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Composition and dynamics of potentially toxic dinoflagellates in a shallow Mediterranean lagoon

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Abstract

The population dynamics of harmful microalgae was investigated in the semi-enclosed shallow lagoon, Tunis northern lagoon (South Mediterranean), during a 1-year period from January to December 2007. A total number of 16 potentially toxic dinoflagellates were identified. Density of each species was analyzed in time and space and in relation to environmental parameters. Some species such as *Alexandrium catenella*, *A. pseudogonyaulax*, *A. tamarense*, *Dinophysis sacculus*, *Prorocentrum lima*, *P. minimum* and *Gonyaulax spinifera* developed high cell concentrations, particularly during spring and summer seasons. Other species such as *A. insuetum*, *A. lee*, *A. minutum*, *Coolia*

monotis, *D. acuminata*, *D. caudata*, *D. fortii*, *Ostreopsis siamensis* and *P. concavum* were detected in this coastal ecosystem, but they were rare or appeared in very low densities.

Densities of the most abundant species were closely associated with spring and summer conditions, being positively correlated with water temperature and salinity. These species performed well in this lagoon, but the specific species-dependent affinity to each of the environment factors determined which organisms proliferated. The spatial distribution of the abundant species exhibited a marked increase towards the western and southern parts of the lagoon represented by sites C, D and E. Examination of the temporal distribution revealed that the species more prone to bloom are those with extensive spatial distribution and frequent presence throughout the year such as *D. sacculus*.

Statistical analysis of *D. sacculus* density dynamics showed a significant difference between summer and winter assemblages. It presented a positive correlation with water temperature and salinity, but it seems to be tolerant to all levels of nutrients.

INTRODUCTION

Coastal ecosystems are ever more becoming susceptible to harmful phytoplankton increases especially in enclosed coastal embayments (Anderson et al. 2002, Hallegraeff 1993). In most cases, these increases are related to anthropogenic activities. This includes nutrient enrichment (Anderson et al. 2002), and enlargement of confined areas is generally associated with construction of jetties, leisure harbors, etc. (Garcés et al. 2000). The increase of harmful phytoplankton events applies to the Mediterranean Sea, where they are mainly associated with locations characterized by a restricted water exchange, such as harbors (Vila et al. 2001), small bays and protected beaches (Garcés et al. 1999), and coastal lagoons (Sarno et al. 1993, Cabrini et al. 1995, Giacobbe et al. 2000).

Especially interesting are the Mediterranean semi-enclosed lagoons, which remain isolated for most of the year and concentrate phytoplankton to a very high biomass.

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Generally, these coastal lagoons have negligible tidal inputs (Britton and Crivelli 1993), but they are occasionally flooded during sea storms, after which they usually recover their confined character without marine exchange (Picot et al. 1990, Quintana et al. 1998, Pérez-Ruzafa et al. 2002). During periods of confinement, water levels tend to decrease, subsequently enhancing the salinity, nutrient levels and phytoplankton concentration.

Mediterranean coastal lagoons may, therefore, act as an accumulator of neritic phytoplankton. Moreover, connections of these lagoons with coastal waters can introduce phytoplankton, which can include harmful species, where they could grow and become more concentrated. However, high variation of environmental characteristics associated with the lagoons' confinement process may lead to significant modifications of the phytoplankton composition (López-Flores 2005). Stability and nutrients' levels are known as determinant factors of the phytoplankton succession in temperate coastal waters (Margalef 1983, Reynolds et al. 2002, Smayda and Reynolds 2003). Nevertheless, little is known about the factors influencing the phytoplankton temporal patterns in confined coastal lagoons (Quintana and Moreno-Amich 2002, López-Flores 2005).

Tunis northern lagoon, located in the neighborhood of the city of Tunis, was undoubtedly one of the most polluted and eutrophic Mediterranean lagoons since it received great amount of waste waters (Zaouali 1977). This eutrophication had led to progressive degradation and ecological disturbances marked by major changes in sediments and lagoon fauna and flora decreasing the species diversity. To address this problem, a project improving the water circulation was elaborated and applied in 1985 (Ben Charrada 1992). The project aimed at improving the ecological factors in particular hydrological conditions. The whole lagoon's water residence time was reduced to about 17–20 days. Furthermore, all wastewater dumping into the lagoon had been stopped, so only urban stormwater runoff is permitted to prevent floods. Following the completion of the restoration in 1988, the decrease in nutrient concentrations and the development of vegetation communities were the most important changes recorded in this ecosystem (Ben Charrada 1992, Ben Maiz 1993).

Researches carried out on harmful phytoplankton communities in this lagoon are few and very recent (Turki 2004, Turki & Balti 2005,

Armi et al. 2009, 2010a) showing the dominance of diatoms and dinoflagellates.

In the present study, the taxonomic structure of phytoplankton populations was evaluated in this lagoon in 2007. The main objectives were to determine composition and dynamics of potentially toxic dinoflagellates in this coastal area and to establish the common water physicochemical variables that favor proliferations of specific harmful species in this ecosystem.

MATERIALS AND METHODS

The study site

Tunis northern lagoon is a shallow water plane located in the North-East of Tunisia (36°45'–36°52'N and 10°10'–10°20'E) at the bottom of the Gulf of Tunis, in the proximity of both Rades and la Goulette harbors and Tunis southern lagoon.

After the establishment of a natural renewal system adapted to the lagoon conditions in 1984–1988 (Ben Charrada 1992), the aspect of Tunis northern lagoon has radically changed in comparison with its status during the last centuries. The surface, previously estimated at 2,800 hectares (Zaouali 1977) was reduced to 2,600 hectares after restoration works (Arjen & Oostinga 1992). Consequently, new hydrological conditions have been created; the depth at the northern part (dredged) varies from 2 to 4 m and reaches 1 m in the southern part. Moreover, physicochemical parameters have been monitored in Tunis northern lagoon in many studies carried out before and after the management. Nowadays, after the construction of the bridge on the navigation channel of Tunis close to the lagoon, the surface was further reduced to 2,400 hectares. In rainy periods of autumn and winter, freshwater runoff can reach the lagoon from point-source origins (Ben Charrada 1995).

Average salinity ranged between 30 and 40 PSU. Maximum water temperature reaches 30°C in summer. The substratum is mainly covered by the seagrass *Ruppia cirrhosa* (Petagna) Grande in the northern part and the green alga *Chaetomorpha linum* (O.F. Müller) Kützinger in the southern part, with other seasonal and ephemeral species sporadically distributed on different sediment types (Trabelsi et al. 2001).

