The study of interaction between fish cage culture and sediment population dynamics of heterotrophic bacteria in the southern of Caspian Sea (Nowshahr region)

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Abstract

The aim of this study was to investigate the interaction between fish cage culture and sediment bacterial contamination in the area southern of Caspian Sea. For this purpose, sampling of sediment was collected in Mazandaran province (Nowshahr region) during the rainbow trout farming period at different distances (shadow, 200 and 1000 m from the cage) in 2017-2018. The results showed that in the whole rearing period, the maximum and minimum mean of total count sediment bacteria (TCB) (246875 CFU/g) and (2500 CFU/g) were observed in the middle and the end of the rearing period at the 200 m distance, respectively. The presence of total coliform and fecal coliform was recorded 12.5% and 2.08%, respectively, and Clostridium perfringens was not observed in any of the samples and stations. Maximum and minimum of pH, Eh and TOM were observed 8.60 and 8.05, -51.3 and -83.4, and 14.18 and 2.45, respectively. The mean of TCB, pH, Eh and TOM% were significantly different between rearing periods ($p<0.05$). It seems that fish cage culture activity in the southern region of the Caspian Sea, due to low breeding capacity, short breeding period, permanent currents, cages place with a suitable distance from the shore, had a relatively small effect on some sediment quality factors and had no significant effect on bacterial communities of sediments around the cage.

Keywords: Bacterial contamination, Fish cage culture, Sediment, Caspian Sea, Nowshahr, Iran

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Introduction

The Caspian Sea is the largest closed body of water on Earth, and the lack of any natural connection with other oceans has made it a very special ecosystem. These unique characteristics make the Caspian Sea vulnerable to external factors such as climate or human change (Barayi, 2010). Aquaculture is growing rapidly due to the significant increase in demand for fish and seafood worldwide as an industry (Gang *et al.*, 2005).

Aquaculture has been the youngest and fastest-growing industry in the last two decades, and has been responsible for maintaining the quality of cage farming. Lake salmon are an important known source of organic waste and nutrients that can increase the levels of organic matter and nutrients in the water column, thereby increasing the growth of bacteria around the cage and enriching sediments at the breeding site. (Karimian *et al.*, 2017).

Marine aquaculture is considered for the optimal use of the sea to provide part of the protein, reconstruction, replenishment of fish stocks, job creation and promotion of tourism. In addition to the growing population of the world and the need of human societies for food resources, the use of inland water resources is of particular importance and basic planning for the optimal use of these resources in most countries has a special priority (Clark *et al.*, 1991). Bacterial communities in marine sediments provide important environmental roles such as nutrient cycling, mineralization, decomposition, and diagenesis (physical and chemical changes during sediment-to-sedimentary rock conversion) (Hornick and Buschmann, 2018). Marine fish farming impacts the marine environment, principally due to the input of a substantial amount of organic matter and nutrients, resulting from uneaten food and fish excretions, which are released into the environment and accumulate in the underlying sediment (Rubio-Portillo *et al.*, 2019). Cages are usually very much in contact with the environment and release the generated waste directly into the environment. The relatively high density of bacteria in breeding cages as well as the spread of food to the environment around the cage affects the microbial population of water (Ling *et al.*, 2007). In general, aquaculture activities, including fish farming in cages, can negatively affect aquatic systems, which are among the major problems caused by aquaculture activities, changes in water quality and sediments.

Therefore, in this study, the bacterial changes (sediment indicator bacteria) and some physical and chemical parameters has been studied in cages in the southern Caspian Sea region (Nowshahr - Mazandaran province) during the years 2017 to 2018.

Materials and methods

Sediment samples were collected from the southern of Caspian sea region of Nowshahr city in Mazandaran province from December 2017 to November 2018 at three stations at each site (cage shade, 200 and 1000m distance) during the rearing periods in four geographical directions (Fig. 1).

