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Scale characteristics of the bloom event: A case study in the Iranian coastal waters of the Southern Caspian Sea

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Abstract

Nutrient enrichment in water and sediments due to excessive anthropogenic activities in recent years has caused excessive algal growth in the Caspian Sea. The current study was conducted to determine the abundance of phytoplankton community, the dominant species and chlorophyll-*a* [Chl-*a*] concentration during algal blooms in the Iranian coastal waters of Caspian Sea through four seasons from 2013 to 2014. The minimum and maximum phytoplankton abundance recorded were 73±31 and 505±55 million cells m^{-3} in summer and winter, respectively. The median concentration of Chl-*a* increased to 5.81 mg m^{-3} in autumn, as compared to the annual median value (2.43 mg m^{-3}). The results indicated that the bloom started in autumn and it continued falling with a low concentration during winter (Chl-*a*: 2.59 mg m^{-3}). The three species *Stephanodiscus socialis*, *Binuclearia lauterbornii* and *Thalassionema nitzschioides* were classified in medium bloom class (100-1000 million cells m^{-3}) in spring, summer and autumn, respectively. While in winter *Pseudonitzschia seriata* (harmful species) and *Dactyliosolen fragilissima* were classified in medium bloom class with high relative frequency. The scaling of bloom abundance revealed that bloom initiation coincided with 10 million cells m^{-3} of the dominant phytoplankton species. The bloom at the regions with more than 100 million cells m^{-3} of total phytoplankton abundance and dominant species was overlapped with the bloom regions based on Chl-*a* concentration.

Keywords: Phytoplankton, Bloom, Scale characteristics, Caspian Sea, Iran

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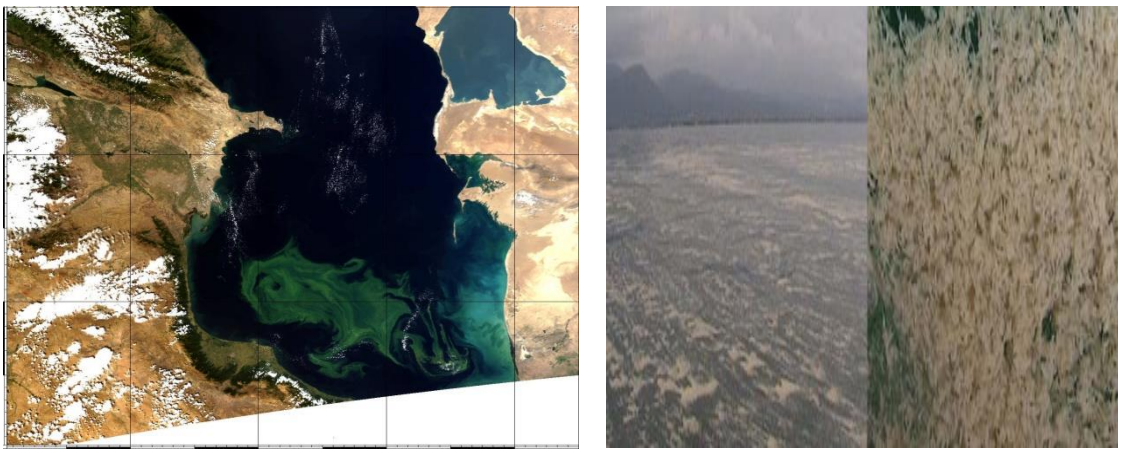
Introduction

Excessive amounts of the majority of nutrients in the water and sediments of Caspian ecosystem were the result of human population growth and anthropogenic impact on the area (Aladin *et al*., 2004; Nasrollahzadeh Saravi *et al*., 2012; Samadi-Maybodi *et al*., 2013; Nasrollahzadeh Saravi *et al*., 2015b; Niyazi *et al*., 2016). Increasing of nutrients obviously led to more proliferation of phytoplankton in the Caspian Sea which is similar to many other ecosystems (Anderson *et al*., 2002; Nasrollahzadeh Saravi *et al*., 2015a). The temporary blooms or high proliferation of phytoplankton under the geochemical cycle is a natural phenomenon and is mostly beneficial to coastal productivity. However, if this event (even in seemingly harmless species) occurs more than the capacity of the system it may bring up negative environmental impacts (Cullen, 2008). Impacts can be acute and severe, or more prolonged and chronic. Until now, almost 300 species of microalgae are known to cause water blooms (Vershinin and Orlova, 2008). In the Iranian coast of the Caspian Sea, the first report of milky tide due to *Nodularia spumigena* bloom was from the Anzali coast in late September of 2005 (CEP, 2006). Then, a red tide of *Heterocapsa* genus (*Pyrrophyta* phylum) blooms was observed from Anzali to Hassanrud coasts in early October 2006 (CEP, 2006). A visible bloom of *Nodularia spumigena* repeated at Tonekabon, Nowshahr and Babolsar transects in late August 2009 and 2010 (Nasrollahzadeh Saravi *et al*., 2011). The abundance of *N. spumigena* reached 112000 and 5830 filaments mL^{-1} at dense blooms areas in 2005 and 2009, respectively (Nasrollahzadeh Saravi *et al*., 2011) (Figs. 1A, 1B). In recent decades, 15 toxic and harmful species and fine size phytoplankton (Maximum Linear Dimension=MLD<10µ) with potential blooms were identified in the Iranian basin of the Caspian Sea (Makhlough *et al*., 2011, 2017).



