

OCCURRENCE OF HARMFUL DINOFLAGELLATES IN THE PUNIC HARBORS OF CARTHAGE (GULF OF TUNIS, TUNISIA) AND THEIR CORRELATIONS WITH THE PHYSICO-CHEMICAL PARAMETERS

Awatef AISSAOUT^{1,2}, S. TURKI² and O. K. BEN HASSINE¹

1- Unité de Recherche Biologie, Ecologie et Parasitologie des organismes aquatiques, Faculté des Sciences de Tunis (FST).

Campus Universitaire. 2092 El Manar, Tunis, Tunisie

2- Laboratoire du Milieu Marin, Institut National des Sciences et Technologies de la Mer (INSTM), 28 rue du 02 mars 1934.

2025 Carthage Salammbô, Tunis, Tunisie.

Aissaoui_awatef@yahoo.com

ملخص

تركيبة الطحالب السامة و علاقتها بالظروف المناخية في محيط شبه مغلق بالمياه الشاطئية بخليج تونس □ لمعرفة تركيبة الطحالب السامة و علاقتها بالظروف المناخية في محيط شبه مغلق بالمياه الشاطئية بخليج تونس تمت مراقبة هذه الطحالب بواسطة التحاليل المجهرية و الفيزيائية و الكيميائية لمياه المواني البونيقية بقرطاج في سبعة محطات و قد امتدت فترة المراقبة من جانفي الى جوان 2006.

دراسة مختلف هذه التحاليل مكنت من التعرف على 66 نوعية من الطحالب من فصيلة ثنائيات الأسواط من بينها 14 نوعية من الطحالب التي تعرف حين ظهورها بتكاثر كبير و سريع يؤدي الى ظهور مياه ملونة و 21 نوعية من الطحالب التي تعرف بإنتاجها لسميات و المرتبة عادة بتسمم المحار و القواقع.

مكنت دراسة الاحصاءيات عن وجود علاقات كبيرة بين التواجد المستمر لهذه النوعيات من الطحالب في المواني البونيقية و درجات الحرارة الملائمة من جهة و كميات الاورطوسفات المتوفرة من جهة اخرى كما تمت مناقشة بعض المتطلبات الايكولوجية لهذه النوعيات.

المفاتيح: الطحالب السامة, المغذيات, خليج تونس

RESUME

Occurrence des dinoflagellés néfastes dans les ports puniques de Carthage (golfe de Tunis, Tunisie) et de leurs corrélations avec les paramètres physico-chimiques : Afin de fournir une meilleure idée sur la structure et la dynamique de la communauté des dinoflagellés et leurs relations avec les paramètres environnementaux dans les zones semi-fermées en communication avec le golfe de Tunis, une étude a été réalisée dans les ports puniques de Carthage afin d'estimer les densités et la dynamique des espèces de dinoflagellés ainsi que les variations des concentrations en nutriments, de janvier à juin 2006. Un nombre de 66 espèces de dinoflagellés ont été identifiés dont 14 espèces sont connues pour être nocives et responsables d'efflorescences nuisibles alors que 21 espèces sont connues pour être productrices de toxines. L'analyse statistique nous a permis de révéler des corrélations significatives entre les densités de nombreuses espèces, telles que : *Dinophysis sacculus*, *D. acuminata*, *Alexandrium spp*, *Gymnodinium. aureolum*, *Gymnodinium impudicum*, *Akashiwo sanguinea*, *Scrippsiella spp* et *Prorocentrum gracile* avec la température de l'eau et la concentration en orthophosphates.

Mots clés : Dinoflagellés, nutriments, ports puniques, Carthage, golfe de Tunis.

ABSTRACT

In order to provide a better understanding of dinoflagellates structure and their relationship with environmental parameters in typical semi-enclosed areas communicating with the gulf of Tunis, a study was carried out to estimate the dinoflagellate assemblage structure and nutrients contents in seven stations during a semi-annual survey (from January to June 2006) in Punic harbors of Carthage (gulf of Tunis, south Mediterranean Sea). 66 species of dinoflagellates were identified among which 14 species are known to be harmful and responsible for harmful blooms and 21 species are known to be producing toxins. Statistical analysis showed significant correlations between many species, such as: *Dinophysis sacculus*, *D. acuminata*, *Alexandrium spp*, *Gymnodinium. aureolum*, *Gymnodinium impudicum*, *Akashiwo sanguinea*, *Scrippsiella spp* and *Prorocentrum gracile* with water temperature and orthophosphate concentrations.

Key words: dinoflagellates, nutrients, punic harbors, Carthage, gulf of Tunis.

INTRODUCTION

Marine coastal areas are important ecosystems and a resource for fishing, aquaculture and tourism. However, they are subject of pollution, eutrophication and harmful algal blooms which cause a widespread

problem (Smayda, 1997). These increases are, mostly, related to anthropogenic activities which include eutrophication and increases of confined areas associated with construction of jetties, leisure harbors (Garcés *et al.*, 2000). It should, however, be

noted that the increasing tendency is partially a consequence of the enhanced monitoring programs.

Increase of harmful algal blooms events applies, also, to the Mediterranean Sea where they are mainly associated with locations characterized by a restricted water exchange, such as harbors (Vila *et al.* 2001; Aissaoui *et al.*, 2007), small bays (Bellakhal *et al.*, 2009) and coastal lagoons (Turki *et al.*, 2006, Armi *et al.*, 2008).

The north coasts of Tunisia, located between the two main basins of the Mediterranean Sea through the Tunisian-Sicily Strait, has many coastal lagoons, lakes and harbors subject to anthropogenic disturbance, where phytoplankton often present mono-specific trends (Ben Rejeb-Jenhani *et al.*, 1991; Armi *et al.*, 2010). The Punic harbors of Carthage consist of two basins, communicating with each other and with the bay of Tunis by channels. The Bay of Tunis is open to the gulf of Tunis on its northern side and Tunis lagoon in the Southwest (Fig 1).

Punic harbors of Carthage are semi-enclosed coastal basins characterized by poor exchange with the seawaters, and is considered to have undergone eutrophication, which is caused by increased nutrient loading as a result of rapidly expanding human population growth in this region since 1990. During the second week of January 2006, the southern basin of the Punic harbors was the area of a significant development of *Ulva lactuca*. The strength of such «green tides» was not encountered, especially after the dredging of this area in 1990 (Hafnaoui, pers. comm.).

In this present work, we provide the first study in the coastal area, Punic harbors of Carthage, aimed to analyze the relationships between the density of potentially harmful phytoplankton species and the abiotic environmental parameters.

MATERIAL AND METHODS

Description of the site

The Punic harbors of Carthage (36°50' N 10°19' E) are represented by two basins. In the north, the military basin which covers an area of approximately 35 600 m² and has the shape of a crown surrounding the small island of admiralty (120 m in diameter). In the south, the mercantile port covers an area of approximately 34 600 m². The two basins are communicating with each other, and with the bay of Tunis by channels. These communications are not adequate enough because of the low tides, the high rate of the sedimentation along these entries. Consequently, water renewal is very slow, which is favorable for the development of aquatic vegetation, the creation of organic mud on the bottom and the appearance of bad smells during hot weather. The west coast of the bay of Tunis was an eutrophic zone (Souissi *et al.*, 2000). In this area the climate is a

Mediterranean type with a hot and dry summer and a soft and rainy winter.

