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# **Dynamics of dinoflagellates and environmental factors** during the summer in the Gulf of Gabes (Tunisia, Eastern Mediterranean Sea)

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SUMMARY: The summer spatial distribution of the dinoflagellate community along an open coastal sea gradient in the Gulf of Gabes (Tunisia, Eastern Mediterranean Sea), together with environmental factors, were studied. The most dominant families were represented by Gymnodiniaceae (32%), Peridiniaceae (20%), Prorocentraceae (15%), Ceratiaceae (13%) and Ebriaceae (10%). The dinoffagellate community was spatially more concentrated along the coast of the gulf than in the open sea. Eight toxic dinoflagellates were recorded, including Karenia cf. selliformis (37% of total toxic dinoflagellates) which was evenly distributed in both the neritic and open sea areas. Dinocysts contributed 33% of the total motile cells and were more abundant along the coast than in the open sea. This high concentration may be ascribed to nitrogen inputs in the coastal waters of Gabes. The Modified Atlantic Water governed dinoflagellate development in the open sea. The degradation of the water quality due to eutrophication in the Gulf of Gabes may have significant socioeconomic consequences. We suggest that a management framework, similar to that used in freshwater ecosystems, should be developed for the Gulf coast in order to drastically reduce urban interferences.

Keywords: Gulf of Gabes, dinoflagellates, dinocysts, nutrients.

RESUMEN: DINÁMICA ESTIVAL DE DINOFLAGELADOS ASOCIADA CON FACTORES AMBIENTALES EN EL GOLFO DE BAGES (TÚNEZ, MEDITERRÁNEO ORIENTAL). - Se estudió la distribución espacial de la comunidad de dinoflagelados junto con los factores ambientales a lo largo de un gradiente desde la costa a mar abierto en el Golfo de Gabes (Túnez, Mediterráneo oriental). Las familias dominantes de dinoflagelados estuvieron representadas por Gymnodiniaceae (32%), Peridiniaceae (20%), Prorocentraceae (15%), Ceratiaceae (13%), and Ebriaceae (10%). La comunidad de dinoflagelados estuvo más concentrada a lo largo de la costa del Golfo que en mar abierto. Se detectaron 8 especies de dinoflagelados tóxicos entre los cuales, *Karenia* cf. selliformis (37% del total de dinoflagelados tóxicos) estuvo homogeneamente distribuida en la zona nerítica y en áreas de mar abierto. Dinocysts contribuyeron en un 33% del total de células móviles y fueron más abundantes a lo largo de la costa que en el mar abierto. Esta alta concentración puede adscribirse a los vertidos de nitrógeno dentro de las agues costeras de Gabes. Las corrientes Atlánticas gobiernan el desarrollo de los dinoflagelados en el mar abierto. La degradación de la cualidad del agua que acompaña la eutrofización del Golfo de Gabes puede tener consecuencias socio-economicas significativas. Nosotros sugerimos que el tipo de gestión de las aguas, similar al usado en ecosistemas de agua dulce sea desarrollado en la zona costera del Golfo, para así reducir de forma drástica las interferencias urbanas.

Palabras clave: Golfo de Gabes, dinoflagelados, dinocysts, nutrientes.

# INTRODUCTION

Dinoflagellates represent a major part of the eukaryotic primary production in marine ecosystems (Parsons et al., 1984; Schnepf and Elbrächter, 1992). The ability of many strains to cause shellfish poisoning and/or to form resting cysts (Wall et al., 1977; Matsuoka et al., 2003), has led to considerable attention being paid to the diversity and distribution of planktonic dinoflagellates in relation to environmental parameters including temperature, salinity and nutrients (Wall et al., 1977; Smayda and Reynolds, 2001). In this respect, the Gulf of Gabes (Southern Tunisia, 35°N and 33°N) which has been put under environmental pressure due to industrial and urban activities (Hamza-Chaffai et al., 1997; Zairi and Rouis, 1999), has experienced a substantial proliferation of microalgae and particularly toxic dinoflagellates (Turki et al., 2006). The proliferation of unwanted microalgae has been widely shown to be an increasing problem in both coastal and estuarine environments (Smayda, 1997; Leong and Taguchi, 2005), causing significant overfishing of demersal resources, and thus degrading benthic habitats (Turki et al., 2006). In addition, fish resources in the Gulf of Gabes have declined as a result of the degradation of Seagrass meadows, Posidonia oceanica. In the open sea, nutrient inputs in the Gulf of Gabes have been shown to be influenced by both the frontal and the Atlantic-Mediterranean water circulation (Font et al., 1995; Estrada et al., 1985). The model by Beranger et al. (2004) shows that the Algerian current brings the upper layer eastwards. It then splits into two branches at the entrance of the Sicily Strait; one branch flows to the Tyrrhenian Sea and the other flows into the Sicily Strait. The latter is composed of two streams, referred to as the Atlantic Ionian Stream (AIS) and the Atlantic Tunisian Current (ATC). These water movements may be crucial in supplementing dinoflagellates with nutrients.

Our aims were to study, from the coast to the open waters, the horizontal and vertical dinoflagellate summer distribution across the Mediterranean waters to the Modified Atlantic Water (MAW) coupled to various environmental factors. As the Gulf of Gabes not only contributes 65% of the national fish production in Tunisia (C.G.P., 1996), but also shelters Djerba island, which, economically, is Tunisia's most important tourist attraction and is a famous habitat for marines turtles such as *Caretta caretta* and *Che*- *lonia mydas* (Baran and kasparek 1989; Maffucci et *al.*, 2006), our study can be useful for managing this ecosystem by helping to plan the best disposal options for anthropogenic wastes and the overall urban interferences.

### MATERIALS AND METHODS

#### **Study site**

This study was carried out in an area of the Gulf of Gabes where the climate is dry (average precipitation: 210 mm) and sunny with strong easterly winds. The study area of the Gulf of Gabes (between 35°N and 33°N) extends from "Ras kapoudia" at the 35°N parallel level to the Tunisian-Libyan border (Fig. 1) and shelters various islands (Kerkennah and Djerba) and lagoons (Bougrara and El Bibane). It opens to the offshore and has a wide continental shelf. Along the Tunisian coast, and during the cold period (winter-spring), the salinity of the MAW is low (37.3 to 37.5 p.s.u), and is very close to that of superficial layers. Conversely, during the warm season, the salinity increases strongly (38 p.s.u) and pronounced local circulation patterns are detected, which are most probably linked to a decline in the MAW-induced advection in the east (Beranger et al., 2004).

