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## Features of phytoplankton community in the southern Caspian Sea, a decade after the invasion of *Mnemiopsis leidyi*

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## Abstract

Phytoplankton study has become more significant in the Caspian Sea due to the occurrence of ecological events such as algal blooms and the introduction of the invader species(Ctenophore, *Mnemiopsis leidyi*). A seasonal study was conducted to investigate the features of phytoplankton community one decade after this invasion in the Iranian coast of Caspian Sea during 2009-2010. According to the results, 195 species in eight phyla of phytoplanktons were identified. In spring, Bacillariophyta and Pyrrophyta were the dominant phyla with 40 and 29% of total abundance, respectively. In summer and winter the dominant phyla were made by Cyanophyta (92%) and Bacillariophyta (94%), respectively. The Bacillariophyta (57%) and Cyanophyta (28%) were the first and second dominant phyla in autumn . It seems that the ctenophore invasion into the Caspian Sea (due to the changes in nutrient levels and decline of phytoplankton grazers) and human's destructive activities play an effective role on phytoplankton community during the period. These changes were mainly accompanied with appearance of new and harmful species (with the ability of severe bloom making) and consequently, displacement of native species in this semi-enclosed ecosystem.

Keywords: Phytoplankton, Diversity, Abundance, Biomass, Caspian Sea

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## Introduction

Historically, phytoplankton data were not evaluated along the Iranian coasts of the Caspian Sea (CS) until 1991 and it started to be consistently gathered and published since 1994 (Pourgholam and Katunin, 1994). The results of the collected data indicated that Bacillariophyta, Pyrrophyta, Cyanophyta, Chlorophyta and Euglenophyta were the main phyla in the mentioned zone from 1994 to 2008 (Fazli et al., 2010; Golaqaei et al., 2012). Moreover, a few species were also observed in the Xantophyta, Cryptophyta and Chrysophyta phyla. Totally, more than 300 species were identified during the periods of study (1994 to 2008) and Bacillariophyta, Pyrrophyta were dominant phyla in terms of abundance and biomass. In general, Bacillariophyta species are reported to form the most abundant and widespread group throughout the Caspian Sea. After Bacillariophyta, chlorophytes and cyanophytes are the abundant groups in the north (since they are chiefly fresh and brackish water forms), while, dinoflagellates (mainly Prorocentrum cordatum) are dominant in the Middle (including eastern) and southern Caspian (Kosarev and Yablonskaya, 1994).

One of the unique characteristics of the Caspian Sea biota is the diversity of its origins. The most abundant species are endemic (about 75% of the total number) and a few from Mediterranean (6%) and the Arctic (3%), and also the immigrants from freshwater . About 15 phytoplankton species have been introduced to the Caspian Sea, as the result of the artificial connecting of the Caspian Sea with the Azov-Black Sea basin by the Volga-

Don Canal since 1952 (Kosarev and Yablonskya, 1994).

Human uses and exploitation of the Caspian Sea resources exposed this semienclosed ecosystem to a high risk range of pollutions such as oil, pesticides, detergents, heavy metals and biological pollutant (Zonn et al., 2010). The invasion of Mnemiopsis leidyi, a ctenophore has been accounted as the main biological pollutant since 2000 in the Caspian Sea. Pollutions reflected on phytoplankton population by causing the decline of some native species and increasing the new comer and harmful species (Makhlough et al., 2012). It is noticeable that algal blooms have recently occurred three times in the southern Caspian Sea. The first bloom formed by a species of Pyrrophyta, however the two others were made by a toxic species of Cyanophyta (CEP, 2006; Makhlough et al., 2011). Apart from its toxicity, it also affects the stability of environment and decreases its resilience against the next ecological events (Sigee, 2004).

Some of the introduced species have negative ecological effects on the ecosystem. Therefore identification and quantification studies of phytoplankton community not only have an important role in the collection of historical data, but are also necessary to understand the polluted and under stress environment of the Caspian Sea. This paper presents the features of diversity, biomass and abundance of phytoplankton during four seasons of 2009-2010 under the light of changing ecosystem over the last fourteen years.

#### Materials and methods

Four seasonal cruises were carried out on board the R/V Guilan during 2009-2010. Eight transects were selected in the Iranian coastal zone of the southern Caspian Sea. Along each transect, five stations were located at water depths of 5,10, 20, 50 and 100 m (Fig. 1). Samplings were carried out through the water column at five water depths: 0(surface), 10, 20, 50 and 100 m depth layers.



Figure 1: Map of the Caspian Sea showing the sampling stations in the southern Caspian Sea

The samples for phytoplankton analysis were collected in 0.5 L bottles and preserved by adding buffered formaldehyde to yield a final concentration of 2%. The samples were let to settle for at least 10 days following which they were concentrated to about 30 ml by sedimentation and centrifugation (APHA, 2005). A subsample of 0.1 ml was analysed under a light microscope (Nikon, AFX-DX, Japan) (coverslip  $22 \times 22$ mm and with magnifications of 100, 200, 400×) (Vollenweider, 1974; Sournia, 1978). Phytoplankton taxonomic identification was

carried out following Proshkina-Lavrenko and Makarova (1968); Prescott (1970); Tiffany and Britton (1971); Habit and Pankow (1976). The geometric volume of each cell was estimated based on the procedure as suggested in APHA, (2005), Vollenweider (1974) and Newell and Newell (1977). The volume values were converted to milligram of biomass. Phytoplankton diversity and evenness were calculated using the Shannon-Weaver diversity and evenness indices (Washington, 1984). The number of species phytoplankton was considered as species richness (Krebs, 1999). order to find dominant species of In

phytoplankton, the ISI (Importance Species Index) was calculated following the equation proposed by Rushforth and Brook (1991):  $ISI-(f_i) \times (D_i)$ 

Where:  $f_i$ = Percent frequency of species I,  $D_i$ = Average relative density of species I.

Generally, photic layer is estimated at three times the transparency depth in the sea (Vilicic et al., 1995; Wetzel and Likens, 2000). During the period of study, maximum photic layer depth (based on maximum transparency) was almost 27 m. So the samples from 50 and 100 m layers were not from the photic layer.

