

## Changes in size and form in the dominant phytoplankton species in the southern Caspian Sea

Makhlough A.<sup>1</sup>; Hassan Nasrollahzadeh Saravi H.<sup>2\*</sup>;  
Eslami F.<sup>3</sup>; Leroy S.A.G.<sup>4</sup>

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### Abstract:

Due to the recent destructive pressures on the Caspian environment, an effect on phytoplankton communities is expected in different aspects such as cell size and form. This paper aims to survey the size and form in the dominant phytoplankton species in the years of 1996, 2001, 2006, 2009, 2011 and 2012 in the Iranian coast of the Caspian Sea. Results of current study showed that the dominant species and along with it their sizes and forms remained quite similar in the fall of different years, but the greatest shift in size and form were observed during spring, summer and winter. It is suggested that these changes reflect an ecological disturbance and instability in the Caspian environment.

**Keywords:** Phytoplankton, Dominant Species, Size, Form, Caspian Sea, Iran

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1, 2-Caspian Sea Ecology Research Center (CSERC), Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), P.O. Box 961, Sari, Iran

3- Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), P.O. Box: 14965/149. Tehran, Iran.

4- Institute for the Environment, Brunel University, Uxbridge UB8 3PH, London, UK

\*Corresponding author' Email: Email: hnsaravi@gmail.com

## Introduction

Many ecological systems can undergo large, sudden and prolonged changes in structure and functions (Scheffer *et al.*, 2001). Eutrophication of water, algal bloom, salinization of soil, destruction of coral marine reefs, overfishing and collapse of fisheries are some of these displacements (Biggs *et al.*, 2012). Ultimately the displacements adversely affect human health and economy. Studies on long-term data in transition systems provide warning signs to researchers (Scheffer *et al.*, 2009). Since algae show rapid responses to environmental changes, they are suitable for environmental assessments (Alves-de-Souza *et al.*, 2008).

Iranian lagoons and coastal regions have moreover been steadily polluted by anthropogenic sources (fertilizers and pesticides used in agriculture and increased nutrient load of river flows due to deforestation of woodland) since the early 1980s (Kideys *et al.*, 2008). The introduction of *Mnemiopsis leidyi* to the Caspian Sea and its tremendous damage and biological pollution is the most important event in the two recent decades in the region (Darvishi *et al.*, 2004; Shiganova *et al.*, 2004; Roohi *et al.*, 2010). A study by Nasrollahzadeh *et al.* (2008) also justified the shift of trophic status of Iranian Coastal of Caspian Sea from oligotrophic to meso-eutrophic from 1997 to 2008 due to increasing nutrients. Pollution reflected on phytoplankton populations by causing a decline of some native species and increasing the population of new comers and harmful species

(Makhloogh *et al.*, 2011). Algal blooms also have recently occurred four times (2005, 2006, 2009 and 2010) in the southern coasts of the Caspian Sea. At all times blooms were formed by a toxic species of Cyanophyta except in 2006 when it was made by species of Pyrrophyta (HAB, 2006; Nasrollahzadeh *et al.*, 2011). A study by Leroy *et al.* (2013) showed that global warming (climate change) and increasing of surface water temperatures had significant positive effects on the dinocysts especially owing to *Lingulodinium machaerophorum*, Deflandre and Cookson 1955, assemblage in terms of relative abundances and concentration since the mid 1960s in the south basin of the Caspian Sea. Its motile form, *Lingulodinium polyedrum*, Stein 1883, Dodge 1989 is reported to cause harmful algal blooms and yessotoxin production (Howard *et al.*, 2009).

It is expected that the destructive factors may also affect phytoplankton communities at different levels, such as cell size and form (colony, chain and filament). Reynolds *et al.*, (2002) noted that the assemblages of different species are based on their tolerances, sensitivities and survival strategies. They highlighted the predominance of the ecological concept over the phylogenetic affinity of the species.

Different research and articles have been done about trophic levels, physico-chemical conditions of water, abundance, biomass and distribution of phytoplankton particularly in the last two decades in the Iranian area of the

Caspian Sea (Nasrollahzadeh *et al.*, 2008; Nasrollahzadeh *et al.*, 2012; Nasrollahzadeh *et al.*, 2013; Nasrollahzadeh *et al.*, 2014). However, there is a lack of published data regarding the size and form of phytoplankton in the southern Caspian Sea. Therefore, the present paper is to survey only the changes of size and form as important aspects in adaptive strategy of the dominant phytoplankton species over six different years from 1996 to 2012 in the Iranian coast of the Caspian Sea.

