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Investigating the possible influence of different environmental factors on the *Alexandrium catenella/tamarense* resting cyst distribution and HAB occurrence within Bizerte lagoon (Mediterranean coast of Tunisia)

Influence des différents facteurs environnementaux sur la distribution des kystes de résistance d'*Alexandrium catenella/tamarense* et de l'absence d'efflorescences toxique de cette espèce dans la lagune de Bizerte (Côtes Méditerranéennes, Tunisie)

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Abstract

Zmerli-Triki H., O. Ben Amor, A. Deidun., O. Kéfi Daly-Yahia – Investigating the possible influence of different environmental factors on the *Alexandrium catenella/tamarense* resting cyst distribution and HAB occurrence within Bizerte lagoon (Mediterranean coast of Tunisia). *Mar. Life,* 18 : 43-53.

The *Alexandrium catenella/tamarense* resting cyst abundance, distribution and vegetative cell toxin profile within sediment from Bizerte lagoon (Western Mediterranean, Tunisia) were studied in Bizerte lagoon. Studies on cyst distribution in Bizerte lagoon sediment are recent and rare (Fertouna-Bellakhal *et al.,* 2014; Zmerli Triki *et al.,* 2014). This species had previously produced toxic blooms in Bizerte lagoon in November 2007. Toxin analysis revealed a toxin level range of 193-322 fmol.cell^{-1}. The emerging toxin profile revealed the presence of GTX-1, 3 and 4 and of GTX6 (B2) and C-1 and 2, with dominance of GTX4, GTX6 and C2 within the three cell cultures used in this study (89.5-93.9 mol% toxin cell). Cyst densities recorded in this study ranged between 0 and 104 cysts.g^{-1} dry sediment; similar densities were responsible for toxic blooms in Thau lagoon in France. The highest cyst concentrations were recorded in the central part of the lagoon and at one peripheral station. Correlations between surface sediment properties and cyst abundance were investigated, but no significant correlations between the two were found, suggesting the existence of other forcing environmental factors influencing cyst distribution within the Bizerte lagoon. The hydrodynamic profile of the lagoon is considered to be the main environmental factor affecting resting cyst distribution. In addition, natural spatial heterogeneity in cyst distribution, sampling design and the fact that the species only recently colonised Bizerte lagoon greatly influence the cyst densities recorded in sediments in this study and explain, at least partially, the recorded distribution of resting cysts and the non-recurrence of the *A. catenella* blooms since 2007, the low densities recorded in Bizerte lagoon and the eventual germination of cysts without regeneration of cyst bank.

KEY-WORDS:
Alexandrium catenella/tamarense, resting cysts, spatial distribution, toxin profile, Bizerte lagoon.

Résumé

Zmerli-Triki H., O. Ben Amor, A. Deidun., O. Kéfi Daly-Yahia – [Influence des différents facteurs environnementaux sur la distribution des kystes de résistance d'*Alexandrium catenella/tamarense* et de l'absence d'efflorescences toxique de cette espèce dans la lagune de Bizerte (Côtes Méditerranéennes, Tunisie)]. *Mar. Life*, 18 : 43-53.

En novembre 2007, *Alexandrium catenella* a engendré une efflorescence toxique dans la lagune de Bizerte causant la contamination des coquillages. Les études relatives à la distribution des kystes dans la lagune de Bizerte sont récentes et très peu nombreuses (Fertouna-Bellakhal *et al.*, 2014; Zmerli Triki *et al.*, 2014). Cette étude, présente la distribution et l'abondance des kystes de résistance d'*Alexandrium catenella/tamarense* ainsi que le profil toxinique des cellules végétatives dans la lagune de Bizerte. Le contenu toxinique varie de 193 à 322 fmol.cell^{-1}. Le profil toxinique révèle la présence de GTX-1, 3 et 4; GTX6 (B2) et C-1 et C-2 à dominance de GTX4, GTX6 et C2 dans les trois souches (89,5 à 93,9 mol% contenu en toxine). La densité des kystes de résistance dans les sédiments de surface varient de 0 à 104 kystes.g^{-1} de sédiment sec. Aucune corrélation significative n'a été retrouvée entre l'abondance des kystes et les caractéristiques du sédiment, suggérant l'existence d'autres facteurs environnementaux qui régissent la distribution des kystes. Les kystes de résistance sont principalement localisés au niveau de la zone Nord-Est de la lagune, là où le bloom d'*A. catenella* a été détecté en 2007. Dans cette zone la présence d'un courant giratoire a permis l'accumulation des kystes, qui se sont formés dans la colonne d'eau lors du bloom, à la surface des sédiments. Néanmoins, les densités de kystes enregistrés dans la lagune de Bizerte sont relativement faibles, cela peut être expliqué par trois hypothèses : (1) le maillage d'échantillonnage mis en place : un maillage d'échantillonnage plus fin pourrait révéler la présence de nouveaux foyers d'accumulation de kystes, (2) l'hétérogénéité de la distribution des kystes au niveau des sédiments, (3) l'appauvrissement des banques de kystes suite à la remise en suspension et germination des kystes.

L'initiation et la récurrence des HAB's sont influencés, en partie, par l'abondance et l'accumulation des kystes au niveau des sédiments. Il est possible que dans le cas de la lagune de Bizerte, les faibles concentrations en kystes enregistrés ne favorisent pas l'efflorescence de cette espèce dans la lagune.

MOTS CLÉS :
Alexandrium catenella/tamarense, distribution spatiale, kystes de résistance, lagune de Bizerte, PSP.

Introduction

Several coastal aquatic ecosystems are bearing the brunt of a variety of environmental pressures associated, directly or indirectly, with harmful algal blooms (HABs). Such harmful outbreaks operate either by producing high-biomass blooms, which in turn result in the deterioration of the coastal landscape amenity value and in changes in aquatic ecosystem community structure, or by producing phycotoxins. Although toxin-producing microalgae normally occur in aquatic systems at very low vegetative cell concentrations, they are biomagnified up the trophic chain, thereby reaching human consumers.

The dynamics of HABs are complex and specific to the affected geographical area and to the causative species (Anderson *et al.*, 2012.). Understanding HAB ecology requires a comprehensive consideration of the various factors involved and of the interactions between these. Nutrient inputs, hydrodynamic factors and also life history of incriminated species are the main factors underlying the dynamics of HABs (Gowen *et al.*, 2008; Hays *et al.*, 2005).

