

Two decades of *Pseudo-nitzschia* spp. blooms and king scallop (*Pecten maximus*) contamination by domoic acid along the French Atlantic and English Channel coasts: Seasonal dynamics, spatial heterogeneity and interannual variability

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Abstract :

King scallop contamination (*Pecten maximus*) by domoic acid, a neurotoxin produced by some species of the diatom *Pseudo-nitzschia*, is highly problematic because of its lengthy retention in the bivalve tissue, leading to prolonged fishery closures. Data collected within the French Phytoplankton and Phycotoxin monitoring network (REPHY) over the 1995–2012 period were used to characterize the seasonal dynamics and the interannual variability of *P.-nitzschia* spp. blooms as well as the contamination of king scallop fishing grounds, in six contrasted bays distributed along the French Atlantic coast and English Channel. Monitoring revealed that these toxic events have become more frequent since the year 2000, but with varying magnitudes, frequencies and timing depending on the bay. Two bays, located in southern Brittany, exhibited both recurrent contaminations and high *P.-nitzschia* abundances. The Brest bay and the Seine bay were intermittently affected. The Pertuis Breton exhibited only one major toxic event related to an exceptionally intense bloom of *P.-nitzschia* in 2010, and the Saint Brieuc bay neither showed significant contamination nor high *P.-nitzschia* abundance. While high *P.-nitzschia* abundance appeared to be correlated to scallop toxicity, this study highlights the difficulty in linking *P.-nitzschia* spp. blooms to king scallop contamination through monitoring. Indeed, *P.-nitzschia* was determined at the genus level and data regarding species abundances and their toxicity levels are an absolute prerequisite to further assess the environmental control of ASP events. As results describe distinct *P.-nitzschia* bloom dynamics along the French coast, this may suggest distinct controlling factors. They also revealed that major climatic events, such as the winter storm

Xynthia in 2010, can trigger toxicity in *P.-nitzschia* over a large spatial scale and impact king scallop fisheries all along the coast.

Highlights

► *Pseudo-nitzschia* blooms occur from May to September along the English Channel/Atlantic French coast. ► Irradiance and temperature play a major role on *Pseudo-nitzschia* blooms initiation in spring. ► Intense *Pseudo-nitzschia* spp. blooms is a prerequisite for king scallop contamination by domoic acid. ► ASP events tend to be more frequent in bays strongly influenced by riverine inputs and/or transitional upwelling. ► Large-scale climatic events could stimulate toxic *Pseudo-nitzschia* blooms and toxicity.

Keywords : *Pseudo-nitzschia* spp., Domoic acid, King scallop, Interannual variability, Seasonal dynamics, Environmental parameters

51 **1. Introduction**

52 The pennate diatom *Pseudo-nitzschia* (Heterokonta, Bacillariophyceae) is a cosmopolitan
53 genus that has been linked to many toxic events worldwide (e.g. Trainer et al., 2012). To date,
54 a dozen species of this genus have been identified as being able to produce domoic acid (DA)
55 (e.g. Bates and Trainer, 2006; Lelong et al., 2012), a potent neurotoxin responsible for
56 Amnesic Shellfish Poisoning (ASP) syndrome in humans after the consumption of
57 contaminated filter feeders (Bates et al., 1998; Wright et al., 1989). Symptoms of this
58 poisoning are more or less severe gastrointestinal and neurological disorders and may even
59 lead to death in the most extreme cases (Jeffery et al., 2004). Toxic *Pseudo-nitzschia* (*PSN*)
60 outbreaks are also responsible for massive mortalities in marine wildlife, such as marine
61 mammals and sea birds, through the trophic transfer of the toxin (e.g. Scholin et al., 2000;
62 Lefebvre et al., 2002a,b; Shumway et al., 2003; Bargu et al., 2012; Lefebvre et al., 2012).
63 Toxic *PSN* blooms therefore represent an expanding risk for both human health and activities
64 such as fisheries and aquaculture, as well as for marine wildlife worldwide (Hallegraeff, 1993;
65 Glibert et al., 2005).

66 Over the last decade, ASP events have strongly impacted fisheries in European waters
67 (EFSA, 2009). Toxic *PSN* blooms have resulted in prolonged closure of king scallop (*Pecten*
68 *maximus*) harvesting in Ireland (Bogan et al., 2007a,b), Scotland (Campbell et al., 2001;
69 Gallacher et al., 2001), Portugal (Vale and Sampayo, 2001), Spain (Arévalo et al., 1998;
70 Fragaet al., 1998) and France (Nézan et al., 2006). King scallop contamination by DA is
71 highly problematic because of its lengthy retention in the bivalve tissue, which in extreme
72 cases can last more than one year (e.g. Doucette et al., 2006; Blanco et al., 2002). In France,
73 *Pecten maximus* fishing grounds are distributed along the French Atlantic and English
74 Channel coasts, with a production ranging between 15 000 and 20 000 tons per year. Since the
75 first detection of DA in mussel tissues in 1998 (i.e. 0.5 $\mu\text{g g}^{-1}$ of wet weight, hereafter ww;

76 Amzil et al., 2001), DA levels are systematically monitored in king scallop tissues before and
77 during harvesting periods, within the framework of the French Phytoplankton and Phycotoxin
78 monitoring network (REPHY). In addition, phytoplankton community composition is
79 monitored at a fortnightly or monthly frequency. When *PSN* densities exceed the alert
80 threshold of $100\,000\text{ cells l}^{-1}$, DA analysis are systematically carried out in king scallop
81 tissues. Since 2004, several ASP events, characterized by DA concentrations well above the
82 European Union regulatory limit (i.e. $> 20\ \mu\text{g g}^{-1}\text{ ww}$), have been observed along the French
83 coast, leading to extended closures of many harvesting sites and causing serious economic
84 losses for the French fleet (Belin et al., 2013).

85 Given its potential impact on local economies and human health, a better knowledge of the
86 spatial and temporal dynamics of *PSN* blooms and ASP events affecting French scallop
87 fisheries is an absolute prerequisite to further assessing the determinism of the *PSN* toxic
88 blooms, and ultimately to developing predictive models of toxigenic *PSN* blooms. Despite the
89 large available dataset from the REPHY monitoring network, seasonal dynamics and
90 interannual variability of *PSN* blooms have not yet been described. This study represents the
91 first step towards an improved understanding of toxic *Pseudo-nitzschia* bloom dynamics and
92 ASP events along the French coast.

93 In this context, the objectives of this study were to (i) describe the seasonal dynamics of
94 *Pseudo-nitzschia* spp. blooms in six contrasted bays scattered along the coast, (ii) to estimate
95 scallop contamination by domoic acid over an interannual time scale and investigate a
96 possible link with the intensity of *PSN* blooms and (iii) to finally discuss the potential role of
97 environmental factors influencing toxic *PSN* outbreaks along the coast.

98

99 **2. Materials and Methods**

100 *2.1. Study sites*

101 This study was conducted in six bays scattered along the French Atlantic and English
102 Channel coasts (Fig. 1). Among the different sampling stations monitored within the REPHY,
103 one station was selected in each bay (Table 1). The choice of these stations was conditioned
104 by (i) the time range and frequency of the available data sets, (ii) the presence of exploited
105 scallop fishing grounds, (iii) the recurring observation of *PSN* within the phytoplankton
106 community and (iv) the representativeness of hydrological features encountered in the bay.
107 The data collected at these selected sampling stations was used throughout the manuscript.
108 For the sake of clarity, the term ‘bay’ will be used thereafter.

109 Despite these common features, the study sites exhibit very different hydro-climatic and
110 biological characteristics (Fig. 1, 2 and Table 2).

111 - The Seine Bay covers 4000 km² with a mean depth around 15 m and is characterized by a
112 megatidal regime, with a tidal range reaching more than 7 m (Guillaud et al., 2000). The bay
113 receives large freshwater inputs mainly from the Seine River (interannual mean flow = 510
114 m³ s⁻¹). The Seine bay exhibits the highest phytoplankton biomass out of the six study sites,
115 with median interannual Chlorophyll *a* (Chl *a*) reaching 4.1 µg l⁻¹ at the chosen sampling
116 station (Table 2). High Chlorophyll *a* levels are regularly observed in the Seine river plume
117 during the productive seasons, with mean values reaching 20 µg l⁻¹ and extreme values
118 peaking at 60 µg l⁻¹ (Cugier et al., 2005). Important freshwater inputs cause high winter
119 nutrient concentrations with median values of 1 µmol l⁻¹, 23 µmol l⁻¹ and 43 µmol l⁻¹ for PO₄
120 (P), SiOH (Si) and dissolved inorganic nitrogen (N) respectively, with N/P always above the
121 Redfield ratio (median of 44.1), highlighting important nitrogen enrichment (Fig. 2). King
122 scallop harvesting in the Seine bay represents 50% to 70% of national production (i.e. 5000 to
123 15000 t year⁻¹).

