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Spatio-temporal distribution of the dominant Diatom and Dinoflagellate species in the Bay of Tunis (SW Mediterranean Sea)

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Abstract

Microphytoplankton composition and its relationships with hydrology and nutrient distributions were investigated over 24 months (December 1993 - November 1995) in the Bay of Tunis (SW Mediterranean Sea). A new index, the 'Specific Preference Index' (SPI) obtained by computing the median value of each parameter weighed by the numerical value of each species density was developed. Using this index, the relationships between each species and temperature, salinity and major nutrients were analysed.

The distribution of chlorophyll a did not show a clear correlation with microplankton abundance suggesting that other factors contribute to chlorophyll concentration, such as smaller phytoplankton size fractions or detritus. The winter-spring diatom blooms did not show a regular pattern during both years. High nutrient inputs in late summer, associated with mild meteorological conditions, contributed to the development of a large diatom bloom in autumn 1995 where significant silicate depletion was witnessed. Generally, diatoms were more stenotherm than dinoflagellates in the Bay, whereas dinoflagellates were more stenohaline than diatoms. The statistical analyses showed that the two species, Bellerochea horologicalis and Lithodesmioides polymorpha var. tunisiense, appeared in a wide range of environmental conditions. An excess of phosphate versus nitrate appeared to be associated with red tides of Gymnodinium spp, whereas Peridinium quinquecorne showed the opposite. Phosphate concentrations appear to be crucial in this coastal environment, where diatom blooms are often limited by low silicate availability.

Keywords: Diatoms; Dinoflagellates; Bay of Tunis; SW Mediterranean Sea; Nutrients; Species Preference Index.

Introduction

Mediterranean coastal ecosystems are undergoing rapid alteration since they are under the combined pressure of climate change and human impact (TURLEY, 1999; BIANCHI & MORRI, 2000; BÉTHOUX *et al.*, 2002). Many studies report that compositional changes in phytoplankton can lead to nuisance and the massive growth of toxic algal blooms (LEGOVIC & JUSTIC, 1997) especially in highly eutrophic areas such as the lagoon of Venice (TOLOMIO *et al.*, 1999) or Aegean coastal waters (GOTSIS-SKRETAS & FRIGILOS, 1990). In addition, mesocosm experiments have been performed to test the response of the Mediterranean phytoplankton community to the increase in nutrient inputs (PITTA *et al.*, 1997; DUARTE *et al.*, 2000; LEBARON *et al.*, 2001).

By the year 2025, the Mediterranean coastal population is predicted to increase to 200–220 million, especially on the southern Mediterranean coasts (BARIC & GASPAROVIC, 1992). This will result in eutrophication and an increased risk of pollution in these areas unless they are well managed. However, information on the response of the phytoplankton to the progressive eutrophication of Mediterranean coastal areas is generally constrained to the European continental side.

The north coast of Tunisia, located between the two main basins of the Mediterranean Sea through the Tunisian-Sicily Strait, has many coastal lagoons and lakes, subject to anthropogenic disturbance, where phytoplankton often present monospecific trends (REJEB-JENHANI *et al.*, 1991). The Bay of Tunis is open to the Gulf of Tunis on its northern side and the Lake of Tunis in the Southwest, and comprises two lagoons separated by an artificial shipping canal. The north lake of Tunis receiving all sewage from the Tunis city area, presents an alteration in both fauna and flora (BELKHIR, 1984; DALY YAHIA-KÉFI, 1998) and an increase of red tide events (GUELORGET, 1992; ROMDHANE *et al.*, 1998; DALY YAHIA-KÉFI *et al.*, 2001).

The purpose of this study is to analyse the response of microphytoplankton, particularly individual species, to nutrient inputs, and to contribute to our knowledge of the understudied southern Mediterranean coast.

Materials and Methods

Field sampling and laboratory analysis

Monthly sampling was carried out (December 1993 to November 1995) at nine stations located in the central waters of the Bay of Tunis (Figure 1). Eight additional stations were included along the coastline in order to

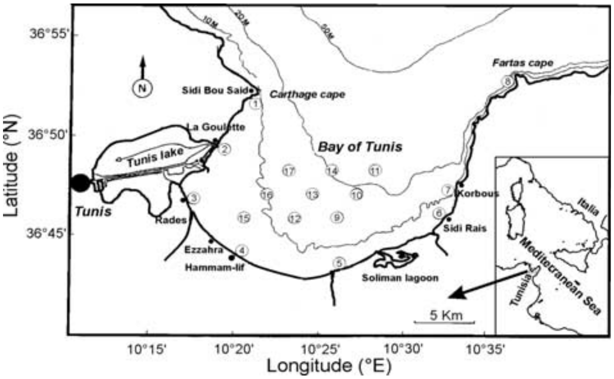


Fig. 1: Map of Tunis Bay showing the different sampling stations for hydrology and plankton analysis, sampled between December 1993 and November 1995.

evaluate nutrient inputs of terrestrial origin. Using a statistical regionalization technique, SOUISSI *et al.* (2000) reported important differences between the central waters of the bay and the coastal stations receiving land-derived inputs.

At each station, sea surface temperature, salinity, ammonium, nitrate, nitrite, phosphate, silicate and chlorophyll a were measured. Analyses of nutrients were carried out using a spectrophotometric method (model Beckman 36 UV-vis) according to FAO (1975) for nitrite, nitrate, phosphate and silicate, and according to AMINOT & CHAUSSEPIED (1983) for ammonium. For chlorophyll a analysis, water samples were filtered through Whatman GF/C filters, frozen and measured with a Spectrophotometer (model Beckman 36 UV – vis.) according to the Lorenzen method described in UNESCO (1966). Surface water samples for phytoplankton analysis were fixed with Lugol's iodine solution and sub-samples of 25 ml were analysed under a Leitz inverted microscope following a sedimentation time of 24 h. Cells were counted along two diametric transects of the bottom plate for dominant species, and by sweeping the whole surface for rare species (THRONDSEN, 1995). Meteorological data (air temperature and precipitation) were monitored daily at the Tunis Airport meteorological station 10 Km from the sampling stations at 4 m above sea level.

Statistical analyses

Calculation of the Specific Preference Index (SPI). The high spatio-temporal variability of the different variables studied in the Bay of Tunis renders the application of classical correlation methods ineffective (i.e. Principal Component Analysis, Factorial Analysis and other regionalisation techniques, see SOUISSI *et al.* 2001 for more details). For the first time, we applied here a very simple and new method in order to calculate the environmental referendum for each species that we will call 'Specific Preference Index' (SPI, hereafter). This index is based on the weighing of a variable value by the abundance of the species in each sample (one station and one date). All samples are used to calculate the weighed median and the interquartile range of the environmental variable for each species. Figure 2 shows an example of calculation of the SPI

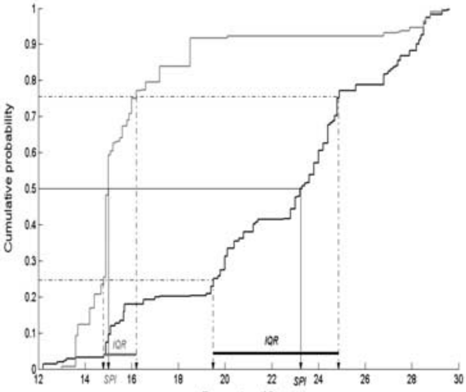


Fig. 2: Example of conditional cumulative probability of two diatom species *Thalassiosira levanderi* (grey curve) and *Cylindrotheca closterium* (black curve). Each curve was obtained by weighting the observed value of temperature in each station and each date by the numerical abundance of the species. This representation allowed to calculate the weighted median (considered as the *SPI*) and the interquartile range (*IQR*). These measures are shown in the figure.

regarding temperature and for two diatom species. The species *Thalassiosira levanderi* occurred during winter and it is characterised by a low SPI for temperature (15°C) and also a low variability (low interquartile range 'IQR', Figure 2). On the other hand, the species *Cylindrotheca closterium* showed a eurytherm response (regular increase of cumulative probability) leading to a high *SPI* (23.3°C) and a high dispersion (Figure 2).