Several of these harmful species produce sexual resting cysts in the water column and settle in the upper layers of bottom sediment pending the return of favorable environmental conditions and the end of their mandatory dormancy period. They are able to persist in bottom sediments for decades, thereby providing a reservoir of diversity of potentially harmful microalgal species that could emerge and bloom when favorable environmental factors are re-instated (Belmonte *et al.*, 1997). The occurrence of resting cysts in bottom sediments can be considered as a marker or indicator of the occurrence of an established population of a given dinoflagellate species. Recently deposited resting cysts normally accumulate in superficial sediments and are mostly linked to fine-grained (clay and silt grain sizes) sediment particle sizes (Joyce *et al.*, 2005; Anglès *et al.*, 2010; Horner *et al.*, 2011). Their transport and distribution within bottom sediments is dependent on several factors, such as bottom currents (Goodman, 1987), the frequency of dredging activities in ports (Dale, 1986) and shipping traffic through the discharge of ballast water and sediments (Hallegraeff, 1998; Lavoie *et al.*, 1999; Niimi, 2004).

A. catenella is a bloom-forming toxic dinoflagellate species associated with harbours and confined waters in the Mediterranean Sea. It blooms in summer when surface temperatures are higher than 20°C and both NO_3^- and NH_4^+ are high (Bravo *et al.*, 2008). The first known bloom of *A. catenella* in the Mediterranean Sea occurred in 1994 in Valencia harbor (Spain); subsequently, blooms of the same species were recorded in several other areas in the Mediterranean, including Thau Lagoon (France) (Lilly *et al*., 2002), the North Lake and Channel of Tunis (Tunisia) (Turki, Balti, 2005), Bizerte lagoon (Tunisia) (Turki *et al*., 2007), Algeria (Frehi *et al*., 2007), Greece (Igniatiades, 2012), Spain (Figueroa *et al*., 2005) and Olbia harbour (Italy) (Luglié *et al*., 2003). In November 2007, a bloom of *A. catenella* was recorded in Bizerte lagoon during the monitoring program (Turki *et al.,* 2007), with observed cell concentrations of 20.10^4 cells.L^{-1}. Mussel tissue was contaminated by paralytic toxins during this event. Since this episode, no further *A. catenella* blooms were recorded in Bizerte lagoon. This sudden disappearance spurred us to assess Bizerte lagoon bottom sediment for its *A. catenella/tamarense* resting cysts distribution, in order to identify areas of high resting cyst density. In addition, we report the toxin profile for the Tunisian *A. catenella* strain using the Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method. This background information is important for *A. catenella* bloom monitoring and for an eventual early warning system. Correlations between sediment grain-size parameters and resting cyst densities were also assessed. The influence of hydrodynamic parameters on resting cyst distribution was also investigated.

Material and methods

Site description

Bizerte lagoon (Northern Tunisian lagoon) is an economically-important lagoon, accomodating 9 mussel farms and producing annually 130183 kg of mussels and 8799 kg of oysters (personal communication with Tunisian Agriculture Ministry). The lagoon is a large depression, with an area of 128 km^2 and two hydrodynamic connections. The first one, known as the navigation channel, connects the lagoon to the Mediterranean Sea, and the second connects the lagoon with an internal lagoon, Ichkeul (ANPE, 1990; Ouakad, 2007). The lagoon receives a freshwater influx from 18 different streams which discharge over the whole of its area and water currents within the lagoon are mostly affected by west-northwesterly winds. The water balance and the lagoon salinity have been considerably affected in recent years by a developing dam infrastructure (Harzallah, 2003). Four main ongoing phenomena influence the bottom sediment composition within the lagoon (Kamens *et al*., 1984): (1) Sand and non-calcareous mud discharged fluvially at Tinja from the Ichkeul lagoon; (2) biotic deposition (calcareous and siliceous skeletons and shells); (3) calcareous and solid materials resulting from soil erosion on cultivated land are also discharged in the lagoon by rivers and run off; (4) silt and clay compounds are deposited in the lagoon by wind (atmospheric deposition, after being picked up from terrestrial areas) and water currents from contiguous Mediterranean areas. Analyses of hydrodynamic data for the lagoon (Harzallah, 2003; Bejaoui, Harzallah, 2005; Bejaoui, 2009) have revealed that waves contribute strongly to the mixing of sediments within the lagoon and to the re-mobilization of the superficial deposits. Bizerte lagoon sediment is mainly composed of the sandy-mud facies and the muddy facies.

A. catenella culture establishment and toxin analysis

Alexandrium catenella culture establishment and a preliminary study of toxin profiles were conducted in order to confirm the toxicity of *Alexandrium catenella* Tunisian strain and to take into account the need to map resting cysts in this area.

A. catenella vegetative cells were collected during the *A. catenella* bloom event in November 2007 from two points within the mussel farm sampling site, located along the southern shore of Bizerte lagoon (37° 12.940'N – 09°55.790'E). Three strains of *A. catenella* cultures were established by micropipette isolation of single 4-cell chains and maintained in flasks containing 25 mL of L1 medium (Guillard, Hargraves, 1993) without the addition of silica, under the following incubation conditions: salinity of 37 psu, 24°C, 90 μmol photon $m^{-2}s^{-1}$, 12h: 12h light:dark cycle. The three *A. catenella* strains were used for the monitoring of vegetative cell growth and for paralytic shellfish poisoning (PSP) toxin analysis. Densities of 1500 cells.mL^{-1} from exponential phase stock cultures for each strain were inoculated on a 750 mL culture medium. Growth rate was monitored every 3 or 4 days for 3 weeks by fixing with iodine solution a 5 mL subsample from each flask culture. Cells were counted in a Sedgewick-Rafter chamber using an inverted microscope. In accordance with Guillard (1973), the specific growth rate (μ; expressed in day^{-1}) was calculated from the slope of a linear regression applied over the entire exponential phase of growth, after logarithmic transformation of the data. Toxin analyses were performed during the late exponential phase, when cell concentrations were 13367, 13887, and 13304 cells.mL^{-1} respectively for S1, S2 and S3, using Reversed Phase High Performance Liquid Chromatography (RP-HPLC), with the Postcolumn Reaction and Fluorimetric Detection methods as described by Franco and Fernandez (1993).