124 - The Saint Brieuc bay is widely open to the Western English Channel. It has a clockwise
125 circulation pattern, with water masses accelerating in the western part of the bay (Garreau,

126 1993). This bay is weakly influenced by freshwater inputs and median interannual Chl *a*
127 reaches $0.7 \mu\text{g l}^{-1}$. This bay displays the lowest phytoplankton biomass recorded out of the six
128 study sites (Fig. 1 and Table 2). Lowest N and Si concentrations are recorded in the Saint
129 Briec Bay, where maximum winter concentrations remain below $20 \mu\text{mol l}^{-1}$ and $12 \mu\text{mol l}^{-1}$
130 respectively (Fig. 2). N/P ratio is also the lowest observed (23), close to the Redfield ratio.
131 The king scallop fishery is one of the major activities of the bay with a landing of 5000 to 10
132 000 t year⁻¹.

133 - The Brest Bay is a semi-enclosed bay of 180 km² connected to the Iroise Sea by a narrow (2
134 km wide) and deep (40 m) strait. The bay is characterized by strong currents (Le Pape and
135 Ménesguen, 1997) and receives relatively high freshwater inputs mainly from the Aulne and
136 Elorn rivers (total interannual mean flow = $35 \text{ m}^3 \text{ s}^{-1}$). Annual median Chl *a* reaches $1.0 \mu\text{g l}^{-1}$.
137 The bay displays high nitrogen enrichment during winter (median N/P of 49) and relatively
138 high P and Si concentrations (median of 0.58 and $12 \mu\text{mol l}^{-1}$ respectively). Around 200 tons
139 of king scallops are harvested every year in the bay.

140 - Concarneau and Quiberon bays, located in the northern Bay of Biscay are characterized by
141 weak tidal currents and receive indirect freshwater inputs, as the Loire and Vilaine river
142 plumes tend to spread towards the NW (Fig. 1) and remain confined along the coast
143 particularly in early spring (Lazure and Jegou, 1998). These freshwater inputs combined with
144 low vertical mixing cause strong haline stratification within the bay (Planque et al., 2004).
145 From spring to mid-September, thermal stratification is superimposed onto haline
146 stratification. In spring low-surface salinity waters induce a significant density gradient over
147 the continental shelf generally oriented northwards ($2\text{-}20 \text{ cm s}^{-1}$). During thermal
148 stratification, W/NW winds induce local transitory upwelling (Lazure and Jégou 1998; Puillat
149 et al., 2004, 2006). In the Concarneau and Quiberon bays annual median Chl *a* reaches $1.1 \mu\text{g}$
150 l^{-1} and $1.3 \mu\text{g l}^{-1}$ respectively (Fig. 1 and Table 2). The two bays differ in their winter nutrient

151 supply, with high median values of P, Si and N concentrations (0.63, 20 and 25 $\mu\text{mol l}^{-1}$
152 ^lrespectively) and high median N/P ratio (41) for Quiberon, versus low median concentrations
153 (P=0.42, Si=9, N =11 $\mu\text{mol l}^{-1}$) and lower median N/P (26) for Concarneau. Around 3000 tons
154 of king scallops are harvested each year in these two bays.

155 - The Pertuis Breton located in the Southern Bay of Biscay is a shallow bay (i.e. <20 m depth)
156 characterized by a macrotidal regime, weak currents (i.e. < 2 knots) and high turbidity (annual
157 median value of 9.1 NTU; Table 2). Only small rivers flow into the bay: the Lay river (20 m^3s^{-1}
158 ^l) and the Seudre-Niortaise river (25 m^3s^{-1}). Phytoplankton biomass is relatively high, with
159 annual median Chl a concentrations of 2.8 $\mu\text{g l}^{-1}$. The bay experiences high nutrient discharge
160 during the winter with median P, Si and N concentrations of 0.66, 18 and 30 $\mu\text{mol l}^{-1}$, and an
161 N/P median ratio of 53. Only 600 to 700 tons of king scallops are landed each year in this
162 area.

163

164

165 2.2. Data collection and sample analysis

166 Since 1987, the REPHY program has been acquiring information on phytoplankton
167 communities along the French coast. Sampling is carried out (i) on a monthly basis from
168 November to March (ii) every two months from April to October and (iii) weekly when
169 potentially toxic species abundances go over specific thresholds. Water samples are collected
170 from sub-surface (1 m) waters using a 5-l Niskin bottle, 1 nautical mile offshore and are timed
171 to coincide with high tide.

172

173 2.2.1. Phytoplankton analysis

174 Phytoplankton biomass was estimated through chlorophyll a concentrations and
175 phytoplankton abundances and diversity were assessed through microscopy numeration

176 within the REPHY program.

177 Samples were collected following three strategies (Belin and Amzil, 2010): (i) FLORTOT,
178 a sampling strategy where all micro-phytoplankton (i.e. $>20\mu\text{m}$, and colonies) present in the
179 sample are counted; (ii) FLORIND, which only counts toxic species of phytoplankton
180 exceeding $100\,000\text{ cells l}^{-1}$; (iii) FLORPAR, which is activated for exceptional blooms or
181 toxic events and induces an increase in the sampling frequency.

182 For chlorophyll *a* concentrations, water samples (200 to 2000 ml) were filtered through
183 glass-fiber filters (Whatman GF/F) and immediately frozen (-20°C) until analysis.
184 Chlorophylleous pigments were subsequently extracted in 5 ml of 90% acetone in the dark at
185 4°C during 12 hours and assayed in a spectrophotometer following Lorenzen (1967).

186 For phytoplankton identification, 1-l water samples were fixed with Lugol iodine solution
187 (2% f.c.) and stored in the dark at 4°C . In the laboratory, samples were gently homogenized
188 before settling in 10-ml sub-samples for $>24\text{ h}$ in Hydro-Bios counting chambers (Utermöhl,
189 1958).

190 Phytoplankton identification was performed using an inverted microscope equipped with
191 phase contrast illumination. In order to guarantee the taxonomic homogeneity of data over
192 time and among sampling sites, some species and some genera were aggregated into taxo-
193 nomic units (TU) corresponding to the lowest taxonomic level possible. This grouping was
194 chosen in order to overcome difficulties of identification between some species or genera us-
195 ing optical microscopy.

196 While *PSN* has been monitored since 1995 within the REPHY monitoring network, species
197 level identification by light microscopy is difficult or even impossible for some species and
198 therefore most of the data refer to the genus scale. Consequently, analyses were carried out
199 using time series of *Pseudo-nitzschia* spp. abundances.

200

201 2.2.2. *Environmental parameters*

202 At each sampling station, salinity, water temperature (°C) and turbidity (NTU) were recorded
203 in subsurface waters (1 m) with YSI 6600 M multiparameter probes (Fondriest).

204 Since 2007, nutrient concentrations have been estimated on a monthly basis over the winter
205 period (i.e. October to March) in each of the six bays within the framework of the European
206 Water Framework Directive. In the Seine, Quiberon and Concarneau bays, monthly sampling
207 has been carried out throughout the year since 2002, 1995 and 1997 respectively.

208 For inorganic nutrients, 100-ml water samples were pre-filtered through 48 μ m mesh
209 directly from the Niskin bottle. For silicate concentrations ($\text{Si} = [\text{Si}(\text{OH})_4]$), water samples
210 were subsequently filtered through 0.45 μ m acetate cellulose membrane and stored at 4°C
211 before analysis. Water samples for the determination of dissolved inorganic nitrogen (i.e.
212 $\text{N} = [\text{NO}_3^- + \text{NO}_2^-] + [\text{NH}_4^+]$) and phosphate concentrations ($\text{P} = [\text{PO}_4^{3-}]$) were stored directly
213 at -20°C. Samples were analyzed within one month after field collection with an auto-analyzer
214 (Technicon III) following standard protocols (K  rouel and Aminot, 1997; Hydes *et al.*, 2010).

