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Multivariate analysis of water quality parameters and phytoplankton composition in the southern of Caspian Sea

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Abstract

This study was conducted to analyze the water quality parameters and biological data of the southern Caspian Sea during two phases, Phase 1 (1996-97) and Phase 2 (2005). The invasion of the southern Caspian Sea by the alien species (*Mnemiopsis leidyi*) occurred between the two phases. This study highlights the advantage of multivariate statistical analyses to better understand a complex water system. On the basis of a canonical discriminant function analysis (CDFA) on water quality parameters and phytoplankton abundance at the euphotic layers, the southern Caspian Sea can be discriminated into four groups that are related to the four seasons of the year. The results also showed that the most significant variables that play the principal role in the classification comprises of temperature, salinity, DO, SD, and nutrient variables. A principle component analysis (PCA) indicated that the variables within each factor (PCs) could vary among seasons. An overall analysis showed that the first factor comprises nitrogen compounds, denoting the importance of nitrogen in this area during the phytoplankton proliferation (in spring and autumn). During Phase 1 and 2 of the study, canonical correspondence analysis (CCA) suggested that the most of the dominant phytoplankton taxon can substantially tolerate fluctuation in water quality variables.

Keywords: Water quality, Biological data, Multivariate analyses, Caspian Sea, Iran

Introduction

Caspian Sea (CS) is the largest lake on the earth in terms of area and volume. It is a landlocked endorheic body of water. CS has recently experienced significant ecosystem changes due to the invasion of the alien ctenophore (*Mnemiopsis leidyi*) in late 1999 (Shiganova et al. 2003). These changes include increased turbidity and the occurrence of Cyanophyta blooms in the western and central region of the sea. To accurately understand these changes we need to understand the temporal patterns in the observed water quality parameters and phytoplankton abundance. Understanding the dynamics of a complex system such as CS, it often requires some degree of simplification (Boyer et al. 1999). To this end, during sampling periods we have used multivariate statistical methods such as the principle component analysis (PCA), canonical discriminant function analysis (CDFA) and canonical correspondence analysis (CCA).

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Over the last 20 years, multivariate statistical methods such as CDFA, PCA and CCA have been abundantly used to characterize physical, chemical and biological parameters and water quality for fresh- and seawater (e.g. Rencher 1992; Gower et al. 1994; Salmaso 1996; Momen and Zehr 1998; Suk and Lee 1999; Yung et al. 2001; Guler et al. 2002; Reghunath et al. 2002; Romano et al. 2004; Anazawa and Ohmori 2005; Valdes et al. 2005; Muxika et al. 2007; Yidana et al. 2008).

In the present work we have applied three multivariate techniques to assess temporal variations in water quality and biological parameters of the southern CS basin during two periods of study: Phase 1 (1996-97, corresponding to the pre-invasion phase, providing background data) and Phase 2 (2005, corresponding to the post-invasion phase).

Materials and methods

Sampling area

Sampling stations (36.99-38.18 N, 49.00-53.80 E), with a maximum depth of 100 m, are located about 10 and 20 nautical miles from the Iranian coast (Figure 1). Four cruises were carried out on board of the R/V Gilan once per seasons. During the two periods of study, samples were taken in spring (May), summer (August), autumn (November), and winter (February). Dissolved inorganic nutrients and phytoplankton samples were taken at the fixed depths of 0, 5, 10, 20, 50 and 100 m (standard levels, SL).

Water quality and phytoplankton analyses

Water quality was assessed from some physical and chemical parameters (transparency, temperature, salinity levels, and all nutrients contents) using the methods detailed in Nasrollahzadeh et al. (2008a).

Phytoplankton samples were kept in 0.5-L bottles and preserved using buffered formaldehyde to yield a final concentration of 2%. Methods of qualitative and quantitative analyses of phytoplankton are detailed in Nasrollahzadeh et al. (2008a). In order to find dominant species of phytoplankton the important species index (ISI) was calculated using the equation proposed by Rushforth and Brock (1991) (see Nasrollahzadeh, 2008 for details). The samples from all sites and years were subject to the same treatment for chemical and biological parameters.

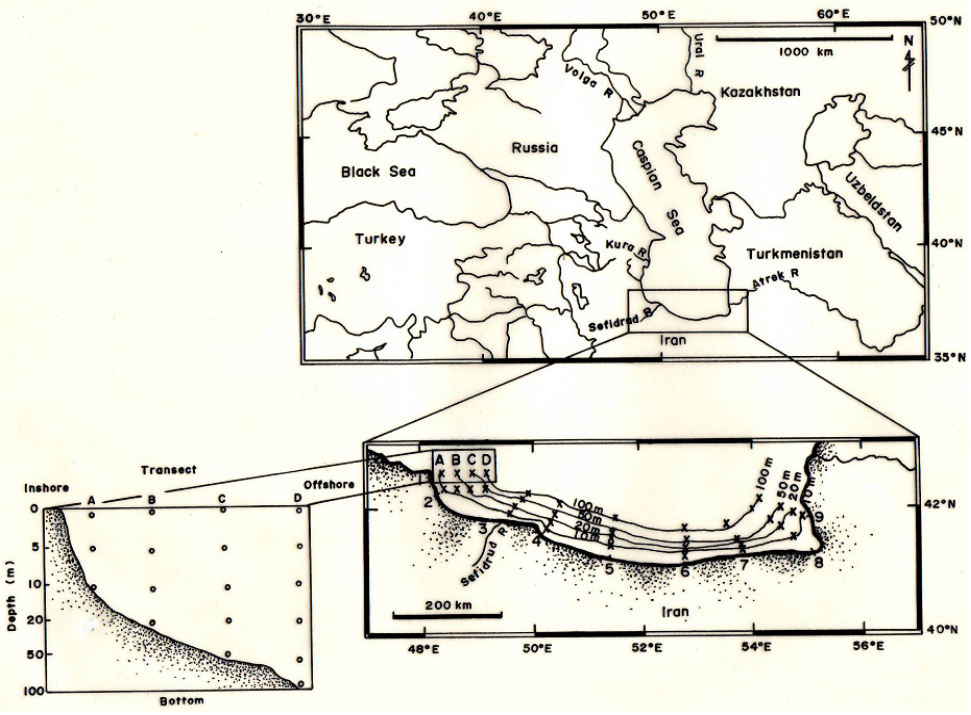

Fig. 1. Map of the southern Caspian Sea showing sites of sampling along nine (1-9) transects (labeled ×) and different depths of sampling.