Sampling

Between January and December 2007, five main sites along this area were selected to collect water samples bi-weekly. The sampling sites (Fig. 1) were located using a Garmin GPS 45. This lagoon was virtually divided into five sections and our sites were located at the central point of each section; site A (mean depth 2.3 m) is characterized by water exchange from the Bay of Tunis; site B (2 m) corresponds to the northern part of the lagoon; site C (2.5 m) is the western part of the lagoon; site D (1 m) represents the southern and the most shallow section of this lagoon and site E (2.5 m) is located opposite site A.

Abiotic parameters measurements

Environmental variables were measured in the field using a WTW multiparameter (Multi 340i) for

water temperature ($^{\circ}\text{C}$) and salinity (PSU), pH and dissolved oxygen concentration (mg l^{-1}). For nutrients analysis, water samples were collected at 30–50 cm under the water surface, in polypropylene bottles of 1 l. Samples were analyzed according to Parsons et al. (1984).

Phytoplankton study

Samples for phytoplankton analysis were collected from the whole water column using a PVC tube sampler of variable length provided with a closing valve (Lindahl 1986).

Aliquots were preserved with acid Lugol solution (1% final concentration), and analyzed using an inverted microscope following Utermöhl's technique (Utermöhl 1958). Cells were expressed as cells l^{-1} (Thronsen 1995).

Dinoflagellates species were generally identified with a BH2 Olympus microscope with a

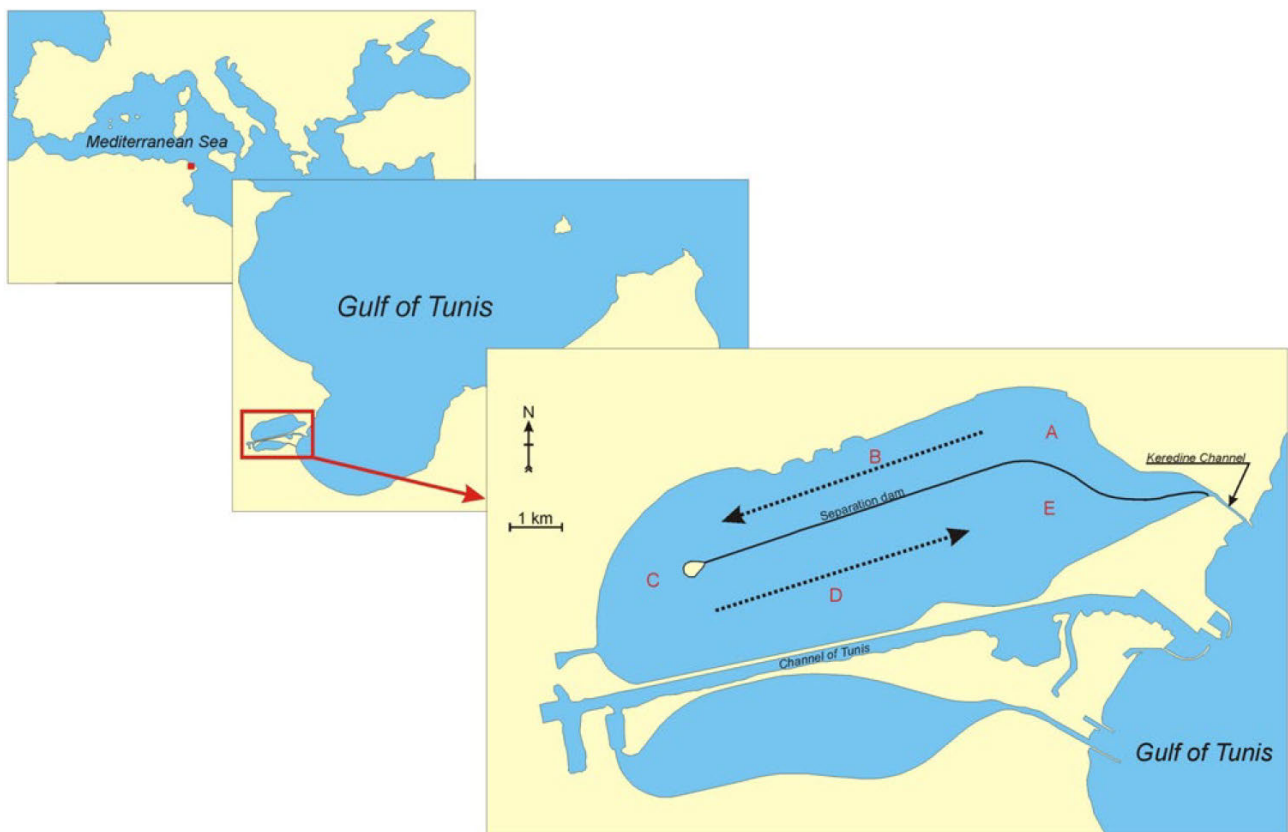


Fig. 1. Location of the sampling sites in Tunis northern lagoon.

magnification of 1000×. Identification was performed according to the descriptions established by Balech (1988), Sournia (1995) and Steidinger and Tangen (1997). *Alexandrium* species were identified according to the work of Balech (1995).

Statistical analysis

Clustering and MDS analysis

The majority of data analysis followed standard community analysis methods described by Clarke (1993) and Clarke and Warwick (2001), using the Plymouth Routines in the Multivariate Ecological Research (PRIMER) software package (PRIMER 5).

Hierarchical cluster analysis was used to establish similarity between dinoflagellate species densities dynamics. Data used in this case consisted of abundance obtained for each species from all sampling sites during the whole study period. Data were log transformed [$\log(x+1)$], the Bray-Curtis index was used as a similarity measure and the group average was selected for the cluster method.

Hierarchical cluster analysis was also used in addition to multivariate data analysis, by non-parametric multi-dimensional scaling (MDS) ordination with the Bary-Curtis similarity measure (performed on square-root transformed *Dinophysis sacculus* species abundance data), in order to establish similarities between samples and months (Table 1).

Table 1

Symbols used in cluster and MDS analyses.

month	code
January	Ja
February	Fe
March	Mr
April	Ap
Mai	Ma
June	Jn
July	Jl
August	Au
September	Sp
October	Oc
November	Nv
December	Dc

Correlation analysis

Possible relationships between potentially toxic dinoflagellates species and environmental parameters were tested by Pearson's correlation analysis,

performed at $p < 0.05$ and $p < 0.001$, using data from all the sampling sites and for the whole period. Data were also log transformed for assessing the homogeneity of variances and assumption. This analysis was performed using the software Statistica (*Statistica 6*, StatSoft).

