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## Changing trends in the abundance and biomass of cyanobacteria in the southern part of the Caspian Sea in 2009 (The stretch between Tonekabon and Amirabad)

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### Abstract

Changes in abundance and biomass of cyanobacteria have been studied in the southern part of the Caspian Sea (the stretch between Tonekabon and Amirabad) in four seasons (spring, summer, autumn, winter) in which samples were taken from five depths of 5, 10, 20, 50 and 100 meters in the stations of Tonekabon, Noushahr, Babolsar and Amirabad. Samples were fixed in formalin (2-2.5%) before transferring to the lab for both qualitative and quantitative analyses. Ten species of cyanobacteria were identified with higher abundance and biomass in the euphotic layer compared to the aphotic layer. Temperature and nutrients have shown significant differences ( $p < 0.05$ ) in different seasons after normalizing the data through the T-test. On the basis of T test on normalized dates differences were meaningful between population of Cyanobacteria in the optical and no optical layers. Statistical analyses have shown significant and positive correlations between abundance and biomass with parameters like temperature, ammonium and phosphate while it was significantly negative for nitrates.

**Keywords:** Cyanobacteria, Abundance, Nutrients, Seasons, The Caspian Sea

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## **Introduction**

The Caspian Sea with an area of 386,400 km<sup>2</sup> and 7,250 km of shoreline considered as the biggest lake in the world has under gone many environmental threats and impacts like sea level fluctuations, overfishing, intrusion of aquatic organisms, industrial, agricultural and municipal pollution by many of its surrounding countries known as developing countries. The southern parts of the Caspian Sea in Iran with a watershed area of 17,700 km<sup>2</sup> constitutes 7% of the total Caspian Sea's watershed area and contributes 5% of total water inflow to this sea (Ministry of Agricultural Jihad with the help of CEP). Almost 71 species out of 449 species of phytoplankton and single-celled algae in the Caspian Sea are living in its southern part (Dehghan, 2005).

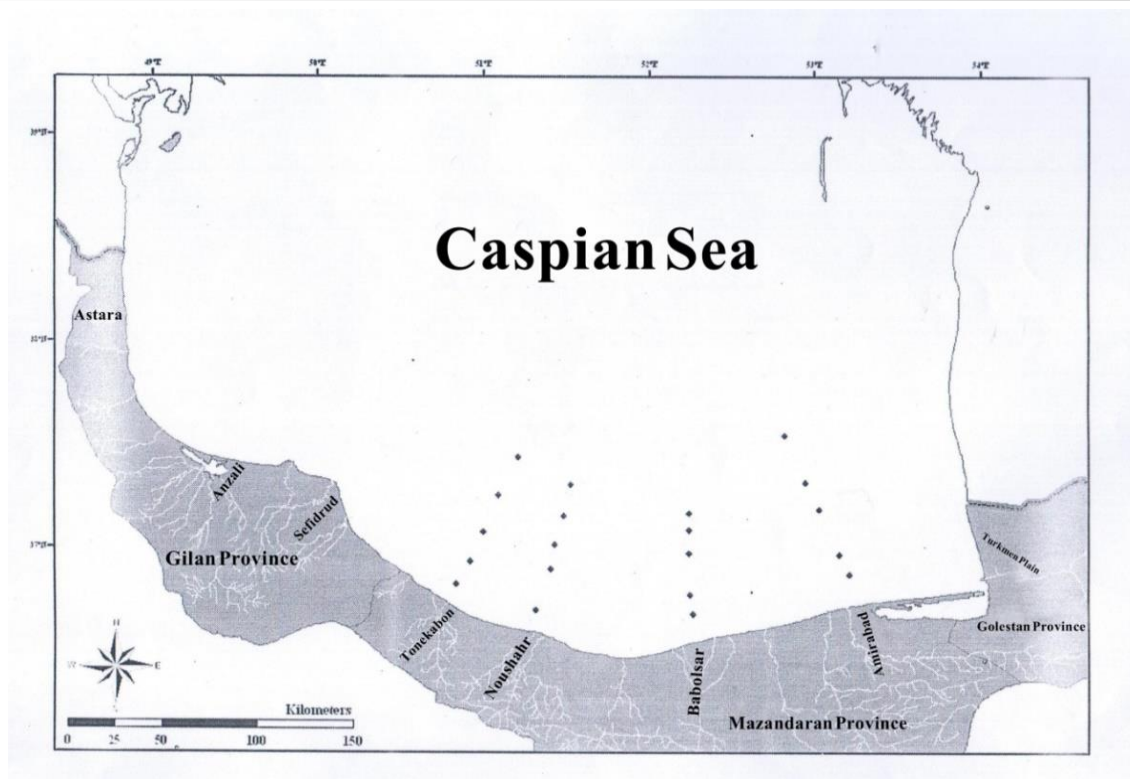
The growth of cyanobacteria depends on environmental factors which could exceed other groups under favorable conditions. Light and other factors like nitrates,