Data treatment and testing for appropriateness

Four requirements should be met before applying canonical discriminant function analysis model (CDFA) to the datasets. The first is sample size: it is preferred to have at least four times as many observations as the number of independent variables. In this study, this was fulfilled. To meet the second requirement, i.e. that data should normally distribution, they were transformed to logarithms, Z-score or to Rankit. The normality of data distribution were tested using the Shapiro-Wilk test (the hypothesis of normality was rejected, $P < 0.05$). In this study, the results according to the Shapiro-Wilk test showed that the hypothesis of normality was not rejected. Using the Box's test, we showed that the datasets fit the third requirement, i.e. homogeneity of variance. Finally, all cases with outliers using box plot graph were eliminated.

To examine the validity and suitability of these data for the PCA, two widely used statistical tests, namely Kaiser–Meyer–Olkin (KMO) test which measure the sampling adequacy and Bartlett's tests, were performed (Anisworth, 2007). In this study, KMO coefficient was equal to 0.58 and to 0.56 during Phase 1 and Phase 2, respectively. Bartlett's test is used to test the null hypothesis that the variables are uncorrelated in the population (Hair et al. 1998).

To run a CCA, environmental data sets should be reduced to a maximum of n-1, where n is the number of sample sites. First, a series of CCAs were run. With each CCA run, the variable with the highest variance inflation factor (VIF) (indicating high collinearity with other variables) was removed from subsequent CCAs (Ter Braak, 1988). To reduce distortion in the CCA ordination, less weight was given to rare species in proportion to their frequency (r). In this study we calculated the ISI for phytoplankton species to eliminate rare species.

Results

Water quality parameters

Temporal variations of water quality parameters in southern CS during Phase 1 and Phase 2 are shown in Tables 1 and 2, respectively. Water temperature followed a seasonal cycle ranging from 10.9 °C (in winter, Phase 2) to 26.5 °C (in summer, Phase 1); seasonal average values over reached 19.0 °C in the four seasons (Tables 1 and 2). Temperature decreased in winter, dropped to less than 13.0 °C, and then gradually increased to over 18.0 °C in spring. Water temperature sharply increased from spring to summer, from about 18.0 °C to 26.0 °C. The annual mean temperature and salinity ranged between 19.1 °C and 19.7 °C and between 11.94‰ and 12.46‰ during Phase 1 and Phase 2, respectively.

The value of pH varied from 8.22 to 8.37 for both sampling periods, and showed slight differences between seasons. Dissolved oxygen (DO) content showed a seasonal cycle ranging from 5.32 mg/l (in summer, Phase 1) to 7.88 mg/l (in autumn, Phase 2), and the seasonal averages ranged between 6.29 to 7.16 mg/l during Phase 1 and Phase 2, respectively (Tables 1 and 2).

Dissolved inorganic nitrogen (DIN) concentration showed an annual cycle characterized by higher and lower values in spring and in summer, respectively, during Phase 1, whereas higher and lower values in autumn and in summer, respectively, during Phase 2 (Tables 1 and 2). NO_3^- and NH_4^+ accounted on average of 45.8% and 53.7% of the total DIN, respectively, during Phase 1 and of 55.4% and 44.3%, respectively, during Phase 2. Dissolved organic nitrogen (DON) concentration lowered to minimum value in summer and reached maximum values in winter during Phase 1 and Phase 2, respectively. Higher dissolved inorganic silicon (DSi) concentrations were recorded during the rainy seasons of spring and autumn during Phase 1 and Phase 2, respectively, whereas lower concentrations were recorded in summer. Seasonal mean dissolved inorganic phosphorus (DIP) concentrations ranged between 0.29 μM and 0.50 μM, being higher during rainy seasons (autumn and winter) as well. Dissolved organic phosphorus (DOP) concentration was low and showed minimal differences among the different seasons.

Phytoplankton structure

Temporal variations of the phytoplankton abundance in southern CS in Phases 1 and 2 are shown in Tables 1 and 2, respectively. In Phase 1, the annual variability of phytoplankton abundance ranged between 4.5 cells m/l (in summer) and 17.9 cells m/l (in winter), whereas in Phase 2, it was between 22.8 cells m/l (in summer) and 54.2 cells m/l (in winter) (Tables 1 and 2). Generally, the phytoplankton community was dominated by Bacillariophyta (diatoms) during both sampling periods. The annual mean percentages of Bacillariophyta (diatoms) were 79.3% and 69.8% during Phase 1 and Phase 2, respectively. Pyrrophyta (18.5%) was the second most abundant group during

Phase 1, while during Phase 2 it was Cyanophyta (25.3%). Other groups were always found in small percentages (less than 10%). During Phase 1, the total phytoplankton abundance was lower than Phase 2, with the annual mean cell abundance of 12.5 cells m/l and 33.3 cells m/l during Phase 1 and Phase 2, respectively.

The important species index (ISI) was calculated to assess dominant species seasonally and annually during both phases. During Phase 2, the three dominant species recorded are similar to those obtained for the ISI index of the year. This suggests that autumn is representative of the general pattern of dominant species in the study area. During Phase 1, two species, namely Exuviaella cordata and Rhizosolenia calcaravis recorded in summer, were similar to the annual species list.