RESULTS

Temporal variation of environmental parameters

Temporal variation of various environmental parameters measured in Tunis northern lagoon is presented in Figure 2. Lowest values of temperature were detected during the winter months, especially in December (7.3°C), the highest value, was 29.1°C unregistered in August at site E. The annual average value in the lagoon was about 19.1°C.

The extreme values of salinity were recorded at sites D and E (33.6°C and 46.8 PSU respectively). Annual averages values of pH and dissolved oxygen were about 8.4 and 7.7 mg l⁻¹ respectively.

Dissolved Inorganic Phosphorus (DIP) concentrations increased during spring months and picked up in March with a maximum concentration of 0.25 µM at site B. The lowest concentration was measured in summer (June 2007) at both sites B and C with a concentration of 0.011 µM.

At the beginning of the rainfall period (September 2007), Dissolved Inorganic Nitrogen (DIN) concentrations were low, but increased during winter and peaked up in October with a maximum concentration of 45 µM at site B. This increase in DIN nutrients concentrations, associated with an increase in the input flow rate, resulted in a maximum nutrient load in the receiving water body during autumn 2007.

The low DIN concentrations (2 µM) were measured in the lagoon in March 2007 at site D.

Potentially toxic dinoflagellates species dynamics

The taxonomic structure of phytoplankton populations in Tunis northern lagoon during this year-long study showed a composition of 58% of Bacillariophyceae (diatoms) mainly represented by *Chaetoceros* and *Navicula* species, 41% of Dinophyceae (dinoflagellates) essentially represented by peridinales and gymnodinales species and 1% of Cyanophyceae, Euglenophyceae, Dictyophyceae, Chlorophyceae and Cryptophyceae.

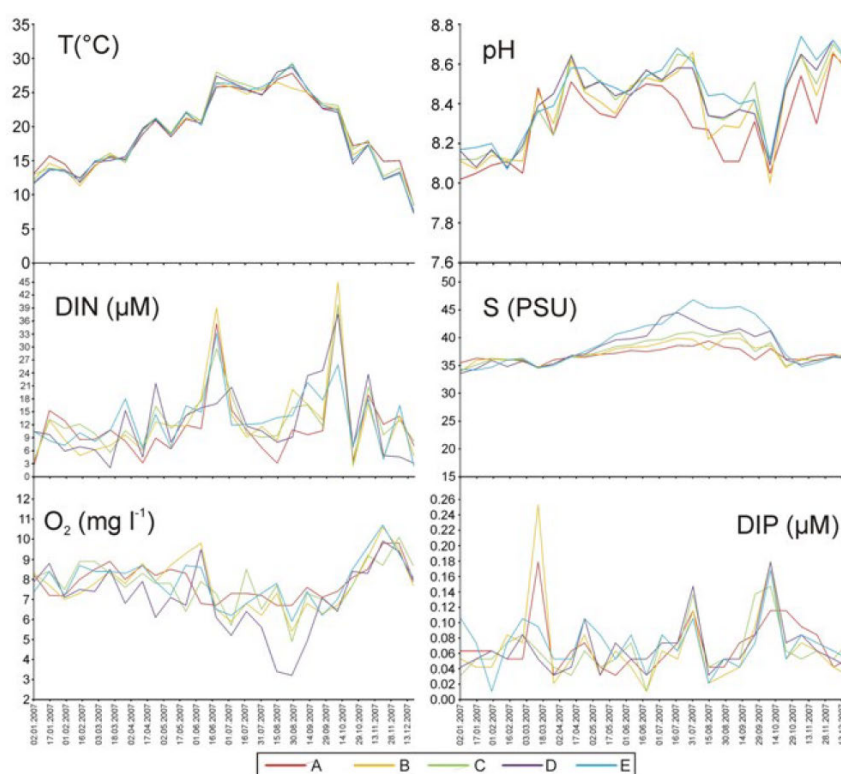


Fig. 2. Temporal variation of environmental parameters within each sampling site in Tunis northern lagoon from January to December 2007.

Most dinoflagellate species detected in the present study have been described in the literature as harmful and/or toxic. Among these species, 16 are potentially toxic (Table 2) out of the total number of 48 dinoflagellates identified in this aquatic ecosystem during the year-long study from January to December 2007.

A pattern of increased abundance during summer was observed for most species (Fig. 3), this being more pronounced in July and August months for all sampling sites.

During this year-long study, the genus *Alexandrium* was represented by 6 different species; *Alexandrium catenella*, *A. insuetum*, *A. lee*, *A. minutum*, *A. pseudogonyaulax* and *A. tamarense*.

In particular, *A. insuetum*, *A. lee* and *A. minutum* were almost totally absent in the lagoon throughout the year, except for late spring and early summer when they were sporadically detected in very small numbers (the maximum detected concentration was about 180 cells l⁻¹ for *A. insuetum* at site E in July 2007).

Table 2

The list of potentially toxic dinoflagellates identified in Tunis north lagoon from January to December 2007, their maximum abundance in cells l⁻¹, and maximum abundance dates.

Species	Impact (Toxins)	Max. abundance (cells l ⁻¹)	Date / Station
<i>Alexandrium catenella</i> Balech	PSP	1.3 × 10 ³	04/12/2007/C
<i>Alexandrium insuetum</i> Balech	PSP	180	17/07/2007/E
<i>Alexandrium lee</i> Balech	PSP	10	22/05/2007/D
<i>Alexandrium minutum</i> Halim	PSP	60	20/11/2007/B
<i>Alexandrium pseudogonyaulax</i> Balech	PSP	2.2 × 10 ³	19/06/2007/E
<i>Alexandrium tamarense</i> Balech	PSP	7 × 10 ³	28/08/2007/C
<i>Coolia monotis</i> Meunier	Cooliatoxin	620	24/04/2007/D
<i>Dinophysis acuminata</i> Claparède & Lachmann	DSP	80	27/03/2007/C
<i>Dinophysis caudata</i> Saville-Kent	DSP	30	11/09/2007/B
<i>Dinophysis fortii</i> Pavillard	DSP	40	05/06/2007/D
<i>Dinophysis sacculus</i> Stein	DSP	4.6 × 10 ⁴	16/01/2007/B
<i>Gonyaulax spinifera</i> Diesing	YTX	10 ³	30/01/2007/A
<i>Ostreopsis siamensis</i> Schmidt	YTX, PLTX	120	27/03/2007/B
<i>Prorocentrum concavum</i> Fukuyo	DSP	40	17/07/2007/B
<i>Prorocentrum lima</i> (Ehrenberg) Dodge	DSP	1.4 × 10 ³	28/08/2007/D
<i>Prorocentrum minimum</i> (Pavillard) Schiller	Neurotoxin	11 × 10 ³	14/08/2007/E

DSP - Diarrhetic shellfish poisoning; PSP - Paralytic shellfish poisoning; PLTX - Palytoxin; YTX - Yessotoxin

A. pseudogonyaulax was more commonly present in the water column particularly in June 2007, when it peaked up at site E with 2.3×10^3 cells l^{-1} .

