INTRODUCTION

The hydrological regime of the Mediterranean Sea has been extensively studied. The water column is well stratified in summer, mixed in winter with transition periods of stratification occurring during late spring and early winter (Béthoux & Prieur 1983). These seasonal dynamics have a manifest influence on the water column nutrient availability. Particularly, the winter mixing is revealed to be a major nutrient input to the surface layer (Estrada et al. 1991, Marty et al. 2002). Moreover, the formation in the western Mediterranean basin of dense water in winter leads to intense vertical mixing; thus nutrients are transported to the surface layer, allowing spring blooms to occur (Béthoux1989). In contrast, due to the strong stratification of the upper layer, the surface waters in the summer are not enriched by deep water nutrients (Marty et al. 2002, Bustillos-Guzman et al. 1995) and the coastal waters become more strongly affected by continental inputs.

The seasonal variations in phytoplankton biomass occur primarily due to changes in environmental variables, such as nutrient and light availability. Some authors suggested that surface waters of the western basin are N-limited (Andersen & Nival 1988, Owens et al. 1989), whilst others suggest that P-limitation is more likely (Béthoux et al. 1992, Béthoux et al. 1998, Thingstad et al. 1998). The evidence for P-limitation of the eastern basin is stronger (Herut et al. 1999, Krom et al 1991) and the limitation in the western basin may shift from N to P, according to the spatio-temporal trophic conditions (Klein et al. 1997). The shifts of phytoplankton biomass in higher trophic levels are also among the factor of regulation of this biomass (Gómez et al. 2001).

Identifying the ecological variables that regulate the seasonal fluctuations of phytoplankton standing stock is essential for understanding the consequences of the potential physical chemical and effects on the biological response of the ecosystem. The phytoplankton community structure has been investigated and discussed in the Gulf of Gabes with regard to the environmental variability during three characteristic periods (Bel Hassen et al. 2008, Bel Hassen et al. 2009a, Bel Hassen et al. 2009b). However, the seasonal transition of the factors regulating the phytoplankton standing stocks, especially the nutrient availability and the potential degradation of phytoplankton biomass, have not yet been approached. The main objective of this work is to investigate the spatial and seasonal patterns of the phytoplankton biomass control in the Gulf of Gabes continental shelf area based on analyses of physical processes, nutrient concentrations, stoichiometric ratios, suspended solid concentrations, and chlorophyll a concentrations.
METHODS

The studied area: The Gulf of Gabes occupies a wide continental shelf area. The water dynamics is influenced by the regional water circulation. Particularly, the dynamics of the Sicily Straits studied by Béranger et al. (2004) showed that the Atlantic Water (AW) in the western Mediterranean enters the Straits of Sicily and splits into two branches: one flowing to the south-eastern Mediterranean and the second flowing to the south and directly affecting the circulation at the mouth of the Gulf of Gabes. These authors pointed out a seasonal variability along the Tunisian coast, observing that the AW was generally stronger during the winter and flowed in the upper 100 m, while they revealed a weakening of the advection during the summer. Similar observations were reported by Poulain & Zambianchi (2007) using Lagrangian drifters data. They particularly showed that, during winter, the AW strongly flowed along the Tunisian coast through the shallow Gulf of Gabes. Recent observations have shown in a study carried out during late winter in the Gulf of Gabes that the AW flows within the continental shelf area between 50 m and 100 m isobaths (Bel Hassen et al. 2009a), whereas it exhibited a weak advection as summer stratification was established (Bel Hassen et al. 2008).