Table 1. Temporal variation of water quality parameters and phytoplankton abundance at the euphotic layer during Phase 1 (1996-97) in the southern Caspian Sea of the Iranian coast

Parameters	Spring	Summer	Autumn	Winter	Entire period
NO_3^- (µM)	1.38 ± 1.34[a]	0.51 ± 0.24	0.67 ± 0.41	0.94 ± 0.91	0.87 ± 0.89
	(0.34 - 6.16)[b]	(0.16 - 1.01)	(0.16 - 1.71)	(0.35 - 4.98)	(0.16 - 6.16)
	135[c]	135	135	135	540
NH_4^+ (µM)	1.49 ± 1.86	0.55 ± 0.32	0.55 ± 0.31	1.47 ± 1.29	1.02 ± 1.10
	(0.11 - 8.63)	(0.08 - 1.21)	(0.10 - 1.59)	(1.05 - 7.55)	(0.08 - 8.63)
DIN (µM)	2.96 ± 2.24	1.05 ± 0.42	1.22 ± 0.60	2.41 ± 0.42	1.90 ± 1.55
	(0.75 - 9.05)	(0.46 - 2.01)	(0.46 - 3.30)	(0.46 - 2.01)	(0.46 - 9.05)
DON (µM)	34.6 ± 16.1	9.6 ± 7.0	34.2 ± 21.5	42.3 ± 24.0	30.2 ± 21.9
	(15.6 - 94.1)	(1.25 - 32.3)	(3.44 - 79.5)	(12.2 - 123.2)	(1.2 - 123.2)
DIP (µM)	0.35 ± 0.18	0.29 ± 0.12	0.33 ± 0.15	0.50 ± 0.64	0.37 ± 0.36
	(0.12 - 1.06)	(0.11 – 0.55)	(0.13 – 0.69)	(0.12 – 4.05)	(0.11 - 4.05)
DOP (µM)	0.61 ± 0.35	0.69 ± 0.51	0.39 ± 0.20	0.52 ± 0.70	0.55 ± 0.49
	(0.15 - 1.39)	(0.11 - 1.77)	(0.06 - 0.70)	(0.13 - 4.42)	(0.06 - 4.42)
DSi (µM)	10.8 ± 5.1	6.6 ± 3.5	7.1 ± 3.3	6.7 ± 2.5	7.8 ± 4.1
	(2.7 - 26.6)	(1.6 – 16.1)	(1.7 – 17.3)	(1.7 – 12.5)	(1.6 – 26.6)
Temp. (^oC)	18.5 ± 2.9	26.5 ± 1.9	18.7 ± 1.0	12.6 ± 0.9	19.1 ± 5.3
	(13.3 - 25.0)	(17.9 - 28.9)	(16.1 – 20.0)	(9.9 - 13.7)	(9.9 - 28.9)
Salinity (%)	12.08 ± 0.29	12.92 ± 0.14	12.60 ± 0.27	12.75 ± 0.23	12.58 ± 0.40
	(11.33 - 12.71)	(12.62 - 13.16)	(11.72 - 12.87)	(12.20 - 13.16)	(11.33 – 3.16)
pH (unit)	8.24 ± 0.03	8.29 ± 0.07	8.37 ± 0.20	8.30 ± 0.06	8.30 ± 0.07
	(8.17 - 8.31)	(8.15 - 8.46)	(8.32 - 8.40)	(8.11 - 8.40)	(8.11 – 8.46)
DO (mg/l)	6.66 ± 0.45	5.32 ± 0.32	6.16 ± 0.20	7.04 ± 0.25	6.29 ± 0.72
	(5.47 - 7.50)	(4.64 - 5.93)	(5.40 - 6.52)	(6.61 - 7.85)	(4.64 – 7.85)
Bacill.	7.80 ± 8.80	2.04 ± 3.62	13.52 ± 23.85	16.22 ± 36.61	9.91 ± 23.51
(cells m/l)	(0.40 – 40.77)	(0.05 – 18.73)	(0.85 – 99.67)	(0.50 – 180.20)	(0.05 – 80.20)
Pyr.	4.51 ± 6.28	1.73 ± 3.10	1.13 ± 1.32	1.81 ± 2.11	2.31 ± 3.94
(cells m/l)	(0.27 – 29.55)	(0.05 – 12.80)	(0.13 – 6.97)	(0.25 – 8.40)	(0.13 – 29.55)
Cyan.	0.17 ± 0.21	0.38 ± 0.50	0.14 ± 0.16	0.15 ± 0.21	0.20 ± 0.29
(cells m/l)	(0.05 – 0.90)	(0.05 – 1.35)	(0.03 – 0.67)	(0.05 – 0.80)	(0.03 – 1.35)
Chlor.	0.58 ± 0.58	5.63 ± 9.45	0.72 ± 0.78	0.82 ± 0.78	1.07 ± 2.77
(cells m/l)	(0.05 – 2.4)	(0.05 – 19.70)	(0.10 – 2.40)	(0.05 – 3.10)	(0.05 – 19.70)
Total phyt.	12.65 ± 11.86	4.48 ± 6.26	14.96 ± 24.48	17.86 ± 38.64	12.49 ± 24.11
(cells m/l)	(0.82 – 52.25)	(0.10 – 27.13)	(1.48 – 101.40)	(0.60 – 182.33)	(0.10 – 82.33)
Total phyt.	122.9 ± 144.2	136.4 ± 258.8	248.1 ± 259.4	52.0 ± 69.6	139.9 ± 209.6
(mg/m^3)	(15.17 – 555.1)	(3.68 – 1265.2)	(7.2 – 1172.3)	(0.92 – 338.5)	(0.92 – 265.2)

[a]Data are Means ± SD, [b]Bracket values indicate ranges of variation, [c] denotes the number of samples. Bacill.= Bacillariophyta; Pyr.=Pyrrophyta; Cyan.=Cyanophyta; Chlor.=Chlorophyta; Total phyt.=Total phytoplankton.