Analysis of variance (ANOVA) was used to determine the statistical significant differences in phytoplankton abundance and biomass between the depths and seasons. T-test was performed to determine the significant difference between layers (photic and below photic). Prior to the analysis, phytoplankton data was ln (x+1) or rank it transformed (Krebs, 1999) to normalize the data set. The normality of data sets was confirmed by Shapiro-Wilk test and Q-Q plot illustrations (Siapatis et al., 2008). The one-way ANOVA was followed by a Tukey and homogeneous tests if a significant difference on a variable tested was observed. Statistical analyses were carried out at a significant level of  $\alpha = 0.05$ (Bluman, 1998; Nasiri, 2010).

## Results

During the sampling period, 195 phytoplankton species were identified from eight major divisions, which were Bacillariophyta (81 species), Pyrrophyta (33), Cyanophyta (28), Chlorophyta (38), Euglenophyta (11), Chrysophyta (2), Haptophyta (1), and Xantophyta (1).

The minimum mean value of phytoplankton abundance recorded in Astara (the westernmost station) at surface, 10, and 20m of layers which were 75, 50 and  $55 \times 10^6$ cells / m<sup>3</sup>, respectively (Table 1), while maximum values in the same layers were in Nowshahr: 370, 176 and  $192 \times 10^6$  cells/m<sup>3</sup>, respectively. The maximum mean value at layers 50 and 100 m observed in Tonekabon  $(133 \times 10^6 \text{ cells/m}^3)$  and Anzali  $(527 \times 10^6 \text{ cells/m}^3)$ cells / m<sup>3</sup>) in that order. The mean abundance showed two peaks in summer and winter (156 and 389 million cells / m<sup>3</sup>) and two falls in spring and fall (49 and  $67 \times 10^6$  cells / m3) at surface and 10 m layers. The mean value of abundance was intensively higher in winter  $(179-229 \times 10^6 \text{ cells / m}^3)$  compared to the other seasons  $(15-66 \times 10^6 \text{ cells / } \text{m}^3)$  at 20 and 50 m of depth layers. At the 100 meters sampling layer, the abundance of phytoplankton was recorded between 2 and  $260 \times 10^6$  cells/m<sup>3</sup> (Table 2).

difference in The phytoplankton biomass was high between surface (968 mg /  $m^3$ ), 10m (1215 mg /  $m^3$ ) of sampling layers of Anzali and mean of other transects (521 and 458 mg /  $m^3$ ) were at same layers (Table 1). Generally, the biomass in winter was 4-5 folds to other seasons at surface and 10 m of sampling layers (Table 2). However, no significant different of abundance occurred between the same layers of different transects and different depths (ANOVA, p > .05). The same ANOVA result obtained for the similar layers of inshore and offshore stations at each transect (p > .05). However, a significant difference in abundance was observed between layers in the water column (ANOVA, p < .05). Based on the ANOVA, water column divided to photic layer (surface, 10 and 20 m) and the layer below photic layer (50 and 100 m). In addition, a significant difference in temperature, pH and salinity were observed between layers in water column (ANOVA, p < .05).

| Transects | a 11 -          | Abun | dance | Biom | SE           94           61           165           274           151           219           530           229           59           111           82           68           161           73           26           188           158           143           195           76           252           340           313           183           84           111           130           120           182 |  |
|-----------|-----------------|------|-------|------|---|--|
| Transects | Sampling Layers | Mean | SE    | Mean | SE  |  |
|           | Surface         | 75   | 15    | 407  | 94  |  |
|           | 10              | 50   | 8     | 353  | 61  |  |
| Astara    | 20              | 55   | 11    | 475  | 165   |  |
|           | 50              | 49   | 25    | 591  | 274   |  |
|           | 100             | 16   | 9     | 264  | 151   |  |
|           | Surface         | 198  | 51    | 969  | 219   |  |
|           | 10              | 121  | 42    | 1215 | 530   |  |
| Anzali    | 20              | 83   | 22    | 473  | 229   |  |
|           | 50              | 39   | 26    | 143  | 59  |  |
|           | 100             | 527  | 481   | 208  | 111   |  |
|           | Surface         | 134  | 33    | 451  | 82  |  |
|           | 10              | 65   | 10    | 292  | 68  |  |
| Sefidrud  | 20              | 70   | 19    | 510  | 161   |  |
|           | 50              | 30   | 7     | 221  | 73  |  |
|           | 100             | 9    | 2     | 71   | 26  |  |
|           | Surface         | 161  | 58    | 634  | 188   |  |
|           | 10              | 162  | 64    | 478  | 158   |  |
| Tonekabon | 20              | 76   | 29    | 369  | 143   |  |
|           | 50              | 133  | 80    | 448  | 195   |  |
|           | 100             | 35   | 29    | 150  | 76  |  |
|           | Surface         | 371  | 129   | 904  | 252   |  |
|           | 10              | 176  | 55    | 864  | 340   |  |
| Nowshahr  | 20              | 192  | 129   | 742  | 313   |  |
|           | 50              | 43   | 22    | 347  | 183   |  |
|           | 100             | 21   | 17    | 128  | 84  |  |
|           | Surface         | 198  | 63    | 428  | 111   |  |
|           | 10              | 143  | 45    | 406  | 130   |  |
| Babolsar  | 20              | 100  | 36    | 317  | 120   |  |
|           | 50              | 41   | 21    | 344  | 182   |  |
|           | 100             | 24   | 14    | 316  | 289   |  |
|           | Surface         | 224  | 58    | 451  | 120   |  |
| Amirabad  | 10              | 82   | 24    | 367  | 135   |  |

Table 1: Phytoplankton abundance (×  $10^6$  cells/m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) at layers of different transects in the southern Caspian Sea in 2009-2010. (For transect location see Figure 1).