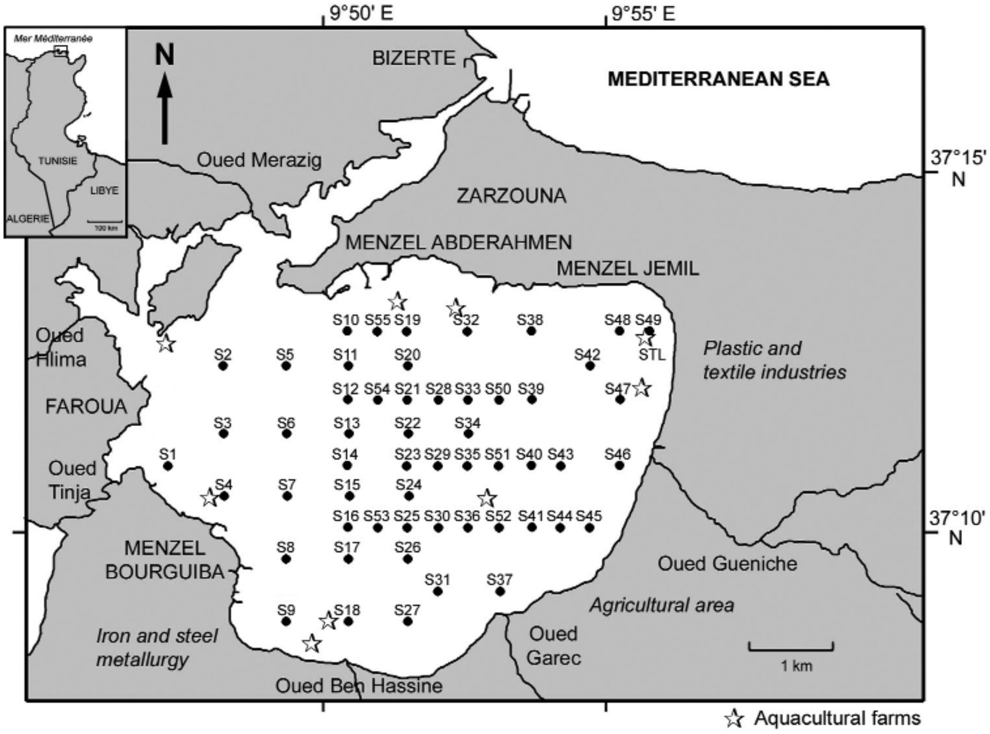

Figure 1
Bizerte lagoon sampling sites.
Localisation des stations d'échantillonnage dans la lagune de Bizerte

Sediment sample collection

The whole lagoon was surveyed in July-August 2012 using two sampling belt transects: a 0.7 km x 0.7 km transect within the muddy facies and a 1.7 km x 1.7 km transect through the muddy-sandy interface (**Figure 1**). Samples were collected as 3 replicates from 55 stations (n = 165), using cylindrical cores (26 cm long, 4 cm diameter) operated by SCUBA divers. The undisturbed upper 3 cm of sediment from three cores for the same station were sectioned and mixed together, and were then stored in total darkness at 4°C to avoid oxidation.

Resting cyst extraction

Duplicate subsamples for each station were used for resting cyst (RCs) quantification. 1 g of wet sediment was suspended in 50 mL Filtered Sea Water (FSW), sonicated for 3 min using an Ultrasonic cleaner bath and then sieved through 100 and 20 µm mesh sieves. The remaining slurry within the upper 20 µm mesh was collected and centrifuged using MIKRO 22R HETTICH centrifuges at 3000 rev / min, 10 min, 4 °C. Resting cyst extraction was done using the gradient density method by adding Polytungstate Solution (PST) (Bolch, 1997). Quantification of dinoflagellate resting cysts was done using sedimentation chambers (3 mL), with an inverted photonic microscope. Densities were expressed as cysts.g^{-1} dry sediment (DS). The PST extraction step was performed consecutively five times on fifteen samples, in order to estimate the accuracy of the PST gradient density method. At each extraction (Extr 1, Extr2, Extr3, Extr4, Extr5), the *A. catenella/tamarense* resting cyst quantification was performed individually and the Efficiency Coefficient (EF) was calculated using this equation: EF = (% total RCs extracted) / (% RCs of Extr 1)

To avoid underestimation during cyst quantification, cyst densities calculated for all samples (using a unique extraction) were multiplied by the EF value using this equation: $N\ (g^{-1}\ DS) = N'\ (g^{-1}\ DS) * EF$, were N' is RC density calculated after one PST extraction in all samples and N is the final RC density

Germination and vegetative cells identification

A. catenella/tamarense RCs were isolated into 96-culture plates (Nunc™ Delta surface), supporting Enriched Natural Sea Water (ENSW) culture medium (Harrison *et al.*, 1980) and incubated at 20°C, 34 psu, 100 µmol photons $m^{-2}\ s^{-1}$ and 12h: 12h light:dark ratio. When germination occurred, the morphology of the emerging vegetative cells and empty cysts was investigated.

Sediment characteristics

Sediment grain-size parameters and organic matter content were quantified in order to investigate any possible correlations between sediment characteristics and RC densities. Cyst densities were expressed in terms of dry sediment so as to reduce possible variability in values due to sediment water content.

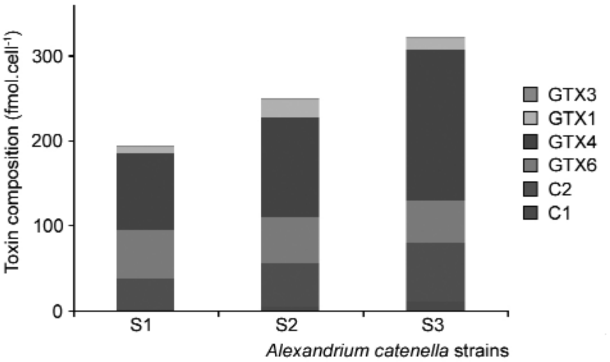

Figure 2
Toxin composition of *Alexandrium catenella* Tunisian strain.
*Profil toxinique de la souche Tunisienne d'*Alexandrium catenella.