Canonical discriminant function analysis (CDFA)

In CDFA, spring, summer, autumn and winter are indicated as 1, 2, 3 and 4 respectively. As there are four seasons to be differentiated, we obtained three CDFs. During Phase 1, CDF1 accounts for the 60.7% of the between-season variance. CDF2 accounts for the 29.5% of the between-season variance. CDF3 accounts for the remaining 9.8% of total between-season variance. Each CDF is a linear combination of the 14 parameters and is orthogonal to the other. The significant canonical correlation between seasons and CDF1, CDF2 and CDF3 were 0.932, 0.874 and 0.719, respectively. During Phase 2, CDF1, CDF2 and CDF3 account for the 78.8, 15.0 and 6.1 percent of the

between-season variance. The significant canonical correlation between seasons and CDF1, CDF2 and CDF3 were 0.948, 0.749 and 0.641, respectively. Overall, more than 98% of the cases are classified correctly during two sampling periods, thus suggesting that the CDFA was satisfactory for the datasets.

Table 2. Temporal variation of water quality parameters and phytoplankton abundance at the euphotic layer during Phase 2 (2005) in the southern Caspian Sea of the Iranian coast

Parameters	Spring	Summer	Autumn	Winter	Entire period
NO_3^- (µM)	2.07 ± 1.56[a]	1.64 ± 0.95	2.02 ± 0.56	1.86 ± 0.38	1.91 ± 1.11
	(0.69 - 7.01)[b]	(0.45 – 3.83)	(1.12 - 3.30)	(1.13 – 2.51)	(0.45 - 7.01)
	74[c]	74	60	74	282
NH_4^+ (µM)	1.01 ± 1.02	1.01 ± 0.79	2.85 ± 1.09	1.79 ± 0.86	1.53 ± 1.19
	(0.12 - 4.62)	(0.07 - 3.41)	(1.13 - 5.17)	(0.56 - 3.91)	(0.11 - 5.17)
DIN (µM)	3.07 ± 2.35	2.65 ± 1.29	4.88 ± 1.45	3.65 ± 1.02	3.45 ± 1.92
	(0.82 - 11.63)	(1.09 - 7.12)	(2.77 – 8.47)	(2.39 – 6.29)	(0.82 - 11.63)
DON (µM)	48.5 ± 9.1	58.0 ± 11.3	42.7 ± 7.8	46.2 ± 6.4	50.2 ± 11.2
	(32.2 – 70.3)	(43.7 – 103.0)	(26.8 – 53.4)	(34.1 - 59.4)	(28.8 - 103.0)
DIP (µM)	0.56 ± 0.25	0.70 ± 0.36	0.97 ± 0.36	0.55 ± 0.14	0.70 ± 0.34
	(0.31 - 1.55)	(0.29 - 1.87)	(0.53 - 1.69)	(0.35 – 0.79)	(0.31 - 1.87)
DOP (µM)	1.09 ± 0.48	1.21 ± 0.41	1.18 ± 0.47	0.75 ± 0.32	1.09 ± 0.47
	(0.34 - 2.27)	(0.45 - 2.18)	(0.27 - 1.83)	(0.34 - 1.45)	(0.27 - 2.27)
DSi (µM)	8.8 ± 4.0	6.0 ± 2.1	10.7 ± 2.4	9.5 ± 2.2	8.3 ± 3.46
	(6.3 - 28.5)	(2.9 – 12.5)	(6.3 - 14.0)	(6.9 - 12.6)	(0.6 - 6.5)
Temp. (^oC)	18.7 ± 1.4	26.3 ± 1.7	18.9 ± 0.7	10.9 ± 0.8	19.7 ± 5.3
	(15.5 - 20.5)	(21.3 – 28.9)	(17.5 - 19.8)	(9.2 - 11.7)	(9.2 - 28.9)
Salinity (‰)	12.23 ± 0.74	12.88 ± 0.75	12.24 ± 0.54	12.50 ± 0.79	12.46 ± 0.71
	(11.70 - 13.51)	(11.00 - 13.63)	(11.82 -12.46)	(11.20 - 12.77)	(11.00 - 13.63)
pH (unit)	8.31 ± 0.11	8.31 ± 0.14	8.23 ± 0.06	8.22 ± 0.19	8.29 ± 0.2
	(7.80 - 8.41)	(7.99 - 8.56)	(8.12 - 8.34)	(7.69 - 8.52)	(0.6 - 6.5)
DO (mg/l)	7.17 ± 0.63	6.66 ± 0.45	7.88 ± 1.25	7.00 ± 0.49	7.16 ± 0.08
	(5.32 – 8.32)	(5.18 – 7.41)	(6.32 – 9.32)	(5.85 - 7.61)	(5.18 - 9.32)
Bacill.	32.30 ± 59.26	5.48 ± 7.12	16.91 ± 17.22	38.16 ± 41.33	23.22 ± 39.70
(cells m/l)	(0.60 – 193.20)	(0.20 – 28.40)	(0.83 – 57.85)	(1.00 – 72.00)	(0.60 – 172.00)
Pyr.	4.78 ± 4.58	1.46 ± 1.12	1.65 ± 3.82	8.01 ± 10.02	3.98 ± 6.67
(cells m/l)	(0.10 – 16.27)	(0.20 – 3.27)	(0.20 – 14.30)	(0.05 – 35.60)	(0.05 – 35.60)
Cyan.	1.57 ± 1.64	15.77 ± 36.02	6.91 ± 12.07	9.32 ± 24.30	8.39 ± 24.12
(cells m/l)	(0.10 – 4.88)	(0.07 – 137.00)	(0.07 – 33.23)	(0.07 – 90.07)	(0.07 – 137.00)
Chlor.	2.12 ± 2.34	1.01 ± 0.74	0.12 ± 0.34	7.20 ± 7.09	2.61 ± 2.82
(cells m/l)	(0.80 – 8.20)	(0.07 – 2.10)	(0.80 – 1.20)	(0.20 – 21.87)	(0.07 – 21.87)
Total phyt.	34.06 ± 60.71	22.75 ± 33.73	22.21 ± 23.85	54.15 ± 51.32	33.29 ± 47.21
(cells m/l)	(0.47 – 203.20)	(0.97 – 139.50)	(1.17 – 78.15)	(2.13 – 200.00)	(0.47 – 203.20)
Total phyt.	120.7 ± 115.4	61.1 ± 41.5	44.9 ± 57.7	116.9 ± 87.3	88.15 ± 87.29
(mg/m^3)	(0.02 – 454.8)	(7.6 – 132.6)	(3.4 – 201.0)	(3.4 – 330.7)	(0.02 – 454.8)

