

Mediterranean Marine Science

Vol 14, No 2 (2013)

Vol 14, No 2 (2013)



Driving factors of the potentially toxic and harmful species of *Prorocentrum Ehrenberg* in a semi-enclosed Mediterranean lagoon (Tunisia, SW Mediterranean)

I. SAHRAOUI, D. BOUCHOUICHA, H. HADJ MABROUK, A. SAKKA HLAILI

doi: [10.12681/mms.338](https://doi.org/10.12681/mms.338)

To cite this article:

SAHRAOUI, I., BOUCHOUICHA, D., HADJ MABROUK, H., & SAKKA HLAILI, A. (2013). Driving factors of the potentially toxic and harmful species of *Prorocentrum Ehrenberg* in a semi-enclosed Mediterranean lagoon (Tunisia, SW Mediterranean). *Mediterranean Marine Science*, 14(2), 353–362. <https://doi.org/10.12681/mms.338>

Driving factors of the potentially toxic and harmful species of *Prorocentrum* Ehrenberg in a semi-enclosed Mediterranean lagoon (Tunisia, SW Mediterranean)

I. SAHRAOUI, D. BOUCHOUICHA, H.H. MABROUK and A.S. HLAILI

Laboratoire de Cytologie végétale et Phytoplanktonologie, Département des Sciences de la Vie, Faculté des Sciences de Bizerte, Université de carthage, 7021 Zarzouna, Bizerte, Tunisie

Corresponding author: ineskalif@yahoo.fr

Handling Editor: Lydia Ignatiades

Received: 3 February 2013; Accepted: 9 May 2013; Published on line: 6 June 2013

Abstract

We analysed the dynamics of the potentially toxic and harmful species of *Prorocentrum* Ehrenberg in Bizerte Lagoon (important aquaculture area, Northern Tunisia), substantiating the possible driving forces (temperature, salinity and nutrients), based on a two-year database. We revealed that *Prorocentrum* spp. blooms of high magnitude (10^4 - 10^5 cells l^{-1}) occurred mostly during the period of late winter to early spring. We found five species of *Prorocentrum*, two of which, namely *P. lima* and *P. cordatum*, the most common during the field, are confirmed agents of Diarrhetic Shellfish Poisoning in various regions of the world. *Prorocentrum* sp., *P. micans* and *P. gracile* were however present only sporadically but with high cell abundances, exemplifying bloom densities. Canonical correspondence analysis revealed that *P. minimum* and *P. lima* were much more abundant in eutrophic waters characterized by high Chl *a* biomass, while *P. gracile* species occurred principally in warm waters. Furthermore, *Prorocentrum* sp. and *P. micans* seemed more likely to proliferate in saline waters with high concentrations of inorganic nutrients (nitrate, ammonia and phosphate). Our study calls attention to a possible intensification of DSP events in Bizerte Lagoon, given the propensity of *Prorocentrum* spp. to proliferate in a eutrophic system.

Keywords: *Prorocentrum* spp., DSP, driving factors, Bizerte Lagoon, Tunisia.

Introduction

Observations of HABs (Harmful Algal Blooms) are being reported with increasing frequency in Mediterranean coastal waters, in relation to human impacts and habitat changes that have occurred during the past few decades (Garcés & Camp, 2012). In fact, a clear trend has been observed towards increasing human population density in Mediterranean coastal areas, which involves major exploitation of the coastline for commercial and recreational purposes. Sites most affected by HABs include bays, harbours, estuaries, lochs and lagoons, which geomorphology are worst impacted by the negative effects of eutrophication. As regards the northern Mediterranean, the bays of the Ebro Delta and Albufera, Valencia (Spain) (Garcés & Camp, 2012), the coastal lagoons of south-eastern France (Collos *et al.*, 2009), Kastela Bay (Croatia) (Ninčević Gladan *et al.*, 2009), and Izmir Bay (Turkey) (Bizsel & Bizsel, 2002), are examples of environments where conditions were found stimulating excessive phytoplankton proliferation. Although not documented as extensively as for the northern Mediterranean, serious HAB incidents have also been reported in southern countries, such as in the coastal waters and lagoons

of the Nile delta in Egypt (Dorgham, 2011), the coastal lagoons of Tunisia (Armi *et al.*, 2010; Sahraoui *et al.*, 2012) and Algeria (Frehi *et al.*, 2007).

Concern about HABs in Tunisian coastal waters (SW Mediterranean) has been growing since the late 1990s, when massive fish deaths (Romdhane *et al.*, 1998) were observed. At the time, more than 700 tons of cultured sea bass and sea bream, and several species of wild fishes were found dead in the lagoons of Bougrara and Ghar El Melh. The causative organisms were *Gymnodinium aureolum* and *Alexandrium minutum*, reaching concentrations of 5.3×10^5 and 2×10^5 Cells l^{-1} , respectively. Since 1996, routine records of phytoplankton samples from almost all major lagoons along the Tunisian coastline have revealed the presence of several toxic and potentially toxic dinoflagellates (Turki, 2004; Armi *et al.*, 2008, 2011). The Bizerte Lagoon zone (northern Tunisian coast), is an economically important area for the shellfish industries, fisheries and tourism, where blooms of *Prorocentrum* spp., potential agents of diarrhetic shellfish poisoning (DSP), were repeatedly noted during the last decade (Turki, 2004; Kacem *et al.*, 2009, 2010). Furthermore, okadaic acid (OA), the toxin responsible of DSP, was found in local shellfish samples (*Mytilus galloprovincialis*), exceed-

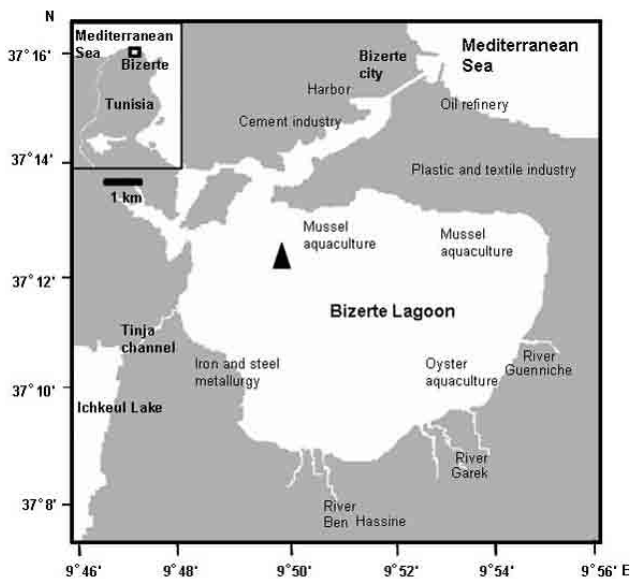


Fig. 1: Map of Bizerte Lagoon, Tunisia, showing the sampling station (▲).

ing the European Union regulatory limit for consumer safety (Kacem *et al.*, 2010).

Managing *Prorocentrum* spp. blooms and DSP events in Bizerte Lagoon requires field investigations in order to understand species dynamics in relation to environmental factors. In this context, we investigated the relationship between *Prorocentrum* species and environmental parameters at a station located near an active mussel aquaculture site in Bizerte Lagoon, during two annual cycles (from March 2004 to March 2005 and from March 2006 to February 2007).