The particle size of the sediment was determined using laser particle sizer Malver Master TM LSE. The sediment water content was evaluated by drying the wet sediment sample (Ww) for 7 days at 60°C. The resulting dry sediment was weighed (Wd), heated to 450°C for 12 hours to combust all organic matter (OM) and then dried again to record (Wd_1).

The sediment water and organic content were calculated as follows: H_2O % = [(Ww - Wd) * 100] / Ww ; OM % = [(Wd - Wd_1) * 100] / Wd_1

Statistical analyses

The relationship between *A. catenella/tamarense* resting cyst distribution and environmental factors (grain-size parameters and the biochemical composition of the sediment) were investigated using the Pearson correlation test and also through Principal Component Analyses (PCA), in order to identify the underlying factors influencing the *A. catenella/tamarense* distribution within Bizerte lagoon. The spatial structure for RCs distribution was investigated using the Moran's I index, a spatial autocorrelation test calculated using R software (available online at: http://www.r-project.org).

Results and discussion

Toxin content and composition

A. catenella is a bloom-forming toxic dinoflagellate, producing Paralytic Shellfish Poisoning (PSP) toxins. Chemically, PSP toxin derivate can be divided into three categories: (1) carbamates, usually considered the most potent toxin category and which include saxitoxin (STX), neosaxitoxin (NEO) and gonyautoxins 1-4 (GTX1, GTX2, GTX3, GTX4); (2) N-sulphocarbamoyl-based toxins (C, B), generally considered to be less potent toxins and (3) decarbamoyl toxins (Turell *et al*., 2007).

Turki *et al.* (2007) reported for the first time the toxicity of Tunisian *A. catenella* species using the mouse bioassay. In this preliminary study, toxin analysis of *A. catenella* strains performed within the late exponential growth phase confirmed the conclusions of this previous study and revealed different toxin levels within different strains. However, toxin composition (mol%) was nearly the same for the three strains. Within the different strains, toxin levels ranged between 193 and 322 $fmol.cell^{-1}$ and toxin composition was mainly represented by GTX4, C2 and GTX6. Growth rate expressed for the different stains was 0.1478, 0.1458 and 0.1482 day^{-1} for S1, S2 and S3, respectively.

Different studies on PSP toxin profiles of *Alexandrium* species at different geographical locations characterised by contrasting environmental conditions revealed that toxin cellular content and profile is affected both by different phonological factors such as species growth rates or growth phases and also by environmental factors such as nutrient levels, temperature and light intensity (Hwang, Lu, 2000; Etheridge, Roesler, 2005; Wang *et al*., 2006; Laabir *et al.*, 2013). Also, the toxin content and composition could vary among *Alexandrium* species and also among strains of the same species (Kim *et al*., 1993; Ichimi *et al.,* 2002; Laabir *et al.*, 2013). These phenomena were also observed in the current study, despite the three strains being collected from the same location and being cultivated under the same abiotic conditions, and the toxin being tested on the exponential phase for all the strains. Chou *et al.,* (2004) suggested that a wide variation of cellular toxin content can occur at the same location and under the same environmental conditions, and that these differences may be due to the intrinsic nature of the toxic algal species involved.

When comparing the toxicity levels of *A. catenella* Tunisian strains with those of other strains isolated from different marine systems, the mean toxin content (254.6 ± 64.7 $fmol.cell^{-1}$) of Tunisian strains is higher than those of Mediterranean, Asian and European Atlantic water strains (Laabir *et al*., 2013), but they contain levels of toxin comparable to some Chilean strains (Varela *et al*., 2012) (**Figure 2**).

The cultured strains of *A. catenella* contained GTX4, GTX6 (B2) and C2 as the major components (89.5 to 93.9 mol% toxin cell) and GTX1, C1 and GTX3 as the minor components. The toxin profiles were similar in the three *Alexandrium* strains analysed, with 51.1 - 59.4 mol% carbamate toxins cell (GTX-1,-3,-4) and 40.6 - 48.9 mol% N-sulfocarbamoyl toxins cell (GTX6, C-1,-2). The following toxins were recorded in decreasing order: GTX4, GTX6, C2, GTX1, C1, GTX3, with GTX3 present in trace amounts. Our

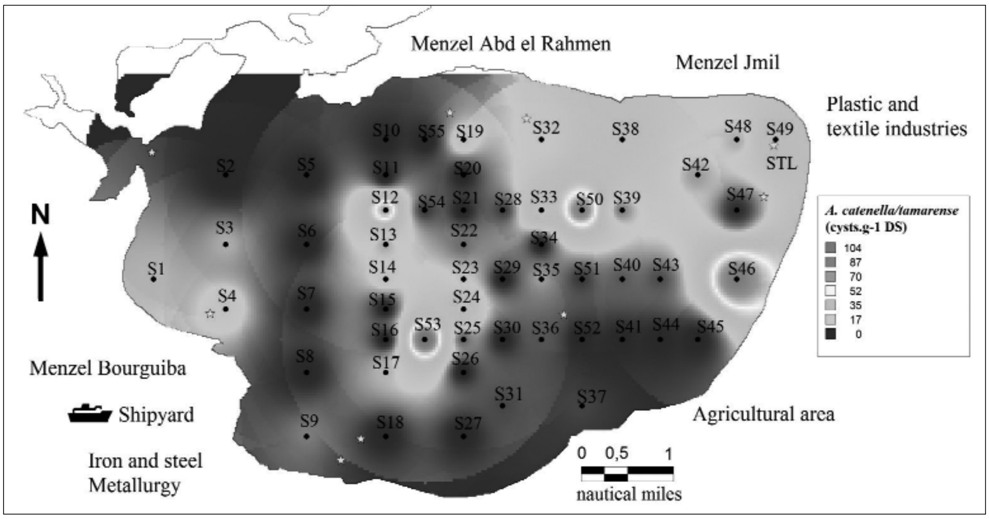

Figure 3
Alexandrium catenella/ tamarense RCs densities distribution.
*Distribution des kystes de résistance d'*Alexandrium catenella/tamarense *dans les sédiments de surface de la lagune de Bizerte.*

toxin analysis revealed that the major toxic component deriving from *A. catenella* cells is GTX-4 (46.5 - 55 mol% toxins cell), which is responsible for high intrinsic toxicity, whereas STX and NeoSTX are absent. These PSP toxin profiles are different from those of the Chilean strains, for which twelve PSP toxins were identified, with the dominance of C2, GTX3, GTX4 and NeoSTX (Varela *et al.*, 2012).