215 Photosynthetic Active Radiations (PAR) data were obtained with the ARPEGE model
216 (M  t   France) and the daily PAR was summed over the five days preceding sampling.

217

218

219 2.2.3. *Domoic acid in king scallops*

220 Routine monitoring of domoic acid (DA) concentration in king scallops has been
221 performed within the REPHY program since 2003 in exploited fishing grounds scattered
222 along the French coast. King scallops are collected fortnightly just before and during the
223 harvesting period (i.e. November to March) as long as the DA concentration remains below
224 the sanitary threshold (i.e. 20 $\mu\text{g DA g}^{-1}$ ww). When toxin levels exceed the sanitary
225 threshold, samples are then collected weekly on the impacted fishing grounds. Scallop tissue

226 samples (i.e. total shellfish meat) are analyzed for DA concentration using a UV diode array,
227 following standard protocols (Lawrence et al., 1991). Only DA concentrations in the total
228 shellfish flesh have been retained in the data analysis.

229 As sampling frequency was not homogenous within and among sampling sites and
230 considering the long retention time of DA in king scallop tissues (sometimes over a year), DA
231 concentrations measured at a particular time may be the result of different contamination
232 periods, and may therefore be linked to different toxic *PSN* blooms. In order to overcome this
233 difficulty, an annual index, (hereafter referred to as the Scallop Toxicity Index (STI), was
234 calculated for each of the six bays over the 2003 to 2012 period. The annual steepest increase
235 in DA concentration was identified in each sampling site. The two dates framing this maximal
236 increase (hereafter referred to as t_i , date of the beginning of the increase and t_f , date ending the
237 steep increase) defined the main period of scallop contamination for a given year. The
238 maximum DA level was then identified among four sampling values: the value at t_f and the
239 values of the 3 sampling dates following t_f . This timeframe was chosen for two reasons: i)
240 while t_f marked the end of the steepest increase of the year, it didn't always correspond to the
241 maximum DA concentrations (i.e. DA concentrations can keep on increasing at a slower rate
242 after t_f) and ii) due to irregular sampling frequencies over time and between sites, this time
243 frame can range from three weeks to several months, which seemed sufficient for the
244 contamination to reach its maximum. Finally, the STI ($\mu\text{g DA g}^{-1} \text{ ww y}^{-1}$) was calculated as
245 the difference between the maximum DA concentration and the lowest DA concentration
246 observed over the period preceding t_i .

247

248 **2.3. Data analyses**

249 A first dataset merging the three strategies (i.e. FLORTOT, FLORIND, and FLORPAR)
250 was used to study the seasonal and interannual dynamics of the genus *Pseudo-nitzschia* in the

251 different bays.

252 A second database built only with the FLORTOT strategy, was used to calculate
253 biodiversity indices such as (i) total micro-phytoplankton abundance, (ii) phytoplankton
254 richness, (iii) Berger-Parker evenness (Berger and Parker, 1970) and (iv) the ratio between
255 Diatoms and Diatoms plus Dinoflagellates. In addition, a rank was assigned for each
256 taxonomic unit (i.e. TU) based on their relative frequencies in decreasing order; the first
257 ranked TU exhibiting the highest relative frequency (Frontier, 1985; Legendre and Legendre
258 1998). Only *PSN* ranks and proportions among the other phytoplankton genus were used in
259 the analysis. In order to standardize the data, abundances were averaged over a 14 day
260 period in both datasets.

261 All analyses were conducted with R software 3.0.0. (R Core Team, 2013). Potential
262 differences and similarities among sampling sites regarding *PSN* blooms were assessed
263 through a Principal Component Analyses (PCA) performed on the phytoplankton biodiversity
264 indices listed above. *PSN* bloom descriptors such as *PSN* absolute, relative abundances and
265 the rank of *PSN* within the phytoplankton community were calculated using the package
266 FactoMineR (Lê et al. 2008). Missing data were previously imputed thanks to the function
267 impute PCA from the package missMDA (Josse et al., 2012). The factor “site” was used as a
268 qualitative supplementary variable and for each site confidence ellipses were drawn around
269 95% of the individuals of each sampling site.

270 Finally, a niche analysis was performed, using environmental data and *PSN* abundances.
271 Niche concept relates the environmental conditions with the presence and development of a
272 specific species. In 1957, Hutchinson defined the ecological niche as the space of
273 environmental conditions in which a species is able to persist. Furthermore, Hutchinson
274 (1957) considered that the environmental factors essential for the existence of a species could
275 be defined as the axes of a multidimensional space. Following this concept, the environmental

276 space occupied by *PSN* was determined using a two-step procedure:

277 *Step 1:* A standardized Principal Component Analysis (PCA) was applied to assess the main
278 variability of environmental data along a reduced number of axes. The PCA analysis
279 integrated the environmental data from the six bays from 2003 to 2012 and included the
280 following variables: temperature, salinity, PAR, turbidity, nutrient concentrations (N, P, Si)
281 and ratios (Si/N/P). Previously to the PCA, particular care was given to select samples with
282 sufficient nutrient data, and the remaining missing data from this selection were imputed as
283 previously mentioned, using the missMDA package. Data kept for the niche analysis
284 amounted to 660 samples, of which 75% came from the Quiberon and Seine bays. All years,
285 from 2003 to 2012, and all months were represented in the analysis. Data selected for the PCA
286 for Concarneau bay, Saint Brieuc Bay and the Pertuis Breton only concerned years 2007 to
287 2012, as nutrient analysis only started in 2007 in these bays, via the European Water
288 Framework Directive protocols. However the Quiberon, Seine and Brest bays have
289 maintained a constant effort in nutrient sampling since 2003. Consequently, data from before
290 2007 used in this analysis came from these three bays only. Environmental data were well
291 distributed throughout the year, except for Saint Brieuc bay and the Pertuis Breton, where
292 only winter data were available for this analysis.

293 *Step 2:* A kernel density calculation, weighed by corresponding *PSN* \log_{10} abundance, was
294 then applied to the first plan of the PCA. This method revealed areas of the plan where the
295 highest abundances were observed, highlighting the most favourable combination of
296 environmental conditions for *PSN* to bloom. Out of these highest abundances, the 5th
297 percentiles were then extracted for each environmental factor in order to determine the
298 ecological niche of *PSN*.

299

300 **3. Results**

301 ***3.1. Seasonal patterns of environmental parameters and phytoplankton***

302 The 6 bays displayed very similar seasonal patterns regarding temperature and cumulated
303 PAR, with maxima being recorded respectively in August and June (Fig. 3A). The highest
304 temperature and cumulated PAR medians were observed in the Pertuis Breton with
305 respectively 21.0°C and 35.3 10³ W m⁻². PAR maxima values were relatively similar in the 5
306 other sites. Some differences were nevertheless observed regarding temperature, particularly
307 during summer. The Seine bay exhibited higher water temperature compared to the other sites
308 (except Pertuis Breton), with a high interannual variability (Fig. 3B). In Concarneau Bay a
309 decrease in water temperature was observed between June and July.

310 With respect to salinity, turbidity and phytoplankton biomass, the six bays showed very
311 distinct seasonal patterns and amplitudes (Fig. 3A). In the Seine bay, salinity is relatively low
312 throughout the year, with median ranging from 31.9 in March to 33.0 in October. Turbidity
313 was relatively high in this bay, with maximum values being commonly observed in winter.
314 Out of the six study sites, the Seine bay exhibited the highest phytoplankton biomass (median:
315 4.12 µg l⁻¹, Fig 3B and Table 2). Phytoplankton blooms start in February/March and the
316 highest Chl *a* concentrations were commonly observed from March to September. Seasonal
317 dynamics of salinity, turbidity and phytoplankton biomass revealed themselves to be highly
318 variable at the interannual time-scale in this semi-enclosed bay (Fig.3B).

319 The Saint Brieuc bay is host to more oceanic conditions, characterized by a constant and
320 relatively high salinity (i.e. 34.8 to 35), low turbidity and low phytoplankton biomass.
321 Moreover, among the 6 bays Saint Brieuc exhibited the lowest phytoplankton biomass, with
322 maximum monthly Chl *a* remaining below 2.5 µg l⁻¹ in May.