The advantage of this simple calculation of SPI is to obtain an index which is very simple and having the units of the environmental variable considered in the analysis and can be plotted using the same scale. If this index is computed for the same species with two environmental variables, the projection of a given species in a bi-dimensional plot of any two variables may identify optimal conditions for this species. This index is used here as a statistical synthesis of a large data set including spatio-temporal variability. It presents the added advantage of plotting species directly in an ecologically meaningful plane, where each axis is represented by one environmental variable. For computation purposes, only species with a presence of >5 % of total samples were considered. The inclusion of scarce or less frequent species can give low statistical significance. The *SPI* was computed on the remaining 32 diatoms and 17 dinoflagellates using temperature and salinity as indicators of seasonality as well as major nutrients (PO_4^{3-}, SiO_2, NH_4 and NO_3+NO_2).

Clustering analysis. Species assemblages have been analysed from the matrix <species x average abundance>. In the second step, a 4th root transformation was applied to the species abundance, then a hierarchical classification was applied using Euclidean distances and a clustering strategy of flexible links with beta set at a value of -0.25 (SOUISSI *et al.*, 2001; ANNEVILLE *et al.*, 2002).

The different steps of the method were programmed with Matlab Software.

Results

Physical variables

The daily air temperature ranged between 9.8 and 36.2 °C in 1994 and 5 and 32.5 °C in 1995 and the monthly sea surface temperature showed a similar pattern with a clear seasonality. In May 1994, the temperature showed a faster increase than in May 1995, followed by a minimum in June. This feature implied that the warming of the water occurred earlier in 1994 (temperature in April 1994 was ~20°C) than in 1995 (around 17°C in April) (Figure 3A-B). The salinity showed a significant inter-annual variability with values around 37.0 (minimum in May 1994), coinciding with the highest precipitation in February 1994 and increasing from June to September. In 1995, a rapid salinity increase occurred in April, the values ranged from 37.2 to 37.6 until November, with a minimum in October (36.81) (Figure 3C). Winter rainfalls in 1994 were concentrated in February, occurring earlier in 1995 (December-January). Rainfall in the autumn began earlier in 1995 than in 1994 (at the end of September) (Figure 3D).

Nutrients

The concentration of nitrate reached a maximum of 1 μM in April and June 1994 and October 1995 (Table 1), whereas lower values were recorded in late summer 1994 and July 1995. Winter 1995 presented low values in comparison with 1994 (with a maximum in October 1995). Ammonium presented higher values than nitrate, being the main form of inorganic nitrogen. The concentration ranged from 2-6 μM in 1994 with lower values in summer and autumn. In 1995, ammonium values were higher in September 1995 (up to 128 μM). Phosphate presented lower values in summer 1994 in comparison with 1995. In the last year the concentration of phosphate decreased in April-May, but high values were recorded during the summer reaching values of up to 1.85 μM in June 1995 (Table 1). The concentration of silicate showed significant inter-annual differences with higher values in 1995. From December 1993 to April 1995 values lower than 4 μM (except a maximum of up to 6 μM in July 1994) were recorded and as of April 1995 increased to >3 μM with a minimum in October 1995.

Based on the results reported by SOUISSI *et al.* (2000), four stations were selected from the more eutrophic zone (2 to 5 in Figure 1) from the eight stations located along the coastline. Station 6 close to the central station of the bay was added. This analysis of the temporal evolution of salinity and nutrients in these coastal stations showed a more pronounced nutrient enrichment in the west and southwestern areas of the Bay of Tunis (Figure 4). Urban and industrial discharges in the southern part of the lake of Tunis reached the bay of Tunis through the Radès channel. The influence of the ports on the western side

Table 1
General characteristics of the minimum, maximum, average and standard deviation of nitrate, ammonium, phosphate and silicate in the central stations (stations 9 to 17) of the Bay of Tunis over the two-year period 1994-1995.

Nutriment	Minimum	Maximum	Mean	Standard Deviation
Nitrate (μM)	0.045	1.075	0.349	0.216
Ammonium (μM)	0.056	128	3.764	12.367
Phosphate (μM)	0.010	1.850	0.202	0.219
Silicate (μM)	0.220	7.562	2.649	1.401

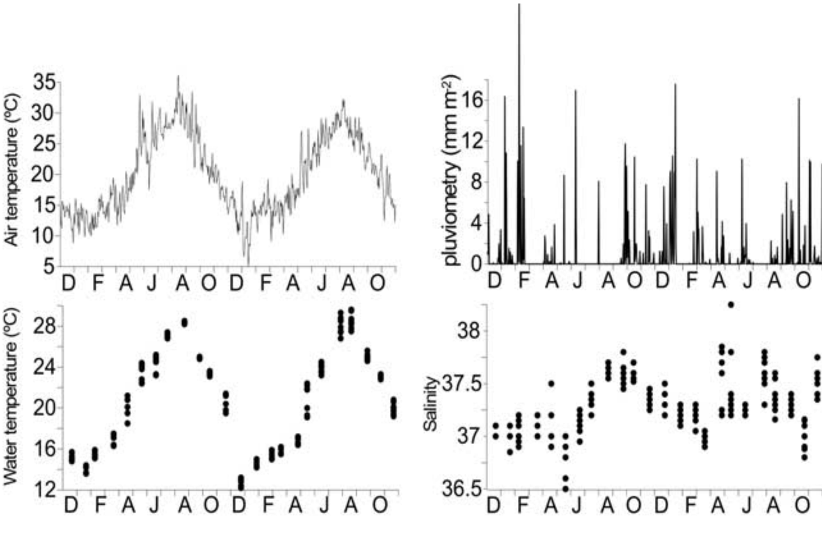


Fig. 3: Temporal evolution of meteorological parameters: (a) air temperature and (b) precipitation during the study period, December 1993 to November 1995, recorded at the Tunis Airport meteorological station. Time evolution of water temperature (c) and salinity (d) in the central stations of the Bay of Tunis sampled monthly.

of the Bay (Radès, la Goulette and Tunis), and also the terrestrial inputs, led to an increase in the trophic state differences between the southwestern side of the Bay and the north and central zones. The effluents along the coastline showed higher values of nitrate during the winter of 1994 with a relative peak in December 1994 and a maximum in October 1995. The concentration of ammonium presented the most variable values, with higher values during the summer (mainly August) reaching the highest values near the Tunis lagoon (up 108 μM in September 1995) and also at station 6 in August 1994 (up 128 μM, Figure 4).