Sediment characteristics

The sediment of Bizerte lagoon contains a high fraction of water (58.89 ± 8.02%). The organic matter distribution within the same lagoon showed a homogeneous sediment water-content pattern for all the sampling stations, with the water content percentage not exceeding 2.28% of dry sediment at all the stations. Sediments were mainly represented by the fine sediment fraction (< 63 m), ranging in weight from 50.48 to 97.6% of total sediment sample weights. The silt fraction and the clay fraction represented 60.56 ± 3.5% and 17.99 ± 8.46%, respectively, of the total fine fraction (Zmerli Triki *et al.*, 2014) (**Table I**).

A. catenella/tamarense resting cysts abundance and mapping

Alexandrium catenella/tamarense cysts accumulated in the surface sediment of the whole lagoon showed different densities as a function of the geographical location of the investigated stations. Resting cysts were mostly observed in the north-eastern and central parts of the lagoon (**Figure 3**). Resting cyst densities calculated using the EF value of 1.5 ranged between values of 0 and 104 RCs.g^{-1} DS with a mean value of 18.06 ± 24.65 cyst.g^{-1} DS (**Table I**). The highest RC abundance was observed at sampling stations 12, 46, 50, 53, with cyst densities ranging between 35-10 cysts.g^{-1} DS.

PCA was performed to investigate any possible correlation between the distribution of RC densities and Bizerte lagoon environmental variables. PCA analysis showed that the two axes comprised 79.4% of sediment parameter information. The first axis (30.945%) is mainly represented by the sediment silty fraction and the second (48.517%) by sediment biochemical properties (**Figure 4**). Neither Pearson correlation test nor PCA analysis showed any significant correlation between cyst accumulation and sediment properties. The *A. catenella/tamarense* cyst distribution in Bizerte lagoon was not spatially correlated. Using 1000 randomizations for the null hypothesis (*i.e.* no spatial relations in the distribution of cyst densities), a Moran I index of P-value = 0.98; = 0.05 indicated that *A. catenella/tamarense* cyst distribution exhibited a strong heterogeneity across the main Bizerte lagoon and no consistent spatial autocorrelation pattern was revealed by the Moran I test (**Table II**).

Factors influencing cyst distribution and abundance

Overall, where *A. catenella/tamarense* blooms having cell densities of around 10^6-10^7 cells.L^{-1} occurred, resting cyst abundance ranged between 600 to 8000 cysts.g^{-1} DS (Anderson *et al.*, 2005; Bravo *et al.*, 2008; Horner *et al.*, 2011 ; Tobin, Horner, 2011), reaching values as high as 220872 ± 148086 cysts.g^{-1} DS in some places, such as Bedford Basin (Lacasse *et al.*, 2013). Compared to these cyst densities, the values recorded from Bizerte lagoon in the current study were relatively low, with the highest recorded value being 104 cysts.g^{-1} DS. Similarly, cyst accumulation recorded in the Thau lagoon sediment was relatively low, with an average value of 20 cysts.g^{-1} DS for the whole lagoon and a peak value of 80 cysts.g^{-1} DS at Crique-de-l'Angle, were blooms occurred. In Puget

	Min	Max	Mean	SD
A. catenella/tamarense	0	104,42	18,06	24,65
Water content (%)	34,81	76,31	59	8,14
MO (%)	0,33	2,28	0,99	0,58
Fine clay (%)	2,61	12,06	7,17	1,93
Coarse clay (%)	3,67	67,65	10,82	8,17
Middle silt (%)	3,61	67,54	51,25	10,15
Coarse silt (%)	7,37	15,94	10,70	2
Fine sand (%)	0,12	23,87	12,20	4,62
Gravel (%)	0,19	36,28	8,12	6,97

Table I
Descriptive statistic of sediment characteristics and *Alexandrium catenella/tamarense* densities in surface sediment of Bizerte lagoon.
*Statistiques descriptives des propriétés sédimentaires et densités kystiques d'*Alexandrium catenella/tamarense *dans la lagune de Bizerte.*

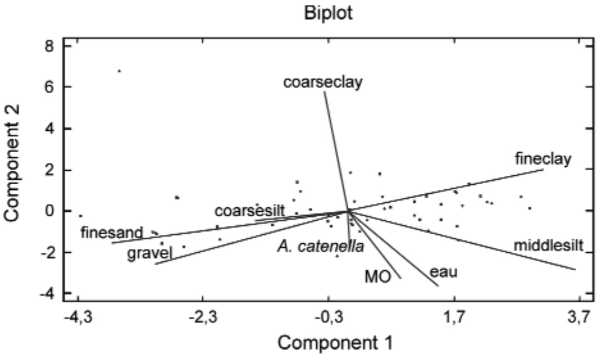

Figure 4
Principal component analysis (PCA) for Bizerte lagoon surface sediment granulometry.
Analyse en composante principale (ACP) des propriétés granulométriques des sédiments de surface de la lagune de Bizerte.

	Water content %	OM %	Fine clay	Coarse clay	Fine silt	Coarse silt	Fine sand	Gravel
Pearson corr.	0.118	– 0.076	0.009	– 0.090	0.012	- 0.023	0.001	0.060
Sig	0.581	0.948	0.515	0.929	0.868	0.993	0.389	0.665
N	55	55	55	55	55	55	55	55

Table II
Pearson correlation test between sediment proprieties and *Alexandrium catenella/tamarense* cysts densities.
*Corrélations de Pearson entre les propriétés des sédiments de la lagune de Bizerte et les densités kystiques d'*Alexandrium catenella/tamarense.

Sound (USA), where paralytic shellfish poison has often occurred historically, cyst counts as low as 12 cysts.g^{-1} DS were reported at some stations (Quartermaster Harbor), but at other sites within the Sound, where PSP was frequently recorded, cyst concentration values often exceeded 50 cysts.g^{-1} DS (Horner *et al*., 2011).

