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Variations in Nutrient Concentration and Phytoplankton Composition at the Euphotic and Aphotic Layers in the Iranian Coastal Waters of the Southern Caspian Sea

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Abstract: Temporal variations and regional distributions of dissolved nutrients and their elemental ratios in the Iranian coastal waters of the Southern Caspian Sea were investigated. The data were collected in 1996-97 (Phase I, as a background data and undisturbed ecosystem) and in 2005 (Phase II, as a disturbed ecosystem) at sampling points (from 10 to 100 m depths). In addition to the two main sampling exercises, additional sample collections were carried out during the period of 1994 to 2004 as a long-term study. This study showed that the dissolved inorganic nitrogen/dissolved inorganic phosphorus (DIN/DIP) ratios in the southern Caspian Sea vary within a very narrow range (4.47 to 5.78) within the euphotic and aphotic layers and is by one order of magnitude lower than what have been reported for several other marine ecosystems. Phytoplankton growth seems to be nitrogen limited while the levels of P and Si always remain high. Factor Analysis/Principal Component Analysis (FA/PCA) of the correlation matrix showed that the nitrogen compounds are associated with the main factor accounting for 25.7-26.2% of the total variance for both the sampling periods. During Phase I, the Chrysophyta were the major group, whereas during Phase II the proportion of Chrysophyta in the total community progressively decreased, while the other groups increased.

Key words: Nutrients, redfield ratios, factor analysis/principal component analysis, phytoplankton composition, Caspian Sea, Iran

INTRODUCTION

The brackish Caspian Sea (CS) is a land-locked water body and its isolation from the world's oceans has enabled it to host several unique animal and plant species. Sea surface area of the CS, situated below sea level is close to 400,000 km^2 and its volume is approximately 78,000 km^3, or about 40% of the world's continental surface water (Leonov and Nazarov, 2001).

Nutrient pattern and elemental ratios in seawater may be used to identify peculiarities in ecosystem dynamics and functioning (Bethoux *et al*., 2002). Redfeld *et al.* (1963) documented that the molar ratios between nitrate, silicate and phosphate in marine phytoplankton is generally constant (N/Si/P = 16/16/1) and is known as the Redfield ratio. Redfeld *et al.* (1963) also elaborated that for optimal phytoplankton growth the N/Si/P ratio should also be 16/16/1, when nutrient levels are sufficient. This ratio has been confirmed empirically by Takahashi *et al.* (1985). Howarth (1988) reported that significant information on nutrient status can be obtained from the N/P ratio. A low value of less than 10 indicates potential N-limitation while high ratios of more than 20 suggests potential P-limitation for phytoplankton growth.

Several approaches such as bioassay experiments, concentrations of dissolved inorganic nutrients and nutrient inputs have been used to evaluate decline in phytoplankton growth due to nutrient limitation. Despite the fact that this approach (nutrient concentrations and their ratios) raises criticisms, it has been used widely by many authors. There is an extensive list of publications in which nutrient concentrations, together with their molar ratios (N/Si/P), have been used to suggest/infer nutrient limitation, as well as changes in the phytoplankton community assemblages (Justic *et al*., 1995; Bethoux *et al*., 2002; Moutin *et al*., 2002; Nedwell *et al*., 2002; Wang *et al*., 2003; Lane *et al*., 2004).

EACS (1996, 1998) and Laloei *et al.* (2002) reported that in the 1990s, the ecosystem of the CS in the Iranian coast was undisturbed and stable with oligotrophic status. Shiganova *et al.* (2003) and Kideys and Moghim (2003) suggested that the trophic status of the CS has suffered considerable changes after the introduction of the ctenophore species, *Mnemiopsis leidyi* in late 1999. Shiganova *et al.* (2003) also reported that under field and experimental conditions increase in phytoplankton abundance (in particular diatoms) in the presence of *M. leidyi* has been noted and *M. leidyi* has also been

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shown to directly affect the various hydrochemical parameters. According to Kremer (1977) and Deason and Smayda (1982), the ctenophore excretes nutrients as well.

A number studies have been carried out on the nutrient fluctuations and limiting factors in the north CS, but not much is known about the south. In this paper, we discuss the variations in the elemental ratio of N, P and Si within the euphotic and aphotic layers of the southern Caspian Sea - Iranian coast. The specific goals of this study are to answer the following questions:

- What is vertical nutrient distribution trend during Phase I and II and long-term study?
- What is the variation in the elemental ratio of N, P and Si within the euphotic and aphotic layers and the possible reasons for the deviations from the Redfield value?
- Does the variation of nutrient ratios affect on phytoplankton compositions during Phase I and II?

MATERIALS AND METHODS

Study area: The coastline of Iran is about 920 km long (Fig. 1). The area includes 3 regions from east to west namely Golestan, Mazandaran and Gilan with a combined population of about 6.4 million which constitutes 10% of the total population of the country. The coastal strip from Astara (Azerbaijan) to the eastern part of Golestan Province (Islamic Republic of Iran) receives more rainfall than any other coastal region of the Caspian Sea (CSN, 2003).

Data: The data for this study were collected during two studies on board the *R/V Gilan* (Ecological Academy of the Caspian Sea) in the Iranian coast of the CS within the framework of the hydrology and hydrobiology of the Southern Caspian Sea in May (spring), August (summer), November (autumn) and February (winter) of 1996-97 (Phase I) and 2005 (Phase II). The selected sampling stations were mostly located close to the coastal slope (Fig. 1). During Phase I, the study area was covered by 36 sampling stations along the West-East transect (9 transects) and during Phase II, the 24 stations were selected along 6 transects from West-East. During Phase I, samples were collected at 0, 5, 10, 20, 50 and 100 m depths (three replicate), but during Phase II, the sampling was focused at 0, 10, 20 and 50 m (three replicate) with additional samples collected at 100 m depths for some transects. In the present study, field data for the main period (Phase II, 2005) are nearly as comprehensive as field data for the Phase I (1996-97), in terms of both numbers of stations and transects occupied