Sediment sampling was performed by grab sampling device. The obtained sediments were poured into a plastic bag and the air was removed and the bags next to the ice were completely sealed and transferred to the Caspian Sea Microbiology Laboratory.

Total count bacteria (TC) were spread on Plate count agar and incubated at 37°C for 24-72 h; Total coliforms (TC) and fecal coliforms (FC) were determined by the membrane filter technique on ECC chrome agar and incubated at 37 and 44°C respectively for 24 h; Clostridium perfringens were measured by inoculating the sample on sps agar and incubating under anaerobic conditions at 22 °C for 48 h. Sediment bacteria density was expressed as Colony Forming Units (CFU) per grams of dry weight. The environmental parameters of sediment such as pH, Eh and TOM% were performed using the standard method introduced and combustion method (Sarda, 1995).

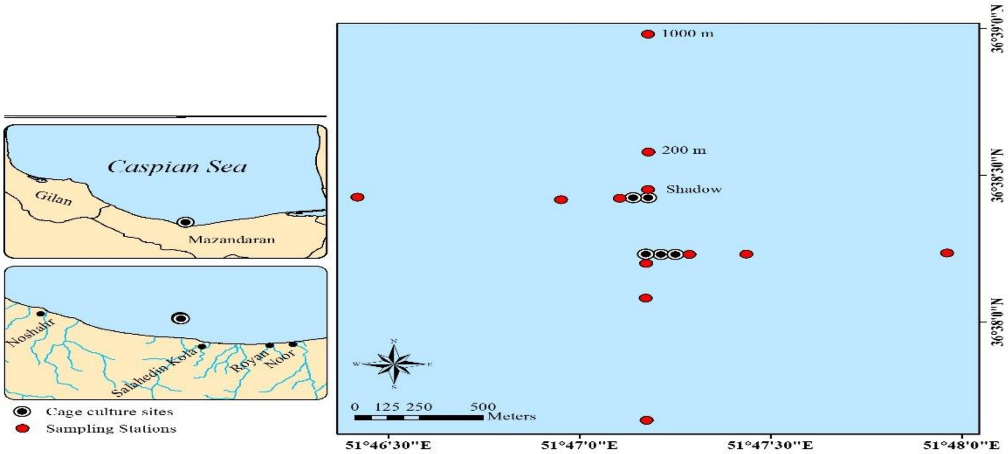


Figure 1: Location, longitude and latitude of different sampling stations in fish cage culture (2017-2018)

Statistical analysis

The data were transferred based on one of the natural logarithm and ranking processes and then confirmed by drawing Q-Q diagram and Shapiro-Wilk test (Siapatis et al., 2008). Parametric tests (ANOVA) on normalized data were used for statistical analysis. Univariate analysis of variance and multivariate analysis (principal component analysis =PCA) was performed.

Also, PCA prerequisite tests were performed to determine the adequacy of the data and Bartlett test was performed to evaluate the status of the correlation matrix between variables (Nasiri, 2009). All means are given with standard error (Mean± SE).

Results

Microbial parameters of sediment

Fig. 1 shows the changes in the mean total count bacteria (TCB) in different seasons (rearing periods) of sediment sampling at the fish cage culture (Nowshahr region). The highest changes in TCB were observed in spring during the rearing period (April) and the lowest at the end of the rearing period (July).

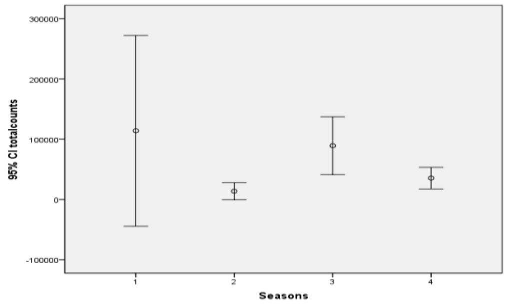


Figure 1: Changes in total count bacterial (CFU / g) of sediments in different seasons of fish cage culture (2017-2018)

Fig. 2 shows changes of the sediment TCB in 4 directions and periods in the fish rearing site (Nowshahr region). The results showed that the maximum and minimum TCB (900000 and 1000 CFU/g) were recorded in the middle and the beginning of the rearing period, respectively. The mean of TCB was 63052±13347 CFU/g. The mean of TCB did not show a significant difference between different breeding periods (p >0.05).