Sampling: The study area encompassed the continental shelf area between 20 m and over 100 m, of depth (Fig. 1). Three cruises aboard the ‘R/V Hannibal’ were conducted during 2006-2007 labelled herein as (May-June), (September) and (March). The first cruise (26 May-6 June) was carried out at the end of spring and the beginning of the summer stratification, the second (7-11 September) coincided with a well-established water column while the third (16-19 March) corresponded to a well-mixed water column. Due to the difference in the hydrological forcing, mainly ascribed to the AW advection, different sampling designs were conducted during the three cruises (Bel Hassen et al. 2008, Bel Hassen et al. 2009a, Bel Hassen et al. 2009b). To highlight the difference in the hydrological regime between the cruises, we have retained in the present study the sampling locations situated along a seaward transect which also characterized the variability across the AW, the main hydrological forcing factor in this area. The station locations along the transect, although not exactly at the same positions for each cruise due to slight differences in the sampling designs, were rather well distributed along the transect.

For each station a vertical profile of temperature and salinity was made with a Seabird conductivity-temperature-depth (CTD) sensor (SBE 9, Seabird Electronics, USA) equipped with a 12l-Niskin rosette. Discrete samples were collected at three depths (2 m, 25 m and near bottom) in stations less than 50 m in depth and from five depths in stations above 50 m in depth (2 m, 10 m, 20 m, 50 m and near bottom). Sub-samples (2l) for pigment analyses were filtered with 47 mm-diameter glass fibre filter Whatman, GF/F. Filters for chlorophyll a analyses were immediately stored at -20° C for subsequent analyses. Samples for nutrient analyses were preserved immediately upon collection (-20° C, in the dark) and analysed within 15 days. Sub-samples (1L) of seawater were filtered with Whatman GF/F membrane filters for determination of the suspended solid concentrations.

The Secchi disk measurements were achieved during daylight. Vertical light attenuation coefficients were estimated according to Holmes (1970), and the euphotic zone depth was calculated assuming that irradiance at the bottom was 1 % of surface irradiance (Cloern 1987).

Sample analysis and criteria for stoichiometric and potential nutrient limitations: Nutrient analyses were performed with an automatic analyser type 3 (BRAN + LUEBBE) using standard methods (Tréguer & Le Corre 1975). The combinations of N, P and Si ratios were established to investigate potential nutrient limitations. Criteria for stoichiometric nutrient limitation were developed based on nutrient requirements of diatoms established by Redfield et al. (1963).

The total suspended solids (TSS) concentrations were based on dry weight filters after drying at 50° C for 48 h. The organic suspended solid (OSS) fraction was determined by weighing after incineration for 5 h at 450° C. Mineral suspended sediment (MSS) was thus deduced as the difference between (TSS) and (OSS) concentrations.

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Chlorophyll-α analyses were performed by HPLC according to Pinckney et al. (2001), the analytical method was fully described in Bel Hassen et al. (2009a).

Statistical analysis: The original data collected were analyzed using the principal component analysis to understand the influence of various factors on the biological component of the system. Physical (temperature, salinity, suspended matter), chemical (Nitrate + Nitrite (NN), phosphate (P) and silicate (Si)) and “biological parameters” (chlorophyll-α) assessed over the three sampled periods were considered. The normality of the sampling distribution was first tested using the Kolmogorov-Smirnov test and the potential relationships between variables were tested by Pearson’s correlation coefficient.

RESULTS

Physical data

The vertical profile of the temperature measured along the transect showed variations between surface and bottom waters of about 6°C in May-June, 10°C in September and 1°C in March. In May-June, the thermocline depth ranged from 10 m in offshore samples to more than 30 m in coastal samples. In September, the water stratification was more pronounced and the thermocline established at a depth of more than 30 m. Surface salinity exhibited a decreased gradient along the transect (Fig. 2). The salinity minima (< 37.5) were observed in the offshore surface water in May-June and March, whereas they were situated between -50 m and -60 m of depth in September.