A number of hypotheses can be proposed to explain the relatively low abundance of accumulated cysts within the Bizerte lagoon bottom sediment. Generally, cyst accumulation shows strong heterogeneity in its distribution, with a high concentration being found in one site and much lower values being reported in contiguous sites; this phenomenon could be influencing the cyst density values reported in our study. On the other hand, the fact that the introduction of *A. catenella/tamarense* within Bizerte lagoon is only recent (Turki *et al.*, 2007), with cyst distribution within the lagoon yet to stabilize, could also be shaping cyst accumulation patterns within the same lagoon.

Alexandrium catenella/tamarense appeared for the first time in Bizerte lagoon between 27 November and 18 December 2006, with cell densities ranging between 100 and 450 cells.L^{-1}. The first bloom for the species occurred within the lagoon during the November-December 2007 period, and no supplementary blooms have been registered since then. Our sediment sampling study was conducted four years later, in 2012. During the autumn season, prevailing winds may be suspending surface sediment containing recently-deposited *A. catenella/tamarense* cysts, promoting vegetative cell germination and thus reducing the volume of RC banks.

A third hypothesis explaining low abundance could revolve around the sampling design. Horner *et al*. (2011) affirm that the cyst count recorded in Quartermaster Harbor within Puget Sound was very different when applying two different sampling scales. The 2005 survey within Puget Sound revealed 12 cysts.g^{-1} DS and the 2006 survey revealed 67 cysts.g^{-1} DS. So the adoption of higher-resolution scale sampling could improve our results and reveal new sites of RC accumulation.

Environmental factors such as sediment grain-size parameters and hydrodynamic forces control the distribution and settlement of dinoflagellate resting cysts (Genovesi *et al*., 2013). The absence of an unambiguous correlation pattern between sediment properties and cyst densities, and also the strong heterogeneity in cyst abundance, suggests that other environmental factors are influencing cyst distribution. The hydrodynamic model of the lagoon developed by Bejaoui (2009) reveals the presence of a hydrodynamic cell located along the north-eastern part of the lagoon, where bottom currents weaken and change direction. The presence of a gyratory cell in this area of the lagoon where *A. catenella* bloom occurred in 2007 could disperse cysts formed in the water column during the bloom episode, to be deposited eventually in surface sediment within other locations of the lagoon (**Figure 3**).

Absence of *A. catenella* HABs in Bizerte lagoon

HAB bloom initiation and recurrence are influenced by several interacting physical, chemical and biological factors (Zingone, Enevoldsen, 2000). Cyst accumulations in surface sediment, the size of cyst beds and endogenous patterns in fact represent important factors in the initiation and recurrence of bloom episodes (Bravo *et al*., 2008). In addition, the relationship between the increase in HAB occurrence on a global scale and the increase in anthropogenic nutrient flux in the last decades has been repeatedly investigated (Montresor, 2001).

In fact, the recurrence of HAB phenomena in different parts of the Mediterranean Sea was observed where high cyst accumulation co-occurred with high levels for both NO_3^- and NH_4^+ (respectively 50 and 20 mol.L^{-1}), with water surface temperatures of 21-25°C and salinities of about 37 psu (Bravo *et al*., 2008). In the North Lake of Tunis in Tunisia, Armi *et al*. (2011) associated *A. catenella* blooms with low temperature (15.9°C), salinity (36.7 psu) and NH_4^+ (8.38 µmol.L^{-1}) values, and with the highest levels of NO_3^- (2.15 µmol.L^{-1}).

Bizerte lagoon *Alexandrium* species are mainly present in autumn (Bouchouicha Smida *et al.,* 2012) and high abundance of these species was correlated with salinity of about 37.5 psu, temperature of about 16°C, average NH_4^+ levels of about 55,45 mol.L^{-1} (Bouchouicha Smida *et al*. 2012) and NO_3^- levels ranging between 0.75 and 6.3 µmol.L^{-1} (Sahraoui *et al.,* 2010). Considering previous results on Bizerte lagoon, we can hypothesize that prevailing environmental factors during much of the year within the lagoon appear to be suitable for the proliferation of *Alexandrium* species. Nevertheless, the relatively low densities of resting cysts encountered in bottom lagoon sediment were not sufficient to reintroduce *A. catenella* HAB into Bizerte lagoon. Complementary studies on *A. catenella/tamarense* hot spot detection, the study of environmental factors controlling their encystment and germination and germling cell viability, are necessary for predicting eventual blooms of *A. catenella/tamarense* within Bizerte lagoon and elsewhere.

Conclusion

A. catenella/tamarense resting cyst distribution was not correlated to sediment properties, suggesting that the main environmental forcing controlling cyst distribution is hydrodynamic in nature. Resting cyst abundance was low (max 104 RCs g^{-1} DS) within surface bottom sediment of Bizerte lagoon and this could be explained by (a) a strong heterogeneity in cyst distribution, or (b) a periodic excystment of resting cysts which re-inoculates the water column with vegetative cells without regenerating cysts beds on surface bottom sediment. While the Bizerte lagoon environmental conditions are suitable for *A. catenella* proliferation, since 2007 no HAB of the same species has been detected. Toxin analysis of *A. catenella* confirms the toxicity of Tunisian strains and suggests that a wide variation of cellular toxin content can exist for cells sampled in the same location and subject to the same environmental conditions. Further studies on the dynamics governing the excystment of *A. catenella/ tamarense* resting cysts and the effect of different combinations of temperature, salinity and light on the growth and toxicity of *A. catenella* are required. Such further studies are important so as to evaluate the potential of this species to trigger new HAB episodes in the Bizerte lagoon and to cause mussel contamination by paralytic toxins when toxin concentrations exceed the threshold of 80 g.100 g^{-1} of flesh accepted by local public health authorities.

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Bibliography

Anderson D.M., C.A. Stock, A. Keafer, A.B. Nelson, B. Thompson, D.J.Jr. Mc Gillicuddy, M. Keller, P.A. Matrai, J. Martin, 2005 - *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. *Deep-Sea Res II*, **52** : 2522-2542.