Sampling

In order to study the hydrobiologic conditions of the site and to characterize the phytoplanktonic indicators of the Punic harbors, 7 sampling stations were selected in this very disturbed ecosystem. In the southern basin where the development of the anarchic proliferations of the macroalgae take place, 4 stations were identified (the average depth is about 2.2 m) and 3 stations were localized in the northern basin (3.3 m) (Fig 1). Stations are located in the way to cover the whole area. At each station, a sample was collected, from the whole water column using a hose with a variable length, for phytoplankton study. This sampling technique allows to overcome the difficulty of the heterogeneous vertical phytoplankton distribution (Lindahl, 1986). In addition, water samples were collected at 0.5 m of depth from the surface to analyze nutrients and chlorophyll *a* (Chl. *a*) contents.

Dissolved oxygen (mg/ l), temperature (°C), salinity (‰) and pH were measured *in situ* using a multiparameter probe (Multi 340i / SET). The transparency of the water column was estimated using a Secchi disc. All samples and measurements were carried twice per month from January to June 2006. Analyses of dissolved inorganic nutrients (N-NO₃⁻, N-NO₂⁻, N-NH₄⁺, P-PO₄³⁻) were performed following the method of Strickland and Parsons (1972). Potential nutrient limitation was rated using the method developed by Justic *et al.* (1995). Water sampling for nutrients analyses were taken from the surface simultaneously with **phytoplankton sampling**. For Chl. *a* quantification, 1 liter of water was filtered on nitrocellulose filters with 0.45 µm pore size. The Chl. *a* was extracted in 10 ml of 90% acetone for 24 h, in the dark at - 4°C, and the extract concentration was analysed spectrophotometrically (UV-Visible Spectrophotometer PU-8800).

Microscopic analysis

Phytoplankton samples (1 l) were concentrated to 100 ml and preserved with neutralized formalin (4%). 10 ml of fixed water samples were settled in a counting chamber for 24 h (Utermöhl, 1958; Andersen and Trondsen, 2003). The entire chamber was scanned at magnifications of 200 - 400 x for quantitative determinations using an IMT2 inverted Olympus microscope. Dinoflagellate species were identified with a BH2 Olympus microscope with a magnification of Gx1000. Species were expressed as Cells L⁻¹ (Thronsen, 1995). The identification of dinoflagellates species was performed according to the descriptions established by Balech (1988), Sournia (1995) and Steidinger and Tangen (1997). *Alexandrium* species were identified according the works of Balech (1995), Daly Yahia-Kéfi *et al.* (2001).

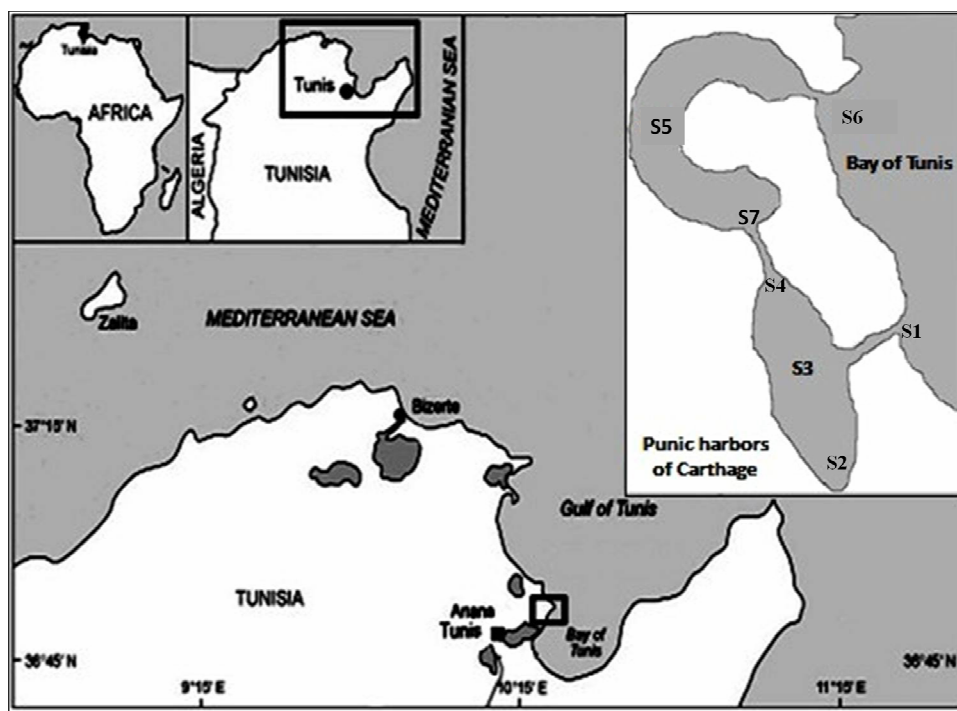


Figure 1. Sampling stations in the Punic harbors of Carthage.

Statistical analysis

A Pearson test performed with StatView was used to determine the correlations between 19 observations composed of dinoflagellate phytoplankton groups and environmental variables.

Results and Discussion

1- Physico-chemical parameters

The variations of the mean water temperature oscillated between 13.2 °C during January to 28.1 °C in June (Fig 2). The average values of salinity varied between 35.5 ‰ in January and 38.5 ‰ in June. At the beginning of April, the increase of the salinity and water temperature coincided with a decline of dissolved oxygen (Fig 2).

The mean dissolved oxygen content increased gradually from 9.7 mg/l in January to 12.8 mg/l in February, and abruptly decreased reaching the values of 4.6 mg/l in May (Fig 2). Averages of pH varied from 8.0 to 8.4 (Fig 3). Averages of turbidity were varying between 15.8 NTU in March to 4.9 NTU in June (Fig 4).

2- Spatio-temporal variations of the nutrients

2-1- Nitrogen compounds

The maximum ammonium (NH_4^+) concentrations were respectively recorded in the southern basin (S3 and S4 with 19.2 $\mu\text{mol/l}$ and 19.0 $\mu\text{mol/l}$), as it is the case in the station S7 of the northern basin which was about 19.8 $\mu\text{mol/l}$. Stirn (1988) noted that aquatic ecosystems located near urban discharges or the rivers are characterized by high ammonium concentrations approximately about 20.0 $\mu\text{mol/l}$, whereas the normal value in sea water is about 0.5

$\mu\text{mol/l}$. In the Punic harbors, the maximum mean concentration was about 16.0 $\mu\text{mol/l}$ (Fig 5). The mean nitrite (NO_2^-) concentrations was indicating rather heterogeneous spatio-temporal fluctuations with a maximum recorded on March in sampling station S2 (2.9 $\mu\text{mol/l}$) (Fig 5).

The maximum nitrate (NO_3^-) concentrations was about 15.7 $\mu\text{mol/l}$ in March and 9.7 $\mu\text{mol/l}$ in June, with a peak of 36.4 $\mu\text{mol/l}$ in station S4 (Fig 5). According to Stirn (1988), the trophic state in Punic harbors was eutrophic to hypereutrophic by taking into the consideration the classification of the Mediterranean waters in which nitrate concentration was up to 0.5 $\mu\text{mol/l}$.

For each basin of the Punic harbors, the physical and chemical parameters were summarized by their average values in Tab I.

2-2- Phosphates compounds

The mean orthophosphate (PO_4^{3-}) concentrations was varying between 0.3 $\mu\text{mol/l}$ in March, to 17.1 $\mu\text{mol/l}$ in late January (Fig 6). This last concentration exceeded by far those recorded in the Mediterranean Sea, ranging between 0 to 4.3 $\mu\text{mol/l}$ (Stirn, 1988). The ratio N/P varied from 0.5 to 51.7, with the great variations during the study period (Fig 8).