|              | Continue Table 1 |     |    |     |     |
|--------------|------------------|-----|----|-----|-----|
|              | 20               | 102 | 30 | 452 | 155 |
|              | 50               | 56  | 26 | 429 | 201 |
|              | 100              | 21  | 17 | 191 | 122 |
|              | Surface          | 133 | 42 | 376 | 104 |
| Bandar       | 10               | 175 | 61 | 450 | 128 |
| Turkman      | 20               | 107 | 29 | 581 | 182 |
| i ui Killali | 50               | 46  | 27 | 222 | 122 |
|              | 100              | 15  | 9  | 121 | 79  |

Table 2: Seasonal phytoplankton abundance (million cells/m<sup>3</sup>) and biomass (mg / m<sup>3</sup>) at different layers in the southern Caspian Sea in 2009-2010.

|                                    | a               | Abun | dance | Biom | ass |
|------------------------------------|-----------------|------|-------|------|-----|
| Season Spring Summer Autumn Winter | Sampling Layers | Mean | SE    | Mean | SE  |
|                                    | Surface         | 56   | 24    | 308  | 95  |
|                                    | 10              | 49   | 26    | 367  | 90  |
| Spring                             | 20              | 41   | 13    | 314  | 40  |
|                                    | 50              | 19   | 4     | 303  | 58  |
|                                    | 100             | 260  | 244   | 371  | 128 |
|                                    | Surface         | 239  | 41    | 296  | 52  |
|                                    | 10              | 157  | 34    | 143  | 22  |
| Summer                             | 20              | 66   | 15    | 70   | 11  |
|                                    | 50              | 5    | 1     | 15   | 3   |
|                                    | 100             | 2    | 1     | 7    | 4   |
|                                    | Surface         | 62   | 12    | 409  | 62  |
|                                    | 10              | 68   | 20    | 318  | 53  |
| Autumn                             | 20              | 55   | 11    | 259  | 40  |
|                                    | 50              | 15   | 4     | 97   | 28  |
|                                    | 100             | 3    | 1     | 17   | 6   |
|                                    | Surface         | 390  | 66    | 1295 | 142 |
|                                    | 10              | 206  | 33    | 1364 | 288 |
| Winter                             | 20              | 229  | 62    | 1312 | 169 |
|                                    | 50              | 179  | 34    | 957  | 145 |
|                                    | 100             | 65   | 13    | 314  | 61  |

Table 3 shows two maxima (winter and summer) and two minima (spring and autumn) of abundance are observed in the photic layer of different depths. Biomass was maximal at 10 m depth station in all seasons and decreased at the stations with higher water depth (50 and 100 m depths), except in winter when inshore and offshore biomass was almost the same. In summer, phytoplankton biomass of the photic zone was 15 times higher than below the photic layers, while the ratio for abundance

obtained was 39. In autumn and winter, the mentioned ratio of abundance and biomass were only 1-5 units; but in the spring, the abundance below the photic layer was higher than photic layer because of high abundance of Haptophyta (Table 4). Meanwhile the coefficient variances of abundance (1.64) and biomass (1.47) were almost similar in the photic layer.

Table 3: Seasonal abundance (million cells/m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) of phytoplankton at photic layer four stations with different water depths in the southern Caspian Sea in 2009-2010. A=Abundance, B=Biomass

|                |           | Season |     |      |     |      |     |      |     |  |  |
|----------------|-----------|--------|-----|------|-----|------|-----|------|-----|--|--|
| Station        |           | Spr    | ing | Sum  | mer | Autu | ımn | Win  | ter |  |  |
| water<br>depth | Parameter | Mean   | SE  | Mean | SE  | Mean | SE  | Mean | SE  |  |  |
| ~              | А         | 99     | 41  | 346  | 115 | 60   | 13  | 432  | 203 |  |  |
| 3              | В         | 254    | 65  | 224  | 48  | 553  | 188 | 1362 | 430 |  |  |
| 10             | А         | 84     | 57  | 168  | 40  | 77   | 37  | 323  | 119 |  |  |
| 10             | В         | 430    | 227 | 320  | 115 | 497  | 112 | 1678 | 582 |  |  |
| 20             | А         | 30     | 13  | 135  | 43  | 74   | 20  | 308  | 69  |  |  |
| 20             | В         | 302    | 81  | 132  | 37  | 328  | 54  | 1187 | 151 |  |  |
| 50             | А         | 18     | 3   | 152  | 40  | 58   | 9   | 281  | 50  |  |  |
| 30             | В         | 272    | 80  | 159  | 31  | 300  | 52  | 1175 | 129 |  |  |
| 100            | А         | 61     | 33  | 159  | 44  | 43   | 7   | 206  | 32  |  |  |
| 100            | В         | 370    | 73  | 174  | 32  | 215  | 32  | 1362 | 154 |  |  |

Annually, the highest percent values of abundance and biomass belonged to Bacillariophyta. Cyanophyta and Pyrrophyta were the second phyla in terms of abundance and biomass respectively. Haptophyta contained 5 and 0.1 percent of total abundance and biomass, respectively. Percentage of abundance and biomass of Euglenophyta, Chrysophyta and Xantophyta were low (Fig. 2).



Figure 2: Percent abundance and biomass of different phytoplankton phyla in the southern Caspian Sea in 2009-2010. (Bac.=Bacillariophyta, Pyr.=Pyrrophyta, Cya.=Cyanophyta, Chl.=Chlorophyta, Eug.=Euglenophyta, Hap.=Haptophyta, Chr.=Chrysophyta,Xan.=Xantophyta)

In the canonical discriminant function analysis (CDFA), the four seasons, namely spring, summer, autumn and winter, are clearly identified. During the sampling period, the first canonical discriminant function (CDF1) accounts for the 80.7% of the between-season variance. The second function canonical discriminant (CDF2) accounts for the 13.3% of the between-season variance. The third canonical discriminant function (CDF3) accounts for the remaining 6.0% of total between seasons variance. The significant canonical correlation between seasons and the first canonical discriminant function (r = 0.87) and second and third CDFs were 0.60 and 0.49, respectively. From the canonical discriminant function scatter plot

and territorial map (Fig. 3), the four seasons form four nearly non-overlapping groups and also the territorial map clearly showed these four groups. The first canonical discriminant function (CDF1, vertical line) separates winter from the other three seasons while the second canonical discriminant function (CDF2, horizontal line) separates spring and other seasons. Result also showed that, among the canonical discriminant coefficient Bacillariophyta and Cyanophyta are the most significant variables in the discriminant function, which means they have the principal role in the classification of the three functions or four groups (seasons).



