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Diazotrophic cyanobacteria signatures and their relationship to hydrographic conditions in the Gulf of Gabes, Tunisia

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Changes in the planktonic cyanobacteria structure, composition and diversity were followed over three consecutive years (2005–2006–2007) in the Gulf of Gabes (Eastern Mediterranean Sea, Tunisia). Cyanobacteria abundances, biomasses and cell lengths were measured together with selected environmental variables (pH, salinity, temperature and nutrients). The space and time variations of the cyanobacteria in relation to the environmental factors showed a close relationship between these plankton communities and the hydrographic structure of the water column. Cyanobacteria developed over semi-mixed conditions (May–June 2006) and during the thermal stratification (July 2005). The cyanobacterial abundance and biomass was evident between 20 and 35 m in inshore stations and between 20 and 25 m in deeper stations during the semi-mixing conditions and stratification. This thermocline level coincided with the euphotic layer (21.85 ± 3.76 m) allowing access of light radiation. The cyanobacteria bloom occurred during May–June 2006 when the N/P ratio (<10) was clearly below the accepted standard molar ratio of N/P = 16/1. Commonalities among cyanobacterial genera include being highly competitive for low concentrations of inorganic P (DIP) and the ability to acquire organic P compounds. Our study showed that both diazotrophic (N_2-fixing) cyanobacteria such as Anabaena *sp.,* Chroococcus *sp.,* Trichodesmium erythraeum, Spirulina *sp. and* Spirulina subsalsa *and non-diazotrophic cyanobacteria such as* Pseudoanabaena *sp. and* Microcystis *display a great flexibility in the N sources which allow formation of blooms.*

Keywords: Gulf of Gabes, diazotrophic cyanobacteria, water column, inshore area, offshore area, mixing conditions, stratification, thermocline, nutrient compounds, N/P ratio

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INTRODUCTION

Primary production is nitrogen-limited in most marine ecosystems (Granéli *et al.*, 1990; Kivi *et al.*, 1993; Tuomainen *et al.*, 2003). Nitrogen fixation, nitrification and denitrification, which are all microbially mediated, are the key processes for determining the availability of nitrogen (Tuomainen *et al.*, 2003; Sorokovikova *et al.*, 2013; Stief *et al.*, 2014). Cyanobacteria are the major biomass producers both in aquatic and terrestrial ecosystems and represent more than 50% of the biomass in many aquatic ecosystems (Häder *et al.*, 2007). Cyanobacteria appear to be responsible for most of planktonic N_2 fixing in aquatic ecosystems and this ability gives a significant competitive advantage to these organisms during periods of nitrogen limitation (Tilman *et al.*, 1982; Howarth *et al.*, 1988; Leppänen *et al.*, 1988; Gallon, 1992; Zehr *et al.*, 2001; Paerl & Otten, 2013). Diazotrophic (nitrogen-fixing) cyanobacteria are important contributors of new nitrogen to oligotrophic environments and greatly influence oceanic productivity (Berman-Frank *et al.*, 2007). The factors controlling N_2 fixation are still poorly known in the Mediterranean Sea (Ridame *et al.*, 2011). As the Mediterranean Sea has been described as a phosphate-depleted basin, phosphorus can be logically suspected to be the limiting nutrient for diazotrophic activity (Ridame *et al.*, 2011). The Mediterranean Sea is strongly impacted by episodic Saharan dust deposition (e.g. Guerzoni *et al.*, 1999; Guieu *et al.*, 2010). It has been shown that new atmospheric nutrients associated with Saharan dust pulses significantly stimulate N_2 fixation in the Mediterranean Sea and that N_2 fixation is a key process in the carbon cycle in oligotrophic environments such as the Gulf of Gabes (Drira *et al.*, 2008; Ridame *et al.*, 2013; Elloumi *et al.*, 2015). Despite being oligotrophic, the Gulf of Gabes accounts for 65% of Tunisian fish production (DGPA, 2005–2009) and is a well-known habitat for marine turtles such as *Caretta caretta* and *Chelonia mydas* (Maffucci *et al.*, 2006; Lotze & Worm, 2009). We hypothesized that, although the Gulf of Gabes is an oligotrophic ecosystem, the diazotrophic

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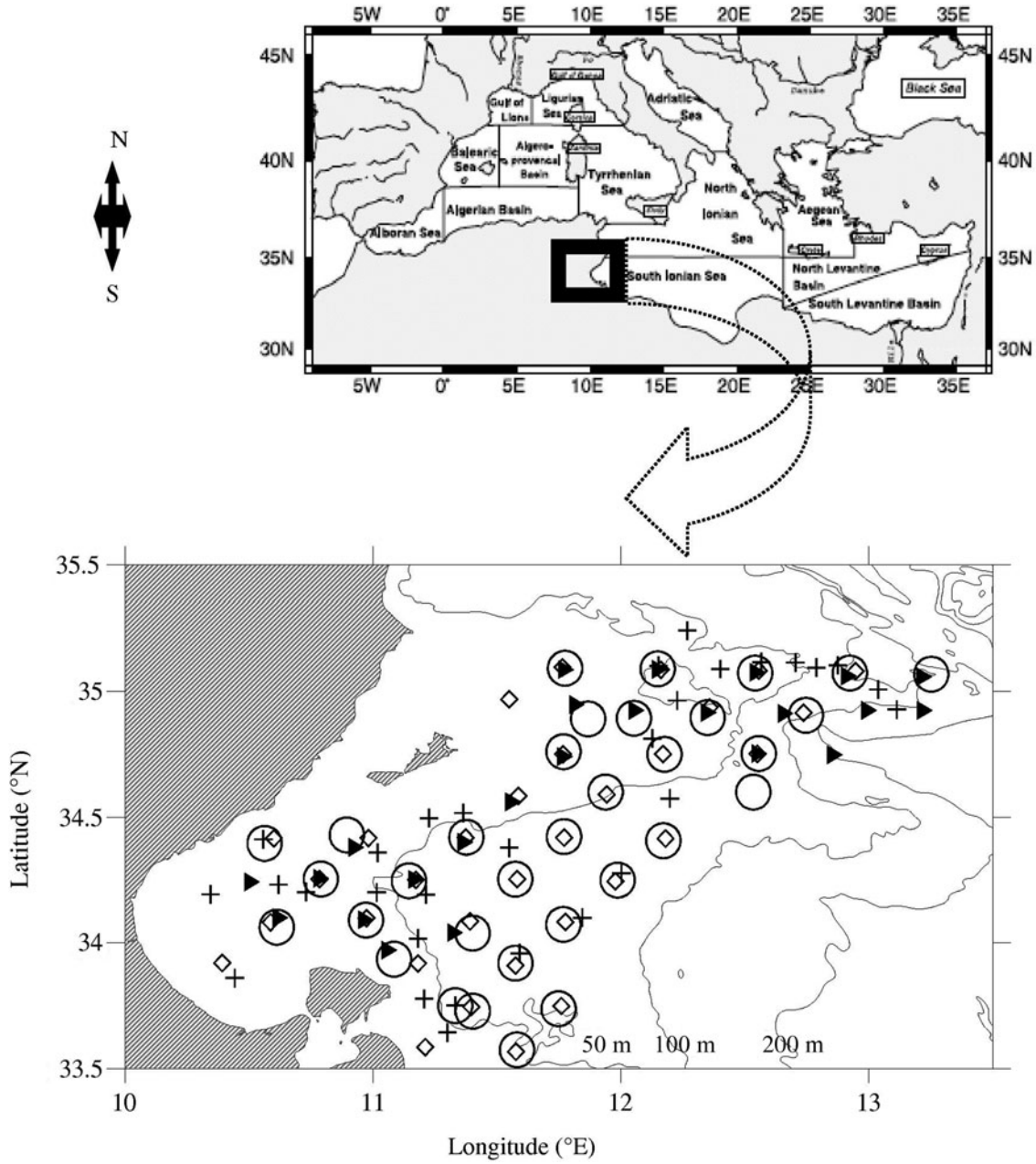