# Sampling

Samples (120) were collected in July 2005 in 33 coast-to-offshore stations on one cruise (Fig. 1). Water samples for physico-chemical analysis and examining phytoplankton were collected at 3 depths (surface, middle of water column and bottom) for stations <50 m deep and at 5 depths (surface, -10 m, -20 m, thermocline and bottom) for stations >50 m deep.

#### **Physico-chemical factors**

In each station, measurements of temperature, salinity, dissolved oxygen and water density were collected with a Conductivity-Temperature-Depth profiler (CTD: SBE 9, Sea-Bird Electronics, USA) equipped with a 12 Niskin bottle rosette sampler lowered from the surface to the near bottom. pH was measured immediately after sampling using a Met Röhm type pH meter. Samples for dissolved inorganic nitrogen (nitrite: NO<sub>2</sub><sup>-</sup>, nitrate: NO<sub>3</sub><sup>-</sup>, ammo-



FIG. 1. – Geographical map focussing on the phytoplankton sampling stations in the Gulf of Gabes.

nium:  $NH_4^+$ ) and orthophosphates:  $PO_4^{3-}$  were stored at -20°C before analysis with an automatic BRAN and LUEBBE type 3 analyzer. Concentrations were determined colorimetrically according to Grasshof (1983).

The concentration of the suspended matter was determined by measuring the dry weight of the residue after filtration through a whatman GF/C membrane.

### **Phytoplankton**

Phytoplankton was identified according to live cells to avoid cell destruction. Phytoplankton enumeration (including dinocysts) was performed with an inverted microscope following the method by Uthermöhl (1958) after fixation with a Lugol (4%) iodine solution (Bourrelly, 1985). Phytoplankton samples were identified according to Tregouboff and Rose 1957; Huber-Pestalozzi, 1968; Dodge, 1973; Dodge, 1975; Dodge, 1982; Dodge, 1985; Balech, 1988; Balech, 1995; Tomas et al., 1993 and Tomas et al., 1996. Biovolumes were estimated from cell dimensions according to Lohman (1908) and converted to carbon biomass with the conversion factor 1  $\mu$ m<sup>3</sup> = 0.12 10<sup>-6</sup>  $\mu$ gC. Samples for chlorophyll-*a* analysis, were filtered by vacuum filtration onto a 0.45  $\mu$ m pore size filter and 47 mm-diameter glass fibre filter Whatman, GF/F. Filters were then immediately stored at - 20°C. Pigment analysis was performed by HPLC according to Pinckney et al. (2001).

The level of community structure was assessed according to the diversity index as described by Shannon and Weaver (1949). The phytoplankton dominance index  $\delta$  was calculated with the formula  $\delta = (n_1 + n_2)/N$ , which expresses the relative contribution of the two most abundant species  $(n_1 + n_2)$  to the total standing stock and N as the total cell abundance.

#### Statistical analysis

The data recorded in this study were submitted to a normalized principal component analysis (PCA) (Dolédec and Chessel, 1989). Simple log (x+1) transformation was applied to data in order to correctly stabilize the variance (Frontier, 1973). A multivariate analysis (PCA and cluster analysis) was used to relate the phytoplankton distribution pattern to environmental variables. Cluster analysis (CA) was performed using PRIMER v5.0 for Windows XP (Clarke and Gorley, 2001) to identify the stations and regroup the samples with similar phytoplankton species composition and nutrient parameters. The results were illustrated by a dendrogram showing the steps in the hierarchical clustering solution and the values of the squared Euclidean distances between clusters.

A Pearson test performed with XL-stat was used to determine the correlations between 19 observations composed of dinoflagellate phytoplankton groups and environmental variables.



FIG. 2. - Contour plots of temperature (a), dissolved oxygen (b), pH (c), salinity (d), water density (e) and suspended matter (f).

### RESULTS

#### **Physico-chemical parameters**

The water temperatures ranged from 16 to 26°C  $(\text{mean} \pm \text{s.d.} = 23.07 \pm 2.47^{\circ}\text{C})$  (Fig. 2a) and had a tendency to increase from the offshore to the coast and from the bottom to surface. The temperatures of coastal waters (mean  $\pm$  s.d. = 24.92  $\pm$  1.65°C) were warmer than the offshore ones (mean  $\pm$  s.d.  $= 21.45 \pm 1.85$ °C) (Fig. 2a). The dissolved oxygen concentrations ranged from 6.4 to 7.7 mg l<sup>-1</sup> (mean  $\pm$  s.d. = 7.06  $\pm$  0.52 mg l<sup>-1</sup>) with the highest concentrations being recorded in the open sea, especially in stations with a depth >50 m (Fig. 2b). The pH ranged from 8.34 to 8.47 (mean  $\pm$  s.d. = 8.40  $\pm$  0.03) with a homogenous distribution of values throughout the monitoring stations, both in the neritic zone (mean  $\pm$  s.d. = 8.41  $\pm$  0.03) and the open sea (mean  $\pm$  s.d. = 8.40  $\pm$  0.03) (Fig. 2c). Salinity ranged from 37.2 to 38 p.s.u (mean  $\pm$  s.d. = 37.52  $\pm 0.29$  p.s.u). The lowest salinity (37.2-37.4 p.s.u) was recorded at a mean depth of 65 m between 11°E and 13°E, and the highest salinity in coastal waters at a mean depth of 33 m between 10.5°E and 11°E. The vertical distribution of salinity showed a surface longitudinal gradient (Fig. 2d). Water density ranged between 24 and 29 kg m<sup>-3</sup> (mean  $\pm$  s.d.  $= 25.6 \pm 0.41$  kg m<sup>-3</sup>) (Fig. 2e). At a depth of 50 m, in Stations 12, 16, 17, 20, 25, 26, 29, 30, and 31, the high water density coincided with low salinity, thus yielding significant negative correlations between the two parameters (Fig. 2d, e). This result indicates that the Gulf of Gabes is subjected to Atlantic water currents which flow between two water layers with similar characteristics (high salinity and water density). However, the coast is characterized by low water density and high salinity probably as a result of water evaporation. The map of depthintegrated density and temperature shows that density was essentially driven by temperature. Furthermore, it decreased from the coast (< 50 m) to the open sea, where strong stratification occurred during the summer. Concentrations in suspended matter ranged from 2 to 588 mg.  $1^{-1}$  (mean  $\pm$  s.d. = 24  $\pm$  70 mg.  $1^{-1}$ ) with high levels recorded between 10 and 20 m in depth (Fig. 2f).