A **B**

Figure 1: A: Algal bloom on MODIS satellite image, 2005, B: Algal bloom in the southern Caspian Sea, 2005 (Photo by Taghipour).

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Chlorophyll-*a* containing organisms are in the producer level in most of the food chains, and the health and abundance of these primary producers affect the integrity of other trophic levels. Chlorophyll-*a* concentrations in the Caspian Sea are influenced by some important factors such as air and seawater temperatures, wind, and discharge of the rivers (Nezlin, 2005). The concentration of chlorophyll-*a* is an important indicator for the occurrence of algal blooms in the region (Thomalla *et al*., 2011).

Abnormal algal blooms can cause mortality in fish populations or problems in seafood safety and human health. Meanwhile, human economic activities such as marine aquaculture and recreational activities may be suppressed because of this unfavorable event (Chorus and Bartram, 1999). Due to the critical role of algal blooms on the ecology and economy of the region, this study was conducted to survey the temporal and spatial variations of algal bloom phenomena in the Iranian coastal waters of the southern Caspian Sea [ICWSCS] based on the following parameters: chlorophyll-*a* concentration, the total abundance of phytoplankton, phyla, and dominant species. By classifying dominant phytoplankton species at different bloom levels this study provides reliable guidelines for predicting the formation and proliferation of algal bloom for further biological, ecological and physiological studies that aim to eliminate or control probable blooms of these species. The results of this paper are useful for the marine aquaculture industry and also for remedial actions in the Caspian Sea.

Materials and methods

The seasonal monitoring was carried out by boat from spring 2013 to winter and 2014 (Fig. 2, Table 1). Along the 4 transects (Anzali, Tonekabon, Nowshahr and Amirabad), three stations located at the depths of 5, 10, and 20m were designated. Water samples were collected with a Niskin 1.7 litter sampler at the surface, 10 and 20m layers.

Figure 2: Map of the locations and depths of sampling stations in Iranian coastal waters of the Caspian Sea (2013-2014).

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Table1: Latitude and longitude of sampling stations in Iranian coastal waters of the Caspian Sea (2013-2014).

		Depth		
Transect		**5m**	**10m**	**20m**
Anzali	Lat.	49 º 29`	49 º 29`	49 º 29`
	Lon.	37 º 29`	37 º 29`	37 º 30`
Tonekabon	Lat.	50 º 54`	50 º 54`	50 º 55`
	Lon.	36 º 49`	36 º 49`	36 º 50`
Nowshahr	Lat.	51 º 30`	51 º 30`	51 º 30`
	Lon.	36 º 40`	36 º 41`	36 º 41`
Amirabad	Lat.	53 º 18`	53 º 17`	53 º 16`
	Lon.	36 º 52`	36 º 53	36 º 56

The samples for identification and enumeration of phytoplankton were collected in 0.5 liter 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 analyzed under a light microscope (Nikon, AFX-DX, Japan) (cover slip 22×22mm and with magnifications of 100, 200, 400×). Phytoplankton taxonomic identification was carried out using the valid identification keys of Proshkina-Lavrenko and Makarova (1968), Tiffany and Britton (1971), Habit and Pankow (1976) and Wehr and Sheath (2003).

To measure Chl-*a*, water samples were filtered with a vacuum pump through Whatman GF/F 0.45 µm pore size glass fiber filter papers. The volume of sample required varied according to the phytoplankton abundance which was 300-1700 ml during the study. The exact volume of filtered water was recorded. The filter papers were ground with a tissue grinder. Then acetone (90%) was added to a constant level (10 ml). The filters were protected from light, and immediately transferred and kept at 4°C overnight. Samples were centrifuged in closed tubes for 20 minutes at 3000 rpm. The absorbance of extracted samples was recorded at 630, 647, 664 and 750nm (turbidity correction). Then chlorophyll-*a* concentration was calculated using the formula according to APHA (2005). The spatial and temporal occurrences of algal bloom were determined based on 4 data sets: total phytoplankton, Bacillariophyta, Pyrrophyta, dominant species abundance and chlorophyll-*a* concentration:

-As a rule of thumb, the total abundance of phytoplankton at bloom periods is more than average for a given region or water body (Schmidt and Schaechter, 2011).