A. catenella species was present at all sites almost throughout the year, with the maximum abundance only during winter. This cell concentration was about 1.2×10^3 cells l^{-1} in December 2007.

The species *A. tamarensis* was also detected at all sampling sites with low densities, except for late August 2007 when it peaked up abruptly with 7×10^3 cells l^{-1} at site C.

Gonyaulax spinifera species was recorded almost throughout the year and in all parts of the lagoon. It reached the highest densities in the winter season with the maximum of 10^3 cells l^{-1} in January at site A.

Prorocentrum minimum was also recorded roughly throughout the year showing a different trend, especially in the summer season where it reached its maximum concentrations. The highest density was detected at site E with more than 10^4 cells l^{-1} in August 2007.

The species *Coolia monotis* was detected especially during the spring season, and reached its maximum concentration in late April at site D (620 cells l^{-1}). On the other hand, *Ostreopsis siamensis*, was detected in very low density throughout the study period with the maximum of only 120 cells l^{-1} in March at site B.

The dynamics of potentially diarrhetic toxin producers, *Prorocentrum* and *Dinophysis* genera, were very diverse in this area. *Prorocentrum conavum*, *Prorocentrum lima*, *Dinophysis acuminata*, *Dinophysis fortii* and *Dinophysis caudata* were rare and recorded only sporadically with low cell densities. The majority of these species had their high abundances during spring and summer seasons, however the maximum density did not exceed 120 cells l^{-1} , except for *P. lima*, which suddenly proliferated with 1.4×10^3 cells l^{-1} in August 2007 at site D (Table 2, Fig. 3).

Dinophysis sacculus Stein 1883 was the most abundant species, which was present in all collected samples, with a peak during winter (4.6×10^4 cells l^{-1} at site B, January 2007).

This species was present in high densities for a long period in the summer season; from June to August 2007, especially at sites A, B and D (Fig. 3).

In the hierarchical cluster analysis (Fig. 4), the potentially toxic species, according to their density dynamics, are divided into several groups. At 60%, only two small groups are distinguished. The species *D. sacculus* and *G. spinifera*, which are the most abundant species, are grouped together (group A); the second group (group B) contains the two species

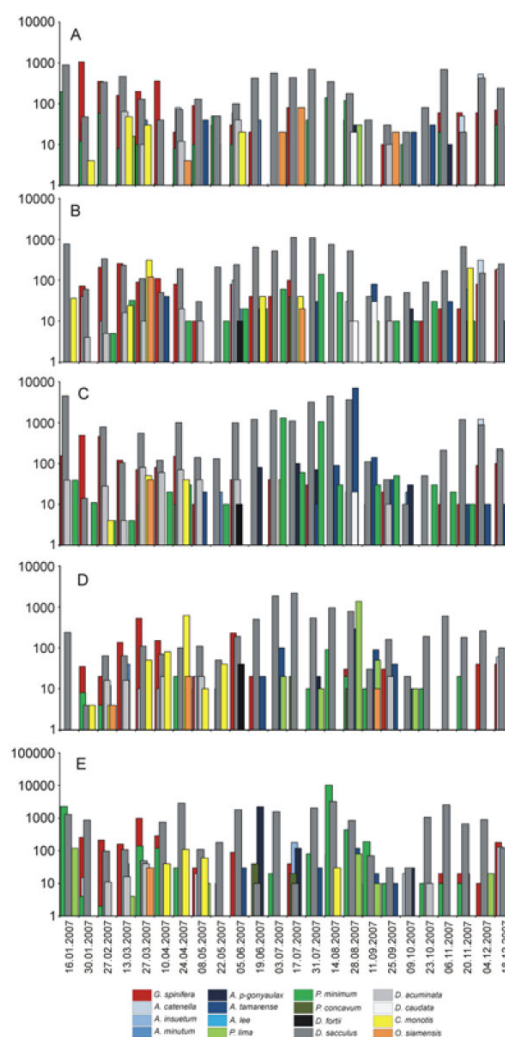


Fig. 3. Temporal variation of cell densities of the potentially toxic dinoflagellate species within each sampling site, exceeding 100 cells l^{-1} , in Tunis northern lagoon from January to December 2007.

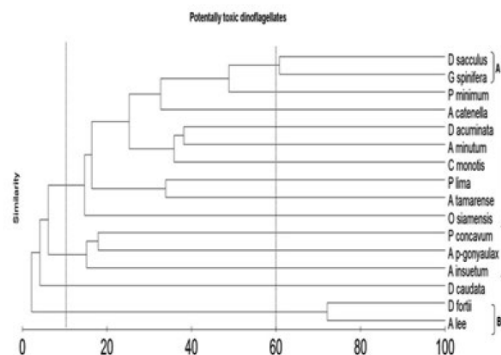


Fig. 4. Cluster analysis of potentially toxic dinoflagellate species. Bary-Curtis similarity and group average were used.

D. fortii and *A. lee*. These two species are nearly absent during the study period. Whereas, all other species are separated in the cluster.

At 10%, the subgroup (A) with the species *P. minimum*, *A. catenella*, *D. acuminata*, *A. minutum*, *C. monotis*, *P. lima*, *A. tamarensis* and *O. siamensis* defined the group (I), whereas the group (II) includes only the species *P. concavum*, *A. pseudogonyaulax* and *A. insuetum* (Fig. 4). These results show that the species composition of the potentially toxic dinoflagellates detected in the present study, and their corresponding densities appeared indeed highly variable and they could not be classified in a single category. There is a high significant difference between species concentrations during this period.

Spatial distribution & relationship with environmental parameters

Correlation analysis between the studied species abundance and the environmental parameters revealed that the increase in the abundance of most potentially toxic species is associated with specific environmental conditions (Table 3).

An exception is observed for *A. catenella*, which was positively correlated with nitrogen nutrients, whereas all other studied species had no significant correlations with nutrients.