Nutrient data

Table I gives the mean nutrient concentrations and standard deviations and Fig. 3 shows the pattern of nutrient distribution along the transect. NN and P concentrated more in May-June than in September and March, while Si concentrations were higher in March than in the other investigated periods. In May-June, NN were mainly recorded in local patches from the surface layer to 20 m of depth. P concentrations were higher in the offshore
Fig. 3 – Vertical profiles of nitrate plus nitrite (µM), phosphate (µM) and Silicate (µM) according to the sampled coast-offshore transect for the three sampled periods.
stations at 30m depth and in bottom water, while Si concentrations were higher in the coastal stations. In September, NN occupied the whole water column in the coastal stations, while they concentrated more at 70 m depth in the offshore stations. P was mainly present in subsurface water in the offshore area; by contrast Si concentrations were higher in the bottom offshore area. In March, NN were mainly present in local zones, in the subsurface coastal area and in bottom offshore areas. A depth-related trend was also observed for P and Si but at varying depth levels (Fig. 3).

The accepted standard molar ratios between N, Si and P for marine diatom biomass growth is Si:N:P = 16:16:1 (Redfield et al. 1963). The molar quotients between the in situ concentrations of potentially limiting nutrients are delimited by the Si:N = 1, N:P = 16 and Si:P = 16 lines (Fig. 4). These define 6 different areas, each one characterized by the potentially limiting nutrients in order of priority (Fig. 4b). The relative proportions of available nutrients evidenced a consistent temporal pattern. In March nutrient ratios fell in the region in which N limitation was most likely to occur. In May-June, nutrient ratio fell within different potential limiting areas. Particularly, in the regions in which Si-limitation, and to a lesser degree N-limitation, were likely to occur. During September strong stratified conditions, nutrient ratios fell in the region in which Si-limitation and to a lesser degree P-limitations were more likely to occur.

**Total suspended sediment and organic suspended sediment**

The TSS concentrations were higher in September than in May-June and March (Table I). The OSS represented about 81.15 %, 78.88 % and 82.50 % of TSS concentrations in May-June, September and March, respectively. TSS vertical distribution along the transect (Fig. 5) showed that the highest concentrations were mainly observed in the offshore stations in May-June and September but at varying depths, between 10 m and 30 m depth and in bottom water in May-June, while between 10 m and 50 m depth in September. In March, at the exception of a local patch observed at 60 m depth in the offshore area; the highest concentrations were mainly recorded in the coastal stations.
Phytoplankton biomasses

Chlorophyll $a$ concentrations were generally less than 0.5 $\mu$g$l^{-1}$ (Table I), the highest value was recorded in May-June (1.2 $\mu$g$l^{-1}$). The coast-offshore transect allowed identification of zones of chlorophyll maxima in both coastal and offshore areas (Fig. 5). In May-June, the euphotic depth deepened to more than 70 m in the offshore stations and local chlorophyll maxima were recorded at surface levels in the coastal area and between 20 m and 40 m depth in the offshore area. In September the chlorophyll maximum was observed in the coastal area at 10m of depth, while in the offshore station concentrations higher than 0.1$\mu$ml$^{-1}$ were recorded at 40 m depth. In March chlorophyll maxima were located in the surface offshore stations and at 50 m depth coinciding with the limit of the euphotic layer.

Relationships between physical, chemical and biological parameters

The PCA of water quality variables for the period May-June indicated that together the first two axes explained 59.75 % of the total variance (Fig. 6). Factor 1 accounted for 34.95 % of the total variance and was associated with positive loadings of temperature and Si and a negative loading of NN. In this factor Si correlated positively with temperature and negatively with NN (Table II). Factor 2 accounted for 24.8 % of the total variance and was associated with a positive loading of chlorophyll $a$ and negative loadings of NN, TSS.
and salinity. In this factor neither the relationship between chlorophyll $a$ and TSS nor between chlorophyll $a$ and NN were statistically significant (Table II).

In September, the PCA results indicated that the two first axes explained 54.25% of the total variance, with factor 1 accounting for 36.68% of the total variance. Factor 1 was associated with positive loadings of temperature and salinity and a negative loading of Si. In this factor Si was negatively correlated with temperature and positively correlated with salinity. Factor 2 accounted for 17.57% of the total variance and was associated with positive loadings of chlorophyll $a$ and TSS and negative loading of NN.