Anderson D.M., A.D. Cembella, G.M. Hallegraeff, 2012 - Progress in understanding harmful algal blooms: Paradigm shifts and new technologies for research, monitoring, and management. *A. Rev. mar. Sci.*, **4** : 143-176.

Anglès S., A. Jordi, E. Garcés, G. Basterretxea, A. Palanques, 2010 - *Alexandrium minutum* resting cyst distribution dynamics in a confined site. *Deep-Sea Res II*, **57**: 210-221.

ANPE, 1990 - *Etude préliminaire de l'écologie du lac de Bizerte*. Rapport, Agence Nationale de Protection de l'Environnement, Tunis, 100 pp.

Armi Z., A. Milandri, S. Turki, B. Hajjem, 2011 - *Alexandrium catenella* and *Alexandrium tamarense* in the North Lake of Tunis: bloom characteristics and the occurrence of paralytic shellfish toxin. *Afr. J. aquat. Sci.*, **36** : 47-56.

Bejaoui B., 2009 - *Développement d'un modèle tridimensionnel couplé dynamique-écologie : application à la lagune de Bizerte*. Rapport de thèse spécialité en génie hydraulique, ENIT, Département hydraulique, 210 pp.

Bejaoui B., A. Harzallah, 2005 - *Modèle hydrodynamique de la lagune de Bizerte avec forçage saisonnier.* Atelier de Modélisation des Écosystèmes Marins, organisé par l'INSTM, 27 et 28 septembre 2005, Tunis.

Belmonte G., A. Miglietta, F. Rubino, F. Boero, 1997 - Morphological convergence of resting stages of planktonic organisms: a review. *Hydrobiologia,* **355** : 159-165.

Bolch C.J.S., 1997 - The use of sodium polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. *Phycologia,* **36** : 472-478.

Bouchouicha Smida D., I. Sahraoui, H. Hadj Mabrouk, A. Sakka Hlaili, 2012 - Dynamique saisonnière du genre *Alexandrium* (dinoflagellé potentiellement toxique) dans la lagune de Bizerte (Nord de la Tunisie) et contrôle par les facteurs abiotiques environnants. *C. r. Biologies,* **335** : 404-416.

Bravo I., M. Vila, M. Maso, R.I. Figueroa, I. Ramilo, 2008 - *Alexandrium catenella* and *Alexandrium minutum* blooms in the Mediterranean Sea: Toward the identification of ecological niches. *Harmful Algae,* **7** : 515-522.

Chou H.G., Y.M. Chen, C.Y. Chen, 2004 - Variety of PSP toxins in four culture strains of *Alexandrium minutum* collected from southern Taiwan. *Toxicon*, **43** : 337-340.

Dale B., 1986 - Life cycle strategies of oceanic dinoflagellates. *UNESCO tech. Pap. mar. Sci.*, **49** : 65-72.

Etheridge S.M., C. Roesler, 2005 - Effects of temperature, irradiance, and salinity on photosynthesis, growth rates, total toxicity, and toxin composition for *Alexandrium fundyense* isolates from the Gulf of Maine and Bay of Fundy. *Deep-Sea Res. II*, **52** : 2491-2500.

Fertouna-Bellakhal M., A. Dhib, B. Béjaoui, S. Turki, L. Aleya, 2014 - Driving factors behind the distribution of dinocyst composition and abundance in surface sediments in a western Mediterranean coastal lagoon: report from a high resolution mapping study. *Mar. Pollut. Bull.*, 84 : 347–362

Figueroa R.I., I. Bravo, E. Garcés, 2005 - Effects of nutritional factors and different parental crosses on the encystment and escystment of *Alexandrium catenella* (Dinophyceae) in culture. *Phycologia*, **44** : 658-670.

Franco J.M., P. Fernandez, 1993 - Separation of paralytic shellfish toxins by reversed phase high performance liquid chromatography with postcolumn reaction and fluorimetric detection. *Chromatographia,* **35** : 613-620.

Frehi H., A. Couté, G. Mascarell, C. Perrette-Gallet, M. Ayada, M.H. Kara, 2007 - Harmful and red-tide dinoflagellates in the Annaba bay (Algeria). *C. r. Biologies*, **330** : 615-628.

Genovesi B., D. Mouillot, T. Laugier, A. Fiandrino, M. Laabir, A. Vaquer, D. Grzebyk, 2013 - Influence of sedimentation and hydrodynamics on the spatial distribution of *Alexandrium catenella/tamarense* resting cysts in a shellfish farming lagoon hit by toxic blooms. *Harmful Algae*, **25** : 15-25.

Goodman D.K., 1987 - Dinoflagellate cysts in ancient and modern sediments. In : *The Biology of Dinoflagellates.* F.J.R. Taylor (ed.), Blackwell Scientific Publications, Oxford, pp : 649-722.

Guillard R.L.R., 1973 - Division Rates. In : *Handbook of Phycological Methods. Cultures Methods and Growth Measurements.* J.R Stein (ed.), Cambridge University Press, Cambridge, UK, pp : 290-311.

Guillard R.R.L., P.E. Hargraves, 1993 - *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia,* **32** : 234-236.

Gow**en R.J., P. Tett, K. Kennington, D.K. Mills, T.M. Shammon, B.M. Stewart, N. Greenwood, C. Flanagan, M. Devlin, A. Wither**, 2008 - The Irish Sea: is it eutrophic? *Estuar. coast. Shelf Sci.*, **76** : 239-254.

Hallegraeff G.M., 1998 - Transport of toxic dinoflagellates via ships' ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Mar. Ecol.-Prog. Ser*., **168** : 297-309.

Harrison P.J., R. Waters, F.J.R. Taylor, 1980 - A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.,* **16** : 28-35.

Harzallah A., 2003 - Transport des polluants dans la lagune de Bizerte simulé par un modèle de circulation de l'eau. *Bull. Inst. natl sci. tech. Mer Salammbô,* **30** : 115-133.

Hays G.C., A.J. Richardson, C. Robinson, 2005 - Climate change and marine plankton. *Trends Ecol. Evol*., **20** : 337-344.

Horner R.A., C.L. Greengrove, K.S. Davies-Vollum, J.E. Gawel, J.R. Postel, A.M. Cox, 2011 - Spatial distribution of benthic cysts of *Alexandrium catenella* in surface sediments of Puget Sound, Washington, USA. *Harmful Algae*, **11** : 96-105.