An increase in the abundance of *A. catenella* was associated with a rise in pH (8.7) ($r = 0.22$; $p < 0.05$), dissolved oxygen (10.1 mg l^{-1}) ($r = 0.21$; $p < 0.05$) and nitrogen nutrients (NO_2^- and NO_3^-) concentrations ($r = 0.32$; $p < 0.001$ and $r = 0.40$; $p < 0.001$

respectively) and a drop in water temperature ($r = -0.27$; $p < 0.05$).

High *A. tamarensis* cell concentration was positively correlated with temperature ($r = 0.21$; $p < 0.05$) and salinity ($r = 0.26$; $p < 0.05$) and low levels of NO_3^- ($r = -0.20$; $p < 0.05$).

A. insuetum was weakly positively correlated with salinity ($r = 0.22$; $p < 0.05$), whereas other *Alexandrium* species (*A. lee*, *A. minutum* and *A. pseudogonyaulax*), as

well as *P. lima*, *D. fortii*, *C. monotis* and *O. siamensis*, had no significant correlations with the environmental factors.

The abundance of the species *G. spinifera* was correlated with a decrease in water temperature ($r = -0.31$; $p < 0.001$) and salinity ($r = -0.29$; $p < 0.05$) and had a low negative correlation with the variable pH ($r = -0.18$; $p < 0.05$).

Both *P. minimum* and *D. acuminata* had a significant correlation with salinity, which was positive ($r = 0.24$; $p < 0.05$) and negative ($r = -0.23$; $p < 0.05$), respectively.

P. concavum was positively correlated with both water temperature ($r = 0.21$; $p < 0.05$) and salinity ($r = 0.27$; $p < 0.05$), whereas the species *D. caudata* was only correlated with the temperature parameter ($r = 0.21$; $p < 0.05$).

The most abundant species, *D. sacculus*, had a significant correlation with temperature ($r = 0.36$; $p < 0.001$) and salinity ($r = 0.45$; $p < 0.001$) and a negative correlation with dissolved oxygen ($r = -0.22$; $p < 0.05$).

The case study of *Dinophysis sacculus*

As evidenced previously, *Dinophysis sacculus* was the most abundant species, in this study period. It was detected at all the sampling sites. Based on these results, we proposed to study the case of this species in the following part of this work.

A general pattern can be deduced from the clustering of the samples data (Figs 5, 6); seasonal groups of samples showed that spring, winter and autumn are clustered together (group I) and samples of the summer season are clustered together (group II). The linkage distance is, on average, indicating low dissimilarity between the two groups (at 75%).

Moreover, at the similarity of 85%, the cluster analysis indicates the separation of smaller groups of samples: the first sub-group includes samples of spring, winter and late autumn (group IA); the second one consists of samples of early autumn

Table 3

Pearson's correlation coefficient for the potentially toxic dinoflagellate species and environmental parameters in Tunis north lagoon from January to December 2007 (N = 120).

	T	S	pH	O ₂	NH ₄	NO ₂	NO ₃	PO ₄
<i>G. spinifera</i>	-0.31**	-0.29*	-0.18*	-	-	-	-	-
<i>A. catenella</i>	-0.27*	-	0.22*	0.21*	-	0.32**	0.40**	-
<i>A. insuetum</i>	-	0.22*	-	-	-	-	-	-
<i>A. lee</i>	-	-	-	-	-	-	-	-
<i>A. minutum</i>	-	-	-	0.21*	-	-	-	-
<i>A. p-gonyaulax</i>	-	-	-	-	-	-	-	-
<i>A. tamarensis</i>	0.21*	0.26*	-	-0.21*	-	-	-0.20	-
<i>P. lima</i>	-	-	-	-	-	-	-	-
<i>P. minimum</i>	-	0.24*	-	-	-	-	-	-
<i>P. concavum</i>	0.21*	0.27*	-	-	-	-	-	-
<i>D. sacculus</i>	0.36**	0.45**	-	-0.22*	-	-	-	-
<i>D. acuminata</i>	-	-0.23*	-	-	-	-	-	-
<i>D. fortii</i>	-	-	-	-	-	-	-	-
<i>D. caudata</i>	0.21*	-	-	-	-	-	-	-
<i>C. monotis</i>	-	-	-	-	-	-	-	-
<i>O. siamensis</i>	-	-	-	-	-	-	-	-

(-) no significance; (*) $p < 0.05$; (**) $p < 0.001$

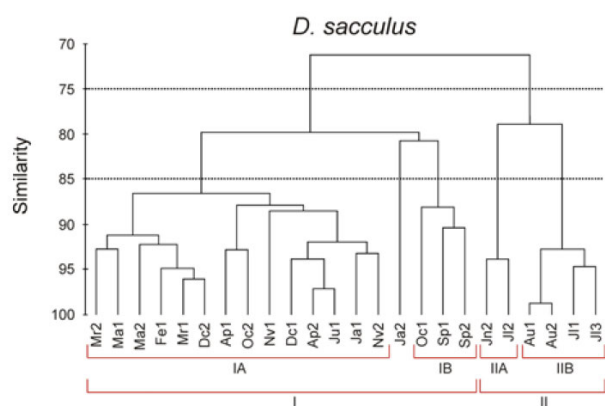


Fig. 5. Cluster analysis of *Dinophysis sacculus* density; classification of the total sampling and seasonal data. Bary-Curtis similarity and group average were used.

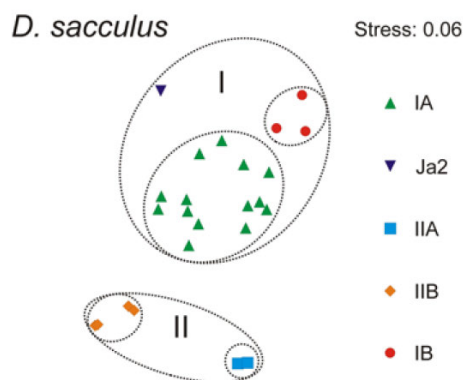


Fig. 6. Non-metric MDS ordination of square-root transformed *Dinophysis sacculus* abundance data from Tunis northern lagoon, from January to December 2007.

(group IB). The two sub-groups constitute the group I.

The samples collected during early summer, except for one sample from early June (Jn₁), are clustered together in the third sub-group (group IIA). On the other hand, the fourth sub-group (group IIB) clustered samples of late summer. The two last sub-groups form the group II.