In March, the two first axes explained 54.18% of the total variance, with factor 1 accounting for 32.80% of the total variance. Factor 1 was associated with positive loadings of temperature, salinity and TSS and negative loadings of chlorophyll $a$ and Si. In this factor temperature was negatively correlated with chlorophyll $a$ and Si. Factor 2 accounted for 21.38% of the total variance and was associated with a positive loading of Si and negative loadings of NN and P.

Table II. – Pearson’s correlation coefficients of biological, chemical and hydrographical variables at all sampled stations for the three cruises. Significant correlations ($P < 0.05$) are reported.

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DISCUSSION

The region showed a high variability level in physical properties at seasonal and spatial scales. The March cruise corresponded to the winter-spring transition with a well-mixed water column, a strong advection of the Atlantic Water (Bel Hassen et al. 2009a) and a reduction of the euphotic layer likely induced by decreased day-length. The May-June cruise coincided with the beginning of the thermal stratification, which was established differently between the coast and the offshore areas. This was more likely explained by the influence of the AW advection, which interfered with the thermocline establishment in the offshore area. The September cruise fell in the well-established summer hydrological conditions. The thermocline deepened to more than 30 m in depth and the Atlantic water occupied the lower layer of the water column. Similar vertical water distribution was reported in this area during July 2005 subjected to a weak advection (Bel Hassen et al. 2008). These authors suspected the AW to be the residual of an enhanced spring circulation having therefore a stronger oligotrophy than the freshly advected water.

NN depletion was revealed during a water mixing condition, whereas general Si and P deficiencies characterized the strong stratification with a subsequent decrease of chlorophyll $a$ concentrations (Table I). The potential resource limitation effect on phytoplankton growth will be discussed in regard to the marine diatoms requirements. During the mixing conditions whereas N-limitation was likely to occur, Si was particularly available suggesting that the potential N-limitation of diatoms growth might explain an incomplete utilization of silica by diatoms. During this period the chlorophyll $a$ concentrations correlated significantly with temperature (Table II), supporting the results that AW, characterised by low temperature and salinity, contributed to the phytoplankton biomass accumulation in this area (Bel Hassen et al. 2009a). With the beginning of stratification, Si-limitation and N-limitation, were likely to occur. These two nutrients exhibited different spatial distributions (Fig. 4). This was also associated with different relationships to temperature (Table II). We infer that the N-limitation occurred at coastal stations whereas Si-limitation occurred at offshore stations. This statement can find support in the scarcity of diatoms during May-June semi-mixed conditions in the offshore compartment and particularly at the deep chlorophyll maximum (Drira et al. 2009a).

The strong stratified conditions were characterized by a general Si deficiency (Table I). The Si was mainly available in the bottom offshore water, thus situated well below the euphotic area. In addition, P depletion was also observed, which was consistent with reports of P limitation in the Mediterranean Sea during the summer (Jacques et al. 1973, Minas et al. 1988, Thingstad & Rassoulzadegan 1995, Thingstad et al. 1998). This situation was also accompanied by low chlorophyll $a$ concentrations. Unexpectedly, the highest chlorophyll $a$ concentrations occurred in the coastal regions particularly marked by the scarcity of the potentially limiting nutrients (i.e., Si and P). This suggests that other factors than nutrient availability, would regulate the phytoplankton standing stock and/or that the phytoplankton composition was adapted to overcome these limiting conditions. Indeed, the microphytoplankton composition structure, studied during this period revealed the dominance of non-siliceous species (Drira et al. 2009a). In addition, associated phytoplankton pigments composition data showed that 87 % of the chlorophyll $a$ was contributed by pico- and nano phytoplankton classes, among them cyanobacteria, prochlorophytes.
and prasinophytes were the most important (Bel Hassen et al. 2009b). This indicates that these small phytoplankton cells were less opportunistic with regard to nutrient requirements than large cells, thus they were better adapted to oligotrophic conditions (Fogg 1991, Gómez et al. 2001).

