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

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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 x 10^5 and 2 x 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 diarrheic 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-

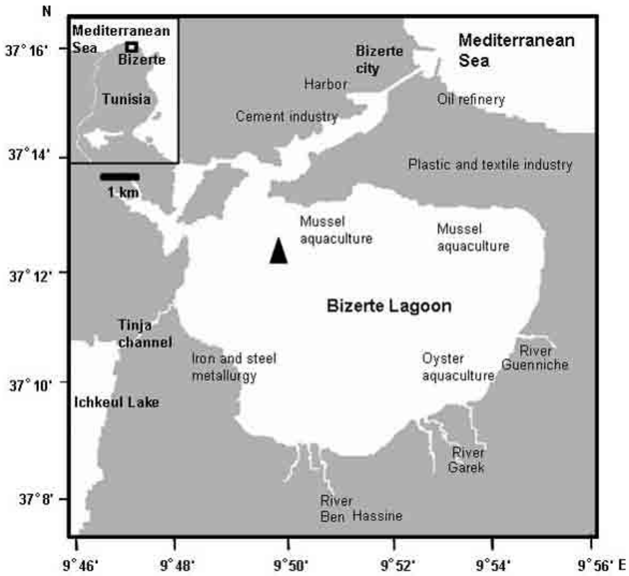

Fig. 1: Map of Bizerte Lagoon, Tunisia, showing the sampling station (▲).

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 km^2, 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); Si $(OH)_4$ (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 Si(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(Ay + 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-