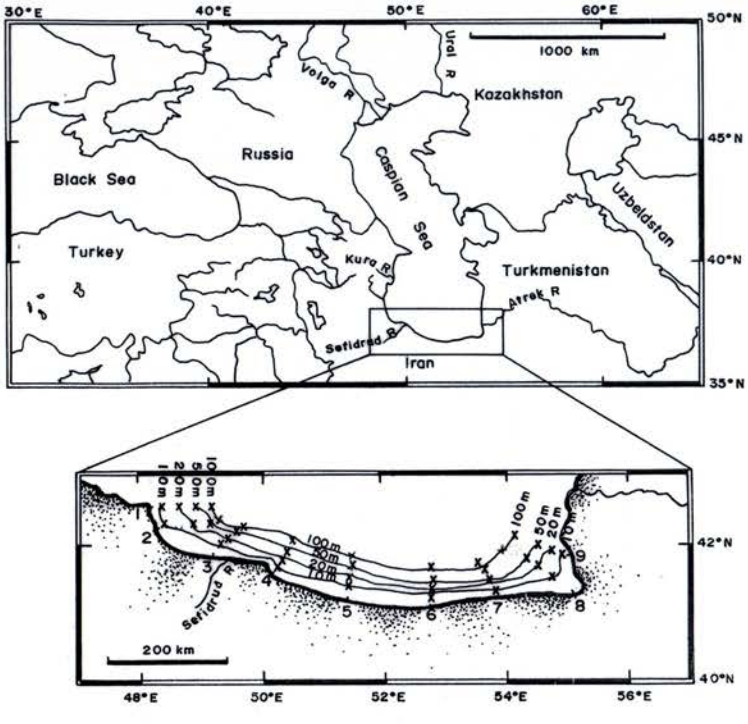

Fig. 1: Map of the study area showing transects and position of sampling stations during the R/V Gilan cruises

and numbers of water quality and biological parameters measured. In addition, data from 16 additional samplings which were carried out on a smaller scale by different research groups at the inshore area (10 m depth) were also used.

The euphotic depth (corresponding approximately to the depth to which 1% of surface irradiance reaches) was calculated as 3 times Secchi depth as suggested by Hayward (1987) and Psarra *et al.* (2000).

Sampling and analyses

Physico-chemical analyses: Water temperature was measured using a reverse thermometer (Hydrobios, Kiel-Holtenau, Germany) while salinity was determined using a salinometer (GM65, Moscow, Russia). Water samples were collected and taken to the laboratory at EACS (Ecological Academy of the Caspian Sea) and FRG (Fishery Research of Gilan) centers for analysis of dissolved oxygen (APHA, 1992) and nutrient contents. Wet combustion for the analysis of total phosphorus and nitrogen and the determination of ammonium nitrogen were carried out according to the methods of Strickland and Parsons (1972), Solorzano, (1969) and Valderrama (1981). Phosphate analysis was carried out according to the methods of Murphy and Riley (1962), while APHA (1992) method was used for the determination of nitrate concentration. Concentration of dissolved silicon (DSi) was determined using the method of Sapozhnikov *et al.* (1988).

Phytoplankton analyses: 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 (Salmanov, 1987). Phytoplankton present in a subsample of 0.1 mL (two replicate) was enumerated using a Sedgewick-Rafter cell under a binocular microscope (Nikon, Japan) (covering slip 24×24 mm and with magnifications of 10X, 20X and 40X) (Vollenweider, 1974; Newell and Newell, 1977; Sournia, 1978). The volume of each cell was computed by measuring its appropriate morphometric characteristics (including diameter, length and width (Vollenweider, 1974; Newell and Newell, 1977; APHA, 1992). Phytoplankton taxonomic classification was carried out based on Zabelina *et al.* (1951), Prescott (1962), Proshkina-Lavrenko and Makarova (1968), Tiffany and Britton (1971) and Habit and Pankow (1976).

Data processing and statistical analyses: Analysis of Variance (ANOVA) and the t-test were used to evaluate differences in environmental and biological parameters among the seasons and between the euphotic and aphotic zones as well as between the two periods and long-term study period. All tests were performed at 5% significance level. Factor Analysis/Principle Component Analysis (FA/PCA) was applied to score and narrow down the selection of parameters in testing similarity and differences between the two periods (Moncheva *et al.*, 2001). The physical, chemical and biological data obtained from the two sampling periods were used for statistical analyses, which were performed using SPSS 11.0 software. Twelve environmental variables were reduced into three and four common factors using the principal component analysis. The eigenvalues, which give the variance of the factor components, were used as criteria for determining significant changes (eigenvalues>1). For easier interpretation, factor axes were modified by factor rotation using varimax (Lau and Lane, 2002).

RESULTS

Physical characteristics of water: During Phase I, salinity varied seasonally between spring: 11.33-12.71, summer: 12.62-13.16, autumn: 11.72-12.78 and winter: 12.20-13.15 ‰. The water temperature for spring, summer, autumn and winter were 13.3-25.0, 17.9-28.9, 16.1-20.0 and 9.9-13.7°C, respectively. Dissolved Oxygen (DO) concentrations recorded for the area ranged between 5.47 and 7.50, 4.64 and 5.93, 5.40 and 6.52, 6.61 and 7.85 mg L^{-1} in spring, summer, autumn and winter, respectively. During Phase II, water salinity changed slightly with values of 11.70-13.51, 11.70-13.63, 11.82-12.64 and 11.20-12.77 ‰, respectively for spring, summer, autumn and winter. Temperature recorded during this period were slightly different with values of between 15.5-20.5, 21.3-28.9, 17.5-19.8 and 9.2- 11.7°C, respectively, for spring, summer, autumn and winter. DO levels recorded were 5.32-8.32, 5.18-7.41, 6.32- 9.93 and 5.85-7.61 mg L^{-1}, respectively, for the same seasons. Lowest concentrations of DO and high salinity were observed in summer during the both periods (Table 1).

The lowest values of transparency (Secchi depth) always prevailed at inshore (ranging from 0.5 to 1.5 m), while the highest values were measured at offshore (ranging from 10 to 13 m). The mean values and standard deviation of Secchi depth was 6.65±3.32 m during Phase I and 5.83±2.26 m during Phase II.

Table 1: Percentile distribution of the original data for the euphotic and aphotic layers during Phase I (1996-97) and Phase II (2005) in the Southern Caspian Sea-Iranian coast