### Material and methods

Surface water samples were collected seasonally during phase 1 (1996, corresponding to the pre-invasion of *Mnemiopsis leidyi*, providing background data) and phase 2 (2001, 2006, 2009, 2011 and 2012, corresponding to the post-invasion phase) along the Iranian coast of the Caspian Sea (Fig. 1 and Table 1). Phytoplankton samples were kept in 0.5-L bottles and preserved using buffered formaldehyde to yield a final concentration of 2%. The samples were left to settle for at least 10 days following which they were concentrated to about 30 ml by sedimentation and centrifugation (APHA, 2005). The quality and quantity analysis of phytoplankton for this study was reported in detail by Nasrollahzadeh *et al.*, (2008). Information of phytoplankton community such as abundance, dominant phyla and species extracted from available papers and reports are referred as data sources in

Table 1. Then the dominant species of each phylum was divided into different groups based on the some morphological characteristics such as form, size and presence/absence of flagella. Size grouping of dominant species was conducted by considering the obtained size fraction (in different phyla) from different references (Lampman and Makarewicz, 1999; Samuelsson and Andersson, 2003; Greisberger *et al.*, 2007; Bellinger and Sigee, 2010), the Maximum Linear Dimension (MLD) of single cells and width range (filamented and chain cells) of the dominant species (in related phylum) in this paper and laboratory equipment (magnification and resolution power of microscope). The single form of cells was defined as large with a linear dimension  $\geq 20 \mu\text{m}$ , small:  $10 \mu\text{m} < \text{linear dimension} < 20 \mu\text{m}$ , and fine: linear dimension  $\leq 10 \mu\text{m}$ . Filamented and chained species were also divided into two groups: thick ( $>10 \mu\text{m}$  wide) and thin ( $<10 \mu\text{m}$  wide).

### Results

The maximum and minimum means of total abundance of phytoplankton were observed in 2001( $330 \pm 52$ ) and 2006 ( $15 \pm 3$ ) (million cells per cubic meter  $\pm$  standard Error), respectively (Fig. 2).

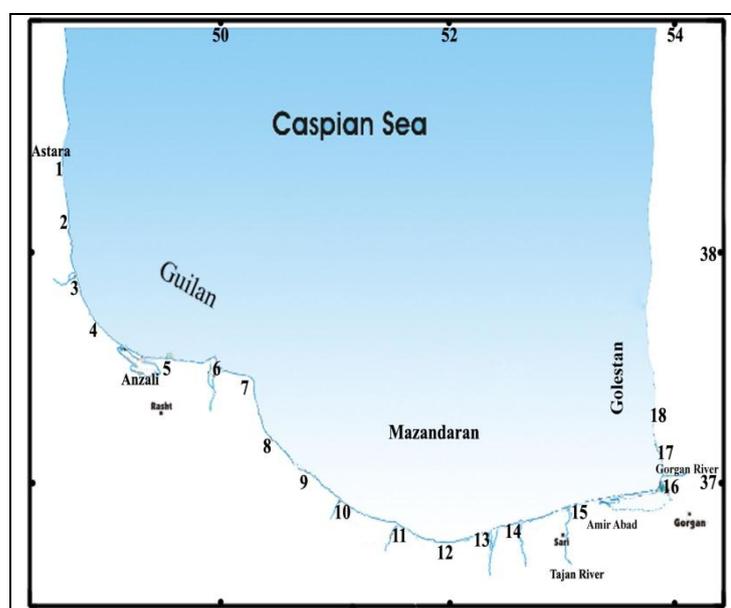


Figure 1: Sampling stations in the Iranian coast of the Caspian Sea during study period 1996 to 2012.