Fig. 1. Geographic map focusing on the planktonic cyanobacteria sampling stations in the Gulf of Gabes during the four cruises between 2005 and 2007.

phenomena as well as the Saharan dust inputs stimulate the primary production in this ecosystem and could explain the important national fish production in Tunisia. The purpose of this study was therefore to examine over space and time variations of the marine nitrogen-fixing cyanobacteria in the Gulf of Gabes in relation to the hydrographic water properties and the nutrient contents during four cruises carried out in the gulf between 2005 and 2007.

MATERIALS AND METHODS

Field sampling

Samplings were carried in the Gulf of Gabes, Tunisia (Eastern Mediterranean Sea, between 35°N and 33°N) aboard the RV 'Hannibal' during four oceanographic cruises: 9–14 July 2005, 27 May – 9 June 2006, 7–10 September 2006 and 16–19 March 2007 (Figure 1). The Gulf of Gabes has a 700 km coastline stretching from the coastal zone of Tunis to the industrial town of Gabes. Its climate is dry (average annual precipitation: 210 mm year^{-1}) and sunny with strong easterly winds resulting in severe aeolian erosion. The Gulf of Gabes opens to the offshore area and has a wide continental shelf. The tide is semidiurnal, with a maximum range of about 2 m. The shallow waters run around Kerkennah, Djerba islands, and the lagoons of Boughrara and El Bibane (Hattour *et al.*, 2010). As the Gulf of Gabes is located between the eastern and western parts of the Mediterranean Sea, the dynamics of its water masses are strongly linked to the general circulation of the Mediterranean Sea.

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To examine spatial trends, 26–34 stations were sampled per cruise encompassing the continental shelf area between 20 and 200 m. In each station, temperature, salinity and density (sigma-t) were measured 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. Water samples for physical and chemical analyses and phytoplankton assessment were collected at three depths (surface, mid-water column and near bottom) in coastal stations less than 50 m in depth and at 5 depths (surface, −10 m, −20 m, thermocline and near bottom) in offshore stations where depth exceeded 50 m. Samples for nutrient analyses (120 mL) were preserved immediately upon collection (−20°C, in the dark), and those for phytoplankton counts (1 L) were preserved with Lugol iodine solution (Bourrelly, 1985) and stored in the dark at low temperature (4°C) until analysis. Water samples (2 L) for chlorophyll *a* analyses were filtered by vacuum filtration onto Whatman GF/F fibreglass filter, and filters were then immediately stored at −20°C.

Samples analysis

The pH was measured immediately after sampling using a Met Röhm® type pH meter. Nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, $Si(OH)_4$, T-N and T-P) were analysed with a BRAN and LUEBBE type 3 autoanalyser and their concentrations were determined colorimetrically using a UV-visible (6400/6405) spectrophotometer (APHA, 1992). We calculated the Ni/Pi = DIN/DIP = $NO_2^- + NO_3^- + NH_4^+ / PO_4^{3-}$. Turbidity was estimated by the measurement of the suspended matter rate (mg L^{-1}) and euphotic layer by using a Secchi disk (m). The concentrations of suspended matter were determined by measuring the dry weight of the residue after filtration of 1 L of seawater onto Whatman GF/C membrane filters. Chlorophyll *a* analyses were performed by HPLC according to Pinckney *et al.* (2001), the analytical method was fully described in Bel Hassen *et al.* (2008).

Cyanobacteria enumeration

Cyanobacteria in sub-samples were counted with an inverted microscope after settling for 24–48 h using the Utermöhl's method (1958). The identification of cyanobacteria taxa was achieved according to Bourrelly (1985) and Baker (1991, 1992). Biovolumes, which were calculated from microscopic measurements of length and width, and assuming simple geometrical shapes (Lohman, 1908; Hillebrand *et al.*, 1999), were converted into carbon biomass using the conversion factors proposed by Menden-Deuer & Lessard (2000): 1 $\mu m^3 = 0.216 \times 10^{-6}$ µg C, for cyanobacteria.

The dominance index for the cyanobacteria was calculated according to the formula $\delta = (n_1 + n_2)/N$, where δ is the dominance index that is equal to the percentage of contribution of the two most important species ($n_1 + n_2$) of the total standing stock and N the total individual abundance.

Statistical analyses

Pearson's rank-correlation was performed using XL-Stat software to determine, for each sampling period, the correlations between the different biological and physico-chemical parameters. To evaluate differences between inshore and offshore areas for each studied biological, physical and chemical parameters with a confidence level of 95%, a non-paired Student's *t*-test was carried out. Differences were considered significant at $P < 0.05$. Analysis of variance (ANOVA) was applied to identify significant differences between the sampled periods. The co-inertia analysis which was a direct extension of multiple regressions to the modelling of a multivariate response matrix (Legendre & Legendre, 1998) was performed to examine the correlation between an array of response variables (in this study the four sampled periods) and of independent explanatory variables (planktonic cyanobacteria communities) conditional to a third matrix (here physical and chemical parameters), keeping the physico-chemical effect constant. The overall canonical relationship between data matrices was tested with a permutation test and the total variation was partitioned into variations due to main cyanobacteria taxa, to physical and chemical variables and to the co-variation between the four sampling periods, following Peres-Neto *et al.* (2006). Computing and graphical displays were performed using R 2.4 (R-Development Core Team, 2006), the packages ade4 1.4.2 (Chessel *et al.*, 2004) and vegan 1.8–3 (Oksanen *et al.*, 2006). In order to prepare the analysis, data matrices were explored using principal component analysis to picture the co-variations between the four sampled periods and between the cyanobacteria data and the physical and chemical parameters.