DISCUSSION

It has been commonly recognized that collection of data on the characteristics, causes and dynamics of HABs, especially toxic species, contributes to the development of appropriate monitoring programs and preventative measures against the occurrence of such harmful events in

coastal ecosystems (Cembella et al. 2005, Ranston et al. 2006, Riegman 1991, Todd 1993, Work et al. 1993). A prerequisite for this is the detailed investigation of the spatial and temporal dynamics of the species involved across the extensive temporal scales along with the corresponding environmental conditions (ICES 2005).

The water of Tunis northern lagoon appears characterized by the high DIN/DIP ratio and greater potential for phosphorus limitation. Overall, N-NH₄⁺ was the most abundant nutrient throughout the lagoon. According to Harrison (1976), Nedwell et al. (2002) and Badylak & Philips (2004), these conditions may favor the growth and proliferation of dinoflagellates species. Collos et al. (2004) and Imai et al. (2006) showed also that deficiency of phosphorus in coastal areas can favor the development of some toxic dinoflagellates, such as the *Alexandrium* genus.

In the present study it was observed that the species *Alexandrium pseudogonyaulax*, *Alexandrium tamarense*, *Coolia monotis* and *Prorocentrum minimum* co-occurred and proliferated mainly during the late spring and summer season as a result of calm weather conditions, stagnant water and relative high water temperature and salinity.

It was evident that higher abundance of potentially harmful dinoflagellate species commonly occurred during spring-summer and early autumn periods, coinciding with an increase in salinity. In fact during this period, high barometric pressure conditions permitted the stability of the shallow water column and likely favored the formation of potentially harmful dinoflagellate assemblages.

The species *D. sacculus* had the highest density in winter at site B (4.6×10^4 cells l⁻¹ in January 2007) but it proliferated also for a long period during spring and summer seasons at all sampling sites. According to Delmas et al. (1992) and Godhe et al. (2002), it has been demonstrated that the dinoflagellate *Dinophysis* spp. was favored by low salinity.

Almost all studied species occurred with extremely low abundance or were completely absent in the main period, whereas at most of the sites the abundant species, *Dinophysis sacculus*, *Gonyaulax spinifera* and *Prorocentrum minimum*, were present in rather high concentrations compared with all other species. The occurrence of these three species throughout the year may be explained by their tolerance to the lagoon hydrological and environmental conditions, whereas *Dinophysis sacculus* and *Prorocentrum minimum* favor summer conditions.

According to Jacobson and Andersen (1994) *D. sacculus* is considered as a mixotrophic species, partly independent of the ambient nutrient conditions.

Benthic potentially toxic dinoflagellates *Coolia monotis*, *Ostreopsis siamensis* and *Prorocentrum lima* were infrequently observed in the water column, and their presence may be attributed to the shallowness of the lagoon and the continuous sediment re-suspension by the blowing winds, because they were often detected at site D, the most shallow section in this lagoon. This result is confirmed by the work of Armi et al. (2010a) describing *Coolia monotis* dynamics in this lagoon during spring 2006.

The environmental conditions enhancing the growth and proliferation of the most abundant species in the lagoon are in accordance with those observed in the previous studies (Annabi-Trabelsi et al. 2003, Armi et al. 2008, Armi et al. 2010b).

The majority of the population sizes of potentially toxic species in Tunis northern lagoon are significantly correlated with physical and weather conditions, such as salinity and temperature, but show no significant correlation with nutrient enrichments. The fact that this trend seems to be recurrent, indicates that environmental conditions in the lagoon favor the proliferation of dinoflagellate species and their suppressed blooms suggest a potential threat to the ecosystem, demonstrated also by the cover-abundance relationship (Dolapsakis et al. 2008).

Most of the potentially toxic species encountered in Tunis northern lagoon appear to have established populations. Some of the dinoflagellates investigated in the present study, such as *Alexandrium* species, *Coolia monotis*, *Ostreopsis siamensis*, *P. concavum* and *P. lima* are known cyst-forming species (Matsuoka and Fukuyo 2000). Dinoflagellate encystment and excystment are known to play a crucial role in the ecology and blooming potential of species (Peña-Manjarrez et al. 2005), and support the view that occurrence and proliferations may have resulted from cyst germination under favorable resource supply and environmental conditions (Anderson and Keafer 1985).

The assumption that the majority of the studied species have established populations in the lagoon is also supported by their continuous presence in the water column throughout the year. One possible explanation for this trend is that species developing dense populations are more likely to have established local populations (possibly widespread) in a given area, thus enhancing their ability to bloom under

favorable conditions. Furthermore, Tunis north lagoon is especially suitable for species establishment and blooming, because it is a semi-closed shallow area, affected by nutrient-rich freshwater inputs during rainfall periods and stagnant climatic conditions for a long time each year (Armi et al. 2008, 2010a), offering a diverse aquatic proliferation environment. The probability of species establishment is further supported by the fact that species' densities used in the current study were averaged not only in space but also in time. Therefore, it seems that species which tend to be present throughout the year (i.e. established species) are likely to proliferate and form blooms.

The cluster analysis of the data set, which consisted of 120 samples distributed throughout the lagoon area, resulted in high dissimilarity related to the studied species densities. In fact, according to the potentially toxic dinoflagellates densities, results of the hierarchical cluster analysis indicated the presence of a few similar groupings, which indicates a large difference and no significant similarity between individuals' concentrations during this year-long period.

D. sacculus sampling cluster analysis showed a significant difference between summer and winter groups. The difference between the summer and winter communities could largely be ascribed to changes in the relative abundance of this species during the two seasons.

CONCLUSION

The results of the survey indicate that the potentially toxic dinoflagellates community structure, species composition and biomass within Tunis northern lagoon conditions are determined essentially by the interactive effects of temperature, salinity, water column stability and nutrients status. The lack of any significant spatial variability in the species densities and dynamics throughout the lagoon can be ascribed to the virtual absence of a vertical gradient in temperature and salinity and daylight due to the shallow area.

It is important to mention that nutrients were not among the most significant factors that previously affected the defined community types when data from all locations were combined. While the analysis of the phytoplankton community structure covering all seasonal successional stages confirmed it to be sensitive to nutrient concentrations at the location level.

This year-long study indicates that phytoplankton community in Tunis northern lagoon was dominated by dinoflagellates in the seasons of spring, summer and early autumn.

It was clear that among these species, the potentially DSP species, *Dinophysis sacculus* is considered as an omnipresent species in this lagoon. It occurred throughout the year at all sampling sites. This species was positively correlated with water temperature and salinity, nevertheless it has no a significant correlation with nutrients levels.

