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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

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ABSTRACT

Species composition and abundance of dinocysts in relation to environmental factors were studied at 123 stations of surface sediment in Bizerte Lagoon. Forty-eight dinocyst types were identified, mainly dominated by *Brigantidinium simplex*, *Votadinum spinosum*, *Alexandrium pseudogonyaulax*, *Alexandrium catenella*, and *Lingulodinium machaerophorum* along with many round brown cysts and spiny round brown cysts. Cysts ranged from 1276 to 20126 cysts g^{-1} dry weight sediment. Significant differences in cyst distribution pattern were recorded among the zones, with a higher cyst abundance occurring in the lagoon's inner areas. Redundancy analyses showed two distinct associations of dinocysts according to location and environmental variables. Ballast water discharges are potential introducers of non-indigenous species, especially harmful ones such as *A. catenella* and *Polysphaeridium zoharyi*, with currents playing a pivotal role in cyst distribution. Findings concerning harmful cyst species indicate potential seedbeds for initiation of future blooms and outbreaks of potentially toxic species in the lagoon.

1. Introduction

Bizerte Lagoon remains the only valuable region of bivalve mollusc production in developing Tunisia. However, over the last 10 years it has suffered from intensive blooms caused by harmful microalgae, mainly dinoflagellates (Turki, 2004; Turki et al., 2007, 2014; Sahraoui et al., 2009), causing massive bivalve mortality. Several technico-administrative entities involving managers, politicians and scientists have therefore provided a weekly assessment of the lagoon's environmental conditions (e.g. oxygen, temperature, ciliates, phytoplankton…) (Sakka et al., 2006; Smida et al., 2012; Turki et al., 2014) in order to develop remedial action when the health of bivalve molluscs and fish appear menaced. Currently, 81 out of 2500 known modern dinoflagellate species are recognised as harmful, causing water discolouration and intoxication to aquatic fauna, and have been known to cause outbreaks of harmful algal blooms (HABs). Many of them can form cysts at some point in their life cycle (Matsuoka and Fukuyo, 1995). Deposited in sediments, these cysts indicate which species of motile stages are present in the water column and can thus provide information about the planktonic populations found there, the cysts being recognised as a seedbed for bloom initiation (Dale, 1983). However, no comprehensive study has been conducted in the area nor have any photographic plates of resting benthic cysts been produced, obscuring our understanding of their putative roles in bloom initiation in the lagoon.

Approximately 200 modern marine dinoflagellate species produce organic walled and calcareous cysts at some point during their sexual or asexual life cycle (Head, 1996). In some toxic dinoflagellate species (e.g. *Alexandrium*), cysts contain more toxins than their vegetative counterparts (Oshima et al., 1992). Similarly, the transfer of resting stages of the toxic dinoflagellates may contaminate previously unaffected coastal regions.

Bizerte Lagoon with its complex habitats may therefore provide a physical environment suitable for a diverse dinocyst community. It contains a maritime zone (MZ) in the northern and western areas, including the docks and navigation channel of Bizerte Harbour, plus an inner zone (IZ) in the central and eastern areas where four oyster and mussel farms are located. In the MZ, dinocyst distribution is expected to be affected by increasing international marine navigation which may introduce non-indigenous

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dinoflagellates, especially cysts, via ballast water since maritime navigation is the greatest dissemination vector for exotic species. In contrast, the IZ is located in a heavily populated area and is subjected to environmental pollution from sewage, industrial and agricultural run-off and other human activities. It is therefore of interest to explore how cysts spatially distribute under these specific conditions and how this translates into terms of cyst richness. Thus, the relationship between dinocysts and environmental conditions has been well documented around the world (Zonneveld, 1997a,b; Marret and Zonneveld, 2003; Radi and de Vernal, 2004; Radi et al., 2007; Shin et al., 2007; Pospelova et al., 2008). In particular, paleo-environmental and ecological studies of dinoflagellate cysts in eutrophicated or industrial areas are being undertaken with increasing frequency (Saetre et al., 1997; Dale et al., 1999; Matsuoka, 1999; Pospelova et al., 2002, 2005; Matsuoka et al., 2003; Dale, 2009). Many dinoflagellates can be extremely toxic and have been known to cause outbreaks of harmful algal blooms (HABs) in coastal waters around the world (Hallegraeff, 1993; Anderson et al., 2012; Feki et al., 2013). For instance, *Alexandrium* cysts contain PSP toxin levels several-fold higher than those in motile cells (Oshima et al., 1992). Many species of this genus are among the most harmful algal blooms to be found in Bizerte Lagoon (Turki et al., 2014), causing paralytic shellfish poisoning that is a threat to human health (Taylor et al., 1995, Anderson et al., 2012, Abdennadher et al., 2012). Unfortunately, the absence of dinocyst studies in the lagoon has left us with no cyst bank to serve as a record of past blooms or as a tool to predict future ones.