RESULTS

Physical and chemical parameters

The mean values of the different physical features such as temperature, salinity, sigma-t, pH and suspended matter determined from inshore (less than 50 m, in depth) and offshore samples (more than 50 m, in depth), during the entire survey period, are given in Table 1. The highest temperature was observed in inshore samples during September 2006 (26.56 ± 1.36°C) and the lowest one was recorded in the offshore sample during March 2007 (16.16 ± 0.29°C). The ANOVA analysis showed that temperature ($F = 5.576$, df = 119) differed significantly ($P < 0.001$) between the sampled periods (Table 1). Salinity, which showed quite similar variations in both inshore and offshore samples during the survey period, varied from 37.38 ± 0.69 in July in the offshore area to 38.15 ± 0.38 detected in September in the inshore area. In the spring season (May–June 2006) the thermocline was at 20 m in depth, while it was located deeper than 25 m depth during the summer period (July 2005) and was even more pronounced and deeper than 30 m in September 2006. The mean salinity profiles were similar in July and September, both in inshore and offshore stations, but they differed markedly in May–June 2006 and March 2007. In May–June 2006, salinity at 20 m was higher in the inshore than in the offshore areas but the reverse case was found in deeper water samples. In March 2007, the water column was well-mixed and the salinity decreased with depth in the inshore area but increased in the open sea. The mean sigma-t values showed a slight difference between the inshore and offshore areas only in May–June 2006. The highest values were recorded in the inshore area during March 2007 and the

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Table 1. Mean values and standard deviation (SD) of physico-chemical and biological parameters in inshore and offshore areas of the Gulf of Gabes during the four cruises between 2005 and 2007. Student's *t*-test was carried out to evaluate differences between inshore and offshore areas for each studied parameters. Analysis of variance (ANOVA) was applied to identify significant differences between the sampled periods with df equal to 119. F values: between-groups mean square/within-groups mean square. *Significant difference between inshore and offshore areas: *($P < 0.05$); **($P < 0.001$); ***($P < 0.0001$).

	July 2005			May–June 2006			September 2006			March 2007			*F* (values)
	Inshore area	Offshore area	|t|(df)	Inshore area	Offshore area	|t|(df)	Inshore area	Offshore area	|t|(df)	Inshore area	Offshore area	|t|(d.f)	
Physical parameters													
Temperature (°C)	24.44 ± 2.09	23.00 ± 1.06	2.18 (31)*	20.85 ± 1.58	18.94 ± 0.96	0.38 (28)	26.56 ± 1.36	23.95 ± 0.91	0.25 (32)	16.42 ± 0.19	16.16 ± 0.29	2.66 (24)*	5.576 **
Salinity	37.68 ± 0.40	37.38 ± 0.69	1.61 (31)	37.80 ± 0.35	37.70 ± 0.14	0.80 (28)	38.15 ± 0.38	38.06 ± 0.15	0.72(32)	37.76 ± 0.66	37.56 ± 0.22	0.85 (24)	0.538
Sigma-t (kg m^{-3})	25.51 ± 0.43	25.88 ± 0.27	2.77 (31)**	26.81 ± 0.26	27.08 ± 0.18	0.85 (28)	25.21 ± 0.28	25.90 ± 0.27	0.86 (32)	27.78 ± 0.49	27.68 ± 0.13	0.55 (24)	1.010
pH	8.42 ± 0.03	8.40 ± 0.04	1.51 (31)	8.44 ± 0.08	8.34 ± 0.08	3.64 (28)***	8.15 ± 0.14	8.01 ± 0.10	3.03 (32)**	8.03 ± 0.39	7.99 ± 0.44	0.27 (24)	29.351***
Suspended matter (mg L^{-1})	33.62 ± 24.43	27.57 ± 29.08	0.05 (31)	20.21 ± 1.61	19.44 ± 1.09	1.42 (28)	27.78 ± 6.01	64.93 ± 30.24	7.21 (32)***	26.31 ± 9.55	28.98 ± 11.66	0.61 (24)	11.936 ***
Secchi disk (m)	17.45 ± 7.90	19.5 ± 6.94	1.08 (31)	18.83 ± 5.37	21.85 ± 3.76	0.26 (28)	16.27 ± 6.66	20.85 ± 3.28	1.92 (32)	17.37 ± 2.87	16 ± 2.94	0.51 (24)	2.167
Chemical parameters													
NO_2^- (μM)	0.37 ± 0.11	0.34 ± 0.07	0.18 (31)	0.23 ± 0.18	0.23 ± 0.14	0.00 (28)	0.20 ± 0.06	0.15 ± 0.05	2.35 (32)*	0.11 ± 0.04	0.12 ± 0.06	0.78 (24)	27.790 ***
NO_3^- (μM)	1.36 ± 0.34	1.42 ± 0.18	1.08 (31)	1.41 ± 0.32	1.39 ± 0.25	0.03 (28)	0.91 ± 0.23	1.00 ± 0.20	1.19 (32)	0.77 ± 0.31	0.87 ± 0.29	0.81 (24)	24.512 ***
NH_4^+ (μM)	0.65 ± 0.25	0.61 ± 0.20	0.04 (31)	0.48 ± 0.25	0.31 ± 0.08	2.24 (28)*	2.26 ± 0.96	1.68 ± 0.66	1.73 (32)	1.77 ± 0.66	1.65 ± 0.72	0.46 (24)	85.276 ***
PO_4^{3-} (μM)	0.09 ± 0.11	0.06 ± 0.02	0.80 (31)	0.40 ± 0.21	0.44 ± 0.21	0.55 (28)	0.10 ± 0.07	0.11 ± 0.06	0.74 (32)	0.23 ± 0.13	0.18 ± 0.04	1.16 (24)	51.592 ***
$Si(OH)_4$ (μM)	1.66 ± 1.26	2.26 ± 1.09	1.93 (31)	1.03 ± 1.26	0.73 ± 0.39	0.49 (28)	0.69 ± 0.19	0.71 ± 0.23	0.13 (32)	2.91 ± 0.92	2.97 ± 1.27	0.01 (24)	33.678 ***
T-N (μM)	12.17 ± 3.17	12.36 ± 0.33	1.01 (31)	8.73 ± 1.39	8.24 ± 1.59	0.92 (28)	10.49 ± 1.59	10.11 ± 1.19	0.64 (32)	10.08 ± 1.41	10.51 ± 0.83	0.95 (24)	2.436
T-P (μM)	2.18 ± 1.74	1.59 ± 0.09	0.18 (31)	1.88 ± 1.01	2.07 ± 0.97	0.57 (28)	1.22 ± 0.70	1.58 ± 0.53	1.66 (32)	1.73 ± 0.45	1.43 ± 0.58	2.18 (24)*	7.464 ***
N/P ratio	41.99 ± 19.92	44.41 ± 8.20	1.27 (31)	7.07 ± 4.45	5.91 ± 5.02	0.94 (28)	59.77 ± 51.49	33.48 ± 22.75	1.90 (32)	23.09 ± 26.66	23.33 ± 10.35	0.95 (24)	43.673 ***
Biological parameters													
Chlorophyll *a* concentration (× 10^{-4} mg L^{-1})	0.50 ± 0.40	0.33 ± 0.22	0.06 (31)	2.64 ± 0.70	2.76 ± 0.52	2.27 (28)*	0.63 ± 0.50	1.16 ± 0.77	2.34 (32)*	1.09 ± 0.54	1.47 ± 0.44	1.86 (24)	19,850 ***
Phytoplankton density (× 10^3 cells L^{-1})	3.18 ± 2.46	2.69 ± 0.97	0.23 (31)	47.63 ± 76.15	9.49 ± 4.83	0.47 (28)	19.96 ± 39.8	19.62 ± 24.38	1.24 (32)	28.32 ± 21.22	18.48 ± 3.7	0.76 (24)	17.564 ***
Cyanobacteria density (10^3 cells L^{-1})	0.95 ± 0.65	1.38 ± 1.41	0.63 (31)	1.79 ± 1.94	1.11 ± 0.99	1.35 (28)	0.08 ± 0.06	0.16 ± 0.19	1.88 (32)	0.59 ± 0.07	0.79 ± 0.22	3.42 (24)**	15.359 ***
Cyanobacteria biomass (× μg C L^{-1})	21.40 ± 11.05	46.59 ± 13.9	1.53 (31)	5.12 ± 5.59	3.66 ± 3.34	0.27 (28)	0.69 ± 0.67	0.56 ± 0.60	0.46 (32)	1.00 ± 0.64	0.68 ± 0.12	0.54 (24)	50.701 ***

