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First evidence of cell deformation occurrence during a *Dinophysis* bloom along the shores of the Gulf of Tunis (SW Mediterranean Sea)

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ABSTRACT

Never before observed or cited in *Dinophysis* studies, deformations in *Dinophysis acuminata* and *Dinophysis sacculus* are reported throughout their cellular division phases (cytokinesis, and sulcal list regeneration) in 5 *in situ* cell cycle studies in the Punic harbors of Carthage (northern Tunisia). Two types of deformation were observed: invaginations in the ventral and dorsal margin and protuberances at the base of the left sulcal list. No virus or bacteria were detected with Syber green stain. *In situ* division rates (μ) varied among seasons and stations for the same species. *D. acuminata* exhibited moderate (0.22 day^{-1}) to high (0.68 day^{-1}) μ rates which were however very low (0.02–0.17 day^{-1}) for *D. sacculus* in autumn and moderate (0.21–0.35 day^{-1}) in late spring. In 2009 the seasonal distribution of *Dinophysis* indicates maximum *Dinophysis cf. ovum* abundance in March and a high number of *D. acuminata* in early June, while in 2010 maximum abundance of the same species was found in mid-June.

Molecular and genetic studies and staining with specific fluorescent strains should be addressed to hopefully explain these *Dinophysis* cell deformations during their *in situ* division.

1. Introduction

The main gaps in knowledge concerning the biology and population dynamics of the genus of *Dinophysis* were reviewed from 1978 to 1998 (Yasumoto et al., 1978, 1980; Hallegraeff and Lucas, 1988; Lassus and Bardouil, 1991; Bravo et al., 1995; Steidinger and Tangen, 1996; Maestrini, 1998). Based on the different results of these studies, many new observations have been recorded since 2001, such as small and intermediate forms (Reguera and González-Gil, 2001), cell-cycle stages (Reguera et al., 2003) and feeding behaviour (Park et al., 2006). Recently, extensive progress has been made thanks to new sampling strategies (GEOHAB, 2008), the application of molecular and analytical techniques and, finally, the successful establishment of mixotrophic cultures of *Dinophysis* fed with the ciliate *Mesodinium rubrum* (Park et al., 2006). In spite of the long history of this genus (Zingone et al., 1998), many difficulties have been encountered in its taxonomic identification. Their identification is therefore principally based on the size, shape and ornamentation of the large hypothecal plates which give the cell its contour and the shape of the left sulcal lists with their three supporting ribs (Larsen and Moestrup, 1992). However, each species of *Dinophysis*, in each biogeographic region, may exhibit different sizes and shapes between the large vegetative specimens and small gamete-like cells, resulting from their polymorphic life cycles with different cell-cycle phases and feeding behaviors (Reguera and González-Gil, 2001; Reguera et al., 2003). Reguera et al. (2012) considered that the "*Dinophysis acuminata* complex", including the morphospecies described as *D. acuminata, Dinophysis sacculus* and *Dinophysis cf. ovum*, is the most common group of *Dinophysis* spp., with strains whose abundance is increasing throughout the world along coasts receiving freshwater input, and over long growing seasons (spring to autumn). In Tunisia, frequent proliferations of *D. sacculus* are associated with diarrheic toxins detected in clams and mussels in the country's northern coastal waters, as in Bizerte Lagoon (Turki et al., 2014) and in Tunis North Lagoon (Armi et al., 2011). In the Punic harbors of Carthage (northern Tunisia) every species of this group bloomed each year from 2008 to 2010 in the same periods, intriguingly exhibiting ventral and dorsal margin deformations. In this study we provide

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the first evidence of *Dinophysis* cell deformations observed during both one seasonal and 5 diel cycles, matching its numerical increase between 2008 and 2010 at three study stations in the Punic harbors.

2. Materials and methods

2.1. Study area

The Punic harbors of Carthage (36°50′ N 10°19′ E) are located in the southern area of the Gulf of Tunis near the Tunis North Lagoon and Radès Harbor. They are composed of two basins that cover an area of about 8 ha, connected to each other and to the Bay of Tunis by channels (Fig. 1). These coastal basins are shallow and enclosed, with an average depth of 3.20 m and only a slight exchange with the Bay of Tunis. They are considered eutrophic as they receive increasing nutrient loads from a human population in the region that has rapidly expanded since the 1990s. According to Souissi et al. (2000), the western shore of the Gulf of Tunis is generally eutrophic, as they observed a relative nutrient enrichment both here and in the harbors (Radès and La Goulette) due to urban and industrial discharges through the Rades channel and the influence of terrestrial input.

