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Dynamics of *Pseudo-nitzschia* spp. and domoic acid production in a macrotidal ecosystem of the Eastern English Channel (Normandy, France)

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Temporal variations in the *Pseudo-nitzschia* assemblage were characterized in the Baie des Veys (Normandy, France) from July 2007 to July 2008. This study describes the succession of *Pseudo-nitzschia* species. Six species were identified, *Pseudo-nitzschia americana*, *P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. multistriata*, and *P. pungens* and their occurrences were related to physical, chemical and biological factors. *Pseudo-nitzschia* spp. were observed during the whole year, the higher abundance (38×10^3 cell L^{-1}) and diversity were found in September while the lowest (0.9×10^3 cell L^{-1}) were observed from October to the end of February. *P. americana* and *P. australis* were only seen in September and were positively correlated with high chlorophyll a, temperature and $Si(OH)_4$ concentrations. *P. delicatissima* and *P. fraudulenta* were more abundant under high NO_3 and low $Si(OH)_4$ concentrations and associated with low chlorophyll a concentrations. *P. multistriata* appeared in October and in November 2007 and its abundance was correlated with NH_4 while *P. pungens* abundance was not related to any environmental factors tested suggesting high acclimation capacities. In September 2007, *P. australis* was observed and domoic acid (7.1 ng DA L^{-1}) was measured in the phytoplankton samples. During this period, the phytoplankton population was mostly limited by PO_4 and NO_3 concentrations. On 17 September a heavy rainfall (15.6 mm) affected the nutrient stocks resulting in $Si(OH)_4$ and NO_3 limitations. Our results suggest that this $Si(OH)_4$ limitation might have promoted the production of domoic acid by *P. australis*. This study presents the first results on *Pseudo-nitzschia* successions and particulate domoic acid concentrations on the French coasts. This work especially highlights the beginning of autumn as a potential risk period for ASP events linked to *P. australis* development in the Baie de Seine.

1. Introduction

The marine diatom of *Pseudo-nitzschia* has a cosmopolitan distribution (Hasle, 2002; Fryxell and Hasle, 2003; Casteleyn et al., 2008). Some *Pseudo-nitzschia* species are known to produce domoic acid (DA), a neurotoxine responsible for amnesic shellfish poisoning (ASP) in sea birds, marine mammals and humans when accumulated through the trophic web (Bates et al., 1989; Bates, 2000; Fehling et al., 2004a). *Pseudo-nitzschia* spp. harmful algal blooms (HABs) are frequently observed in North America: California, Washington, Bay of Fundy, Prince Edward Island, British Colombia (Hallegraeff, 2003). More recently, *Pseudo-nitzschia* HAB events were described in Europe: Western Spain (Miguez et al., 1996), Western Scotland (Campbell et al., 2001; Gallacher et al., 2001; Fehling et al., 2004a), Ireland (Cusack et al., 2002), and France (Nezan et al., 2006). These events are frequently observed in coastal areas and have deep socio-economic impacts on shellfish farming or harvesting and fishermen.

The French shellfish farming production (oysters, mussels, scallops, and clams) is one of the most important in Europe with an annual production of 200,000 tonnes. About a quarter of this production comes from the Baie de Seine in the English Channel (45,000 tonnes year^{-1}) mainly from the Baie des Veys (BDV), which is a small bay within the Baie de Seine, where the production of oysters and mussels represent 10,700 tonnes per years. In autumn 2004, the French phytoplankton monitoring network (REPHY) detected domoic acid (DA) above the EU-regulatory limit of 20 μg DA g^{-1} wet weight of tissue in king scallops (*Pecten maximus*) from the Baie de Seine. Scallops harvest (7000–10,000 tonnes per years) in this area represents 50–70% of the national production. Shellfish harvesting sites were closed for several months during the annual king scallops fishing period. In November 2004, Nezan et al. (2006) found a few empty frustules of *Pseudo-nitzschia* species in sediment of many affected sites in the Baie de Seine whereas neither in bottom waters nor at the surface *Pseudo-nitzschia* species were observed. Three species were

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identified in the sediment samples: *P. pungens*, *P. multiseries* and *P. australis*. As a consequence, since this event, *Pseudo-nitzschia* has focused of much attention in this coastal area.