Dissolved inorganic nitrogen (DIN) and orthophosphate concentrations were higher near the coast than in the open sea (Fig. 3a, b, c and d). High nitrate (1.42  $\pm$  0.26  $\mu$ mol l<sup>-1</sup>) and ammonium (0.61  $\pm$  0.21  $\mu$ mol l<sup>-1</sup>) concentrations were obtained at the bottom and thermocline (25 m) together with orthophosphates (0.06  $\pm$  0.03  $\mu$ mol l<sup>-1</sup>), whereas nitrite (0.36

 $\pm$  0.26 µmol 1<sup>-1</sup>) was concentrated chiefly in coastal waters (Fig 3a, b, c and d).

N/P: DIN (DIN =  $NO_2^- + NO_3^- + NH_4^+$ ) to DIP (DIP =  $PO_4^{3-}$ ) ratio varied from 10.87 to 106 (mean  $\pm$  s.d. = 43.61  $\pm$  17.98) (Fig 3e). This average was higher than the Redfield ratio (16), which suggests potential P limitation. N/P ratios in coastal waters varied between 20.20 and 79.28 (mean  $\pm$  s.d = 45.02  $\pm$  15.29) but decreased in the open sea with levels ranging between 14.06 and 53.03 (mean  $\pm$  s.d = 38.66  $\pm$  9.80). This indicates that the coast was supplied with more DIN than the open sea.



FIG. 3. – Spatial distribution of nitrate (a), ammonium (b), nitrite (c), and orthophosphorus (d) concentrations and N/P ratios (e) along a longitudinal gradient in the 0-120 m layer.



FIG. 4. – Spatial distribution of total phytoplankton abundance (a) contour plots of chlorophyll-*a* concentrations (ng. 1<sup>-1</sup>) along a longitudinal gradient in the 0-120m layer (b) diversity index (bits. cell<sup>-1</sup>) for total phytoplankton (c) and relative abundance and biomass of the different phytoplankton groups (d)

## Phytoplankton community structure

The phytoplankton community of the Gabes Gulf consisted of five groups: Dinophyceae, Bacillariophyceae, Cyanobacteria, Dictyophyceae and Euglenophyceae, among which Dinophyceae and Bacillariophyceae were the most diversified with a total of 78 and 31 species respectively. Total phytoplankton abundance varied from 2.1  $10^3$  (in Station 2 at 52 m) to 2.3  $10^5$  cells  $1^{-1}$  (in Station 29 at 19.5 m) (mean  $\pm$  s.d =  $2.3 \times 10^4 \pm 4.0 \times 10^4$  cells  $1^{-1}$ ) (Fig. 4a).

Chlorophyll-*a* concentrations ranged from 0 to  $2.6 \times 10^2$  ng l<sup>-1</sup> (mean  $\pm$  s.d. = 48.4  $\pm$  54.2 ng l<sup>-1</sup>) with a Deep Chlorophyll maximum (DCM) at 10 m, especially in Stations 3 and 9 (Fig 4b). In the Gulf of Gabes, the highest chlorophyll-*a* concentrations were

TABLE 1. – Correlation matrix (Pearson test) made with XL-stat for physical, chemical and biological variables under study in the Gulf of Gabes during summer 2005 (\* p<0.05; \*\* p<0.001, \*\*\* p<0.0001; number of parameters = 19 and number of analyzed samples: n = 120).

	Dinocysts	Prorocentraceae	Gymnodiniaceae	Peridiniaceae	Dinoflagellates	Phytoplankton
Salinity	0.441*	-0.409*	0.346*	-0.050	-0.080	0.017
Temperature	0.456*	-0.403*	0.379*	0.015	-0.058	0.065
Water density	0.439*	-0.407*	0.335	-0.063	-0.082	0.001
NO2-	0.357*	-0.240	0.422*	0.286	0.540*	0.180
NO3-	0.540**	0.010	0.296	0.171	0.289	0.108
NH4+	0.361*	-0.110	0.343	0.270	0.293	-0.081
PO43-	0.161	0.054	0.357*	0.200	0.200	0.031
N-T	0.598**	-0.047	0.365*	0.215	0.280	0.142
P-T	0.565*	-0.140	0.302	0.056	0.230	0.020
N/P	0.298	-0.042	0.382*	0.255	0.597*	0.150
Si(OH)4	0.245	-0.138	0.418*	-0.027	0.136	0.451
Depth (m)	0.340	-0.299	0.115	-0.221	-0.070	-0.186
Suspended matter	-0.129	-0.164	-0.172	-0.026	0.412*	-0.107
Chlorophyll-a	0.383*	-0.074	0.259	0.487	0.719***	0.832***



FIG. 5. – Spatial distribution of the total Dinophyceae group (a) relative abundance and biomass of its different families (b) and Distribution of the diversity index for Dinophyceae (c)

recorded along the coast (130 ng l<sup>-1</sup>), and correlated with dinoflagellate development (r = 0.719, n = 120, p<0.001) (Table 1). In the open sea, chlorophyll-*a* concentrations decreased (average: 30 ng l<sup>-1</sup>).