323 In the Brest bay, salinity increased from 33.6 in March to 35.0 in August and turbidity
324 remains very low (i.e < 2 NTU) throughout the year (Fig. 2A). Phytoplankton blooms occurred
325 from March to September, with the highest monthly Chl *a* concentrations being recorded in

326 May ($3.0 \mu\text{g l}^{-1}$).

327 Quiberon and Concarneau bays displayed similar seasonal patterns. Salinity regularly
328 increased from March and reached its highest values in August. Turbidity decreased from
329 February to reach very low level in April. Phytoplankton biomass started increasing in March
330 and reached the highest level in May. Some differences in terms of amplitude were
331 nevertheless observed between these two sites, with Quiberon bay displaying a lower salinity,
332 higher turbidity and higher phytoplankton biomass than Concarneau bay.

333 The strongest salinity variations were observed in the Pertuis Breton, where salinity
334 increased from 30.0 in January to 35.0 in August (Fig. 3A). The highest turbidity levels were
335 recorded in this shallow bay with the highest levels (i.e. > 15 NTU) being observed from
336 December to April. Phytoplankton bloom was observed in March with Chl *a* concentrations
337 remaining relatively high until September.

338

339 ***3.2. Pseudo-nitzschia blooms along the Atlantic and English Channel coasts of France***

340 *3.2.1. Seasonal dynamics of Pseudo-nitzschia blooms*

341 The genus *Pseudo-nitzschia* was observed from March to November in the six study sites
342 (Fig. 4A). Considering seasonal patterns as well as the amplitude of *PSN* blooms, three
343 groups of bays were identified.

344 The highest *PSN* abundances were observed in Quiberon and Concarneau bays. Blooms
345 occurred as of March and reached their maxima in June. *PSN* Abundances decreased quickly
346 thereafter in Quiberon, to reach very low values in July. In contrast, abundances remained
347 relatively high in Concarneau throughout the summer and a second bloom was generally
348 observed in September at this sampling site.

349 In the Seine and Brest bays, the intensity of *PSN* blooms was lower, with a maxima
350 commonly reached in May. Abundances remained relatively high until July in Brest bay,

351 whereas a slow decrease was observed in the Seine bay. A secondary bloom could be
352 observed at both sampling sites: in August in the Seine bay and in September in the Brest bay.

353 Saint Brieuc and the Pertuis Breton exhibited very low *PSN* abundances throughout the
354 year compared to the four other sites. Maxima were observed in May in Saint Brieuc and in
355 May-June in the Pertuis Breton.

356 As shown in the Figure 4B, the intensity of *PSN* blooms may greatly vary from one year to
357 another for each of the study sites. This variability was particularly high in the Concarneau,
358 Quiberon and Seine bays. The highest variability of *PSN* abundances was observed in June in
359 the Quiberon and Concarneau bays.

360

361 3.2.2. Interannual variability of *Pseudo-nitzschia* blooms

362 Figure 5 shows *Pseudo-nitzschia* spp. abundances at the 6 sampling sites over the 1995 to
363 2012 period (data were not available before 1997 for the Seine bay).

364 Since 1995, high *PSN* abundances were consistently observed in Quiberon and Concarneau
365 bays. Concarneau bay was the most concerned by *PSN* blooms, as abundances exceeded the
366 sanitary threshold of 1×10^5 cells l^{-1} every year. Moreover, in 2006 and 2008 abundances
367 reached the highest values, with 9×10^6 cells l^{-1} and 14×10^6 cells l^{-1} respectively. Very high
368 abundances were recorded every year in Quiberon bay (Fig. 5), except in 2003, 2006, 2007
369 and 2010 when *PSN* abundances remained below 1×10^6 cells l^{-1} (Fig. 5).

370 In the Brest and Seine bays, *PSN* blooms were less intense than those observed in the
371 Concarneau and Quiberon bays. In the Brest bay cell numbers exceeded the sanitary threshold
372 in June 2004, 2007, 2009, and more than 10^6 cells l^{-1} were observed in June 2008. In the Seine
373 bay, except in 2010 and 2011 when low abundances were observed, since 2003 *PSN*
374 abundances have regularly exceeded the sanitary threshold. The interannual variability was
375 particularly high in the Seine bay. The highest abundances (i.e. $> 10^6$ cells l^{-1}) were recorded

376 in May-June 2003, 2005 and 2009. In 2005 and 2012, the bloom was particularly long as *PSN*
377 abundances remained high from April to August (Fig. 5).

378 In Saint Brieuc bay *PSN* abundances rarely exceeded the sanitary threshold. The highest
379 abundances were recorded in May 2007 and 2008, with respectively 1.33×10^5 and 1.11×10^5
380 cells l^{-1} (Fig. 5).

381 In the Pertuis Breton, except in 2006 and 2010 when *PSN* abundances reached the highest
382 values of 1.2×10^5 cells l^{-1} and 7.7×10^5 cells l^{-1} respectively, the threshold of 1×10^5 cells l^{-1}
383 was never reached in 18 years of monitoring. While the highest *PSN* abundances were usually
384 recorded in May-June (Fig. 4A), in 2010 *PSN* bloom occurred in March. A second bloom of
385 *PSN* was observed in May-June following the exceptional and early bloom of March 2010
386 (Fig. 5).

387

388 3.2.3. *Pseudo-nitzschia* within the phytoplankton community

389 A Principal Component Analysis (PCA) was performed on phytoplankton biodiversity
390 indices and *PSN* descriptors to assess the differences and similarities regarding the structure
391 of the phytoplankton communities during *Pseudo-nitzschia* blooms in the different bays
392 (Fig.6A). The first two axes explain nearly 48% of the variance. Axis 1 (28.02%) is mainly
393 structured by *PSN* descriptors such as the *PSN*-rank (*PSN*-Rank: 68.7%; $p < 0.0001$), *PSN*
394 frequency within the community (%*PSN*: 74.3%; $p < 0.0001$) and *PSN* abundance (*PSN*:
395 61.7%; $p < 0.0001$). The second axis is explained by the total phytoplankton abundance (TOT:
396 67.2%; $p < 0.0001$) and the richness of the phytoplankton community (S: 57.6%; $p < 0.0001$).

397 Three groups of sampling sites were identified along the axis 1 (Fig. 6B). Located on the
398 left side of the axis 1, Saint Brieuc bay and Pertuis Breton are characterized by low absolute
399 and relative abundances of *PSN*, which never dominate the phytoplankton community (i.e.
400 higher rank). In contrast, Concarneau and Quiberon bays, located on the right hand side of

401 axis 1, displayed higher absolute and relative abundances of *PSN*, where it occupied a lower
402 rank within the phytoplankton community. The Seine and Brest bays did not show any
403 significant different from each other for these descriptors and were not well discriminated
404 along the first axis.

405

406 3.2.4. Ecological niche of *Pseudo-nitzschia* spp.

407 PCA on environmental variables corresponds to the first step in determining the ecological
408 niche of *Pseudo-nitzschia*. As shown on Figure 7, the first two axes, representing 56% of the
409 explained variability, showed the seasonal patterns of environmental variables: (i) high
410 nutrient concentration and high turbidity during the autumn-winter period, corresponding to
411 well-mixed conditions and high freshwater input, i.e. high turbidity and nutrient
412 concentration, on the right side of the first axis and (ii) a decrease in nutrient concentration
413 over the spring and summer when the water temperature and irradiance increase, on the left
414 side of the first axis. While the second axis of the PCA is mainly driven by the N/P ratio, Si/N
415 and Si/P ratios are not well represented on the first two axis of the analysis ($\cos^2_{Si/N} = 3.7 \cdot 10^{-2}$,
416 $\cos^2_{Si/P} = 6.05 \cdot 10^{-5}$, where \cos^2 measures the quality of a variable representation on the
417 factor map).

418 The niche approach used here provided evidence of the environmental occupancy of *Pseudo-*
419 *nitzschia* through the analysis of their occurrences. A main region of dense occurrence
420 (weighted by the abundance) was observed on the left side of the PCA analysis and mainly
421 over the first axis (Fig. 7). *PSN* exhibited a niche characterized by a relatively high irradiance,
422 water temperature and salinity and conversely, low nutrient concentration and turbidity, which
423 corresponds to spring/summer conditions.