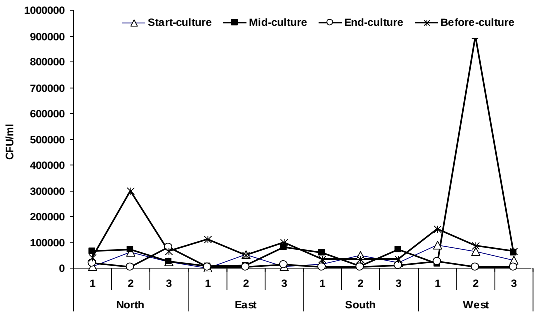


Figure 2: Changes Total count bacterial (CFU /g) sediment in 4 directions and period at the cage culture (2017-2018)

Fig. 3 shows the changes in the TCB at different sampling distances of sediment at the fish rearing site (Nowshahr region). The highest changes of TCB were observed at a distance of 200 m from the cage and the lowest in the shadow of the cage.

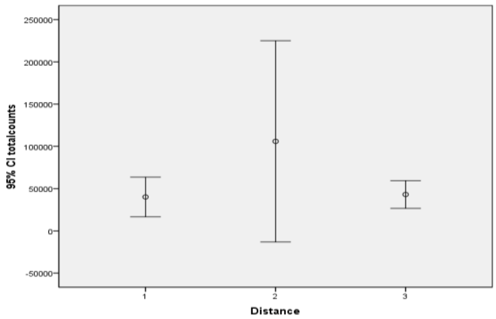


Figure 3: Changes in the total bacterial count (CFU /g) of sediments at different distances of fish cage culture (2017-2018)

While in the cage shadow station, the maximum average TCB was 83750 CFU/g before the rearing period and the minimum average was 12500 CFU /g at the end of the rearing period. At stations 100 and 200m, the maximum and minimum average of TCB were observed 24875 and 2500 CFU/g in the middle and the end of the rearing period, respectively. At the station 1000 m, the maximum and minimum average TCB were 66250 and 21250 CFU/g before and beginning the rearing periods, respectively (Fig. 4). One-way analysis of variance to compare the mean of TCB at different distance did not show significant differences (p>0.05).

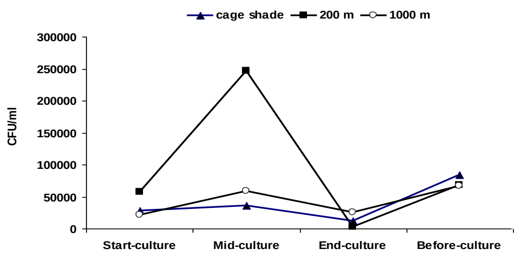


Figure 4: Changes in the average distance total bacteria (CFU / g) of sediment of fish cage culture (2017-2018)

The range of changes in the TCB from 1000 to 900000 CFU/g was shown in Table 1. The maximum TCB was observed in 200 in the middle-culture and the minimum total count bacteria was observed in cage shadow and the beginning of the rearing period.