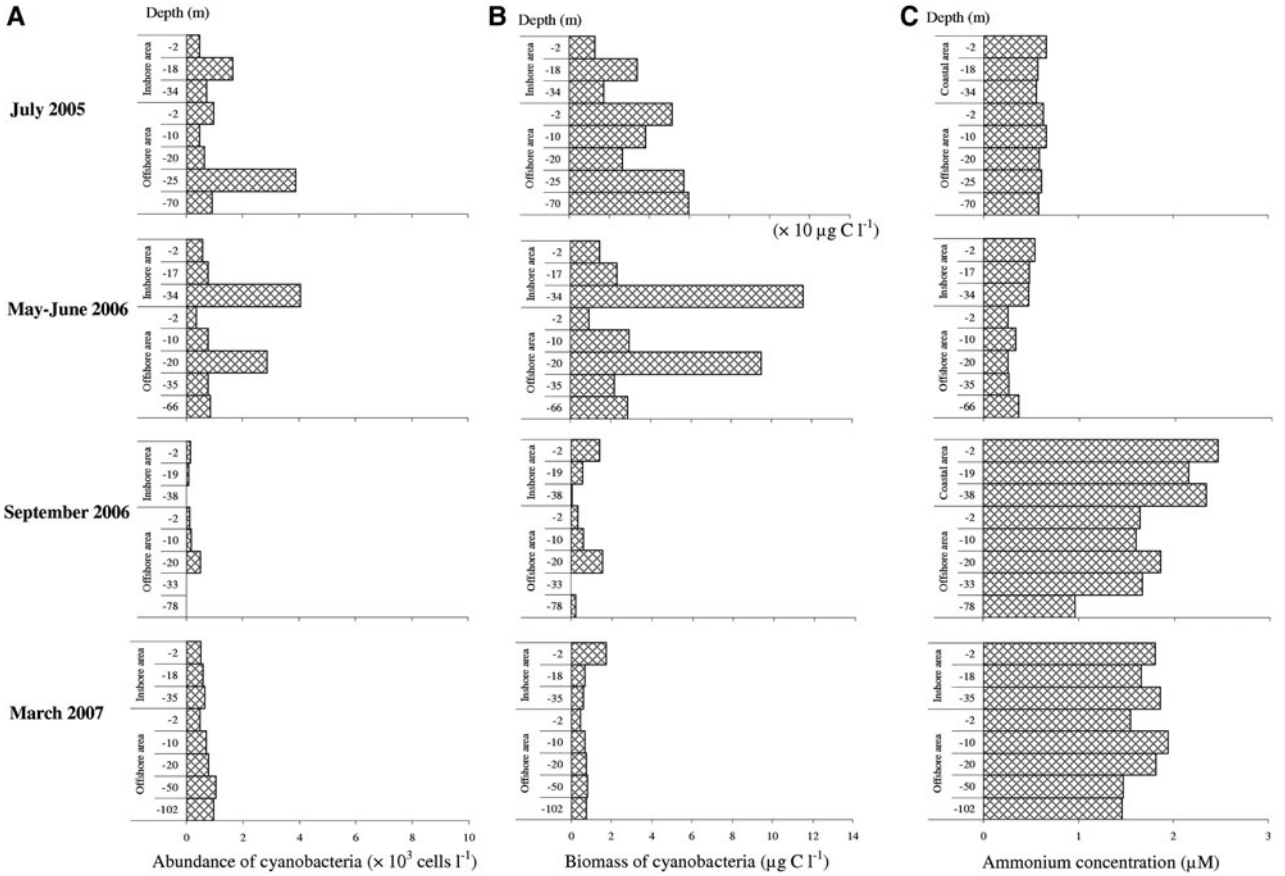

Fig. 2. Average vertical distribution of cyanobacteria abundance (A), biomass (B) and ammonium concentration (C) in coastal and open sea areas in the Gulf of Gabes between 2005 and 2007.

lowest ones at the inshore area in September 2006. Sigma-t was negatively correlated with temperature at the beginning of the water stratification in May–June ($r = -0.745$, $P < 0.0001$, df = 28), July ($r = -0.825$, $P < 0.0001$, df = 31) and September ($r = -0.903$, $P < 0.0001$, df = 32). However, sigma-t and salinity of inshore waters were positively correlated ($r = 0.992$, $P < 0.05$, df = 24) under the well-mixed conditions in March 2007. In the offshore region, sigma-t was correlated with both temperature ($r = 0.416$, $P < 0.05$, df = 24) and salinity ($r = 0.967$, $P < 0.05$, df = 24).