Material and Methods

Study area and sampling station

Bizerte Lagoon (SW Mediterranean Sea) (Fig. 1) has an average depth of 8 m and an area of 150 km², and is connected to the sea through a 300 m-wide and 12 km-long channel. Hydrological and circulation patterns are mainly influenced by wind intensity and freshwater discharge (Harzallah, 2003). The lagoon ecosystem is considered to have undergone eutrophication (Sakka Hlaili *et al.*, 2008) as a result of poor circulation and continuous inputs of nutrients and organic matter from internal (developed shellfish farming) and external (urban, industrial and agricultural) sources.

Sampling was conducted at a fixed station (37°13.24' N, 09°54.36' E), situated near an *Mytilus galloprovincialis* active aquaculture farm, for 2 years (March 2004-March 2005 and March 2006-February 2007) on roughly a monthly basis (22 sampling occasions). Water was collected at 2 m below surface (depth of the chlorophyll maximum (Sakka Hlaili *et al.*, 2006)) using a HYDRO-BIOS (Kiel-Holtenau, Germany) sampler.

Water analysis

Temperature and salinity (Practical Salinity Scale) were determined *in situ* using a Microprocessor Conductivity Meter (WTW-LF-197, Brives B.V., Germany). For chlorophyll *a* (Chl *a*), seawater samples (1000 ml) were filtered through Whatman GF/F filters. Pigment concentrations were determined using a standard spectrophotometric method (Parsons *et al.*, 1984), following extraction with 10 ml 90% acetone overnight at 4°C in the dark. Samples for nutrients (1000 ml) were filtered through Whatman GF/F filters. The filtrates were collected in acid-washed vials and kept frozen (-20°C) until analysis. Nutrient concentrations were determined by spectrophotometric methods [NO_3^- and NO_2^- (Wood *et al.* 1967); PO_4^{3-} (Murphy & Ryley, 1962); NH_4^+ (Aminot & Chaussepied, 1983); $\text{Si}(\text{OH})_4$ (Mullin & Riley, 1955)]. Detection limits of the analytic methods are 0.01, 0.02 and 0.1 μM , for nitrite, phosphate and silicate, respectively, and 0.05 μM for nitrate and ammonia.

Microscopic analysis

Identification and enumeration of the phytoplankton were performed under oil immersion (x100 objective) using an inverted microscope on 24-hour settled volumes of 50 ml lugol-iodine preserved (3% final concentration) water subsamples (Utermöhl, 1958). The literature references used for taxonomic identification of dinoflagellate species of the genus *Prorocentrum* Ehrenberg included: Dodge (1982); Sournia (1986) and Steidinger & Tangen (1996).

Statistics

Canonical Correspondence Analysis (CCA) was performed using Canoco v. 4.5 (ter Braak & Smilauer, 2002). The quantitative, independent environmental variables examined were $\text{Si}(\text{OH})_4$, PO_4^{3-} , NO_3^- , NO_2^- , NH_4^+ , salinity, temperature and Chl *a*. These variables are coded in table 1 and figure 6 as Si, P, NO3, NO2, NH4, Sal, Temp and Chl, respectively. CCA was performed on log-transformed [$\ln(\text{Ay} + \text{B})$; A = 1, B = 1] calculated species cell densities from each sample. In order to reveal statistically significant relationships between species and environmental variables, a biplot of t-values (approximate t-ratios of the regression coefficients of the multivariate regression) was constructed according to the Van Dobben method (ter Braak & Looman, 1994). Positive Van Dobben circles were constructed with diameters defined by the line-segment joining the t-values of the environmental variables and the origin. Negative circles were obtained by mirroring the positive circles in the tangent at the origin. Species that have their t-value coordinates inside these circles are inferred to react positively or negatively to the environmental variables.

Results

Environmental factors

The monthly profiles of physicochemical variables at the studied station are presented in Fig. 2. Water temperature (Fig. 2b) exhibited typical seasonal pattern for temperate lagoons, with minima occurring in December-January (12.2 °C, January 2005) and maxima in July-August (28.8 °C, July 2006). Furthermore, typical salinity profiles for the dry and rainy seasons were observed, with a minimum value of 28.9, registered in July 2004, and a maximum value of 39.4, recorded in November 2004 (Fig. 2a). DIN, DIP and silicate concentrations did not show a consistent seasonal pattern, however, an interannual variability seems to exist, with higher values in 2006

($p < 0.001$, Student's *t* test) (Fig. 2 c, d, e). The lowest DIN concentration was detected in July 2006 (8.81 μM) and the highest in November 2006 (70.70 μM) (Fig. 2 c). Ammonia was the dominant form of DIN with a mean value of 17.42 μM . Nitrate concentrations were, on average, less than 3 μM , and nitrite concentration was just 3.3 % of total DIN, with average concentrations of 0.35 μM . The DIP concentrations varied between 0.03 μM (June 2004) and 1.22 μM (December 2006) (Fig. 2d), and the silicate between 0.53 (December 2004) and 12.48 (February 2007) (Fig. 2e).

Taxonomy

Gross morphological features viewed by LM allowed small (16-20 μm long to 10-12 μm wide) oval to trian-

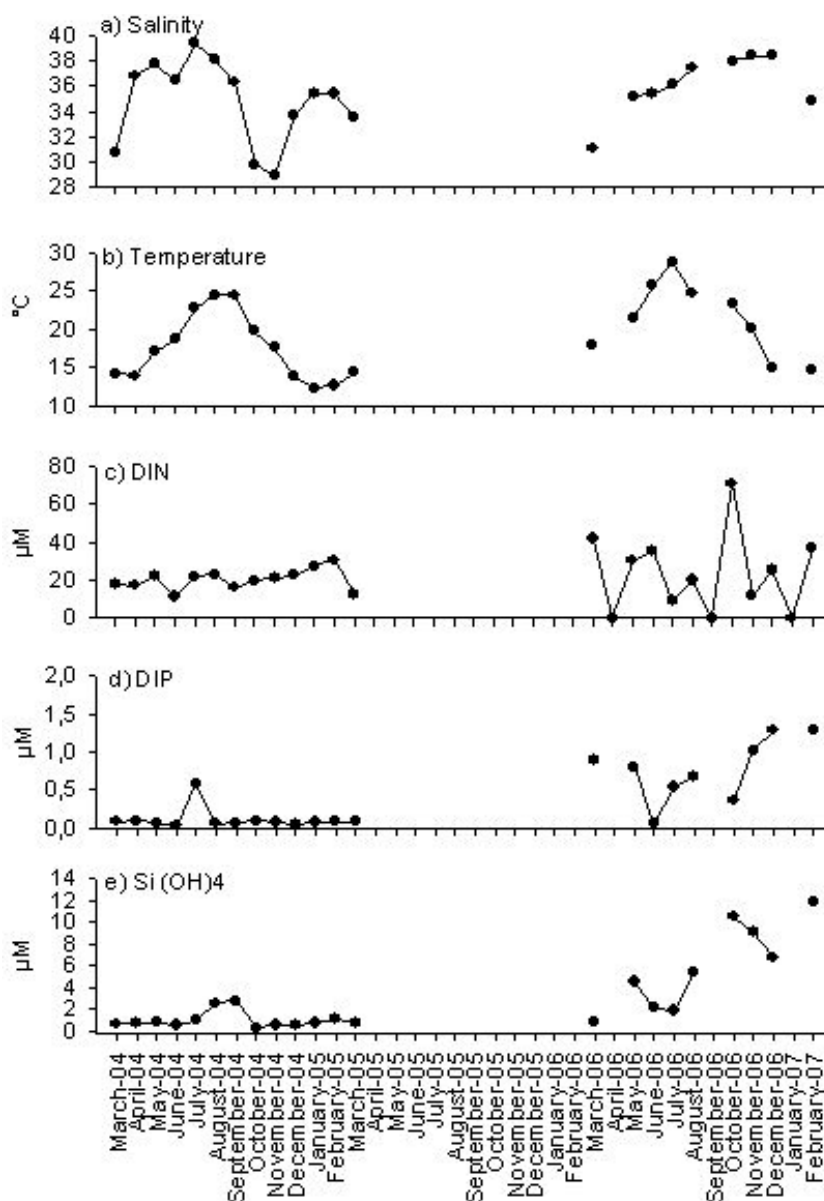


Fig. 2: Monthly profiles of the physicochemical variables at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007): (a) temperature; (b) salinity; (c) dissolved inorganic nitrogen; (d) dissolved inorganic phosphorus; (e) Si(OH)_4 .