3- Chlorophyll a

Chlorophyll a (Chl. a) and the phaeopigments have fluctuated between 1,61 mg m^{-3} - 4,87 mg m^{-3} and 6,77 mg m^{-3} - 12,1 mg m^{-3} (Fig 7). The concentrations of the chlorophyll a were comparable to those registered at the level of the bay of Tunis by Souissi

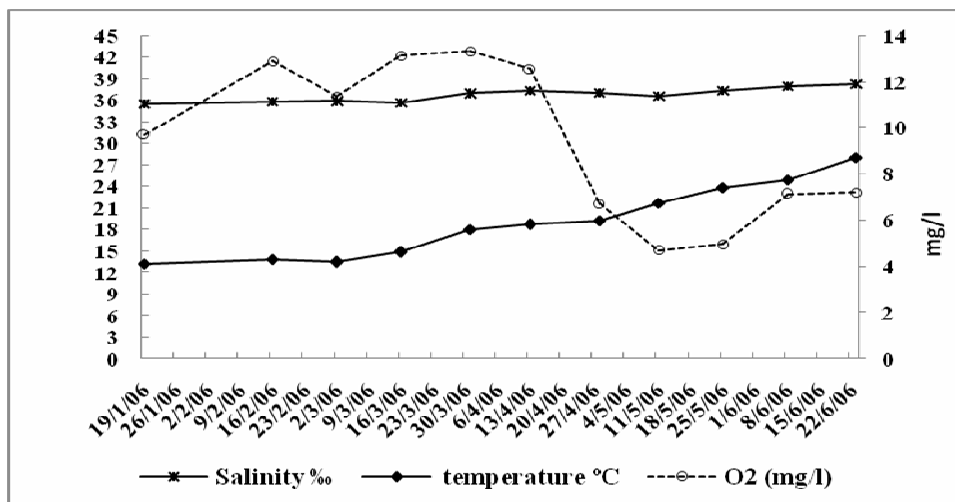


Figure 2. Mean water temperature, salinity and dissolved oxygen in the Punic harbors of Carthage (January 06 - June 06).

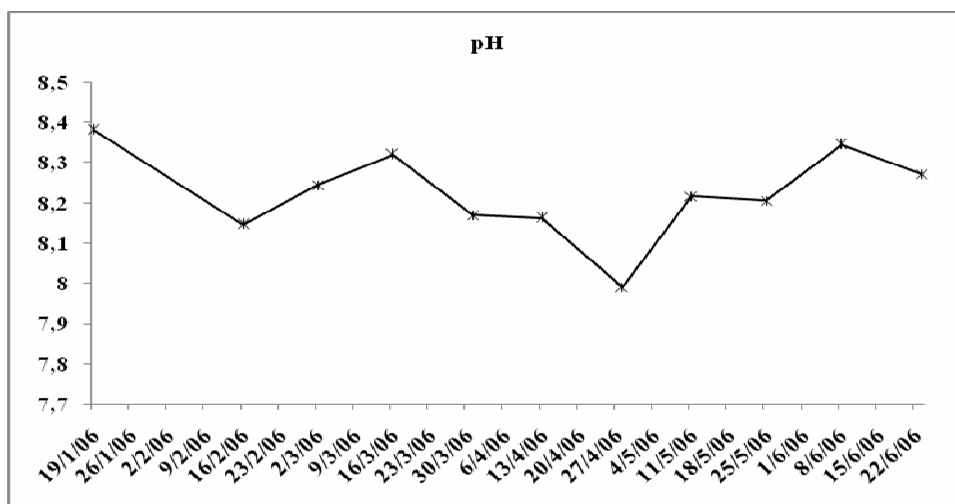


Figure 3. Mean pH in the Punic harbors of Carthage (January 06 - June 06).

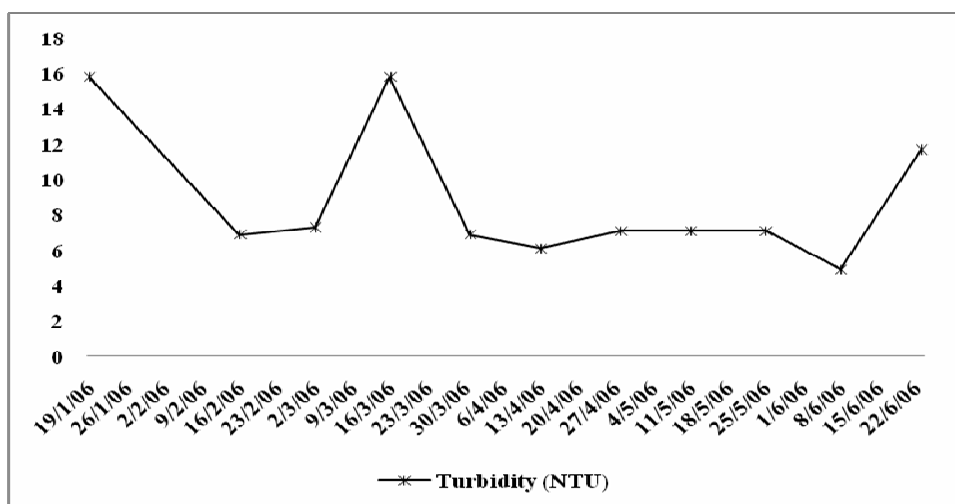


Figure 4. Mean turbidity in the Punic harbors of Carthage (January 06 - June 06).

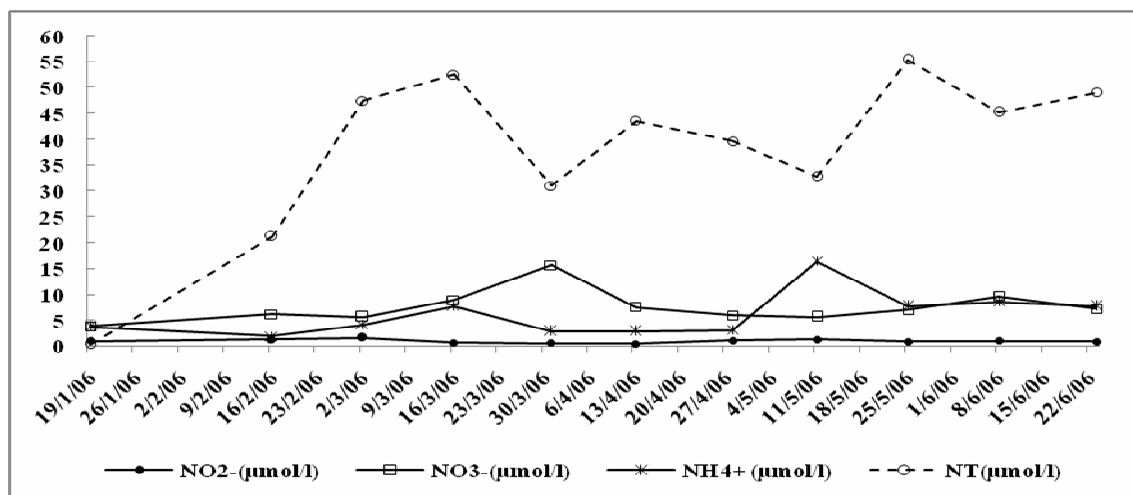


Figure 5. Variations of average nitrogen concentrations in the Punic harbors of Carthage (January 06 - June 06).

Table I. The physicochemical parameters in the Punic harbors of Carthage.

Parameters	North Basin	South Basin
Salinity ‰	36.79	36.73
Temperature of water C°	19.39	18.78
Dissolved oxygen (mg/l)	9.23	9.59
pH	8.20	8.23
Turbidity (NTU)	8.54	7.65
NO ₂ ⁻ (µmol/l)	1.01	1.02
NO ₃ ⁻ (µmol/l)	7.51	7.56
NH ₄ ⁺ (µmol/l)	5.18	7.04
PO ₄ ³⁻ (µmol/l)	0.80	2.81
NT (µmol/l)	35.08	39.24
PT (µmol/l)	1.53	1.30

et al (2000), which is considered as a very eutrophic ecosystem in the Mediterranean Sea.