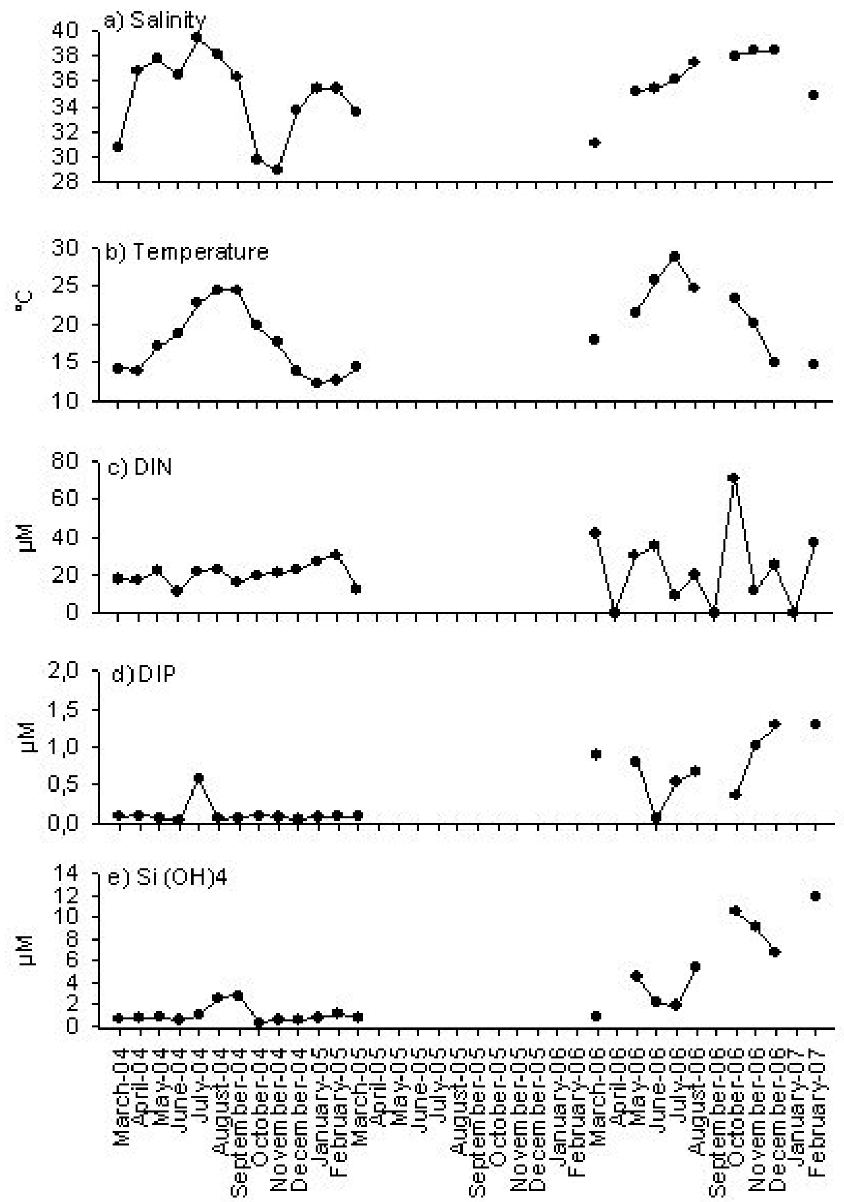

Fig. 2: Monthly profiles of the physicochemical variables at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007): (a) temperature; (b) salinity; (c) dissolved inorganic nitrogen; (d) dissolved inorganic phosphorus; (e) $Si(OH)_4$.

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).

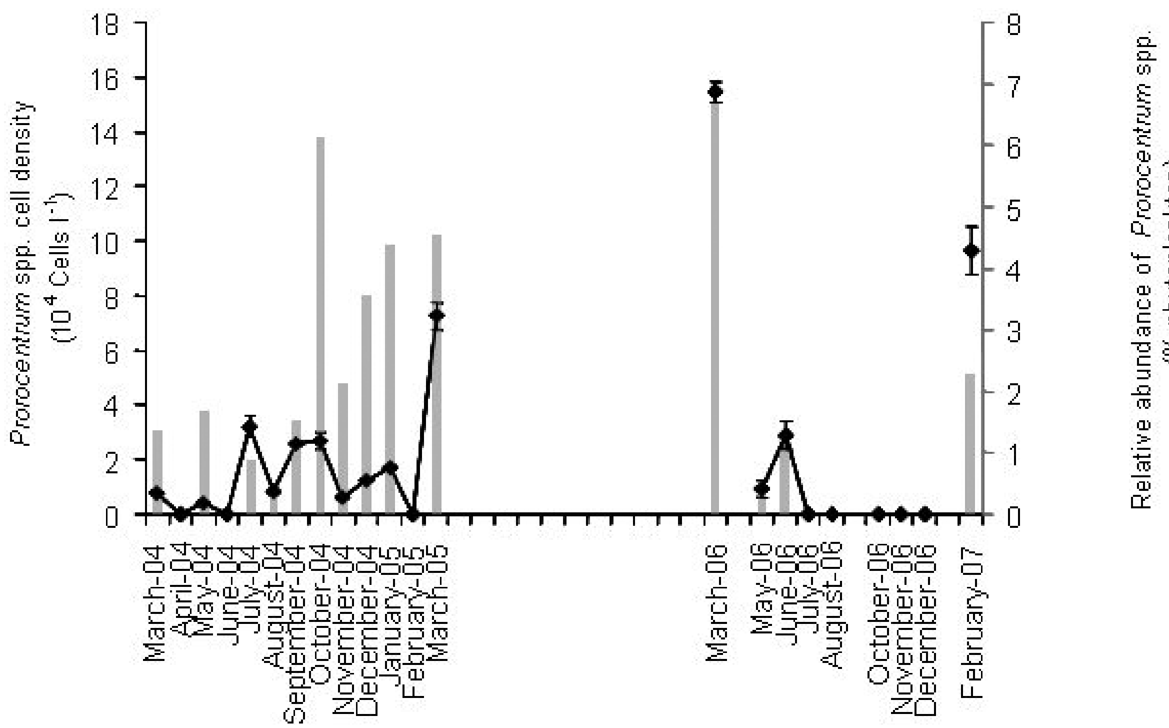

Fig. 3: Monthly variation in total (Line and scatter plot, Mean ± SD, n=3) and relative abundance (grey bars) of *Prorocentrum* spp. at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007).