The mean pH values were generally more alkaline in the inshore samples than in the offshore samples, suggesting a more pronounced photosynthetic activity along the coast mainly during May–June 2006 (Student's *t*, $P < 0.0001$). The mean concentrations of the suspended matter ranged from 19.44 mg L^{-1} (May–June 2006) to 64.93 mg L^{-1} (September 2006) in offshore area and from 20.21 ± 1.61 mg L^{-1} (May–June 2006) to 33.62 ± 24.43 mg L^{-1} (July 2005) in the inshore stations. The Secchi disk values showed highest values in the offshore area during the beginning of stratification in May–June 2006 (21.85 ± 3.76 m) and the lowest ones during the mixing period in March 2007 in the offshore area (16 ± 2.94 m). The mean nutrient values were more important in inshore than in offshore sampled stations during all the survey period. The concentrations of $NO_3^- + NO_2^-$ were significantly higher during May–June 2006 and July 2005 than during September 2006 and March 2007, whereas NH_4^+ concentrations exhibited a reverse trend. PO_4^{3-} concentrations were low (0.06–0.1 µM) during the well-stratified conditions (July 2005 and September 2006), but reached a maximum of 0.44 ± 0.21 µM during May–June in inshore area. So, N/P ratios were higher (>10) during stratification but were lower than the Redfield ratio (16), suggesting a potential N limitation during May–June 2006 (<10).

Biological parameters

The mean chlorophyll-*a* concentrations determined during the thermal stratification from both inshore and offshore samples were $< 3 \times 10^{-4}$ mg L^{-1} (Table 1). They were high during May–June 2006 with a sub-surface chlorophyll maximum found up to 15 m in the inshore area, and a deep chlorophyll maximum, DCM (5×10^{-4} mg L^{-1}), found up to 40 m in the offshore area. The phytoplankton community consisted of Dinophyceae (120 taxa), Bacillariophyceae (40 taxa), Cyanobacteria (seven taxa) and other groups such as Dictyochophyceae, Euglenophyceae, Coccolithophorideae and Chlorophyceae were represented by one species. The phytoplankton abundance varied from 2.69×10^3 to 47.63×10^3 cells L^{-1}, these being recorded respectively in July 2005 in offshore area and May–June 2006 in inshore area. The highest cyanobacteria abundance was recorded in May–June 2006 in the inshore area ($1.79 \times 10^3 \pm 1.94 \times 10^3$ cells l^{-1}), with *Pseudoanabaena* sp. contributing to 10% of the total phytoplankton and 68% of total cyanobacteria abundances (Table 1, Figures 2A & 3B). The lowest cyanobacteria abundance was observed in September 2006

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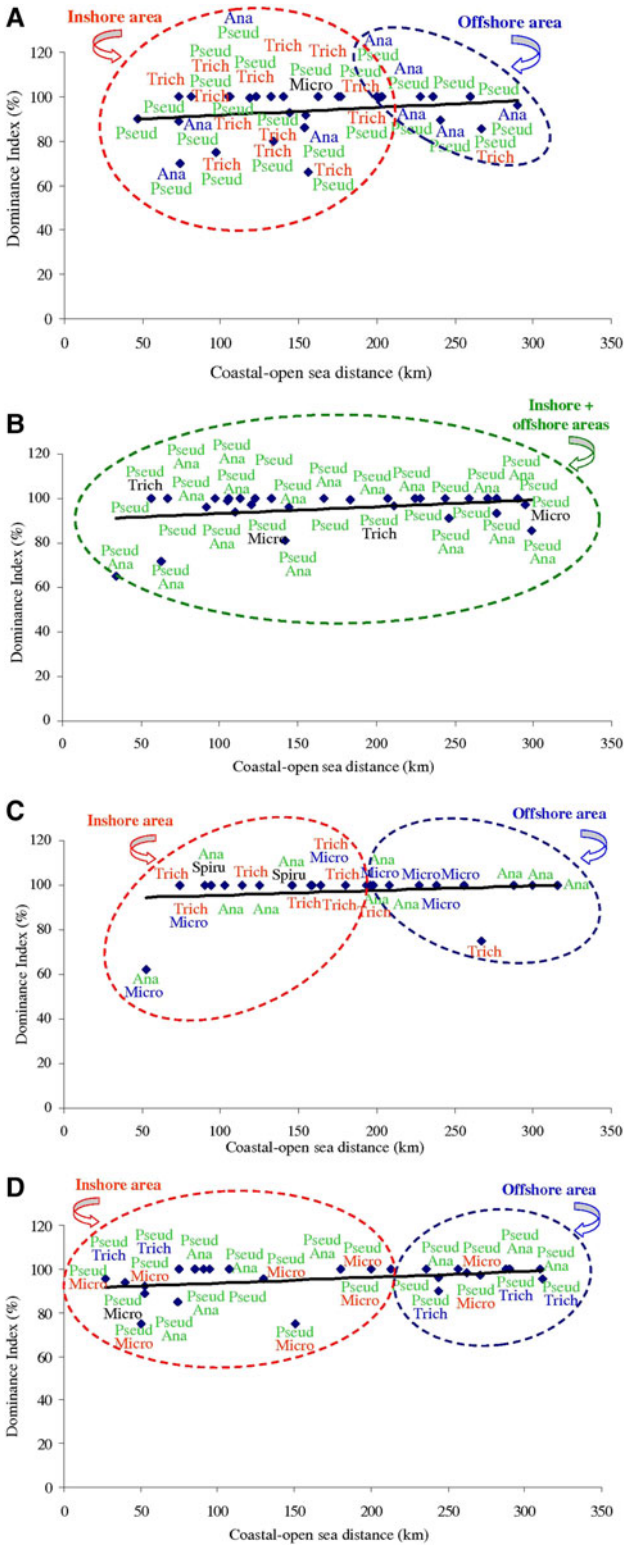

Fig. 3. Relationships between the dominance index of the planktonic cyanobacteria species and distance to the coast during July 2005 (A), May–June 2006 (B), September 2006 (C) and March 2007 (D) (Ana: *Anabaena* sp., Pseudo: *Pseudoanabaena* sp., Tricho: *Trichodesmium erythraeum*, Micro: *Microcystis* sp., Spiru: *Spirulina subsalsa*).