4- Dinoflagellates community structure

The dinoflagellates community occurred in Punic harbors was belonging to four families: *Prorocentraceae*, *Peridiniaceae*, *Dinophysiaceae* and *Gymnodiniaceae* (Tab III). During our study period, *Prorocentraceae* was the dominant group (Fig 9). The proliferations were announced at the beginning of March until the end of May all over the sampling stations, reaching their maximum on May, 25th 2006 (2.41×10^5 cells L⁻¹ at stations S5 and S6)

The species attributed to the *Peridiniaceae* and *Dinophysiaceae*, which were perennial, were characterized by high densities in April and May (Fig 9). The max. concentrations were recorded in the sampling stations S5 and S6.

The densities of *Gymnodiniaceae* were more significant in the southern basin of which the higher density (1.3×10^4 cells L⁻¹) was recorded in June at the station S3 (Fig 9).

The analysis of average relative abundances and the frequencies of the various species of dinoflagellates were reported in Tab I.

4-1- Species responsible for colored water and known to be harmful

In the Punic harbors, 14 species are recorded likely to cause colored water. Certain species are associated with ichthyotoxins production. The most significant species responsible for water coloration are: *Akashiwo sanguinea*, *Gymnodinium aureolum*, *Gyrodinium spirale*, *Prorocentrum micans*, and *P. triestinum*; their maximum densities are mentioned in Tab III.

4-2- Potentially toxic species

During this study, 21 potentially toxic species were identified. The most abundant species were: *Dinophysis acuminata*, *D. sacculus*, *Alexandrium pseudogyaulax*, *A. margalefii* and *Coolia monotis*. All detected potentially toxic species are presented in Tab III.

The total abundance of dinoflagellates presented a very significant correlation with the content of Chlorophyll a. But only *P. triestinum* was highly correlated with this parameter. Indeed the maximum density of *P. triestinum* (1.77×10^5 cells L⁻¹) recorded in the station S5 (April 2006) corresponding to a content of chlorophyll a of about 4.8 mg m⁻³.

The variations in the temperature have direct impact on the variation of the phytoplanktonic density. Significant correlations were founded between the temperature and the species of dinoflagellates : *A. sanguinea*, *G. aureolum*, *G. impudicum*, *Alexandrium spp*, and *D. acuminata*. In fact, during May and June we noticed relatively important densities of these species, *D. acuminata*: 3.04×10^4 cells L⁻¹, and *A. sanguinea*: 7.24×10^3 cells L⁻¹ respectively for water temperatures of about 26.2 °C and 24.7 °C (Fig 2).

Other species such as *Scrippsiella spp*, *Prorocentrum gracile*, *Prorocentrum micans*, and *Prorocentrum triestinum* proliferated independently of the water temperature variations , which could explain the presence of these species during all the study period.

Highly significant relations were found between the salinity and the densities of *A. sanguinea* and *G. aureolum*. Indeed, the maximum concentrations of these two species which are respectively about 7.24×10^3 cells L⁻¹ and 6.41×10^3 cells L⁻¹ were recorded on June 8th in the station S3 for a salinity of about 37.9 ‰ (Fig. 2). *G. impudicum* and *Alexandrium spp* presented significant relationships with salinity (Tab IV).

Salinity has a great influence for the phytoplanktonic communities, the strong densities increase the chlorophyll biomass and stimulate the growth of the phytoplankton. The effect of salinity is especially striking in the coastal ecosystems which receive a fresh water flow varying during seasons. The seasonal variations of salinity in these mediums cause physiological and ecological conditions very different from one season to another and consequently, led to

an important variability in the taxonomic structure of the phytoplankton (Daly Yahia- Kéfi, 1998).

Significant negative correlations were found between the dissolved oxygen content and the densities of *D. sacculus*, *D. acuminata* and *Scrippsiella spp* (Tab IV). The great diversity of dinoflagellates in the Punic harbors of Carthage was associated with calm water and low oxygen content (4.6 mg /l entregistred in May).

The absence of correlation between nutrient loading and *Dinophysis* species density fits well with other studies (Delmas *et al.*, 1992; Blanco *et al.*, 1998; Aubry *et al.*, 2000; Caroppo *et al.*, 2001; Smayda and Reynolds, 2001; Godhe *et al.*, 2002) and this may be attributed to the potential mixotrophic character of *Dinophysis* cells and to the tendency of this species to accumulate in low depths.

Variations of densities of the species of the genus *Prorocentrum* and *Alexandrium* are independent of the dissolved oxygen content.

Highly significant correlations were found between the content of orthophosphates and the total density of dinoflagellates *D. sacculus*, *D. acuminata*, *P. triestinum*, *P. gracile*, and *P. micans* (Tab IV). This confirms that orthophosphate is an essential component for the development of dinoflagellates. This proves that the deficit in this element involves a severe production limitation.

Daly Yahia-Kéfi *et al.* (2005) reported that, in the Bay of Tunis, red tides of athecate forms such as *Gyrodinium impudicum* and *Akashiwo sanguinea*, primarily considered as autotrophic species, appeared under high phosphate levels versus nitrogen (nitrogen limited conditions). In comparison with other groups, dinoflagellates presented higher contents of nucleic acids that could imply higher phosphorus requirements (Costas and López-Rodas, 1991).

A high negative significant correlation was found between the density of dinoflagellates and the total nitrogen content. In fact, dinoflagellates present more diversified trophic behaviour than diatoms, being more independent of the nutrient levels. Several species of dinoflagellates appeared at very low nutrient concentrations and consequently a mixo-heterotrophic behavior can be expected (Daly Yahia - Kéfi *et al.*, 2005). Heterotrophy has been reported in *Akashiwo sanguinea* (Bockstahler et Coasts, 1993a; 1993b), *Prorocentrum* and mixotrophy in *Dinophysis* species (Jacobson et Anderssen, 1994; Berland *et al.*, 1995; Park *et al.*, 2006; Park *et al.*, 2008). Thus, it seems that most species of this class can multiply in water with low nitrogen content on the contrary to *G. impudicum* which presented a highly significant relationship to the content nitrates and *G. spirale* which is significantly correlated with the content ammonium. Indeed, ammonium is absorbed twice more quickly

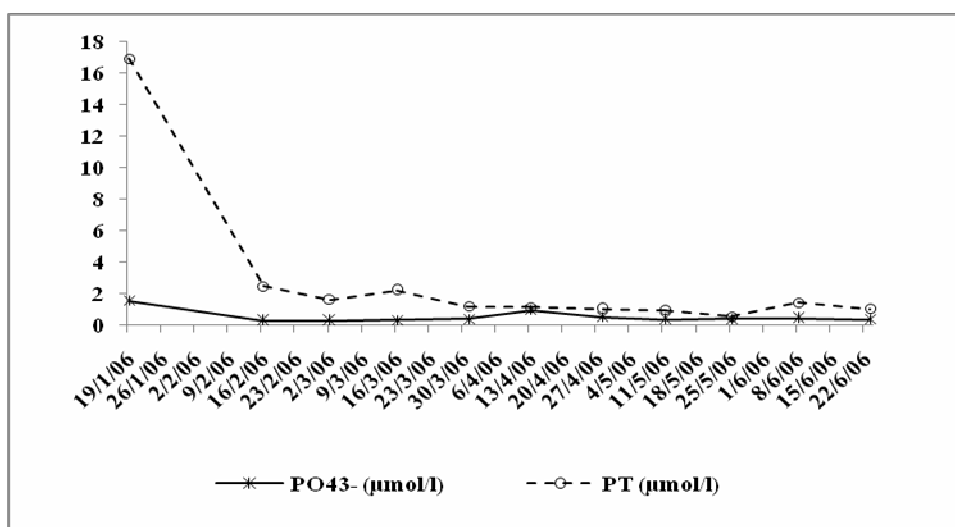


Figure 6. Variations of average phosphate concentrations in the Punic harbors of Carthage (January 06 - June 06).