Chlorophyll and microphytoplankton

In the central waters of the Bay of Tunis, concentrations of chlorophyll *a* showed values below 0.2 μg l^{-1} except in December 1993 and July 1994 with a clear maximum in October 1994. The peaks in the distribution of chlorophyll *a* and microphytoplankton abundance did not display similar patterns. For example, the peak in October 1995 was not associated with a proportional increase in the chlorophyll concentration (Figure 5A).

During this study diatom maxima were observed in December 1993 reaching values of up to 131 cells ml^{-1} in an assemblage mainly composed of *Asterionellopsis glacialis* (44 cells ml^{-1}), *Chaetoceros tortissimus, Lauderia annulata, Leptocylindrus* spp, *Pseudo-nitzschia 'seriata'* and *Skeletonema costatum*. Other peaks occurred in January 1995 also dominated by A. glacialis (34 cells ml-1) followed by Chaetoceros fragilis and Thalassiosira levanderi. In October 1995, all stations showed a maximum composed of *A. glacialis*, *Leptocylindus danicus, Pseudo-nitzschia 'seriata'* and *Guinardia striata*. In August 1995, the highest maximum 257 cells ml-^{1} was almost totally composed of *Chaetoceros fragilis.* Several taxa presented perennial distributions such as the diatoms *Bellerochea* spp, *Chaetoceros fragilis, Lithodesmioides polymorpha* and *Cylindrotheca closterium* (Figure 6).

Dinoflagellates showed their lowest values in autumn-winter with an increase during the summer with *Scrippsiella* spp as the most abundant species. Secondary peaks were observed due to *Peridinium quinquecorne* (up 52 cells ml^{-1}) in June 94 and to *Scrippsiella* spp, *Prorocentrum triestinum* and *Ceratium furca* in May 1995. Several species were ubiquitous throughout the whole year such as *Ceratium furca, C. fusus, Gyrodinium spirale* or *Scrippsiella* spp. Among the potential red tide taxa *Alexandrium* reached its highest abundance in the summer and early autumn with highest values (3.9 cells ml^{-1}) in July 1994. Several species of *Gymnodinium* spp were dominant in July 1995 (up 47 cells ml-1). *Gyrodinium impudicum* was observed exclusively in summer and *Gymnodinium sanguineum* presented higher values in later summer (up 2 cells ml^{-1}). Dinophysiales did not reach high abundances, with maximum values in May-June (up 1.2 cells ml^{-1}) for *Dinophysis cf. acuminata* (Figure 6).

Microplankton assemblages

The presence of associations among common species was investigated by using an ordination technique that shows several subgroups of species with similar ecological trends in their distributions (Figure 7). The composition of groups and their homogeneity (diatoms or dinoflagellates) were considered in order to choose the cut-off level of the dendrogram. Group A was composed of the most common dinoflagellate species, but excluded the most common species, *Scrippsiella* spp, was associated with the bloom-forming diatoms. Species belonging to the genera *Gonyaulax* and *Alexandrium* (A1) appeared together. The A2 subgroup was composed of *Prorocentrum micans, Ceratium fusus* and *C. furca*.

The subgroups B and C were composed of the diatoms that were responsible for sporadic blooms, reaching high abundance values. The D subgroup was composed of typical winter-spring blooming diatoms. Between these last

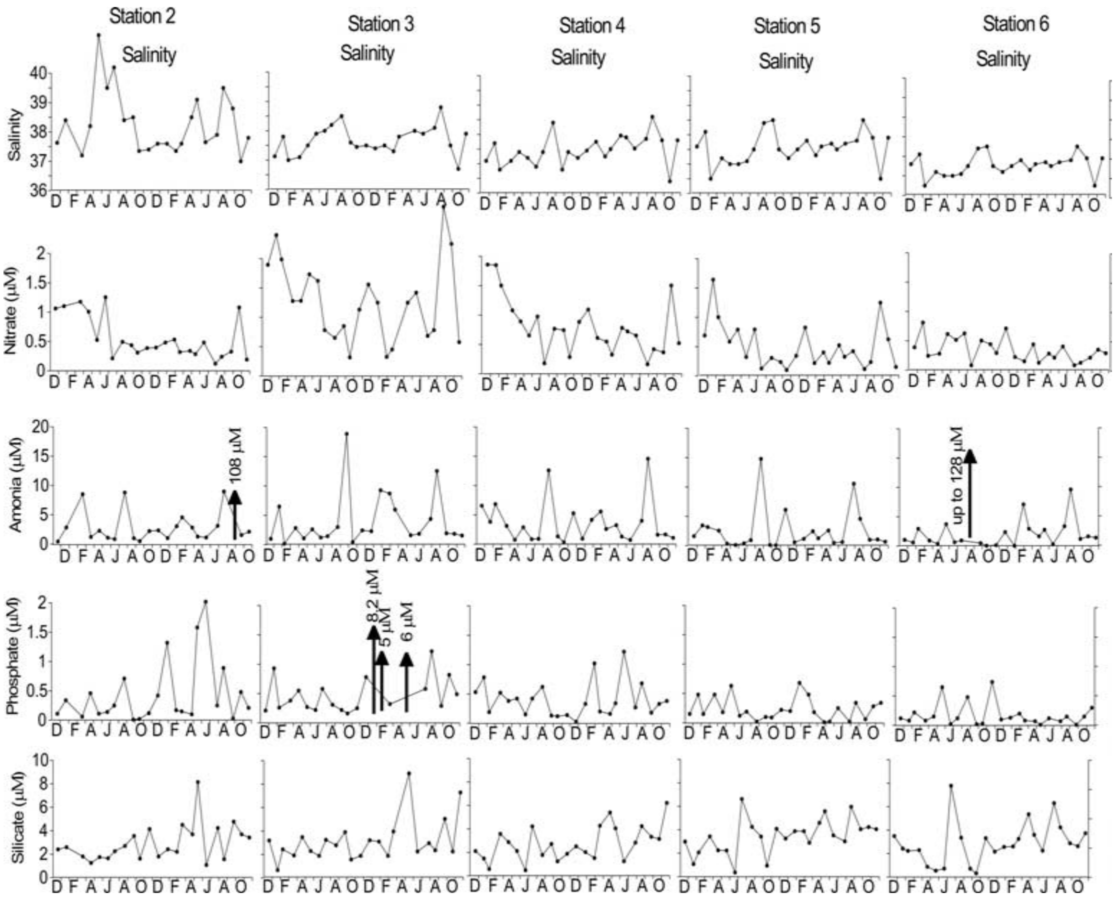


Fig. 4: Temporal evolution of salinity and nutrients (nitrate, ammonium, phosphate and silicate) in selected south-western stations 2 to 6 (see Figure1). The arrow shows the highest values exceeding the Y-axis scale limits.

groups *Asterionellopsis glacialis* appeared, being the dominant species in the diatom blooms of the Bay. Group E was composed of species observed over the entire study period, especially group F1 that presented several perennial diatom species. In spite of their ubiquitous distribution, these taxa never reached high abundances or dominance in the phytoplankton community. The subgroup F2 includes dinoflagellate species often observed during the summer. This subgroup also included the diatom *Pseudosolenia calcar-avis*.