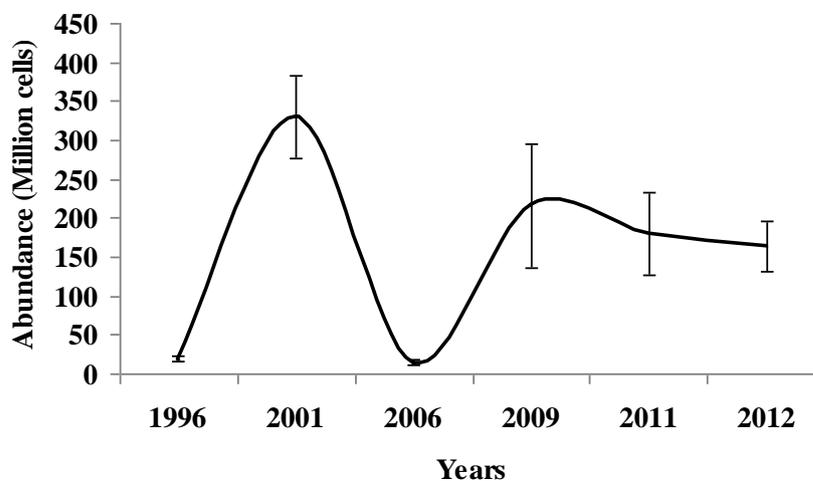
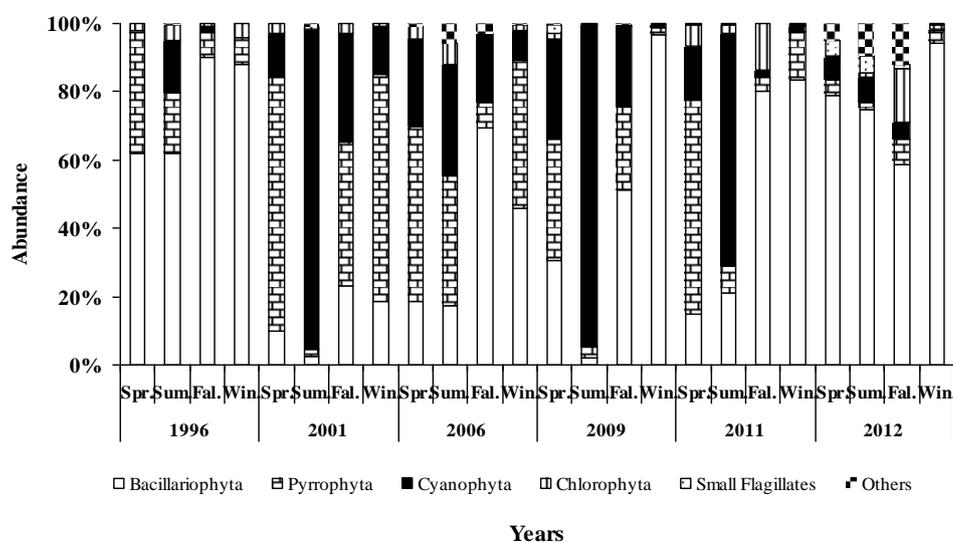


Figure 2: Mean of total phytoplankton abundance in the Iranian coast of the Caspian Sea from 1996 to 2012.

Bacillariophyta, Pyrrophyta and Cyanophyta were the most abundant, i.e. more than 70% of the total phytoplankton abundance, in all seasons from 1996 to 2012 (Fig. 3). A few species of Chlorophyta were rarely able to form a significant abundance in some seasons (especially fall). However, the percent abundance of other phyla

(Euglenophyta, Cryptophyta and Xantophyta) was less than 1% of the total phytoplankton abundance.

Table 2 shows that the species contributed to more than 70 percent of the total abundance of phytoplankton in the years of study periods.



**Figure 3: Percent abundance of dominant phytoplankton phyla in the Iranian coast of the Caspian Sea from 1996 to 2012.**

**Table 1: Distribution of sampling characteristics over six different years from 1996 to 2012 in the Iranian coast of the Caspian Sea.**

Year	Depths (meter)	Transects*	Number of samples	Data sources
1996	10, 20	1-18	142	Fazli <i>et al.</i> , 2010, Nasrollahzadeh <i>et al.</i> , 2013
2001	5, 10, 20	10, 15, 16	35	Fazli <i>et al.</i> , 2010, Nasrollahzadeh <i>et al.</i> , 2013
2006	5, 10, 20	2, 5, 7, 10, 15, 16	57	Fazli <i>et al.</i> , 2010, Nasrollahzadeh <i>et al.</i> , 2013
2009	5, 10, 20	1, 5, 7, 9, 10, 15, 16, 17	96	Makhlough <i>et al.</i> , 2012
2011	5, 10, 20	5, 10, 15	33	Unpublished data
2012	5, 10, 20	9, 10, 15, 16	72	Makhlough <i>et al.</i> , 2014

\*Transects are shown in Fig. 1.