[a]Data are Means ± SD, [b]Bracket values indicate ranges of variation, [c] denotes the number of samples. Bacill.= Bacillariophyta; Pyr.=Pyrrophyta; Cyan.=Cyanophyta; Chlor.=Chlorophyta; Total phyt.=Total phytoplankton.

For the both sampling periods the results showed that temporal CDFA is satisfactory for the datasets. Since eigenvalues are significant, percentage of variance greater than 5 is significant, cumulative to approximately 75% is significant, a canonical correlation of greater than 0.60 and a probability p value ($P < 0.05$) is significant. In the CDF scatter plot, seasons form four non-overlapping groups, as shown by the territorial map (Figure 2). Temperature, DO and DON are the most significant variables in the discriminant function, which indicates their role in the classification of the three functions or four groups during Phase 1. During Phase 2, the most significant variables in the discriminant function are temperature, DO, NH_4^+ and NO_3^-, which indicates their role in the classification of the three functions or four groups.

Temporal canonical discriminant coefficients of phytoplankton datasets for three functions during Phase 1 showed that Chlorophyta, followed by Bacillariophyta, were the most significant variables in the discriminant function, which indicates their role in the classification of the three functions or four groups. In this phase,

Euglenophyta reach the minimum CDF coefficient. During Phase 2, the most significant variables in the discriminant function are Cyanophyta, followed by Bacillariophyta, which indicates their role in the classification of the two functions or three groups. During this period, the Chlorophyta reach the minimum CDF coefficient.

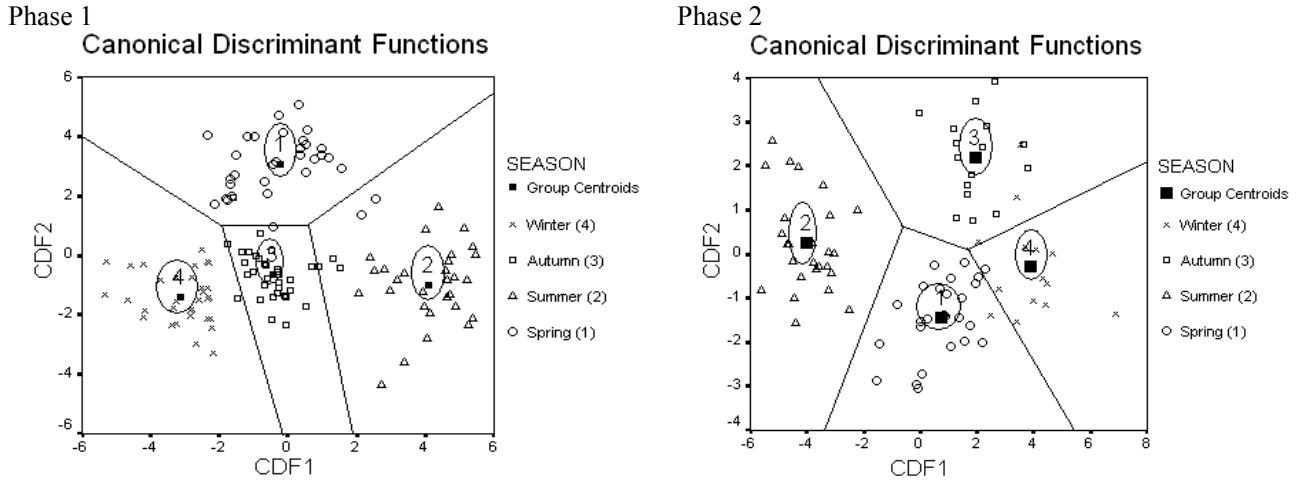

Fig. 2. Scatter plot and territorial map (indicated by lines) of temporal canonical discriminate function analysis based on physico-chemical parameters in different seasons during Phase 1 and Phase 2 in the southern Caspian Sea of the Iranian coast

Principle Component Analysis (PCA)

PCA was applied to the 11 variables collected during four seasons (Temperature, salinity, DO, pH, SD, NO_3^-, NH_4^+, DON, DIP, DOP, DSi) (Table 3). The result of annual PCA test showed that three main factors were enough to explain more than 60.0% of cumulated variance during Phase 1. PC1 and PC2 account for 29.5% and 19.2% of the total variance, respectively. PC1 explained organic and inorganic nitrogen compounds with physical (temperature and salinity) and biological/photosynthesis (DO) factors, while PC2 included DSi and DIP. PC3 explained relatively lower variance (11.2%) with a moderate negative loading on biological/photosynthesis factor (pH). The annual PC during Phase 2 showed that PC1 (26.5% of the variance) depends on inorganic nitrogen compounds and moderate loading on DSi. PC2 (16.6% of the variance) is characterized by physical (temperature) and biological/photosynthesis (SD) factors. PC3, explaining about 15.7% of variance, has a strong negative loading on DIP. The loading of biological/photosynthesis factor (DO) is 0.598 in PC4.