-The thresholds blooms of 750 million cells m^{-3} of total phytoplankton and 500 millioncells m^{-3} of Bacillariophyta and Pyrrophyta were considered (Revilla *et al*., 2009).

-The blooms species was classified in small (10-100 million cells m^{-3}), medium (100-1000 million cells m^{-3}) and large (more than 1000 million cells

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m^{-3}) threshold blooms (Anderson *et al*., 2010).

-At bloom periods, the median concentrations of chlorophyll-*a* are more than median for a given region or water body (Thomalla *et al*., 2011).

The one way analysis of variance (ANOVA) was used to determine the statistically significant difference in the phytoplankton abundance and biomass between the transect depths, layers and seasons. Prior to the analysis, phytoplankton data was transformed through rank cases (Krebs, 1999) to normalize the data sets.

Results

Seven phytoplankton phyla including Bacillariophyta, Pyrrophyta, Cyanophyta, Chlorophyta, Euglenophyta, Chrysophyta, Xantophyta and fine size group (MLD<10µ) were identified during the sampling period. In this study, data from 25 and 25 - 75 percentiles shows that the phytoplankton abundance varied between <22 million cells m^{-3} and 22-300 million cells m^{-3}, respectively. Most of the winter samples which collected at surface and 10m layers (in 10 and 20m depths) classified in 75-100 percentile with phytoplankton abundance >300 million cells m^{-3} (Fig. 3).

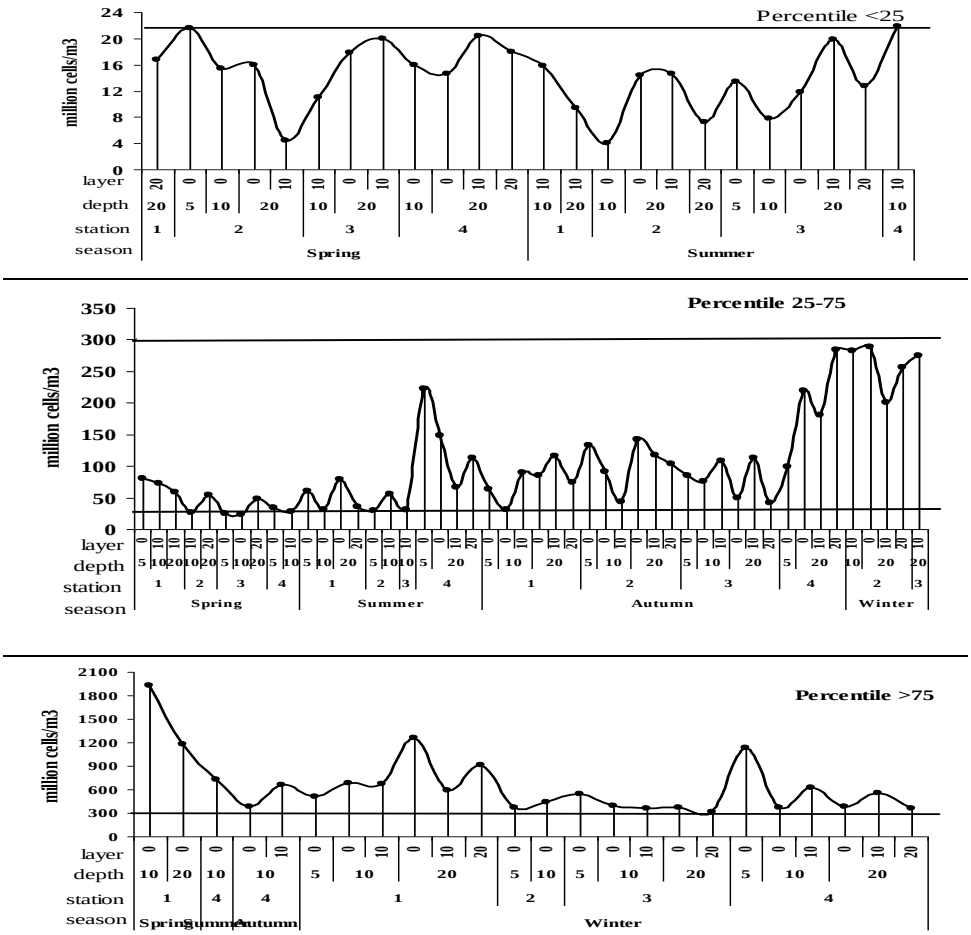

Figure 3: Phytoplankton abundance percentiles in the southern coasts of the Caspian Sea (2013-2014). 1: Anzali, 2: Tonekabon, 3: Nowshahr and 4: Amirabad.