It seems that temperature and salinity influenced the distribution of the majority of potentially toxic dinoflagellates species. It was evident that higher abundance of potentially toxic dinoflagellates species commonly occurred during spring, summer and early autumn, the periods coinciding with an increase in salinity and temperature in this area. During this period, essentially climatic conditions favored the proliferation of dinoflagellate assemblages, including the toxic species.

Detailed analyses and comparisons of ecosystems are needed to understand why only specific lagoons are susceptible to development of harmful microalgae events.

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REFERENCES

- Anderson D.M. and Keafer B.A. 1985. Dinoflagellate cyst dynamics in coastal and estuarine waters. In: Anderson D.M., White A.W. and Baden D.G. (eds), Toxic dinoflagellates. Elsevier, New York, pp 219-224.
- Anderson D.M., Glibert P.M. and Burkholder J.M. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25, 704-726.
- Annabi-Trabelsi N., Trabelsi E.B., Daly Yahia M.N., Ben Maiz N. and Romdhane M.S. 2003. Preliminary evaluation of the trophic status of the north lake of Tunis waters: application of the Trophic index (TRIX). *Water Waste and Environment Research* 4, 39-46.
- Arjen H. and Oostinga H. 1992. North Lake of Tunis and its shores: restoration and development. *Terra e Aqua* 23-32.
- Armi Z., Turki S., Trabelsi E. and Ben Maiz N. 2008. Nutrient loading and occurrence of potentially harmful phytoplankton species in the North Lake of Tunis (Tunisia). *Cahiers de Biologie Marine* 49, 311-321.
- Armi Z., Turki S., Kacem I., Ben Maiz N., Bouaicha N. and Hajjem B. 2009. Accidentally Appearance of the dinoflagellate *Kryptoperidinium foliaceum* (Stein 1883) in Tunis north lagoon. *Revue de la Faculté des Sciences de Bizerte* 6, 35-42.
- Armi Z., Turki S., Trabelsi E. and Ben Maiz N. 2010a. First recorded proliferation of *Coolia monotis* (Meunier, 1919) in the North Lake of Tunis (Tunisia). *Environmental Monitoring and Assessment* 164, 423-433.
- Armi Z., Trabelsi E., Turki S., Béjaoui B. and Ben Maiz N. 2010b. Seasonal phytoplankton responses to environmental factors in a shallow Mediterranean lagoon. *Journal of Marine Science and Technology* 15, 417 - 426.
- Badylak S. and Philips E.J. 2004. Spatial and temporal patterns of phytoplankton composition in a subtropical coastal lagoon, the Indian River Lagoon, Florida, USA. *Journal of Plankton Research* 26, 1229-1247.
- Balech E. 1988. Los Dinoflagelados del Atlantico sudoccidental. *Publicaciones Especiales Instituto Español de Oceanografía* 1, 1-31.
- Balech E. 1995. The Genus *Alexandrium* Halim (Dinoflagellata). Sherkin Island Marine Station, Sherkin Island Co., Cork, Ireland, 151 pp.
- Ben Charrada R. 1992. Le lac de Tunis après les aménagements. Paramètres physico-chimiques de l'eau et relation avec la croissance des macro-algues. *Marine Life* 1, 29-44.
- Ben Charrada R. 1995. Impact des aménagements de restauration sur la qualité des eaux et des peuplements benthiques du lac de Tunis. *Marine life* 5, 51-64.
- Ben Maiz N. 1993. Evolution des peuplements phytobenthiques après les travaux d'assainissement du lac nord de Tunis. In: Boudouresque, C.F., Pergent-Martini, C., (Eds.), Qualité du milieu marin, indicateurs biologiques et physicochimiques. GIS Posidonie publication, France, pp. 89-104.
- Britton R.H. and Crivelli A.J. 1993. Wetlands of southern Europe and North Africa: Mediterranean wetlands. In: Whigman D.F., Dykxjová D. and Hejný S. (eds), Wetlands of the World I: Inventory, Ecology and Management. Handbook of Vegetation Science. Kluwer Academic Publishers, The Netherlands, pp 129-194.
- Cabrini E., Cataletto B., Umani S.F. and Ramani P. 1995. Identification of a lagoon phytoplankton community by multivariate analysis. In: Ravera O. and Anelli A. (eds), VI Congr. Naz. Società Italiana di Ecologia, Venezia, pp 181-185.
- Cembella A.D., Ibarra I.A., Diogene J. and Dahl E. 2005. Harmful algal blooms and their assessment in fjords and coastal embayments. *Oceanography* 18, 158-171.
- Clarke K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian journal of Ecology* 18, 117-143.
- Clarke K.R. and Warwick R.M. 2001. Changes in marine communities: an approach to statistical analysis and interpretation, 2nd edition. Plymouth, UK, Plymouth Marine Laboratory, PRIMER-E.
- Collos Y., Gagne C., Laabir M., Vaquer A., Cecchi P. & Souchu P. 2004. Nitrogenous nutrition of *Alexandrium catenella* (Dinophyceae) in cultures and in Thau lagoon, southern France. *J. Phycol.* 40: 96 - 103.
- Delmas D., Herbland A. and Maestrini S.Y. 1992. Environmental conditions which lead to increase in cell density of the toxic dinoflagellates *Dinophysis* spp. in nutrient-rich and nutrient-poor waters of the French Atlantic coast. *Marine Ecology Progress Series* 89, 53-61.
- Dolapsakis N.P., Tzovenis I., Kantourou P., Bitis I. and Economou-Amilli A. 2008. Potentially harmful microalgae from lagoons of the NW Ionian sea, Greece. *Journal of Biological Research-Thessaloniki* 9, 89-95.

