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## ***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<sup>-1</sup>) to high (263 pg mL<sup>-1</sup>) 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<sup>-1</sup>). 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.

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## Highlights

► Low *Pseudo-nitzschia* sp. diversity (three species) in the southern North Sea. ► Domoic acid levels reached 263 pg mL<sup>-1</sup> in May 2012, 83% as dissolved DA. ► Strong species seasonality driven by temperature and nutrient availability (DIN and silicates). ► Autumnal DA concentrations could be attributed to *P. pungens*. ► DA concentration was not systematically correlated to *Pseudo-nitzschia* spp. abundance.

**Keywords** : *Pseudo-nitzschia*, Southern North Sea, Domoic acid, *Phaeocystis globosa*, Harmful algal blooms (HAB)

## 39 **1 Introduction**

40 The cosmopolitan diatom genus *Pseudo-nitzschia* sp. comprises more than 50 species  
41 amongst which 24 are known to produce Domoic Acid (DA; Gai et al., 2018; Lundholm,  
42 2018), a potent neurotoxin responsible for Amnesic Shellfish Poisoning (ASP). Since the first  
43 reports of ASP in 1987 at Prince Edward Island, shellfish stocks have been systematically  
44 monitored to prevent any outbreaks of ASP among humans. Since then, alert indicators such  
45 as DA concentration and/or *Pseudo-nitzschia* sp. abundance monitoring have been shown to  
46 be efficient tools, as no human poisoning has ever been reported (Trainer et al., 2012).  
47 However, DA poisoning of marine mammals and seabirds can still occur when they feed on  
48 DA-contaminated planktivorous prey (e.g. anchovies and sardines; Du et al., 2016; Gible et  
49 al., 2018; Jensen et al., 2015; Lefebvre et al., 2002; Louw et al., 2018; Scholin et al., 2000;  
50 Sierra-Beltran et al., 1997; Stauffer et al., 2012). The trend of increasing abundance of  
51 *Pseudo-nitzschia* over the past decade has raised scientific awareness and concerns about  
52 public health (e.g. Hernández-Fariñas et al., 2014; Lefebvre et al., 2014; Lundholm et al.,  
53 2010; Parsons et al., 2002; Trainer et al., 2012) emphasizing the need to assess its dynamics,  
54 and investigate potential DA production in exploited coastal areas. In the English Channel,  
55 occurrence of DA in king scallop (*Pecten maximus*) has been reported several times from  
56 south western regions such as the Bay of Seine and the Bay of Brest (Husson et al., 2016). In  
57 the Eastern English Channel-North Sea (EEC-NS), reported ASP events were mainly  
58 concentrated in the northern regions (Scotland, Norway, Denmark; Trainer et al., 2012)  
59 although *Pseudo-nitzschia* sp. cells are present year-round in the southern regions (Bresnan et  
60 al., 2015; Hernández-Fariñas et al., 2014; Schapira et al., 2008; Seuront et al., 2006). Thus,  
61 reports of DA and shellfish closures were not recorded in the EEC-NS until 2014, when king  
62 scallop stocks off the Bay of Somme (France) were contaminated with DA, which represented  
63 the northernmost ASP alert of France (Lefebvre A., pers. com.). Existing studies and

64 monitoring surveys often limit the detection of *Pseudo-nitzschia* sp. to the genus level or to  
65 morphological features measurable by optical microscopy, *i.e.* valve width, distinguishing  
66 large species (width > 3 µm, *Pseudo-nitzschia* complex *seriata*), from narrow ones (width < 3  
67 µm, *P. delicatissima* complex). This distinction by size categories has already made possible  
68 seasonal studies within this genus (Bresnan et al., 2015; Brown and Bresnan, 2008; Fehling et  
69 al., 2004; Thorel et al., 2017) as well as allowing for analysis of the short and long-term  
70 changes in *Pseudo-nitzschia* with regard to environmental parameters (Díaz et al., 2014;  
71 Husson et al., 2016; McKibben et al., 2015).

72 In the Eastern English Channel and Southern Bight of the North Sea, phytoplankton studies  
73 have largely focused on spring bloom periods, revealing that diatoms and the  
74 prymnesiophytes *Phaeocystis globosa* form the bulk of phytoplankton biomass (Bonato et al.,  
75 2015; Schapira et al., 2008; Seuront et al., 2006). However, there is still a clear lack of  
76 knowledge in this region regarding *Pseudo-nitzschia* species diversity and seasonality in  
77 relation to environmental parameters and DA levels. Finally, studies defining *Pseudo-*  
78 *nitzschia* species nutrient and physical requirements and biotic interactions at the regional  
79 scale are scarce but crucial to predict proliferation and associated toxicity. Recently, Husson  
80 et al. (2016) identified the environmental niche of *Pseudo-nitzschia* sp. in six distinct bays  
81 from Brest to the Seine estuary. In this study, additional data focusing on *Pseudo-nitzschia*  
82 seasonality and diversity in the EEC-NS ecosystem over an annual cycle is provided. The aim  
83 is to define their importance in the seasonal phytoplankton succession and to estimate  
84 associated DA concentrations with regard to environmental forcing and hydro-biological  
85 conditions.

## 86 **2 Material and Methods**

### 87 **2.1 Sampling strategy**

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

95 Temperature (°C) and salinity were measured with an Aanderaa Instruments probe, turbidity  
96 (Turb) with an Eutech instruments waterproof probe, pH with a HANNA pH probe, and  
97 dissolved oxygen (dO<sub>2</sub>, mg L<sup>-1</sup>) with a Handy Polaris Oxyguard probe at each sampling date.  
98 Seawater samples were collected at one meter depth using a Niskin bottle. For inorganic  
99 nutrients, seawater samples (frozen at -20 °C until analysis) were analyzed by either  
100 fluorimetry (NH<sub>4</sub><sup>+</sup>; Trilogy, Turner Designs; Holmes et al., 1999) or by the use of an  
101 autoanalyzer (Alliance Integral Futura) for NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, Si(OH)<sub>4</sub> following standard  
102 protocols (Bendschneider and Robinson, 1952; Mullin and Riley, 1955; Murphy and Riley,  
103 1962). The surface solar irradiance (ssi) was derived from the bi-directional reflectance  
104 measured by MSG (METEOSAT Second Generation) and provided by the OSI SAF (Le  
105 Borgne et al., 2006). Winds (hourly measurements) and rainfall (daily) data were obtained  
106 from the Dunkirk Meteo France station ([www.meteofrance.fr](http://www.meteofrance.fr)).

## 107 2.2 Phytoplankton standing stock and community composition

108 Phytoplankton biomass was assessed from chlorophyll *a* and pheopigment concentrations.  
109 Seawater samples (250 to 500 mL) were filtered on glass fiber filters (Whatman GF/F) under  
110 low vacuum and frozen at -20 °C until analysis (within 2 months after collection). Pigments  
111 were extracted in 90 % acetone overnight at 4°C and chlorophyll *a*, and pheopigments  
112 concentrations were estimated following Lorenzen (1966) using a pre-calibrated (chlorophyll

113 *a* from *Anacystis nidulans*, Sigma) fluorometer (Trilogy, Turner designs). For phytoplankton  
114 community analyses, 250 mL samples were preserved in the field with lugol/glutaraldehyde  
115 fixative (2 % final concentration, Verity et al., 2007) and stored at 4 °C in the dark until  
116 analysis (within 3 months after collection). At the laboratory, 5 to 10 mL subsamples were  
117 settled in Hydrobios counting chambers. Phytoplankton cells were identified and enumerated  
118 using an inverted microscope (Nikon Eclipse TE2000-S, magnification ×200, ×400) under  
119 phase contrast illumination. On average  $942 \pm 568$  cells per sample were identified and  
120 counted. For *Phaeocystis globosa*, total cells were enumerated without stage distinction (i.e.  
121 isolated, colonial stages). Under optical microscopy, *Pseudo-nitzschia* cells were categorized  
122 on the basis of cell width. More specifically, *Pseudo-nitzschia* cells with frustule width up to  
123  $3\mu\text{m}$  were placed in the *P. delicatissima* complex while those with frustules above  $3\mu\text{m}$  were  
124 placed in the *P. seriata* complex as was done in several studies (Bresnan et al., 2015; Fehling  
125 et al., 2004; Hasle and Syvertsen, 1997; Thorel et al., 2017).