Table 1: The range of changes in total bacteria count (CFU / g) sediment at different distances and periods of fish cage culture (2017-2018)

Station	Cage shade	200m	1000 m
Start-culture	(3000-8000)	(2500-16000)	(4300-21000)
Mid-culture	(500-20000)	(1000-40000)	(2000-40000)
End-culture	(2000-15000)	(2000-6000)	(2000-30000)
Before-culture	(4000-12000)	(3000-40000)	(15000-18000)

The presence of total coliform and fecal coliform in sediment samples was recorded as 12.5% and 2.08%, respectively. A maximum total coliform count of 3500 CFU/g sediment, west station of cage shade was observed at the beginning of rearing period and minimum total count coliform sediment of 30 CFU/g, south station of 200 m cage was observed before rearing period. Fecal coliform was recorded CFU/g 2000, east station of the cage shadow station at the beginning of the rearing period (Fig 5).

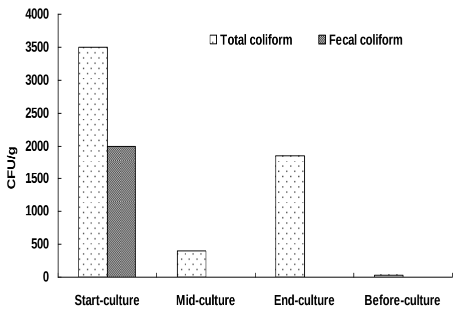


Figure 5: Percentage of sediments contaminated (g) with total coliforms and fecal coliforms at different sampling stations

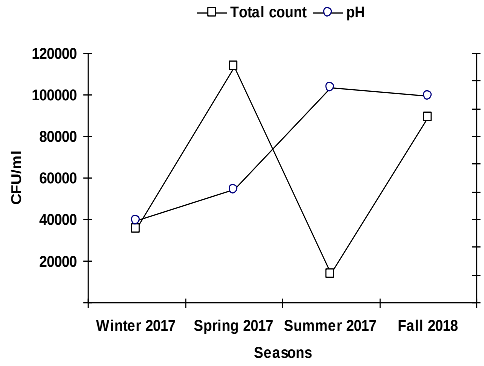
Clostridium perfringens (spore-forming anaerobes) sediment was not observed at any of the sampling stations.

Physicochemical factors

The results showed that the maximum and minimum pH of sediment were recorded (8.05 and 8.60) at the end and before the rearing period, respectively. The average pH of the sediment was 8.29±0.09. Based on one-way analysis of variance, the mean pH showed a significant difference between different periods ($p<0.05$) (Fig 6 - A).

The result was shown that the maximum and minimum of Total sediment organic matter sediment (TOM%) were recorded (14.18 and 2.45) at the end and before the rearing period, respectively. The mean TOM% of sediment was 4.45±1.97. Based on one-way analysis of variance, the mean TOM of sediment showed a significant difference between different periods ($p<0.05$) (Fig 6 - B).

The results showed that the maximum and minimum Eh of sediment were recorded (-51.3 and -83.4), respectively, at the beginning and the end of the rearing period. The mean Eh was -65.2±5.5. Based on one-way analysis of variance, the mean Eh deposition showed a significant difference between different periods ($p<0.05$) (Fig 6 - C).



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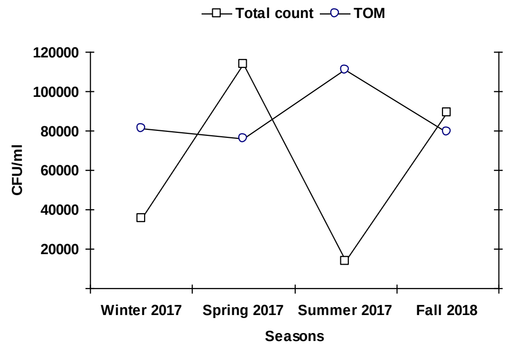

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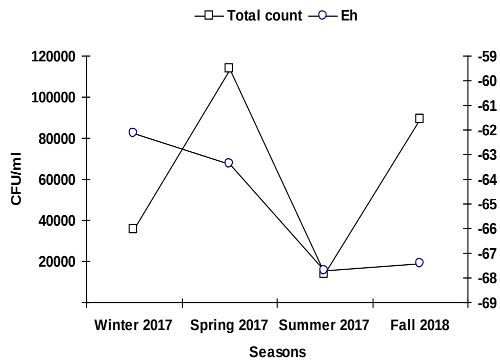