	Phase I (1996-97)						Phase II (2005)					
Factors	5%	15%	25%	50%	75%	95%	5%	15%	25%	50%	75%	95%
NO_3^-	0.26	0.35	0.41	0.61	0.98	2.27	0.71	0.89	1.07	1.70	2.21	3.87
	0.22	0.41	0.49	0.79	1.12	4.02	0.57	0.86	1.12	1.65	2.21	4.63
NH_4^+	0.17	0.29	0.44	0.83	1.39	2.99	0.14	0.37	0.45	1.18	1.98	3.93
	0.16	0.28	0.40	0.79	1.29	2.62	0.13	0.31	0.38	0.67	1.26	3.63
DIN	0.56	0.78	1.03	1.50	2.24	5.60	1.15	1.54	1.95	2.95	4.06	7.13
	0.68	0.94	1.15	1.64	2.63	5.21	0.88	1.25	1.69	2.58	3.48	7.68
DON	3.35	7.54	14.71	23.96	45.45	74.65	35.13	39.08	43.62	49.22	55.39	69.08
	3.51	8.51	11.93	21.34	45.91	64.03	34.32	43.00	45.73	55.63	66.24	81.80
TN	4.36	8.22	15.59	27.26	48.00	73.17	38.59	43.52	46.12	53.12	59.00	74.15
	4.52	10.19	13.43	23.63	48.54	65.71	38.23	47.60	49.24	57.82	68.02	85.03
DIP	0.14	0.18	0.21	0.31	0.43	0.71	0.32	0.38	0.45	0.57	0.80	1.55
	0.13	0.16	0.18	0.32	0.46	0.75	0.26	0.33	0.37	0.55	0.75	1.24
DOP	0.13	0.18	0.25	0.45	0.69	1.38	0.40	0.61	0.69	1.09	1.43	1.86
	0.07	0.17	0.23	0.41	0.67	1.34	0.44	0.64	0.80	1.19	1.53	2.19
TP	0.37	0.43	0.52	0.77	1.12	1.99	1.05	1.27	1.38	1.95	2.44	3.72
	0.29	0.43	0.50	0.74	1.16	2.01	0.91	1.17	1.52	1.84	2.46	3.20
DSi	2.27	3.53	4.73	7.52	9.62	16.41	4.32	5.20	6.44	7.58	10.03	13.49
	2.75	4.31	5.23	7.81	10.43	18.12	3.26	5.33	6.27	7.62	10.04	12.46
DIN/DIP	1.64	2.38	3.09	5.38	7.95	18.85	1.97	2.33	3.41	5.39	7.31	16.57
	1.61	2.34	3.33	5.87	9.40	25.25	1.49	2.50	2.80	4.47	8.74	18.44
DSi/DIP	4.63	9.56	12.98	27.89	43.35	66.93	5.28	7.57	9.53	13.24	22.41	30.90
	5.02	9.27	15.24	27.32	45.08	88.38	4.65	7.95	10.71	15.17	21.43	35.17
Trans.	1.05	1.80	2.85	5.50	8.50	12.00	1.00	1.55	2.00	3.50	5.00	8.05
Temp.	12.01	12.88	13.54	18.63	24.59	27.84	10.66	11.67	17.60	19.65	25.33	28.00
	8.12	9.01	10.10	12.30	13.60	22.74	10.12	11.03	11.40	16.90	19.00	26.70
DO	5.08	5.41	5.76	6.29	6.90	7.25	6.05	6.48	6.59	6.92	7.43	8.76
	5.22	5.61	5.83	6.18	6.70	7.40	5.50	5.78	6.00	6.64	7.04	8.62
DO (%)	97.00	99.00	101.00	103.00	105.00	112.00	92.00	104.00	109.00	122.00	130.00	146.00
	73.00	80.00	85.00	94.00	101.00	107.00	82.00	88.00	90.00	106.00	118.00	137.00
Salinity	11.89	12.13	12.29	12.70	12.85	13.07	10.70	11.33	11.56	12.85	12.44	13.39
	12.15	12.43	12.51	12.74	12.85	13.13	10.83	11.41	11.69	12.17	12.49	13.36
pH	8.19	8.22	8.25	8.30	8.37	8.40	7.99	8.18	8.25	8.30	8.38	8.46
	8.09	8.14	8.18	8.26	8.31	8.38	8.10	8.18	8.22	8.30	8.37	8.46

Units in μM where applicable; Temp. (°C), Salinity (‰), Oxygen (%Saturation), DO (mg L^{-1}), Transparency (m). Numerator; Euphotic and Denominator; aphotic

Distribution of inorganic nutrients: Figure 2 shows the vertical distribution of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorus (DIP) concentrations at different seasons during Phase I and Phase II. In these figures the solid line denotes the trendline of each parameter.

Differences in the DIN concentrations between seasons during the two sampling periods were found to be significant ($p<0.05$). Differences in the DIN concentrations between depths during Phase I were also found to be significant ($p<0.05$) but not so for Phase II. Figure 2 shows that during Phase I, the DIN concentrations increase with depth during all seasons except winter, while during Phase II the DIN concentrations exhibit a descending trend, with the higher concentrations at the surface. During Phase II, the DIN concentrations were found to increase significantly (compared to Phase I) in summer, autumn and winter ($p<0.05$) but was quite stable in spring.

Differences in the DIP concentrations between seasons during the two periods were found to be significant ($p<0.05$). During Phase II, the differences in the DIP concentrations between depths were also found to be significant ($p<0.05$) but not so for the Phase I. During Phase I, the DIP concentrations show ascending trend with depth in summer and autumn while in spring and winter the changes were minimal (Fig. 2). During Phase II, the situation was somewhat reversed (decrease with depth) in spring, summer and winter while in autumn the values remained generally unchanged. If the Phase I and Phase II are compared by seasons, the results also show that the DIP concentrations (except for winter) had significantly increased.

The differences in the Dissolved Silicon (DSi) concentrations between seasons during both periods were found to be significant but not so for depths. During Phase I, the DSi concentrations increase with depth for all seasons but during Phase II, the increase was only significant in winter (Fig. 3). If the Phase I and Phase II are compared by seasons, the results also show that the DSi concentrations had significantly decreased.

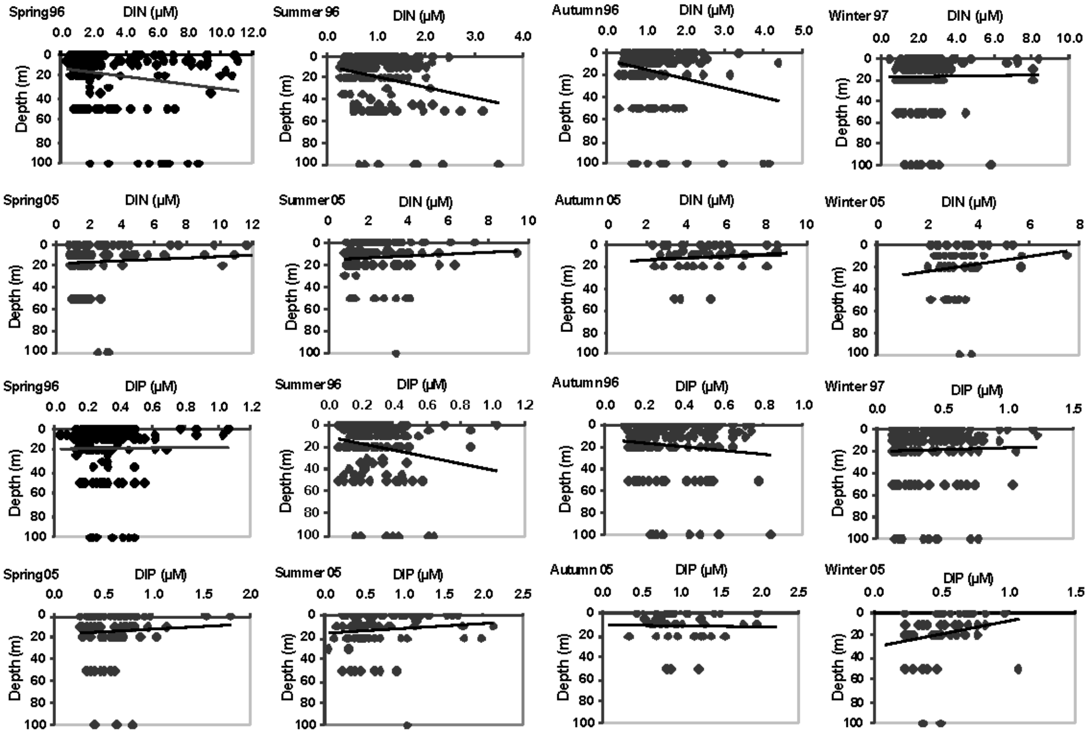

Fig. 2: Vertical distribution of DIN and DIP concentrations (µM) at four seasons during Phase I (1996-97) and Phase II (2005) in the southern Caspian Sea - Iranian coast. The trendlines show their linear regression