phosphates, silica and so on may restrict the photosynthesis process in oceans and seas (Karehi, 2004). In addition to nutrients, season is considered another factor in determining the abundance rate of cyanobacteria to other phytoplankton in the environment (Chaichi, 2005).

In this study, the changing trends in abundance and biomass of cyanobacteria have been analyzed by considering the ecological influences in the southern part of the Caspian Sea (the stretches between Tonekabon and Amirabad) during different seasons.

## **Materials and methods**

Four vertical transect lines were considered for the study of cyanobacteria in the south central part of the Caspian Sea. The area between Tonekabon and Amirabad and each transect was sampled in five different depths of 0, 10, 20, 50 and 100 meter during the summer and autumn of 2009 (Fig.1).



**Figure 1: The Caspian Sea mapped with five littoral countries and sampling stations in the southern Caspian Sea – Iranian coast.**

The 5, 10, 20, 50 and 100 m labels show the isobaths where the stations are located, and the numbers show the four sampled transects.

Samples were taken with a Rutner water sampler and 500 ml fixed in formalin (2%) in glass bottles before transferring to the lab for quantitative analysis (APHA, 2005). Water temperature (0.01 °C precision) was recorded using the Rutner reversing internal thermometer. Water transparency was measured by averaging the two reading numbers of Secchi Disk. The two euphotic and aphotic layers were also separated using Secchi readings.

To settle the plankton, samples were kept in darkness for 10 days prior to siphoning and centrifugation to the final volume of 20-25 ml. Each sample was studied two times qualitatively

(identification) and once quantitatively (abundance) by placing 0.1 ml of the sample on a 24×24 mm cover slip and observing by a Nikon optical microscope with three magnification values of 100X, 200X and 400X. Phytoplankton species were identified using identification keys like Eaton *et al.*, 2005; Kasimov, 2004; Wetzel and Likens, 2000; Carmelo, 1997 and Proshkin, 1951. Statistical analysis of ANOVA was done using SPSS software. After normalizing ( $\log_N$ ), all data and Tukey test were used when necessary.

#### *Sampling and nutrients measurement*

All samples were analyzed using American and Russian standard methods (APHA, 2005; Sapozhnikov *et al.*, 1988). Water temperature was recorded in situ using a

mercury thermometer installed inside the Rutner sampler (0.1 precision).

Nutrients (N, P and NH<sub>3</sub>) were analyzed using a Cecil spectrophotometer after the samples were taken as below (Sapozhnikov, 1988).

Sampled water was transferred to the lab under the specific condition in 1 Litter plastic bottles. Nitrite was measured by the method of Sapozhnikov (1988) in which the absorption rate was recorded for the chromatic nitrite ion at a wavelength of 543 nm. Nitrate was measured using cadmium reduction method (Sapozhnikov, 1988). Phosphate was measured at a wavelength of 885 nm (Sapozhnikov, 1988). Ammonium was measured in terms of µg/lit at a wavelength of 630 nm and to a precision of 0.01 µg/lit (phenate method).

#### Statistical analysis

#### Results

Ten cyanobacteria species were identified during this one year study in the presence of *Nodularia* sp., *Lingbya* sp., *Osillatoria* sp., *Spirulina* sp., *Anabaena* sp., *Microcystis*

All data collected were recorded in log sheets before transferring to the Excel software for further calculations and plotting of the graph. In this study, two groups of independent (stations, seasons, year, transects and depth) and dependent parameters (plankton density, physical parameters and nutrients) were transformed using either log<sub>N</sub>, log<sub>10</sub>, Z-score, square or cubic root and their normality was verified through diagrams before using ANOVA and T-test for comparison of environmental and biological parameters in different euphotic and aphotic layers of water. All the tests were conducted at 5% significant level.

Minimum recorded transparency was 9 m and therefore up to the depth of 30 m was considered euphotic and from 50 m downward was considered aphotic layers (euphotic layer is considered as almost 2.5-3 times the transparency depth).