SPI projection in the temperature-salinity plane

The SPI projection of selected species in the temperature-salinity plane presented a net gradient with a high dispersion around the intermediate values where three groups can be distinguished (Table 2). The first 15 diatom species showed a SPI in the ranges 14.8-15.8 and 37.0-37.1 of temperature and salinity, respectively. The lowest values of the interquartile range (*IQR* <1) for both temperature and salinity (Table 2) were obtained for the species *Chaetoceros decipiens, Hemiaulus hauckii, Lauderia annulata, Odontella mobiliensis, Skeletonema costatum* and *Dactyliosolen fragilissimus*. On the other hand, the highest values of SPI for temperature and salinity were obtained for the dinoflagellate species *Gyrodinium impudicum* and *Pyrophacus horologicum* (Figure 8, Table 2). The SPI of the other species was projected around the median values of environmental variables. This analysis, based only on the average *IQR* values, revealed that diatoms were more stenotherm than dinoflagellates in the Bay, whereas the dinoflagellates can be considered more stenohaline than diatoms (Table 2).

Calculation of the SPI values for the major nutrients

The concentrations of nutrients and abundances of the main taxa were investigated by using the *SPI* in order to evaluate the range of nutrients appearing optimal for the observation of each taxon (Table 2). The oxidised forms of nitrogen (nitrate+nitrite)

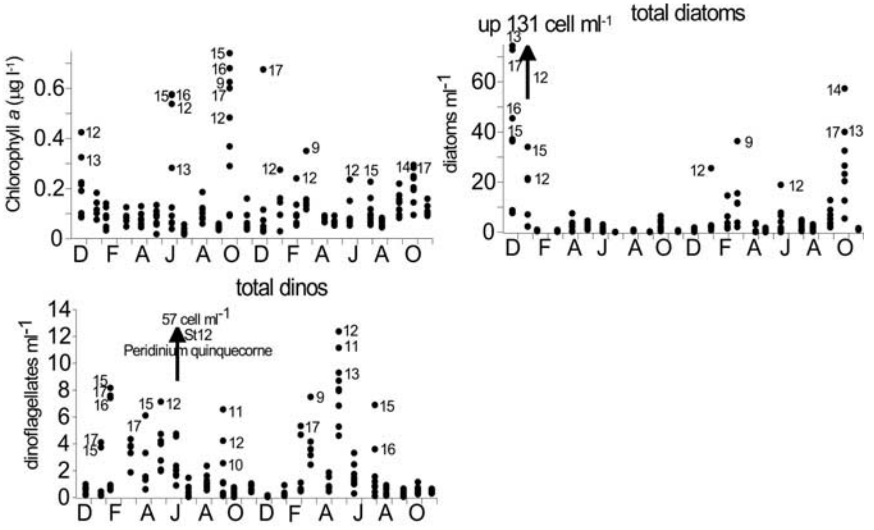
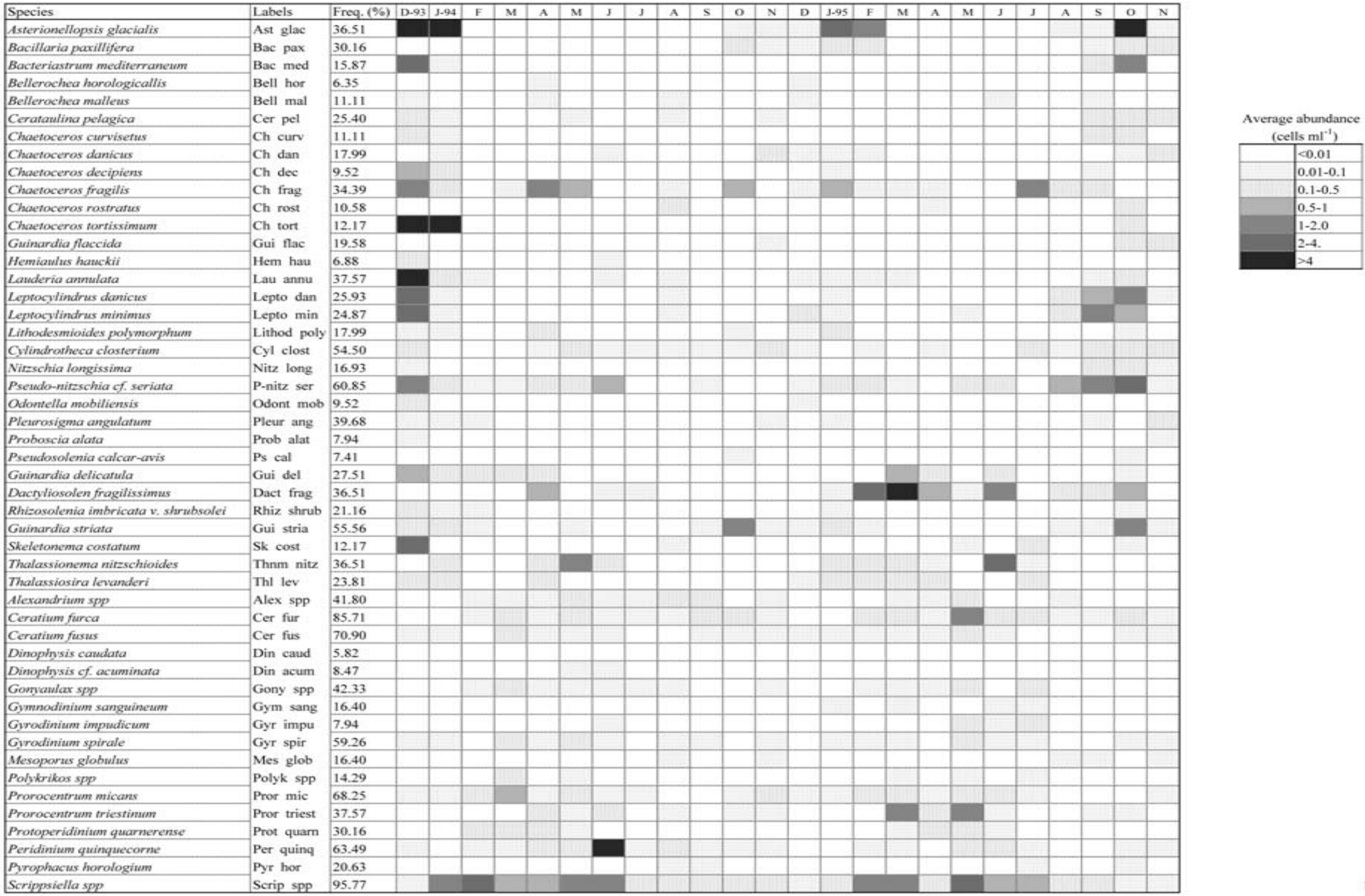


Fig. 5: Temporal evolution in chlorophyll (a), total diatoms (b) and total dinoflagelates (c) in the 9 central stations of the Bay of Tunis.