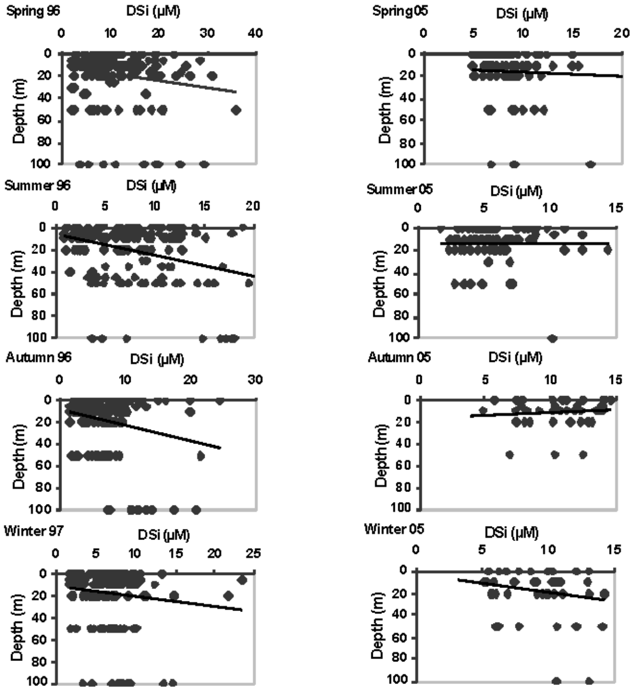

Fig. 3: Vertical distribution of dissolved silicon (DSi) concentration (µM) at four seasons during Phase I (1996-97) and Phase II (2005) in the Southern Caspian Sea-Iranian coast. The trendlines show their linear regression

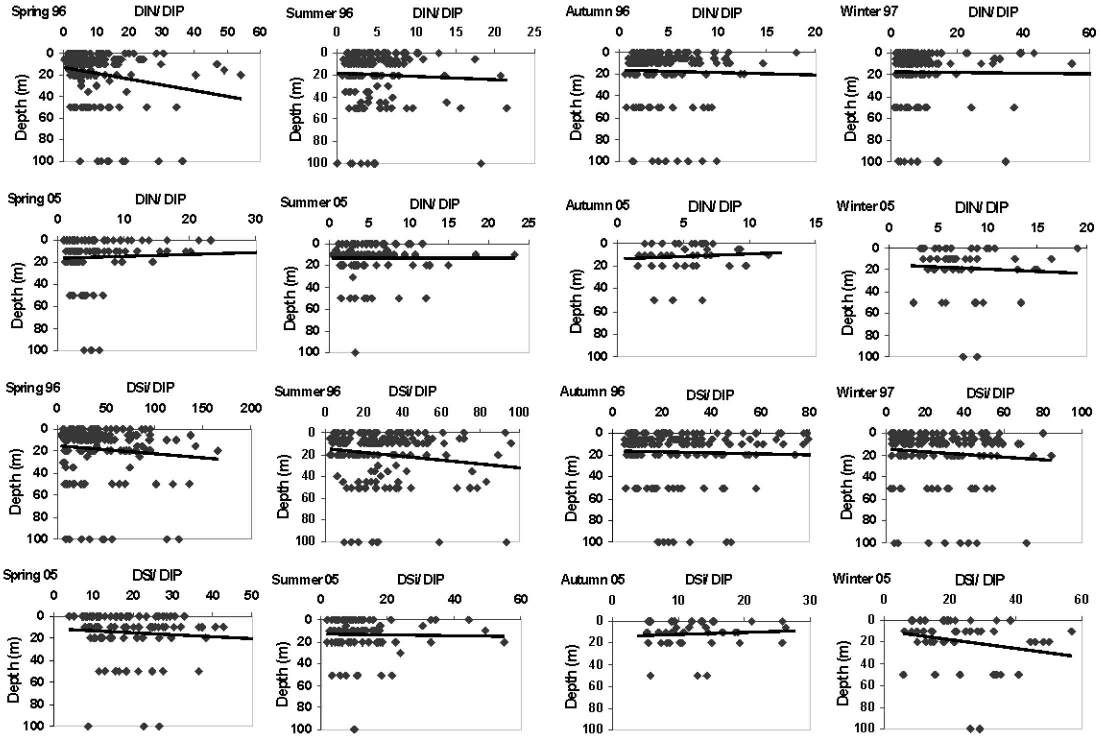

Fig. 4: Vertical distribution of DIN/DIP and DSi/DIP ratios at four seasons during Phase I (1996-97) and Phase II (2005) in the southern Caspian Sea - Iranian coast. The trendlines show their linear regression

Figure 4 shows the DIN/DIP and DSi/DIP ratios during different seasons and at different water depths. The differences in the DIN/DIP ratios were found to be significant between seasons during both the sampling periods ($p<0.05$) while between depths it was not significant ($p>0.05$). The same figure suggests that the DIN/DIP ratio increases with depth in spring 97 while during the other seasons there were only slight changes. The data also revealed that most of the ratios were less than the Redfield criteria especially during Phase II. During this period the DIN/DIP and DSi/DIP ratios were also found to be quite stable in value. However, the DIN/DIP ratio showed significant variations in spring.

The differences in the DSi/DIP ratio was found to be significant between seasons during both the sampling periods while between depths it was found to be significant only during Phase I ($p<0.05$). During Phase I, the DSi/DIP ratio increased with depth during spring and winter while in summer and autumn there were only minimal changes (Fig. 4). During Phase II, this ratio also increased with depth during spring, autumn and winter. Comparing the two periods by seasons, we found that these ratios significantly decreased (except in winter) from the Phase I to Phase II ($p<0.05$).

Scatter plots of the Phase I and Phase II for molar ratios DIN/DIP and DSi/DIN within the euphotic layer in the Southern CS are shown in Fig. 5. Stoichiometric N-limitation is observed for about 90% of the samples during Phase I (DIN/DIP<10, DSi/DIN>1). The data also indicate stoichiometric P-limitation in about 4% of samples and Si-limitation in about 3% (DIN/DIP>20, DSi/DIN<1). During Phase II, the DIN/DIP and DSi/DIN ratios indicate that about 92% of samples experience N-limitation (DIN/DIP<10, DSi/DIN>1) and less than 2% suffers P-limitation (DIN/DIP>20). As with the Phase I; the phytoplankton do not seem to have any problem with Si supply (DSi/DIN<1).