These species are classified based on the size (small, large, thick or thin), morphotype (unicellular, colonies, chains or filament) and the presence of flagella.

The species having the highest overall abundances among dominant species in each season of the years of the study period are shown in Table 3.

In general the solitary, non-flagellated and relatively large-sized species from Bacillariophyta

(*Thalassionema nitzschoideae*, (Grunow) Grunow ex Hustedt 1932) and flagellated, small-sized Pyrrophyta (*Exuviaella cordata*, (Ostenfeld) Dodge 1975) showed the highest abundance in the fall for the different years of study (Table 2 and Fig. 4).

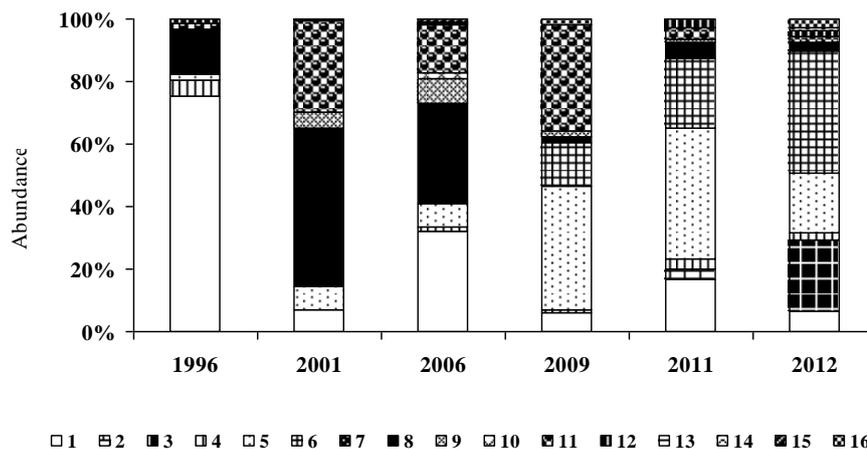
**Table 2: Size and form data for the dominant species in six different years from 1996 to 2012 in the Iranian coast of the Caspian Sea.**

Code number	Phylum	Form	Size	Flagella	Taxa
1	Bacillariophyta	Single	Large	-	<i>Thalassionema nitzschoideae</i> (Grunow) Grunow ex Hustedt 1932, <i>Nitzschia acicularis</i> , (Kützing) W. Smith 1853, <i>Synedra ulna</i> Ehrenberg 1832, <i>Pseudosolenia calcaravis</i> (Schultze) B.G. Sundström, 1986
2	Bacillariophyta	Single	Large	-	<i>Chaetoceros peruvianus</i> Brightwell, 1856
3	Bacillariophyta	Single	Small-Fine	-	<i>Chaetoceros subtilis</i> Cleve 1896, <i>Chaetoceros thronsenii</i> (Marino, Montresor & Zingone) Marino, Montresor & Zingone, 1991
4	Bacillariophyta	Single	Small	-	<i>Cyclotella meneghiniana</i> Kuetzing , 1844, <i>Nitzschia</i> sp.
5	Bacillariophyta	Chain	Thick	-	<i>Skeletonema costatum</i> (Greville) Cleve, 1873, <i>Dactyliosolen fragilissimus</i> (Bergon) Hasle 1996 , <i>Stephanodiscus hantzschii</i> Grunow, 1880, <i>Stephanodiscus socialis</i> Makarova & Proshkina, 1958, <i>Cerataulina pelagica</i> (Cleve) Hendey, 1937
6	Bacillariophyta	Chain	Thin	-	<i>Skeletonema subsalsum</i> (Cleve-Euler) Bethge, 1928, <i>Pseudonitzschia seriata</i> (Cleve) H.Peragallo, 1899
7	Bacillariophyta	Chain	Thin	-	<i>Chaetoceros socialis</i> Lauder, 1864
8	Pyrrophyta	Single	Small	+	<i>Exuviaella cordata</i> (Ostenfeld) Dodge, 1975
9	Pyrrophyta	Single	Large	+	<i>Prorocentrum scutellum</i> Schröder 1900, <i>Prorocentrum obtusum</i> Ostenfeld, 1908, <i>Prorocentrum proximum</i> Makarova, 1967
10	Pyrrophyta	Single	Large	+	<i>Glenodinium behningii</i> , (Lindemann, 1927) Kisseleva, <i>Gymnodinium variabil</i> E.C.Herdman, 1924, <i>Goniaulax polyedra</i> Stein, 1883
11	Cyanophyta	Filament & Colony	Mostly thin	-	<i>Oscillatoria limosa</i> Agardh ex Gomont, 1892, <i>Oscillatoria</i> sp., <i>Lyngbya</i> sp., <i>Lyngbya limnetica</i> , Lemmermann, 1898
12	Chlorophyta	Filament	Mostly thin	-	<i>Anabaena aphanizomenoides Forti</i> , 1911, <i>Anabaena</i> sp., <i>Synechococcus</i> sp.
13	Chlorophyta	Single	Large	-	<i>Binuclearia lauterbornii</i> (Schmidle) Proshkina-Lavrenko, 1966, <i>Binuclearia</i> sp.
14	Chlorophyta	Single	Small-Fine	-	<i>Ankistrodesmus arcuatus</i> Korshikov, 1953
15	Alternative	Single	Fine	+	<i>Schroederia setigera</i> (Schröder) Lemmermann, 1898
					Small Flagellate