## Figure 3: Scatter plot and territorial map (shown with lines) of canonical discriminate function analysis based on abundance of three major phytoplankton phyla at different seasons in the photic layer in the southern Caspian Sea.

The seasonal values of abundance and biomass of phytoplankton phyla in the photic and below photic layers showed that Cyanophyta (in summer) and Bacillariophyta (in autumn and winter) were the predominant phyla. Bacillariophyta and Pyrrophyta, accompanied by Cyanophyta, formed most of the phytoplankton population in spring. Pyrrophyta was the dominant phylum in seasonal phytoplankton biomass except in winter, when they were replaced by Bacillariophyta (Table 4).

|          |       | Ba      | c.     | Ру     | r.    | Су      | a.     | Cl      | ıl.   | Eu   | g.   | Ha     | ap.    | Tot  | al  |
|----------|-------|---------|--------|--------|-------|---------|--------|---------|-------|------|------|--------|--------|------|-----|
| Season   | Layer | Mean    | SE     | Mean   | SE    | Mean    | SE     | Mean    | SE    | Mean | SE   | Mean   | SE     | Mean | SE  |
|          |       |         |        |        |       |         | A      | bundanc | e     |      |      |        |        |      |     |
| Service  | р     | 196.53  | 91.39  | 142.75 | 16.93 | 47.23   | 5.88   | 7.66    | 3.14  | 0.36 | 0.10 | 102.64 | 81.79  | 491  | 131 |
| Spring   | bp    | 22.42   | 6.45   | 123.13 | 20.88 | 30.79   | 9.16   | 3.21    | 1.11  | 0.58 | 0.28 | 812.58 | 812.50 | 990  | 81  |
| <b>C</b> | р     | 47.05   | 10.10  | 65.21  | 8.30  | 1557.76 | 211.06 | 3.73    | 1.02  | 0.81 | 0.33 | 9.95   | 6.75   | 1681 | 810 |
| Summer   | bp    | 441.17  | 73.69  | 81.50  | 14.96 | 68.33   | 25.10  | 14.25   | 7.14  | 0.00 | 0.00 | 0.33   | 0.33   | 40   | 30  |
| A        | р     | 441.17  | 73.69  | 81.50  | 14.96 | 68.33   | 25.10  | 14.25   | 7.14  | 0.00 | 0.00 | 0.33   | 0.33   | 620  | 211 |
| Autumn   | bp    | 57.43   | 18.76  | 13.00  | 3.96  | 42.09   | 13.44  | 2.61    | 1.47  | 0.00 | 0.00 | 0.04   | 0.04   | 120  | 34  |
| Winter   | р     | 2724.22 | 338.37 | 105.33 | 9.59  | 43.06   | 5.33   | 9.15    | 2.10  | 1.82 | 0.29 | 1.52   | 0.38   | 2881 | 9   |
| winter   | bp    | 1359.38 | 252.27 | 13.58  | 2.35  | 24.25   | 6.66   | 14.08   | 8.62  | 0.42 | 0.21 | 0.50   | 0.37   | 1410 | 250 |
|          |       |         |        |        |       |         | Bior   | nass    |       |      |      |        |        |      |     |
| с ·      | р     | 105.08  | 25.91  | 211.85 | 31.13 | 10.02   | 7.33   | 1.38    | 0.74  | 0.26 | 0.08 | 0.82   | 0.65   | 329  | 51  |
| Spring   | bp    | 20.93   | 8.66   | 296.55 | 55.09 | 0.51    | 0.13   | 0.81    | 0.78  | 0.45 | 0.35 | 6.50   | 6.50   | 326  | 33  |
| <b>C</b> | р     | 15.38   | 4.96   | 148.83 | 23.29 | 21.24   | 2.85   | 2.23    | 1.01  | 0.30 | 0.13 | 0.08   | 0.05   | 188  | 56  |
| Summer   | bp    | 2.59    | 1.08   | 8.81   | 2.09  | 1.02    | 0.31   | 0.01    | 0.00  | 0.02 | 0.02 | 0.00   | 0.00   | 13   | 21  |
| A        | р     | 56.29   | 9.00   | 263.84 | 23.14 | 3.75    | 0.79   | 18.29   | 13.33 | 0.36 | 0.27 | < 0.01 |        | 342  | 25  |
| Autumn   | bp    | 23.18   | 11.14  | 48.50  | 16.35 | 0.80    | 0.27   | 0.19    | 0.11  | 0.00 | 0.00 | 0.00   | 0.00   | 73   | 119 |
| Winter   | р     | 964.25  | 109.88 | 355.24 | 30.11 | 2.97    | 1.80   | 0.98    | 0.63  | 0.73 | 0.13 | 0.01   | 0.00   | 1323 | 3   |
| winter   | bp    | 697.45  | 111.76 | 44.45  | 10.32 | 0.33    | 0.08   | 0.09    | 0.05  | 0.26 | 0.14 | < 0.01 |        | 743  | 116 |

Table 4: Mean±SE of abundance (×100000cells/m<sup>3</sup>) and biomass (mg/m<sup>3</sup>) of phytoplankton phyla at photic (p) and below photic (bp) layers in the southern Caspian Sea in 2009-2010. Abbreviation as Figure 2.

The Bacillariophyta showed the highest number of species among the phytoplankton phyla in all seasons; however its lowest number of species occurred in summer (42). The value for Cyanophyta was the same from spring to autumn (16); however it was slightly lower in winter (14). The highest number of species for Chlorophyta (25) and Pyrrophyta (28) was observed in spring and summer, respectively (Table 5). In total, the number of species in the photic layer was higher than below the photic layer in all phyla, and Bacillariophyta had the highest number of species both in the photic and below the photic layers.

