DIVERSITY AND TOXICITY OF *PSEUDO-NITZSCHIA* FROM TUNISIAN WATERS (SW MEDITERRANEAN)

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Abstract
The occurrence of potentially toxic diatoms was investigated in the northern Tunisian waters from 2011 to 2013. These microalgae were present over almost all the sampling period and exhibited pronounced blooms in spring, summer or autumn. Several species of *Pseudo-nitzschia* (*P. calliantha, P.* cf *seriata* and *P. mannii)* were identified as the causative organisms of the blooms. Some *Pseudo-nitzschia* species (*P. fryxelliana, P. hasleana and P.* cf. *seriata*) were observed for the first time in Mediterranean waters. The ability of identified strains to produce domoic acid in culture was assessed by LC-MS/MS. The toxicity of *P. calliantha* was confirmed whereas our investigation represents the first report that *P. hasleana* and *P. mannii* produce DA, bringing the total number of toxic *Pseudo-nitzschia* species to 21

Keywords: Biodiversity, South-Western Mediterranean, Blooms, Diatoms, Toxins

Introduction
Toxic and potentially toxic (PT) diatoms that mainly belong to the genus *Pseudo-nitzschia* have received considerable scientific attention, because 19 of the 45 known species can produce the potent neurotoxin, domoic acid (DA) [1]. Blooms of toxic diatoms are increasing in frequency and magnitude in several coastal Mediterranean waters and thus, may harm human health and represent a serious threat to the economy of aquacultured and wild shellfish industries. Therefore, the occurrence of toxic diatoms, their diversity and toxicity were investigated in economically important shellfish culture areas in the northern Tunisia

Materials and Methods
Diatom samples were collected monthly, from August to October 2011 and from March 2012 to April 2013 in the Lagoon, the Bay and the Channel of Bizerte. Several strains of PT diatoms were isolated, from seawater, and cultured in f/2 medium. The batch cultures were maintained at 20 °C, 100 µmol photons m^{-2} s^{-1} and 12h light:12h dark. The isolated strains were examined by ETM. Furthermore, in some cases strains were characterized molecularly. DA analyses were carried out on stationary-phase cells of strains established in culture, using the rapid resolution LC-MS/MS method (detection limit @0.02 ng DA ml^{-1}).

Results and discussion
Blooms of PT diatoms were found in spring (April), summer (July) and autumn (September or October), with pronounced peak (10^5–10^6 cells l^{-1}), as was previously observed in French and Italian coastal waters. Five *Pseudso-nitzschia* species were distinguished. *P. calliantha* dominated the blooms in September 2011(>10^5 cells l^{-1}). The species has a widespread distribution in the Mediterranean Sea, where it has been shown to be toxic. Our investigations confirm also its toxicity (1.56 ng DA L^{-1}). *P. fryxelliana* and *P. hasleana* were reported in September and January 2012, respectively, but at relatively low concentrations (10^3 cells l^{-1}). Both species have not been reported previously in Mediterranean waters, thus expanding our knowledge of their distribution. *P. mannii,* which was newly observed along the Catalan coast of Spain [2], was observed only in spring 2012. The LC-MS/MS technique revealed the toxicity of *P. hasleana* (1.28–7.29 ng DA l^{-1}) and *P. mannii* (5.73 ng DA l^{-1}). Previously, both species have been reported as non-toxic [2; 3] Thus, this is the first report that *P. hasleana* and P. mannii are toxigenic, bringing the total number of toxic *Pseudo-nitzschia* species to 21. The presence of *P.* cf *seriata* in Tunisian waters was unexpected, as the species is associated with cold waters [1]. P. cf. *seriata* has bloomed at Bizerte Bay in April 2013 (>10^5 cells l^{-1}), when the temperature was low (@10ºC). Although the species had been found to be toxic in others areas, no DA was detected by LC-MS/MS in strains isolated from northern Tunisian waters.

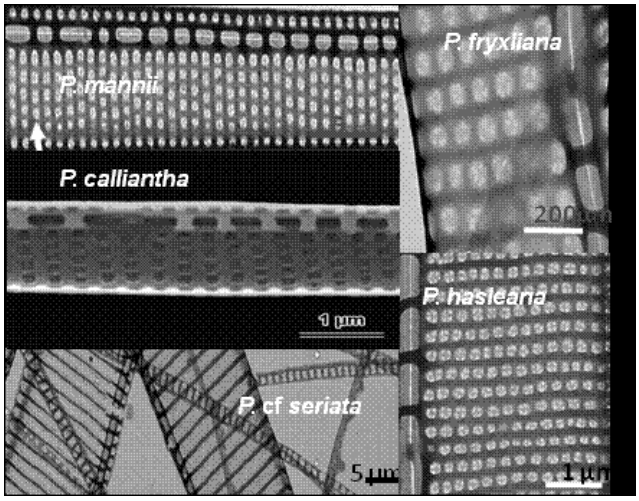

Fig. 1. *Pseudo-nitzschia* species identified in northern Tunisian waters

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