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The maximum and minimum means of total phytoplankton abundance were recorded as 505±55 and 73±31 million cells m^{-3} in winter and summer, respectively. Mean total phytoplankton abundance was observed as 156±91 and 140±28 million cells m^{-3} during spring and autumn, respectively (Fig. 4). The abundance of Bacillariophyta formed more than 75% of the total abundance during all seasons (except summer). Chlorophyta with 8% contribution in the abundance was in the second order. In winter, the aforementioned values were 3 to 6 folds that in other seasons. The abundance of Chlorophyta stayed at a maximum during summer. The maximum abundance of fine size phytoplankton was indicated during autumn, but it was a major phylum during summer at Tonekabon and Amirabad transects (Fig. 5). The highest abundance of Cyanophyta in spring was observed in the Anzali transect. Mean phytoplankton abundance was not significantly different between layers and depths (ANOVA, $p>0.05$), while, mean phytoplankton abundance (total abundance, Bacillariophyta, Pyrrophyta, Cyanophyta, Chlorophyta and fine size phytoplankton) were significantly different between seasons (ANOVA, $p<0.05$).

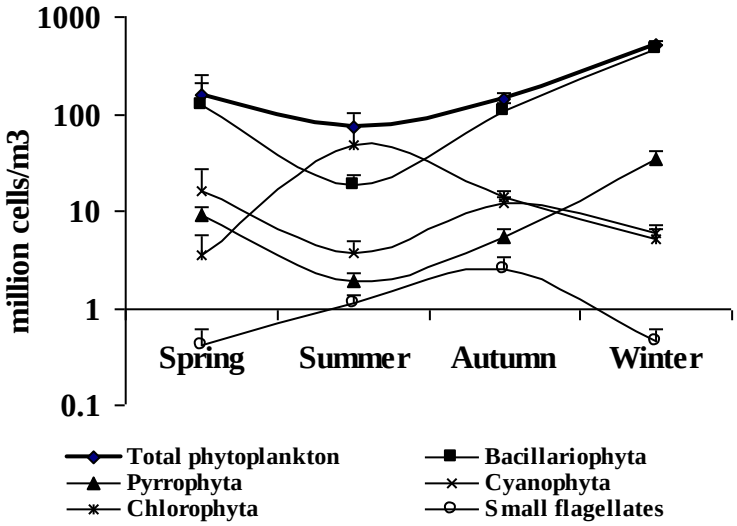

Figure 4: Mean total abundance of phytoplankton and major phyla (log scale) during different seasons in the southern coasts of the Caspian Sea (2013-2014).

In this study, 147 phytoplankton species were identified. The maximum number of species was recorded during spring (108 species) and in the Anzali transects (111 species) (Table 2).

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Table 2: Number of species of phytoplankton phyla at different seasons and transects in the southern coasts of the Caspian Sea (2013-2014).

Transect	Bacillariophyta	Pyrrophyta	Cyanophyta	Chlorophyta	Euglenophyta	*Other phyla	Annual
Anzali	51	13	19	20	5	3	111
Tonekabon	43	16	7	7	4	2	78
Nowshahr	37	18	9	8	3	2	76
Amirabad	36	16	15	8	5	2	81
Season							
Spring	51	14	18	17	6	2	108
Summer	40	16	12	9	2	2	81
Autumn	36	19	10	7	3	3	78
Winter	30	16	13	10	5	1	75
Annual	68	22	26	21	7	3	147

*Chrysophyta, Xantophyta

In total, sixteen species along with fine size phytoplankton contributed to more than 70% of phytoplankton abundance at different seasons and transects (Fig. 5).