The diversity species index of the photic layer (2.39) was higher than below the photic layer (2.04). The highest and lowest values of species diversity and evenness indices of spring (2.50, 0.51) were higher than summer (0.96, 0.21) (Table 6).

Table 5: Number of species in different phyla in seasons and layers in the southern Caspian Sea in 2009-2010. Abbreviation as Fig.2.

|              | Phylum |      |      |      |      |      |      |      |  |  |  |
|--------------|--------|------|------|------|------|------|------|------|--|--|--|
|              | Bac.   | Pyr. | Cya. | Chl. | Eug. | Chr. | Xan. | Нар. |  |  |  |
| Season       |        |      |      |      |      |      |      |      |  |  |  |
| Spring       | 53     | 27   | 16   | 25   | 7    | 1    | 1    | 1    |  |  |  |
| Summer       | 42     | 28   | 16   | 13   | 7    | -    | -    | 1    |  |  |  |
| Autumn       | 55     | 23   | 16   | 14   | 5    | -    | -    | 1    |  |  |  |
| Winter       | 53     | 26   | 14   | 16   | 6    | 1    | 1    | 1    |  |  |  |
|              |        |      | Lay  | /er  |      |      |      |      |  |  |  |
| Photic       | 80     | 32   | 28   | 36   | 11   | 2    | 1    | 1    |  |  |  |
| Below photic | 37     | 23   | 10   | 14   | 5    | -    | -    | 1    |  |  |  |
| Water column | 81     | 33   | 28   | 38   | 11   | 2    | 1    | 1    |  |  |  |

 Table 6: Shannon diversity species and evenness indices and species richness in the southern

 Caspian Sea in 2009-2010.

| Season       | Shannon index | Evenness | Species richness |
|--------------|---------------|----------|------------------|
| Spring       | 2.50          | 0.51     | 131              |
| Summer       | 0.96          | 0.21     | 107              |
| Autumn       | 2.39          | 0.50     | 115              |
| Winter       | 1.69          | 0.36     | 118              |
|              | Laye          | er       |                  |
| Photic       | 2.39          | 0.45     | 192              |
| Below photic | 2.04          | 0.45     | 90               |

Based on the ISI, the most dominant species belonged to the Bacillariophyta in each season except in summer (Table 7). Thereafter, species of Pyrrophyta and Cyanophyta were in the dominant species list. *Binuculeria lauteriburni* was the most frequent and abundant species of Chlorophyta. Based on the ISI, *Oscillatoria* sp., *Exuviaella cordata* and *Chrysochromulina* sp. were the dominant species in the photic layer in spring. *Oscillatoria* sp. was the predominant species in summer. In autumn, *Oscillatoria* sp. still was in the dominant species list but the ISI of *Thalassionema nitzschioides* was a little higher than it. Finally, in winter *Pseudonitzschia seriata* and *Cerataulina pelagica* were dominant and co-dominant species respectively (Table 7).

|   |      | Spring |      |      | Summer |      |        | Fall  |       |         | Winter  |        |  |
|---|------|--------|------|------|--------|------|--------|-------|-------|---------|---------|--------|--|
| Species   | ISI  | Mean   | SE   | ISI  | Mean   | SE   | ISI    | Mean  | SE    | ISI     | Mean    | SE     |  |
| Cerataulina pelagica(Cleve)Hendey                               | 0.76 | 3.13   | 0.87 | 0.01 | 0.21   | 0.15 | 0.00   | 0.19  | 0.15  | 19.74   | 646.65  | 169.19 |  |
| Chaetoceros convolutes Castracane                               | 0.21 | 0.66   | 0.17 | 0.09 | 1.18   | 0.29 | 1.80   | 10.88 | 2.39  | 0.23    | 8.80    | 1.76   |  |
| Chaetoceros mirabilisMakarova                                   | 0.00 | 0.00   | 0.00 | 0.00 | 0.00   | 0.00 | 0.51   | 4.34  | 1.75  | < 0.001 | 0.21    | 0.21   |  |
| Chaetoceros peruvianusBrightwell                                | 0.22 | 1.19   | 0.37 | 0.22 | 1.95   | 0.35 | 4.37   | 29.43 | 6.98  | 0.36    | 12.65   | 3.27   |  |
| Chaetoceros socialisProschkina-Lavrenko                         | 1.12 | 4.83   | 3.46 | 0.00 | 0.00   | 0.00 | 0.34   | 3.56  | 1.24  | 0.30    | 11.28   | 3.15   |  |
| Chaetoceros throndsenii Zingone in Marino et al.                | 0.57 | 2.13   | 0.81 | 0.05 | 1.11   | 0.27 | 0.06   | 0.28  | 0.10  | 0.34    | 11.34   | 4.00   |  |
| Cyclotella meneghinianaKützing                                  | 3.26 | 12.99  | 3.83 | 0.87 | 8.86   | 2.34 | 0.79   | 4.36  | 1.44  | 0.74    | 18.60   | 5.15   |  |
| Dactyliosolen fragilissima (Bergon) Hasle                       | 0.57 | 3.67   | 0.92 | 0.02 | 0.26   | 0.14 | 0.27   | 2.15  | 0.74  | 16.90   | 453.75  | 83.49  |  |
| Navicula sp.  | 0.80 | 2.87   | 1.13 | 0.06 | 1.38   | 0.82 | 0.06   | 0.45  | 0.16  | 0.09    | 2.38    | 0.96   |  |
| Nitzschia acicularis(Kützing). Smith                            | 4.27 | 18.47  | 8.99 | 0.52 | 5.78   | 0.86 | 8.46   | 51.67 | 15.95 | 2.54    | 80.46   | 16.72  |  |
| Nitzschia reversa Smith   | 1.52 | 8.04   | 5.19 | 0.04 | 0.53   | 0.17 | 0.48   | 2.65  | 0.70  | 0.06    | 1.80    | 0.53   |  |
| Nitzschia tenirustrisMereschkowsky                              | 1.78 | 11.09  | 9.31 | 0.02 | 0.27   | 0.09 | 0.30   | 1.83  | 0.41  | 0.02    | 0.56    | 0.16   |  |
| Pseudonitzschia seriata Cleve Peragallo in H.<br>& M. Peragallo | 0.65 | 3.00   | 0.84 | 0.00 | 0.06   | 0.05 | 8.54   | 62.49 | 24.18 | 45.81   | 1348.35 | 156.30 |  |
| <i>Pseudosolenia calcar-avis</i> (Schultze)<br>Sundström        | 0.77 | 4.87   | 1.17 | 0.00 | 0.13   | 0.06 | <0.001 | 0.01  | 0.01  | 0.54    | 14.11   | 1.42   |  |
| Sceletonema costatum (Greville) Cleve                           | 1.08 | 3.81   | 1.09 | 0.15 | 1.85   | 1.36 | 0.32   | 2.32  | 0.98  | 3.02    | 59.74   | 18.72  |  |