gular shaped cells in valve view with short apical spine to be attributed to *Prorocentrum minimum* (Pavillard) (Sournia, 1986; Steidinger & Tangen, 1996). Obovate cells, broadest postmedially with central pyrenoid and posterior nucleus, of 30-35µm in length and 20-23µm in width, were attributed to *Prorocentrum lima* (Ehrenberg). Heart shaped cells, sized 35-40 µm long and 20-27 µm wide, rounded anteriorly, pointed posteriorly, and broadest around the middle with a well developed apical spine (≈ 10 µm long) and a length-width ratio usually less than two were attributed to *Prorocentrum micans* (Ehrenberg) (Tomas, 1997). Small to medium-sized (42-56 µm in length and 23-31 µm in width) elongate to lanceolate cells with more than twice as long as broad and a strong winged apical spine were attributed to *Prorocentrum gracile* (Sournia, 1986). Broadly ovate cells of 30-32 µm in width and 32-35 long, with central pyrenoid and posterior nucleus, were identified as *Prorocentrum* sp.

Occurrence and densities of *Prorocentrum* spp. in Bizerte Lagoon

The genus *Prorocentrum* was always present throughout the studied period of 2004-2005 whereas it was less observed in 2006-2007 (Fig. 3). Allocation to total phytoplankton density was low (never exceeded 6.72 %) and significantly variable over time ($P < 0.05$). Contribution to total algae was the highest during the autumn 2004-winter 2005 period (2.28 - 6.14 %) and in March 2006 (6.72 %).

In terms of cell number, abundances of *Prorocentrum* spp. ranged between a minimum value of 4.30×10^3 cells per litre (May 2004) and a maximum value of 1.54×10^5 cells l⁻¹ (March 2006) (Fig. 3). Peaks of cell density were found in spring (7.25×10^4 and 1.54×10^5 cells l⁻¹ in March 2005 and March 2006, respectively) and also in winter (9.64×10^4 cells l⁻¹ in February 2007).

Five morphospecies of *Prorocentrum* spp. were found in this study (Fig. 4); *Prorocentrum lima*, *P. minimum*, *P. micans*, *P. gracile* and *Prorocentrum* sp. The primary *Prorocentrum* species was *P. lima*, detected in ~ 50 % of the samples. It was observed mostly during the 2004-2005 study period (Fig. 4), when it contributed from 53.33 % (October 2004) to 100% (May and August 2004 and March 2005) to total *Prorocentrum* spp. abundance. The highest cell abundance of this species was found in March 2005 (7.25×10^4 cells l⁻¹) (Fig. 5). The second most important taxon was *P. minimum*, present in ~ 32 % of the samples. The species was also mostly present during the 2004-2005 study period, when it formed from 24.16 % (September 2004) to 46.66 % (October 2004) of total *Prorocentrum* spp. abundance (Fig. 4) The highest abundance of this species was recorded in March 2006, with 1.31×10^5 cells l⁻¹ (Fig. 5). *Prorocentrum* sp. and *P. micans* were present in only ~ 5 % of the samples and occurred only sporadically in February 2007, with cell densities of 9×10^4 and 6.45×10^3 cells l⁻¹, respectively (Fig. 5). *P. gracile* was also found in only ~ 5 % of the samples and was present only sporadically (in June 2006) but with high cell density (2.90×10^4 cells l⁻¹) (Fig. 5).

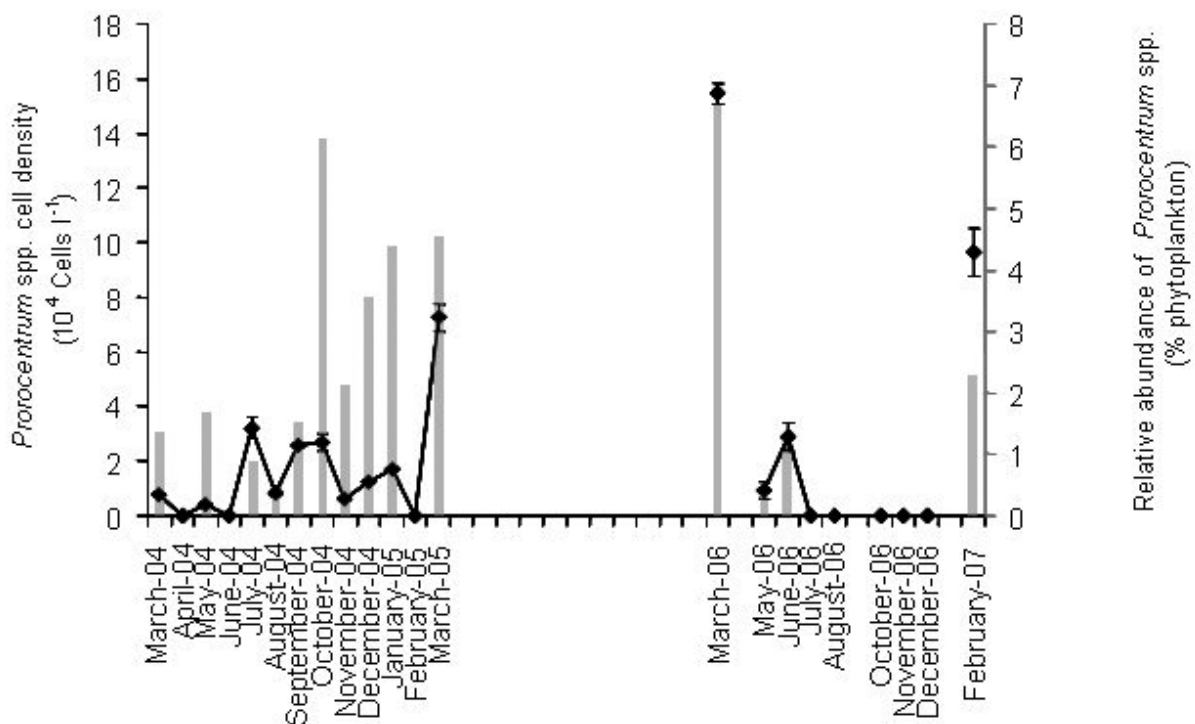


Fig. 3: Monthly variation in total (Line and scatter plot, Mean \pm SD, n=3) and relative abundance (grey bars) of *Prorocentrum* spp. at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007).