126 When *Pseudo-nitzschia* sp. cell concentrations were higher than  $10^3 \text{ cell L}^{-1}$ , Scanning  
127 Electron Microscopic (SEM) analyses were carried out for species identification, covering the  
128 whole study period (April 4, May 2, May 16, June 1, September 5, October 3). In short, 10 to  
129 100 mL of homogenized preserved samples were filtered on polycarbonate filters (24 mm  
130 diam.;  $0.8 \mu\text{m}$  porosity) and rinsed with Milli-Q water to eliminate salts. Filters containing  
131 diatom frustules were acid cleaned (2 - 3 mL HCl 37%) in Teflon beakers overnight at room  
132 temperature and rinsed in a Milli-Q water bath. Acid and water baths were filtered once again  
133 using same filter to limit cell loss during the acidification and rinsing steps. Thereafter, filters  
134 were placed on aluminium stubs with double sticky carbon tabs and air-dried during 12 h  
135 under laminar flow. Lastly, dried filters were metallized under argon flow with Au/Pd during  
136 90 s (Polaron SC 7620). Quantified measures based on literature review (e.g., valve length  
137 and width, number of fibulae and of interstriae in  $10 \mu\text{m}$ ) allowed for the discrimination of the

138 species within *Pseudo-nitzschia seriata* complex (width > 3  $\mu\text{m}$ ) and *P. delicatissima*  
139 complex (width < 3  $\mu\text{m}$ ; see Table 1), but species variety could not be inferred from our  
140 study. At least 50 *Pseudo-nitzschia* sp. cells per sample were identified via SEM analyses.  
141 When present in lower numbers, the entire filter was scanned for *Pseudo-nitzschia* frustules  
142 and identification was carried out on available material. Dissolved and particulate domoic  
143 acid (DA;  $\text{pg mL}^{-1}$ ) concentrations were measured using ASP ELISA kits (Biosense, Bergen,  
144 Norway) following the provided procedure. Total DA concentration (tDA *i.e.*, dissolved +  
145 particulate DA) was measured from 5 mL of natural seawater samples stored at -20  $^{\circ}\text{C}$  until  
146 analysis. For dissolved DA, collected seawater samples (5 mL) were filtered under low  
147 vacuum pressure (< 50 mm Hg) on 0.2  $\mu\text{m}$  cellulose acetate filters before being frozen (-20  
148  $^{\circ}\text{C}$ ). On the day of the analysis, samples were thawed at 4  $^{\circ}\text{C}$  and dissolved DA samples were  
149 directly processed. For total DA samples, sonication on ice was carried out in order to disrupt  
150 cells, and samples were filtered on 0.2  $\mu\text{m}$  cellulose acetate before the assay. Particulate DA  
151 was obtained by subtracting dissolved DA from total DA. Given the limit of *Pseudo-nitzschia*  
152 cell counts via microscopy, DA cell quota were computed only when *Pseudo-nitzschia* cell  
153 abundance reached  $10^3$  cell  $\text{L}^{-1}$  (*i.e.* at least 5-10 *Pseudo-nitzschia* cells were counted).

### 154 2.3 Data analyses

155 In order to efficiently synthesize the environmental context of the survey and identify forcing  
156 parameters, a Principal Component Analysis (PCA) was applied to the environmental data  
157 matrix (11 parameters x 16 dates, Legendre and Legendre, 1998). Wind speed data from three  
158 days before the sampling date were averaged. Rainfalls were summed over 3 days before the  
159 sampling date in order to take into account watershed run-offs.

160 Nutrients concentrations of DIN (*i.e.* the sum of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations),  $\text{HPO}_4^{2-}$   
161 and  $\text{Si(OH)}_4$  concentrations were depicted in the PCA plots as N, P and Si, respectively.

162 Nutrient ratios (S/P, Si/N and Si/P) were considered as illustrative variables to highlight



163 potential nutrient limitations. Prior to the analysis, all data were tested for normality (Shapiro  
164 test) and  $\log(x+1)$  transformed.

165 *Pseudo-nitzschia* complex raw abundances were plotted on each PCA graph to give a picture  
166 of species seasonality. Only variables significantly correlated to at least one principal  
167 component (PC) of the PCA are presented and discussed (Spearman test, p-value <0.05).  
168 Correlation coefficients will be referred to as  $r'_{PC1}$ ,  $r'_{PC2}$ ,  $r'_{PC3}$  for the first three principal  
169 components of the PCA (Table 2). Additionally, correlations between *Pseudo-nitzschia*  
170 complexes abundances and domoic acid concentrations have been assessed. Since data did not  
171 fit the normality, correlations were tested using Spearman test (significance threshold was set  
172 at p-value<0.05). Statistics were performed using R software packages (R Core Team, 2012)

### 173 **3 Results**

#### 174 3.1 Environmental conditions

175 The present study began in the cold snap of February 2012 (temperature of minus 10°C) that  
176 had not occurred in the area since 1991 (MeteoFrance data; regional bulletin,  
177 [www.meteofrance.fr](http://www.meteofrance.fr)). The following spring was cooler (-1.0 °C) and drier (half as much  
178 rainfall) than average (Figure 2A, 2C). Stormy weather dominated the summer months (June-  
179 July) and autumn. Autumn 2012 was characterized by strong southwestern and northeastern  
180 winds (up to 100 km h<sup>-1</sup>), excess precipitations (+30%) and a rapid drop in temperature.  
181 Seawater surface temperature followed a typical seasonal evolution increasing from 4.9 °C in  
182 February to 19.0 °C in August. By contrast, as measured by flood tide, salinity remained  
183 stable with an average of  $34.2 \pm 0.3$  (min: 33.4, max = 35.0; Figure 2A). The mean annual pH  
184 value was  $8.1 \pm 0.3$  and decreased continuously over the study period from February (8.6) to  
185 November (7.6; Figure 2B). The mean annual dissolved oxygen concentration (dO<sub>2</sub>) was  $8.1 \pm$   
186  $1.3 \text{ mg L}^{-1}$  with the highest values recorded in winter and late autumn, and the lowest ( $6.4 \text{ mg}$   
187  $\text{L}^{-1}$ ) in late summer (Figure 2B). A slight increase in dO<sub>2</sub> concentrations coincided with the

188 spring bloom development in May. Turbidity averaged at  $6.9 \pm 3.2$  NTU (Figure 2C). The  
189 lowest turbidity values were measured in April and June (2.9 NTU) and the highest in May  
190 and September (14.3 and 12.1 NTU, respectively).  
191 With the exception of  $\text{NH}_4^+$ , all concentrations of nutrients decreased rapidly to reach their  
192 annual minima of 0.01, 0.35 and  $0.30 \mu\text{mol L}^{-1}$  for  $\text{NO}_2^- + \text{NO}_3^-$ ,  $\text{HPO}_4^{2-}$  and  $\text{Si}(\text{OH})_4$ ,  
193 respectively, in April (Figure 3). Thereafter,  $\text{HPO}_4^{2-}$  concentration varied between 0.5 and  $1.0$   
194  $\mu\text{mol L}^{-1}$  and the maximal values followed storm events (July and November).  $\text{NO}_2^- + \text{NO}_3^-$   
195 concentrations remained stable below  $5 \mu\text{mol L}^{-1}$  from April to October and increased to high  
196 values at the end of the survey ( $11.7 \mu\text{mol L}^{-1}$ ) although they did not reach the previous winter  
197 maxima of  $22.5 \mu\text{mol L}^{-1}$ . Silicate concentrations decreased from 7.5 to 0.3 between February  
198 and late March. A second minimal value ( $0.2 \mu\text{mol L}^{-1}$ ) was recorded in June. The silicate  
199 concentrations increased continuously during summer and reached  $7.5 \mu\text{mol L}^{-1}$  at the end of  
200 the survey.  $\text{NH}_4^+$  concentrations were highly variable from February to June ranging between  
201 1.0 and  $3.8 \mu\text{mol L}^{-1}$ . Thereafter, concentrations remained between 2.0 to  $4.0 \mu\text{mol L}^{-1}$ .

### 202 3.2 Phytoplankton standing stock, seasonality and domoic acid levels

203 The phytoplankton bloom, started at the end of February 2012 and lasted until early June. It  
204 was characterized by three successive peaks in chlorophyll *a* concentrations (from 4.0 to  $9.5$   
205  $\mu\text{g chl}a \text{ L}^{-1}$ ; Figure 4A), and phytoplankton abundance (from  $8.2 \times 10^5$  to  $3.6 \times 10^6$  cells  $\text{L}^{-1}$ ;  
206 Figure 4B). From July to November, chlorophyll *a* concentrations ( $2.3 \pm 0.7 \mu\text{g L}^{-1}$ ) along  
207 with phytoplankton abundance ( $1.9 \pm 0.7 \times 10^5$  cells  $\text{L}^{-1}$ ) remained relatively stable and low.  
208 Pheopigment concentrations followed the same pattern as chlorophyll *a* (Figure 5A) and  
209 represented 22 to 49 % of total pigments. Four periods could be identified based on  
210 phytoplankton community composition (Figure 4C): Period 1 (P1, February 20 – April 5) was  
211 characterized by the dominance of small diatoms (5-20  $\mu\text{m}$  in width/length; *Thalassiosira* sp.,  
212 *Skeletonema costatum*, *Asterionellopsis glacialis* and *Chaetoceros* sp.), either as isolated cells

213 or in forming colonies, and phytoplankton abundance peaked at  $8.2 \times 10^5$  cells  $L^{-1}$ . Period 2  
214 (P2, April 5 to May 20) corresponded to the *P. globosa* bloom with abundances ranging from  
215  $1.8$  to  $3.6 \times 10^6$  cells  $L^{-1}$  (Figure 4B). This bloom co-occurred with high abundances of  
216 *Chaetoceros* sp. ( $8.7 \times 10^4$  cells  $L^{-1}$ , 64 % of the diatom community at the beginning of P2),  
217 and *Pseudo-nitzschia* sp. ( $2.1 \times 10^5$  cells  $L^{-1}$  equivalent to 25% and 86% of the phytoplankton  
218 and the diatom communities, respectively; at the end of P2). Period 3 is the post-bloom period  
219 (P3, from May 20 to September 8). Both fine walled summer diatoms such as *Guinardia* sp.  
220 (i.e. *G. delicatula*, *G. flaccida*, and *G. striata*), and highly silicified spring diatoms such as  
221 *Chaetoceros* sp. and *Thalassiosira* sp. composed the bulk of the diatom community in  
222 association with small Cryptophytes. Period 4 (P4, from September 8 to November 16)  
223 revealed the occurrence of intermediate sized diatoms (30 - 40  $\mu m$  in length/width; *Delphineis*  
224 sp.) as well as diatoms in forming colonies (e.g., *Thalassiosira* sp.) which remained in low  
225 number ( $1.2 \pm 0.5 \times 10^5$  cells  $L^{-1}$ ).