Table 3. Temporal PCA results from correlation matrix during Phase 1 and Phase 2 at the euphotic layer in the southern Caspian Sea of the Iranian coast

Phase 1				Phase 2			
Eigenvalue	VFs	Loading	Total Variance (%)	Eigenvalue	VFs	Loading	Total Variance (%)
3.56	NH4+	0.779	29.5	3.63	NH4+	0.825	26.5
	DON	0.701			NO3-	0.720	
	DIN	0.699			DIN	0.725	
	Temperature	-0.659			DSi	0.637	
	Salinity	-0.622					
	DO	0.672					
				3.09	Temperature	-0.796	16.6
2.85	DSi	0.806	19.2		SD	0.789	
	DIP	0.660			Salinity	-0.622	
2.05	pH	-0.566	11.2	2.17	DIP	0.871	15.7
					DON	0.774	
				1.80	DO	0.598	8.4
			60				67.2

Canonical corresponding analysis (CCA)

The Canonical Correspondence Analysis (CCA) was done on nine environmental variables, namely nutrient variables (NO_3^-, NH_4^+, DON, DIP, and DSi), biological variables (DO and SD) and physical variables (salinity and temperature) during both phases (Figures 3 and 4). These were the variables with the strongest loading factors in the PCA and thus the most relevant for differentiating samplings.

During Phase 1, CCA was run with eight variables. Among these variables the temperature and DO comprised the highest variance inflation factor (VIF). DO was eliminated instead of temperature because, on the one hand, CS temperature is more important than DO and, on the other, DO concentration is high meaning oversaturation. Therefore, the remaining variables could effectively explain the greatest amount of variance in the species datasets. The result showed scores scale by species and VIF for the remaining variables with stable canonical coefficient and variables contain VIF less than 10. In this period, the DON had highest weighted mean, followed by temperature and salinity. The minimum weighted mean belongs to DIP.

During Phase 2, CCA was run with five variables. All five variables explained the greatest amount of variance in the species datasets. The result showed scores scale by species and VIF for variables with stable canonical coefficient and variables contain VIF less than 10. In this period, temperature reached the highest weighted mean, followed by DSi, whereas the minimum weighted mean was reached by NH_4^+.

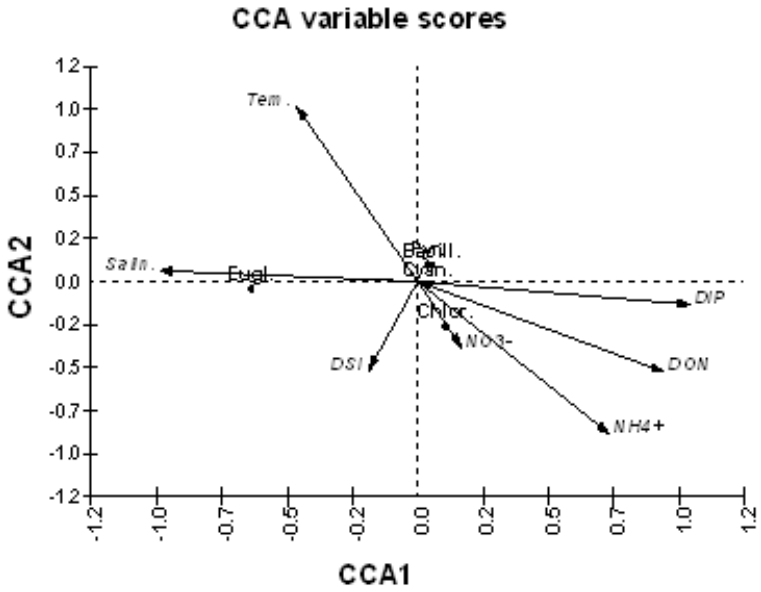

Fig. 3. Canonical correspondence analysis ordination showing the five groups of phytoplankton with respect to the environmental variables, relatively to axes 1 and 2 during Phase 1 in the southern Caspian Sea of the Iranian coast. Cumulative percentage variance explained by axes: Species-I = 19.2% and I+II = 28.0%. Key Notes: Refer to Tables 1 and 2.

During Phase 1, when seven important variables were analyzed, CCA confirms the importance of salinity (r with axis1 (CCA1) = -0.67) and DIP (r with axis1 (CCA1) = 0.51), in explaining the variance in the phytoplankton groups data. Axis 2 (CCA2) is negatively correlated with NH_4^+ (r = -0.60) and DSi (r = -0.57), whereas it is positively correlated with temperature (r = 0.60). Species-environment correlations are almost high, 0.66 and 0.56 for the first and the second axis, respectively. The Bacillariophyta, Pyrrophyta and Cyanophyta were placed near the origin of the ordination diagram, meaning that these phytoplankton taxa were belonged to all samples and were not associated to any environmental variable. Chlorophyta seemed to be related to sites and seasons with lower temperature and higher NO_3^-, NH_4^+, DON and DIP. Euglenophyta were positively correlated with salinity. During Phase 2, when five important variables considered were, CCA confirms the importance of all variables except SD with a high correlation coefficient (r with 0.65 to 0.84) in CCA1, in explaining the variance in the phytoplankton data. Axis 2 (CCA2) is negatively correlated with SD (r = -0.51). The five phytoplankton taxa and the five environmental variables for CCA in axis 1 (0.95) and axis 2 (0.51) were correlated. Bacillariophyta, Pyrrophyta and Cyanophyta were placed near the origin of the ordination diagram, meaning that these taxa were present in all

samples and were not associated to any environmental variable. Chlorophyta seemed to be related to sites and seasons with lower NH_4^+. Euglenophyta were found to be positively correlated with temperature.