+ : Flagella, - : No Flagella, Fine: Linear Dimension  $\leq 10 \mu\text{m}$  , Small:  $10 \mu\text{m} < \text{Linear Dimension} < 20 \mu\text{m}$  , Large: Linear Dimension  $\geq 20 \mu\text{m}$  · Thin: wide  $< 10 \mu\text{m}$  · Thick : wide  $\geq 10 \mu\text{m}$ .

**Table 3: Species having high overall abundances during each season in the Iranian coast of the Caspian Sea from 1996 to 2012.**

Years Seasons	1996	2001	2006	2009	2011	2012
Spring	<i>Exuviaella cordata</i> , <i>Cyclotella meneghiniana</i> , <i>Thalassionema nitzschooidae</i>	<i>Exuviaella cordata</i> , <i>Prorocentrum proximum</i>	<i>Exuviaella cordata</i> ,	<i>Stephanodiscus</i> , Small-Fine size phytoplankton, <i>Exuviaella cordata</i>	<i>Skeletonema costatum</i> , <i>Exuviaella cordata</i>	<i>Chaetoceros thronsdonii</i>
Summer	<i>Exuviaella cordata</i> , <i>Pseudosolenia calcaravis</i>	<i>Oscillatoria</i> sp.	<i>Exuviaella cordata</i> , <i>Oscillatoria</i> sp.	<i>Oscillatoria</i> sp.	<i>Lyngbya</i> sp., <i>Oscillatoria</i> sp., <i>Cyclotella meneghiniana</i>	<i>Chaetoceros thronsdonii</i>
Fall	<i>Thalassionema nitzschooidae</i>	<i>Oscillatoria</i> sp., <i>Exuviaella cordata</i> , <i>Thalassionema nitzschooidae</i>	<i>Thalassionema nitzschooidae</i> , <i>Oscillatoria</i> sp.	<i>Thalassionema nitzschooidae</i> , <i>Oscillatoria</i> sp.	<i>Thalassionema nitzschooidae</i> , <i>Skeletonema costatum</i>	<i>Thalassionema nitzschooidae</i>
Winter	<i>Thalassionema nitzschooidae</i>	<i>Exuviaella cordata</i>	<i>Exuviaella cordata</i> , <i>Thalassionema nitzschooidae</i> ,	<i>Cerataulina pelagica</i> , <i>Pseudonitzschia seriata</i> , <i>Dactyliosolen fragilissimus</i>	<i>Skeletonema costatum</i> , <i>Skeletonema subsalsum</i>	<i>Pseudonitzschia seriata</i> , <i>Skeletonema costatum</i>



**Figure 4: Percent abundance of dominant phytoplankton species of six years from 1996 to 2012 in the Iranian coast of the Caspian Sea. Legend numbers are shown in Table 2.**

However, in the fall of 2001, the filament form and thin size of Cyanophyta (*Oscillatoria* sp.) appeared as co-dominant species. In 1996, the dominant species were in unicellular form (*Cyclotella meneghiniana* Kuetzing 1844, *Thalassionema*

*nitzschooidae*, *Exuviaella cordata*), from Bacillariophyta and Pyrrophyta in all seasons.