*sp.* and *Merismopedia* sp. in all four seasons. Figure 2 shows the percentage of cyanobacteria in the southern part of the Caspian Sea.

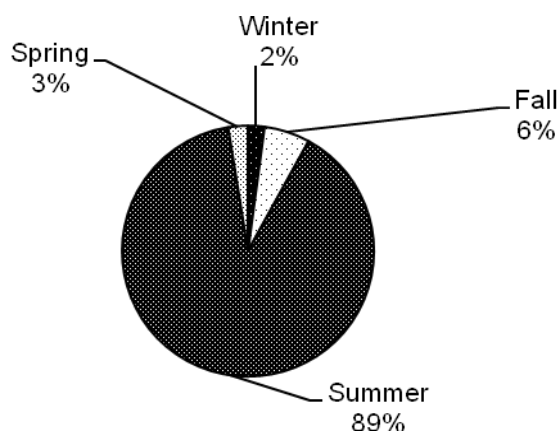


Figure 2: Percentage of cyanobacteria in the southern part of the Caspian Sea (area between Tonekabon and Amirabad) in 2009.

In summer, the biomass of cyanobacteria reached a maximum in both the euphotic and aphotic layers. In other seasons, the biomass shows highest at euphotic layer.

The lowest biomass in both layers was recorded in the spring and winter seasons (Fig. 3).

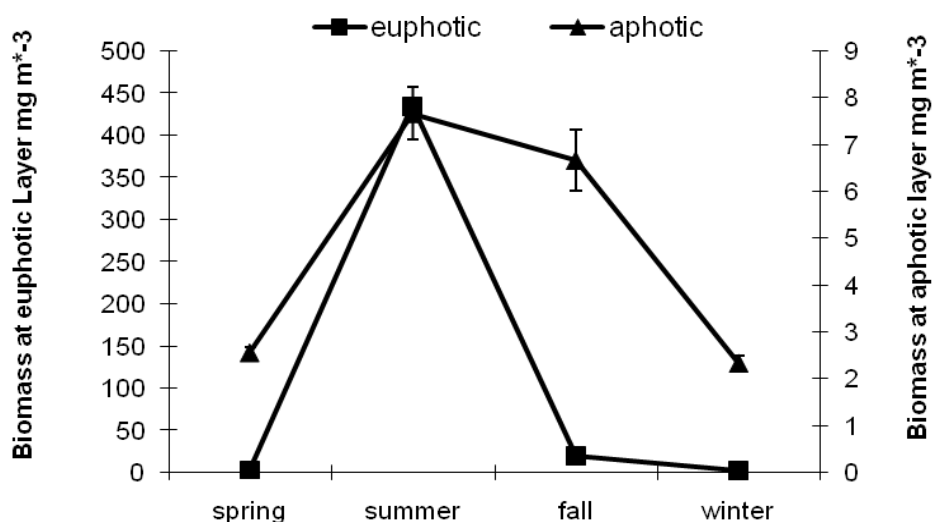


Figure 3: Biomass of cyanobacteria (area between Tonekabon and Amirabad) in 2009.

The highest cell densities were also recorded at the euphotic layer for each season with the highest rate in summer. The densities in the aphotic layer were lower

than the euphotic layer in all four seasons. The lowest cell density in both layers was observed in winter (Fig.4).

Table 1: Changes in abundance and biomass of cyanobacteria have been studied in the southern part of the Caspian Sea (the stretch between Tonekabon and Amirabad, , 2009).

Season	Spring		Summer		Fall		Winter	
	Cells density (10 <sup>6</sup> cells m <sup>-3</sup> )	Biomass (mg m <sup>-3</sup> )	Cells density (10 <sup>6</sup> cells m <sup>-3</sup> )	Biomass (mg m <sup>-3</sup> )	Cells density (10 <sup>6</sup> cells m <sup>-3</sup> )	Biomass (mg m <sup>-3</sup> )	Cells density (10 <sup>6</sup> cells m <sup>-3</sup> )	Biomass (mg m <sup>-3</sup> )
Tonekabon	6660	2.015	176.607	91.257	275.084	15.93	0.497	2.541
Noushahr	110.267	3.662	503.154	248.846	16920	15.93	7520	3.689
Babolsar	119.734	3.937	765.214	248.846	9526.667	4.897	3.727	1.539
Amirabad	107.534	3.456	8018.267	490.849	4612.667	25.89577	5.307	2.537

Maximum mean temperature of 26.18°C was recorded at the euphotic layer during summer and the minimum at 9.50°C was

observed at the aphotic layer in winter (Fig. 5).