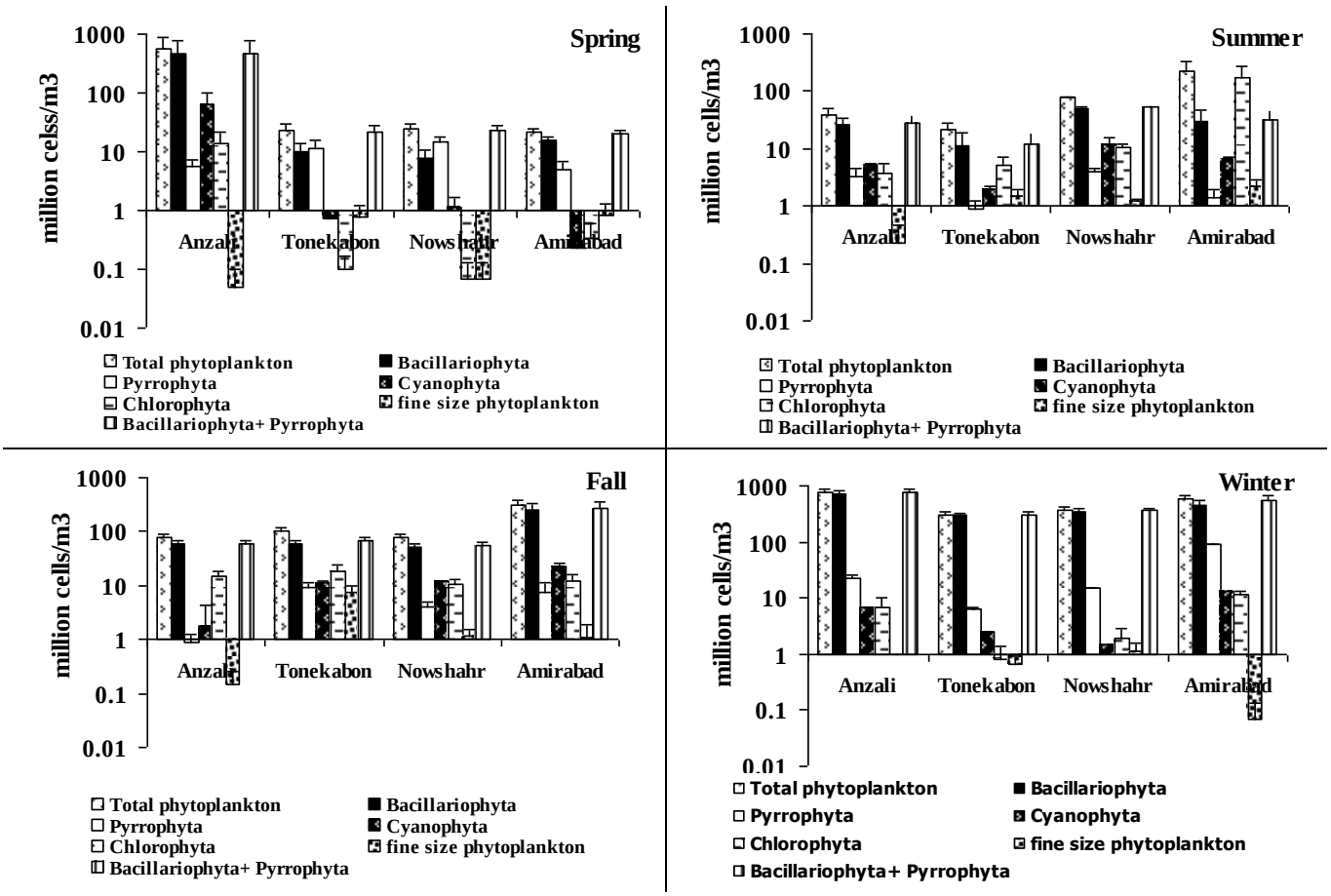

Figure 5: Seasonal mean total abundance of phytoplankton and major phyla (log scale) at different transects in the southern of Caspian Sea (2013-2014).

Bacillariophyta had the greatest number of dominant species in all seasons. Other phyla were represented by only 1 to 2 species in the dominant phytoplankton species list. The abundances of some dominant species (Fig. 6) were in threshold algal blooms category (Table 3). As shown in the

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table, some of the species such as *Oscillatoria* sp. (in spring), *Chaetoceros throndsenii* (in summer), *C. peruvianus* (in autumn), *Pseudonitzschia seriata* and *Cerataulina pelagica* (in winter) have harmful or toxigenic potential: The high threshold (>1000 million cells m^{-3}) blooms was represented by *Stephanodiscus socialis* with relative frequency of less than 4%. *P. seriata* was classified at medium threshold (100-1000 million cells m^{-3}) with the highest relative frequency (100%). The relative frequency of other dominant species in medium threshold blooms were from 4 to 17%.