(80 ± 60 cells L^{-1}). The ANOVA analysis showed that cyanobacteria abundance differed significantly ($F = 15.359$, df = 119, $P < 0.0001$) between the sampled periods (Table 1). The study of the marine planktonic cyanobacteria species throughout the sampling period showed the presence of seven different species (Table 2). The cyanobacteria were distributed throughout the water column in both inshore and offshore areas (Figure 2A) but they exhibited no clear trend in their vertical distribution. They were more abundant during the water mixing period (March 2007) and preferentially during the semi-mixing period (May–June 2006) mainly in the inshore area at 34 m in depth (Figure 2A). At this latter period a high proliferation of planktonic cyanobacteria was observed at the offshore stations near the thermocline level coinciding with the euphotic layer (21.85 ± 3.76 m) allowing access of light radiation. During this period, we also noted the lowest ammonium concentration decreasing with depth but the planktonic cyanobacteria was weakly correlated with the NH_4^+ amount ($r = 0.098$, $P < 0.05$, df = 28).

The mean values of cyanobacteria biomass varied from 0.56 ± 0.60 μg C L^{-1} in September 2006 in the offshore area to 46.59 ± 13.9 μg C L^{-1} in July 2005 in the offshore area (Table 1, Figure 2B). This important biomass in July 2005 was due to the large size of the dominant species that were present at this time, such as *Trichodesmium erythraeum* (134.3 ± 123.85 μm) and *Pseudoanabaena* sp. (82.41 ± 53.20 μm) with an abundance varying from 1 to 5% of total phytoplankton and representing 41 and 50% of total cyanobacteria abundance, respectively (Table 2). During the semi-mixed period (May–June 2006), the biomass of cyanobacteria was still important with large-sized opportunist species such as *Pseudoanabaena* sp. (128.75 ± 66.12 μm) (300 ± 129 μm) accounting for 68% of the total cyanobacteria abundance (Table 2). The lowest biomass recorded in March 2007 in the offshore area was due to small-sized opportunist species such as *Anabaena* sp. (35.11 ± 5.05 μm) and *Spirulina subsalsa* (54 ± 12.72 μm) (Table 2). The ANOVA analysis showed that cyanobacteria biomass differed significantly ($F = 50.701$, df = 119, $P < 0.0001$) between the sampled periods (Table 1). During all the survey period, the dominance index showed that the biodiversity of cyanobacteria increased gradually along the inshore-offshore gradient (Figure 3). This index increase depended on a coastal–open sea distance during the stratified period (July 2005 and September 2006), the mixing period (March 2007) and at the beginning of stratification (May–June 2006). In fact, during July 2005, *Trichodesmium erythraeum* dominated in the inshore zone, while *Anabaena* sp. proliferated in the offshore zone. *Pseudoanabaena* sp. was found in substantial amounts between the inshore and offshore areas (Figures 3A & 4). During May–June 2006, we noted the dominance of *Pseudoanabaena* sp. and *Anabaena* sp. in substantial amounts between the inshore and offshore areas (Figures 3B & 4). During September 2006, the inshore zone was dominated by *Trichodesmium erythraeum* while the offshore zone was characterized rather by *Microcystis* sp. *Anabaena* sp. was found in substantial amounts between the inshore and offshore areas (Figures 3C & 4). During March 2007, the inshore zone was dominated by *Microcystis* sp. while the offshore zone was characterized rather by *Trichodesmium erythraeum*. *Pseudoanabaena* sp. and *Anabaena* sp. proliferated at the intermediate zone (Figures 3D & 4).

The co-inertia plot illustrated a close relationship between the composition of planktonic cyanobacteria species and the physico-chemical features of the water column during the four sampling periods (Figure 5A). The overall model

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Table 2. List, abundance (Abun), mean density (D) and length (L) of the marine cyanobacteria species observed between 2005 and 2007 (V = very abundant. 30–100%; A = abundant. 10–30%; C = common. 5–10%; R = rare. 1–5%; X = present occasionally. 0–1; – Not detected).

Species	July 2005			May–June 2006			September 2006			March 2007		
	Abun (%)	D ($\times 10^2$ cells L^{-1})	L (µm)	Abun (%)	D ($\times 10^2$ cells L^{-1})	L (µm)	Abun (%)	D ($\times 10^2$ cells L^{-1})	L (µm)	Abun (%)	D ($\times 10^2$ cells l^{-1})	L (µm)
Anabaena sp. (Bornet & Flahault, 1886)	R	3.3 ± 7	35.11 ± 32.16	R	4.11 ± 12.81	187.5 ± 159.09	R	0.61 ± 1.76	109.11 ± 93.46	X	0.27 ± 0.40	35.11 ± 5.05
Chroococcus sp. (Fremy, 1930)	–	–	–	–	0.38 ± 0.10	–	X	0.02 ± 0.17	–	–	–	–
Microcystis sp. (Lemmermann, 1907)	X	0.48 ± 1.12	–	X	0.35 ± 1.46	–	X	0.10 ± 0.27	–	X	0.28 ± 0.44	240 ± 120
Trichodesmium erythraeum (Ehrenberg ex Gomont, 1892)	C	17.2 ± 65.9	134.3 ± 123.85	X	0.19 ± 0.53	–	X	0.32 ± 1.32	55 ± 7.07	X	0.33 ± 0.76	126.30 ± 87.38
Pseudoanabaena sp. (Lauterborn, 1915)	C	21.2 ± 33.4	82.41 ± 53.20	C	10.5 ± 22.7	128.75 ± 66.12	X	0.01 ± 0.03	300 ± 129	C	5.69 ± 3.26	66.11 ± 22.78
Spirulina sp. (Turpin ex Gomont, 1892)	–	–	–	X	0.01 ± 0.06	–	–	–	–	–	–	67.5 ± 38.76
Spirulina subsalsa (Oersted, 1842)	X	0.12 ± 0.69	67.5 ± 62.51	–	–	–	X	0.03 ± 0.09	56.66 ± 5.77	X	0.02 ± 0.09	54 ± 12.72

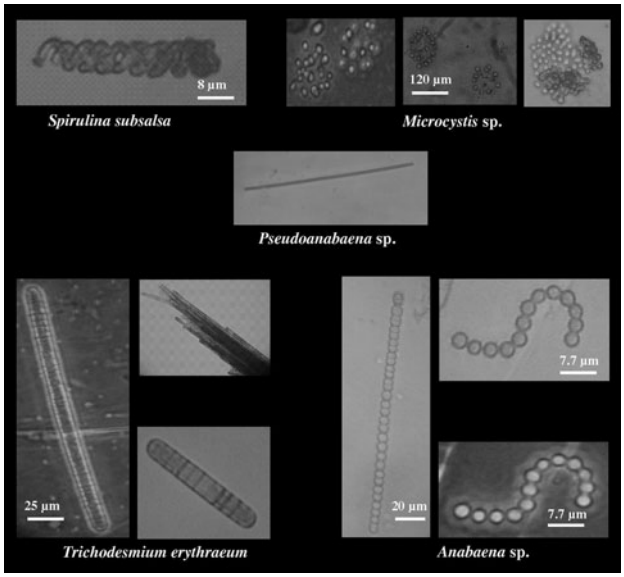

Fig. 4. The main cyanobacteria species taken with inverted microscopy (40×) recorded in the Gulf of Gabes during the survey period (2005–2007).