Species	Labels	Freq. (%)
Asterionellopsis glacialis	Ast glac	36.51
Bacillaria paxillifera	Bac pax	30.16
Bacteriastrum mediterraneum	Bac med	15.87
Bellerochea horologicallis	Bell hor	6.35
Bellerochea malleus	Bell mal	11.11
Cerataulina pelagica	Cer pel	25.40
Chaetoceros curvisetus	Ch curv	11.11
Chaetoceros danicus	Ch dan	17.99
Chaetoceros decipiens	Ch dec	9.52
Chaetoceros fragilis	Ch frag	34.39
Chaetoceros rostratus	Ch rost	10.58
Chaetoceros tortissimum	Ch tort	12.17
Guinardia flaccida	Gui flac	19.58
Hemiaulus hauckii	Hem hau	6.88
Lauderia annulata	Lau annu	37.57
Leptocylindrus danicus	Lepto dan	25.93
Leptocylindrus minimus	Lepto min	24.87
Lithodesmioides polymorphum	Lithod poly	17.99
Cylindrotheca closterium	Cyl clost	54.50
Nitzschia longissima	Nitz long	16.93
Pseudo-nitzschia cf. seriata	P-nitz ser	60.85
Odontella mobiliensis	Odont mob	9.52
Pleurosigma angulatum	Pleur ang	39.68
Proboscia alata	Prob alat	7.94
Pseudosolenia calcar-avis	Ps cal	7.41
Guinardia delicatula	Gui del	27.51
Dactyliosolen fragilissimus	Dact frag	36.51
Rhizosolenia imbricata v. shrubsolei	Rhiz shrub	21.16
Guinardia striata	Gui stria	55.56
Skeletonema costatum	Sk cost	12.17
Thalassionema nitzschioides	Thnm nitz	36.51
Thalassiosira levanderi	Thl lev	23.81
Alexandrium spp	Alex spp	41.80
Ceratium furca	Cer fur	85.71
Ceratium fusus	Cer fus	70.90
Dinophysis caudata	Din caud	5.82
Dinophysis cf. acuminata	Din acum	8.47
Gonyaulax spp	Gony spp	42.33
Gymnodinium sanguineum	Gym sang	16.40
Gyrodinium impudicum	Gyr impu	7.94
Gyrodinium spirale	Gyr spir	59.26
Mesoporus globulus	Mes glob	16.40
Polykrikos spp	Polyk spp	14.29
Prorocentrum micans	Pror mic	68.25
Prorocentrum triestinum	Pror triest	37.57
Protoperidinium quarnerense	Prot quarn	30.16
Peridinium quinquecorne	Per quinq	63.49
Pyrophacus horologium	Pyr hor	20.63
Scrippsiella spp	Scrip spp	95.77

Figure 6

Fig. 6: Map of the bi-annual evolution of 49 selected species (frequency > 5%) of diatoms and dinoflagellates in the central stations of the Bay of Tunis. The average abundance per species is represented by a grey scale according to 7 value classes.

Table 2

Species Preference Index *(SPI)* and Inter-quartile range values *(IQR)* for selected diatom and dinoflagellate species (see Figure 6). The *IQR* is a robust estimate of the spread of data obtained from the weighted vectors (see Figure 2).

Species label	Temperature (°C)		Salinity		PO_4 (μM)		SiO_2 (μM)		NH_4 (μM)		NO_2+NO_3 (μM)	
	SPI	**IQR**	**SPI**	**IQR**	**SPI**	**IQR**	**SPI**	**IQR**	**SPI**	**IQR**	**SPI**	**IQR**
Ast glac	15.0	8.10	37.1	2.35	0.15	0.13	1.22	1.77	3.12	2.00	0.50	0.34
Bac pax	20.8	8.10	37.3	1.20	0.09	0.14	2.08	1.54	0.96	2.27	0.37	0.39
Bac med	15.0	8.10	37.1	2.80	0.15	0.11	2.12	2.63	4.2	24.81	0.39	0.51
Bell hor	14.8	1.60	37.0	2.50	0.25	0.08	3.43	0.46	1.83	0.39	0.39	0.45
Bell mal	24.	12.80	37.3	1.10	0.32	0.29	2.61	0.72	1.44	4.02	0.51	0.48
Cer pel	23.0	4.20	37.2	0.60	0.12	0.07	2.61	2.00	3.25	4.57	0.51	0.30
Ch curv	23.0	1.80	37.0	0.60	0.13	0.04	2.24	1.39	3.25	3.11	0.56	0.16
Ch dan	20.1	3.50	37.4	1.00	0.12	0.05	2.26	2.21	1.06	1.25	0.49	0.36
Ch dec	14.9	0.10	37.1	0.80	0.15	0.03	0.50	0.13	4.70	24.28	0.31	0.25
Ch frag	22.5	13.60	37.3	1.70	0.24	0.21	2.34	2.47	2.83	2.56	0.25	0.23
Ch rost	23.2	5.23	37.1	1.53	0.12	0.14	2.12	1.29	3.12	2.85	0.71	0.50
Ch tort	14.8	0.10	37.0	2.00	0.22	0.10	1.08	2.94	1.83	2.39	0.39	0.18
Gui flac	22.8	3.20	37.2	0.30	0.12	0.02	2.84	2.63	2.36	2.29	0.58	0.26
Hem hau	14.9	0.20	37.1	0.80	0.15	0.12	3.12	2.83	2.25	2.39	0.39	0.08
Lau annu	14.9	0.20	37.1	0.80	0.18	0.10	1.25	2.79	2.89	3.89	0.39	0.10
Lepto dan	22.8	8.30	37.1	2.60	0.14	0.04	2.26	2.72	3.25	24.81	0.39	0.37
Lepto min	15.0	8.40	37.1	2.70	0.15	0.11	2.61	2.72	3.25	10.04	0.35	0.23
Lithod poly	20.8	6.60	37.2	0.10	0.33	0.44	1.25	1.24	3.88	2.11	0.71	0.21
Cyl clost	23.3	5.30	37.3	1.00	0.13	0.21	2.26	2.07	1.25	2.81	0.36	0.29
Nitz long	24.8	2.60	37.2	0.70	0.08	0.06	2.61	0.54	1.63	5.40	0.39	0.12
P-nitz ser	23.0	5.30	37.1	1.20	0.14	0.07	2.26	2.13	3.12	4.22	0.54	0.42
Odont mob	14.9	0.20	37.1	0.90	0.18	0.10	0.50	1.94	3.00	3.41	0.39	0.18
Pleur ang	19.4	5.50	37.3	1.50	0.12	0.10	3.02	2.21	1.12	3.44	0.40	0.16
Prob alat	15.1	4.50	37.1	2.10	0.13	0.08	2.84	2.91	0.99	2.95	0.33	0.25
Ps cal	23.1	0.28	37.1	1.43	0.11	0.13	1.35	0.32	3.12	3.97	0.58	0.51
Gui del	15.4	1.20	37.1	1.85	0.13	0.12	1.97	2.23	1.83	2.24	0.49	0.37
Dact frag	15.8	0.60	37.0	0.80	0.13	0.10	2.30	0.41	1.83	0.65	0.28	0.20
Rhiz shrub	15.7	8.10	37.1	2.70	0.14	0.07	2.12	1.94	2.28	2.59	0.43	0.32
Gui stria	23.1	0.40	37.2	1.60	0.11	0.16	1.51	0.98	1.83	3.03	0.39	0.52
Sk cost	14.9	0.10	37.1	0.80	0.17	0.10	0.50	2.83	2.89	3.89	0.32	0.22
Thnm nitz	24.2	0.70	37.2	0.60	0.42	0.39	2.58	0.78	0.89	0.36	0.36	0.24
Thl lev	15.0	1.40	37.1	1.40	0.18	0.17	2.03	2.50	2.25	2.55	0.39	0.33
Alex spp	24.4	5.20	37.4	1.60	0.09	0.21	2.22	1.70	0.89	1.17	0.33	0.35
Cer fur	21.9	4.30	37.3	1.00	0.08	0.15	3.65	1.97	1.56	2.06	0.33	0.24
Cer fus	19.8	6.00	37.3	0.80	0.11	0.10	2.61	2.55	1.85	2.50	0.41	0.43
Din caud	23.1	0.30	37.5	1.40	0.05	0.10	1.43	1.80	0.73	3.01	0.28	0.39
Din acum	24.2	0.50	37.0	0.50	0.05	0.09	2.32	0.49	0.75	1.61	0.23	0.36
Gony spp	22.2	11.00	37.2	1.00	0.18	0.31	3.02	1.53	1.39	1.74	0.33	0.31
Gym sang	23.5	12.90	37.3	0.40	0.25	0.25	2.89	0.99	1.39	1.68	0.28	0.31
Gyr impu	25.2	6.90	37.8	2.10	0.36	0.32	3.12	1.04	1.39	0.50	0.25	0.15
Gyr spir	21.8	8.00	37.1	1.10	0.12	0.21	2.25	1.86	1.35	1.79	0.36	0.32
Mes glob	24.6	8.30	37.5	1.68	0.12	0.19	3.02	1.99	0.83	0.67	0.4	0.19
Polyk spp	20.8	11.30	37.2	0.60	0.12	0.34	2.61	1.53	1.39	1.19	0.28	0.35
Pror mic	17.4	5.80	37.2	0.60	0.13	0.16	2.56	1.60	1.56	1.33	0.40	0.45
Pror triest	19.3	5.60	37.2	1.40	0.05	0.08	4.14	2.27	1.83	1.67	0.33	0.17
Prot quarn	19.1	5.40	37.2	1.00	0.18	0.27	2.58	1.90	1.61	1.46	0.53	0.55
Per quinq	25.0	0.00	37.2	0.00	0.06	0.00	2.01	0.00	1.67	0.00	0.78	0.00
Pyr hor	28.2	3.75	37.6	1.80	0.12	0.30	2.58	1.98	1.35	1.15	0.29	0.32
Scrip spp	21.2	8.80	37.2	1.20	0.16	0.28	2.22	2.00	1.44	1.33	0.39	0.49