424 This visual impression was confirmed by the extraction of the occurrences with the highest
425 densities (defined as the 5% contour, see Fig. 7, Table 3). The 5% *PSN* highest abundance

426 window displayed an ecological niche (Table 3) corresponding to the highest salinity waters
427 (not less than 34.02), relatively high water temperature (between 15.9 and 17.9°C) and
428 irradiance (between 21791 and 28227 Wm⁻²). This confirmed that *PSN* bloom occur mostly
429 during spring and early summer time. Turbidity should not exceed 1.35 NTU. Nutrient values
430 for the 5% window showed minimum values (P = 0.03 μmol l⁻¹, Si = 0.33 μmol l⁻¹, N = 0.06
431 μmol l⁻¹) corresponding to relatively low concentrations, particularly for N. The N/P ratio for
432 the 5% window showed values lower than the Redfield ratio (16) and the second axis did not
433 appear to have any impact on the maximum *PSN* abundances, even if there was slightly more
434 *PSN* data on the upper second axis (higher N/P).

435

436 **3**

437 ***King scallop contamination by domoic acid along the French Atlantic and English***
438 ***Channel coasts***

439 ***3.3.1. Interannual variability of toxic events***

440 The first king scallop contaminations by domoic acid were recorded in 2004 in the Seine
441 and Brest bays (Fig.8A). Since then, ASP events in *Pecten maximus* fishing grounds have
442 become more frequent and widespread along the French coast. At the interannual time scale
443 the frequency of these toxic events differs widely between sites (Fig. 8A).

444 Saint Brieuc bay was never affected by ASP events; DA concentration in king scallops
445 never exceeded the sanitary threshold (i.e. 20 μg DA g⁻¹ ww) and STI remained negligible
446 over the study period (<3 μg DA g⁻¹ y⁻¹ ww y⁻¹). In the Pertuis Breton, high STI was only
447 recorded in 2010 (i.e. 194 μg DA g⁻¹wwy⁻¹).

448 The Brest and Seine bays were irregularly concerned by DA contamination. High STI
449 values were recorded in 2004, 2007, 2008 and 2009 in the Brest bay with a maximum of 180
450 μg DA g⁻¹ wwy⁻¹ in 2007. In the Seine bay STI were particularly high in 2004, 2005, 2011 and

451 highest values were recorded in 2012 ($287 \mu\text{g DA g}^{-1}\text{wwy}^{-1}$).

452 Concarneau and Quiberon bays were both regularly impacted by significant DA
453 contaminations as high STI have been observed each year since 2006 and 2004 respectively.

454 An exceptional contamination in terms of intensity and spatial extension was observed in
455 2010 in the bay of Biscay (i.e. the Pertuis Breton, Quiberon bay and Concarneau bay) with a
456 maximum STI of $445 \mu\text{g DA g}^{-1}\text{wwy}^{-1}$ in Quiberon bay.

457

458 3.3.2. STI and annual maximum *Pseudo-nitzschia* spp. abundance

459 STI calculated over the 2003-2012 period in each study sites was plotted against annual
460 maximum *PSN* abundance (Fig.8B) in order to investigate a possible link between *PSN* bloom
461 intensity and STI.

462 In Saint Brieuc bay, over the study period maximum concentrations of *PSN* remained
463 below, or very close, to the sanitary threshold (i.e. $< 10^5 \text{ cellsL}^{-1}$) and negligible STI were
464 always observed. Similarly low maximal concentrations of *PSN* and low STI were usually
465 recorded in the Pertuis Breton. Nevertheless, in 2010 maximum *PSN* abundance greatly
466 exceeded the sanitary threshold in the Pertuis Breton and the highest STI was recorded this
467 particular year. In 2011 and 2012, while STI remained low, annual maximum abundance of
468 *PSN* was relatively low.

469 In the Brest and Seine bays, high STI were intermittently observed. Since 2004, high STI
470 always matched high *max PSN* in the Brest bay. In contrast, in the Seine bay *max PSN*
471 regularly exceed the sanitary threshold without major STI levels.

472 Prior to 2004 in Concarneau and to 2006 in Quiberon, high *max PSN* were observed in
473 both bays without high STI. Since then, *PSN* concentrations have always reached values
474 above 10^5 cellsL^{-1} and high STI were regularly observed in both bays.

475

476 4. Discussion

477 4.1 Seasonal patterns of *Pseudo-nitzschia* sp. blooms along the French coast

478 The genus *Pseudo-nitzschia* was a common member of the diatom community and was
479 represented throughout the year at the six study sites (Fig. 4). In contrast with previous
480 observations suggesting that *PSN* blooms were mainly observed from January to May in
481 European waters (Hasle et al., 1996; Lelong et al., 2012), results show that *PSN* blooms
482 occurred from May to September along the French Atlantic and English Channel coasts. A first
483 bloom was observed in spring (i.e. May/June) in the six study sites and was followed by
484 another smaller peak at the end of summer in the Concarneau, Brest and Seine bays (Fig.4).
485 These results were congruent with previous studies reporting *PSN* blooms in spring and at the
486 end of the summer in very diverse ecosystems such as the Gulf of Mexico (Dortch et al.,
487 1997; Parsons and Dortch, 2002; Parsons et al., 2013), western Scottish waters (Fehling et al.,
488 2006), the North Sea (Bresnan et al., 2015) or the Mediterranean Sea (Mercado et al., 2005).

489 Despite the diversity of environmental constraints encountered among the six bays, a clear
490 synchronism was nevertheless observed regarding *Pseudo-nitzschia* spp. spring blooms,
491 suggesting that large-scale factors might trigger *PSN* blooms along the coast during this
492 period. Much of the seasonal variability of *PSN* blooms has indeed been attributed to regular
493 shifts in environmental conditions such as wind, irradiance, temperature or river flow (e.g.
494 Trainer et al., 2012 and references therein). In this study, the *PSN* spring blooms started
495 between March (for Quiberon and Concarneau bays) and April (other bays) under various
496 conditions of salinity (and related river flow), turbidity and phytoplankton biomass (Table 2;
497 Fig. 2A). However, similar ranges of photosynthetic active radiation (PAR) and relatively
498 similar ranges of temperature were commonly observed along the coast during this period.
499 These observations suggest that irradiance and temperature may play a major role on *PSN*
500 bloom initiation in spring along the French Atlantic and English Channel coasts. A first spring

501 *PSN* maximum was reached between May and June for all sites corresponding to maximum
502 PAR but not to maximum temperature (Fig.2). This was confirmed by the niche analysis (Fig.
503 7, Table 3).

504 Regarding nutrient availability, although the lack of regular data made it difficult to draw
505 conclusions, March/April are known to be favorable months for diatom growth as high winter
506 nutrient concentrations were still substantial and day length started to increase to increase
507 (Gohin et al 2003).

508 In contrast, the discrepancy observed among sampling sites regarding the occurrence of a
509 second *Pseudo-nitzschia* spp. bloom at the end of summer suggests that these late blooms
510 were driven by more local meteorological phenomenon and, as such, should be more strongly
511 related to the intrinsic environmental characteristics of each bay. Moreover, the occurrence of
512 these late blooms was highly variable at the interannual time scale for the 3 bays concerned
513 (i.e. Seine, Brest and Concarneau bays; Fig.4), supporting the hypothesis of non-recurring
514 local events controlling *PSN* blooms during this period. The Seine and Brest bays are well-
515 mixed semi-enclosed areas receiving direct freshwater inputs which are highly variable during
516 the summer. These freshwater inputs bring an influx of nutrients during the season when they
517 are naturally at their lowest in the marine environment, and could therefore stimulate late *PSN*
518 blooms as previously shown in the Seine bay (Klein et al., 2010) and in other coastal areas
519 (e.g. Bates et al., 1996; Schnetzer et al., 2007; Liefer et al., 2013). Strong wind events that mix
520 a stratified water column and transitory upwelling are also responsible for nutrient enrichment
521 and have been shown to trigger *PSN* blooms in many coastal areas (e.g. Dortch et al., 1997;
522 Lund-Hansen et al., 2004; Trainer et al., 2007; Schnetzer et al., 2013). This may also be the
523 case for the triggering of the *PSN* bloom observed in September in Concarneau bay. Results
524 underline the difficulty in fully assessing the environmental cues that control *PSN* blooms that
525 can be unique to the bays where they occur. Moreover, additional environmental data, such as

526 nutrient concentrations are lacking and would be necessary in order to fully understand the
527 dynamics of *PSN* blooms along the French Atlantic and English Channel coasts. Specific
528 seasonal surveys are needed to thoroughly identify the main factors controlling *Pseudo-*
529 *nitzschia* spp. blooms within each bay.