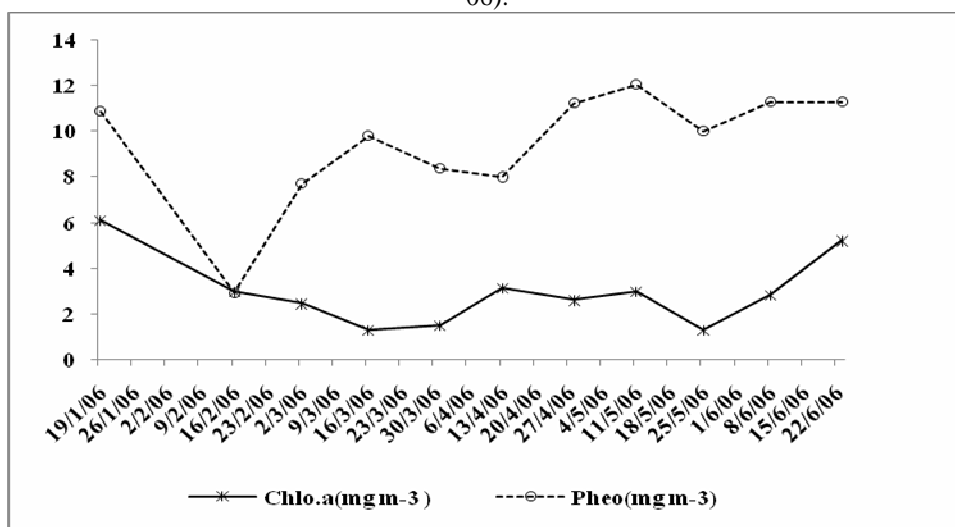


Figure 7. Spatio-temporal variations of the chlorophyll a and phaeopigments in the Punic harbors of Carthage (January 06 - June 06).

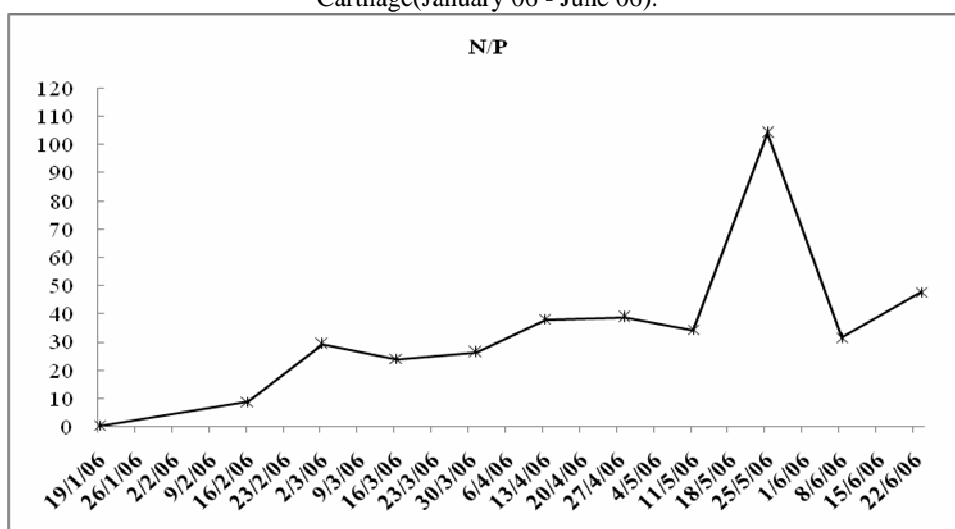


Figure 8. Spatio-temporal variations of the ratio N/P in the Punic harbors of Carthage (January 06 - June 06).

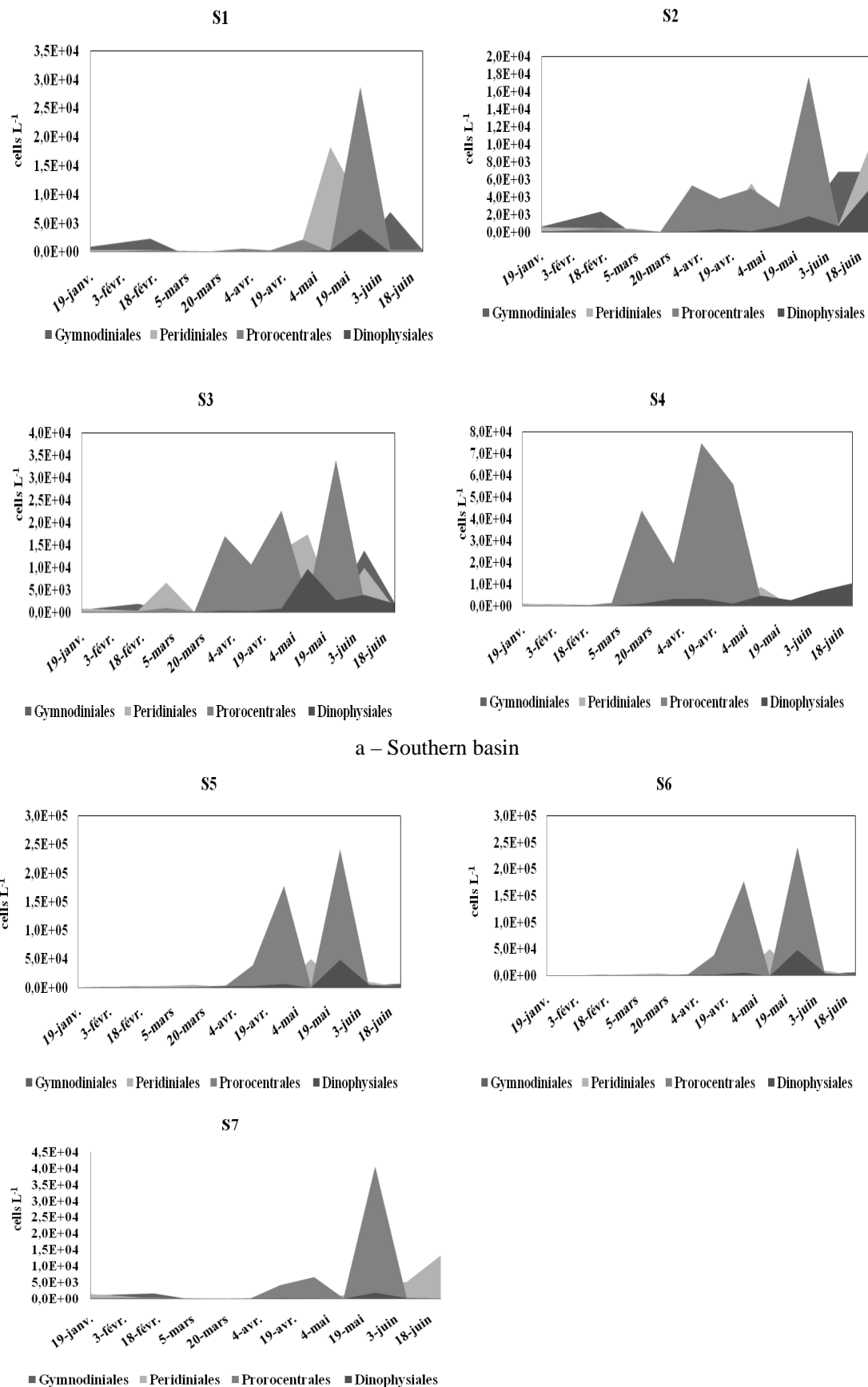


Figure 9. Spatio-temporal variations of dinoflagellates populations in the Punic harbors of Carthage.