226 SEM analyses revealed a low diversity of *Pseudo-nitzschia* sp. in the area with three distinct  
227 species namely *P. delicatissima* (belonging to the *P. delicatissima* complex), *P. fraudulenta*,  
228 and *P. pungens* (both belonging to the *P. seriata* complex, see Table 1 and Figure 5). No  
229 overlap between the two complexes was found in terms of species morphological features  
230 since the width of *P. delicatissima* width was below 2  $\mu m$ , and the widths of *P. pungens* and  
231 *P. seriata* were above 2.7  $\mu m$  (Table 1). *P. delicatissima* represented up to 99 % of SEM-  
232 identified frustules during the *P. globosa* bloom period (P2, Mid-May) and outnumbered  
233 other diatoms. By contrast, at the beginning of the post-bloom period (P3), the percentage of  
234 *Pseudo-nitzschia* sp. to total diatoms decreased to 10 % of total diatoms. *P. pungens* markedly  
235 dominated (54 % of the SEM-identified frustules) over *P. fraudulenta* and *P. delicatissima*  
236 (33 % and 13 %, respectively). In autumn (P4), all the identified *Pseudo-nitzschia* sp.

237 frustules belonged to the *P. pungens* species ( $< 10^3$  cells L<sup>-1</sup> representing  $< 1\%$  of total  
238 diatoms).  
239 Domoic acid was mostly present as dissolved DA throughout the survey averaging  $83 \pm 16 \%$   
240 of the total DA (tDA). Particulate domoic acid (pDA) was detected in May (end of P2) and  
241 was still measurable at the end of P4. pDA punctually represented 10 to 40 % of tDA (Figure  
242 6). tDA concentration followed *Pseudo-nitzschia* sp. dynamics, particularly during the *P.*  
243 *globosa* bloom and post-bloom periods (P2 and P3, Figure 7). tDA maxima ( $263 \text{ pg mL}^{-1}$ )  
244 corresponded to the co-occurrence of *P. seriata* complex and *P. delicatissima* complex,  
245 reaching  $2.8 \times 10^4$  and  $7.5 \times 10^5$  cells L<sup>-1</sup> in early May (P2), respectively. Thereafter, tDA  
246 decreased to 118 and 110  $\text{pg mL}^{-1}$  at the end of P2 and beginning of P3, which coincided with  
247 the highest abundance of *P. delicatissima* complex and *P. seriata* complex, respectively. In  
248 autumn, tDA concentrations remained high ( $102 \pm 39 \text{ pg mL}^{-1}$ ) despite low abundances of *P.*  
249 *seriata* complex (*i.e.* *P. pungens*), as suggested by the absence of correlation between tDA  
250 and *Pseudo-nitzschia* abundances. When measurable, DA cell quota ranged between 0.016  $\text{pg}$   
251  $\text{cell}^{-1}$  in May and 7.9  $\text{pg cell}^{-1}$  in September.

### 252 3.3 Abundance of *Pseudo-nitzschia* in relation to environmental factors

253 PCA confirmed the clear seasonal pattern of environmental variables and allowed to  
254 synthesize their relation with the temporal variability of *Pseudo-nitzschia* (Figure 7). The first  
255 three principal components (PC) explained 75.82 % of the total variance (PC1: 35.01 %, PC2:  
256 28.77 %, PC3: 12.04 %). Considering coefficients in the linear combination of variables,  
257 period P1 was mainly characterized by high DIN ( $r'_{\text{PC1}} = 0.88$ ) and dO<sub>2</sub> ( $r'_{\text{PC2}} = -0.75$ )  
258 concentrations, high N/P ratios ( $r'_{\text{PC1}} = 0.66$ ;  $r'_{\text{PC2}} = -0.50$ ) and low temperature and salinity  
259 ( $r'_{\text{PC2}} = 0.73$  and 0.64, respectively; Table 2). P2 (bloom period) was more related to high ssi  
260 ( $r'_{\text{PC1}} = -0.74$ ), pH ( $r'_{\text{PC2}} = -0.64$ ), and increases in temperature and salinity ( $r'_{\text{PC2}} = 0.73$  and  
261 0.64), low nutrient stocks, particularly Si(OH)<sub>4</sub> and HPO<sub>4</sub><sup>2-</sup> ( $r'_{\text{PC1}} = 0.69$  and 0.87), and to a

262 lesser extent low Si/P ratio ( $r'_{PC2}=0.44$ , Table 2). Additionally, punctual storm events in May  
263 characterized by high rainfalls ( $r'_{PC3}=0.61$ ) and by strong winds ( $r'_{PC3}=0.43$ ) led to a prompt  
264 increase in turbidity (Figures 3 and 7B). High temperature, DIN limitations illustrated by low  
265 N/P, and increasing Si/P ratio and  $\text{Si(OH)}_4$  concentrations characterized periods P3 and P4  
266 (Table 2).

267 The plotting of *Pseudo-nitzschia* abundance on the PCA graphs illustrated the importance of  
268 high rainfall, increasing pH, ssi in the initiation of the *P. delicatissima* complex bloom  
269 (Figures 7A and 7B). The seasonality of the *P. seriata* complex was essentially shown by  
270 their high abundance in June (beginning of P3) and their disappearance in August and  
271 September (end of P3; Figures 7C and 7D). The highest abundance of *P. seriata* complex was  
272 observed at low nutrient levels (particularly DIN and  $\text{Si(OH)}_4$ ), increasing surface solar  
273 irradiance and high pH. From September to November 2012 (P4), rainfalls and wind speed  
274 decreased, and *P. seriata* complex persisted at low abundance in increasing concentrations of  
275  $\text{Si(OH)}_4$ , decreasing pH and concentrations of  $\text{dO}_2$ . Finally, as illustrated by the distribution of  
276 abundance values on the PCA plots, both complexes were present under  $\text{Si(OH)}_4$  limitation  
277 and the *P. seriata* complex seemed to tolerate higher variable environmental conditions (e.g.  
278 nutrients) as compared to the *P. delicatissima* complex.

## 279 **4 Discussion**

### 280 4.1 Specificity of the 2012 spring bloom

281 In the southern North Sea - Eastern English Channel ecosystem, the phenology of  
282 phytoplankton communities is generally characterized by a phytoplankton spring bloom  
283 dominated by *Phaeocystis globosa* (Gentilhomme and Lizon, 1998; Lefebvre et al., 2011;  
284 Schapira et al., 2008). In 2012, the spring bloom appeared to be of low magnitude and short  
285 duration compared to other studies ( $\sim 20 \mu\text{g L}^{-1}$  in Breton et al., 2000 and Kesaulya et al.,  
286 2008 ;  $\sim 50 \mu\text{g L}^{-1}$  in Lefebvre et al., 2011; Seuront et al., 2006), as exemplified by low

287 chlorophyll *a* values ( $9.5 \mu\text{g L}^{-1}$ , Figure 4A). This small bloom magnitude was confirmed by  
288 the low *P. globosa* abundance (between  $0.019$  and  $3.7 \times 10^6 \text{ cell L}^{-1}$  in this study vs  $5.5$  to  
289  $100 \times 10^6 \text{ cell L}^{-1}$  in Gómez and Souissi, 2008; Seuront et al., 2006; Widdicombe et al., 2010)  
290 and its low contribution to total phytoplankton abundance ( $50\%$  vs  $74\text{-}90\%$  between 1992 and  
291 2011, Hernández-Fariñas et al., 2014). *P. globosa* dynamics can exhibit high interannual  
292 variability (Lamy et al., 2006; Lefebvre et al., 2011) and the year 2012 appeared as a low  
293 *Phaeocystis* year. The co-occurrence of the *P. delicatissima* complex and *P. globosa*  
294 development (Figure 4; Period P2) is often described: *P. delicatissima* complex is a co-  
295 occurring species of *P. globosa* bloom (Grattepanche et al., 2011; Hernández-Fariñas et al.,  
296 2014; Monchy et al., 2012; Sazhin et al., 2007) and may serve as solid substrate during the  
297 transitional phase from free-living cells to colonial stages (Peperzak and Gäbler-Schwarz,  
298 2012; Rousseau et al., 2007; Verity et al., 2007).

#### 299 4.2 Environmental factors drive *Pseudo-nitzschia* diversity and seasonality.

300 Three species of *Pseudo-nitzschia* were identified in 2012 in the Southern North Sea (*P.*  
301 *delicatissima*, *P. pungens* and *P. fraudulenta*) by using SEM imagery and measurements.  
302 These results are congruent with recent published data from adjacent areas (Eastern English  
303 Channel - Thorel et al., 2017, Northern North Sea - Bresnan et al., 2015). Identification was  
304 based on a literature review and best matches with species morphometric issues are specified  
305 in Table 1. The identification of *Pseudo-nitzschia* species is a tricky issue since some species  
306 can have very similar morphological characteristics (e.g. *P. delicatissima* with *P. dolorosa*, *P.*  
307 *decipiens* or *P. fraudulenta* with *P. subfraudulenta*, Amato et al., 2007; Orsini et al., 2004;  
308 Quijano-Scheggia et al., 2009). Further studies are therefore needed to confirm these  
309 identifications using molecular tools (e.g. Pugliese et al. 2017).