explained 77% of the total variation (permutation test, $P = 0.24$, 1000 replicates). This variation was due to cyanobacteria taxa (22%) and to physico-chemical variability (17%) (Figure 5B). The May–June 2006 and March 2007 sampling periods showed close links between sigma-t, NO_3^-, PO_4^{3-} and T-P concentrations and the phytoplankton composition which was illustrated by the position of cyanobacteria species such as *Pseudoanabaena* sp., *Anabaena* sp., *Microcystis* sp., *Spirulina* sp. and *Chroococcus* sp. around the Y axis (Figure 5A). In contrast, during July 2005 and September 2006, this component axis was surrounded by the numerically dominant *Trichodesmium erythraeum* and *Spirulina subsalsa*. To summarize, the co-inertia indicated the clear differences between the four studied periods for the distribution of planktonic cyanobacteria species. In fact, we recorded a clear dominance of *Pseudoanabaena* sp. as opportunistic species during the mixing (March 2007; 68% of the total cyanobacteria abundance) and the begging of stratification (May–June 2006; 88% of the total cyanobacteria abundance). This species characterizing the period of bloom showed a significantly negative correlation with the nitrate concentration ($r = -0.09$; $P < 0.05$; $df = 28$) probably indicating nitrate consumption. *Trichodesmium erythraeum* dominated the cyanobacteria community when the water column was well stratified (July 2005: 41% of the total cyanobacteria abundance and September 2006: 21% of the total cyanobacteria abundance).

DISCUSSION

As described in previous studies (Drira *et al.*, 2009, 2014a, b), the hydrological features showed that during early spring (March 2007) the water column is well mixed and that thermal stratification of the water column starts in late spring (May–June 2006) until its complete establishment in summer (July 2005 and September 2006). The nitrogen availability in July 2005 was similar to that in May–June 2006 with a dominance of nitrate over ammonium concentrations.

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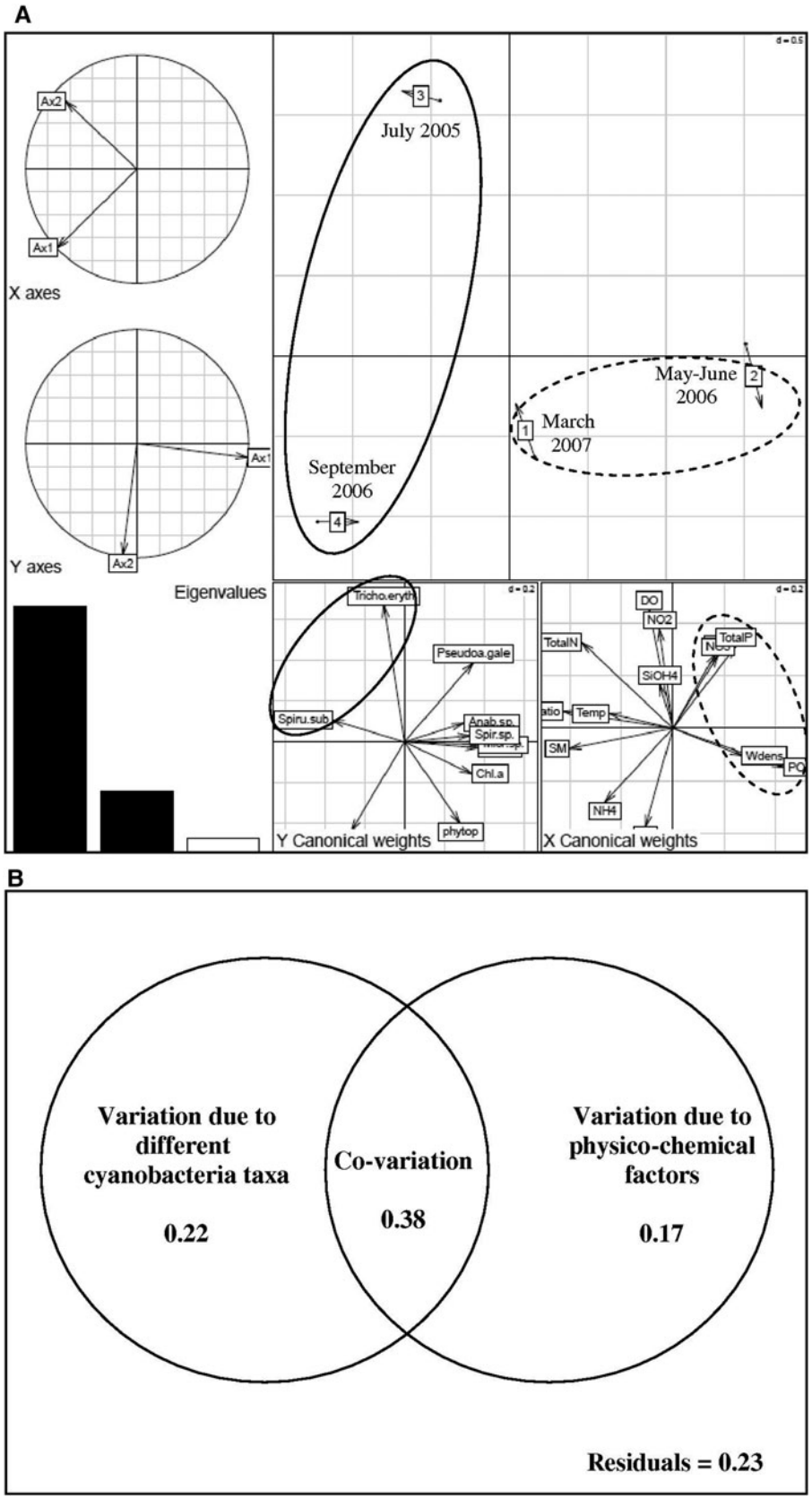

Fig. 5. Co-inertia plot for the biotic and abiotic parameters changes for the four sampling periods (A) and partition of cyanobacteria species and physico-chemical features.