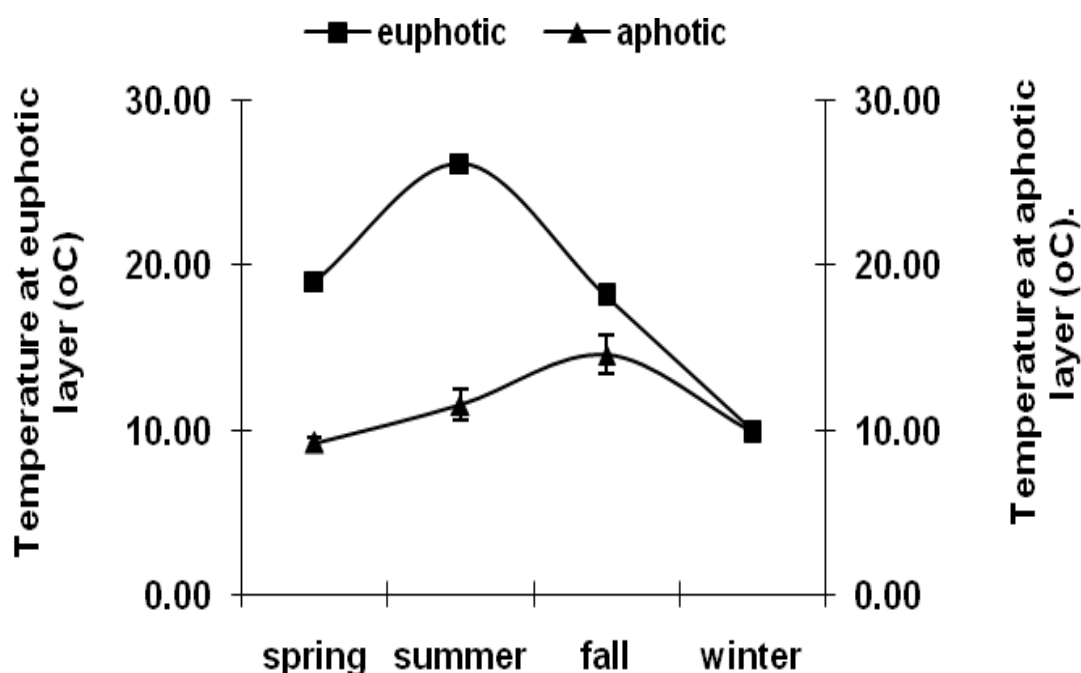


Figure 5: Water mean temperature (area between Tonekabon and Amirabad) in 2009.

The maximum mean concentration of ammonium in both euphotic and aphotic layers was observed during autumn (Fig. 6).

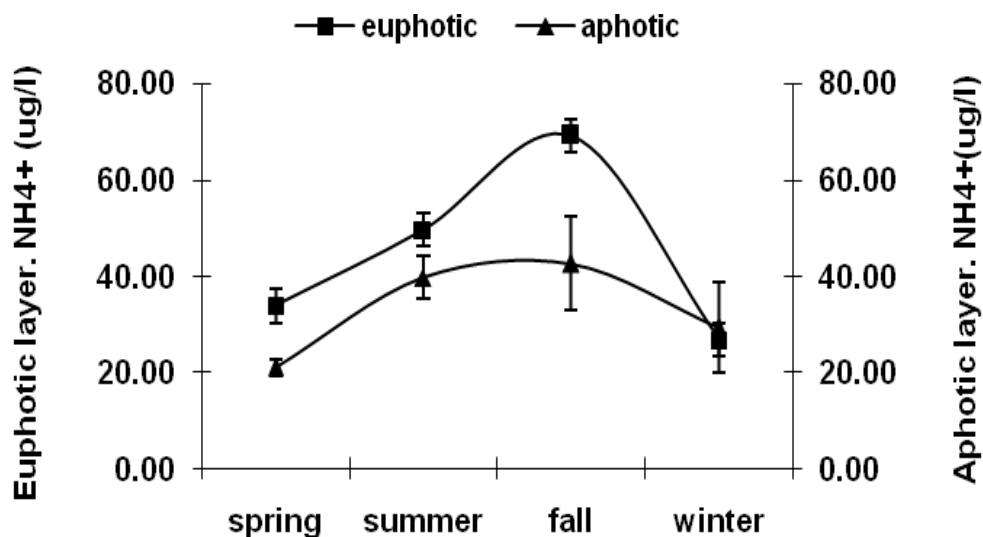


Figure 6: Ammonium mean concentration (area between Tonekabon and Amirabad) in 2009.