310 The seasonality of *Pseudo-nitzschia* species within the Eastern English Channel – North Sea  
311 (EEC-NS) ecosystem resulted in two distinct periods of abundance (spring and autumn)

312 during which a very small number of *Pseudo-nitzschia* species was observed. The three  
313 identified species (*P. delicatissima*, *P. pungens*, and *P. fraudulenta*) are considered to be  
314 cosmopolites (see Table 3; Hasle 2002 and Lelong et al. 2012 for reviews). They are also  
315 known to have different but variable hydrological preferences depending on their ecosystem  
316 inhabited, and genetic strains. Temperature and salinity values recorded in 2012 (4.9-19.0°C  
317 and 33.6-35.0, respectively; Figure 2A) are consistent with the euryhaline features of *Pseudo-*  
318 *nitzschia* spp. Most species are indeed able to grow at salinities comprised between 1 and >  
319 30, occurring and growing more efficiently at high salinity (Cho et al., 2001; Husson et al.,  
320 2016; Thessen et al., 2005). Prior studies have also demonstrated a wide temperature range for  
321 *Pseudo-nitzschia* spp. (Dortch et al., 1997; Louw et al., 2016; Tas and Lundholm, 2016). In  
322 particular, *P. delicatissima*, which was dominant in May (P2, 86% of the diatom community  
323 and 99 % of identified frustules) seemed to better grow at temperatures ranging between 10-  
324 12 °C (Table 3; Fehling et al. 2006). *P. pungens* and *P. fraudulenta* were commonly present  
325 in summer (June) and autumn (October) when days were longer and temperatures higher  
326 (Table 3). However, *P. pungens* seems to have a wider environmental tolerance than *P.*  
327 *fraudulenta* (Klein et al., 2010) as it can peak at temperatures up to 21.0 °C (Terenko and  
328 Terenko, 2012). The increasing relative contribution of *P. pungens* to *Pseudo-nitzschia*  
329 species between June (54 %) and October (100 %) may be explained by two non-conflicting  
330 hypotheses. Firstly, the *P. pungens* population may comprise different varieties exhibiting  
331 different temperature preferences (e.g. Kim et al. 2015). Secondly, it can present varying  
332 responses to temperature increases (Thessen et al., 2009).

333 *P. delicatissima* development began in February (P1) under high DIN concentrations, high  
334 N/P ratios and low temperature (i.e. late winter conditions; Figure 7A, 7B). It is well known  
335 that high abundances of *Pseudo-nitzschia* spp. are found in areas enriched with nutrients  
336 (Parsons et al., 2002; Tas et al., 2016) which can originate from upwelling (Díaz et al., 2014),

337 mixing events and riverine inputs (Trainer et al., 2012). Paradoxically, the highest abundance  
338 of *P. delicatissima* in May (P2) occurred after a decrease in DIN concentrations and rather  
339 suggested nutrient limiting conditions. Pulses of DIN (Figure 3A) following rainfall events in  
340 May (Figure 2C) could have favored the species rapid growth and bloom, a phenomenon  
341 which has already been shown in estuarine systems (Tas et al., 2016; Tas and Lundholm,  
342 2016). The results of this study also demonstrated that the maximum abundance of the *P.*  
343 *delicatissima* complex occurred at high pH levels and dO<sub>2</sub> concentrations (P2, Figure 7).  
344 Lundholm et al. (2004) suggested that high pH values (8.7 - 9.1) can inhibit the growth of  
345 most *Pseudo-nitzschia* species. Such high values were never recorded during our study (7.6 to  
346 8.6; Figure 3) and no significant relationships could be established between pH values and  
347 *Pseudo-nitzschia* abundance. Rather than being associated solely with the *P. delicatissima*  
348 complex, variability in dO<sub>2</sub> is likely associated with the photosynthetic activity of the whole  
349 phytoplankton community including the *Phaeocystis* sp. bloom (P2).  
350 High amount of particulate matter (i.e., high turbidity) linked to water mixing and river inputs  
351 can limit light availability and consequently inhibit photosynthesis and growth. This light  
352 attenuation can result in the limitation of both the spatial and temporal distribution of *Pseudo-*  
353 *nitzschia* as demonstrated in estuarine and coastal systems (Dursun et al., 2016; Tas et al.,  
354 2016). These studies are consistent with the *P. seriata* complex distribution pattern in the  
355 Southern Bight of the North Sea (SBNS), the species being abundant when turbidity was low  
356 (e.g. in June, Figure 2C, 7D). *P. delicatissima* could have benefited from the storm event  
357 (high wind and rainfall) which occurred in May and particularly from the subsequent pulses of  
358 DIN and Si(OH)<sub>4</sub> (Figures 3A and 7B).  
359 The *P. seriata* species complex was the most abundant (beginning of P3) in low DIN and  
360 SiOH<sub>4</sub> concentrations resulting in low Si/N ratios (figure 7C). These conditions may have  
361 been caused by the lag effect of *P. delicatissima* and the associated *P. globosa* bloom having



362 already drawn down the nutrients. The *P. seriata* species complex was still persisting at low  
363 abundance in the area from the end of the summer to the fall (end of P3 and P4). PCA plots  
364 suggest that the growth of the *P. seriata* complex may benefit from nutrient pulses of silicate,  
365 phosphate and DIN but that it tends to be limited at that time of the year by decreasing  
366 temperature and irradiance (Figure 7C, 7D).

367 Silicate limitations were already proven to increase *Pseudo-nitzschia* abundance and toxicity  
368 (Anderson et al., 2010; Thorel et al., 2017). For instance, *P. multiseriata* was able to  
369 outcompete other phytoplankton species when silicate was low compared to nitrogen  
370 (Sommer, 1994). Laboratory culture conditions conducive to DA production generally include  
371 limitation by silicate or phosphate during the stationary phase (reviewed by Bates et al., 1989;  
372 Trainer et al., 2012, 2008). This could explain this study findings of the high domoic acid  
373 concentration in spring (P2, May, Figure 6) which coincided with low Si and DIN  
374 concentrations along with low Si/P ratios (Figure 7A- C). However, no significant correlation  
375 could be demonstrated between nutrients stocks and DA concentrations. Consequently, more  
376 targeted studies (e.g., nutrient uptake experiments) are required in order to properly evaluate  
377 the nitrogen nutritional status and nitrogen preference (i.e.,  $\text{NO}_2^- + \text{NO}_3^-$  vs  $\text{NH}_4^+$  vs urea) for  
378 *Pseudo-nitzschia* populations, and thus the potential influence of nitrogen on bloom dynamics  
379 and toxicity in the EEC-NS.

#### 380 4.3 Domoic acid concentrations and *Pseudo-nitzschia* species

381 The existence of toxigenic and non-toxigenic strains of the same species has been mentioned  
382 recurrently for several *Pseudo-nitzschia* species (e.g. Sahraoui et al. 2011; Thessen et al.  
383 2009; Villac et al. 1993) Sahraoui et al., 2011; Thessen et al., 2009; Villac et al., 1993). Total  
384 DA concentrations measured during this study (max.  $263 \text{ pg mL}^{-1}$ ) were higher than several  
385 published values in the EEC-NS ecosystem (up to  $7 \text{ pg mL}^{-1}$ ; Downes-Tettmar et al. 2013;  
386 Klein et al. 2010) but remained lower than recent measurements in the Bay of Seine ( $1.4 \times 10^3$

387 pg mL<sup>-1</sup>, Thorel et al. 2017), and other ecosystems such as Monterey Bay (10×10<sup>3</sup> pg mL<sup>-1</sup>;  
388 Walz et al. 1994), the Gulf of Mexico (3.3×10<sup>3</sup> pg mL<sup>-1</sup>, Liefer et al. 2013) or Luanda Bay,  
389 Angola (1.4×10<sup>3</sup> pg mL<sup>-1</sup>, Blanco et al. 2010).

390 Although *Pseudo-nitzschia* abundance matched high DA concentration in spring, this  
391 observation did not hold for the end of summer and autumn periods (Figure 6). Other species  
392 (i.e. *Nitzschia navis-vargnica* and *N. bizertensis*) are known to produce DA in south-east Asia  
393 and Tunisia, respectively (Bouchouicha-Smida et al., 2015; Kotaki et al., 2000; Lundholm and  
394 Moestrup, 2000; Tan et al., 2016). Since neither species has been detected in North Atlantic  
395 waters (including the English Channel and the North Sea) or been observed during this 2012  
396 survey, it is likely that DA originate exclusively from *Pseudo-nitzschia* species.

397 Consequently, this result supports other studies where DA concentration in seawater  
398 (particulate and dissolved) or in shellfish does not always depend on *Pseudo-nitzschia*  
399 abundance (e.g. Bates et al. 1998; Bresnan et al. 2017; Klein et al. 2010; Lelong et al. 2012;  
400 Walz et al. 1994). During P2 and P3, high tDA concentrations coincided with the co-  
401 occurrence of two or three *Pseudo-nitzschia* species (i.e., *P. delicatissima* and *P. pungens*  
402 during P2 and *P. delicatissima*, *P. pungens* and *P. fraudulenta* during P3). Since these three  
403 species have been proven at least once to produce DA (Lelong et al., 2012), the formal  
404 identification of the species responsible for DA concentration cannot be addressed with  
405 certainty. Consistent with Downes-Tettmar et al. (2013), *P. pungens* was certainly toxic as it  
406 was the only *Pseudo-nitzschia* sp. present in autumn when DA concentrations reached 101 pg  
407 mL<sup>-1</sup>. *P. delicatissima* and *P. fraudulenta* were demonstrated to be weakly toxigenic in  
408 different ecosystems (Almandoz et al., 2017; Parsons et al., 2012; Rhodes et al., 1998).