Overall, we found higher phytoplankton abundance in the nearshore than in the open sea. This also translated into a higher community diversification (H' max: 4.2 bits cell<sup>-1</sup>) (mean  $\pm$  s.d. = 3.04  $\pm$  0.74 bits. cell<sup>-1</sup>) due to the simultaneous presence of several dinoflagellate species (H' = 2.2 to 3 bits. cell<sup>-1</sup>, 17 species) (Fig. 4c). Although Dictyophyceae were only represented by one genus (*Dictyocha* sp.), they were the most abundant group (41% of total abundance), followed by Dinophyceae (25%), Bacillariophyceae (16%), Cyanobacteria (17%) and Euglenophyceae (1%) (Fig. 4d). In terms of biomass, Diatoms and Dinophyceae contributed 45 % and 25% of the total carbon biomass respectively (Fig. 4d).

### **Dinoflagellate community structure**

Dinoflagellate abundance ranged from  $1.6 \times 10^3$  to  $26 \times 10^3$  cells l<sup>-1</sup> (mean  $\pm$  s.d = 6.8  $10^3 \pm 4.7 \ 10^3$  cells.l<sup>-1</sup>) (Fig. 5a). The most dominant families were

Gymnodiniaceae (32%), Peridiniaceae (20%), Prorocentraceae (15%), Ceratiaceae (13%), Ebriaceae (10%) and 'others' (10%) (Table 2, Fig. 5b). However, the Ceratiaceae family was the largest contributor to the total Dinophyceae carbon biomass (78%) (Fig. 5b). The diversification of Dinophyceae increased from the open sea (H' = 2.4 to 3 bits cell<sup>-1</sup>, 15 species) in Station 4 to coastal waters, where H' reached a maximum of 4.0 bits. cell<sup>-1</sup> in Station 28 (mean  $\pm$ s.d. =  $2.4 \pm 0.5$  bits cell<sup>-1</sup>), due to the simultaneous presence of 20 different species (Fig. 5c). The Dinophyceae, like the entire phytoplankton community, was more concentrated along the Gulf coast than in the open sea, especially in Stations 23 and 27. Indeed, a positive and significant correlation (r = 0.41, n = 120, p<0.05) was found between the dominance index and the distance from the coast (Fig. 6). The coastal zone was dominated by Ceratium and the offshore by Protoperidinium. We also recorded eight toxic dinoflagellates (6% of the total Dinoflagellates), among which, Karenia cf. selliformis (37% of total toxic dinoflagellates) was distributed homogeneously in both the neritic zone and open sea (Figs. 6, 7). Dinocysts contributed 33% of the total motile cells and

Class	Order	Family	Relative percentage(%)	Genus	Species
Dinoflagellates	Gymnodinial	Gymnodiniaceae	32	Gymnodinium	sp., filum, abbreviatum, marinum, gliba, sanguinum, venificum, catenatum
				Gyrodinium Amphidinium Cochlodinium	sp., <i>fusiforme, spiralea, falcatum</i> sp., <i>ovoideum, cataerae, sphenoïdes</i> sp.
		D 1-1-1'1-	2	Karenia	mirabile, cf selliformis, pappillionacea
		Polykrikaceae	2	Polykrikos	sp.
		Warnowiaceae	0.5	Warnowia Buono contrum	sp.
		Prorocentraceae	15	Prorocentrum	micans, triestinum, concavum, gracile,
		Dinophysaceae	1	Dinophysis	accuminata, rotundata
		1 5		Phalocroma	sp.
				Metaphalocroma	sp.
				Ornithocercus	sp.
		Ebriaceae	10	Ebria	sp.
				Hermesinium	sp.
		Protodiniferacea	e 0.5	Pronoctiluca	sp.
		Peridiniaceae	20	Protoperidinium	sp., depressum, thoranum, minitum, globulum, diabolus, ovum, curvipes, diabolum, splendes divergens auinauecorne teristinum
				Scrippsiella	sp., supselsa
				Peridinium	sp.
				Heterocapsa	sp.
		Oxytoxaceae	0.5	Oxytoxum	sceptrum, constrictum, scolopax
		Heterdiniaceae	0.5	Heterodinium	milneri
		Goniodomatacea	e 0.5	Gniodoma	polydricum
		Gonyaulacaceae	2	Alexandrium	sp.
				Gonyaulax	sp., polygrama, polyedra, spinifera
				Protoceratium	reticulum
		Ceratiaceae	13	Ceratium	falcatum, macroceros, candelabrum, tripos, furca, fusus, lineatum, extensum
		Ceratocorvacea	0.5	Ceratocorvs	SD.
		Podolampadacea	e 2	Podolampas	sp. palmines
		. suonampudaeea		. caorampas	SP., P

TABLE 2. – Taxon	omic of the	Dinophyceae	group phytoplanktor	contributions.
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were more abundant along the coast than in the open sea (Fig. 8a). This was particularly clear in the 50 m isobaths in coastal Stations 20, 30 and 31 (Fig. 8a). In coastal waters, motile cells and cysts increased slightly with depth (Fig. 8b), whereas in the open sea their numbers did not change at the surface, at -10 m, or -20 m and at the bottom except in the thermocline where the highest Dinoflagellate and Dinocyst abun-



Neritic-opean sea distance (km)

FIG. 6. – Relationships between the dominance index and distance from the coast. The two most dominant species are indicated for each sampling station.



FIG. 7. - Relative abundance of different toxic dinoflagellate species.

dances were recorded  $(3.36 \times 10^4 \text{ and } 2.19 \times 10^4 \text{ cells})$ 1<sup>-1</sup> respectively (Fig. 8b).

The principal component analysis (PCA) (first plot) allowed two groups to be discriminated around the F1 and F2 axes (Fig. 9). Axis F1, which represents 57.55% of the variability and to which the environmental variables are small and equal contributors, positively selects group G1 which is composed of total phytoplankton and nutrient parameters (nitrate, nitrite, ammonium, orthophosphorus, N/P ratio, total nitrogen: Total-N and total phosphorus: Total-P). Axis F2 explains 12.7% of the variability and negatively selects group G2 formed by abiotic variables (temperature, salinity, water density and silicate) and depth. This association confirms our previous observations that phytoplankton abundance decreases with depth, and is more concentrated in coastal rather than open sea areas. In addition, the phytoplankton density was dependent on nutrient



FIG. 8. – Spatial distribution of Dinocysts (a) spatial and vertical distribution of abundance of Dinocysts and total dinoflagellates (b)

availability (nitrate, nitrite, ammonium, orthophosphates, Total-N, Total-P) and especially on N/P ratios, which seemed to be the deterministic regulator of dinoflagellate dynamics.