Table II. Relative Average abundances and frequencies of dinoflagellates species in the Punic harbors of Carthage.

Species	Frequency %		Abundance %	
	South basin	North basin	South basin	North basin
<i>Akashiwo sanguinea</i>	88,64	87,88	6,66	1,09
<i>Gyrodinium aureolum</i>	79,55	63,64	3,02	0,84
<i>Gyrodinium impudicum</i>	34,09	45,45	0,18	0,59
<i>Gyrodinium spirale</i>	47,73	45,45	0,27	0,60
<i>Diplopsalis lenticula</i>	77,27	75,76	1,25	3,36
<i>Scripsiella subsalsa</i>	100	96,97	15,90	13,27
<i>Peridiniella sphaeroidea</i>	20,45	33,33	3,82	1,69
<i>Prorocentrum gracile</i>	43,18	57,58	0,57	32,44
<i>Prorocentrum triestinum</i>	38,64	33,33	31,08	27,61
<i>Prorocentrum micans</i>	100	90,91	24,68	7,77
<i>Dinophysis sacculus</i>	93,18	81,82	2,94	3,01
<i>Dinophysis acuminata</i>	65,91	75,76	7,45	6,06

than the two other inorganic nitrogen forms, therefore the use of nitrates takes place only when ammonium is exhausted (Parsons *et al.*, 1977; Valiela, 1984).

Nutrients have been considered as one of the major factors controlling the composition and abundance of phytoplankton community, and also the occurrence of the blooms. It has been generally thought that dissolved silicate and dissolved nitrogen played important roles on population dynamic and blooms of diatoms (Eppley, 1977; Hodgkiss and Lu, 2004), while dissolved phosphorus and N/P ratio on dinoflagellates blooms (Riegman, 1995; Escaravage *et al.*, 1996; Hodgkiss and Ho, 1997; Yutaka *et al.*, 1998). In a Sicilian lagoon, Giacobbe *et al.* (1996) reported that *Alexandrium* occurrence being associated with nitrate limited waters.

Among the red tide species, the eutrophication and the modification of the habitats seem to be associated with the increase and expansion of red tide events in the Mediterranean Sea (Garcés *et al.*, 2000). Moreover, fish mortality has been reported in the north coastal lagoon of Tunis associated with the presence of blooms of *Gyrodinium aureolum* and *Alexandrium minutum* (Romdhane *et al.*, 1998).

CONCLUSION

Punic harbors constitute a great archaeological site. This semi enclosed site was mainly characterized by reduced communications with the gulf of Tunis. The nutrients contents revealed an hypereutrophic state associated with macroalgal proliferations, such as *Ulva lactuca* which invaded the total surface of the southern basin, recorded four times during all the study period. Within This work, many harmful phytoplanktonic species were recorded, some of them could be the origin of discolored water like the species: *Scripsiella trochoidea* and *Prorocentrum micans*. Then, macroalgal proliferations and harmful

microalgae blooms were representing two eutrophic situations in this ecosystem.

The study of the correlation established between the various physicochemical parameters and the variations of the densities of the dinoflagellates species revealed a close relationship between the development of the phytoplanktonic densities and the variations of salinity, temperature and dissolved oxygen content. Thus, the content of ion orthophosphates shows a significant relation with the harmful species of dinoflagellates in particular.

A. sanguinea such as *Alexandrium* species seem to thrive in water relatively salt, hot, with low dissolved oxygen and alkaline pH. Significant correlations between abundances of this species and the abiotic parameters can explain its dominant presence in the Punic harbors.

Dinophysis species seems to prefer water with low oxygen content, rich in orthophosphates and with high temperatures. The development of the species of the kind *Prorocentrum* (*P. micans*, *P. triestinum*) seems to be independent of the variations of salinity, the temperature and oxygen. However, these microalgae would require mediums rich in orthophosphates.

The results of this survey indicate that the potentially toxic dinoflagellates species and densities within the Punic harbors of Carthage conditions were determined essentially by the interactive effects of temperature, salinity, water column stability and nutrients status. While the analysis of the phytoplankton community structure covering all seasonal stage successions, we suggest that more detailed analyzes as well as comparisons of ecosystems will be necessary to understand why specific Mediterranean coastal ecosystems are subject to develop harmful microalgae events.

Table III. Harmful dinoflagellates species occurred in the Punic harbors of Carthage and their maximum concentrations (January - June 2006).

Order	Species	Max. Concentration (Cell L ⁻¹)	Date
<i>Dinophysiiales</i>	<i>Dinophysis acuminata</i>	3.04 x 10 ⁴	25.05.2006
	<i>D. sacculus</i>	1.7 x 10 ⁴	25.05.2006
	<i>D. caudata</i>	7.7	19.01.2006
	<i>D. fortii</i>	16.5	23.06.2006
	<i>D. rotundata</i>	38.3	31.03.2006
	<i>D. rapa</i>	3.01	31.03.2006
	<i>D. acuta</i>	2.4	16.03.2006
<i>Peridiniales</i>	<i>Alexandrium catenella</i>	280.7	19.01.2006
	<i>A. minutum</i>	54.08	11.05.2006
	<i>A. pseudogyaulax</i>	2.3 x 10 ³	23.06.2006
	<i>A. tamarense</i>	271.4	23.06.2006
	<i>A. margalefii</i>	3.8 x 10 ³	23.06.2006
	<i>A. peruvianum</i>	9	16.02.2006
	<i>A. insuetum</i>	2.5	16.02.2006
	<i>A. foedum</i>	3.4	23.06.2006
	<i>Coolia monotis</i>	2.3 x 10 ³	23.06.2006
	<i>Ostropsis siamensis</i>	126.3	23.06.2006
	<i>Protoperidinium depressum</i>	23,5	19.01.2006
	<i>Scripsiella trochoidea</i>	28,31	11.05.2006
	<i>G. spinifera</i>	1,2 x 10 ³	08.06.2006
	<i>G. polyedra</i>	163,6	25.05.2006
	<i>G. polydramma</i>	8,2	31.03.2006
	<i>Amylax triacantha</i>	222,1	11.05.2006
	<i>C. furca</i>	3,6	28.04.2006
	<i>C. lineatum</i>	3,1	16.03.2006
	<i>C. fusus</i>	13,8	13.04.2006
<i>Prorocentrales</i>	<i>Prorocentrum lima</i>	11.27	19.01.2006
	<i>P. concavum</i>	2.6	19.01.2006
	<i>P. compressum</i>	5.9	16.02.2006
	<i>P. minimum</i>	12.9	19.01.2006
	<i>P. micans</i>	1,86 x 10 ⁴	13.04.2006
	<i>P. triestinum</i>	1,77 x 10 ⁵	28.04.2006
<i>Gymnodiniales</i>	<i>Akashiwo sanguinea</i>	7,2 x 10 ³	08.06.2006
	<i>Gyrodinium aureolum</i>	6,4 x 10 ³	08.06.2006
	<i>Gyrodinium spirale</i>	1,9 x 10 ³	16.02.2006