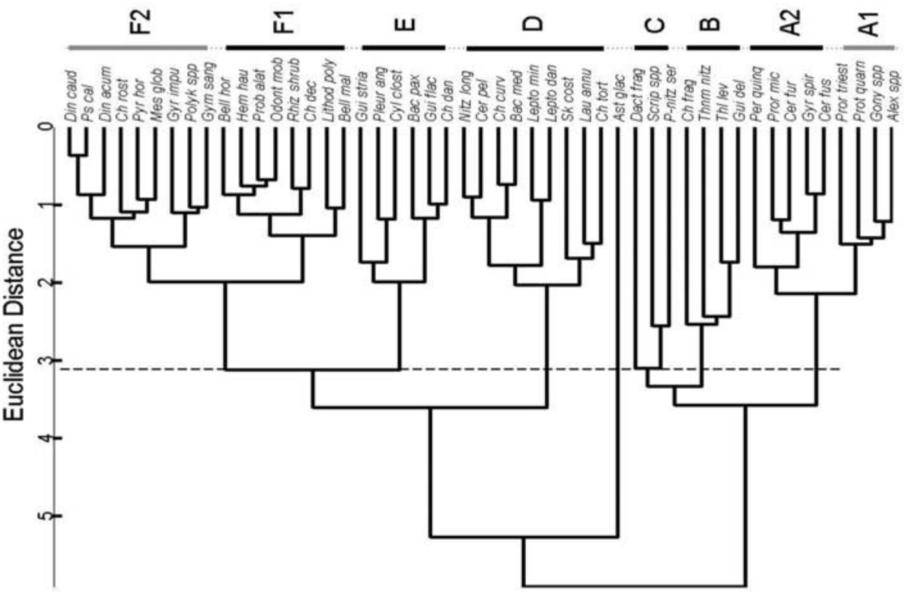


Fig. 7: Dendrogram showing the result of the hierarchical classification of the selected species using the average abundance of figure 6 and Euclidean distance. The species labels corresponded to those of figure 6. The discontinuous line separate 7 groups of species (A to F) and a single species Ast glac.

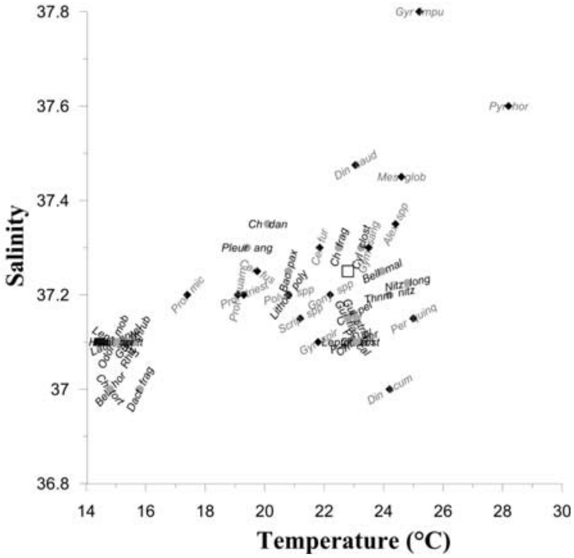


Fig. 8: Projected values of the specific preference index (SPI) for abundant diatoms (grey filled circles and black labels) and for abundant dinoflagellates (black filled diamonds and grey labels) in the temperature-salinity plane. The species labels in each plane correspond to those used in Figure 6. The square corresponds to the co-ordinates of the median values of the environmental factors.

versus the phosphate showed that taxa such as *Dinophysis caudata, D. acuminata* or *Prorocentrum micans* appeared in a range of very low nutrient concentrations. Taxa such as *Gyrodinium impudicum* and *Thalassionema nitzschioides* appeared under higher phosphate concentrations and low nitrate levels, whereas *Peridinium quinquecorne* was the only taxon that presented the inverse trend. Species such *Lithodesmioides polymorpha* var. *tunisiense* and *Bellerochea horologicalis* appeared isolated, from the rest of the phytoplankton in the statistical analysis (Table 2). Some species like *Chaetoceros decipiens* and *Bacteriastrum mediterraneum* appeared associated with higher values of ammonium.