Table 7: Seasonal ISI and mean abundance (×100000 cells/m<sup>3</sup>) of dominant species in the photic layer of the southern Caspian Sea in 2009-2010.

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## **Continue Table 7:**

| Sceletonema subsalsum (Cleve-Euler) Bethge | 0.80   | 15.37 | 14.24 | 0.01    | 0.31   | 0.29  | 0.09  | 1.46   | 0.69  | 0.03   | 1.31  | 0.66 |
|--|--------|-------|-------|---------|--------|-------|-------|--------|-------|--------|-------|------|
| Stephanodiscus hantzschii Grunow in P.T.   | 1.26   | 62.80 | 62.97 | 0.00    | 0.00   | 0.00  | 0.17  | 4 27   | 2 00  | -0.001 | 0.21  | 0.15 |
| Cleve &Grunow                              | 1.20   | 02.89 | 02.87 | 0.00    | 0.00   | 0.00  | 0.17  | 4.37   | 3.88  | <0.001 | 0.21  | 0.15 |
| Thalassionema nitzschioides (Grunow)       | 0.16   | 4.02  | 1.46  | 1.50    | 16.65  | 6.01  | 07.04 | 150 50 | 27.04 | 1.62   | 41.50 | 5.24 |
| Grunow ex Hustedt                          | 2.16   | 4.83  | 1.46  | 1.53    | 16.65  | 6.01  | 27.06 | 170.73 | 37.06 | 1.63   | 41.58 | 5.34 |
| Exuviaella cordataOstenfeld                | 18.55  | 76.19 | 11.42 | 1.58    | 21.71  | 3.48  | 1.30  | 6.89   | 1.14  | 1.02   | 22.60 | 5.11 |
| Glenodinium behningii LindemannKisseleva   | 0.39   | 1.64  | 0.52  | 0.05    | 0.70   | 0.23  | 0.38  | 2.30   | 0.57  | 0.11   | 2.50  | 0.48 |
| Goniaulax polyedra Stein                   | 1.72   | 5.73  | 1.06  | 0.45    | 5.33   | 0.81  | 0.84  | 4.32   | 0.99  | 0.06   | 1.06  | 0.27 |
| Gymnodinium variabileHerdman               | 0.18   | 0.85  | 0.24  | 0.04    | 0.59   | 0.17  | 0.01  | 0.13   | 0.09  | 0.01   | 0.33  | 0.12 |
| Heteraulacus polyedricus(Pouchet) Drugg &  | 0.20   | 1 1 2 | 0.00  | 0.04    | 0.00   | 0.21  | 0.04  | 0.51   | 0.21  | 0.01   | 0.21  | 0.17 |
| Loeblich                                   | 0.29   | 1.13  | 0.90  | 0.04    | 0.66   | 0.31  | 0.04  | 0.51   | 0.31  | 0.01   | 0.31  | 0.17 |
| Heterocapsa triquetra(Ehrenberg) Stein     | 0.70   | 3.54  | 1.19  | 0.09    | 1.14   | 0.46  | 0.02  | 0.13   | 0.08  | 0.01   | 0.48  | 0.17 |
| Peridinium achromaticum (Levander,)        | 2.96   | 9.09  | 2.14  | 0.11    | 1.64   | 0.44  | 0.22  | 1.11   | 0.25  | 0.14   | 3.07  | 0.64 |
| Peridinium trochoideum (Stein) Lemmermann  | 0.17   | 0.73  | 0.27  | 0.13    | 1.81   | 0.46  | 0.02  | 0.14   | 0.06  | 0.05   | 1.32  | 0.55 |
| Prorocentrum proximum Makarova             | 4.34   | 20.60 | 4.76  | 1.73    | 20.69  | 4.32  | 9.04  | 42.86  | 4.43  | 2.68   | 63.39 | 5.86 |
| Prorocentrum scutellum Schroder            | 2.05   | 8.43  | 2.08  | 0.54    | 6.82   | 1.66  | 2.08  | 9.89   | 2.62  | 0.08   | 2.06  | 1.09 |
| Protoperidinium sp.                        | 1.23   | 5.19  | 1.71  | 0.08    | 0.83   | 0.28  | 0.07  | 0.43   | 0.22  | 0.00   | 0.44  | 0.22 |
| Protoperidinium sp.2.                      | 1.19   | 5.75  | 2.87  | 0.00    | 0.07   | 0.05  | 0.00  | 0.00   | 0.00  | 0.00   | 0.16  | 0.13 |
| Anabaena spiroides Klebahn                 | <0/001 | 0.09  | 0.06  | < 0.001 | 0.04   | 0.04  | 0.13  | 0.86   | 0.43  | 0.00   | 0.00  | 0.00 |
| Lyngbya limnetica Lemmermann               | 1.54   | 5.51  | 1.43  | 0.43    | 6.08   | 1.72  | 0.40  | 1.68   | 0.42  | 0.05   | 1.17  | 0.27 |
| <i>Lyngbya</i> sp.                         | 0.54   | 2.56  | 0.98  | 4.44    | 114.49 | 78.02 | 0.43  | 3.74   | 1.59  | 0.73   | 14.88 | 3.72 |