- Garcés E., Masó M. and Camp J. 1999. A recurrent and localized dinoflagellate bloom in a Mediterranean beach. *Journal of Plankton Research* 21, 2373-2391.
- Garcés E., Masó M., Vila M. and Camp J. 2000. HABs events in the Mediterranean Sea: are they increasing? A case study the last decade in the NW Mediterranean and the genus *Alexandrium*. *Harmful Algal News* 20, 1-11.
- Giacobbe M.G., Penna A., Ceredi A., Milandri A., Poletti R. and Yang X. 2000. Toxicity and ribosomal DNA of the dinoflagellate *Dinophysis sacculus* (Dinophyta). *Phycologia* 39, 177-182.
- Godhe A., Svensson S. and Rehnstam-Holm A.S. 2002. Oceanographic settings explain fluctuations in *Dinophysis* spp. and concentrations of diarrhetic shellfish toxin in the plankton community within a mussel farm area on the Swedish west coast. *Marine Ecology Progress Series* 240, 71-83.
- Hallegraeff G.M. 1993. A review of harmful algal blooms and their apparent Global increase. *Phycologia* 32, 79-99.
- Harrison W.G. 1976. Nitrate metabolism of the red tide dinoflagellate *Gonyaulax polyedra* Stein. *Journal of Experimental Marine Biology and Ecology* 21, 9-209.
- ICES 2005. Report of the ICES-IOC Working Group of Harmful Algal Bloom Dynamics (WGHABD), 4-7 April 2005, Flodevigen, Norway. C03 Re. ACME, ACE.
- Imai I., Yamaguchi M. & Hori Y. 2006. Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. *Plankt. Benthos Res.*, 1 (2): 71 - 84.
- Jacobson D.M. and Andersen R.A. 1994. The discovery of mixotrophy in photosynthetic species of *Dinophysis* (Dinophyceae): light and electron microscopical observations of food vacuoles in *Dinophysis acuminata*, *D. norvegica* and two heterotrophic dinophysoid dinoflagellates. *Phycologia* 33, 97-100.
- Lindahl O. 1986. A divided hose for phytoplankton sampling. ICES, C. M. L 26, Annex 3.
- López-Flores R. 2005. Phytoplankton dynamics in permanent and temporary waters of Empordà salt marshes (NE Spain). PhD Thesis. Universitat de Girona, Spain.
- Margalef R. 1983. Limnologia. Omega, SA, Barcelona, Spain, 1010 pp.
- Matsuoka K. and Fukuyo Y. (2000) Technical guide for modern dinoflagellate cyst study. WESTPAC-HAB/WESTPAC/IOC.
- Nedwell D.B., Dong L.F., Sage A. and Underwood G.J.C. 2002. Variations of the nutrients loads to the mainland U.K. estuaries: correlation with catchment areas, urbanization and coastal eutrophication. *Estuarine, Coastal and Shelf Science* 54, 951-970.
- Parsons T.R., Maita Y. and Lalli C.M. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford.
- Peña-Manjarrez J.L., Helenes J., Gaxiola-Castro G. and Orellana-Cepeda E. 2005. Dinoflagellate cysts and bloom events at Todos Santos Bay, Baja California, Mexico, 1999-2000. *Continental Shelf Research* 25, 1375-1393.
- Pérez-Ruzafa A., Gilbert J., Gutiérrez J.M., Fernández A.I. and Marcos C. 2002. Evidence of a planktonic food web response to changes in nutrient input dynamics in the Mar Menor coastal lagoon, Spain. *Hydrobiologia* 359-369, 475-476.
- Picot B., Pena G., Casellas C., Bondon D. and Bontoux J. 1990. Interpretation of the seasonal variations of nutrients in a mediterranean: Etang de Thau. *Hydrobiologia* 207, 105-114.
- Quintana X.D. and Moreno-Amich R. 2002. Phytoplankton composition of Empordà salt marshes, Spain and its response to freshwater flux regulation. *Journal of Coastal Research* 36, 581-590.
- Quintana X.D., Moreno-Amich R. and Comín F.A. 1998. Nutrient and plankton dynamics in a Mediterranean salt marsh dominated by incidents of flooding. Part. I. Differential confinement of nutrients. *Journal of Plankton Research* 20, 2089-2107.
- Ranston E.R., Webber D.F. and Larsen J. 2006. The first description of the potentially toxic dinoflagellate, *Alexandrium minutum* in Hunts Bay, Kingston Harbour, Jamaica. *Harmful Algae* 16, 29-47.
- Reynolds C.S., Huszar V., Kruk C., Naselli-Flores L. and Melo S. 2002. Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research* 24, 417-428.
- Riegman R. 1991. Mechanisms behind eutrophication-induced novel algal blooms. *Neth. Inst. Journal of Sea Research* 9, 1-51.
- Sarno D., Zingone A., Saggiomo V. and Carrada G.C. 1993. Phytoplankton biomass and species composition in a Mediterranean coastal lagoon. *Hydrobiologia* 271, 27-40.
- Smayda T.J. and Reynolds C.S. 2003. Strategies of marine dinoflagellate survival and some rules of assembly. *Journal of Sea Research* 49, 95-106.
- Sournia A. 1995. Red tide and toxic marine phytoplankton of the world ocean: an inquiry into biodiversity. In: Lassus P., Arzul G., Erard E., Gentien P. and Marcaillou-Le Baut C. (eds) *Harmful marine algal blooms*, pp 103-112.
- Steidinger K.A. and Tangen K. 1997. Dinoflagellates. In: Tomas C.R. (eds), *Identifying Marine Phytoplankton*, Academic Press, St. Petersburg, Florida, USA, pp 387- 584.
- Thronsdon J. 1995. Estimating cell numbers. In: Hallegraeff G.M., Anderson D.M., Cembella A.D. and Enevoldsen H.O. (eds), *Manual on Harmful Marine Microalgae*, IOC Manual and Guides No. 33. UNESCO, Paris, pp 63-80.
- Todd E.C.D. 1993. Domoic Acid and Amnesic Shellfish Poisoning - A Review. *Journal of Food Protection* 56, 69-83.
- Trabelsi E.B., Shili A. and Ben Maïz N. 2001. Marine flora of the north lake of Tunis 12 years after its restoration. In: Özhan (eds), *Proceedings of the fifth international conference on the Mediterranean coastal environment, MEDCOAST 01*. Middle East Technical University, Ankara 2, 916-92.
- 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 Technologie de la Mer de Salammbô* 31, 83-96.
- Turki S. and Balti N. 2005. Detetion of toxic *Alexandrium catenella* (Whedon & Kofoid) Balech in clam production zone of North Lake and Channel, Tunisia. *Harmful Algae News* 28, 1-2.
- Utermöhl H. 1958. Zur vervollkommnung der qualitativen Phytoplankton metodik. *Mitteil Inter Verein Limnologie* 9, 1-38.
- Vila M., Camp J., Garcés E., Masó M. and Delgado M. 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. *Journal of Plankton Research* 23, 497-514.
- Work T.M., Barr B., Beale A.M., Fritz L., Quilliam M.A. and Wright J.L.C. 1993. epidemiology of domoic acid poisoning in Brown Pelicans (*Pelecanus occidentalis*) and Brandt Cormorants (*Phalacrocorax penicillatus*) in California. *Journal of Zoo and Wildlife Medicine* 24, 54-62.
- Zaouali J. 1977. Le lac de Tunis: facteurs climatiques, physicochimiques et crises dystrophiques. *Bulletin de l'Office National des Pêches de Tunisie* 1, 37-49.