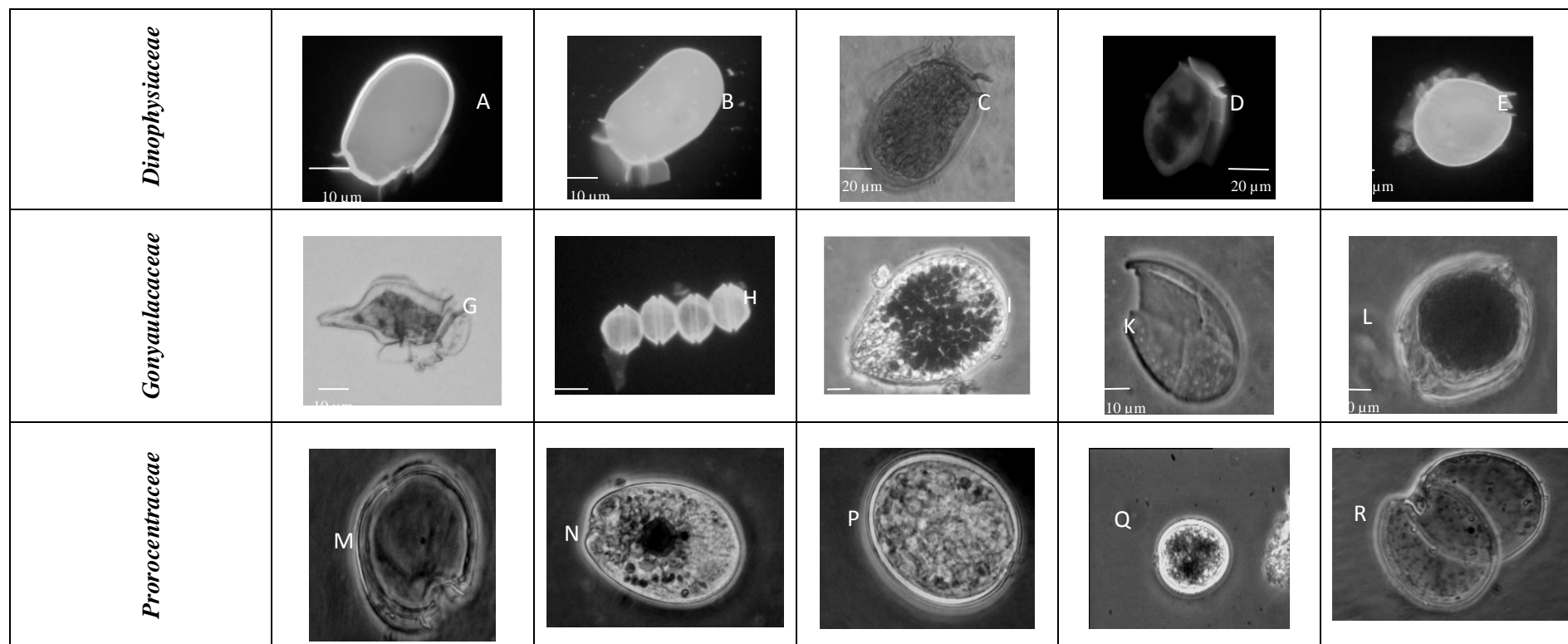


Figure 10. Microphotographies of harmful phytoplankton species detected in the Punic harbors of Carthage. A, B, D, and H: Specimens stained with Calcofluor. A: *Dinophysis acuminata*; B: *Dinophysis sacculus*; C: *Dinophysis fortii*; D: *Dinophysis acuta*; E: *Dinophysis rotundata*; F: *Dinophysis caudata*; G: *Alexandrium catenella* Chain of 4 cells; H-I: *Oestropsis ovata*. I hypothecal view K-L: *Coolia monotis*, L plates and Po ; M: *Prorocentrum lima*; N: *Prorocentrum compressum* ; P: *Prorocentrum minimum*, Q: Hypothecal and epithecal views of *Prorocentrum emarginatum*.

Table IV. Correlations study between dinoflagellates species and abiotic parameters in the Punic harbors of Carthage.

	Dinof	<i>G. impudicum</i>	<i>A. sanguinea</i>	<i>G. aureolum</i>	<i>G. spirale</i>	<i>Scrippsiella.spp</i>	<i>Alexandrium spp</i>	<i>P. gracile</i>	<i>P. micans</i>	<i>P. triestinum</i>	<i>D. sacculus</i>	<i>D. acuminata</i>
T	0.24*	0.32**	0.47**	0.38**	-0.23*	0.15	0.32**	0.13	-0.08	0.03	0.35**	0.31**
S	0.18	0.23*	0.46**	0.35**	-0.16	-0.01	0.27*	0.06	-0.03	0.08	0.17	0.21*
O ₂	0.29*	-0.09	-0.26*	-0.11	0.22*	-0.37**	-0.12	-0.16	0.17	-0.14	-0.32**	-0.31**
pH	-0.19	0.07	0.26*	0.22*	-0.21*	-0.06	0.07	-0.01	-0.17	-0.26*	0.01	0.04
NO ₃ ⁻	-0.14	0.44**	0.06	0.20*	-0.01	-0.14	-0.01	-0.10	-0.15	-0.03	-0.13	-0.11
NH ₄ ⁺	-0.06	-0.09	0.00	-0.01	0.24*	-0.19	-0.02	0.09	-0.05	-0.04	-0.05	-0.04
PO ₄ ³⁻	0.44**	-0.06	-0.10	-0.11	-0.04	-0.03	-0.05	0.40**	0.39**	0.36**	0.37**	0.39**
NT	0.34**	0.00	0.02	0.14	-0.08	0.04	-0.06	-0.21*	-0.16	-0.27*	-0.16	-0.22*
Chl. <i>a</i>	0.36**	-0.06	0.01	-0.04	0.02	0.07	-0.03	0.16	0.22*	0.31**	0.19	0.25*