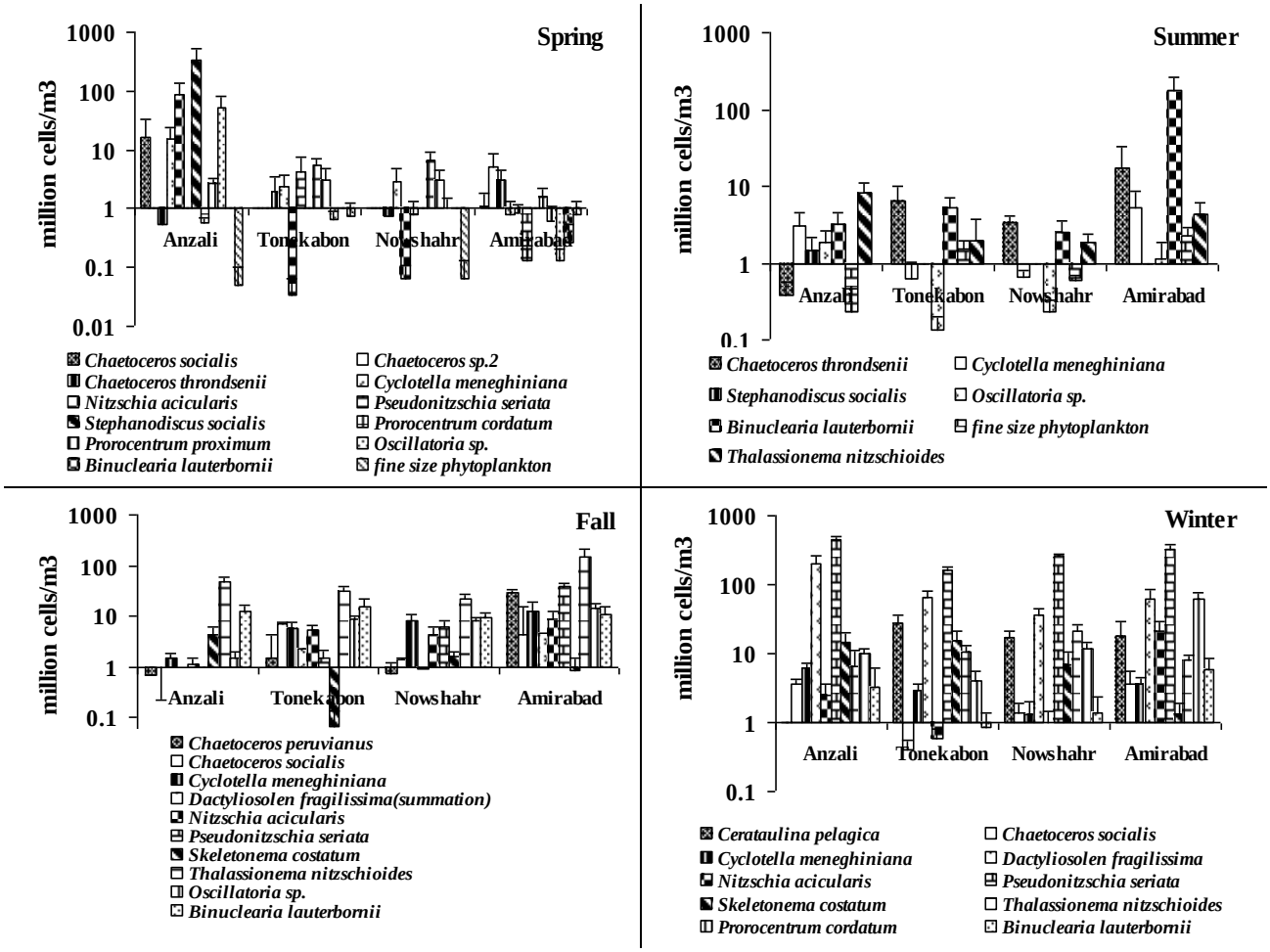

Figure 6: Seasonal mean abundance of dominant phytoplankton species (log scale) at different transects in the southern of Caspian Sea (2013-2014).

Table 3: Observed species in each (different) threshold blooms in the southern of Caspian Sea (2013-2014).

		Small Threshold (10-100 million cells m^{-3})		Medium threshold (100-1000 million cells m^{-3})		Large threshold (>1000 million cells m^{-3})	
Seasons	**Species**	**Transect**	**RPF**	**Transect**	**RPF**	**Transect**	**RPF**
Spring	*Chaetoceros socialis*	Anzali	8	-	-	-	-
	Nitzschia acicularis	Anzali	13	Anzali	8	-	-
	Cyclotella meneghiniana	Anzali	13	-	-	-	-
	Oscillatoria sp.	Anzali	8	-	-	-	-
	Stephanodiscus socialis	Anzali	13	-	-	Anzali	4
Summer	*Chaetoceros throndsenii*	Amirabad	8	-	-	-	-
	Binuclearia lauterbornii	Amirabad	17	Amirabad	13	-	-
Autumn	*Chaetoceros peruvianus*	Amirabad	17	-	-	-	-

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Table 3 continued:

	Cyclotella meneghiniana	Amirabad	8	-	-	-	-
	Pseudonitzschia seriata	Amirabad	33	-	-	-	-
	Thalassionema nitzschioides	Four transects	79	Amirabad	13	-	-
	Oscillatoria sp.	Tonekabon, Amirabad	38	-	-	-	-
	Binuclearialauterbornii	Anzali, Tonekabon, Amirabad	46	-	-	-	-
	Cerataulina pelagica	Tonekabon, Nowshahr, Amirabad,	46	-	-	-	-
	Dactyliosolen fragilissima	Four transects	75	Anzali	17	-	-
	Nitzschia acicularis	Amirabad	17	-	-	-	-
Winter	*Pseudonitzschia seriata*	-	-	Four transects	100	-	-
	Skeletonema costatum	Anzali, Tonekabon	33	-	-	-	-
	Thalassionema nitzschioides	Anzali, Tonekabon	33	-	-	-	-
	Prorocentrum cordatum	Tonekabon, Nowshahr	38	-	-	-	-

RPF=relative percent frequency

The annual median concentration of Chl-*a* was determined as 2.43 mg m^{-3}. The median concentration of Chl-*a* was higher than 2.43 mg m^{-3} at all transects in autumn, but in summer it was less than 2.43 mg m^{-3} at all transects. In spring and winter, the median concentration of Chl-*a* was higher than the annual median (2.43 mg m^{-3}) only in the Anzali transect (Fig. 7).