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| Continue Table 7:  |       |        |       |       |         |        |       |        |       |         |       |      |
|--|-------|--------|-------|-------|---------|--------|-------|--------|-------|---------|-------|------|
| <i>Nodularia spumigena</i> Mertens ex Bornet &<br>Flahault | 0.01  | 0.06   | 0.05  | 0.07  | 0.68    | 0.37   | 0.01  | 0.23   | 0.14  | 0.002   | 0.09  | 0.07 |
| Oscillatoria limosa Agardhex Gomont                        | 0.00  | 0.00   | 0.00  | 0.76  | 68.02   | 53.61  | 0.63  | 15.66  | 12.77 | < 0.001 | 0.08  | 0.08 |
| Oscillatoria sp.   | 12.87 | 37.65  | 4.82  | 80.24 | 1360.63 | 183.06 | 22.55 | 149.35 | 29.97 | 0.81    | 22.79 | 3.68 |
| Binuclearia lauterbornii (Schmidle)<br>Proschkina-Lavrenko | 0.72  | 2.88   | 1.11  | 0.03  | 0.73    | 0.35   | 2.19  | 10.85  | 2.36  | 0.30    | 6.83  | 2.05 |
| Chrysochromulina sp.                                       | 5.04  | 102.64 | 81.79 | 0.22  | 9.95    | 6.75   | 0.08  | 0.45   | 0.17  | 0.05    | 1.52  | 0.38 |

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|                             | Percent | Frequency | Percent<br>I | Abundance of<br>ohylum | Perce<br>of | nt Biomass<br>phylum |
|-----------------------------|---------|-----------|--------------|------------------------|-------------|----------------------|
|                             |         | Year      |              | Year                   |             | Year                 |
| Species                     | 1997    | 2009-2010 | 1997         | 2009-2010              | 1997        | 2009-2010            |
| Cyclotella meneghiniana     | 31.0    | 50.0      | 15.0         | 1.2                    | 10          | 0.7                  |
| Pseudosolenia calcar-avis   | 56.0    | 35.0      | 7.9          | 0.6                    | 75.5        | 27                   |
| Dactyliosolen fragilissima  | 3.70    | 36.0      | 0.3          | 9                      | < 0.01      | 30                   |
| Thalassionema nitzschioides | 68.0    | 65.0      | 68.0         | 6.7                    | 6.3         | 2                    |
| Cerataulina pelagica        |         | 20.0      |              | 18.3                   |             | 9.8                  |
| Pseudonitzschia seriata     |         | 43.0      |              | 43.5                   |             | 20.5                 |
| Bacillariophyta             | 99.0    | 97.0      | 77.0         | 58.3                   | 93          | 53.8                 |
| Exuviaella cordata          | 80.0    | 82.0      | 89.0         | 31                     | 48.6        | 2.4                  |
| Goniaulax polyedra          | 2.0     | 44.0      | 0.5          | 4                      | 1.6         | 1_9                  |
| Prorocentrum proximum       | 0.7     | 83.0      | 0.07         | 37                     | 0.8         | ٧٢٩                  |
| Pyrrophyta                  | 89.0    | 97.0      | 19           | 6.7                    | 5.9         | 43.5                 |
| Oscillatoria limosa         | 4.0     | 2.0       | 46           | 5                      | 2.4         | 5                    |
| Oscillatoria sp.            |         | 95.0      |              | 86                     |             | 53                   |
| Nodularia spumigena         |         | 6.0       |              | 0.07                   |             | 1.3                  |
| Cyanophyta                  | 14.0    | 97.0      | 1.4          | 29.3                   | 0.5         | 1.6                  |
| Binuclearia lauterbornii    | 12.0    | 37.0      | 88           | 57                     | < 0.01      | < 0.01               |
| Chlorophyta                 | 14.0    | 41.0      | 2.6          | 0.6                    | 0.05        | 0.9                  |
| Chrysochromulina sp.        |         | 0.02      |              | 100                    |             | 100                  |

# Table 8:Comparison of percent abundance and frequency of some dominant, native and dwellphytoplankton species in the southern Caspian Sea in 1997 versus 2009-2010.

Percent abundance and frequency of some dominant, native and dwell species of the Caspian Sea are compared for the period between 1997 and 2010. The decrease of frequency and abundance of some native and dwell species (*Thalassionema nitzschioides* and *Pseudosolenia calcar-avis*) are shown in the Table 8.

## Discussion

During the period of study, the highest and lowest surface sea temperatures (SST) were observed in summer (26.46±0.12°C) and winter (9.59±0.31°C), respectively; while the values were 20.76±0.25°C and 17.88±0.20°C in spring and autumn, respectively (Nasrollahzadeh et al., 2012).The seasonal mean of SST was almost similar to data in

1997 (Nasrollahzadeh, 2008). It has been accepted that climate changes (Gomez and Souissi, 2003, 2007) and increase in nutrients are important factors in the increase of phytoplankton reproduction (Auer et al., 2004). Increases of detergents, fertilizers and nutrients from enhanced outlet of domestic, industrial and agricultural sewages to the wetlands and coastal water of Caspian Sea and increase of deforestation (Kideys et al., 2008), are important factors for nutrient sources. A study by Nasrollahzadeh et al. (2008<sub>a</sub>) also justified the shift of trophic status of Iranian Coastal of Caspian Sea from oligotrophic to mesoeutrophic from 1997 to 2008 due to increasing of nutrients.