The second plot allowed two groups to be discriminated around the F1 and F2 axes (Fig. 9). Both axes represent 58.04% of the variability and posi-



FIG. 9. – Principal Component Analysis (PCA) (Axis I and II) of the total phytoplankton abundance and that of the different dinoflagellate families and selected environmental variables (arrows) in the Gulf of Gabes during July (summer).



FIG. 10. - Dendrogram of the Euclidean distance between the sampling stations (a). The three identified clusters are highlighted on the map (b).

tively select group G1 which is composed of all phytoplankton, with dominant Dinoflagellates families (Gymnodiniaceae, Peridiniaceae), Dinocysts and nutrient parameters (nitrate, nitrite, ammonium, orthophosphates, Total-N, Total-P and N/P ratio). The F2 axis, which explains 15.01% of the variability, negatively selects group G2 formed by abiotic variables (temperature, salinity, density and water density) and depth. This association confirms our previous observations on the close links between the distribution of Dinoflagellates and Dinocysts (r = 0.597, n = 120, p<0.05) and between dinoflagellates and nutrients. We also found a significant correlation between nitrate concentrations and dinoflagellate abundance (r = 0.540, n = 120, p<0.05) (Table 1). Dinoflagellate abundance was higher along the nutrient-rich coast than in the open sea.

The dendrogram from the cluster analysis emphasizes 3 natural groupings. Cluster 1 groups the open sea stations (3, 4, 6, 10 and 12), Cluster 2 is composed of stations situated between coastal and open sea areas (5, 9, 11, 16, 18, 21, 22 and 26), and Cluster 3 exclusively groups the coastal monitoring stations (14, 15, 19, 20, 23 and 24) (Fig. 10a, b). Among these, Stations 23 and 24 near Djerba Island were home to enhanced dinoflagellate growth which coincided with high nitrogen availability.

DISCUSSION

groups: Dinophyceae, Bacillariophyceae, Cyanobacteria, Dictyophyceae and Euglenophyceae, among which Dinophyceae and Bacillariophyceae were the most diversified groups. In addition, we found higher cell abundances along the coast than in the open sea, which also translated into higher chlorophyll-a concentrations and community diversification. The range of total phytoplankton density found in the Gulf of Gabes was close to that reported in various temperate environments (Casotti et al., 2000; Denis et al., 2000, Gomez and Gorsky, 2003; Moran et al., 2001; Turki et al., 2006). This is specifically evident for the distribution of dinoflagellates, which showed the same density pattern as the total phytoplankton but with more pronounced coastal-open sea gradients. The highest Dinoflagellate abundance (2.6  $\times$ 10<sup>4</sup> cells. 1<sup>-1</sup>) and species diversity (4 bits. cell<sup>-1</sup>) were recorded in the coastal sea areas near Djerba Island (Stations 23, 27, 28) (Fig. 1, 8a, 9) with Ceratium furca and C. fusus as dominant species, while offshore Protoperidinium spread abundantly. Ceratium and Protoperidinium were found to be dominant in November 1969 (Borgne-David, 1975) and Octo-

The results indicate that the summer phytoplank-

ton community of the Gabes Gulf consisted of five

ber 2000 (Turki et al., 2006) in the Gulf of Gabes. They were also the most abundant dinoflagellates in summer 2001 in lake Bizerte (Northern coast of Tunisia) (Turki et al., 2006). Ceratium furca and C. fusus were repeatedly observed in other Mediterranean marine environments (Revelante and Gilmartin, 1976). This might be ascribed to nitrogen inputs to the coastal waters of the Gabes Gulf. In addition, the continuous consumption of phosphate by growing phytoplankton resulted in low concentrations of this element. We found significant correlations between the dinoflagellate abundance and N/P ratios (r = 0.597, n = 120, p<0.05) and nitrate concentrations (r = 0.540, n = 120, p < 0.05), but no significant correlations with phosphate. This highlights the crucial and openly accepted role played by nitrogen in sustaining the dinoflagellate proliferation in marine ecosystems (Fanning, 1992; Reul et al., 2005), and in enhancing its storage to face nitrogen shortage (Glibert et al., 2001; Badylak and Phlips, 2004). The low chlorophyll-a concentrations and higher than 'normal' oceanic Redfield ratio of 16:1 recorded in the Gulf of Gabes indicate that this ecosystem is very highly-oligotrophic (Vollenweider et al., 1992). This seems in accordance with the oligotrophic status, given that the Ionian Sea is based both on low nutrient concentrations (Bregant et al., 1992) and low primary production derived from chlorophylla concentrations (Magazzù and Decembrini, 1995; Boldrin et al., 2002; Moutin and Rainbault, 2002). Growing evidence indicates that the Eastern Mediterranean basin is an "ultra-oligotrophic" ecosystem (Krom et al., 1991; Heurt et al., 2005) characterized by dinoflagellate dominance as shown for the Gulf of Gabes (Gomez et al., 2000). Moreover, due to the lack of deep mixing along the coast, which gives an advantage to motile cells over non-motile ones (e.g., diatoms) (Paerl, 1997), dinoflagellates spread along the coastal sea. Similar observations were reported in the Gulf of Tunis (Daly-Yahia, 1998); in the Bay of Ville-Franche (NW Mediterranean Sea) (Gomez and Gorsky, 2003); in hypertrophic costal waters of Tokyo Bay (Matsuoka et al., 2003) where C. furca was among the dominant Dinoflagellates (Matsuoka et al., 2003) followed by Protoperidinium. Ceratium was also among the dominant phytoplankton species observed in summer in Chinese lakes from 1991 to 1999 (Chen et al., 2003). Large phytoplankton cells tend to dominate in nutrient-rich coastal waters, while small cells are likely to be characteristic of oligotrophic oceanic systems (Kang et al., 2004). This