Marked correlations are significant at $p < 0.05$

BIBLIOGRAPHY

- Aissaoui A., Turki S., Akrouf F., Sammari, C. 2007. Toxic planktonic microalgae in Punic harbors of Carthage. Proceedings of the 8th Edition of TJASSST, pp. 12 - 18.
- Andersen P., Throndsen J. 2003. Estimating cell numbers. In : G. M. Hallegraeff, D. M. Anderson & A. D. Cembella (Eds.), pp. 99-130. Manual on harmful marine microalgae. Monographs on oceanographic methodology 11, Paris, UNESCO, 793 p.
- 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, 64: 423-433.
- Armi Z., Turki S., Trabelsi E., 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.
- Aubry F.B., Berton A., Bastianini M., Bertaggia R., Baroni A., Socal G. 2000. Seasonal dynamics of *Dinophysis* in coastal waters of the NW Adriatic Sea (1990 - 1996). Botanica Marina, 43: 423 - 430.
- 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 p.
- Bellakhal M., Daly-Yahia Kefi O., Bellakhal Fartouna M., Daly-Yahia, N. 2009. Effet d'une pollution thermique et d'une eutrophisation côtière sur la distribution du phytoplancton de la baie de Sousse, Tunisie. REDVET Rev. electrón. Vet, 10 : 1695 - 7504.
- Ben Rejeb-Jenhani A., Kartas F., Lemoalle J., Amblard C. 1991. Phytoplancton du lac Ichkeul (Tunisie) : Composition, structure et activité photosynthétique. Hydrobiologia, 76 : 241-256.
- Berland B.R., Maestrini S.Y., Grzebyk D., Thomas, P., 1995. Recent aspects of nutrition in the dinoflagellate *Dinophysis cf. acuminata*. Aquatic Microbial Ecology, 9 : 191-198.
- Blanco J., Morono Á., Pazos Y., Maneiro J., Mariño J. 1998. Trends and variations of the abundance of main PSP and DSP producing species in the Galician Rias: environmental and biological influences. In: B. Reguera, J. Blanco, M.L. Fernández, T. Wyatt (Eds), pp. 204-207. *Harmful Algae*. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO. 635 p.
- Bockstahler K.R., Coasts D.W., 1993a. Spatial and temporal aspects of mixotrophy in Chesapeake Bay dinoflagellates. Journal of Eukaryotic Microbiology, 40 : 49-60.
- Bockstahler K.R., Coasts D.W., 1993b. Grazing of the trophic dinoflagellate *Gymnodinium sanguineum* on ciliate populations of Chesapeake Bay. Marine Biology, 116 : 477-487.
- Caroppo C., Congestri R., Bruno M. 2001. Dynamics of *Dinophysis* sensu lato species (Dinophyceae) in a coastal Mediterranean environment (Adriatic Sea). *Continental Shelf Research*, 21: 1839-1854.
- Costas E., Lopez-Rodas V. 1991. A comparative study of DNA content in six dinoflagellate species. Scientia Marina, 55: 557 - 561.
- Daly Yahia-Kéfi O. 1998. Le phytoplancton de la baie de Tunis: analyse systématique, biogéographique, quantitative et synécologique des Diatomés et des Dinoflagellés. Ph.D. Thesis, Faculté des Sciences de Tunis, 242 p.
- Daly Yahia-Kéfi O., Nézan E., Daly Yahia M.N. 2001. Sur la présence du genre *Alexandrium Halim* (Dinoflagellés) dans la baie de Tunis (Tunisie). Oceanologica Acta, 24: S17 - 25.
- Daly Yahia-Kéfi O., Souissi S., Gómez F., Daly Yahia M.N. 2005. Spatio-temporal distribution of the dominant Diatom and Dinoflagellate species in the Bay of Tunis (SW Mediterranean Sea). Mediterranean Marine Science, 6: 17 - 34.
- Delmas D., Herbland A., 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.
- Eppey R.W. 1977. The growth and culture of diatoms. In : Werner D. (Eds.). The Biology of Diatoms. Bio. Monogr., 13: 24 - 64.
- Escaravage V., Prins T.C., Smaal A.C., Peeters J.C.H. 1996. The response of phytoplankton communities to phosphorus reduction in mesocosm experiments. Journal of Experimental Marine Biology and Ecology, 198: 55 - 79.
- Garces E., Maso M., Vila M., Camp J. 2000. Harmful algae events in the Mediterranean: are they increasing? Harmful Algae News, 20: 10 - 11.
- Giacobbe M.G., Oliva F.D., Maimone G. 1996. Environmental factors and seasonal occurrence of the dinoflagellate *Alexandrium minutum*, a PSP potential producer, in a Mediterranean

- lagoon. *Estuarine Coastal and Shelf Science*, 42: 539 - 549.
- Godhe A., Svensson S., 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.
- Hodgkiss I.J., Ho K.C. 1997. Are changes in N:P ratios in coastal water key to increase red tide blooms? *Hydrobiologia*, 352 :141-147.
- Hodgkiss I.J., Lu S.H. 2004. The effects of nutrients and their ratios on phytoplankton abundance in Junk Bay, Hong Kong. *Hydrobiologia*, 512 : 215-229.
- Jacobson D.M., 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 : 97-110.
- Justic D., Rabalais N.N., Turner R.E., Dortch Q. 1995. Changes in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences. *Estuarine, Coastal and Shelf Science*, 40 : 339-356.
- Lindahl O. 1986. A divided hose for phytoplankton sampling. *ICES*, 26, Annex 3.
- Park M.G., Kim S., Kim H.S., Myung G., Kang Y.G., Yih, W. 2006. First successful culture of the marine dinoflagellate *Dinophysis acuminata*. *Aquatic Microbial Ecology*, 45: 101 - 106.
- Park M.G., Park J.S., Kim M., Yih W. 2008. Plastid dynamics during survival of *Dinophysis caudata* without its ciliate prey. *Journal of Phycology*, 44: 1154 - 1163.
- Parsons T. R., Takahashi M., Hargrave B. 1977. *Biological oceanographic processes*. Pergamon Press, Oxford, 332 p.
- Riegman R., 1995. Nutrient related selection mechanisms in marine phytoplankton communities and the impact of eutrophication on the planktonic food web. *Water Sci.Technol.*, 32 : 63-75.
- Romdhane M.S., Eilertsen H.C., Daly Yahia-Kéfi O., Daly Yahia, M.N. 1998. Toxic dinoflagellate blooms in Tunisian lagoons: causes and consequences for aquaculture. p. 80-83. In : B. Reguera, J.
- Smayda T.J., 1997. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. *Limnol. Oceanogr.*, 42 :1137-1153.
- Smayda T.J., Reynolds C.S. 2001. Community assembly in marine phytoplankton: application of recent models to harmful dinoflagellate blooms. *Journal of Plankton Research*, 23 : 447-461.
- Souissi S., Daly Yahia-Kéfi O., Daly Yahia M.N. 2000. Spatial characterization of nutrient dynamics in the Bay of Tunis (south western Mediterranean) using multivariate analyses: consequences for phyto- and zooplankton distribution. *Journal of Plankton Research*, 22 : 2039-2059.
- Sournia A. 1995. Red tide and toxic marine phytoplankton of the world ocean : an inquiry into biodiversity. In : P. Lassus, G. Arzul, E. Erard, P. Gentien, C. Marcailou-Le Baut C. (Eds), pp. 103-112. *Harmful marine algal blooms*. Paris, UNESCO, 878 p.
- Steidinger K.A., Tangen K. 1997. Dinoflagellates. In: C.R Tomas (Ed.), pp. 387-584. *Identifying Marine Phytoplankton*. Academic Press, St. Petersburg, Florida, USA, 858 p.
- Stirn J. 1988. Eutrophication in the Mediterranean Sea : receiving capacity and monitoring of long-term effects. UNESCO Bologna, Italy, pp. 161 - 188.
- Strickland J.H.D., Parsons T.R. 1972. A practical handbook of seawater analysis. *Fisheries Board of Canada Bulletin*, 167, 311 p.
- Thronsdon J. 1995. Estimating cell numbers. In : G.M. Hallegraeff, D.M. Anderson, A.D. Cembella, H.O. Enevoldsen (Eds), pp. 63-80. *Manual on Harmful Marine Microalgae*. IOC Manual and Guides 33. UNESCO, Paris, 858 p.
- Turki S., Sammari C., Harzallah A. 2006. Occurrence of harmful dinoflagellates in two different Tunisian ecosystems: the lake of Bizerte and the gulf of Gabès. *Cahiers de Biologie Marine*, 47: 253 - 259.
- Utermöhl H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-methodik. *Comm. Assoc. Int. Limnol. Theor. Appl.*, 9 : 1-38.
- Valiela I., 1984. *Marine ecological processes*. New York, 546 p.
- Vila M., Garcés E., Masó M., Camp J. 2001. Is the distribution of the toxic dinoflagellate *Alexandrium catenella* expanding along the NW Mediterranean coast? *Mar. Ecol. Prog. Ser.* 222 : 73-83.
- Yutaka H., Miyahara K., Nagai S. 1998. Relationships between the dominant phytoplankton and DIN:DIP ratios in Osaka Bay and Harima-Nada. *Nippon Suisan Gakkaishi*, 64 (2) : 243-248