Long-term variation in nutrient concentrations: During the period from 1994 to 1997, the average DIP concentrations for the study area was found to be relatively high compared to the DIN value, leading to relative N deficiency for phytoplankton growth. During the decline stage of DIN/DIP ratio in 1996-97, both the DIN and DIP also decreased. The DIN/DIP ratio dropped sharply from 9 in 1994-95 to 4 in 1998-1999 and this value remained rather stable until 2005. The differences in the DIN/DIP and DSi/DIN ratios were not significant between

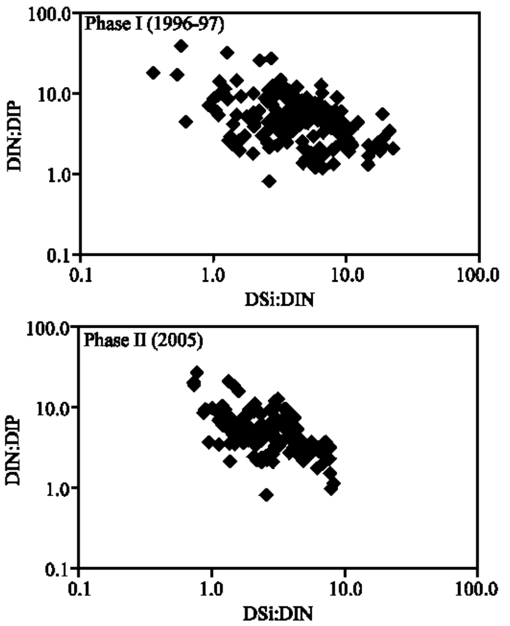

Fig. 5: Scatter diagrams of molar nutrient ratios within the euphotic layer during Phase I (1996-97) and Phase II (2005) in the Southern Caspian Sea-Iranian coast. The trendlines show their linear regression

the before the introduction of the ctenophore and after the introduction of the ctenophore (p>0.05). During this period, the DIN and DIP concentrations significantly increased after the introduction of the ctenophore (p<0.05).

The data from the long-term study showed that the DSi concentrations varied from 8.2 to 14.3 μM. During this period, minimal variation in the DSi concentrations were observed. The DSi/DIP ratios fluctuated from 11 to 24 which are higher than the Redfield criteria. Figure 6 shows that the highest DSi concentration was observed in 1998-99 and the highest DSi/DIP ratio was recorded in 1994-95. The lowest DSi concentration was recorded in 1996-97 while the lowest ratio was observed in 2005. During this period, the DSi/DIP ratios decreased significantly from the before the introduction of the ctenophore to after the introduction of the ctenophore (p<0.05).

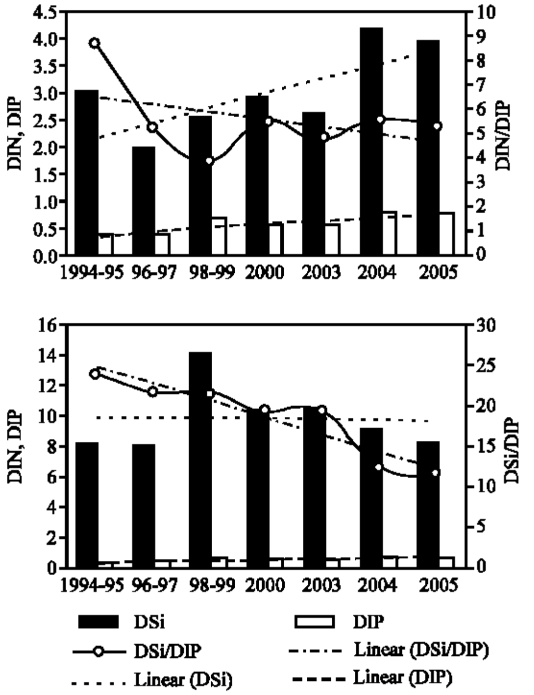

Fig. 6: Long-term changes in the DIN, DIP and DSi (μM) and their ratios (at 10 m depth) in the Southern Caspian Sea-Iranian coast. The data from 1994 to 1997 were adopted from EACS (1996, 1998), data from 1998 to 1999 from Laloei *et al.* (2002), data from 2000 to 2003 from Hashemian *et al.* (2004) and data from 2003 to 2004 from Tabari *et al.* (2005). The dashed lines show their linear regression

The phytoplankton community structure: During Phase I, the Chrysophyta made up more than 60% of the total phytoplankton population within the euphotic layer during spring, autumn and winter. During summer, however, the Chrysophyta was only dominant within the surface layer, making up more than 60% of the total phytoplankton population. At other depths within the euphotic layer (from 5 to 20 m) the group made up less than 30% of the population (Fig. 7). During this period, the Pyrrophyta was second in dominance at the different depths, followed by Chlorophyta. During Phase II, the relative dominance of the phytoplankton was quit different. In autumn the Chrysophyta made up almost 40 to 80% of the population at different depths while during the other seasons the relative abundance was from 10 to 50% only. During the same period, the second dominant group was Chlorophyta in spring and winter but in summer it was taken over by Cyanophyta. Unlike during the Phase I, the Pyrrophyta population was found to be very low in numbers (Fig. 7).

During Phase I, the differences in abundance and biomass of the total phytoplankton, Chrysophyta, Pyrrophyta and Chlorophyta (except Cyanophyta) between seasons within the euphotic layer were found to be significant (p<0.05). The data show that the abundance of total phytoplankton, Chrysophyta and Pyrrophyta decrease from spring to summer and increase in autumn and as well as winter. The abundance of Cyanophyta and Chlorophyta increases from spring to summer and this is followed by a decline in autumn and then increase again in winter (Table 2).