The maximum mean concentration of Nitrite in both euphotic and aphotic layers was observed during autumn (Fig.7)

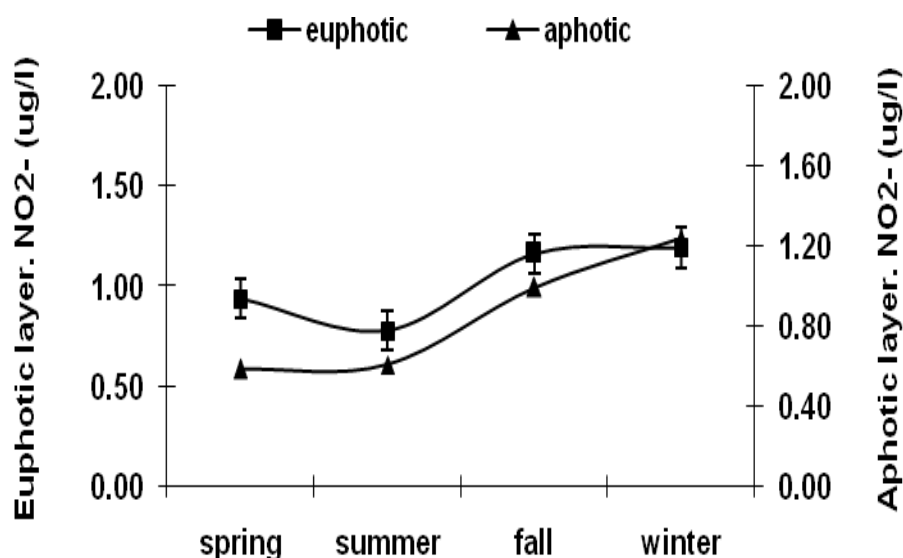


Figure 7: Nitrite mean concentration (area between Tonekabon and Amirabad) in 2009.

The maximum mean concentration of Nitrate in both euphotic and aphotic layers was observed during autumn (Fig.8).

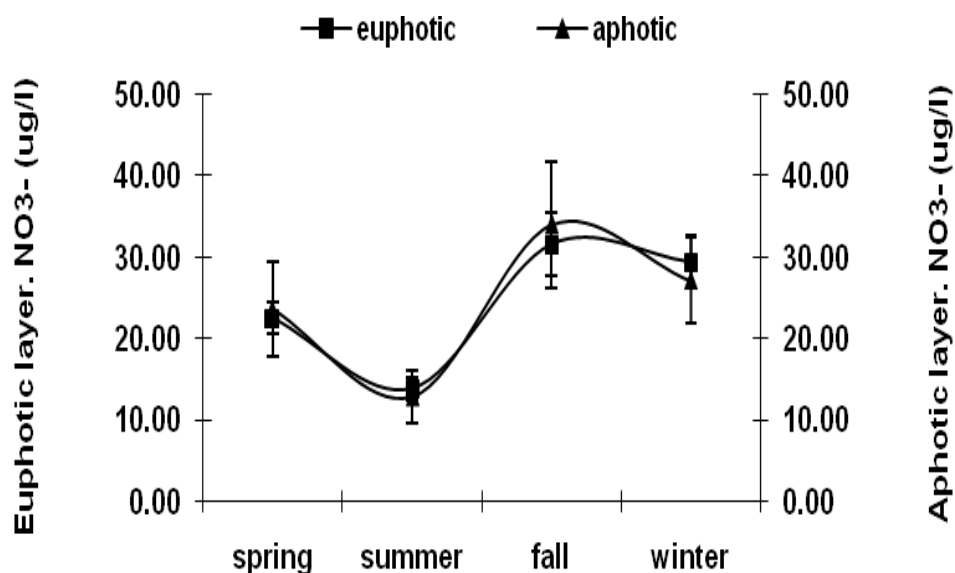


Figure 8: Nitrate mean concentration (area between Tonekabon and Amirabad) in 2009.

The maximum mean concentration of Phosphate in both euphotic and aphotic

layers was observed during autumn (Fig. 9).