In 2001, the dominant species were formed by the unicellular form of Pyrrophyta (*Exuviaella cordata*, *Prorocentrum proximum*, Makarova

1967) and filament form of Cyanophyta (*Oscillatoria* sp.). The pattern of dominant species was very similar in the years of 2001 and 2006. In 2009, Cyanophyta and Bacillariophyta phyla occupied the two most dominant positions and they mostly appeared as the form with filaments (*Oscillatoria*) and thick (*Stephanodiscus hantzschii* Grunow 1880, *Cerataulina pelagica* (Cleve) Hendey, 1937), thin (*Pseudonitzschia seriata* (Cleve) H. Peragallo, 1899) size of chains. Temporary and snap shot increasing of single form and fine sized (Small Flagellate) species were observed in the spring of 2009. Chain form of Bacillariophyta and filaments type of Cyanophyta were maintained as dominant species in 2011, however a solitary form of Pyrrophyta (*Exuviaella cordata*) was also observed in spring. The dominant species in the spring and summer of 2012 was small-fine sized and solitary form of Bacillariophyta, which clearly showed different features as compared to the previous years. However, in winter the same as for 2011, chain form (thick and thin) species of Bacillariophyta (*Pseudonitzschia seriata*, *Skeletonema costatum* (Greville) Cleve 1873) showed the highest abundances.

### Discussion

Bacillariophyta species are reported to form the most abundant and widespread group throughout the Caspian Sea (Kosarev and Yablonskaya, 1994, Ganjian *et al.*, 2010). It might be due to the absence of silica limitation in this

area (Kasymov, 2004; Nasrollahzadeh *et al.*, 2012). Pyrrophyta is always known as one of the top two dominant phyla, particularly in the summer and fall, which is coincident with thermal stratification of water and low surface nutrients in the South basin (Salmanov, 1987) and the Iranian coast of the Caspian Sea (Nasrollahzadeh, 2008; Fazli *et al.*, 2010). The percent abundance of Cyanophyta severely decreased in the summer of 2012 compared to that in 2001-2009. Small colonies of *Syneococcus* were the dominant species of Cyanophyta in 2012 (Makhloogh *et al.*, 2014). There are some unpublished reports on the presence of this species in the Iranian coast of the Caspian Sea in the late 1990s and early 2000s. This species was observed in low numbers in 2009 and 2011 (Makhloogh *et al.*, 2014). The species is one of the important producers in the ocean carbon cycle and it is able to support the fine sized flagellate growth (Christaki *et al.*, 2002). There has been no report for the occurrence of fine sized flagellates in the Iranian coast of the Caspian Sea before 2009. Then, after observing a low abundance in 2011, it was relatively well recorded with a high abundance in 2012, which was much higher than Cyanophyta abundance.

A study in the south of the Black Sea from 2000 to 2006 showed that the percentage of Bacillariophyta decreased in spring, summer and autumn, while the percentage of small flagellated Chrysophyta increased. The evaluation of phyla sequences in the interior basin

of the Black Sea also showed that the dominance of Bacillariophyta in spring and summer, continued with the dominance of small flagellates in fall in the years 1994-1985 (Nesterova *et al.*, 2008). This event was accompanied by an increase of heterotrophic dinoflagellates and toxic species of the *Pseudonitzschia*. It reflected features of irregular pattern of species succession and an ecological instability in the area.