409 Additionally, in the northern North Sea, *P. delicatissima* cells have not been observed to  
410 produce DA (Scottish Waters, (Fehling et al., 2006, 2004). The DA cell quotas estimated for  
411 the three samples in which particulate DA was associated with *Pseudo-nitzschia* abundance >

412  $10^3$  cell L<sup>-1</sup> ranged between 0.016 and 7.9 pg. cell<sup>-1</sup>. These DA cell quotas are comparable to  
413 values obtained for batch cultures of *P. fraudulenta* (0.03 pg.cell<sup>-1</sup>, Rhodes et al. 1998; 0.16  
414 pg.cell<sup>-1</sup>, Thessen et al. 2009) and *P. pungens* (0.2 to 10 pg. cell<sup>-1</sup>; Lelong et al. 2012). They  
415 are also congruent with DA cell quotas inferred from *in situ* surveys for *P. fraudulenta* (0.002  
416 to 0.120 pg. cell<sup>-1</sup>; Almandoz et al. 2017) and *P. delicatissima* (1.52 to 5.54 pg. cell<sup>-1</sup>, Parsons  
417 et al. 2012).

#### 418 4.4 DA implications in the ecosystem and challenges for future surveys

419 In this study, the high proportion of dissolved domoic acid year round (~83%) may have two  
420 main implications regarding phytoplankton communities succession and marine resources.

421 Dissolved domoic acid can indeed act as an effective Fe chelator, increasing its bioavailability  
422 and providing a competitive advantage to *Pseudo-nitzschia* sp. over other phytoplankton  
423 species for Fe assimilation (Prince et al., 2013; Rue and Bruland, 2001; Trick et al., 2010;  
424 Zabaglo et al., 2016). Other studies suggest that DA synthesis requires available Fe (e.g.  
425 Sobrinho et al. 2017). In both cases, accumulated iron in the polysaccharidic matrix of *P.*  
426 *globosa* (Schoemann et al., 2005) could have benefitted *Pseudo-nitzschia* sp. growth and DA  
427 production and could well explain its appearance every year, during and after the *P. globosa*  
428 bloom.

429 Regarding marine resources, dissolved DA is reported to alter bivalve larvae growth (Liu et  
430 al., 2007) and immunity (De Rijcke et al., 2015) and these effects could spread to population  
431 recruitment and picking activities. Dissolved DA is also reported to inhibit herbivorous  
432 feeding as shown for krill (Bargu et al., 2006) and copepod species (Tammilehto et al., 2015)  
433 modifying matter and energy fluxes within the trophic web. These elements suggest the  
434 potential for dissolved DA to impact marine resources throughout the year and to trigger long-  
435 term effects regarding growth and recruitment.

436 Despite its absence in spring, particulate DA appeared at significant level (10-40%) over a  
437 wide temporal window (May to December). During this period, *Pseudo-nitzschia* sp. could  
438 provide a non-negligible food source from which particulate DA could be directly transferred  
439 to higher trophic levels such as exploited filter feeders namely mussels or king scallops in the  
440 EEC-NS (Ambiaud and Lefebvre, 2017). Phytoplankton consumers such as *Noctiluca*  
441 *scintillans* and copepods could act as DA vectors/inducers (Escalera et al., 2007; Fock and  
442 Greve, 2002; Leandro et al., 2010; Maneiro et al., 2005; Tammilehto et al., 2015) and were  
443 shown to dominate the zooplankton community when particulate DA reached 40% of total  
444 DA in the summer. Our data suggest that the SBNS could be an at-risk area regarding DA  
445 contamination and/or exposure of marine resources to dissolved DA: the phytoplankton  
446 abundance alert threshold was reached at least once for the *P. delicatissima* complex during  
447 the 2012 survey (i.e. >300 000 cells L<sup>-1</sup>, (EC) No 853/2004 2004; (EC) No 854/2004 2004)  
448 concomitantly with significant DA concentrations in seawater. However, high domoic acid  
449 concentration was not always associated with high *Pseudo-nitzschia* abundance. This study at  
450 the seasonal scale allowed the identification of risky periods regarding species diversity and  
451 DA forms and concentrations in the SBNS ecosystem. Given the inter-annual variability of  
452 *Pseudo-nitzschia* species occurrence (e.g. Thorel et al. 2017), long term monitoring is  
453 fundamental to assess whether the species seasonality persist over years and whether it is  
454 always associated to the same toxicogenic species and environmental parameters.

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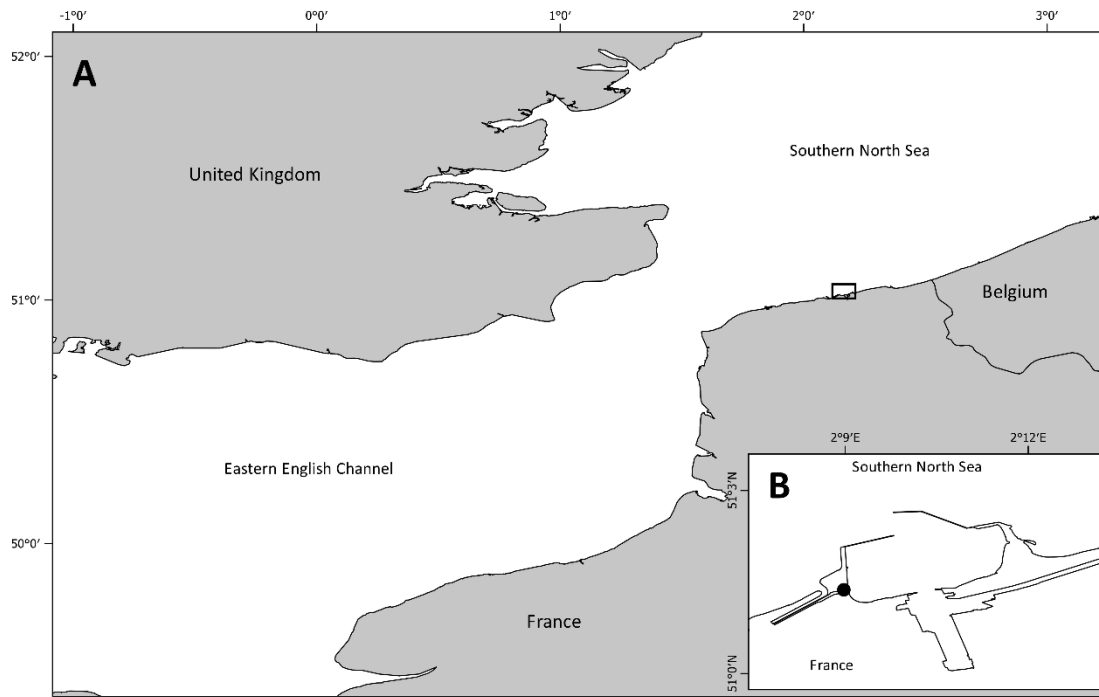
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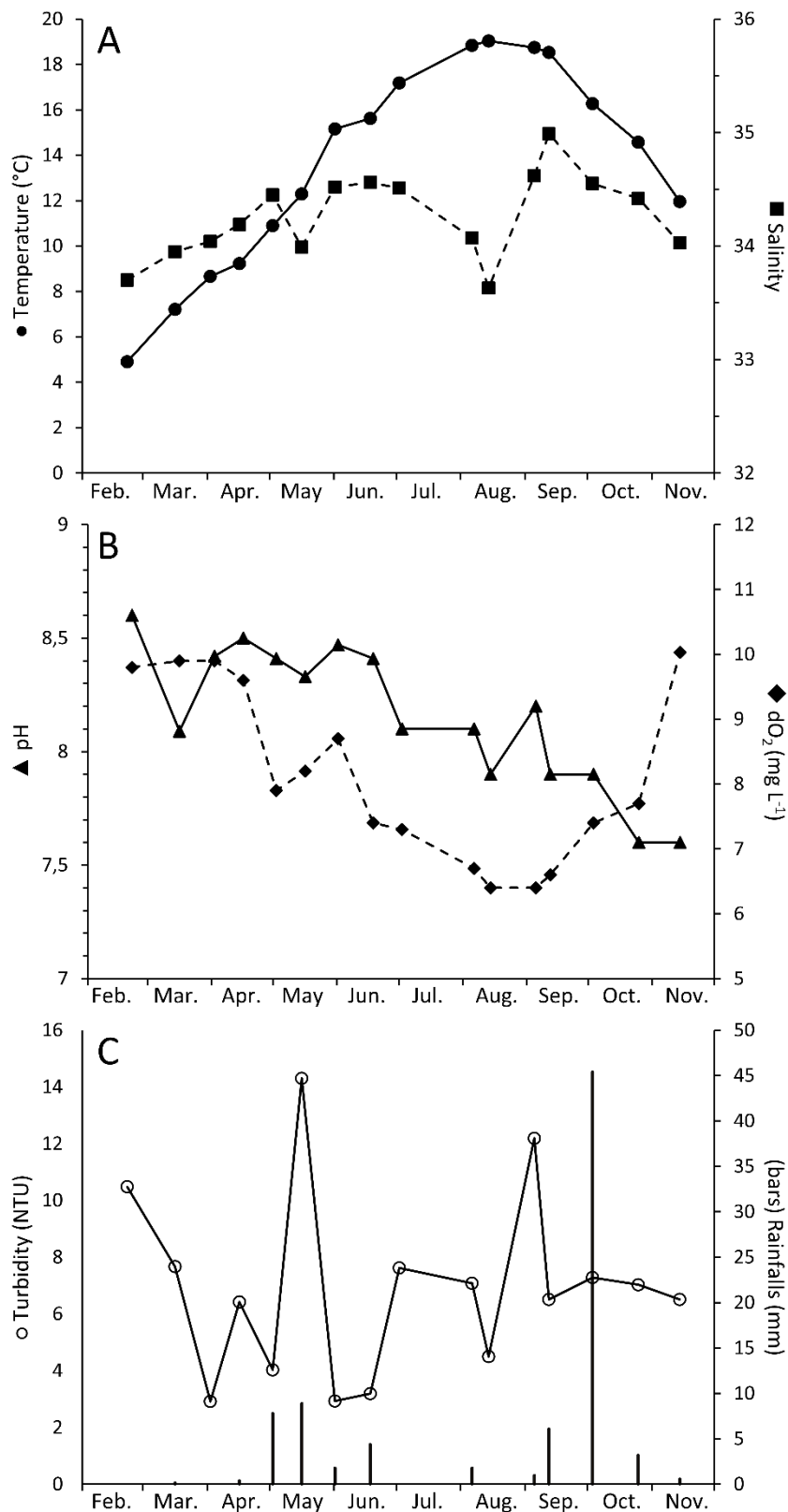
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793 Figure 1. A – Location of the study area. B – Location of the sampling station (black dot) in  
 794 Dunkirk harbor (Southern Bight of the North Sea).