Discussion

Use of SPI analysis

Our investigation demonstrated for the first time a spatio-temporal distribution of the dominant Diatom and Dinoflagellate species related to the physico-chemical parameters in the Bay of Tunis (SW Mediterranean Sea).

The data-base was performed in order to collect enough information related to the phytoplankton dynamics. The analysis of the literature showed a great lack in the comprehension of the phytoplankton dynamics and ecology in the Bay of Tunis. The study of the patterns of the main diatom and dinoflagellate species revealed a high disturbance level probably due to nutrient loading. Our investigation points to the existence of heterogeneity in the ecosystem, associated with the impact of a high gradient of nutrient loaded from SW regions of the Bay. Our scientific approaches to evaluate the phytoplankton dynamics in the Bay of Tunis showed many difficulties; especially the use of standard spatio-temporal techniques (SOUISSI *et al.*, 2001). SOUISSI *et al.* (2000) demonstrated the presence of spatial regionalisation linked to the quality of nutrients. This demonstration was developed by the use of an adequate and simple methodology adapted to such databases (SOUISSI *et al.*, 2000). This investigation was performed in order to verify the hypothesis of the existence of a similar hydrologic region. Previous approaches have many weaknesses related to the low number of data and the high variability of the studied system. In order to resolve the difficulties we used a new index called Specific Preference Index (SPI). This index applied to dominant diatoms and dinoflagellates pointed out clearly the seasonal patterns of these species. The SPI identifies the apparent ecological niches of the selected species characterized by temperature, salinity and nutrients. This index could be used for other databases obtained in different coastal ecosystems.

Offshore influences: Atlantic modified current

The Gulf of Tunis, located on the southern side of the Tunisian-Sicily Strait, is influenced by the Modified Atlantic Water (MAW), which can be traced by its low salinity values. The current is composed of two major tongues, one branching into the Tyrrhenian Sea and the other one continuing eastwards along the African coast. Open Tyrrhenian waters occasionally flush into the Bay causing the renewal of the coastal waters associated with the Atlantic Modified Water current. The absolute salinity minimum is generally found near the Tunisian coast (SAMMARI *et al.*, 1999). ASTRALDI *et al.* (2002) reported lower values of salinity (<36.8) in 1995 compared to data of 1994, in the Modified Atlantic Water along a Tunis-Sicilian transect. By contrast, our results report another observation on water salinity related to this phenomena in the Bay of Tunis (Figure 2D).

LECAL (1957) reported species such as *Guinardia delicatula, Detonula pumila* and *Eucampia zoodiacus* as the typical species of the Atlantic current. BERNARD & TALEB (1970) reported the existence of many species; *Thalassiosira rotula, Chaetoceros decipiens, Ch. affinis* and *Guinardia delicatula* as indicators of the influence of Atlantic waters. In the Bay of Tunis, these taxa were not common whereas other neritic species such as *Skeletonema costatum, Guinardia flaccida, Asteronellopsis glacialis* and *Thalassionema nitzschioides* were frequently observed and sometimes dominant in the study area and in the Algerian coastal waters (LECAL, 1957). Despite a diatom maximum occurring in winter in the Bay of Tunis, it seems that there was limited influence of the offshore assemblages associated with the Atlantic current in the community structure of the bay. The terrestrial nutrient inputs have a higher importance than the fertilisation induced by the Atlantic current in the phytoplankton in the Bay of Tunis.

Autumnal blooms

Our results showed a clear autumnal maximum, often higher than the winter-spring bloom (Figure 5). The autumnal blooms are a common feature in the western Mediterranean coastal areas (ZINGONE *et al.*, 1995). The land-derived nutrient inputs (enhanced by autumnal rain or untreated discharges) followed by mild meteorological conditions have usually been considered as responsible for these blooms. This period of mild weather, the so-called 'St. Martin's summer', is a short interruption of the shift from autumn to winter characterised by mild dry high pressure frequent in the Western Mediterranean basin (ZINGONE *et al.*, 1995). The autumnal diatom maximum in October 1995 is observed after a strong ammonium and nitrate input, which was associated with low salinity, indicating terrestrial inputs (Figure 4). In October 1994, the average atmospheric pressure was 1014 mb whereas in 1995 it was 1019 mb. The number of hours of sunlight was 1/3 higher in October 1995 than 1994 (data not shown from Tunis Airport Meteorological station). The high diatom bloom observed in October 1995, in comparison with October 1994, is probably related to rainfalls registered during this same period.

Both October blooms (1994 and 1995) showed similar [NO_2+NO_3]/PO_4 ratios at all stations with values higher than 4. Despite concentrations of phosphate always being high, during the increase in biomass, phosphate seemed to be more affected by the development of phytoplankton blooms. Both maxima presented some differences. Thus, during the bloom in autumn in 1994, chlorophyll reached high values, but low abundance of diatoms, whereas the opposite trend was observed in 1995. During the summer of 1995, silicate concentration exceeded 3 μM SiO_2, whereas in the summer of 1994 values were generally < 3 μM (except in July). Both autumnal blooms were associated with minima in silicate concentrations as could be expected. The different availability of silicate prior to the development of the bloom, could have previously limited the development of the diatom bloom in 1994, whereas the limitation appears after the development of the bloom in 1995. It could explain the differences and also the decrease in the concentration of silicate in October 1995 compared to previous months (Figure 3). Diatoms have been observed to dominate the phytoplankton community when silicate concentration is higher than a threshold concentration of approximately 2 μM SiO_2 (EGGE & AKSNES, 1992).

The lower values of silicate seemed to be related to typical-bloom forming diatoms such as *Skeletonema costatum* and *Chaetoceros decipiens*, whereas the dinoflagellates appeared to be associated with high values. Diatoms presumably consumed the silicate stock with clear depletion in October 1995. Consequently, the growth of the diatom bloom is limited by the lack of silicate and non-siliceous organisms such as dinoflagellates and flagellates, frequently associated with harmful consequences, would further replace the bloom. This shows the essential role played by silicates, when nitrogen and phosphates are supplied in excess, as in most eutrophic coastal areas.

The evolution of nitrate concentration presented differences between both blooms. In 1994, nitrate presented a relatively low minimum (<0.3 μM) whereas in 1995 it showed a relative maximum with values >0.4 μM, associated with the previous strong nitrate and ammonium inputs in September-October 1995. The bloom in October 1994 was probably limited by nitrogen.

Species response to nutrient variability

Diatoms. An inter-annual periodicity in the distribution of phytoplankton was not observed, probably due to the strong variability induced by the irregular nutrient inputs. Human activities have significantly increased the input of nitrate and phosphate nutrients into coastal waters, while the silicate concentration has remained constant or even decreased in terrestrial freshwater discharges. This has affected the distribution of diatoms, FOUILLAND *et al.* (2002), demonstrated an increase of small picophytoplankton and diatom biomass in the enriched inorganic nitrogen treatments in a Mediterranean lagoon. EGGE (1998) reported that diatoms were not able to dominate when phosphate was deficient, although silicate and nitrate were in excess.

Several diatoms appeared separated in their optimal environmental ranges from the remaining taxa. *Thalassionema nitzschioides* seemed to be favoured when there are high values of phosphate and possible nitrate-limited conditions. We have the same observation with *Chaetoceros fragilis* (Table 2).