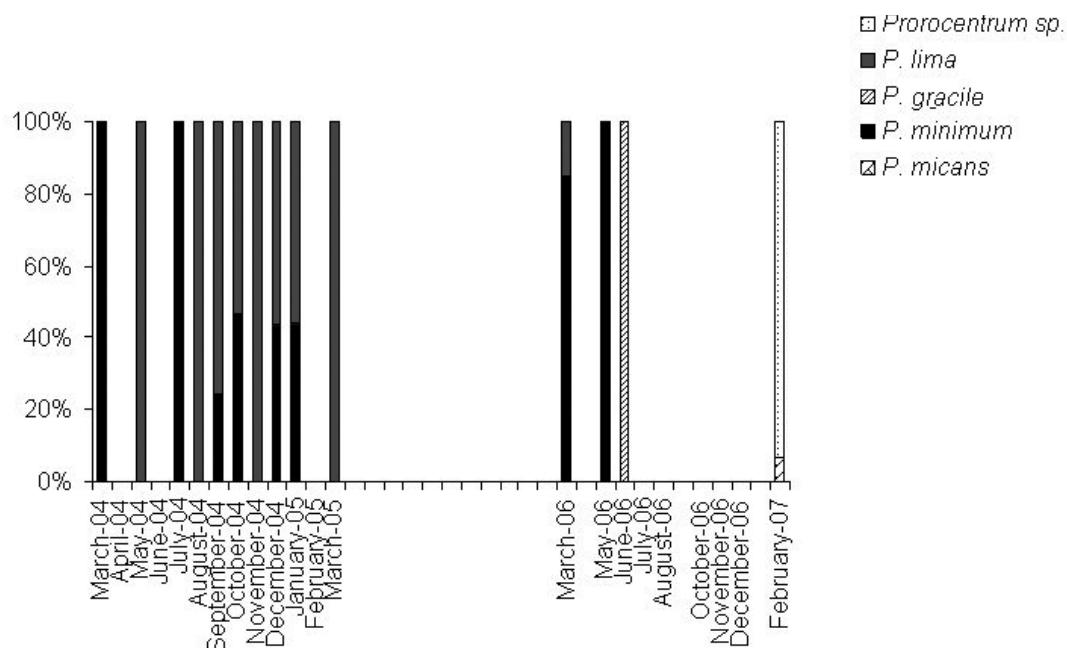


Fig. 4: Monthly variation in the composition of the genus *Procoentrum* at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007).

Procoentrum species and environmental interactions

CCA was used to relate the distribution of *Procoentrum* species to environmental variables. The first three extracted ordination axes of the CCA explained 39.8, 35.9 and 12.9 % respectively of the variance in the species data (total 88.6%). Of the variance extracted in

the ordination axes, each axis extracted 44.9, 40.5 and 14.6 % respectively, of the explainable variance (total 100 %). Our CCA analysis thus explains all the variance in the three extracted axes. Monte Carlo permutation tests found the first axis alone statistically significant ($P < 0.005$), as well as all the canonical axes ($P < 0.005$). The analysis did not produce significant canonical coef-

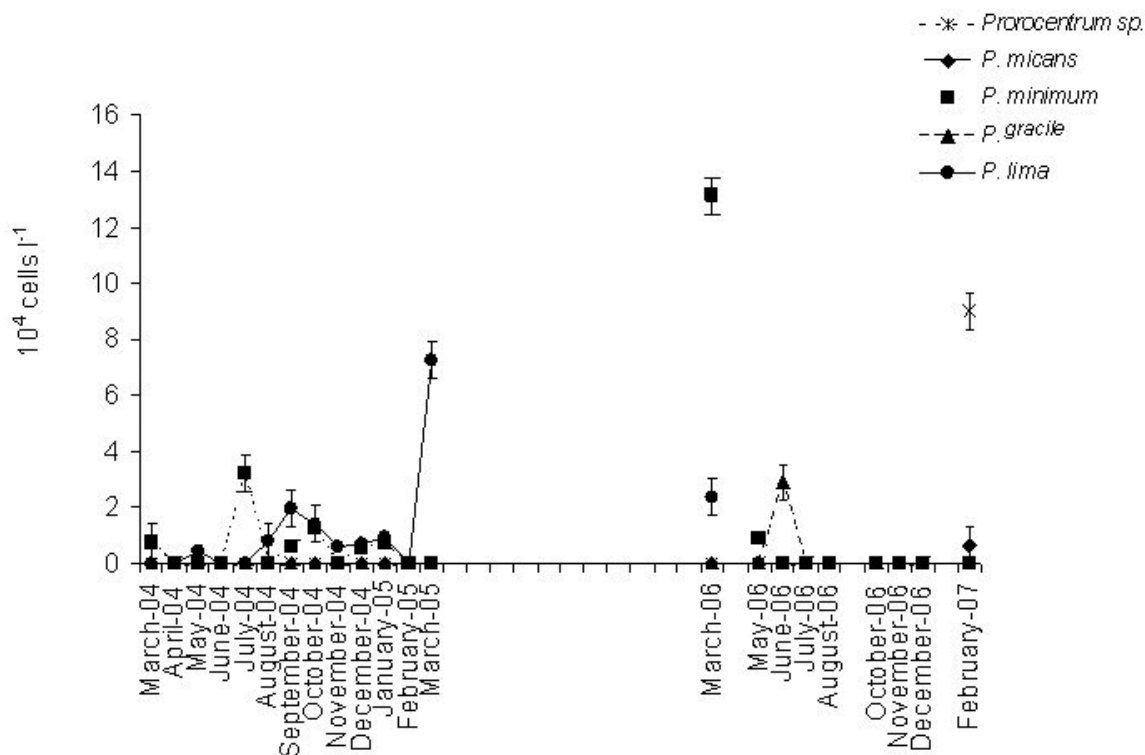


Fig. 5: Fluctuations of *Procoentrum* species abundances at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007) (Mean \pm SD, n=3).

ficients for any of the environmental variables in the third ordination axes. Our discussion of results will therefore be restricted to the first two ordination axes.

Figure 6 illustrates the CCA analysis in the form of a classical biplot of species scores and gradient vectors for the quantitative environmental variables. Of the 8 quantitative environmental variables evaluated, 5 variables (NH_4^+ , PO_4^{3-} , Temperature, Salinity and Chl *a*) were found to be statistically significant in terms of effects on species abundance (Table 1). The positions of species scores (Fig. 6) revealed that two major groups of species were separated by the first (horizontal) ordination axis. On the one hand, *Prorocentrum micans* and *Prorocentrum* sp. had positive first axis scores. On the other hand, *P. gracile*, *P. minimum* and *P. lima* all had negative first axis scores. In the second ordination vertical axis, *P. gracile* was clearly separated from the remaining *Prorocentrum* species. The results suggest, therefore, that *P. gracile* occurred principally in warm waters, whereas *P. minimum* and *P. lima* were much more abundant in relatively warm

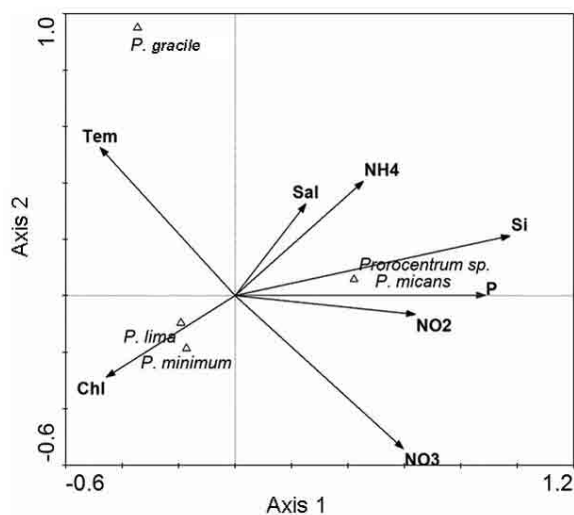


Fig. 6: Canonical correspondence analysis (CCA) ordination diagram showing the relationships between environmental variables (abbreviations in the Material and Methods section) and *Prorocentrum* species distribution.