result applies to the Gulf of Gabes in which coastal Ceratium had a biovolume ranging from 1.17 to  $60 \times$  $10^3 \,\mu\text{m}$  (mean ± s.d. =  $2.23 \times 10^2 \pm 18.56 \,\mu\text{m}$ ), whereas exclusively oceanic (>100 km) Protoperidinium cells were small, ranging from 10 to 85  $\mu$ m (mean ± s.d. =  $41.26 \pm 18.56 \mu m$ ). While dinoflagellate development followed a coastal-open sea gradient, toxic species and especially Karenia cf. selliformis (37% of total toxic dinoflagellates) spread throughout the Gulf. This corroborates the observations reported by others in the Gulf of Gabes in October 2000, which show the presence of 10 toxic taxa, among which Karenia cf. selliformis was the most significant toxin producer (Turki et al., 2006). However, the distribution of this group is poorly indicated since, to date, there have not been any thorough studies carried out in this ecosystem. Earlier studies were mostly conducted in a restricted zone without depth samplings (Balkis, 2003), so an attempt to compare them with other published results to assess how communities are changing, is impossible. Nonetheless, Chang et al. (2003) reported lower numbers of toxic Karenia offshore than inshore in a north-eastern New Zealand Gulf during the summer. In the Gulf of Gabes, we detected high numbers of dinocysts blocked in the thermocline. These may have been at least partially brought by a fraction of the Modified Atlantic Water (MAW) which detaches from the south of Lampedusa Island (Italy), invades the Gulf of Gabes and recirculates anticyclonally on the Tunisian shelf (Lecombe and Tchernia, 1972). The MAW may inoculate the Gulf of Gabes with phytoplankters, toxic Dinoflagellates and their cysts (Reul et al., 2002; Gomez, 2003; Gomez, et al., 2004). Additional dinoflagellate cyst inocula may originate from other areas as they can survive being transported in ballast water from ships (Hallegraeff, 1993; Yoshida et al., 1996; Hallegraeff, et al., 1997).

#### CONCLUSION

Dinophyceae (both motile non-toxic and toxic cells and cysts) seems to be an important component of the phytoplankton community in the Gulf of Gabes. They occur throughout the coastal-open sea gradient. The abundance of harmful species is of great concern because their presence can lead to a significant impact on the edibility and marketability of marine foodstuffs. In addition, monitoring Stations 23 and 24 near the socio-economically impor-

tant Djerba Island reveal high numbers of toxic *K. selliformis* that coincides with enhanced DIN/P ratios (25.25). This suggests that excess reactive nitrogen is derived primarily from fertilizer applications, animal wastes and fuel combustion. The dinoflagellate ecology in the Gulf of Gabes is complex due to the interaction of various factors (water movements, urban interferences, marine traffic...) and needs to be clarified through long term studies. Treating urban and industrial wastes is the essential cornerstone for controlling aquatic eutrophication in the coastal waters of the Gulf. In the open sea, the MAW occurrence is the main deterministic factor of dinoflagellate development.

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#### REFERENCES

- Badylak, S. and E.J. Phlips. 2004. Spatial and temporal patterns of phytoplankton composition in a subtropical coastal lagoom, the Indian River Lagoon, Florida, USA. J. Plankton Res., 26(10): 1229-1247.
- Balech, E. 1988. Los dinoflagelados del Atlantico sudoccidental. Instituto Español de Oceanografia (Publicaciones especiales), Madrid.
- Balech, E. 1995. *The genus Alexandrium Halim (Dinoflagellata)*. Special publication, Sherkin Island Marine Station, Cork.
- Balkis, N. 2003. Seasonal variations in phytoplankton and nutrient dynamics in the neritic water of Buyukçekmece Bay, Sea of Marmzarra. J. Plankton Res., 25(7): 703-717.
  Baran, I. and M. Kasparek. 1989. On the whereabout of immature
- Baran, I. and M. Kasparek. 1989. On the whereabout of immature sea turtles (*Caretta caretta* and *Chelonia mydas*) in the eastern Mediterranean. Zool. Middle East, 3: 31-36.
- Beranger, K., L. Mortier, G.P. Gasparini, L. Gervasio, M. Astraldi and M.Crepon. – 2004. The dynamics of the sicily strait: a comprehensive study from observations and models. *Deep Sea Res.*, 51: 411-440.
- Boldrin, A., S. Miserocchi, S. Rabitti, M.M.Turchetto, V. Balboni and G. Socal. – 2002. Particulate matter in the southern Adriatic and Ionian Sea: Characterisation and downward fluxes. J. Mar. Syst., 33: 389-410.
- Borgne-David. 1975. Bacillariales et Dinophcées de la partie sudoccidentale du bassin oriental de la méditerranée. *Rev. Trav. Inst. Pêches marit.*, 39(3): 235-247.
- Bourrelly, P. 1985. Les Algues d'Eau Douce. Initiation à la Systèmatique. Tome II. Les Algues bleues et rouges. Les Euglénins, Peridiniens et Cryptomonadines. Société Nouvelle des Editions

Boubée.