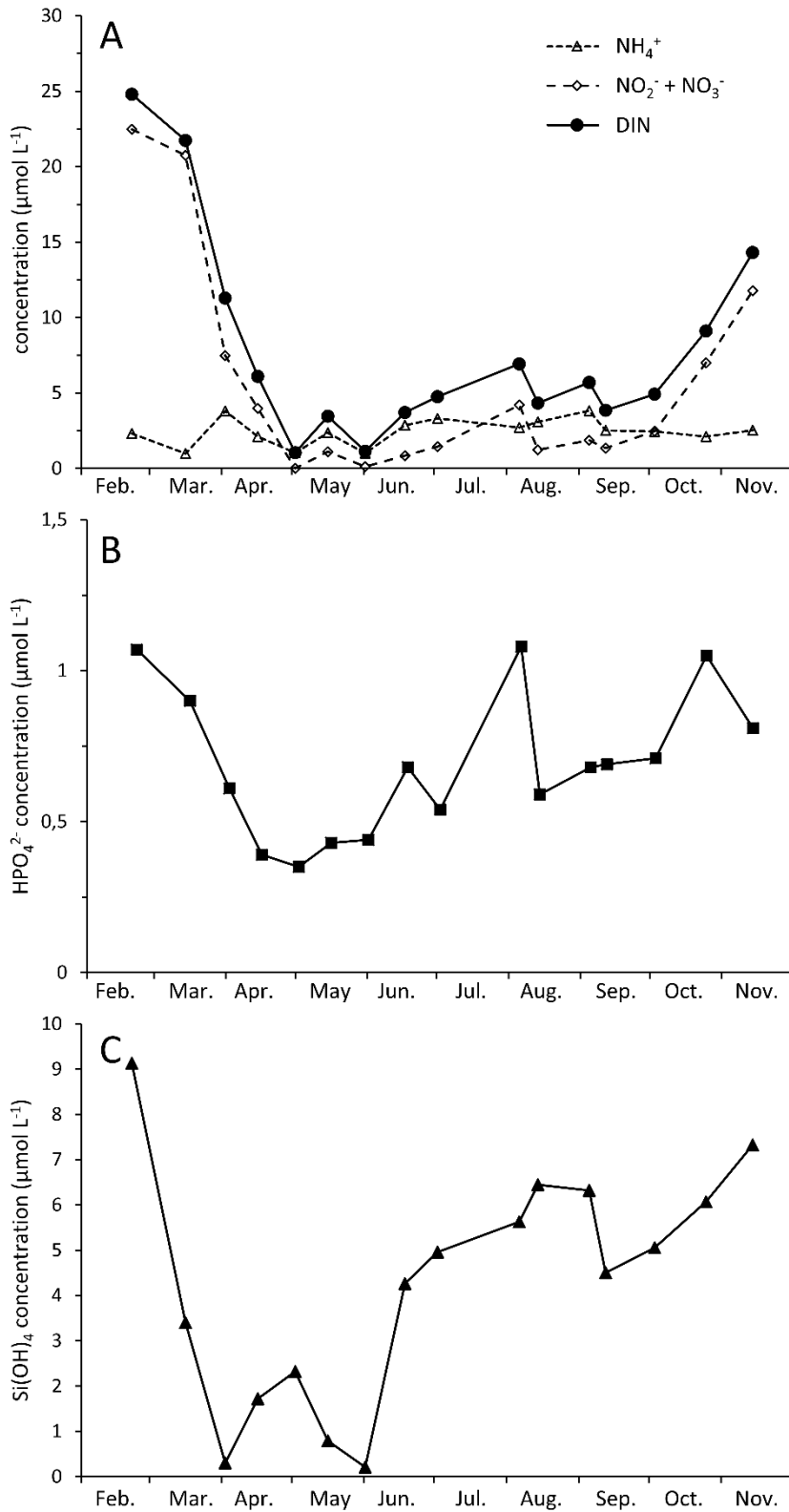
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797 Figure 2. Time series of meteorological and hydrological parameters. A. Temperature (°C)  
 798 and salinity, B. pH and dO<sub>2</sub> (mg L<sup>-1</sup>), C. Turbidity (NTU) and cumulated rainfalls over three  
 799 days (bars, mm) during the 2012 survey.

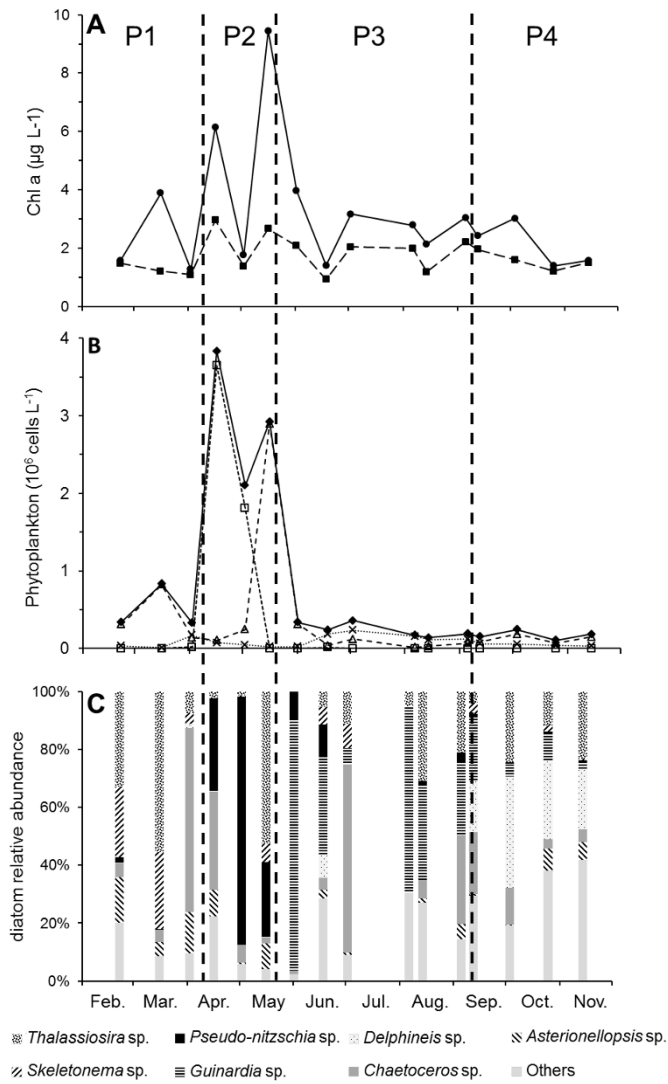
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802 Figure 3. time series of nutrient concentrations ( $\mu\text{mol L}^{-1}$ ). A.  $\text{NO}_2 + \text{NO}_3$ ,  $\text{NH}_4^+$  and total  
 803 dissolved inorganic nitrogen ( $\text{DIN} = \text{NO}_2 + \text{NO}_3 + \text{NH}_4^+$ ). B.  $\text{HPO}_4^{2-}$ . C.  $\text{Si(OH)}_4$ , during the  
 804 2012 survey.