Recent studies (Kaczmarska et al., 2005, 2007; Schnetzer et al., 2007; Almandoz et al., 2008) have shown the importance of a variety of factors such as salinity (Thessen et al., 2005), nutrient concentrations (Bates et al., 1993; Caroppo et al., 2005; Spatharis et al., 2007), pH (Lundhlom et al., 2004) or photoperiod (Fehling et al., 2006) on *Pseudo-nitzschia* growth and distribution. All *Pseudo-nitzschia* species are not able to produce DA. Toxic ones do not synthesize DA all the time. Currently, 11 or 12 species of *Pseudo-nitzschia* were identified as potentially toxic (Bates and Trainer, 2006). Environmental factors influence largely DA production. Silicates ($Si(OH)_4$) or phosphates (PO_4) limitations are known to increase DA production in some species (Bates et al., 1991; Pan et al., 1996a,b; Fehling et al., 2004b) while nitrates (NO_3) limitation seems to decrease it (Bates et al., 1991). Other studies, performed on cultures, demonstrated that iron and copper limitations, as well as elevated copper concentrations, induce DA production in both *P. multiseries* and *P. australis* (Maldonado et al., 2002; Wells et al., 2005). Many scenarii can then explain *Pseudo-nitzschia* bloom events and DA production in natural ecosystem. Furthermore, a same species can react differently to environmental factors. For example, *P. calliantha* was observed in various sites at different seasons and temperatures. In Adriatic Sea, *P. calliantha* bloom appeared during February when the water temperature was between 9 and 11 °C (Caroppo et al., 2005); in the Danish North sea from July to November when the water temperature was between 16 and 19 °C (Lundholm et al., 1997); and in southern France from May to August when the water temperature was between 15 and 20 °C (Quiroga, 2006). These few examples demonstrate that specific and detailed studies have to be performed to understand the occurrences of *Pseudo-nitzschia* spp. in a particular area. Concerning the Baie de Seine at the site of the BDV, previous studies regarding phytoplankton population dynamics were conducted since 2002 (Jouenne et al., 2005, 2007; Pannard et al., 2008). Jouenne et al. (2007) established the seasonal pattern of microalgal succession in the BDV and identified more than 150 species. They observed that the local phytoplankton population was largely dominated by diatoms (60%) and by Dinophyta (19%). Jouenne et al. (2005) and Pannard et al. (2008) showed how short-term events like wind or rain can influence phytoplankton dynamics and production in this shallow Bay. Moreover, Marin Leal et al. (2008) using isotopic signatures established that phytoplankton was the larger contributor of oyster's (*Crassostrea gigas*) feeding before microphytobenthos and detritus, in the BDV.

In this context, the occurrence of *Pseudo-nitzschia* species and DA production were studied as a function of environmental parameters during one year between July 2007 and July 2008 in the BDV. The annual succession of *Pseudo-nitzschia* was established with a focus on a toxic event observed in September 2007.

2. Materials and methods

2.1. Study area and sampling

Samples were collected in the Baie des Veys (BDV) in Normandy (North of France) in the western part of the Baie de Seine (Fig. 1). The BDV is a macrotidal estuary with a maximal tidal range of 8 m and a small intertidal area of 35 km^2 (Ducrotoy and Sylvand, 1991). Freshwater inputs derive from the discharge of four rivers, notably the main river Vire with an annual mean discharge of 15 $m^3 s^{-1}$. Previous studies (Jouenne et al., 2007) showed that the sampling station represents properly the dynamic of the whole BDV and that the main source of nutrients in this bay comes from rivers. Samples were collected three times a week during three weeks in autumn (September 2007) and spring (June 2008); and monthly between both periods (July 2007 to July 2008, except during March 2008). Sampling was always performed at high tide at the same location

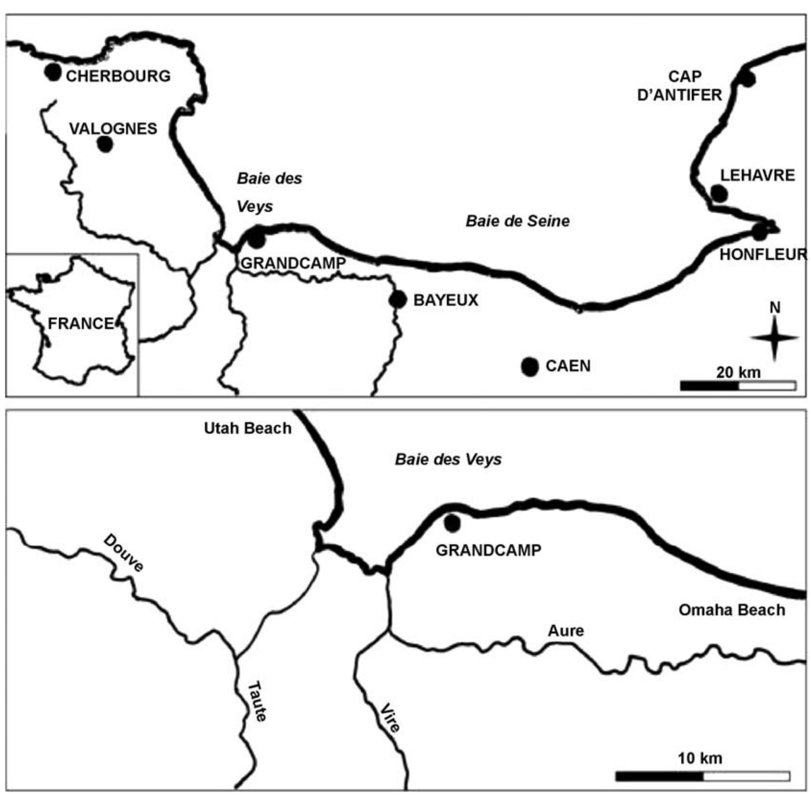

Fig. 1. Map of the Normandy region and the Baie des Veys.