In an undisturbed ecosystem, various parameters (nutrient, temperature, pH, climate, and grazing pressure) have an impact on phytoplankton population through natural cycles and sources. However, today, some unnatural and new factors interplay in the Caspian Sea: sources of nutrient, regional warming and predatory relationships. They may lead to favour situations of algal blooms in different seasons and species. Cyanophyta are living organisms from the natural ecosystem. However, human activity and organic matter caused an increase in population and distribution of Cyanophyta. A comparison with the results of previous studies (Laloei, 2005; Farabi et al., 2009) in Amirabad (transect nearby the Neka Power Plant) indicates the dramatic increase of Cyanophyta population. High Cyanophyta population is an alarm of eutrophication in this area. Heat from the cooling water system of the Neka Power Plant near the Amirabad transect, nutrient matters from lysis of the huge mass of M.leidyi (Shiganova et al., 2003; Nasrollahzadeh et al.,2008<sub>b</sub>), discharge of sewage to the sea or river tributaries and global warming are the main unnatural causes of increase in the phytoplankton population. It is noticeable that the Cyanophyta bloom in 2009 in Nowshahr and Tonekabon transects, has made this semienclose ecosystem more instable to ecological events (Nasrollahzadeh et al., 2011). In this view, it is important to identify the potential of blooming harmful species. Because bloom disturbs the ecosystem even if it happens in valuable species of the food chain. Massive blooms of Exuviaella cordata during the early 1980 (after invasion *M.leidyi*) in the Black Sea (Sorokin, 1999) increased the development of eutrophication in the area.

In summer, high population of *M. leidyi* in the Caspian Sea decreased the grazers of phytoplankton (i.e. zooplankton) and indirectly increased the phytoplankton abundance (Shiganova et al., 2003). Meanwhile a combination of suitable conditions has led to the bloom of Nodularia spumigena mainly offshoreTonekabon and it was felt in the Nowshahr transect too (Nasrollahzadeh et al., 2011). The bloom took place some days before summer cruises and crashed a few hours before sampling. Spare strips of bloomed algae were observed on the water surface at the sampling time. Inspite of bloom breaking, the high population of Cyanophyta in Tonekabon transect indicated the persistence of suitable conditions for other species of Cyanophyta reproduction.

In winter, massive precipitation of mucus, dead ctenophores (Roohi et al., 2010;

Mokarami et al., 2012) and enormous phytoplankton biomass (Makhlough et al., 2012) entered nutrients to the bottom. Then, upwelling processes injected high nutrients to the water column that became available for phytoplankton reproduction (Kamburska et al., 2006).

Phytoplankton abundance showed two maxima in spring and autumn in 1997. The two peaks of seasonal abundance shifted in 2010 (about a decade after the invasion of M. leidyi to the Caspian Sea) to summer and winter due to more available nutrients. The canonical discriminant coefficient of data showed that Bacillariophyta and Pyrrophyta had the main role in the classification of seasons in 1997. However, this study indicated the dominance of Bacillariophyta and Cyanophyta in all seasons except in some samples from Anzali in spring when Haptophyta exceeded Cyanophyta. So in the canonical discriminant coefficient, Bacillariophyta and Cyanophyta are the most significant variables in the discriminant function.

Evenness index was low in summer (0.21) and winter (0.36), while it was higher in spring and autumn (0.50) in 2010. It seems that some environmental conditions that were suitable for the reproduction of a larger number of phytoplankton species in the spring and fall occurred. Increase of photic hours, temperature and nutrients are the main effective factors in phytoplankton increment in spring. In autumn, temperature was set between the high values of summer and the low ones winter. In this season the thermal

stratification with a thermocline began to degrade and was instable (Nasrollahzadeh et al., 2012). So, the water body was in a transient situation. In other words, fall was between the shifting of certain characteristics of summer (warm and calm weather, stratified water) to winter (cold and turbulent water). The combination of these factors along with low grazers (due to high abundance of *M.leidyi*) (Kideys, 2008; Mokarami et al., 2012) provided suitable conditions for species of different phytoplankton phyla.

As mentioned, warm (more than 20 °C), calm and stratified water in summer are favourable for *Oscillatoria* sp. reproduction (Chorus and Bartram, 1999; Nasrollahzadeh et al., 2008<sub>c</sub>). *Oscillatoria* sp. is one of dwelling species of the Caspian Sea. However, this large abundance of the species in 2009 inserted *Oscillatoria* sp. in the list of "harmful and potential bloom species" in the area (Makhlough et al., 2012).

In winter, turbulent water, upwelling and high nutrient sources provided good conditions for extreme reproduction of three species of Bacillariophyta. The population of dominant species was high as much as the bloom in some samples in winter. It led to low values of Shannon and evenness indices (Brower et al., 1998).

The list of dominant species is almost different to that of the previous years. As Nasrollahzadeh et al. (2008<sub>a</sub>) showed, the dominant species contained *Exuviaella cordata*, *Thalassionema nitzschioides*, *Cyclotella meneghiniana*, *Pseudosolenia calcar-avis* and *Oscillatoria* sp. from 1997 to 2004. In 2009-2010 not only some new and harmful species appeared in the phytoplankton community of the Caspian Sea, but also they contributed to the dominant species list: Oscillatoria sp., Pseudonitzschia seriata, Thalassionema nitzschioides, Cerataulina pelagica, Chrysochromulina sp. and Exuviaella cordata. The study in north and middle of the Caspian Sea also indicated to presence of invader species such as Pseudonitzschia seriata, Cerataulina pelagica in recent decade (Shiganova et al., 2005). As the results showed the abundance percentages of some dwell or native (Pseudosolenia calcar-avis and Cyclotella meneghiniana) species decreased, while frequency and abundance percentage of some species such as Oscillatoria sp. and fragilissimus Dactyliosolen increased noticeably in 2010. Unusual diatoms changed the phytoplankton structure of the Caspian Sea. In other words, the abundance and composition of phytoplankton are driven by unnatural environment factors and stress (Makhlough et al., 2012) as well as natural factors.

Two peaks of phytoplankton abundance were shifted from spring and autumn (before the invasion of *M.leidyi*) to summer and winter (one decade after the invasion of *M.leidyi*) due to the suitable warmer climate and the new availability nutrient. The peaks were formed by *Oscillatoria* sp. (filament form) and *Pseudonitzschia seriata* (chain form) species from Cyanophyta and Bacillariophyta phyla. Opportunistic reproduction, toxin production and harmful characteristics of dominant species in the two seasons are the evidences of the under stress features of the Caspian Sea. The next sequence of this phenomenon will set the Caspian Sea in an unprecedented ecological situation.

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