In 2012, fine-sized flagellates (with a high ratio of surface to volume, S/V) were observed in diverse phyla. For example, *Apedinella spinifera* (Chrysophyta), that was reported for first time in the area, showed high percent abundance and frequency (even more than Cyanophyta) mainly in summer and fall. *Chaetoceros thronsdonii* (fine-size from Bacillariophyta) did not show considerable abundance in 2009. However, its percent frequency reached above 60% and was the predominant species in the spring and summer of 2012. There are some reports of extreme abundance and even bloom event of *Chaetoceros thronsdonii* due to urban waste and litter fall in a coastal lagoon of Mexico (Ake-Castillo and Vazquez, 2008; Livingston, 2002). So, in the present study, the high abundance of this species perhaps is an indicator of low water quality in spring and summer. Decomposition of phytoplankton mass (in the previous seasons) provides a valuable nutrient source for the bloom of nanoflagellates, small diatoms and Pico cyanobacteria (Hajdu *et al.*, 2007). Therefore, if

Cyanophyta showed an important role in the expression of water quality and ecological status of the Caspian Sea in the years 2001-2009s, fine sized and flagellated species (from different phyla), were an important characteristic of water quality from spring to fall in 2012. In these seasons (spring to fall), the absence of large colonies, chained and filament species and morphological characteristics of dominant species such as fine-small sized, high S/V indicated regeneration of nutrients or entrainment of nutrient-rich deep layer of water (Dahl *et al.*, 2005).

There are various opinions on how fine-small sized and flagellated species increased in the water column. The study of Silkin *et al.* (2011) showed that *Chaetoceros thronsdonii* (as an invader species) increases in areas with a low rate of water exchange in the water column during summer. Flagellated phytoplankton is able to rapidly swim vertically to the bottom (near the floor) for nutrient intake, and back to the upper layers for light absorption. The flagellum is a good adjusting element for such a small flagellated C-strategy pattern organisms because C-strategy pattern organisms have invasive proliferation with suitable amount of nutrients. It means that they are not as much resistant as S-strategy pattern organisms (such as small colonies of *Synechococcus*) to nutrient deficiency in the upper layer during summer time. Therefore, sensitivity of *Chrysochromulina* sp. and *Apedinella spinifera* (Thronsdon) Thronsdon, 1971, species to low nutrient content of the

upper layer is covered by the possession of a flagellum and mobility character (Hajdu *et al.*, 2007). On the other hand, fine sized and small flagellated species are able to absorb high concentrations of nitrogen and phosphorus (high growth and photosynthesis efficiency) due to their high cell S/V (Dahl *et al.*, 2005; Reynolds, 2006; Greisberger *et al.*, 2007). So, small flagellates have more chances to proliferate compared to some flagellated S-strategy pattern organisms than Pyrrophyta which have low S/V (Sigeo, 2004). It was also found that a high abundance of the C-strategy pattern species (fine-small sized species with or without flagella from different phyla) may indicate increased levels of nutrients, especially from anthropogenic sources (Moncheva *et al.*, 2001).

It should be noted that nowadays *Acartia* (Copepod) is a major organism in the zooplankton abundance in the Caspian Sea particularly from spring to autumn. Grazing by *Acartia* creates a selective pressure on the phytoplankton community, causing elimination of large sized phytoplankton species (Roohi *et al.*, 2010, Nasrollahzadeh *et al.*, 2012). As a result, small sized phytoplankton species dominate in phytoplankton communities. Besides the aforementioned reasons, global warming also has a negative effect on inter and intraspecific size changes of phytoplankton species (Atkinson 1994; Atkinson *et al.*, 2003; Sommer *et al.*, 2003; Allan 2007; Forster *et al.*, 2011; Yvon-Durocher *et al.*, 2011; Peter and Sommer, 2012).

In the winter of 2012, *Pseudonitzschia seriata* (chain form and fairly long length) had a dominant abundance in the phytoplankton structure while the dominant species of other seasons were in the unicellular forms. This shifting pattern in size and form is a clear sign of increased nutrients in the water column (Dahl *et al.*, 2005). It was probably brought by vertical mixing of water from deeper layers.

In spite of insufficient and uneven sample collection during the study period, which makes long-term evaluation very difficult, our study nevertheless produced helpful suggestions from the available data. The study emphasizes phytoplankton size shifting over recent years in the Iranian area of the Caspian Sea and showed that a morph type conception accompanied with phytoplankton identification and enumeration is useful for the prediction and determination of ecological status. There is doubt whether the Caspian Sea is able to overcome the instability of ecological conditions and if its situation would go back to that in the 1990s. However, it is obvious that the input of nutrients from external sources (mainly anthropogenic activities) and the insufficient management of the water body would expose the ecosystem to more disturbance, instability and unfavourable ecological events.

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