Table 2. Summary of t-value biplot/Van Dobben analyses of the impact of environmental variables (abbreviations in the Material and methods section) on five species of *Prorocentrum* found in the studied Bizerte Lagoon station (March 2004 - March 2005/ March 2006- February 2007). “+”, “-” and “0” indicate that environmental variables have significant positive, negative or no effect on *Prorocentrum* species distribution, respectively.

Variable	<i>Prorocentrum lima</i>	<i>Prorocentrum minimum</i>	<i>Prorocentrum sigmoides</i>	<i>Prorocentrum micans</i>
NO3	0	-	0	+
NO2	0	0	-	+
NH4	-	-	0	+
Si	0	-	-	0
P	-	0	0	-
Temp	0	0	+	0
Sal	-	-	0	+
Chl	+	+	-	0

Table 1. Canonical correspondence analysis (CCA) regression coefficients of the physical and chemical variables collected during the field study, in Bizerte Lagoon. Significant coefficients shown in bold.

Variable	Axis 1	Axis 2
NO3	0.0915	-0.4404
NO2	-0.0341	0.2325
NH4	-0.1228	0.8405
Si	0.8202	1.1785
P	0.2087	-1.5023
Temp	-0.1178	0.3237
Sal	-0.0247	-0.1489
Chl	0.0253	-0.2345

waters with high Chl *a* biomass. Furthermore, *Prorocentrum* sp. and *P. micans* seemed more likely to proliferate in saline waters with high a concentration of inorganic nutrients (nitrate, ammonia and phosphate). The statistical significances of all these species-variables associations were confirmed by t-value biplot analysis, using the Van Dobben method (Table 2).

Discussion

DSP is the most recurrent threat for shellfish industries worldwide (Garcia *et al.*, 2003). It is principally associated with polycyclic ether toxins: okadaic acid, dinophysistoxins, and pectenotoxins (Bowden, 2006). The syndrome is non lethal with symptoms including adverse effects such as nausea, vomiting, abdominal cramps, and diarrhoea. However, some of the polyether toxins involved may promote stomach tumours (Suganuma *et al.*, 1988) and thus produce serious problems in shellfish consumers. The known distribution of DSP toxins in the Mediterranean includes Italy (Draisci *et al.*, 1995; 1998), Spain (Bravo *et al.*, 2001), Greece (Mouratidou *et al.*, 2004; Ciminiello *et al.*, 2006), France (Lassus *et al.*, 1985), Morocco (Elgarch *et al.*, 2008) and also Tunisia (Bizerte Lagoon, Turki, 2004; Kacem *et al.*, 2010).

Although DSP and several related potentially toxic species (including *P. lima*, *P. concavum*, *P. mexicanum* and *P. minimum*) were reported for the Bizerte Lagoon (Turki, 2004), very little is known about the relationship between particular species and the environmental conditions that favour their growth. This study is the first to investigate the potential relationships between species of the genus *Prorocentrum* and environmental factors in Tunisian waters. We revealed the potentially toxic dinoflagellates of the genus *Prorocentrum* as significant members of the phytoplankton community of the Bizerte Lagoon surface waters. In fact, the genus was present with high cell abundances (max: 1.54×10^5 cells l⁻¹), comparable with those previously reported by Turki (2004) in the same area (max: 1.1×10^6 cells l⁻¹). Furthermore, our investigation confirmed previous observations on the preference of *Prorocentrum* species for enclosed, nutrient rich water bodies (Dolapsakis *et al.*, 2008; Spatharis *et al.*, 2009; Xu *et al.*, 2010).

Five species of *Prorocentrum* were recorded, two of which, namely *Prorocentrum lima* and *P. minimum*, are potential producers of DSP toxins (Lundholm, 2011). *Prorocentrum lima* was the best represented species of the genus *Prorocentrum*, present in ~50 % of the subsurface water samples and reaching high concentrations (> 10⁴ cells l⁻¹) in March 2005. *P. lima* is a confirmed producer of OA in cultures (Marr *et al.*, 1992; Bravo *et al.*, 2001) and is considered as the main agent for DSP in many regions (e.g. Galician coasts, NW Spain (Bravo *et al.*, 2001); Gulf of California, Mexico (Heredia-Tapia *et al.*, 2002)). The species occurs worldwide in coastal areas mostly in benthic and epiphytic habitats (Faust *et al.*, 1999). In this study, however, high concentrations of *P. lima* were found free-living in subsurface waters. In eastern Canada, this species has also been found in substantial concentrations in the water column (Bates, 1997; Lawrence *et al.*, 2000). This species has been frequently found in Mediterranean waters during the last decade (Simoni *et al.*, 2004; Aligizaki *et al.*, 2006; Ingarao *et al.*, 2007). Our data on cell densities are comparable with those reported by Ingarao *et al.* (2009) for the western Adriatic, exceeding, however, those previously reported for Bizerte Lagoon (max: > 10³ cells l⁻¹, Turki, 2004) and other ecosystems (max: < 330 cells l⁻¹ along the Catalan coast, NW Mediterranean, Vila *et al.*, 2001; max: < 900 cells l⁻¹ in the Gulf of St Lawrence, Canada, Levasseur *et al.*, 2003 and max: > 500 cells l⁻¹ in the Black Sea, Morton *et al.*, 2009).

P. minimum was also an important representative of the genus *Prorocentrum* during the field study (present in ~32 % of the samples), reaching bloom densities in March 2006. The seasonality of *P. minimum* in Bizerte Lagoon is in agreement with the findings of Zingone *et al.* (2006), who noted that this species commonly bloomed in March along the coasts of the Campania region (South Tyrrhenian Sea, Mediterranean Sea). Moreover, maxi-

imum cell densities found in this study (> 10⁵ cells l⁻¹) are comparable with those found in Chesapeake Bay (USA) (Tango *et al.*, 2005), where *P. minimum* blooms were responsible for serious aquaculture impacts (US \$25,000-30,000 for a single grower). High density blooms of *P. minimum* (> 10⁸ cells l⁻¹) have regularly occurred in the Black Sea, causing mass mortality of fish, molluscs and crustaceans (Velikova & Larsen, 1999). This species has generated increasing concern worldwide with evidence of toxin production and impacts on human health due to shellfish consumption from bloom waters (Heil, 2005).

P. minimum and *P. lima* were significantly positively correlated to Chl *a* concentrations and temperature. These species did in fact proliferate principally in spring (March 2005, 2006) after important precipitation events, and in line with progressive temperature increases. It is also suggested that the potential river discharge of organic nutrient rich waters triggers the growth of *P. minimum* and *P. lima* in Bizerte Lagoon waters. The potential relationship between both species and the nutrient load may support the hypothesis of Glibert *et al.* (2008) concerning an apparent link between *P. minimum* and eutrophication, based on several site-specific long-term databases and a review of its global spreading. Ignatiades & Gotsis-Skretas (2010) also found that *P. minimum* bloom incidents in Greek coastal waters were closely related to anthropogenically-induced eutrophication conditions. Furthermore, Heil (2005) demonstrated that terrestrially derived dissolved organic carbon fractions play an active role in the stimulation of *P. minimum* growth. Grzebyk & Berland (1996) have also noted that *P. minimum* blooms generally occur in zones affected by freshwater inputs (large deltas, estuaries, fjords, lagoons) and/or anthropogenic inputs. For the Baltic Sea, Pertola *et al.* (2006) have suggested that the relatively recent invasion of *P. minimum* could have been enhanced by dissolved organic nitrogen enrichment. Additionally, high concentrations of *P. lima* were found in a eutrophic area considerably influenced by runoff from nearby rivers in the western Adriatic (Ingarao *et al.*, 2009). The link between *P. minimum*, *P. lima* and temperature is in accordance with the findings of Grzebyk & Berland (1996), who demonstrated that temperature considerably affected the growth of a Mediterranean clone of *P. minimum*. Furthermore, the occurrence of *P. lima* was positively correlated with temperature in the western Adriatic (Ingarao *et al.*, 2009).