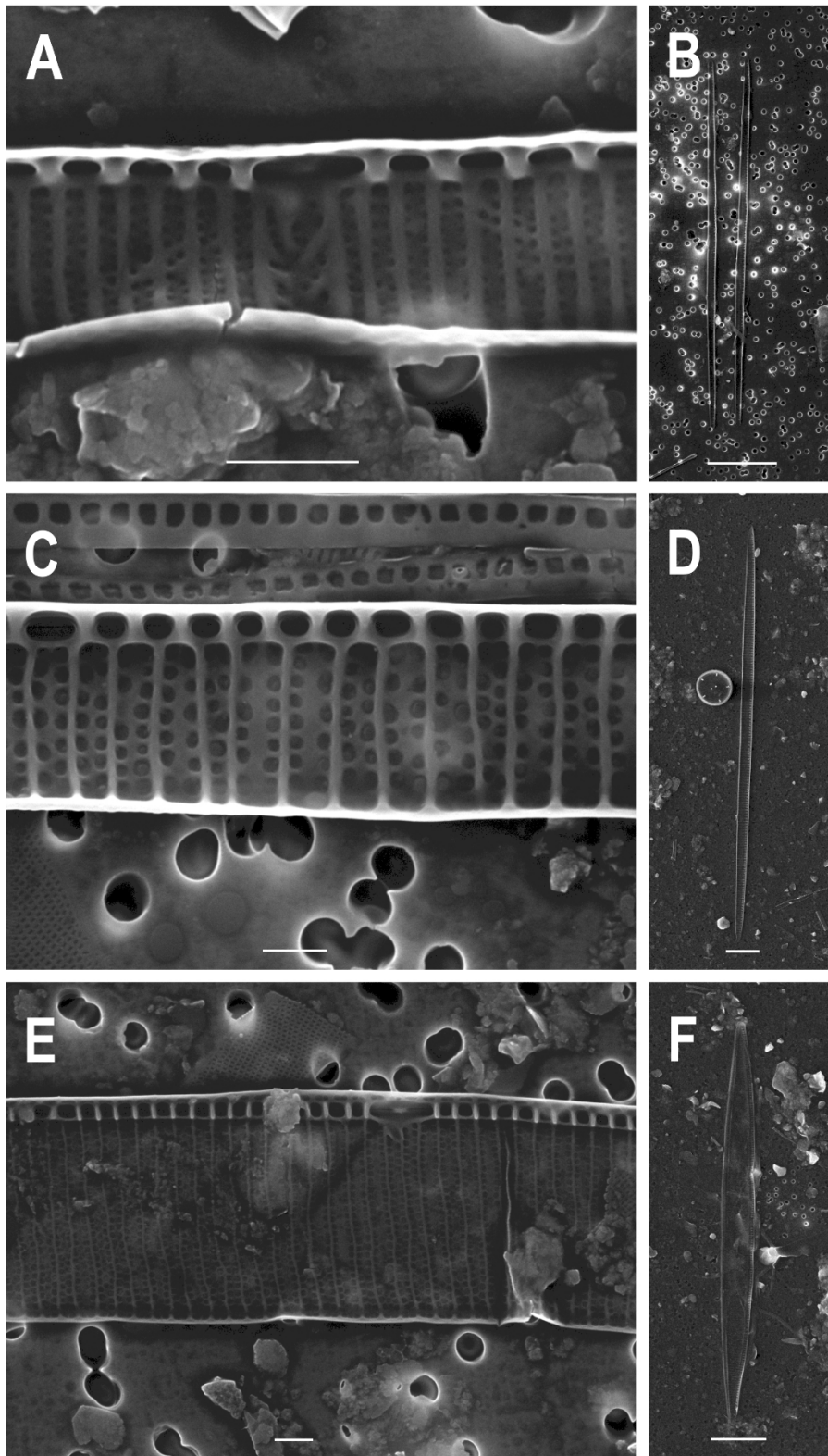
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807 Figure 4. Time series of A. Chlorophyll *a* (●) and pheopigment (■) concentrations ( $\mu\text{g L}^{-1}$ );  
 808 B. Abundance ( $\text{cell L}^{-1}$ ) of total phytoplankton (◆), diatoms (Δ), *Phaeocystis globosa* (□) and  
 809 other phytoplankton groups (×); and C. relative abundance of diatom genus (%) during the  
 810 2012 survey.

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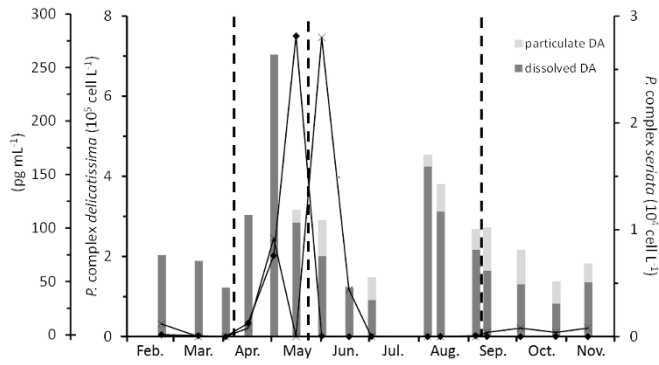


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814 Figure 5. SEM micrographs of the three identified *Pseudo-nitzschia* species. A-B: *Pseudo-*  
 815 *nitzschia delicatissima*; C-d: *Pseudo-nitzschia pungens*; E-F: *Pseudo-nitzschia fraudulenta*.  
 816 Scale bars: A, C, E = 1 $\mu$ m, B, D, F = 10 $\mu$ m.

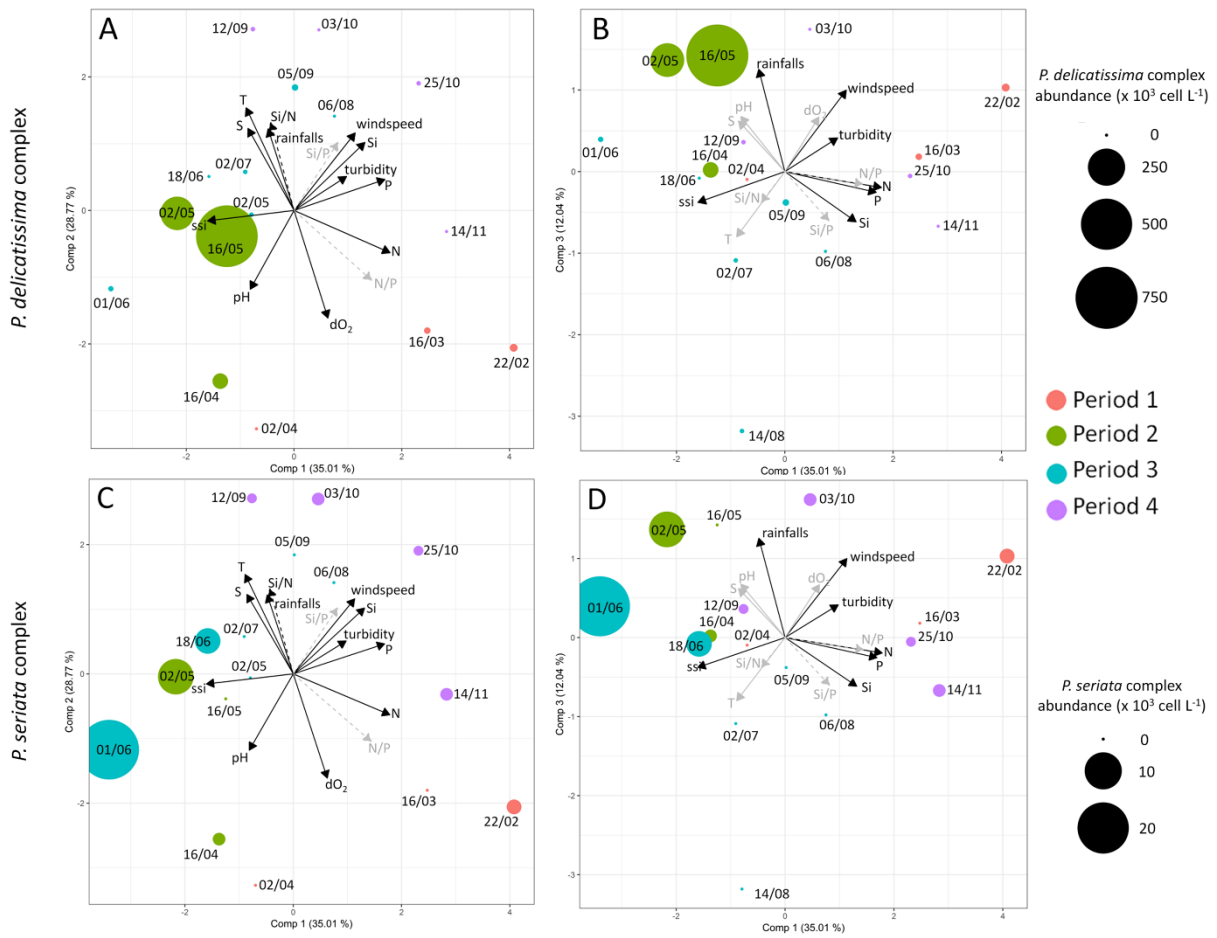




817

818 Figure 6: Time series of *Pseudo-nitzschia* complex *delicatissima* (◆) and *P. complex seriata*  
 819 ( $\times$ ) abundances (cell L<sup>-1</sup>), and particulate and dissolved domoic acid (DA, pg mL<sup>-1</sup>) during the  
 820 2012 survey. Stars below the x axis indicate the dates of SEM analyses for *Pseudo-nitzschia*  
 821 sp. diversity.

822



823

824 Figure 7: PCA plots of environmental variables. A and C. Plane defined by the two first axes  
 825 of the PCA (PC1 and PC2) B and D. plane defined by the first (PC1) and third axis (PC3) of  
 826 the PCA (B and D). Points correspond to sampling dates. The colors of the circles correspond  
 827 to the periods (P1 to P4) determined from phytoplankton communities composition. Circles  
 828 size is proportional to *Pseudo-nitzschia* abundance (cell L<sup>-1</sup>) for *P. complex delicatissima* (A  
 829 and B) and *P. complex seriata* (C and D). Black arrows represent variables significantly  
 830 correlated to at least one represented PC.