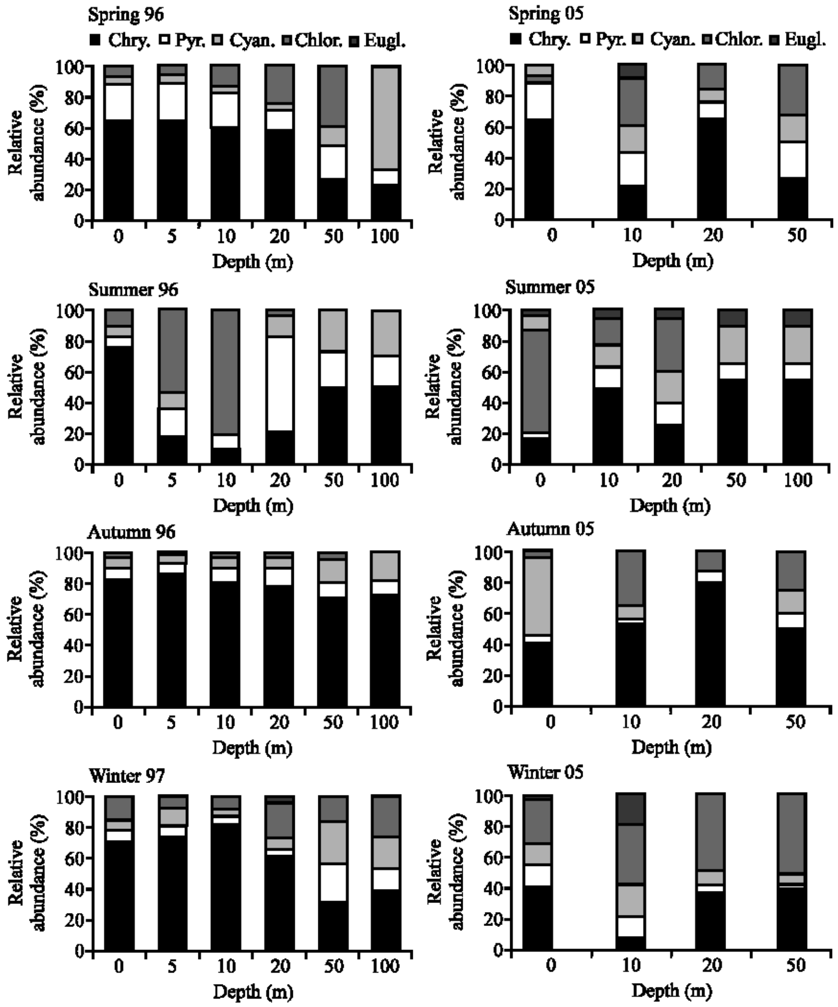

Fig. 7: Relative phytoplankton abundance during four seasons at different depths during Phase I (1996-97) and Phase II (2005) in the Southern Caspian Sea-Iranian coast

Table 2: The mean phytoplankton abundance (cells L^{-1}) and biomass (mg m^{-3}) within the euphotic layer at different seasons during Phase I (1996-97) and Phase II (2005) in the Southern Caspian Sea-Iranian coat

	Spring		Summer		Autumn		Winter	
Phytoplanktons	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
Chry.	7321	21194	2329	7334	11807	17146	14083	34769
	106.3	72.5	58.3	28.5	231.0	21.8	41.9	43.4
Pyrro.	4625	5237	2471	1634	1076	2100	1624	6319
	13.9	58.9	9.4	32.9	5.61	23.9	6.6	69.5
Cyano.	450	1730	970	11818	640	4917	450	3864
	2.8	0.62	2.70	12.4	0.30	5.4	5.7	8.9
Chloro.	1390	2994	4893	1647	202	8680	1948	8683
	0.03	1.4	0.3	5.2	0.09	13.0	0.08	14.52
Total phyt.	13786	31157	10664	22433	13149	32846	18106	53635
	123.0	133.0	71.0	80.0	237.0	64.0	54.0	136.0

Chry.: Chrysophyta, Pyrro: Pyrrophyta, Cyano.: Cyanophyta, Chloro.: Chlorophyta, Total phyt.: Total phytoplankton, Numerator, abundance and Denominator, biomass

During Phase II, the differences in the total abundance of phytoplankton, Chrysophyta, Pyrrophyta and Cyanophyta within the euphotic layer between seasons were found to be significant ($p<0.05$), but not so for Chlorophyta. Only differences in biomass by seasons of Chrysophyta and Pyrrophyta were found to be significant. The total abundance of phytoplankton, Chrysophyta, Pyrrophyta and Chlorophyta were found to

Table 3: Results of Principal Component Analysis (FA/PCA) are shown as factor loadings for the first four and three principle components after varimax rotated matrix during Phase I (1996-97) and Phase II (2005) in the Southern Caspian Sea-Iranian coast

	Phase I (1996-97)				Phase II (2005)		
	Factor loadings				Factor loadings		
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3
NH_4^+	0.67				0.80		
NO_3^-	0.74				0.71		
DIN	0.94				0.97		
DIP			0.85			- 0.82	
DSi		0.93				0.47	0.71
DON				0.82			-0.60
DOP			0.87				-0.58
DIN/DIP	0.85					0.95	
DSi/DIN	-0.40	0.71			-0.85		
DSi/DIP		0.85				0.92	
Temperature				-0.84			-0.73
Salinity	-0.34			-0.41		0.40	
Cumulative Variances (%)	26.20	45.50	59.30	72.90	25.70	51.30	68.60
Eigenvalues	3.40	2.52	1.79	1.77	3.08	3.07	2.08
N	539.00				254.00		

Loading with a magnitude >0.30 are shown. N: No. of data included in analysis

decrease from spring to summer which it increased in autumn and as well as in winter. During the same period, the abundance of Cyanophyta increased from spring to autumn and then declined in winter (Table 2). Just like during Phase I there was no clear trend in the changes in phytoplankton biomass during Phase II.

Comparing Phase I and Phase II by seasons, the results show that the total abundance of the phytoplankton and four other taxa had significantly increased (Table 2).

Statistical interpretation and analysis: In order to highlight the relationships existing between environmental parameters and the biological populations, data from a total of 539 samples within the euphotic layer for the pre-invasion period and 254 for the post-invasion period were statistically processed (Table 3).

To order the variables, Principal Components Analysis (FA/PCA) was applied using 12 environmental variables. The factor loadings, as correlations between the original variables and the principal components, indicated four and three separate factors for the Phase I and Phase II, respectively (Table 3). During Phase I, four factors (principal components) were extracted, which explained about 26.2, 19.3, 13.8 and 13.6% of the total variance with eigenvalues of 3.40, 2.52, 1.79 and 1.77, respectively. During Phase II, three factors explained 25.7, 25.6 and 17.3% of the total variance with eigenvalues of 3.08, 3.07 and 2.08, respectively.

The contribution of each variable during the pre-invasion period is shown in Table 3. The first factor, inorganic nitrogen (NH_4^+, NO_3^- and DIN) and its ratios (DIN/DIP and DSi/DIN) have high positive loadings. Salinity was shown to produce significant reverse influence (as shown by the negative loading). Positive loadings are shown by the DSi and its ratios (DSi/DIP and DSi/DIN) in Factor 2 and by the DIP and DOP in Factor 3. Strong negative loading for temperature and positive loading for DON are seen in Factor 4.

During Phase II, inorganic nitrogen and its ratios were the first factor as was also observed during Phase I (Table 3). During this period, the DIP is negatively represented in Factor 2, while the DSi has reduced in importance where it now represents Factor 3. The organic compounds (DON and DOP) with positive relationship with temperature are also represented in Factor 3. During Phase I, the organic compounds contributed to both Factor 3 and 4. During Phase II, salinity is shown to positively contribute to Factor 2.