In the deep Mediterranean waters the concentration of ammonia was negligible compared to nitrate (L'HELGUEN *et al.*, 2002). However, our data and a previous report (GIACOBBE *et al.*, 1996) demonstrated the dominance of ammonia in the Bay of Tunis. The assimilation of ammonium by phytoplankton requires a lower metabolic energy than nitrate (COLLOS *et al.*, 1997). EPPLEY *et al.* (1969) asserted that ammonium levels higher than 2 μM induced almost maximal inhibition of nitrate uptake by phytoplankton. High levels of ammonium was related to species such as *Chaetoceros decipiens, Bacteriastrum mediterraneum, Lithodesmioides polymorpha* var. *tunisiense* as well as *Cerataulina pelagica, Chaetoceros curvisetus, Chaetoceros rostratus, Leptocylindrus* spp and *Asterionellopsis glacialis* or *Pseudosolenia calcar-avis*. Lower values of ammonia appeared to be associated with *Guinardia flaccida, Rhizosolenia shrubsolei, Hemiaulus hauckii, Proboscia alata* or *Chaetoceros danicus*.

The low silicate levels were usually associated with *Odontella, Skeletonema, Chaetoceros, Asterionellopsis*. By contrast, *Guinardia flaccida, Proboscia alata, Hemiaulus hauckii* appeared under higher concentrations. The first group of typical blooming species was characterised by the presence of siliceous protuberances or appendices, implying higher silicate requirements versus the second group composed of less silica-containing taxa. This feature could be responsible for the higher consumption and the observation of these species associated with almost silicate-depleted water as in October 1995.

Among the diatoms, a mechanism that could contribute to a more diverse range of nutrient conditions is the presence of endosymbionts capable of fixing molecular nitrogen (KIMOR *et al.*, 1992). *Pseudosolenia calcar-avis* (that also bloomed in both October peaks), *Hemiaulus hauckii* and *Rhizosolenia hebetata* could present symbionts under oligotrophic conditions, releasing them under favourable conditions. Species such as *Hemiaulus hauckii, H. sinensis, Chaetoceros rostratus, Proboscia alata*, and *Rhizosolenia calcar-avis* are typical components of the last stage of succession (MARGALEF, 1967; IGNATIADES, 1969) and were candidates to be associated with symbionts under oligotrophic conditions in the waters of the Bay of Tunis.

Despite dinoflagellate species being the most frequently reported as toxic, more recently some diatom taxa have been identified as toxic species. *Pseudo-nitzschia 'seriata'*, a common species in the Bay of Tunis, is reported here as a collective name (HASLE, 1972) of a group of species of difficult identification. OUNISSI & FRÉHI (1999) reported high abundances of *Pseudo-nitzschia multiseries* (>1000 cells ml^{-1}) on the eastern coast of Algeria. Recently, ORSINI *et al.* (2002) confirmed the presence of toxic species of *Pseudo-nitzschia* in the Tyrrhenian coasts. This genus requires further investigation in the Bay of Tunis.

Diatoms such as *Bellerochea horologicalis* and *Lithodesmioides polymorpha* var. *tunisiense* appeared separated from the rest of the diatoms, as well as from apparently similar taxa such as *Bellerochea malleus* that is frequent in the Mediterranean (for example in the Algerian coast, OUNISSI & FRÉHI, 1999). *Bellerochea horologicalis* and *L. polymorpha* var. *tunisiense* have never been reported in the Mediterranean Sea and these taxa are probably of boreal origin because of their capacity to adapt to wide ranges of environmental conditions in comparison with the Mediterranean flora (DALY YAHIA-KÉFI *et al.*, 2005).

Dinoflagellates. Dinoflagellates present more diversified trophic behaviour than diatoms, being more independent of the nutrient levels. Several species of dinoflagellates appeared at very low nutrient concentrations (Table 2) and consequently a mixo-heterotrophic behaviour can be expected. Heterotrophy has been reported in *Ceratium furca, Gymnodinium sanguineum* (BOCKSTAHLER & COATS, 1993a, b), *Prorocentrum* (STOECKER *et al.*, 1997) and mixotrophy in *Dinophysis* species (JACOBSON & ANDSERSEN, 1994; BERLAND *et al.*, 1995).

Among the red tide species, the eutrophication and the modification of the habitats seem to be associated with the increase and expansion of red tide events in the Mediterranean Sea (GARCÉS *et al.*, 2000). Moreover, fish mortality has been reported in the north coastal lagoon of Tunis associated with the presence of blooms of *Gyrodinium aureolum* and *Alexandrium minutum* (ROMDHAME *et al.*, 1998). Daly Yahia-Kéfi et al. (2001) reported 10 species of *Alexandrium* in the Bay of Tunis. GIACOBBE *et al.* (1996), in a Sicilian lagoon reported *Alexandrium* occurrence being associated with nitrate-limited waters in concordance with our observations.

In the Tunisian-Sicily Strait, *Gyrodinium impudicum* presented maximum abundance during the autumn (GIACOBBE *et al.*, 1995). Red tides of athecate forms such as *Gyrodinium impudicum* and *Gymnodinium sanguineum*, primarily considered as authotrophic species, appeared under high phosphate levels versus nitrogen (nitrogen limited conditions) in the Bay of Tunis. In comparison with other groups, dinoflagellates presented higher contents of nucleic acids that could imply higher phosphorus requirements (COSTAS & LÓPEZ-RODAS, 1991). In the Greek coastal waters, the red tide of *Gymnodinium breve* appeared associated with nitrogen-limited waters with an excess of phosphate (SATSMADJIS & FRILIGOS, 1983).

The analysis of the oxidised forms of nitrogen (NO_3+NO_2) versus phosphate showed that the estuarine-brackish dinoflagellate *Peridinium quinquecorne*, typical taxon of polluted waters [as in the lagoon of Venice (TOLOMIO & MOSCHIN, 1995)] was observed under high values of nitrate but quasi-depleted phosphate levels. The presence of a chrysophyte endosymbiont could represent an advantage in comparison with marine dinoflagellates (HORIGUCHI & PIENNAR, 1991), being more competitive and in bloom under these conditions.

Conclusions

The investigation reported a high disturbance of the ecosystem surveyed in the Bay of Tunis and points out the responses of common diatoms and dinoflagellates to this environment. The responses of these species were evaluated by the use of a new index called

'SPI: Specific Preference Index'. It is a contribution to developing new indices that may be useful for understanding coastal systems' functioning and to enhance global studies in the Mediterranean Sea, because such information in the Bay of Tunis and more generally on the southern coast of the Mediterranean Sea is scarce. The ecosystem of the Bay of Tunis receives several inputs from surrounding urban and natural discharges. Even if the enrichment of this area is irregular, it concerns the south-western zone of the bay in communication with the Tunis Lake. However, the diversity of Diatoms and Dinoflagellates remains high compared to eutrophicated coastal areas. This study showed that several potential harmful species are present and dominant in the Bay of Tunis. On the other hand, the study showed a clear inter-annual variability between 1994 and 1995. It is clear that the preliminary conclusions obtained here should be confirmed by developing long-term monitoring programmes.

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