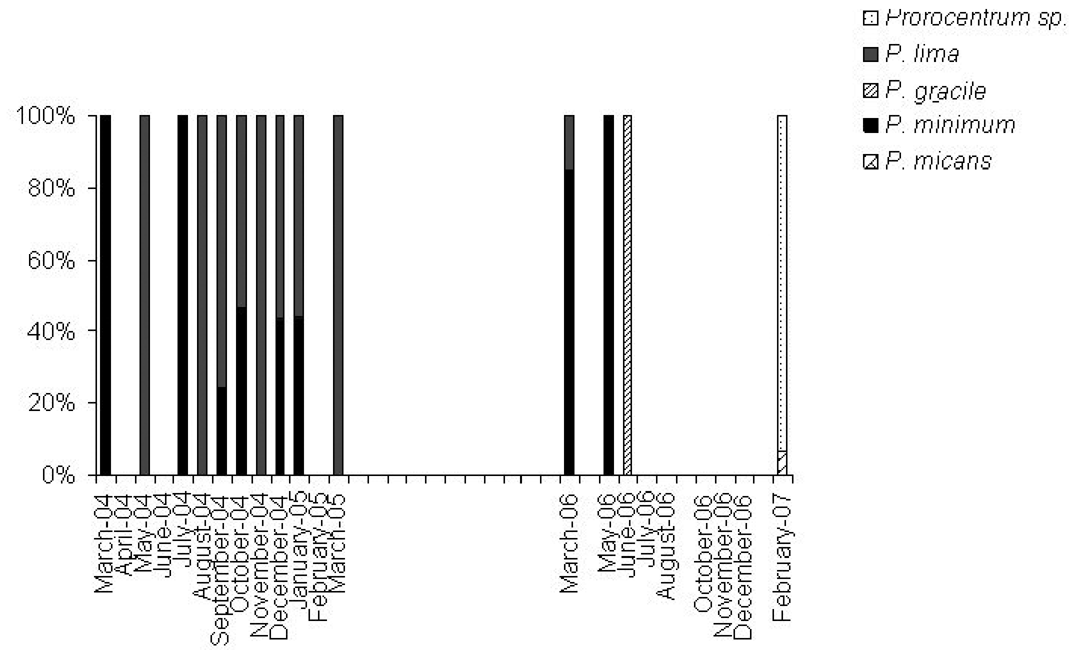

Fig. 4: Monthly variation in the composition of the genus *Prorocentrum* at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007).

Prorocentrum species and environmental interactions

CCA was used to relate the distribution of *Prorocentrum* 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-