C

Figure 6: Changes in the mean factors of pH, TOM, Eh and bacterial total count (CFU /g) of sediments in fish cage culture (2017-2018)

Table 2 presents the results Principal Component Analysis (PCA) of TCB and some sediment environmental parameters. Based on the results of this analysis, the changes in KMO index were 0.50 and the Barttlet test showed a significant difference ($p<0.05$). In PCA, based on the scree curve and the specific value greater than one unit, the two main components with a total variance of 75.7% of the total variance were located. Component one (PC1) accounted for 50.1% of the total variance with pH and Eh parameters. Component two (PC2) participated with 25.6% of the total variance, TCB parameters and the percentage of TOM%.

Table 2: Multivariate statistical analysis of principal component test for total count and some environmental parameters of surface sediments around fish cage culture of Nowshahr region of the Caspian Sea (2017-2018)

	PC1 (50.1%)	PC2 (25.6%)
Special amount	2.00	1.02
Cumulative variance	50.1	75.7
Total bacteria (TCB)		-0.71
TOM%		0.72
pH	-0.998	
Eh	0.998	

Discussion

Over the years, marine fish farming cages using coastal waters have flourished in many countries and has had a growing trend. Salmon caged farming is a well-known source of organic waste and nutrient production that increases the levels of nutrient and organic material in the water column and subsequently increases the growth of bacteria in the sediments around the cage (Caruso, 2014).

Zmyslowska *et al*. (2001) noted that uneaten foods and feces added to water and sediment nutrients promote the rapid growth of bacteria. The above study also showed the highest TCB in the middle of the breeding period. In current study, Total organic matter percentage (TOM%) was determined to be below 6% for all the sediments analyzed. The maximum mean of TOM% was observed at end of breeding period but for TCB at middle of breeding period which coincident with aforementioned study. Also, the TCB populations are strongly correlated to the TOM content (in PCA test), suggesting involvement in degradation of organic matter in sediments of our study site.

Physicochemical properties (e.g., pH, redox) in marine sediments commonly change around the cage culture. The electronegative redox (Eh) values measured in the first 5 cm of the sediments in all near sites of fish cage, suggested that oxygen was depleted by microbial respiration as common reported for this layer in shallow marine sediments (Aranda et al., 2015). Gorlach-Lira et al. (2013) reported that pH and nutrients are factors influencing the bacterial population. In the current study, there is not correlated between pH and TCB. It is because that changes in mean sediment pH were very low (less than 0.1 units). Similar results have been obtained for the Eh variable.

Clostridium perfringens (spore-forming anaerobes) was not observed at any of the sampling stations.

The effects of fish cage culture mostly depend on various factors such as breeding conditions (breeding species, density, nutritional management) and physicochemical and biological characteristics of the area. Hydrological conditions of the water column such as depth, currents or water retention, temperature, and breeding place (coastal areas or open seas) (Karimian *et al*., 2017).

Finaly, low breeding capacity, short breeding period, permanent currents, breeding place with a suitable distance from the shore, were all important factors that led to relatively minor environmental effects of fish farming activities in the study area.

References

An, Y.J., Kampbell, D.H. and Beidenbach, G.P., 2002. Escherichia coli and total coliforms in water and sediments at lake marinas. *Environ. Pollution*, 120(**3**), 771-778.

AOAC., 2002 Official methods of analysis of the association of official analytical chemists (14th Ed). Washington, DC. Chalamaiah, M., Yu, W., and Wu, J., 2018. Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. *Food Chemistry*, 245, 205-222.