- Bregant, D., G. Civitarese and A. Luchetta. 1992. Chemical parameters distribution in the Ionian Sea during POEM-06 cruise (October 1991). *Rapp. P. V. Reun. C.I.E.S.M.*, 1-395.
- C.G.P. 1996. Annuaire des statistiques des pêches en Tunisie. Ministère de l'agriculture, Tunisie.
- Casotti, R., C. Brunet, B. Aronne and M. Ribera d'Alcalà. 2000. Mesoscale features of phytoplankton and planktonic bacteria in a coastal area as induced by external water masses. *Mar. Ecol. Prog. Ser.*, 195: 15-127.
- Chang, E.H., J. Zeldis, M. Gall and J. Hall. 2003. Seasonal and spatial variation of phytoplankton assemblages, biomass and cell size from spring to summer across the north-eastern New Zealand continental shelf. J. Plankton Res., 25(7): 737-758.
  Chen, Y., B. Qin, K. Teubner and M.T. Dokulil. – 2003. Long-term
- Chen, Y., B. Qin, K. Teubner and M.T. Dokulil. 2003. Long-term dynamics of phytoplankton assemblages: Microcystis-domination in Lake Taihu, a large shallow lake in China. J. Plankton Res., 25 (1): 445-453.
- Clarke, K.R. and R.N. Gorley. 2001. PRIMER v5: user manual/ tutorial. PRIMER-E, Plymouth.
- Daly Yahia, O. 1998. Le phytoplancton de la Baie de Tunis (analyse systématique, bio-géographie quantitative et synécologie des diatomées et dinoflagellés). Ph. D. thesis, Univ. Tunisia.
  Denis, M., V. Martin and V. Andersen. 2000. Short term varia-
- Denis, M., V. Martin and V. Andersen. 2000. Short term variations of the vertical distribution of cyanobacteria in the open Mediterranean Sea. Sci. Mar., 64: 157-163.
- Dodge, J.D. 1973. *The fine structure of algal cells*. Academic Press, London and New York.
- Dodge, J.D. 1975. The Prorocentrales (Dinophyceae). II. Revision of taxonomy within the genus *Prorocentrum. Bot. J. Linn. Soc.*, 71: 103-125.
- Dodge, J.D. 1982. Marine Dinoflagellates of the British Isles. Her Majesty's Statonery Office, London.
- Dodge, J.D. 1985. Atlas of dinoflagellates. A scanning Electron Microscope Survey. Ferrand Press, London.
- Dolédec, S. and D. Chessel. 1989. Rythmes saisonniers et composantes stationnelles en milieu aquatique. II. Prise en compte et élimination d'effets dans un tableau faunistique. Acta Oecol. Oec. Gen., 10: 207-332.
- Estrada, M., F. Vives and M. Alcarez. 1985. Life and productivity of the open sea. In: R. Marglef, (eds.), *Western. Mediterranean*, pp. 148-197, Pergamon Press, Oxford.
- Fanning, K.A. 1992. Nutrient provinces in the Sea: concentration ratios, reaction rate ratios, and ideal covariation. J. Geophys. Res., 97: 5693-5712.
- Font, J., E. Garcia-Ladona and E.G. Gorriz. 1995. The seasonality of mesoscale motion in the Northern Current of the Western Mediterranean: several year of evidence. *Oceanol. Acta.*, 18: 207-219.
- Frontier, S. 1973. Etude statistique de la dispersion du zooplancton. J. Exp. Mar. Biol. Ecol., 12: 229-262.
  Glibert, P.M., R. Magnien, M.W. Lomas, J. Alexander, C. Fan, E.
- Glibert, P.M., R. Magnien, M.W. Lomas, J. Alexander, C. Fan, E. Haramoto, M. Trace and T.M. Kana. – 2001. Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: comparison of 1997, 1998, and 1999 events. *Estuaries*, 24(6): 875-883.
- Gomez, F. 2003. The toxic dinoflagellate *Gymnodinium catenatum*: An invader in the Mediterranean Sea. Acta Bot. Croat., 62(2): 65-72.
- Gomez, F. and G. Gorsky. 2003. Annual microphytoplankton cycles in the Villefranche Bay, Ligurian Sea, NW Mediterranean. J. Plankton Res., 25: 323-339.
- Gomez, F., F. Echevarria, C.M. Garcia, L. Prieto, J. Ruiz, A. Reul, F. Jimenez-Gomez and M.J. Varela. – 2000. Microplankton distribution in the Strait of Gibraltar: coupling between organisms and hydrodynamic structures. J. Plankton Res., 22(4): 603-617.
- Gomez, F., G. Gorsky, E. Garcia-Gorriz and Picheral. 2004. Control of the phytoplankton distribution in the strait of Gibraltar by wind and fortnightly tides. *Estuar. Coast. Shelf Sci.*, 59: 485-497.
- Grasshof, K.M. 1983. Determination of nitrate, in Methods of Seawater Analyses. In : K.M. Grasshoff, K. Ehradt and K. Kremling (eds.), pp. 143-150. Verlag Chemie, Weinheim, Germany.
- Hallegraeff, G.M. 1993. A Review of harmful algal blooms and their apparent global increase. *Phycologia.*, 32(2): 79-99.