530

531 ***4.2. Pseudo-nitzschia* spp. blooms and ASP event frequency along the French coast**

532 The magnitude and characteristics of *Pseudo-nitzschia* spp. blooms as well as the
533 frequency of ASP events greatly varied between the bays and the 3 groups identified (Fig. 6
534 and Fig. 8A). The strongest *PSN* blooms occurred in the Quiberon and Concarneau bays,
535 where the highest abundances were recorded and where this genus recurrently dominated the
536 phytoplankton community (Fig. 6). Furthermore, king scallop fishing grounds were also
537 chronically impacted by domoic acid contamination in both these bays: since 2004 in
538 Concarneau and 2006 in Quiberon (Fig. 8). These results suggest recurring local events to be
539 at play in stimulating toxic *PSN* blooms every year in the Northern Bay of Biscay. The
540 coincidence of *PSN* dominance and ASP events has been shown in the North Sea (Bresnan et
541 al. 2015). Indeed, the Scottish site where diatom cell abundance was dominated by the *PSN*
542 was affected by toxicity, whereas in German sites, where diatom blooms were not dominated
543 by *PSN* no toxic events have been observed.

544 At the other end of the spectrum are the Pertuis Breton and Saint Brieuc bay, where ASP
545 events were shown to be scarce and where *PSN* blooms were, most of the time, very limited in
546 terms of absolute and relative abundance (Fig 6 and Fig. 8). Despite very distinct
547 environmental constraints, this implies the prevalence of unfavorable conditions for toxic *PSN*
548 blooms in both these coastal ecosystems. Between these two extremes in *PSN* bloom
549 magnitudes as well as ASP event frequency, the Seine and Brest bays exhibited relatively high
550 *Pseudo-nitzschia* spp. abundances, and king scallops fishing grounds were intermittently

551 affected by domoic acid contamination in both these bays. This suggests that non-recurring
552 local events may control toxic *PSN* bloom outbreaks in these well-mixed shallow bays.

553 These results suggest a strong link between the magnitude of *Pseudo-nitzschia* spp.
554 blooms, the importance of this genus within the phytoplankton community and the frequency
555 of ASP events impacting king scallop fishing grounds. In addition, the discrepancies and
556 similarities of environmental constraints among and within the different groups of bays could
557 give rise to a hypothesis regarding the frequency of ASP events along the coast.

558

559 *4.2.1 Chronic ASP events*

560 Located in the northern Bay of Biscay, Concarneau and Quiberon bays have emerged as
561 perennial “hot spots” for *Pseudo-nitzschia* spp. blooms and ASP events along the French
562 coast. In winter and early spring, this coastal zone receives large freshwater inputs from the
563 Loire and Vilaine rivers (e.g. Loyer et al., 2006; Guillaud et al., 2008), whilst in summer, local
564 upwelling can form along the coast following W/NW wind episodes (Lazure and Jégou 1998;
565 Puillat et al., 2004, 2006). The combination and/or the succession of both these hydrological
566 phenomena could trigger intense and recurrent toxic *PSN* blooms in both these bays. Indeed,
567 nutrient inputs from several sources, including riverine inputs and upwelling or mixing
568 events, have been shown to stimulate *PSN* blooms in a variety of coastal areas (e.g. Trainer et
569 al., 2000; Parsons et al., 2002; Kudela et al., 2005; Anderson et al., 2008) when temperature
570 and irradiance are favorable. In particular, the unbalance in the delivery of nitrogen with
571 respect to silica and/or phosphorus has been shown to be associated with DA production (Pan
572 et al., 1996; Fehling et al., 2004; Parsons and Dortch, 2002; Anderson et al., 2006; Trainer et
573 al., 2007). This is the case particularly for the Quiberon and Concarneau bays, and in fact for
574 all bays excepting Saint Brieuc which hasn't shown toxic *PSN*, figure 2. In the northern Bay
575 of Biscay, the succession, or combined effect, of freshwater inputs from the Loire and Vilaine

576 rivers and local upwelling may therefore contribute to the recurrent development of toxic
577 *Pseudo-nitzschia* blooms and contamination of king scallop fishing grounds in these open
578 bays.

579

580 *4.2.2. Episodic ASP events*

581 The Seine and Brest bays are well-mixed semi-enclosed areas receiving direct freshwater
582 inputs. Anthropogenic nutrients have been shown to strongly affect the functioning in both
583 these eutrophic coastal zones (e.g. Del Amo et al., 1997; Cugier et al., 2005; Romero et al.,
584 2013) and fluxes of silicate, phosphate and nitrate into the bays are strongly controlled by
585 river discharge at the interannual and seasonal scales (e.g. Billen et al., 2001; Romero et al.,
586 2013). In addition, in these well-mixed shallow bays benthic fluxes may also influence
587 phytoplankton community structure. Previous studies carried out in the Brest bay have shown
588 the role of benthic fluxes of dissolved silicic acid in controlling the structure of the
589 phytoplankton community (Del Amo et al., 1997; Fouillaron et al., 2007). Many chemical,
590 physical and biological processes may influence the nutrient supply from the sediments to the
591 pelagic zone (Andrieux-Loyer et al, 2008; Sakamaki et al., 2006) and this autochthonous
592 nutrient supply is likely to vary at both the seasonal and interannual scales. N/P ratios are high
593 in winter during flood events (over the Redfield ratio). This emphasizes observations made by
594 Klein et al (2010), who in September 2007 reported a bloom dominated by *P. australis* and
595 associated with significant levels of particulate domoic acid. This toxic bloom occurred after
596 high nitrogen inputs following a sudden rainfall event resulting in a potential limitation by Si
597 and/or P.

598

599 *4.2.3. Exceptional ASP events*

600 The same causes may explain the exceptional ASP event observed in 2010 in the Pertuis

601 Breton and in the Northern Bay of Biscay (Fig. 6). In winter 2010 the European windstorm
602 “Xynthia” hit the French coast on the 28th of February, causing an exceptional storm surge
603 and consequent flooding along the Southwestern part of the French Atlantic coast (Bertin et
604 al, 2014). Sea water flooding as well as high precipitation may have led to unusually high
605 nutrient loads which could have triggered the intense bloom of *PSN* observed as early as
606 March in the Pertuis Breton and the subsequent contamination of king scallop fishing grounds
607 (Fig.8). Moreover, the highly toxic species *Pseudo-nitzschia australis* was identified in March
608 2010 in the Pertuis Breton (Nézan et al., 2010). This environmental context has already been
609 described in other areas, where *PSN* toxic blooms have appeared after a period of heavy
610 rainfall resulting in nutrient-rich freshwater inputs, in places such as the eastern Canadian
611 coast (Smith et al., 1990; Bates et al., 1998b), the northern Gulf of Mexico (Parsons et al.,
612 2013; Macintyre et al., 2011), Monterey Bay (Bird and Wright, 1989), the Californian coast
613 (Trainer et al., 2000), San Diego bay (Busse et al., 2006) and the Gulf of Kalloni in Greece in
614 2004 (Spatharis et al., 2007). While nutrient concentration values in the water column
615 following windstorm Xynthia are lacking to confirm this hypothesis, results show that major
616 climatic events can trigger toxic *Pseudo-nitzschia* outbreaks in previously non-impacted
617 coastal waters. In addition, they show that large-scale climatic events could stimulate toxic
618 *Pseudo-nitzschia* blooms over a large spatial scale and impact king scallop fisheries all along
619 the Bay of Biscay coastline. In the context of sea level rise and increases in extreme weather
620 events such as winter storms, an improved knowledge of the potential consequences of large-
621 scale climatic events on toxic phytoplankton blooms is an absolute prerequisite for risk
622 assessment.