DISCUSSION

During the long-term study it was found that the oxygen concentrations were very high within euphotic layer, where the DO was found to be oversaturated (105-122% saturation). The DO oversaturation at this layer was most likely caused by biological production where high population of phytoplankton was recorded. Below the 50 m depth the DO concentrations were about 80-100% saturated. Peeters *et al.* (2000) reported that at the deepest points of the south and central basin of the Caspian Sea, the DO concentrations are strongly undersaturated due to high oxygen consumption and can reach as low as about 2 and 3 mg L^{-1}, respectively (20 to 40% saturation at 800 m depth). The DO concentrations recorded during the long-term study in the southern CS-Iranian coast (EACS, 1996) agree with the values as reported by Peeters *et al.* (2000).

High NH_4^+ concentrations at the euphotic layer and low concentrations at the bottom were observed at most stations during Phase I and Phase II, which may be ascribed to the biological metabolism in the euphotic layer (Karl *et al*., 2001). During Phase I, the high concentration of NO_3^- observed at the bottom can be associated with the high level of dissolved oxygen which may cause oxidation of the NH_4^+ and NO_2^- (saturation>91%) (Liu *et al*., 2003). Low NO_3^- concentration at the euphotic layer on the contrary maybe due to the uptake by phytoplankton as reported by EACS (1996). During Phase II, the NO_3^- concentrations at the two layers did not change very much.

During Phase I, after vertical mixing which normally occurs in winter, the DIN concentrations increased during spring but declined in summer probably because of phytoplankton proliferation. In autumn, the DIN concentrations did not change even though there was vertical mixing. This probably happened because the increase in DIN concentrations was accompanied by an increase in phytoplankton growth. Similar observations were reported for the long-term study (1994-1999) and this was associated with the undisturbed condition of the Caspian Sea. A different scenario was observed during Phase II, where it was found that the DIN concentrations did not change very much during spring, autumn and winter, but increased slightly in summer. The mean concentrations of DIP and DSi throughout the water column were found to be almost stable. Furthermore, there was a marked difference between seasons in the depth profiles in the euphotic layer and no notable seasonal variation in the aphotic layer.

In the area where *M. leidyi* dwells, Shiganova *et al.* (2003) documented that the ratio of the organic compounds (especially nitrogen) to its mineralized forms will normally be high. Mucous, which is persistently excreted from the body surface of *M. leidyi* (a product of its vital activity) will cause an increase in the suspended organic matter in the water. In our study, data from 25 to 75 percentile shows that the DON/DIN ratio during Phase II varied from 21.5 to 34.4 as opposed to 10.4 to 17.4 for Phase I (Table 1). For organic phosphorous, the DOP/DIP ratio was found to vary from 2.1 to 2.3 during Phase II and from 1.23 to 1.5 during Phase I. Apparently, after the introduction of the ctenophore, the DON/DIN and DOP/DIP ratios increased which was probably associated with mucus excretion and secretion from *M. leidyi* as suggested by Kremer (1977) and Shiganova *et al.* (2003). In the present study, we also found that the water transparency decreased during Phase II (Table 1). This is again probably due to the increase in suspended organic compound resulting from the mucus excretion and secretion. The increase in the DON/DIN and DOP/DIP ratios were also found to be influenced by the discharge of the largest river in the Southern district (Sefidrud River) into the area which was found to decrease from 4 km^3 $year^{-1}$ (1958-1995, before the invasion) to less than 2 km^3 $year^{-1}$ (2002-2005, after the invasion) as reported by Lahijani (2004) and Momeni (2005).

Criteria for stoichiometric nutrient limitation were developed based on the nutrient requirements of Chrysophyta (Redfeld *et al*., 1963; Brzezinski, 1985). In present study, the Chrysophyta were dominant during most of the seasons during the long-term study period. Thus, this stoichiometric ratio is a good estimate to show the limitation of nutrients in the southern CS-Iranian coast. Moreover, studies on nutrient uptake kinetics have suggested that when the ambient molar ratio of dissolved DIN/DIP<10 and DSi/DIN>1, it indicates stoichiometric N-limited (Healey and Hendzel, 1979; Brzezinski, 1985; Levasseur and Therriault, 1987). In contrast, a DSi/DIN ratio<1 and DSi/DIP ratio<10 is indicative of Si-limitation (Harrison *et al*., 1977; Levasseur and Therriault, 1987), while a DIN/DIP ratio>20-30 suggests P-limitation (Goldman *et al*., 1979; Healey and Hendzel, 1979).

Harrison *et al.* (1977) and Nelson and Brzezinski (1990) proposed that stoichiometric limitation (N/P, Si/N and Si/P) should be considered together with the threshold nutrient uptake. Based on their studies on nutrient uptake kinetics, the concentration of DSi = 2.0 μM, DIN = 1.0 μM and DIP = 0.10 μM were selected as threshold values. During Phase I, it was found that about 12% of the DIN concentrations were less than the threshold value in summer and autumn but during Phase II, it was only 1%. The DIP and DSi concentrations were found to be more than the threshold values during both sampling periods. Therefore, DIN, DIP and DSi concentrations with its ratios have been used suitably to indicate nutrient deficiency for this area. In brackish water, Tamminen (1982) documented that the TN/TP-ratio is not a good indicator of the annual succession of the plankton community. This is because the organic forms of the nutrients are not directly utilizable by the plankton. And in the case of the study area, the organic form of the nutrients make up between about 50 to 90% of the total concentration.

Values of nutrient ratios have been used to infer nutrient deficiency (Howarth, 1988). During Phase I, the mean annual DIN/DIP, DSi/DIN and DSi/DIP ratios measured at the euphotic zone were 6.96, 5.63 and 29.81, respectively and these declined to 5.98, 3.08 and 15.24, respectively during Phase I. This decrease is mainly due to the relative increase in the mean values of DIN, DIP and DSi concentrations which were calculated to be 74, 85 and 0.8%, respectively. For the same reason, the DSi/DIN and DSi/DIP ratios were also found to significantly decrease from Phase I to Phase II.

Based on the DIN/DIP ratios calculated, we are of the opinion that phytoplankton growth in the area is N-limited and this is true for both sampling periods. Since the DSi/DIP ratios were always higher than the criteria value, Si is never found to be limiting.

During the long-term study, the DIN/DIP ratios did not show much fluctuation because the increase in DIN concentrations was also accompanied by an increase in DIP concentrations. Similar trend was also observed for the area after the introduction of the ctenophore. The DSi concentrations show a slight decline probably associated with a decrease in river input. The DSi/DIP ratios declined sharply (from 24 to 11) from 1994 to 2005 mainly due to the increase in DIP concentrations.