P. gracile, *P. micans* and *Prorocentrum* sp. were the minor representatives of the genus *Prorocentrum* during the field study. Sporadic occurrences of these species took place abruptly, as bloom events, which are defined here through high cell abundance (10³-10⁴ cells l⁻¹), suggesting an opportunistic behaviour for these dinoflagellates, as reported in other studies (Reynolds, 2006). In fact, in Bizerte Lagoon, intensive shipping activities may have introduced many species through ballast water discharge during the last decades. Invasive non-native species growing outside

their natural range generally manifested a rapid growth in chemically disturbed habitats (Villac *et al.*, 2005).

P. gracile growth was significantly stimulated by elevated temperatures, which is in agreement with the observations of La Barbera-Sánchez *et al.* (2004) who found that this species was the major blooming species during summer at Margarita Island, Venezuela. In the Nanji Islands National Nature Reserve (East China), Li *et al.* (2011) have also found that *P. gracile* proliferated only in August, when the water temperatures were highest.

Prorocentrum sp. and *P. micans* seemed likely to proliferate in saline nutrient rich waters (nitrate, ammonia and phosphate). These results are in agreement with Steidinger & Tangen (1996), who found that *P. micans* species can tolerate very high salinity. In fact, populations have been reported from hypersaline salt lagoons (> 90 ‰) in the Caribbean islands. Moreover, the species is described to prefer higher salinity coastal areas (Johnson and Allen, 2005). *Prorocentrum micans* cultures were also found to reach the highest total biovolume under high dissolved nutrient concentrations (Eker-Develi *et al.*, 2006). Additionally, this species is reported to form extensive blooms and is suspected to have caused shellfish deaths in Portugal (Pinto & Silva, 1956) and South Africa (Horstman, 1981).

Conclusion

During the time of this investigation, *Prorocentrum* spp. were commonly present with substantial cell abundances near an active Mussel farm in Bizerte Lagoon (> 10⁴ cells l⁻¹). The main representative species were *P. lima* and *P. minimum*, both confirmed producers of OA and responsible for several DSP events worldwide. Blooms of these species were significantly associated with eutrophic waters (characterized here by elevated chl *a* concentrations). *Prorocentrum* sp. and *P. micans* were furthermore found in high salinity waters rich in inorganic nutrients, while *P. gracile* abundance was significantly related to water temperature. The progressive warming and salinization of Bizerte Lagoon waters (Ouakad, 2007) and the cultural eutrophication of Bizerte Lagoon waters may accentuate the severity of local DSP events. The occurrence of toxic and potentially toxic *Prorocentrum* spp. blooms under existing eutrophic Bizerte Lagoon conditions represents significant challenges for water quality and living resource recovery efforts. Nutrient management strategies have to be adequately planned in order to improve water quality and algal biomass conditions.

Acknowledgements

We gratefully thank Dr. Richard E. Furnas (Microcomputer Power, NY, USA), for providing us with a personal Canoco 4.5 trial version.

References

- Aligizaki, K., Nikolaidis, G., 2006. The presence of the potentially toxic genera *Ostreopsis* and *Coolia* (Dinophyceae) in the North Aegean Sea, Greece. *Harmful Algae*, 5, 717-730.
- Aminot, A., Chausspié, M., 1983. Manuel des analyses chimiques en milieu marin. Centre national pour l'exploitation des océans. Brest. ISBN: 2.902721.10.2.
- Armi, Z., Turki, S., Ben Maiz, N., Trabelsi, E., 2008. Nutrient loadings and occurrence of harmful phytoplankton species in the North Lake of Tunis (Tunisia). *Cahiers de Biologie Marine*, 49, 311-321.
- Armi, Z., Turki, S., Trabelsi, E., Ben Maiz, N., 2010. First recorded proliferation of *Coolia monotis* (Meunier, 1919) in the North Lake of Tunis (Tunisia) correlation with environmental factors. *Environmental Monitoring and Assessment*, 164, 423-433.
- Armi, Z., Milandri, A., Turki, S., Hajjem, B., 2011. *Alexandrium catenella* and *Alexandrium tamarense* in the North Lake of Tunis: bloom characteristics and the occurrence of paralytic shellfish toxin. *African Journal of Aquatic Science*, 36 (1), 47-56.
- Bates, S.S., 1997. Toxic phytoplankton on the Canadian east coast: implications for aquaculture. *Bulletin of the Aquaculture Association of Canada*, 97 (3), 9-18.
- Bizsel, K.C., Bizsel, N., 2002. New records of toxic algae *Heterosigma* cf. *akashwo* and *Gymnodinium* cf. *mikimotoi* in the hypereutrophic Izmir Bay (Aegean Sea): coupling between organisms and water quality parameters. *Israel Journal of Plant Sciences*, 50, 33-44.
- Bowden, B.F., 2006. Yessotoxins - polycyclic ethers from dinoflagellates: relationships to diarrhetic shellfish toxins. *Toxin Reviews*, 25, 137-157.
- Bravo, I., Fernández, M.L., Ramilo, I., Marínez, A., 2001. Toxin composition of the toxic dinoflagellate *Prorocentrum lima* isolated from different locations along the Galician coast (NW Spain). *Toxicon*, 39, 1537-1545.
- Ciminiello, P., Dell'Aversano, C., Fattorusso, E., Forino, M., Magno, G.S. *et al.*, 2006. The Genoa 2005 outbreak determination of putative palytoxin in Mediterranean *Ostreopsis ovata* by a new liquid chromatography tandem mass spectrometry method. *Analytical Chemistry*, 78, 6153-6159.
- Collos, Y., Bec, B., Jauzein, C., Abadie, E., Laugier, T. *et al.*, 2009. Oligotrophication and emergence of picocyanobacteria and a toxic dinoflagellate in Thau lagoon, southern France. *Journal of Sea Research*, 61, 68-75.
- Dodge, J.D., 1982. *Marine Dinoflagellates of the British Isles*. Her Majesty's Stationery Office, London. 303 pp.
- Dolapsakis, N.P., Tzovenis, I., Kantourou, P., Bitis, I., Economou-Amilli, A., 2008. Potentially harmful microalgae from lagoons of the NW Ionian Sea, Greece. *Journal of Biological Research*, 9, 89-95.
- Dorgham, M.M., 2011. Eutrophication Problem in Egypt. p. 171-194. In: *Eutrophication: causes, consequences and control*, Ansari, A.A., Gill, S.S., Lanza, G.R., Rast, W. (Eds). Springer, Netherlands.
- Draisci, R., Lucentini, L., Giannetti, L., Bori, P., Stacchini, A., 1995. Detection of diarrhetic shellfish toxins in mussels from Italy by liquid chromatography-mass spectrometry. *Toxicon*, 33, 1591-1603.
- Draisci, R., Giannetti, L., Lucentini, L., Marchiafava, C., James,

