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Invited Feature

Pseudo-nitzschia sp. diversity and seasonality in the southern North Sea, domoic acid levels and associated phytoplankton communities

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ABSTRACT

The diversity, toxicity and seasonality of *Pseudo-nitzschia* sp. were investigated from February to November 2012 in the southern Bight of the North Sea (SBNS) along the French coast. The identification of *Pseudo-nitzschia* species in this area was addressed for the first time in this study. Our results revealed a low species richness (3 distinct species) in association with moderate (102 pg mL^{-1}) to high (263 pg mL^{-1}) domoic acid (DA) levels in autumn and spring, respectively.

Pseudo-nitzschia succession corresponded to the dominance of *P. delicatissima* in April–May (86% of total diatoms) as a co-occurring species of the *Phaeocystis globosa* bloom. Following the *Phaeocystis* bloom (May–September), *P. pungens* dominated markedly over *P. fraudulenta* and *P. delicatissima* and was the only species present in autumn, although at low abundance (< 1000 cell L^{-1}). The results of this study support the idea that *Pseudo-nitzschia* seasonality in the SBNS relies principally on temperature and nutrient availability (DIN and silicates), which, in turn, depend on locally fluctuating environmental conditions (rainfalls and winds). This study highlights the potential for the SBNS to be a potential risk area in regard to the possible impacts of DA on marine resources and the DA transfer through marine food webs. This is of particular concern since DA concentration in seawater was not systematically correlated to potentially toxic *Pseudo-nitzschia* abundance.

1. Introduction

The cosmopolitan diatom genus *Pseudo-nitzschia* sp. comprises more than 50 species amongst which 24 are known to produce Domoic Acid (DA; Gai et al., 2018; Lundholm, 2018), a potent neurotoxin responsible for Amnesic Shellfish Poisoning (ASP). Since the first reports of ASP in 1987 at Prince Edward Island, shellfish stocks have been systematically monitored to prevent any outbreaks of ASP among humans. Since then, alert indicators such as DA concentration and/or *Pseudo-nitzschia* sp. abundance monitoring have been shown to be efficient tools, as no human poisoning has ever been reported (Trainer et al., 2012). However, DA poisoning of marine mammals and seabirds can still occur when they feed on DA-contaminated planktivorous prey (e.g. anchovies and sardines; Du et al., 2016; Gibble et al., 2018; Jensen et al., 2015; Lefebvre et al., 2002; Louw et al., 2018; Scholin et al., 2000; Sierra-Beltran et al., 1997; Stauffer et al., 2012). The trend of increasing abundance of *Pseudo-nitzschia* over the past decade has raised scientific awareness and concerns about public health (e.g. Hernández-Fariñas et al., 2014; Lefebvre et al., 2014; Lundholm et al., 2010; Parsons et al., 2002; Trainer et al., 2012) emphasizing the need to assess its dynamics, and investigate potential DA production in exploited coastal areas. In the English Channel, occurrence of DA in king scallop (*Pecten maximus*) has been reported several times from south western regions such as the Bay of Seine and the Bay of Brest (Husson et al., 2016). In the Eastern English Channel-North Sea (EEC-NS), reported ASP events were mainly concentrated in the northern regions (Scotland, Norway, Denmark; Trainer et al., 2012) although *Pseudo-nitzschia* sp. cells are present year-round in the southern regions (Bresnan et al., 2015; Hernández-Fariñas et al., 2014; Schapira et al., 2008; Seuront et al., 2006). Thus, reports of DA and shellfish closures were not recorded in the EEC-NS until 2014, when king scallop stocks off the Bay of Somme (France) were contaminated with DA, which represented the northernmost ASP alert of France (Lefebvre A., pers. com.). Existing studies and monitoring surveys often limit the detection of *Pseudo-nitzschia* sp. to the genus level or to morphological features measurable by optical microscopy, *i.e.* valve width, distinguishing large species (width > 3 µm, *Pseudo-*

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nitzschia complex *seriata*), from narrow ones (width < 3 µm, *P. delicatissima* complex). This distinction by size categories has already made possible seasonal studies within this genus (Bresnan et al., 2015; Brown and Bresnan, 2008; Fehling et al., 2004; Thorel et al., 2017) as well as allowing for analysis of the short and long-term changes in *Pseudo-nitzschia* with regard to environmental parameters (Díaz et al., 2014; Husson et al., 2016; McKibben et al., 2015).

In the Eastern English Channel and Southern Bight of the North Sea, phytoplankton studies have largely focused on spring bloom periods, revealing that diatoms and the prymnesiophytes *Phaeocystis globosa* form the bulk of phytoplankton biomass (Bonato et al., 2015; Schapira et al., 2008; Seuront et al., 2006). However, there is still a clear lack of knowledge in this region regarding *Pseudo-nitzschia* species diversity and seasonality in relation to environmental parameters and DA levels. Finally, studies defining *Pseudo-nitzschia* species nutrient and physical requirements and biotic interactions at the regional scale are scarce but crucial to predict proliferation and associated toxicity. Recently, Husson et al. (2016) identified the environmental niche of *Pseudo-nitzschia* sp. in six distinct bays from Brest to the Seine estuary. In this study, additional data focusing on *Pseudo-nitzschia* seasonality and diversity in the EEC-NS ecosystem over an annual cycle is provided. The aim is to define their importance in the seasonal phytoplankton succession and to estimate associated DA concentrations with regard to environmental forcing and hydro-biological conditions.