The DIN/DIP ratios calculated for various regions of the Caspian Sea indicate that P primary production is limited in the zone of influence of the Volga runoff, P and N primary production is limited for the other northern parts of the sea and N primary production mainly limited in the middle and south Caspian Sea (Leonov and Stygar, 2001; Shiganova *et al*., 2003). Long-term (1935-1988) changes in nutrient ratios (DIN/DIP) in the fore delta of the north Caspian Sea was reported to be from 29 to 67 (Kosarev and Yablonskaya, 1994). Semenov (1984) reported that the DSi/DIP ratios for the middle and south Caspian Sea varies between 8 and 100 which suggests no Si-limitation. The conditions of nutrient primary production vary in other seas as well. For instance, the ratio of mineral N/P in the Gulf of Riga (the Baltic Sea) is greater than 20 (the limiting element is P), whereas in the waters of the Baltic Sea proper and the Gulf of Finland, the primary production processes are controlled mainly by N (N/P~9). As shown by experiments, phytoplankton development in the Black Sea coastal waters P deficiency is more pronounced than that of N (Leonov and Stygar, 2001). In present study, P and Si were found to be in excess while phytoplankton growth was N-limited as similarly reported by Semenov (1984) and Leonov and Stygar (2001).

Sladecek (1963) stated that biological parameters vary over the long-term period, whereas the chemical variables are related only to the point and time of the sampling (short-term period). Thus we must try to find the relationship between these variables.

It is known that nitrate is an important nitrogen source for phytoplankton growth but phosphorous concentration should also be considered in determining possible limitations in primary production (Pearsall, 1932). In present study, all groups of phytoplankton growth (except Cyanophyta) were found to be positively correlated with nitrate but there was no significant correlation between phosphorus and phytoplankton density, which suggests that in the Southern CS-Iranian coast phytoplankton growth is only limited by nitrogen.

We have drawn attention to the fact that the process of eutrophication is accompanied by a shift in the existing qualitative and quantitative relationship between the major phytoplankton taxa. In more general terms, this signifies a relative decrease in the number of Chrysophyta and a relative increase in Pyrrophyta and green and blue-green algae. Another general change in the phytoplankton community is the increase in abundance of the smaller species during eutrophication (Turkoglu and Koray, 2002). The results of our study show that the Southern CS-Iranian coast ecosystem shifted from oligotrophic to meso-eutrophic from Phase I to Phase II (Nasrollahzadeh, *et al*., 2006a, b, c). During Phase II this event (changes in phytoplankton composition) was observed and the proportion of Chrysophyta in the total community progressively decreased, while the other groups (with smaller species) increased.

Variation in the nutrient ratios can cause changes to the phytoplankton community structure. Justic *et al*. (1995) reported that in the Baltic Sea, Kattegate, Dutch Waden Sea, North Sea and the Black Sea, significant bloom of non-siliceous algae was observed during periods of decline in DSi/DIP ratios. It is assumed that with decreasing Si/N and/or Si/P ratios, more N and P remain available for the growth of non-diatom biomass because silicate sets the limit for diatom growth (Sommer, 1994). During the long-term observation for this study, with decreasing of DSi/DIP ratios (From 25 to 11) abundance of the non-siliceous algae (Cyanophyta and Chlorophyta) increased as similarly reported by Sommer (1994) and Justic *et al*. (1995).

Pearsall (1932) reported that Cyanophyta are able to develop at very low concentrations of inorganic nutrients and this has been confirmed by Hutchinson (1967). This does not necessarily contradict to Vollenweider (1968) generalization that blue-green algae tend to be abundant in water bodies containing high amount of nutrients. This seasonal occurrence of nitrogen-fixing blue-green algae in the water body is consistent with the hypothesis that low DIN/DIP ratios typically favour the growth of blue-greens over other phytoplankton groups (Smith, 1983; Smith and Bennett, 1999). Based on Pearsall hypothesis, during Phase I when the CS stands in undisturbed condition, the DIN concentrations from spring to summer decreased within the euphotic layer with decreasing DIN/DIP ratio. There was also an increase in the abundance of the blue-green groups (5%) although the Chrysophyta remained the dominant group. This was similarly reported by

Makhlough and Nasrollahzadeh (2003). During Phase II, the DIN concentrations and DIN/DIP ratio slightly increased within the euphotic layer from spring to summer. There was also an increase in the abundance of the blue- green which was second in dominance (25%) after Chrysophyta. This increase is mainly associated with nutrient enrichment (N and P) as reported by Vollenweider (1968). During Phase II, a bloom of one species of Cyanophyta was reported by Soloviev (2005) in late August (summer) of 2005, which further confirmed the above discussion. Overall, during Phase II the abundance and diversity of phytoplankton increased within the euphotic layer.

FA/PCA identified four composite variables for the Phase I and three for the Phase II (Table 3). During Phase I, the first factor was composed of inorganic nitrogen and its ratios which were inversely related to salinity. The high input of water from the rivers seems to be the driving force for this relationship. In contrast, this relationship was not very important during Phase II because of the depleting water input from the rivers. In this case salinity was represented only in Factor 2. FA/PCA analysis also revealed that the nitrogen compounds, as expected, were the first factor with 25.7-26.2% variance for both periods as nitrogen is the limiting factor for phytoplankton growth in the area. During Phase I, the DON was found to be inversely related to temperature and this is because high rate of mineralization of organic compounds normally occurs with increasing temperatures. In contrast, during Phase II, the DON concentrations were found to be positively correlated to temperature. This is because the high temperature resulted in an increase in the abundance of *M. leidyi* which released more mucus secretion (and DON) into the water as reported by Kremer (1977) and Shiganova *et al*. (2003).

CONCLUSION

Significant differences in the mean nutrient concentrations were detected within the euphotic and aphotic layers of the southern CS-Iranian coast between the samples collected during Phase I and Phase II. During Phase II, DSi (natural source) concentrations were found to be quite stable but not so for nitrogen and phosphorous. The DSi/DIN and DSi/DIP ratios were found to be significantly reduced after the introduction of the ctenophore mainly as a result of increase in inorganic nitrogen and phosphorous. There was not much variation in the DIN/DIP ratio because generally the increase in DIN concentration was accompanied by an increase in DIP concentration as well. As in most other marine environment, phytoplankton growth in the Southern CS-ranian coast is greatly influenced by nitrogen concentration. FA/PCA analysis also revealed that the nitrogen compounds are the main factor influencing the growth of the phytoplankton.

Results of this study suggest that changes in phytoplankton, abundance and diversity can be associated with the introduction of the ctenophore, *M. leidyi*, as earlier reported by researchers for the middle and South Caspian Sea. Results of the long-term study showed that the Chrysophyta was almost always found to be the dominant group. This is because apart from the N and P being aplenty, the high supply of Si to the area resulted in the DSi/DIP ratio being always greater than the critical level of Redfield ratio. During Phase II, the dominance of the Chrysophyta was replaced by Chlorophyta and Cyanophyta which was due to the low availability of N.

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