623

624 **4.3. Environmental control of toxic *Pseudo-nitzschia* spp. blooms and ASP events**

625 Environmental pressures act both on species phenology and on toxin synthesis (Trainer et

626 al., 2012). Nitrogen enrichment and silicate or phosphorus limitation are known to stimulate
627 DA production (Bates et al., 1998a; Fehling et al., 2004b; Parsons et al. 2013; Van de Waal et
628 al. 2014). Among the different environmental factors that have been shown to be associated
629 with toxic *Pseudo-nitzschia* blooms (e.g. Trainer et al., 2012; Lelong et al., 2012 and
630 references therein), different degrees of nitrogen enrichment from river discharge, vertical
631 mixing or upwelling could partly explain the discrepancies observed along the French Atlantic
632 and English Channel coasts, regarding the magnitude of *Pseudo-nitzschia* blooms and the
633 frequency of ASP events. Indeed, results have shown that ASP events tend to be more
634 frequent in bays strongly influenced by riverine inputs and/or transitional upwelling such as
635 the Concarneau, Quiberon, Brest and Seine bays. In contrast, in the Saint Brieuc bay where
636 freshwater inputs are very limited, no ASP event has been observed in the 2003 to 2012
637 period in local king scallop fishing grounds. This observation tends to support the hypothesis
638 of an increasing risk of toxic *Pseudo-nitzschia* outbreaks along a gradient of nutrient inputs
639 from anthropogenic or natural origins..

640 However, the lack of intense *PSN* blooms in the Pertuis Breton under typical
641 environmental conditions tends to invalidate this hypothesis. Indeed, despite substantial
642 freshwater inputs from the Lay ($20 \text{ m}^3 \text{ s}^{-1}$) and Sèvre-Niortaise rivers ($25 \text{ m}^3 \text{ s}^{-1}$), *Pseudo-*
643 *nitzschia* abundances rarely exceed the sanitary threshold in the Pertuis Breton. King scallop
644 stocks remained safe from domoic acid contamination, except in 2010 following the Xynthia
645 windstorm. This suggests that other environmental factors could control the growth of
646 *Pseudo-nitzschia* cells in this shallow embayment. In particular, this bay exhibited higher
647 temperature, irradiance and turbidity than the five other sites (Fig. 2). Increased temperature
648 and irradiance have been shown to stimulate growth rates and DA production in *Pseudo-*
649 *nitzschia australis* (Thorel et al., 2014). The high turbidity observed in this shallow bay
650 especially during the spring was likely to reduce light availability and therefore limit the

651 growth of *Pseudo-nitzschia* as well as DA production.

652 While the comparison of *PSN* bloom magnitudes and ASP event frequencies in six
653 contrasted ecosystems allowed us to formulate some hypotheses regarding the environmental
654 factors driving the discrepancies observed along the coast, nutrient concentration data are
655 crucially lacking to validate this hypothesis. Systematic sampling for nutrient concentrations
656 during routine monitoring surveys is an absolute prerequisite to further assessing the role of
657 nutrients on toxic *PSN* blooms along the French Atlantic and English Channel coasts..

658 Different hypotheses could be raised to explain the discrepancies between toxicity and
659 yearly maximum *PSN*. While several *PSN* blooms may occur over a year (Fig.5), not all of
660 them necessarily lead to scallop contamination, depending on: i) the presence and dynamics
661 of toxic *Pseudo-nitzschia* species and environmental conditions favoring DA synthesis in
662 toxic species and (ii) the pathway between *PSN* toxic blooms to king scallop contamination,
663 considering transport and sedimentation.

664

665 4.4. A matter of diversity

666 *The Pseudo-nitzschia* genus was common member of the phytoplankton community and
667 was present in the water column from spring to autumn in the different studied sites. The risk
668 period for king scallop fishing grounds extends from March to September depending on the
669 bay considered. The sampling frequency as well as the strategy implemented for the survey of
670 king scallop contamination by DA, did not permit an accurate identification of the date of
671 king scallop contamination over this long timeframe. It was therefore impossible to precisely
672 identify the bloom responsible for a given ASP event, or which species was responsible for
673 this bloom.

674 Thirteen different *Pseudo-nitzschia* species have been identified along the French coast
675 (Belin et al 2012). Considering the difficulty in precisely identifying *Pseudo-nitzschia* species

676 under light microscopy, most data only refers to the genus level and the assumptions made
677 previously only concern the genus *Pseudo-nitzschia* spp.. Among the different species
678 identified along the French coast, the highly toxic species *P. australis* and also *P. multiseriata*
679 have been linked to major ASP events along the French coast (Nézan et al., 2006,
680 2010). Several species of *PSN*, with different optimal growth constraints might thrive over the
681 year, e.g. six species have been identified in the Seine bay over an entire year (Klein et al.,
682 2010). In Stonehaven (Scotland), Bresnan et al (2015) showed that, *P. australis* and *P. seriata*,
683 which are highly toxic, were only observed in late summer and autumn whereas in spring *P.*
684 *delicatissima*, *P. pseudodelicatissima* and *P. fraudulenta* were dominant. As different species
685 can demonstrate distinct correlations with environmental factors (e.g. Trainer et al., 2012;
686 Lelong et al., 2012), further research is needed to identify the different species of *PSN*
687 succeeding over the year within each bay in order to identify the environmental cues
688 triggering their growth and toxicity.

689

690 4.5. King scallop contamination vector by domoic acid

691 Contamination of benthic bivalves depends on a variety of physical (e.g. transport,
692 sedimentation of *PSN*) and biological (e.g. physiological state, cellular DA levels) parameters.
693 In particular, the transport and sedimentation of *PSN* blooms over king scallop fishing
694 grounds may be particularly important in bays where king scallop beds are located far from
695 phytoplankton monitoring sampling points, such as in the Seine bay. The optimal way to
696 accurately identify the timing of toxic *Pseudo-nitzschia* blooms, which is an absolute
697 prerequisite to identify the triggering factors, would be to carry out synchronized
698 measurements of (i) *Pseudo-nitzschia* spp. abundance, (ii) domoic acid concentrations in the
699 water column and in the vicinity of king scallop fishing grounds and (iii) DA concentrations
700 in king scallops.

701 Another source of discrepancy between *PSN* abundance and STI could be the origin of
702 contamination. Indeed, the accumulation of dense *PSN* biomass on the seafloor provides a
703 vector for DA accumulation in a variety of benthic fauna (Lefebvre et al. 2002b; Kvittek et al.,
704 2008; Sekula-Wood et al., 2009). As significant levels of domoic acid have been measured in
705 sediment traps long after *PSN* blooms have died out on the surface (e.g. Sekula-Wood et al.,
706 2009), the period of king scallop contamination is therefore likely to extend well beyond the
707 period of *PSN* blooms. If contaminated sediments became re-suspended following mixing
708 events (e.g. storm, spring tides currents, dredging...) they could constitute a ‘secondary’
709 source of contamination for king scallops. An improved knowledge of contamination
710 processes in king scallops as well as additional data regarding the wane of domoic acid in the
711 sediment are crucially needed to fully assess the threat of toxic *Pseudo-nitzschia* blooms on
712 *Pecten maximus* fisheries.

713

714 **5. Conclusion**

715 This work is one of the first studies describing *Pseudo-nitzschia* spp. blooms and Amnesic
716 Shellfish Poisoning (ASP) events in *Pecten maximus* fishing grounds along the French
717 Atlantic and English Channel coasts. The genus *Pseudo-nitzschia* is a common member of the
718 diatom community which can bloom from May to September along the coast. The magnitude
719 and characteristic of *Pseudo-nitzschia* spp. blooms as well as the frequency of ASP events
720 greatly vary along the coast. Different degrees of nitrogen enrichment from river discharge,
721 vertical mixing or upwelling could partly explain the discrepancies observed along the French
722 Atlantic and English Channel coasts. Results suggest the frequency of ASP events, i.e.
723 chronic, episodic and exceptional, to be linked to the frequency of physical and hydrological
724 processes (i.e. river discharge, upwelling, vertical mixing) controlling nutrient ratios over the
725 seasonal and interannual time scale. In addition, this study has shown that large-scale climatic

726 events, such as the ‘Xynthia’ windstorm in 2010, could stimulate toxic *Pseudo-nitzschia*
727 blooms and impact king scallop fisheries over a large spatial scale. In addition, these results
728 highlight the difficulty in fully assessing the environmental cues that control *PSN* blooms that
729 can be unique to the bays where they occur. Additional environmental data, such as nutrient
730 concentrations are lacking to fully understand the dynamics of *PSN* blooms along the French
731 Atlantic and English Channel coasts. Specific seasonal surveys are therefore needed to
732 thoroughly identify the main factors controlling *Pseudo-nitzschia* spp. blooms within each
733 bay.

734 Finally, different climate change scenarios (i.e. sea level rise, increases in storminess, deep
735 changes in river discharge and increased anthropogenic pressure along river watersheds) make
736 it necessary to both improve our knowledge of the environmental factors controlling toxic
737 phytoplankton blooms and to ameliorate surveys, in order to provide information for risk
738 management in exploited coastal waters.