Hwang D.F., Y.H Lu, 2000 - Influence of environmental and nutritional factor on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. *Toxicon*, **38** : 1491-1503.

Ichimi K., T. Suzuki, A. Ito, 2002 - Variety of PSP toxin profiles in various culture strains of *Alexandrium tamarense* and change of toxin profile in natural *A. tamarense* population. *J. expl mar. Biol. Ecol.,* **273** : 51-60.

Igniatiades L., 2012 - Mixotrophic and heterotrophic dinoflagellates in eutrophic coastal waters of the Aegean Sea (eastern Mediterranean Sea). *Botanica mar*., **55** : 39-48.

Joyce L.B., G.C. Pitcher, A.D. Randt, P.M.S. Monteiro, 2005 - Dinoflagellate cysts from surface sediments of Saldanha Bay South Africa: an indication of the potential risk of harmful algal blooms. *Harmful Algae*, **4** : 309-318.

Kamens J., O. Pilkey, P. Whalting, 1984 - Etude sédimentologique des lacs de Bizerte et de Tunis. *Notes Serv. géol. Tunisie,* **49** : 5-25.

Kim C.H., Y. Sako, Y. Ishida, 1993 - Comparison of toxin composition between populations of *Alexandrium spp*. from geographically distant areas. *Nippon Suisan Gakkaishi,* **59** : 641-646.

Laabir M., Y. Collos, E. Masseret, D. Grzebyk, E. Abadie, V. Savar, M. Sibat, Z. Amzil, 2013 - Influence of Environmental Factors on the Paralytic Shellfish Toxin Content and Profile of *Alexandrium catenella* (Dinophyceae) Isolated from the Mediterranean Sea. *Mar. Drugs,* **11** : 1583-1601.

Lacasse O., A. Rochon, S. Roy, 2013 - High cyst concentrations of the potentially toxic dinoflagellate *Alexandrium tamarense* species complex in Bedford Basin, Halifax, Nova Scotia, Canada. *Mar. Pollut. Bull*., **66** : 230-233.

Lavoie D.M., L.D. Smith, G.M. Ruiz, 1999 - The potential for intracoastal transfer of non-indigenous species in the ballast water of ships. *Estuar. coast. Shelf Sci*., **48** : 551-564.

Lilly E.L., D.M. Kulis, P. Gentien, D.M. Anderson, 2002 - Paralytic shellfish poisoning toxins in France linked to a human-introduced strain of *Alexandrium catenella* from the western Pacific: evidence from DNA and toxin analysis. *J. Plankt. Res.,* **24** : 443-452.

Luglié A., M.G. Giacobbe, A. Sannio, F. Fiocca, N. Sechi, 2003 - First record of the dinoflagellate *Alexandrium catenella* (Dinophyta), a potential producer of paralytic shellfish poisoning, in Italian waters (Sardinia, Tyrrhenian Sea). *Bocconea,* **16** : 1045-1052.

Montresor M., 2001 - To what extent are dinoflagellate life histories important for HABs? In : *LIFEHAB Life histories of microalgal species causing harmful blooms*. E. Garcés, A. Zingone, M. Montresor, B. Reguera, B. Dale (eds), Report of a European workshop, 24-27 October 2001, at Calvià, Majorca, Spain, pp : 18-21.

Niimi A.J., 2004 - Role of container vessels in the introduction of exotic species. *Mar. Pollut. Bull*., **49** : 778-782.

Ouakad M., 2007 - *Genèse et évolution des milieux laguno-lacustres du Nord-Est de la Tunisie (Garaet el Ichkeul, Lagunes de Bizerte et de Ghar el Melh*. Thèse de Doctorat d'Etat sciences Géologiques. Université de Tunis El Manar, Faculté des Sciences de Tunis, 461 pp.

Sahraoui I., 2010 - *Ecologie, diversité et écotoxicité des diatomées du genre* Pseudo-nitzschia *H. Peragallo dans la lagune de Bizerte*. Thèse de doctorat. Faculté de science de Bizerte, Tunisie, 267 pp.

Tobin E.D., R.A. Horner, 2011 - Germination characteristics of *Alexandrium catenella* cysts from surface sediments in Quartermaster Harbor, Puget Sound, Washington, USA. *Harmful Algae*, **10** : 216-223.

Turell E.A., J.P. Lacaze, L. Stobo, 2007 - Determination of paralytic shellfish poisoning (PSP) toxins in UK shellfish. *Harmful Algae,* **6** : 438-448.

Turki S., N. Balti, 2005 - Detection of toxic *Alexandrium catenella* (Whedon & Kofoid) Balech in clam production zone of North Lake and Channel, Tunisia. *Harmful Algae News,* **28** : 1-2.

Turki S., N. Balti, H. Ben Jannet, 2007 - First bloom of dinoflagellate *Alexandrium catenella* in Bizerte Lagoon (northern Tunisia). *Harmful Algae News,* **35** : 7-9.

Varela D., J. Paredes, C. Alves-de-Souza, M. Seguel, A. Sfeir, M. Frangopulos, 2012 - Intraregional variation among *Alexandrium catenella* (Dinophyceae) strains from southern Chile: Morphological, toxicological and genetic diversity. *Harmful Algae*, **15** : 8-18.

Wang D.Z., S.G. Zhang, H.F. Gu, L.L. Chan, H.S. Hong, 2006 - Paralytic shellfish toxin profiles and toxin variability of the genus *Alexandrium* (Dinophyceae) isolated from the Southeast China Sea. *Toxicon*, **48** : 138-151.

Zingone A., H.O. Enevoldsen, 2000 - The diversity of harmful algal blooms: a challenge for science and management. *Ocean coast. Mgmt*, **43** : 725-748.

Zmerli Triki H., O. Kefi Daly-Yahia, D. Malouche, Y. Komiha, A. Deidun, M. Brahim, M. Laabir, 2014 - Distribution of resting cysts of the potentially toxic dinoflagellate *Alexandrium pseudogonyaulax* in recently-deposited sediment within Bizerte Lagoon (Mediterranean coast, Tunisia). *Mar. Pollut. Bull.*, **84** : 172-181.

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