- K.J. *et al.*, 1998. Isolation of a new okadaic acid analogue from phytoplankton implicated in diarrhetic shellfish poisoning. *Journal of Chromatography A*, 798, 137-145.
- Eker-Develi, E., Kideys, A.E., Tugrul, S., 2006. Effect of nutrients on culture dynamics of marine phytoplankton. *Aquatic Sciences*, 68, 28-39.
- Elgarch, A., Vale, P., Rifai, S., Fassouane, A., 2008. Detection of diarrhetic shellfish poisoning and azaspiracid toxins in Moroccan mussels: comparison of the LC-MS method with the commercial immunoassay kit. *Marine Drugs*, 6, 587-594.
- Faust, M.A., Larsen, J., Moestrup, Ø., 1999. Potentially toxic phytoplankton. 3. Genus *Prorocentrum* (Dinophyceae), p. 24. In: *ICES identification leaflets for plankton*, Lindley, J.A. (Ed.). ICES, Copenhagen.
- Frehi, A., Couté, G., Mascarell, C., Perrette-Gallet, M., Ayada, M. *et al.*, 2007. Dinoflagellés toxiques et/ou responsables de blooms dans la baie d'Annaba (Algérie). *Comptes Rendus Biologies*, 330, 615-628.
- García, C., Pereira, P., Valle, L., Lagos, N., 2003. Quantitation of diarrhetic shellfish poisoning toxins in Chilean mussel using pyrenyldiasomethane as fluorescent labeling reagent. *Biological Research*, 36, 171-183.
- Garcés, E., Camp, J., 2012. Habitat changes in the Mediterranean Sea and the consequences for harmful algal blooms formation. p. 519-541. In: *Life in the Mediterranean Sea: A Look at Habitat Changes*. Stambler, N. (Ed.). Nova Science Publishers, Inc.
- Glibert, P.M., Mayorga, E., Seitzinger, S., 2008. *Prorocentrum minimum* tracks anthropogenic nitrogen and phosphorus inputs on a global basis: application of spatially explicit nutrient export models. *Harmful Algae*, 8, 33-38.
- Grzebyk, D., Berland, B., 1996. Influence of temperature, salinity and irradiance on growth of *Prorocentrum minimum* (Dinophyceae) from the Mediterranean Sea. *Journal of Plankton Research*, 18, 1837-1849.
- Harzallah, A., 2003. Transport de polluants dans la lagune de Bizerte simulé par un modèle de circulation de l'eau. *Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô*, 30, 121-133.
- Heil, C.A., 2005. Influence of humic, fulvic and hydrophilic acids on the growth, photosynthesis and respiration of the dinoflagellate *Prorocentrum minimum* (Pavillard) Schiller. *Harmful Algae*, 4(3), 603-618.
- Heil, C.A., Glibert, P.M., Fan, C., 2005. *Prorocentrum minimum* (Pavillard) Schiller. A review of a harmful algal bloom species of growing worldwide importance. *Harmful Algae*, 4, 449-470.
- Heredia-Tapia, A., Arredondo-Vega, B.O., Nñez-Vázquez, E.J., Yasumoto, T., Yasuda, M. *et al.*, 2002. Isolation of *Prorocentrum lima* (Syn. *Exuviaella lima*) and diarrhetic shellfish poisoning (DSP) risk assessment in the Gulf of California, Mexico. *Toxicon*, 40, 1121-1127.
- Horstman, D.A., 1981. Reported red-water outbreaks and their effects on fauna of the west and south coasts of South Africa, 1959-1980. *Fisheries Bulletin (South Africa)*, 15, 71-88.
- Ignatiades, L., Gotsis-Skretas, O., 2010. A Review on Toxic and Harmful Algae in Greek Coastal Waters (E. Mediterranean Sea). *Toxins*, 2, 1019-1037.
- Ingarao, C., Lanciani, G., Verri, C., Pagliani, T., 2007. First record of *Prorocentrum lima* on Abruzzo Region coast, W Adriatic. *Harmful Algae News*, 35, 10-12.
- Ingarao, C., Lanciani, G., Verri, C., Pagliani, T., 2009. First record of *Prorocentrum lima* (Dinophyceae) inside harbor areas and along the Abruzzo region coast, W Adriatic. *Marine Pollution Bulletin*, 58(4), 596-600.
- Johnson, W.S., Allen, D.M., 2005. *Zooplankton of the Atlantic and Gulf Coasts: A Guide to Their Identification and Ecology Baltimore*: Johns Hopkins University Press.
- Kacem, I., Hajjem, B., Bouaïcha, N., 2009. First evidence of okadaic acid in *Mytilus galloprovincialis* mussels, collected in a Mediterranean lagoon, Tunisia. *Bulletin of Environmental Contamination and Toxicology*, 82 (6), 660-664.
- Kacem, I., Hajjem, B., Bouaïcha, N., 2010. Comparison of okadaic acid profiles in mussels and oysters collected in Mediterranean lagoon, Tunisia. *International Journal of Biology*, 2 (2), 238-245.
- La Barbera-Sánchez, A., Soler, J.F., Rojas de Astudillo, L., Chang-Yen, I., 2004. Paralytic Shellfish Poisoning (PSP) in Margarita Island, Venezuela. *Revista de Biología Tropical*, 52 (1), 89-98.
- Lassus, P., Bardouil, M., Truquet, I., Truquet, P., Le Baut, C. *et al.*, 1985. *Dinophysis acuminata* distribution and toxicity along the southern Brittany coasts (France): correlation with hydrological parameters. p. 159-162. In: *Toxic Dinoflagellates*, Anderson, D.M., White, A.W., Baden, D.G. (Eds). Elsevier, North-liolland.
- Lawrence, J.E., Grant, J., Quilliam, M.A., Bauder, A.G., Cembella, A.D., 2000. Colonization and growth of the toxic dinoflagellate *Prorocentrum lima* and associated fouling macroalgae on mussels in suspended culture. *Marine Ecology Progress Series*, 201, 147-154.
- Levasseur, M., Couture, J.Y., Weise, A.M., Michaud, S., Elbrachter, M. *et al.*, 2003. Pelagic and epiphytic summer distributions of *Prorocentrum lima* and *P. mexicanum* at two mussel farms in the Gulf of St. Lawrence, Canada. *Aquatic Microbial Ecology*, 30, 283-293.
- Li, Y., Lü, S., Jiang, T., Xiao, Y., You, S., 2011. Environmental factors and seasonal dynamics of *Prorocentrum* populations in Nanji Islands National Nature Reserve, East China Sea. *Harmful Algae*, 10 (5), 426-432.
- Lundholm, N., 2011. Bacillariophyta. In: *Taxonomic reference list of harmful micro algae*. Lundholm, N. (Ed.). IOC-UNESCO, Available online at http://www.marinespecies.org/HAB/aphia.php?p=taxlist&pid=148899&tRank=220&rComp=%3D&context_in=30&vOnly=1.
- Marr, J.C., Jackson, A.E., McLachlan, J.L., 1992. Occurrence of *Prorocentrum lima*, a DSP toxin-producing species from the Atlantic coast of Canada. *Journal of Applied Phycology*, 4, 17-24.
- Morton, S.L., Vershinin, A., Smith, L.L., Leighfield, T.A., Pankov, S. *et al.*, 2009. Seasonality of *Dinophysis* spp. and *Prorocentrum lima* in Black Sea phytoplankton and associated shellfish toxicity. *Harmful Algae*, 8, 629-636.
- Mouratidou, T., Kaniou-Grigoriadou, I., Samara, C., Koumtzis, T., 2004. Determination of okadaic acid and related toxins in Greek mussels by HPLC with fluorimetric detection. *Journal of Liquid Chromatography and Related Technologies*, 27 (14), 2153-2166.
- Mullin, J.B., Riley, J., 1955. The spectrophotometric determination of nitrate in natural waters with particular reference to sea water. *Analytica Chimica Acta*, 12, 464-480.
- Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31-36.