Aranda, C.P., Valenzuela, C., Matamala, Y., Godoy, F.A. and Aranda, N., 2015. Sulphur-cycling bacteria and ciliated protozoans in a Beggiatoaceae mat covering organically enriched sediments beneath a salmon farm in a southern Chilean fjord. *Marine Pollution Bulletin*, 100, 270–278.

Barayi, A., 2010. Environmental challenges of the Caspian Sea and the presentation of solutions. Ports and Maritime Organization. 14 P. http://www.pmo.ir/fa/article/177

Buchanana, J.b., 1984. sediment analysis. In Methods for the study of marine benthos. N. A. MC. In tyre (eds). Black well Secintific publication, oxford.

Caruso, G., 2014. Effects of aquaculture activities on microbial assemblages. *Oceanography*, 2: 107.

Gorlach-Lira, K., Pacheco, C., Carvalho, L.C.T., Melo Júnior, H.N. and Crispim, M.C., 2013. The influence of fish culture in floating net cages on microbial indicators f water quality. *Brazilian Journal of Biology*, 73(**3**), 457-463.

Holme, N.A. and McIntyre, D.A. (Editors), 1984. Methods for the study of marine benthos. IBP Handbook No. 16 (2nd ed). Oxford: Blackwell Scientific Publications. 387 P.

Hornick, K.M. and Buschmann, A.H., 2018. Insights into the diversity and metabolic function of bacterial communities in sediments from Chilean salmon aquaculture sites. *Annals of Microbiology*, 68(**2**), 63-77.

Karimian, A., Zakeri, M., Farabi, S.M., Haqi, M.V. and Kochenin, P., 2017. The effect of rainbow trout (*Oncorhynchus mykiss*) breeding in floating cages on the zooplankton population structure of Abbasabad region, in the south of the Caspian Sea. Journal f Aquaculture Development, Year 11, No. 3.

Mohamad, N., Mustafa, M., Amal, M.N.A., Saad, M.Z., Md Yasin, I.S. and Al-saari, N., 2019. Environmental factors associated with the presence of Vibrionaceae in tropical cage-cultured marine fishes. *Journal of Aquatic Animal Health*.

Moncada, C., Hassenrück, C., Gärdes, A. and Conaco, C., 2019. Microbial community composition of sediments influenced by intensive mariculture activity. *FEMS Microbiology Ecology*, 95(**2**), fiz006.

Rubio-Portillo, E., Villamor, A., Fernandez-Gonzalez, V., Antón, J. and Sanchez-Jerez, P., 2019. Exploring changes in bacterial communities to assess the influence of fish farming on marine sediments. *Aquaculture*, 506, 459-464.

Sarda, R., 1995. Life cycle. Demography and production of marenzeleria viridis in sait marsh of southern new England. J. Mer. Biol. U. k, 75, 725-738.

Shakouri, M., 2003. Impact of cage culture on sediment chemistry a case study in mjoifjordur. The United Nations University. Fisheries Training Programme. 44 P.

Siapatis, A., Giannoulaki, M., Valavanis, V., Palialexis, A., Schismenou, E., Machias, A. and Somarakis, S., 2008. Modelling potential habitat of the invasive ctenophore *Mnemiopsis leidyi* in Aegean Sea. *Hydrobiology*, 612: 281–295.

.

Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C. and Yam, V., 1994. Impact of marine fish farming on water quality and bottom sediment: A case study in the sub-tropical environment. *Marine Environmental Research*, 38(**2**), 115-145.

Yang, Z.F., Chen, L.Q., Zhou, Z.L., Chen, Y. and Wu, L.K., 2003. Effects of fishery development on water environment and its countermeasures in Taihu Lake. *Chinese Journal of Eco-Agriculture*, 11, 156-158.

Zmyslowska, I., Harnisz, M. and Lewandowska, D., 2001. Sanitary and bacteriological evaluation of water quality during cage culture of wels (*Silurus glanis* L.) in cooling water. *ARCHIWUM RYBACTWA POLSKIEGO*, 9(**2**), 191-200