Recent studies have been undertaken in an attempt to understand the relationship between environmental conditions and dinoflagellates in the various confined coastal regions around the world (Zonneveld, 1997a,b; Zonneveld et al., 2000; Marret and Zonneveld, 2003; Radi and de Vernal, 2004; Radi et al., 2007; Shin et al., 2007; Pospelova et al., 2008). The composition of dinoflagellate resting cyst assemblages in recent sediments has been examined in only a few studies in the Mediterranean Sea (Rubino et al., 2000, 2002; Giannakourou et al., 2005; Zonneveld et al., 2009; Satta et al., 2013). In this region, incidence of dinoflagellate blooms has increased in the recent past due to increased human interference, aquaculture and naval activities (Saetre et al., 1997; Dale et al., 1999; Matsuoka, 1999; Pospelova et al., 2002, 2005; Matsuoka et al., 2003; Dale, 2009) and many harmful blooms of species belonging to the genus *Alexandrium* have been reported. The complex habitats in this lagoon may provide a physical environment suitable to support bloom dynamics (Turki et al., 2014).

In this context, we investigated dinocysts in Bizerte Lagoon in relation to both environmental factors and hydrodynamic conditions. Our purpose is to: (1) give detailed descriptions and illustrations of cysts and also to seek the presence of non-indigenous morphotypes possibly introduced by ship ballasts (Ruiz and Carlton, 2003), (2) provide information on the distribution and abundance of dinocysts, (3) assess seedbeds in potential risk areas and (4) identify the potential driving factors of cyst distribution.

Our intent in this study is also to provide the first high resolution mapping of cysts in Bizerte Lagoon, so as to help aquaculture farm managers and to serve as a basis for future studies of dinoflagellate dynamics in the lagoon.

2. Materials and methods

2.1. Study area

Bizerte Lagoon is a semi-enclosed area located in northern Tunisia (37°8′–37°14′N; 9°46′–9°56′E) (Fig. 1). Connected to the Mediterranean Sea through a 6 km-long channel, and to Ichkeul Lake to the west through the 5 km-long Tinja River, the lagoon has a surface area of 130 km^2 and a mean depth of 8 m. It receives freshwater input (20 $Mm^3\,yr^{-1}$) from several surrounding rivers and from Ichkeul Lake. The lagoon is under stress from a plethora of human activities such as naval and commercial shipping, increasing industrial activity (cement factory, steel mill, tyre factories, etc.) and open-air waste-dumping sites on its shores.

During summer, the surface sediments of the northern and eastern sectors of Bizerte Lagoon exhibit a facies range of silty–sandy–muddy to sandy–silty–muddy (fraction > 63 μm represents 80–90% of the total). The percentage of the fine fraction increase in sediments in the area of the river mouths (the Tinja, for example) exhibits a facies of muddy–silty–sandy (the fine fraction representing 64% of the total). The western and central areas of the lagoon exhibit a muddy–silty to muddy facies throughout the year, with the fine fraction representing up to 98% of the total (Ben Garali et al., 2008).

2.2. Sampling

Sampling was carried out in June 2012 at 123 stations, 1 km equidistant (Fig. 1 and Table 1). Stations 1–40 and 41–123 were located in the MZ and the IZ, respectively. Dinocyst distribution was expected to be heavily influenced by marine navigation in the MZ and by environmental pollution in the IZ. Sediment was collected from the top 3 cm with a Van Veen Grab Sampler (Yamaguchi et al., 1995) and the water column was sampled by means of a submersible pump. Temperature, salinity and pH were measured with a WTW 25i multiparameter probe. Sediment samples were placed in dark plastic containers, hermetically sealed to prevent any germination and stored in the dark at 4 °C. Laboratory analysis of nutrients (ammonium, nitrite, nitrate, phosphate and silicate) were by means of a BRAN and LUEBBE type 3 autoanalyser while concentrations were determined colourimetrically using a UV–visible (JENWAY 6705) spectrophotometer (APHA, 1992). Water samples for chlorophyll *a* analysis were filtered by vacuum filtration onto Whatman GF/F glass fibre filters (pore size: 0.45 μm) which were immediately stored at −20 °C. Chlorophyll *a* analyses were performed by HPLC according to Pinckney et al. (2001).

To identify the impact of hydrodynamics on cyst distribution, current measurements were achieved concordantly by the deployment of two current-meters: an Argonaut (MD-1500 kHz) and an ADCP (Acoustic Doppler Current Profiler; 500 kHz). The Argonaut was deployed at 4 m depth opposite Menzel Abderrahmen Harbour, so as to estimate the average current speed in the layer 0–2 m. The ADCP was deployed at 10 m depth in the eastern area near the mussel aquaculture station STL. Measurements were taken in 4 cells of 2 m each. Through these current measurements we sought to better understand the circulation in the inner part of the lagoon and its potential impact on cyst diversity. Furthermore, a comparison between our current measurements and simulations shows that the model already computed reproduces our current measurements well (Béjaoui, 2009; MeHSIP-PPIF, 2011). Additional details concerning model equations, current measurements and validation are found elsewhere (INSTM, 2002; Harzallah, 2003; Béjaoui et al., 2008).

2.3. Cyst processing and indentification

Dinocysts were separated from the sediment fraction according to the modified gradient density method using Ludox CLX as described in Genovesi et al. (2007) modified from Erard-Le Denn and Boulay (1995) and Yamaguchi et al. (1995). We checked only the surface sediment and empty cysts were not considered. Subsamples (1 g) were suspended in 20 ml of sucrose 24% and sonicated for 3 min at 100 Hz in a Wiseclean DAIHAN sonicator. The sonicated suspension was sieved through 120 μm and 20 μm mesh