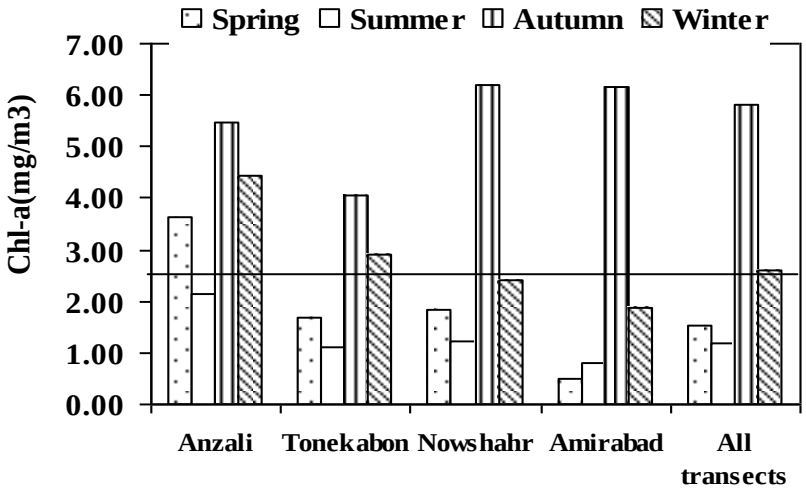

Figure 7: Median concentration of Chl-*a* (mg m^{-3}) in different seasons and transects in the southern of Caspian Sea (2013-2014) (vertical line is median threshold of Chl-*a* concentration (2.43 mg m^{-3}).

Discussion

The frequency, intensity, and distribution of HABs have increased during the past several decades mostly due to extra anthropogenic activities in

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the coastal waters (Anderson *et al*., 2002).

In the present study, a bloom once occurred at the Anzali transect (770 million cells m^{-3}) during spring based on total phytoplankton abundance and a bloom was formed twice at Anzali (756 million cells m^{-3}) and Amirabad (545 million cells m^{-3}) transects during winter based on Bacillariophyta and Pyrrophyta according to Revilla *et al*. (2009) definition. Chorus and Bartram (1999) stated that 200 million cells m^{-3} abundance of species is a sign of an early Cyanophyta bloom event. In the current study, the abundance of all Cyanophyta species was lower than 200 million cells m^{-3}. So no bloom phenomenon of Cyanophyta was recorded at all transects and seasons.

Most of the bloom species classified at small threshold blooms (10-100 million cells m^{-3}) and the highest relative frequency (>70%) belonged to *Thalassionema nitzschioides* and *Dactyliosolen fragilissima* in autumn and winter, respectively (Table 3). In general, the spatial distribution of bloom species in autumn and winter was more than in spring and summer. The species which were classified in medium threshold (100-1000 million cells m^{-3}) blooms in spring, summer, autumn and winter were (*Nitzschia acicularis* and *Stephanodiscus socialis*), (*Binuclearia lauterbornii*), (*T. nitzschioides*) and (*Pseudonitzschia seriata* and *D. fragilissima*), respectively. In the Amirabad transect there was at least one species with medium bloom category in all seasons (except in spring).

Some of the blooms species such as *T. nitzschioides* and *B. lauterbornii* are native and inhabitants of the Caspian Sea and their positive role in trophic chains is beneficial for the ecosystem (Pourgholam and Katunin, 1994). Only in spring, *S. socialis* was the single species in the large threshold blooms (>1000 million cells m^{-3}) at the Anzali transect. This might be related to the high nutrient concentration due to the influence of the Anzali Wetland and river inflows (Bagheri *et al*., 2014).

Chaetoceros throndsenii has a potential to bloom because of the influences of untreated urban wastewater (Livingston, 2002). Therefore, the increase of this species at the Amirabad transect may be a sign of degrading water quality during summer. At the Amirabad transect, warm coastal water produced from the cooling process of the Neka Power Plant and increasing amounts of nutrients from Gohar-baran river inflows (Pourgholam, and Katunin, 1994; Makhlough *et al*., 2012) probably influenced the increasing abundance of *C. throndsenii* and *Oscillatoria* sp. during summer and autumn, respectively.