- Ninčević Gladan, Ž., Marasović, I., Grbec, B., Skejić, S., Bužančić, M. *et al.*, 2009. Inter-decadal variability in phytoplankton community in the middle Adriatic (Kaštela Bay) in relation to the North Atlantic oscillation. *Estuaries and Coasts*, 33, 376-383.
- Ouakad, M., 2007. *Genèse et évolution des milieux laguno-lacustres du Nord-Est de la Tunisie (Garaet el Ichkeul, Lagunes de Bizerte et Ghar el Melh)*. Thesis. University of Carthage, Tunisia, 461 pp.
- Pertola, S., Kuosa, H., Olsonen, R., 2006. Is the invasion of *Prorocentrum minimum* (Dinophyceae) related to the nitrogen enrichment of the Baltic Sea? *Harmful Algae*, 4 (3), 481-492.
- Pinto, J.S., Silva, E.S., 1956. The toxicity of *Cardium edule* L. and its possible relation to the dinoflagellate *Prorocentrum micans* Ehr. *Notas e Estudos do Instituto de Biologia Marítima*, 12, 1-20.
- Reynolds, C.S., 2006. *The Ecology of Phytoplankton*. Cambridge University Press, Cambridge, UK. 535 pp.
- Romdhane, M.S., Eilertsen, H.C., Yahia, O.K.D., Yahia, M.N., 1998. Toxic dinoflagellate blooms in Tunisian lagoons: causes and consequences for aquaculture. p. 80-83. In: *Harmful Algae*. Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. (Eds). Xunta de Galicia and Intergovernmental Oceanographic Commission of United Nations Educational, Scientific and Cultural Organization, Paris, France.
- Sakka Hlaili, A., Chikhaoui, M.A., El Grami, B., Hadj Mabrouk, H., 2006. Effects of N and P supply on phytoplankton in Bizerte Lagoon (western Mediterranean). *Journal of Experimental Marine Biology and Ecology*, 333, 79-96.
- Sakka Hlaili, A., Grami, B., Niquil, N., Gosselin, M., Hamel, D. *et al.*, 2008. The planktonic food web of the Bizerte lagoon (South-Western Mediterranean) during summer: I. Spatial distribution under different anthropogenic pressures. *Estuarine Coastal and Shelf Science*, 78, 61-77.
- Sahraoui, I., Grami, B., Bates, S.S., Bouchouicha, D., Chikhaoui, M.A. *et al.*, 2012. Response of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) populations and domoic acid to environmental conditions in a eutrophied, SW Mediterranean coastal lagoon (Tunisia). *Estuarine Coastal and Shelf Science*, 102-103, 95-104.
- Simoni, C., Di Paolo, L., Lepri, G., Lepri, L., Mancino, A. *et al.*, 2004. Further investigation on blooms of *Ostreopsis ovata*, *Coolia monotis*, *Prorocentrum lima* on the macroalgae of artificial and natural reefs in the Northern Tyrrhenian Sea. *Harmful Algae News*, 26, 5-7.
- Sournia, A., 1986. *Atlas du Phytoplancton Marin. Vol. I: Introduction, Cyanophycées, Dictyochophycées, Dinophycées et Raphidophycées*. CNRS (Eds), Paris.
- Spatharis, S., Dolapsakis, N.P., Economou-Amilli, A., Tsirtsis, G., Danielidis, D.B., 2009. Dynamics of potentially harmful microalgae in a confined Mediterranean Gulf— Assessing the risk of bloom formation. *Harmful Algae*, 8 (5), 736-743.
- Steidinger, K.A., Tangen, K., 1996. *Dinoflagellates*. p. 387-584. In: *Identifying marine diatoms and dinoflagellates*. Tomas, C.R. (Ed). Academic Press, San Diego.
- Suganuma, M., Fujuki, H., Suguri, H., Yoshizawa, S., Hirota, M. *et al.*, 1988. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. *Proceedings of the National Academy of Sciences of the United States of America*, 85, 1768-1771.
- Tango, P. J., Magnien, R., Butler, W., Lockett, C., Luckenba, M. *et al.*, 2005. Impacts and potential effects due to *Prorocentrum minimum* blooms in Chesapeake Bay. *Harmful Algae*, 4, 525-531.
- ter Braak, C.J.F., Looman, C.W.M., 1994. Biplots in reduced-rank regression. *Biometrical Journal*, 36, 983-1003.
- ter Braak, C.J.F., Šmilauer, P., 2002. *Canoco reference manual and CanoDraw for Windows user's guide: software for canonical community ordination* (version 4.5). Microcomputer Power, Ithaca, New York, USA.
- Tomas, R.C., 1996. *Identifying Marine Diatoms and Dinoflagellates*. Academic Press. Sandiego. 263 pp.
- Turki, S., 2004. Suivi des microalgues planctoniques toxiques dans les zones de production, d'élevage des mollusques bivalves et d'exploitation des oursins du Nord de la Tunisie. *Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô*, 31, 83-96.
- Turki, S., El Abed, A., 2001. On the presence of potentially toxic algae in the lagoons of Tunisia. *Harmful Algae News*, 22. pp. 12.
- Utermöhl, H., 1958. Zur vervollkommnung der quantitativen Phytoplankton Methodik. *Mitteilungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 9, 1-38.
- Velikova, V., Larsen J., 1999. The *Prorocentrum cordatum*/*Prorocentrum minimum* taxonomic problem. *Grana*, 38, 108-112.
- Vila, M., Camp, J., Garcés, E., Maso, M., Delgado, M., 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. *Journal of Plankton Research*, 23 (5), 497-514.
- Vila, M., Giacobbe, M.G., Maso, M., Gangemi, E., Penna, A. *et al.*, 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. *Harmful Algae*, 4, 673-695.
- Villac, M.C., Melo, S., Menezes, M., Rivera, D., 2005. *Pseudo-nitzschia brasiliensis* (Bacillariophyceae), an opportunistic diatom on the coast of the state of Rio de Janeiro, Brazil. *Atlântica Rio Grande*, 27, 139-145.
- Wood, E.D., Armstrong, F.A.J., Richards, F.A., 1967. Determination of nitrate in sea water by cadmium-copper reduction to nitrite. *Marine Biological Association of the UK*, 47, 23-31.
- Xu, H., Min, G.S., Choi, J.K., Zhu, M., Jiang, Y. *et al.*, 2010. Temporal population dynamics of dinoflagellate *Prorocentrum minimum* in a semi-enclosed mariculture pond and its relationship to environmental factors and protozoan grazers. *Chinese Journal of Oceanology and Limnology*, 28 (1), 75-81.
- Zingone, A., Siano, R., D'Alelio, D., Sarno, D., 2006. Potentially toxic and harmful microalgae from coastal waters of the Campania region (Tyrrhenian Sea, Mediterranean Sea). *Harmful Algae*, 5, 321-337.