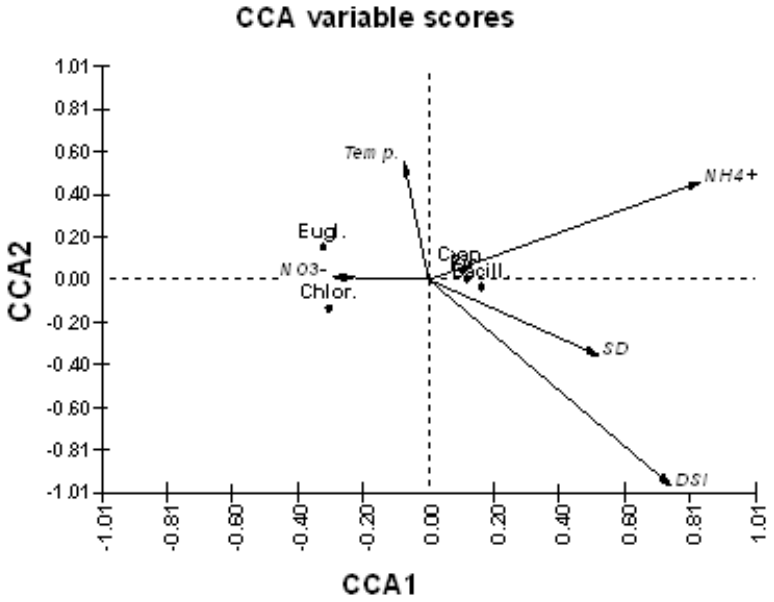

Fig. 4. Canonical correspondence analysis ordination showing the five groups of phytoplankton with respect to the environmental variables, relatively to axes 1 and 2 during Phase 2 in the southern Caspian Sea of the Iranian coast. Cumulative percentage variance explained by axes: Species-I = 37.3% and I+II = 43.5%. Key Notes: Refer to Tables 1 and 2.

Discussion

In this study, nutrients showed a marked difference depending on the season and they were significantly increased from Phase 1 to Phase 2 ($P < 0.05$). DIP and DSi appeared to be sufficient at the euphotic layer, rarely limiting the growth of phytoplankton. Conversely, DIN level was low and limited.

In the southern CS, many factors are key-drivers for changes in nutrient concentrations. In addition, the CS has undergone nutrient enrichment caused especially by increased nutrient as a result of the rapidly expanding population of *M. leidyi* in the region (Shiganova et al. 2003; Nasrollahzadeh et al. 2008 a, b). This study showed that similar changes in ecosystem and increased nutrients occur in southern CS in recent years.

Similar changes in both ecosystem and phytoplankton community took place. Southern CS was characterized by low phytoplankton abundance during Phase 1 (EACS 1996), while phytoplankton abundance sharply increased during Phase 2 (Phase 1; 12.5 cells m/l and Phase 2; 33.3 cells m/l). During the pre-invasion period (Phase 1), Bacillariophyta dominated phytoplankton, followed by Pyrrophyta, Chlorophyta, and Cyanophyta, while Bacillariophyta were followed by Cyanophyta, Pyrrophyta, and Chlorophyta during the post-invasion period (Phase 2). Meanwhile, the temporal population dynamics of Bacillariophyta (diatoms) superposed that of total phytoplankton. The Bacillariophyta (diatoms) occurred abundantly under all temperatures and salinities within the year during both sampling periods. This is because DSi concentration is not a limiting factor for phytoplankton growth as aforementioned.

Perez-Ruzafa et al. (2002) reported that small phytoplankton, such as flagellate algae (Pyrrophyta), should out-compete large phytoplankton when nutrients are scarce, while larger phytoplankton, such as diatoms and Pyrrophyta, should out-compete small phytoplankton when nutrient level increases. During Phase 1, when the system was stable, the Bacillariophyta (*Rhizosolenia calcaravis*, *Thalassionema nitzschiodes)* were the dominant species with high nutrient concentration in spring, autumn and winter, while in summer small phytoplankton species (Pyrrophyta*; Exuviaella cordata*) were dominant. Similar finding was reported by Perez-Ruzafa et al. (2002). During Phase 2, when the system was unstable, Bacillariophyta (*Chaetoceros* sp., *Thalassionema nitzschiodes*) and

Pyrrophyta (*Exuviaella cordata*) were dominant species during high nutrient concentration in spring, autumn and winter, while in summer Cyanophyta (*Oscillatoria* sp.) was dominant with low nutrient concentrations.

Based on the number of species (ISI), Bacillariophyta was found to be the dominant taxon during Phase 1 followed by species of Pyrrophyta. During Phase 2, while Bacillariophyta was still the dominant taxon, they were followed by Cyanophyta instead of Pyrrophyta. During Phase 2, four species of Cyanophyta, never recorded during Phase 1, namely *Lyngbya* sp.*, Oscillatoria limosa, Oscillatoria* sp., *Spirulina laxissma*, appeared. In CS, the appearance and increase in abundance of some species of Cyanophyta (especially *Spirulina laxissma*) during Phase 2 suggests that this ecosystem moves towards unstable or disturbed conditions as explained by Shiganova et al. (2003) and Kideys and Moghim (2003) for the north and middle areas of CS. Additionally, one potentially toxic species of Pyrrophyta (*Prorocentrum micans*) appeared in Phase 2, which suggests that the environments moves towards an unstable or disturbed condition, as reported by Faust and Gulledge (2002).