The striking findings of this work were the high concentrations, throughout the cruises, of suspended matter with high proportions of organic components. Surprisingly, the highest concentrations were recorded in stratified offshore stations, while we expected higher concentrations in the coastal stations, mainly due to the water column vertical mixing. As the Gulf of Gabes has one of the largest trowable shelf areas in the SE Mediterranean, the trawling-induced re-suspension of sediment (Durrieu de Madron et al. 2005) was a likely factor justifying the high TSS concentrations. In addition, the physical mechanisms associated with the formation of the AW at bottom layer depth during enhanced stratification, although it is not yet well elucidated and documented, could also have an impact on shelf sediment erosion as suggested by Bourrin et al. (2008) in the Gulf of Lions. These physical processes tended to generate high TSS concentrations in the bottom layer depth. However, the highest concentrations were recorded at subsurface levels and at the thermocline depth inferring that the TSS depth-related distribution might not depend on purely physical processes. Indeed, almost 80 % of TSS had organic origin which suggests that many other important biological factors considerably influenced the TSS vertical distribution patterns.

TSS and chlorophyll $a$ vertical distributions exhibited general opposition patterns (Fig. 5). These opposing trends were also reflected in the PCA plots during the three cruises (Fig. 6). This suggests that high TSS concentrations might limit chlorophyll $a$ biomass by constraining sunlight penetration through the water column (Cloern 1987, Irigoien & Castel 1997). Nevertheless, this is unlikely to occur due to the deepening of the eutrophic layer well below the depth of the highest TSS concentrations. Moreover, these opposing trends between TSS and chlorophyll $a$ is a very relevant information and could contribute to the calibration of a remote sensing algorithm in this area, since most of the algorithms used at the Mediterranean basin scale are not always appropriate for the Gulf of Gabes water (Bosc et al. 2004).

The absence of significant relationships between chlorophyll $a$ and TSS indicates that phytoplankton biomass did not directly contribute to the TSS. However, zooplankton organisms were particularly abundant during summer stratification corresponding to the highest TSS concentrations (Drira et al. 2009b). These metazoans were mainly represented by copepods, averaging 80 % of the total zooplankton biomass (Drira et al. 2009b). Furthermore, the study of the ciliate community showed that ciliates also concentrated in the coastal area of the Gulf of Gabes (Hannachi et al. 2009). We suspect predation by zooplankton and protozoan ciliates as being among the most important factors regulating the phytoplankton biomass in this area and this could therefore justify the inverse trend between TSS and Chlorophyll $a$. Moreover, zooplankton ingestion is followed by egesting of faecal pellets, which could significantly increase the TSS concentrations (Krivtsov et al. 2001).

The results of the present study show a seasonal pattern of potential nutrient limitation on the phytoplankton biomass. Particularly, Si deficiency which started in early stratification and increased with well-established stratification should be carefully considered and monitored as it could mark the transition to a non-siliceous phytoplankton-dominated ecosystem with its long-term effects on water quality and on the economical value of the resources.

The bottom down processes seems to play a major role in shaping the phytoplankton composition structure in this area. Nevertheless, the top down processes, mainly the grazing by protozoan ciliates and zooplankters, although not deeply investigated, were suspected to play a significant role in regulating the phytoplankton standing stocks. Therefore, their functional roles in the trophic transfer within the food chain remain to be determined.

ACKNOWLEDGMENTS. – This work was supported by the Tunisian funded project POEMM (LR02INSTM04), which was conducted in the National Institute of Marine Sciences and Technologies (INSTM). The authors wish to thank the crew of the ‘RV Hannibal’ for their assistance.

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