2. Material and methods

2.1. Sampling strategy

The sampling station (~10 m depth) was located along the French coast of the southern North Sea, in Dunkirk harbor (51°1′12″ N, 1°9′0″E, Fig. 1). The inner part of this harbor forms a small semi-enclosed embayment where semi-diurnal macrotidal tides prevail and enable important water exchanges with coastal waters of the southern North Sea. Sampling was conducted fortnightly from February 22 to November 14 2012 during flood (within 2 h before high tide) to consider only neritic plankton communities although benthic communities could be re-suspended during high mixing periods.

Temperature (°C) and salinity were measured with an Aanderaa Instruments probe, turbidity (Turb) with an Eutech instruments waterproof probe, pH with a HANNA pH probe, and dissolved oxygen (dO_2, mg L^{-1}) with a Handy Polaris Oxyguard probe at each sampling date. Seawater samples were collected at 1 m depth using a Niskin bottle. For inorganic nutrients, seawater samples (frozen at −20 °C until analysis) were analysed by either fluorimetry (NH_4^+; Trilogy, Turner Designs; Holmes et al., 1999) or by the use of an autoanalyzer (Alliance Integral Futura) for NO_2^-, NO_3^-, HPO_4^{2-}, $Si(OH)_4$ following standard protocols (Bendschneider and Robinson, 1952; Mullin and Riley, 1955; Murphy and Riley, 1962). The surface solar irradiance (ssi) was derived from the bi-directional reflectance measured by MSG (METEOSAT Second Generation) and provided by the OSI SAF (Le Borgne et al., 2006). Winds (hourly measurements) and rainfall (daily) data were obtained from the Dunkirk Meteo France station (www.meteofrance.fr).

2.2. Phytoplankton standing stock and community composition

Phytoplankton biomass was assessed from chlorophyll *a* and pheopigment concentrations. Seawater samples (250–500 mL) were filtered on glass fiber filters (Whatman GF/F) under low vacuum and frozen at −20 °C until analysis (within 2 months after collection). Pigments were extracted in 90% acetone overnight at 4 °C and chlorophyll *a*, and pheopigments concentrations were estimated following Lorenzen (1966) using a pre-calibrated (chlorophyll *a* from *Anacystis nidulans*, Sigma) fluorometer (Trilogy, Turner designs). For phytoplankton community analyses, 250 mL samples were preserved in the field with lugol/glutaraldehyde fixative (2% final concentration, Verity et al., 2007) and stored at 4 °C in the dark until analysis (within 3 months after collection). At the laboratory, 5–10 mL subsamples were settled in Hydrobios counting chambers. Phytoplankton cells were identified and enumerated using an inverted microscope (Nikon Eclipse TE2000-S, magnification × 200, × 400) under phase contrast illumination. On average 942 ± 568 cells per sample were identified and counted. For *Phaeocystis globosa*, total cells were enumerated without stage distinction (i.e. isolated, colonial stages). Under optical microscopy, *Pseudo-nitzschia* cells were categorized on the basis of cell width. More

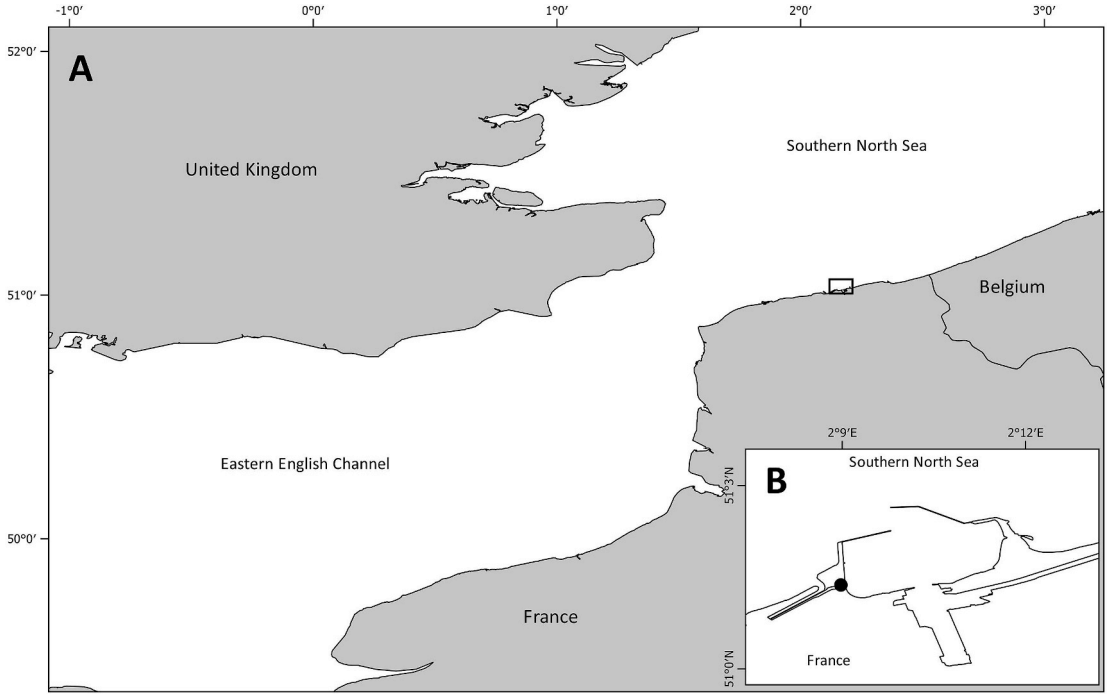

Fig. 1. A – Location of the study area. B – Location of the sampling station (black dot) in Dunkirk harbor (Southern Bight of the North Sea).