- Hallegraeff, G.M., J.P. Valentine, J.A. Marshall and J.B. Christopher. – 1997. Temperature tolerances of toxic dinoflagellate cysts: application to the treatment of ships' ballast water. *Aquat. Ecol.*, 31 : 47-52.
- Hamza-Chaffai, A., C. Amiard-Triquet and A. El Abed. 1997. Metallothionein-like protein, is it an efficient biomarker of metal contamination? A case study based on fish from the Tunisian coast. Arch. Environ. Contam. Toxicol., 33: 53-62.
- Heurt, B., T. Zohary, M.D. Krom, R. Fauzi, C. Mantoura, P. Pitta, S. Psarra, F. Rassoulzadegan, T.Tanaka and T.F. Thingstad. – 2005. Response of East Mediterranean surface water to Saharan dust: On-board microcosm experiment and field observations. *Deep-Sea Res. II*, 52: 3024-3040.
- Huber-Pestalozzi, G. 1968. Das phytoplankton des Susswassars, 1. Halfte, Cryptophyceae, Chloromonadophyceae, Dinophyceae. E. Schweizerbart Verlag, Stuttgart.
- Kang, J.H., W.S. Kim, K.I. Chang and J.H. Noh. 2004. Distribution of plankton related to the mesoscale physical structure within the surface mixed layer in southwestern East Sea, Korea. J. Plankton Res., 26(12): 1515-1528.
- Krom, M.D., S. Brenner, N. Kress and L.I. Gordon. 1991. Phosphorus limitation of primary productivity in the E. Mediterranean Sea. *Limnol. Oceanogr.*, 36: 424-432.
- ranean Sea. Limnol. Oceanogr., 36: 424-432. Lecombe, H. and P. Tchernia. – 1972. Caractères hydrologiques et circulation des eaux en Méditerranée. In: D. Stanley (eds.), *Mediterranean Sea*, pp. 25-36. Dowden, Hutchinson and Ross, Stroudsburg.
- Leong, S.C.Y. and S. Taguchi. 2005. Optical characteristics of the harmful dinoflagellate Alexandrium tamarense in response to different nitrogen sources. *Harmful Algae.*, 4: 211-219.
- Lohman, H. 1908. Untersuchungen zur Feststellung des Vollständigen Gehaltes des Meeres an Plankton. Wiss. Meeresunters., 10: 131-370.
- Maffucci, F., W.H.C.F. Kooistra and F. Bentivegna. 2006. Natal origin of loggerhead turtles, *Caretta caretta*, in the neritic habitat off the Italian coasts, Central Mediterranean. *Biol. Conserv.*, 127: 183-189.
- Magazzù, G. and F. Decembrini. 1995. Primary production, biomass and abundance of phototrophic picoplankton in the Mediterranean Sea: A review. *Aquat. Micobial Ecol.*, 9: 97-104.
   Matsuoka, K., L.B. Joyce, Y. Kotani and Y. Matsuyama. 2003.
- Matsuoka, K., L.B. Joyce, Y. Kotani and Y. Matsuyama. 2003. Modern dinoflagellate cysts in hypertrophic costal waters of Tokyo Bay, Japan. J. Plankton Res., 25(12): 1641-1470.
- Moran, X.A., I. Taupier-Letage, E.S. Väzquez-Dominguez, L. Arin, P. Raimbault, M. Estrada. – 2001. Physical-biological coupling in the Algerian Basin (SW Mediterranean): Influence of mesoscale instabilities on the biomass and production of phytoplankton and bacterioplanktonm. *Deep-Sea Res. I*, 48: 405-437. Moutin, T. and P. Rainbault. – 2002. Primary production, carbon
- Moutin, T. and P. Rainbault. 2002. Primary production, carbon export and nutrients availability in western and eastern Mediterranean Sea in early summer 1996 (MINOS cruise). J. Mar. Syst., 33: 273-288.
  Paerl, H.W. 1997. Coastal eutrophication and harmful algal
- Paerl, H.W. 1997. Coastal eutrophication and harmful algal blooms: importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. *Limnol. Oceanogr.*, 42: 1154-1165.
- Parsons, T.R., M. Takahashi and B. Hargrave. 1984. Biological Oceanographic Processes. Pergamon Press, Oxford.
- Pinckney, J.L., T.L. Richardson, D.F. Millie and H.W. Paerl. 2001. Application of photopigment biomarkers for quantifying microalgal community composition and in situ growth rates. Org. Geochem., 32: 585-595.

- Reul, A., J.M. Vargas, F. Jimenez-Gomez, F. Echevarria, J. Garcia-Lafuente, J. Rodriguez. – 2002. Exchange of planktonic biomass through the Strait of Gibraltar in the late summer conditions. *Deep-Sea Res.*, 49: 4131-4144.
- Reul, A., V. Rodriguez, F. Jiménez-Gomez, J.M. Blanco, B. Bautista, T. Sarhan, F. Guerrero, J. Ruiz and J. Garcia-Lafuente. – 2005. Variability in the spatio-temporal distribution and size structure of phytoplankton across an upwelling area in the NW-Alboran Sea, (W-Mediterranean). *Cont. Shelf Res.*, 25: 589-608.
- Revelante, N. and M.Gilmartin. 1976. Temporal succession of phytoplankton in the northern Adriatic. *Neth. J. Sea Res.*, 10: 377-396.
- Schnepf, E. and M. Elbrächter. 1992. Nutritional strategies in dinoflagellates. A review with emphasis on cell biological aspects. *Europ. J. Protistol.*, 28: 3-24.
- Shannon, C.E. and G. Weaver. 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana, Chicago, IL.
- cago, IL. Smayda, T.J. – 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.*, 42: 1137-1153.
- Smayda, T.J. and C.S. Reynolds. 2001. Community assembly in marine phytoplankton; application of recent models to harmful dinoflagellate blooms. J. Plankton Res., 23: 447-461.
- Tomas, C.R., G.R. Hasle, A.K. Steidinger, E.E. Syvertsen and C. Tangen. – 1996. Identifing marine diatoms and dinoflagellates. Academic Press, Inc.
- Tomas, C.R., J. Throndsen and B.R. Heimdal. 1993. Marine phytoplankton, a guide to naked flagellates and coccolithophorids. Academic Press, Inc.
- Tregouboff, G. and Rose, M. 1957. Manuel de planctonologie méditerranéenne. CNRS, Paris, Vol II.
  Turki, S., A. Harzallah and C. Sammari. – 2006. Occurrence of
- Turki, S., A. Harzallah and C. Sammari. 2006. Occurrence of harmful dinoflagellates in two different Tunisian ecosystems: the lake of Bizerte and the gulf of Gabes. *Cah. Biol. Mar.*, 47.
- Utermöhl, H. 1958. Zur Vervollkommung der quantitativen Phytoplankton Methodik. Mitteilungen Internationale Vereinigung für Theoretische und Angewandte. *Limnol.*, 9: 1-38.
- Vollenweider, R.A., R. Marchetti and R. Viviani. 1992. Marine Coastal Eutrophication. The Response of Marine Transitional Systems to Human Impact: Problems and Perspectives for Restoration. Science of the Total Environment. Elsevier Science, Amsterdam, The Netherlands.
- Wall, D., B. Dale, G.P. Lohman and W.K. Smith. 1977. The environmental and climatic distribution of dinoflagellate cysts in modern marine sediments from regions in the North and South Atlantic Oceans and adjacent seas. Mar. *Micropaleontol.*, 2: 121-200.
- Yoshida, M., Y. Fukuyo, T.Murase and T. Ikegama. 1996. Onboard observations of phytoplankton viability in ship's ballast tanks under critical light and temperature conditions. In: T. Yasumoto, Y.Oshima and Y. Fukuyo (eds.), *Harmful and Toxic Algal Blooms*, pp. 205-208. IOC, Paris.
   Zairi, M. and M.J. Rouis. 1999. Impacts environnementaux du
- Zairi, M. and M.J. Rouis. 1999. Impacts environnementaux du stockage du phosphogypse à Sfax (Tunisie). Bulletin des Laboratoires des Ponts et Chaussées., 219: 29-40.

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