The results showed that *P. seriata* was able to survive (with low abundance) in the warm seasons (spring and summer) of 2013-2014 in contrast to 2004-2010 years (Makhlough *et al*., 2011). Reproduction of *P. seriata* increased in autumn and winter. In cold seasons, vertical turbulence injected internal sources of nutrients to the water column (Kamburska *et al*., 2006; Nasrollahzadeh Saravi *et al*., 2014; Niyazi *et al*., 2016) that became

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available for psychrophilic phytoplankton such as *Pseudonitzschia* and *Cerataulina* (Skov *et al*., 1999; Makhlough *et al*., 2011). Increase in anthropogenic inputs such as agricultural fertilizers from the basin area might cause an increase in *P. seriata* and the abundance of other harmful species (Bates and Strain, 2006) in the Caspian Sea.

The proposed thresholds of blooms of other ecosystems are useful guidelines, but appropriate classifications are usually adopted according to local knowledge and prior monitoring history (Cullen, 2008). In the present study, the year of 1995-96 was considered as a reference value because the Caspian Sea environment was in an undisturbed condition according to several studies based on biotic and abiotic parameters (Nasrollahzadeh Saravi *et al*., 2015a, 2016; Pourang *et al*., 2016). So, in the present study (2013-2014), about 73 percent of the annual phytoplankton abundance, 50 percent of spring and 100 percent of abundance data in other seasons were considered as blooms with comparison to the reference value of 1995-96 (Table 4). In fact, the increasing trend of phytoplankton started in early 2001 (Makhlough *et al*., 2012) and has continued until now (Nasrollahzadeh Saravi *et al*., 2015a).

Table 4: Mean (±SE) of phytoplankton abundance (million cells m^{-3}) in the Iranian coast of the Caspian Sea.

	Spring	Summer	Autumn	Winter	Annual
1995-96	22±3	7±2	39±10	29±8	24±3
2013-14	156±91	73±31	140±28	505±55	219±33

However, considering the seasonal mean of phytoplankton abundance in 2013-14 (Table 4), useful and applicable results were achieved. The findings indicated that 8, 21, 25 and 40 percent of samples in spring, summer, autumn and winter were higher than seasonal means of phytoplankton abundance, which are considered as bloom conditions according to Schmidt and Schaechter (2011). The abundance of dominant species was equal or more than 10 million cells m^{-3} in bloom samples (Fig. 6). Most of the bloom species from regional scales were similar to blooms species (Table 3) in Anderson *et al*. (2010) method. Therefore, the Caspian Sea bloom data were supported by all classes of bloom thresholds (small, medium and large) proposed by Anderson *et al*. (2010).

The median concentrations of Chl-*a* in autumn were more than the annual median value (2.43 mg m^{-3}) at all transects. Therefore, seasonal bloom started in autumn, and continued during winter in the Anzali and Tonekabon transects. Based on the median concentration of Chl-*a*, the algal bloom in spring only happened in the Anzali transect. The small threshold blooms in Anderson *et al*. (2010) classification did not support any bloom event based on Chl-*a* concentration. In other words in the Caspian Sea, the overlapping of the two methods (median of Chl-*a* concentration and dominant species abundance) of bloom events was

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observed from the medium threshold blooms of Anderson *et al*. (2010) classification.

Chl-*a* concentration of water is affected by many physicochemical and hydrobiological factors (Nezlin, 2005) such as transparency depth and nutrient availability as well as phytoplankton composition and Chl-*a* content of dominant species (Reynolds, 2006). In this study, the median of Chl-*a* in winter was less than in autumn, while the abundance of total phytoplankton and dominant species in winter were higher than the values in autumn. This probably happened because of the low content of Chl-*a* in dominant species in winter, *P. seriata* (Hagstrom *et al*., 2011) compared with the dominant species *Thalassionema nitzschioides* in autumn, and less transparency depth in winter (Nasrollahzadeh Saravi *et al*., 2015c).

The study showed that the species which have an abundance of more than 10 million cells m^{-3} were in the bloom species lists based on the Caspian Sea data (Fig. 6) as well as Anderson *et al*. (2010) method (Table 3). Meanwhile, the bloom occurrence in a sample with species abundance of more than 100 million cells m^{-3} was justified by chlorophyll-*a* value. In addition, the high relative frequency and abundance of bloom species has played a significant role in the severity of the blooms as well as the high Chl-*a* concentration. There was a broad spatial and temporal distribution of *Pseudonitzschia* in the study. It seems that the highest risk of harmful algal blooms in the Caspian Sea was from *P. seriata*. Meanwhile, the density of other species in the community with harmful, toxic and invasive growth potential (such as *Oscillatoria* sp., *C. pelagica*, *C. throndsenii*, *C. peruvianus*) should be considered as a health risk to the Caspian Sea. In a conductive condition, a small but continuous seasonal abundance of the species may act as a seed for blooms to happen. It is important to point out from this paper that recurrence of *T. nitzschioides* in the dominant phytoplankton list of 2013-14 in the Caspian Sea is a positive point for the recovery of the Caspian Sea after ecological disturbances in several recent decades.

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