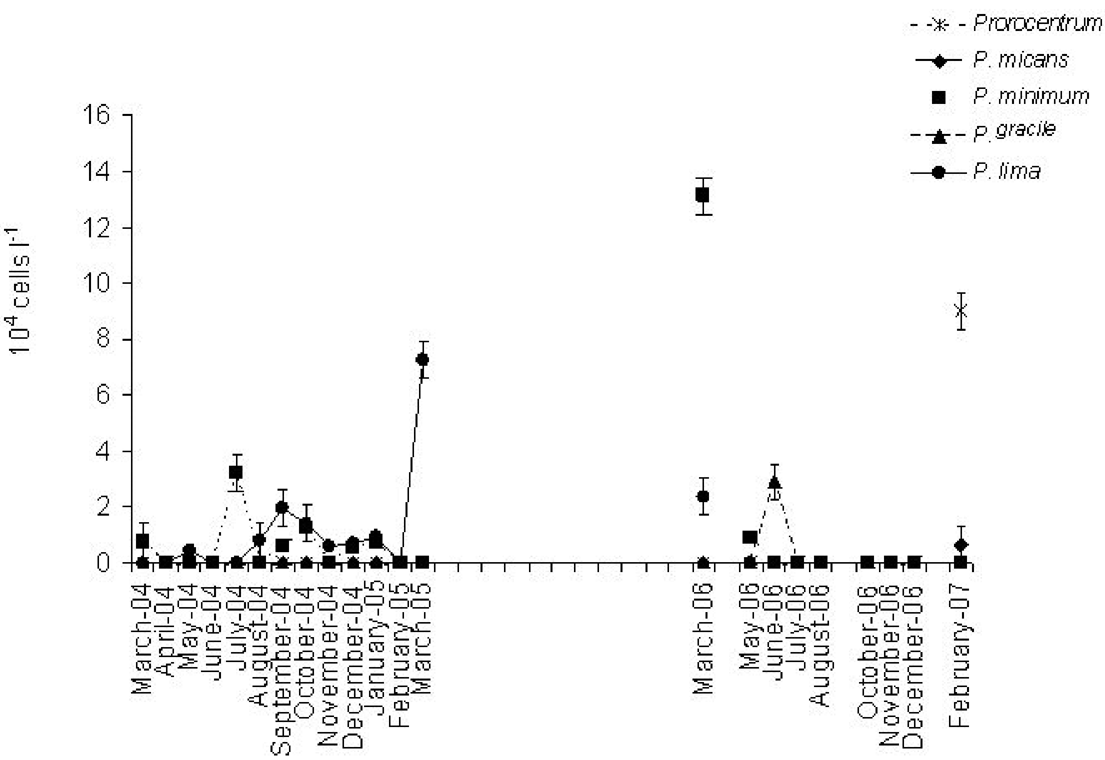

Fig. 5: Fluctuations of *Prorocentrum* species abundances at the sampling station, Bizerte Lagoon (March 2004 - March 2005/ March 2006 - February 2007) (Mean ± SD, n=3).

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 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).

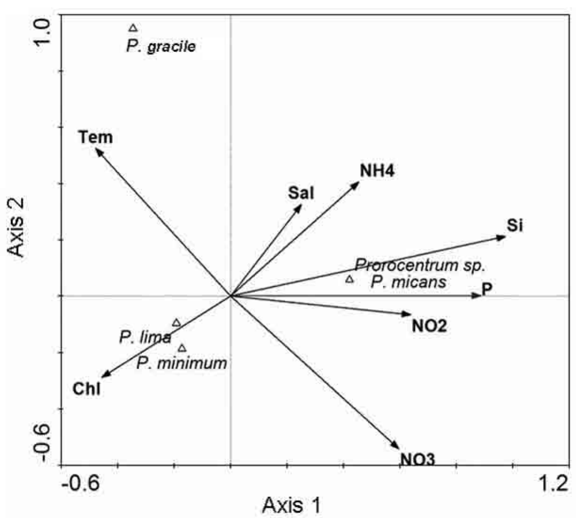

Fig. 6: Canonical correspondence analysis (CCA) ordination diagram showing the relationships between environmental variables (abbreviations in the Material and Methods section) and *Prorocentrum* species distribution.

Table 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

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).

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 - March2005/ March 2006- February 2007). "+", "-" and "0" indicate that environmental variables have significant positive, negative or no effect on *Prorocentrum* species distribution, respectively.

Variable	*Prorocentrum lima*	*Prorocentrum minimum*	*Prorocentrum sigmoides*	*Prorocentrum micans*
NO3	0	-	0	+
NO2	0	0	-	+
NH4	-	-	0	+
Si	0	-	-	0
P	-	0	0	-
Temp	0	0	+	0
Sal	-	-	0	+
Chl	+	+	-	0

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 (> 10^4 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: > 10^3 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, maximum cell densities found in this study (> 10^5 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* (> 10^8 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 (10^3-10^4 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 (> 10^4 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.

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