also achieved in studies of Bagheri *et al.* (2016) and Afraei Bandpei *et al.* (2016).

Bagheri and Makaremi (2018) reported that abundance of diatoms was significantly higher than that of other phytoplankton at the station near fish cages farms, all of which were fed by predominantly *M. leidyi* with a length of less than 5 mm in Caspian Sea (Finenko *et al.*, 2006; Sullivan, 2010). Also in this study, the size of *M. leidyi* length groups was consistent with the studies conducted by other researchers (Kideys and Moghim, 2003; Shiganova *et al.*, 2004).

Findings displayed that *Beroe ovata* was observed at stations near marine fish farming (47 ind. m⁻³, Fig. 3). In addition

Roohi *et al.* (Unpublished findings) reported high abundance of *B. ovata* at the site of cage fish farming in southeastern Caspian Sea in September 2019. The occurrence of non-native *B. ovata* has indicated an increase in *M. leidyi* abundance in the cage fish farming site in the present study (Fig. 3), and has provided the conditions for appearance of *B. ovata*.

In this study, changes in the structure of *M. leidyi* at the cage fish farming site in 2018-2020 on the coast of Guilan Province, Caspian region were examined. In present study, an attempt was made to show changes in the composition and abundance of ctenophores in the area of cage fish farming site. The results showed that the abundance of Ctenophora near the cage fish farming have increased due to increased nutrients and chl-*a* concentrations. Therefore, any development of fish farming cage sites in Caspian Sea should be done considering the extent of its environmental impact on valuable native species of Caspian Sea and any haste should be avoided.

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