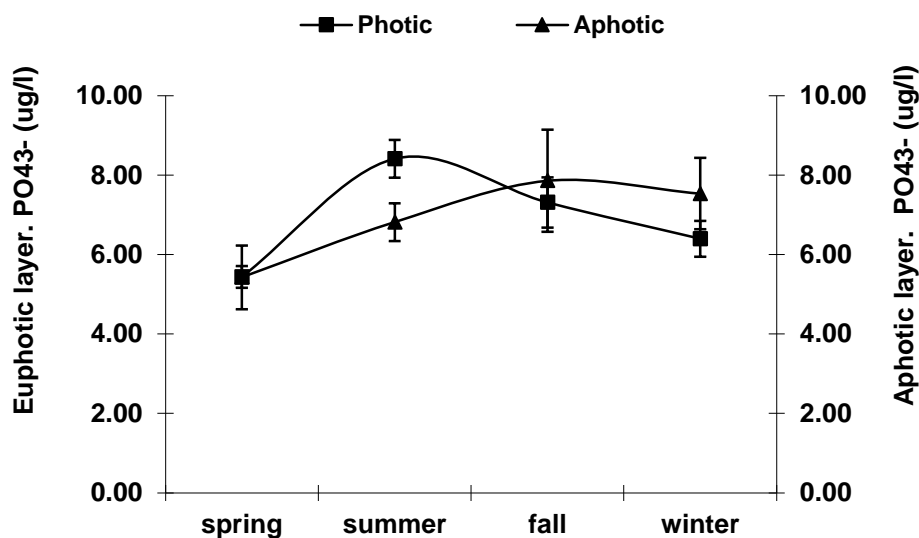


Figure 9: Phosphate mean concentration (area between Tonekabon and Amirabad) in 2009.

### Discussion

Blue-green algae or cyanobacteria are considered the smallest type of phytoplankton which may make their identification a difficult task. Phytoplanktons which can also be found in seawater and known as photosynthesizing organisms are in fact single-celled plants with their most primary forms in seas and oceans as many other organisms' life are depending on them (Manshouri, 2009). These organisms with non-uniform distributions in water have vertical movements in the water column to find suitable living conditions like nutrients, temperature and so on. The population of blue-green algae increases in June and July as temperature rises in the air and water (Sze, 1986).

Maximum cell density and biomass were observed in the euphotic layer with significant differences between euphotic and aphotic layers ( $p < 0.05$ ). Previous studies in the southern part of the Caspian Sea have also shown the same results (Khosravi, 1999). The euphotic layer accommodated the highest number of phytoplankton which gradually decreased by moving toward the aphotic layer (Nasrollahzadeh, 2001). Cyanobacteria population increases as the light increases (Stewart, 2003). Hadju (2007) found that 40-50% of biomass in the first 20 meters of the euphotic layer is composed of cyanobacteria confirming our results. One way ANOVA test shows significant differences for population number of cyanobacteria between seasons ( $p < 0.05$ ).



Highest population density of cyanobacteria in summer could be due to increase in temperature and nutrients (nitrate, ammonium and phosphate) following the increase in use of fertilizers by farmers and their drainage returning to rivers and seas (Khosravi, 1999) as significant differences ( $p < 0.05$ ) were observed for these factors between seasons. Minimum population density in winter which was observed simultaneously with decreases in temperature, increases in nitrate (in both euphotic and aphotic layers) and decrease in ammonium (in both euphotic and aphotic layers) shows that population density has a positive correlation with temperature and ammonium and a negative correlation with nitrate concentrations as it was demonstrated in previous studies (Ahmadi Kamarposhti, 2006). Statistical analysis shows significant differences ( $p < 0.05$ ) in density and biomass between seasons and layers (euphotic and aphotic), though it was not significant ( $p > 0.05$ ) between transects.

In summer, the highest temperature, ammonium, nitrite and phosphate was observed in Amirabad transect while Noushahr transect showed the highest amounts of nitrate with the highest density and biomass of cyanobacteria in this season. In autumn, the highest temperature, nitrate and nitrite was recorded in Tonekabon transect while Amirabad transect had the highest concentrations of ammonium and phosphate. In winter, the highest and lowest population density and biomass in the euphotic layer was recorded in Noushahr and Babolsar transects respectively, whereas at the aphotic layer, it was the highest in Amirabad transect.

Statistical analyses show strong positive correlations in population density and biomass with parameters like temperature, ammonium and phosphate which corresponds with the previous studies showing that not only nutrients but also other factors like temperature are considered important in controlling the growth of cyanobacteria (Khosravi Rineh, 2011).

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