Sophisticated statistical techniques may often find significant relationships in large datasets (Luoma and Bryan 1982). When a process in nature is controlled by a suite of inter-relating variables, classic way such as correlations between more than two parameters are not easy to establish at a glance (Luoma and Bryan 1982). Therefore, multivariate analyses for the interpretation of large environmental and biological datasets have been used in plankton research to identify relationships between abiotic and biotic factors, and community interpretation (Matta and Marshall 1984; Pagou and Ignatiades 1988; Varis 1991; Marshall and Alden 1995; Del Giorgio et al. 1997).

In present study, results from temporal CDF analysis showed that, based on water quality parameters, the southern CS is discriminated into four groups. This means that the differences among seasons are significant, indicating seasonal variability during both sampling periods. This results also showed that for both phases of the study the most significant variables are physical (temperature and salinity), biological (DO, SD) and nutrient variables. It is apparent that most parameters vary with seasons.

PCA was used to obtain temporal changes in water chemistry and to find the main pattern to be extracted. In summary: the most important variables in PC1 with the highest factor loading, irrespective of the nutrient ratios, were NH_4+ in spring, DOP in summer, and NO_3^- in autumn and DIP in winter during Phase 1. During Phase 2, the most important variables in PC1 were DIN in spring and autumn, DIP in summer, and NH_4+ in winter. Although the variables within each factor (PCs) seasonally were not always the same during both sampling periods, the overall analysis showed that in PC1 at least one kind of nitrogen compound is always present, suggesting the importance of this compound for this area during the phytoplankton proliferation (in spring and autumn). As Nasrollahzadeh et al. (2008 a, b) noted that the southern CS is nitrogen limiting which agrees with our PCA results.

Nutrients have been considered as one of the major factors controlling the composition and abundance of phytoplankton community (Bianchi et al. 2003). It has been generally thought that DSi and DIN played important roles on the population dynamics of Bacillariophyta (diatoms) (Eppley 1977; Hodgkiss and Ho 1997; Hodgkiss and Lu 2004), while DIP and N:P ratio are important for Pyrrophyta (dinoflagellate) (Riegman 1995; Escaravage et al. 1996; Hodgkiss and Ho 1997). The phytoplankton community in the southern CS was dominated by Bacillariophyta (diatoms) due to the high nutrient levels and sufficient DSi. However, as the development of the Bacillariophyta (diatoms) occurred, large amounts of DSi and DIN were used up and caused the temporal exhaustion of DSi and a decline of DSi/DIN ratio, which led to a succession of phytoplankton from Bacillariophyta (diatoms) with high silica demands to non-siliceous phytoplankton, such as Pyrrophyta (dinoflagellate) and Cyanophyta. In our study, high abundances of Pyrrophyta (motile phytoplankton, dinoflagellate) and Cyanophyta (nitrogen fixing) occurred followed by the Bacillariophyta (diatoms) proliferation, when DSi and DIN were temporarily exhausted from the water column (euphotic layer) in summer. Meanwhile, in summer, during both periods phosphorus compounds are important variables (PCA analysis) with declining DSi.

Three PCs, annually explaining 60-67% of the total variance, was estimated on the basis of a Kaiser (1960) criterion of the eigenvalues greater or equal to 1 and from a screen plot (Cattell 1966), respectively. During Phase 1, a negative sign of temperature loading indicates its inverse relationship to NH_4+ and DON. It can be simply explained by their oxidation to nitrate (not nitrification through biochemical reactions) which depends on the temperature. The DON concentration was found to be low in summer when temperature was high which is in agreement with the PCA results. Temperature and DO also showed an inverse relationship. This is expected because solubility of gases such as DO will decrease with increased temperature. Oxygen concentrations were found to be high within the euphotic layer, where the DO was found to be oversaturated (104-123% saturation). The DO oversaturation at this layer was most likely caused by biological production as high abundance of phytoplankton was recorded. Salinity had a negative sign as compared to the nutrient variables. Salinity is one of the main factors in PCA analysis but during Phase 2 it loses its importance. It can be explained that the river inflow during Phase 1

was more than Phase 2, which can affect water salinity (Nasrollahzadeh et al. 2008b). DSi was also one of the main factors during both sampling periods. This is because Bacillariophyta, which consume silicon in the water, are most abundance in the southern CS. During Phase 2, all inorganic nitrogen and DSi are in PC1 and temperature is one of the variable in PC2 which has positive loading with negative loading on SD. Transparency was found to be inversely related to phytoplankton abundance. As a consequence, increased temperature will result in decreased SD. In this period, DIP concentration and DO was not related to other variables and were in PC3 and PC4.

To examine the effect of a particular environmental variable on phytoplankton, one can use the canonical correspondence analysis (CCA) in order to find relationships between the phytoplankton community and water quality variables. During phases 1 and 2, CCA analysis suggests that the majority of phytoplankton taxon have substantial tolerance to different environmental variables. In the ordination diagram, environmental variables are represented by arrows pointing to the direction of maximum change and the arrow length indicates the importance of the environmental variable (Palmer 1993; Gower et al. 1994). In particular, the controlling factors influencing phytoplankton community varied from year (Phase 1) to year (Phase 2). Thus, it seems, phytoplankton assemblage was highly affected by salinity and NH_4^+ concentration during Phase 1, and temperature and DSi concentration during Phase 2.

To compare between phases, we consider all water quality variables which are more important in Phase 1 for CCA analysis of species are also important during Phase 2. We found that temperature and DSi still contain long length of arrow, which indicates that these two water quality variables are more important during Phase 2.

Conclusions

The result of the present study showed that the PCA was 100% successful, as almost all variables in CDF1 appeared in PC1 with high loading factors. CCA analysis suggests that the controlling factors influencing phytoplankton community varied from year (Phase 1) to year (Phase 2). It is apparent from the CCA diagram that the Bacillariophyta, Pyrrophyta and Cyanophyta group were close to the centre of this diagram. Thus, these three divisions were not associated to one or more particular environmental variables during both sampling periods.

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