**Table 1.** Morphological features of *Pseudo-nitzschia* species and comparison with literature values (min-max). \* correspond to one frustule analysed and presenting outliers in poroids features (among >300 identified frustules)

Species	Width µm	Length µm	Central Interspace	Fibulae 10 µm <sup>-1</sup>	Interstriae 10 µm <sup>-1</sup>	Rows of poroids	Poroids 1 µm <sup>-1</sup>	Reference
<i>P. delicatissima</i>	1.3-2	N/A	+	22-29	33-43	2	8-11	Amato et al., 2005
	1.7-2.8	N/A	+	20-27	24-40	2	8-14	Fehling et al., 2006
	1.1-2	40-78	+	19-25	36-40	2	10-12	Skov et al., 1999
	1.0-1.5	42-66	+	20-23	env 40	2	10-12	Hasle et al., 1996
	1.7-2.3	17-48	+	18-36	37-44	2	7.5-13.4	Kaczmarek et al., 2008
	1.3-1.6	48-49	-	22-23	36-40	2	8-9	Klein et al., 2010
	1.5-2.0	19-76	N/A	19-26	35-40	2	12	Lundholm et al., 2006
	1.3-1.6	34-46	+	24-28	41-45	2	7-11	Moschandreou and Nikolaidis, 2010
	1.0-2.4	19-78	+	19-30	33-42	2	8-12.5	Quijano-Scheggia et al., 2010
	1.4-2.6	31-48	+	20-28	38-41	2	8-12	Fernandes et al 2014
	<b>1.1-1.7</b>	<b>40.7-67.1</b>	<b>+</b>	<b>18-27</b>	<b>34-44</b>	<b>2(3*)</b>	<b>10-14(18*)</b>	<b>This study</b>
<i>P. fraudulenta</i>	4.3-5.1	N/A	+	20-24	22-26	2-3	5-6	Fehling et al., 2006
	4.5-10	50-119	+	12-24	18-24	2-3	5-7	Skov et al., 1999
	5-6	73-117	+	19-23	19-23	2-3	4-5	Hasle et al., 1996
	3-4.5	63-99	+	15-17	14-15	2	N/A	Hernández-Becerril, 1998
	5.1-5.6	56.8-81	-	21-22	22-24	2-3	6-7	Klein et al., 2010
	4-7	71-101	N/A	18-30	19-28	2-3	6-7	Ljubešić et al., 2011
	4.4-5.8	48-80	+	22-24	21-24	2	5-6	Moschandreou and Nikolaidis, 2010
	2.9-7.1	38.9-131.5	+	12-24	18-24	2	4-7	Quijano-Scheggia et al., 2010
	5.2-6.2	64-84	+	20-23	22-23	2	5-6	Fernandes et al. 2014
	<b>5.2-6.1</b>	<b>75.0-98.8</b>	<b>+</b>	<b>19-23</b>	<b>17-23</b>	<b>2-3</b>	<b>6-7</b>	<b>This study</b>
<i>P. pungens</i>	3.1	N/A	-	12	12	2	3-4	Fehling et al., 2006
	2.4-5.3	74-174	-	9-16	9-16	1-2	3-4	Skov et al., 1999
	2.9-4.5	74-142	-	9-15	9-15	2	3-4	Hasle et al., 1996
	3-4	94-109	-	10-11	10-12	2	N/A	Hernández-Becerril, 1998
	2.9-3.3	61-106	-	11-13	11-14	2-3	2-4	Klein et al., 2010
	3.9-5.7	88-105	-	10-17	8-15	2	2-4	Ljubešić et al., 2011
	2.5-3.6	77-101	-	11-13	11-13	2	2-4	Moschandreou and Nikolaidis, 2010
	2-4.8	37-156	-	9-13	9-15	2	1-5	Quijano-Scheggia et al., 2010
	4-4.5	75-130	-	9-13	N/A	2	3-4	Stonik et al., 2001
	3.0-4.1	72-149	-	11-13	10-12	2	3-4	Fernandes et al 2014
		<b>2.7-4.7</b>	<b>95.9-135.5</b>	<b>-</b>	<b>10-15</b>	<b>10-15</b>	<b>2-3</b>	<b>2-4</b>

Table 2. Spearman correlation coefficient between principal components (PCx), environmental parameters, *Pseudo-nitzschia* complexes abundances and domoic acid concentration.

Parameters	PC1	PC2	PC3
<i>Main variables</i>			
Temperature (T)	-0.18	0.73**	-0.45 <sup>+</sup>
Salinity (S)	-0.39	0.64**	0.13
pH	0.43 <sup>+</sup>	-0.64**	0.29
dO <sub>2</sub>	0.22	-0.75***	0.26
Turbidity	0.498*	0.22	0.15
Rainfalls	-0.29	0.55*	0.61*
Windspeed	0.61*	0.51*	0.47
Surface Solar Irradiance (ssi)	-0.74**	-0.06	-0.15
DIN	0.88***	-0.29	-0.25
PO <sub>4</sub> <sup>2-</sup>	0.87***	0.28	-0.15
Si(OH) <sub>4</sub>	0.69***	0.37	-0.38
<i>Supplementary variables</i>			
Si/P	0.26	0.44	-0.38
Si/N	-0.23	0.69**	-0.19
N/P	0.66**	-0.50*	-0.20
<i>Pseudo-nitzschia and DA</i>			
<i>P. delicatissima</i> complex	-0.27	-0.33	0.47 <sup>+</sup>
<i>P. seriata</i> complex	-0.31	-0.07	0.50*
tDA	-0.30	0.12	0.20
(* p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001)			

**Table 3.** Environmental features of *Pseudo-nitzschia* spp. identified during the 2012 survey and literature values.

Species	Location	Temperature °C	Salinity	DIN µmol L <sup>-1</sup>	PO4 µmol L <sup>-1</sup>	Si µmol L <sup>-1</sup>	Abundance cells L <sup>-1</sup>	DA	Reference
<i>P. delicatissima</i>	Southern Adriatic Sea	8.79-29.91, R<0	R>0	no significant pattern			nd	nd	Caroppo et al., 2005
	Western Scotland	~8°C	nd	~3µM	~0.3µM	~2.5µM	1.6·10 <sup>5</sup>	nd	Fehling et al., 2006
	Louisiana	nd	nd	no significant pattern			1·10 <sup>6</sup>	5.5pg cell <sup>-1</sup>	Parsons et al., 2013
	NE Spanish Coast	R>0	36.8±1.1, R<0	R>0	nd	nd	17.6±5.2·10 <sup>5</sup>	nd	Quijano-Scheggia et al., 2008
	Bay of Fundy	nd	nd	R>0	no significant correlation		nd	nd	Kaczmarek et al., 2007
	Gulf of maine	nd	nd	nd	nd	nd	nd	111-390 pg mL <sup>-1</sup>	Fernandes et al., 2014
	Black Sea	16-17.5	14.5-15.7	nd	nd	nd	1.5-3.1·10 <sup>6</sup>	nd	
<i>P. delicatissima</i> complex	Western English Channel	déc-15	34.9±0.4, R>0	nd	R<0	nd	2,5·10 <sup>5</sup>	0.2-0.4 pg mL <sup>-1</sup>	Downes-Tettmar et al., 2013
	Galicia – Spain	~ 13.1	~35.6	~2.1	~0.22	~3	6.6·10 <sup>5</sup>	not detected	Díaz et al., 2014
	<b>Southern Bight of the North Sea</b>	<b>9-12</b>	<b>34.2±0.2</b>	<b>3.5±2.5</b> decreasing	<b>0.39±0.04</b>	<b>1.6±0.8</b>	<b>33·10<sup>5</sup>-7.5·10<sup>6</sup></b>	<b>92-229pg mL<sup>-1</sup></b>	<b>This study</b>
<i>P. pungens</i>	Louisiana	nd	nd	R<0	R>0	no corr	50000	nd	Parsons et al., 2013
	NE Spanish Coast	nd	nd	no pattern	nd	nd	nd	nd	Quijano-Scheggia et al., 2008
	Gulf of Maine	nd	nd	Nd	nd	nd	nd	40-1100 pg mL <sup>-1</sup>	Fernandes et al., 2014
	Bay of Fundy	nd	nd	R<0	R>0	nd	nd	nd	Kaczmarek et al., 2007
	Western English Channel	12-16	34.9±0.4	R<0	R<0	R<0	25000	0.2-0.4 pg mL <sup>-1</sup>	Downes-Tettmar et al., 2013
	Sea of Marmara, Turkey	11.6-22.8	21.6	nd	nd	1.8	4.8·10 <sup>5</sup>	nd	Tas and Lundholm, 2016
	Sea of Marmara, Turkey	7.4-26.3	15.9-19.9	Nd	Nd	Nd	1.6-1.7·10 <sup>4</sup>	1.0pg mL <sup>-1</sup>	Tas et al., 2016
<i>P. fraudulenta</i>	California	18.5-26.5	nd	0-3	nd	2.9-35.5	5-13 10 <sup>5</sup>	detected	Gárate-Lizárraga et al., 2007
	North Western Mediterranean Sea	nd	R>0	R<0	nd	nd	nd	nd	Quijano-Scheggia et al., 2008
<i>P. seriata</i> complex including <i>P. pungens</i> , <i>P. fraudulenta</i>	Western Scotland	12-13	nd	~0.3	~0.25	~3	110000	nd	Fehling et al., 2006
	<b>Southern Bight of the North Sea</b>	<b>15.4±0.3</b>	<b>34.50±0.03</b>	<b>2.4±1.8</b> decreasing	<b>0.56±0.17</b>	<b>2.2±2.8</b> increasing	<b>9.2·10<sup>5</sup>-2.8·10<sup>6</sup></b>	<b>82-108pg mL<sup>-1</sup></b>	<b>This study</b>

Min-max, mean ± standard deviation, R<0 and R>0: negative and positive correlation with diatom abundance, respectively., nd = no data