2.2. Sampling and processing

The present study is based on samples from the study of 5 diel cycles carried out essentially in early summer (June 3rd and 4th, 2008; June 15th and 16th, 2009; June 26th and 27th, 2010), and mid-autumn (November 2nd and 3rd, 2008; October 30th and 31st, 2009). Due to the shallow harbor depths, samples were collected by vertical net hauls from the entire water column using a 20-μm mesh at the three stations: station S1 (mean depth, 5.5 m), situated at the point of water exchange between the Gulf of Tunis and the north basin (larger than the southern point of connection), station S2 (3 m), corresponding to the north basin and station S3 (2.3 m), in the south basin. Temperature and salinity were simultaneously measured at each station. Chlorophyll *a* was extracted in 10 ml of 90% acetone for 24 h, in the dark at −4 °C and the extract concentration was analyzed spectrophotometrically (UV-visible spectrophotometer PU-8800).

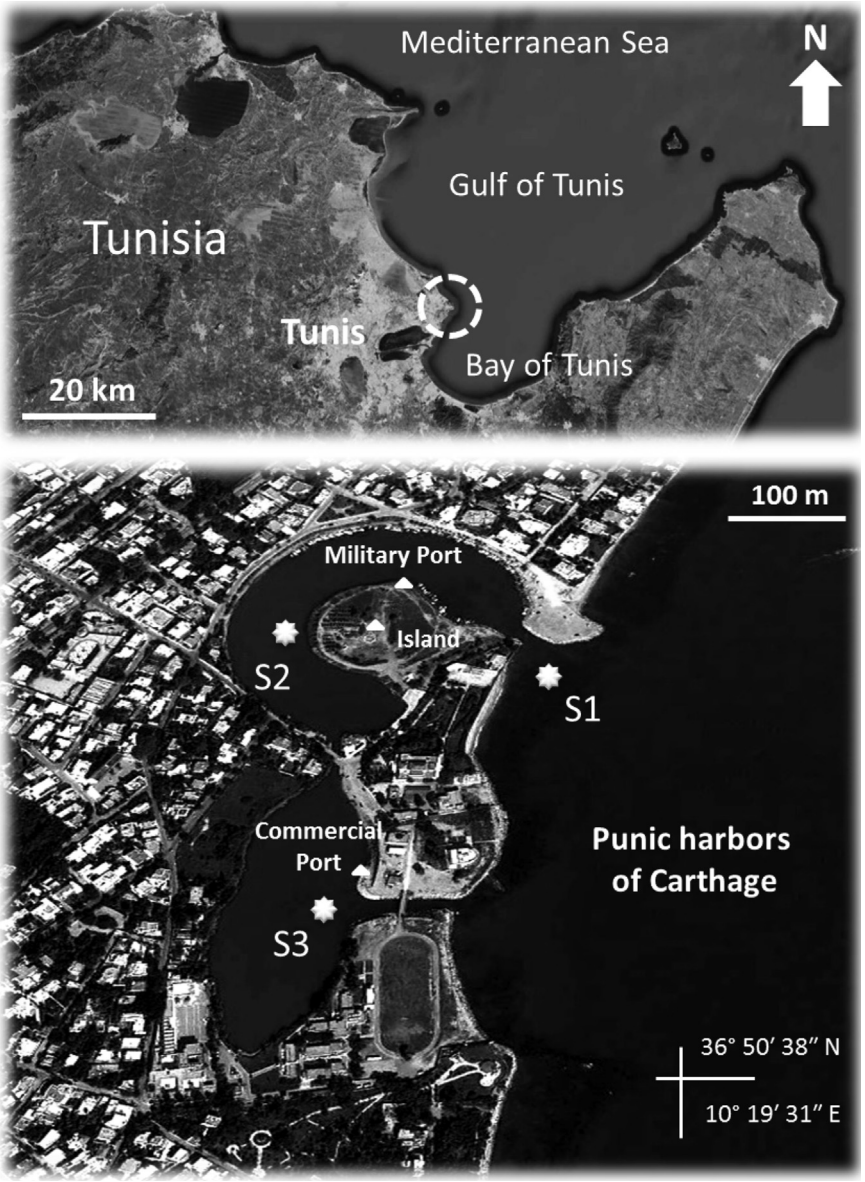

Fig. 1. Sampling site.