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Moreover, in July 2005, the low phosphate concentration was similar to that in September 2006. If it is assumed that the July cruise constituted the continuum between May–June and September, it could be inferred that phosphate was the first limiting nutrient factor in the Gulf of Gabes, as reflected by the high N/P ratio during the strong stratification period in July and September. This strong stratification resulted in a shift in nitrogen sources from nitrates to ammonium as well as to phosphate depletion (<2.5 μM) and to a decrease in silicate concentrations (<3 μM) (Bel Hassen *et al.*, 2009; Drira *et al.*, 2009). N/P ratio was lower in May–June 2006 (<16) than in September 2006 (>16). In May–June 2006, when the cyanobacteria abundance was highest, the N/P ratio was clearly below the Redfield standard molar ratio of 16/1. The same result was observed in the Baltic Sea where cyanobacteria were favoured by low N/P-ratios and P was probably in excess during the bloom formation (Westman *et al.*, 2003; Vahtera *et al.*, 2007; Walve *et al.*, 2014). This situation was also observed in the Mediterranean Sea in two sites on the Sicilian coastline in 1993 during which low N/P ratios (<10:l) coincided with high development of planktonic cyanobacteria in the subsurface layer (Giacobbe *et al.*, 1995). In addition, high values of N/P ratio widely exceeding the Redfield ratio (N/P > 20), suggesting an overall phosphate depletion during July, September and March cruises, are consistent with other reports of P limitation in the Mediterranean Sea (Jacques *et al.*, 1973; Minas *et al.*, 1988; Thingstad & Rassoulzadegan, 1995; Thingstad *et al.*, 1998). During our survey, cyanobacteria were found throughout the water column and were more concentrated at the thermocline within the deeper zone as commonly observed (Ribera d'Alcalà *et al.*, 2004). Cyanobacteria proliferated during the semi-mixed conditions (May–June 2006 and July 2005) mainly in the coastal area. They seemed to be governed by the nitrogen availability in the offshore area of the Gulf of Gabes favoured by the presence of a thermocline between 20 and 25 m (Drira *et al.*, 2009). In fact, cyanobacteria developed throughout the whole water column with a high density recorded at the thermocline and associated with *Trichodesmium erythraeum* and *Pseudoanabaena* sp. which contributed to 41 and 50% of the total cyanobacterial abundance, respectively. In these layers, cyanobacteria abundance was correlated with both NO_3^- ($r = 0.343$, $P < 0.05$, df = 119) and NH_4^+ ($r = 0.344$, $P < 0.05$, df = 119), as also demonstrated by Drira *et al.* (2008) and a significant correlation was recorded between cyanobacteria and N/P ($r = 0.566$, $P < 0.05$, df = 119). This indicates that the cyanobacterial growth was probably induced by the nitrate replenishment of the upper stratified layers after release by the thermocline.

Our study showed that cyanobacteria in the Gulf of Gabes was made up of seven different species, with some of them being N_2-fixing such as *Anabaena* sp., *Chroococcus* sp., *Trichodesmium erythraeum, Spirulina* sp. and *Spirulina subsalsa*, and others being non N_2-fixing such as *Pseudoanabaena* sp. and *Microcystis* sp. (Berman-Frank *et al.*, 2007; O'Neil *et al.*, 2012; Paerl & Otten, 2013). All these cyanobacteria species were also recorded in the Gulf of Gabes in summer (July) 2005 (Drira *et al.*, 2010). These species, except *Microcystis* sp., were also observed in the coast of Chebba (Gulf of Gabes, East of Tunisia) during a summer cruise (August 2011) (Mabrouk *et al.*, 2014). In addition, *Anabaena spherica* and *Microcystis aeruginosa* developed in both inshore and offshore stations in July 2006 during the summer water stratification in the Gulf of Hammamet (Tunisia, eastern Mediterranean Sea) (Hannachi *et al.*, 2011). Furthermore, a preferential occurrence of cyanobacteria under the semi-mixed conditions was also reported in a coastal north-western Mediterranean site (Bustillos-Guzman *et al.*, 1995; Marty *et al.*, 2002). In fact, the recorded species were generally large colony-forming prokaryotes such as the filamentous *Anabaena* and *Pseudoanabaena*, which were able to overcome sedimentation losses under stratified conditions (Drira *et al.*, 2009). *Anabaena* sp. was also the dominant species (36% of total cyanobacteria abundance) at 20 nearshore stations of the Gulf of Gabes during a 10-year survey with the greatest abundance (5.3×10^4 cells L^{-1}) being recorded in 1997 (Feki *et al.*, 2013). In our study the highest cyanobacteria abundance ($1.79 \times 10^3 \pm 1.94 \times 10^3$ cells L^{-1}) was recorded during May–June 2006 in the inshore area with *Pseudoanabaena* sp. contributing to 10% of the total phytoplankton and 68% of total cyanobacteria abundances. The development of cyanobacteria near the thermocline (−25 m) was associated with high nitrate concentrations ($r = 0.312$, $P < 0.05$, df = 31) suggesting that nutrients played an important role in governing their vertical distribution (Drira *et al.*, 2009).

The bloom of cyanobacteria in the Gulf of Gabes during May–June 2006 was associated with the lowest ammonium concentration decreasing with depth. For this reason, we suggest that the blooms of cyanobacteria consumed the full amount of available ammonium. In fact, we recorded a weak correlation between cyanobacteria with the NH_4^+ amount ($r = -0.202$, $P < 0.05$, df = 28). During May–June 2006, we noted the dominance of *Pseudoanabaena* sp. and *Anabaena* sp. in substantial amounts between the inshore and offshore areas. In the Gulf of Gabes, the summer season is characterized by high temperature and salinity which promote the stratification of the water column and induce the appearance of phytoplankton blooms with prominent presence of such cyanobacterial taxa. The phenomenon of bloom occurred frequently in several regions of the Gulf of Gabes: near Kerkennah Islands in offshore areas (Hamza & Ben Maiz, 1990; Fremy & Feldmann, 1935) and in Bougrara Lagoon and inshore areas due to high overgrowth *of Trichodesmium erytreum* (Turki *et al.*, 2006). This important proliferation of *Trichodesmium erytreum*, which is termed a harmful species, was responsible for algal bloom-induced large-scale mortality and stranding of large quantities of fish, eels, cuttlefish, etc. in the southern Tunisian coasts (Hamza & Ben Maiz, 1990; Turki & El Abed, 2001; Hansen *et al.*, 2004; Turki *et al.*, 2006).

CONCLUSION

In conclusion, the nutrient availability and the water column stability seemed to be among the major factors determining cyanobacteria dynamics in the Gulf of Gabes. Indeed, cyanobacteria were prominent near the thermocline during the period of strong stratification. Cyanobacteria were found throughout the water column and were more concentrated at the thermocline within the deeper zone. The bloom of cyanobacteria recorded in May–June 2006 was promoted by low N/P-ratios. The factor limiting the diazotrophic activity during the study period (2005–2007) was shown to be

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variable in the Gulf of Gabes: phosphorus as DIP was shown to be the limiting nutrient of N_2 fixation and strongly influenced the cyanobacterial bloom dynamics.

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