739

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746

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1101

1 **Figures captions**

2

3 **Figure 1.** Location of the six studied bays scattered along the French coast and annual
4 average chlorophyll *a* concentrations ([Chl *a*]) over the 1998-2012 period (color bar). From
5 the North to the South: (i) Seine bay, (ii) St Brieuc bay, (iii) Brest bay, (iv) Concarneau bay,
6 (v) Quiberon bay and (vi) Pertuis Breton. Major rivers influencing the six bays are shown in
7 italic. Annual average Chl *a* concentrations were estimated from ocean color using OC5
8 algorithm and daily composite product merging three satellites Chlorophyll *a* derived data
9 (i.e. MERIS, MODIS/Aqua and SeaWiFS) over the 1998-2012 period (Saulquin et al., 2010).

10

11 **Figure 2:** Box plots of the winter variation of nutrients PO₄, SiOH and DIN (μmol l⁻¹), and
12 their ratio. Horizontal lines symbolize the median value for each bay, boxes represent lower
13 and upper quartiles, and dots are outliers.

14

15 **Figure 3:** A) Monthly median of chlorophyll *a* concentrations (μg l⁻¹), salinity, temperature
16 (°C), turbidity (NTU) and Photosynthetic Active radiation (PAR; 10³ w m⁻²) calculated over
17 the 1998 to 2012 period in each bay. B) Box plots of the annual variation of chlorophyll *a*
18 concentrations (μg l⁻¹), salinity, temperature (°C), turbidity (NTU) and Photosynthetic Active
19 radiation (PAR; 10³ w m⁻²) calculated over the 1998 to 2012 period in each bay. For B)
20 horizontal lines symbolize the median value for each bay, boxes represent lower and upper
21 quartiles, and dots are outliers.

22

23 **Figure 4 :** Seasonal patterns of *Pseudo-nitzschia* spp. blooms for each of the 6 study sites. (A)
24 Median abundance and (B) lower (Q1) and upper (Q2) quartiles regions calculated for each
25 site over the 1995 to 2012 period.

26
 27 **Figure 5:** Principal Component Analysis (PCA) conducted on phytoplankton biodiversity
 28 index and *Pseudo-nitzschia* spp. descriptors from the 6 studied bays. (A) Variables vector map
 29 and (B) Individuals factor map. ‘PSN-Rank’: rank of *Pseudo-nitzschia* spp. within the
 30 phytoplankton community; ‘S’: phytoplankton richness; ‘J’: Berger-Parker evenness; ‘TOT’:
 31 total phytoplankton abundances; ‘%Diat’: the proportion of diatoms versus diatoms plus
 32 dinoflagellates; ‘PSN’: *Pseudo-nitzschia* spp. abundances; ‘%PSN’: relative abundance of
 33 *Pseudo-nitzschia* spp. within the phytoplankton community.

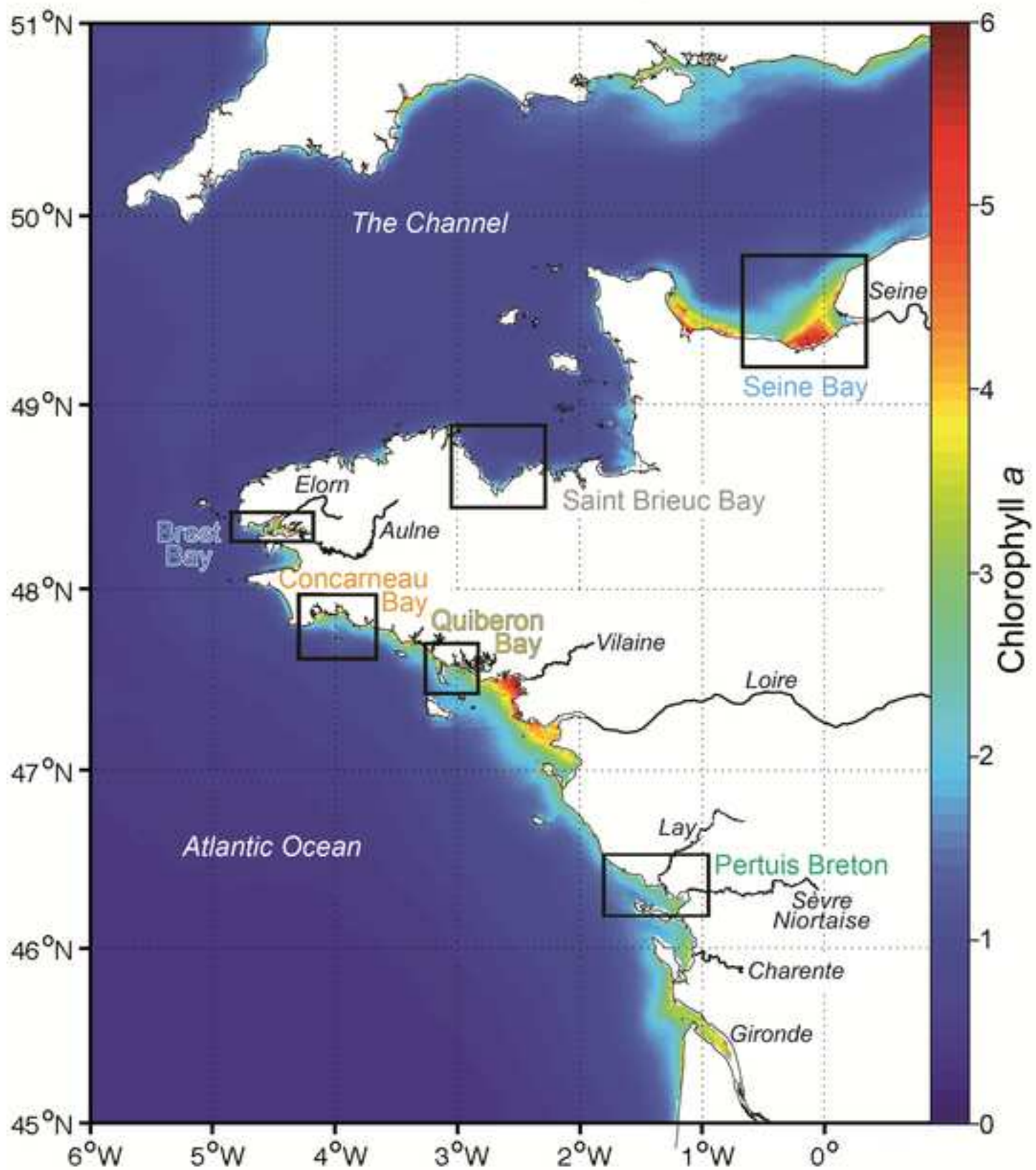
34
 35 **Figure 6:** *Pseudo-nitzschia* spp. abundances (log (Abund. + 1)) over the 1995 to 2012 period
 36 at the six study sites; (A) Seine bay, (B) Saint Brieuc bay, (C) Brest bay, (D) Concarneau bay,
 37 (E) Quiberon bay and (F) Pertuis Breton. For the Seine bay (A), data were not available
 38 before 1997.

39
 40 **Figure 7:** Environmental niche of *Pseudo-nitzschia*. SALI: salinity; TEMP: temperature (°C);
 41 TURB.: turbidity (NTU); PO4 : phosphates ($\mu\text{mol l}^{-1}$); SiOH: silicate ($\mu\text{mol l}^{-1}$) ; DIN :
 42 dissolved inorganic nitrogen ($\mu\text{mol l}^{-1}$) ; N/P : DIN/PO4 ratio ; Si/N : SiOH/DIN ratio ; Si/P :
 43 SiOH/PO4 ratio ; PAR: Photosynthetic Active Radiation (W m^{-2}). Gradient from white to
 44 black shows increasing abundances of *Pseudo-nitzschia*, as calculated from the weighed
 45 Kernel density. Contours mark respectively 25%, 50% and 75% of highest densities of
 46 *Pseudo-nitzschia*.

47
 48 **Figure 8:** A) Scallops Toxicity Index (STI; $\mu\text{g DA g}^{-1} \text{ ww y}^{-1}$) and (B) annual maximum
 49 *Pseudo-nitzschia* spp. abundances ($10^6 \text{ cells l}^{-1}$) over the 2003 to 2012 period at the 6 study
 50 sites.

51

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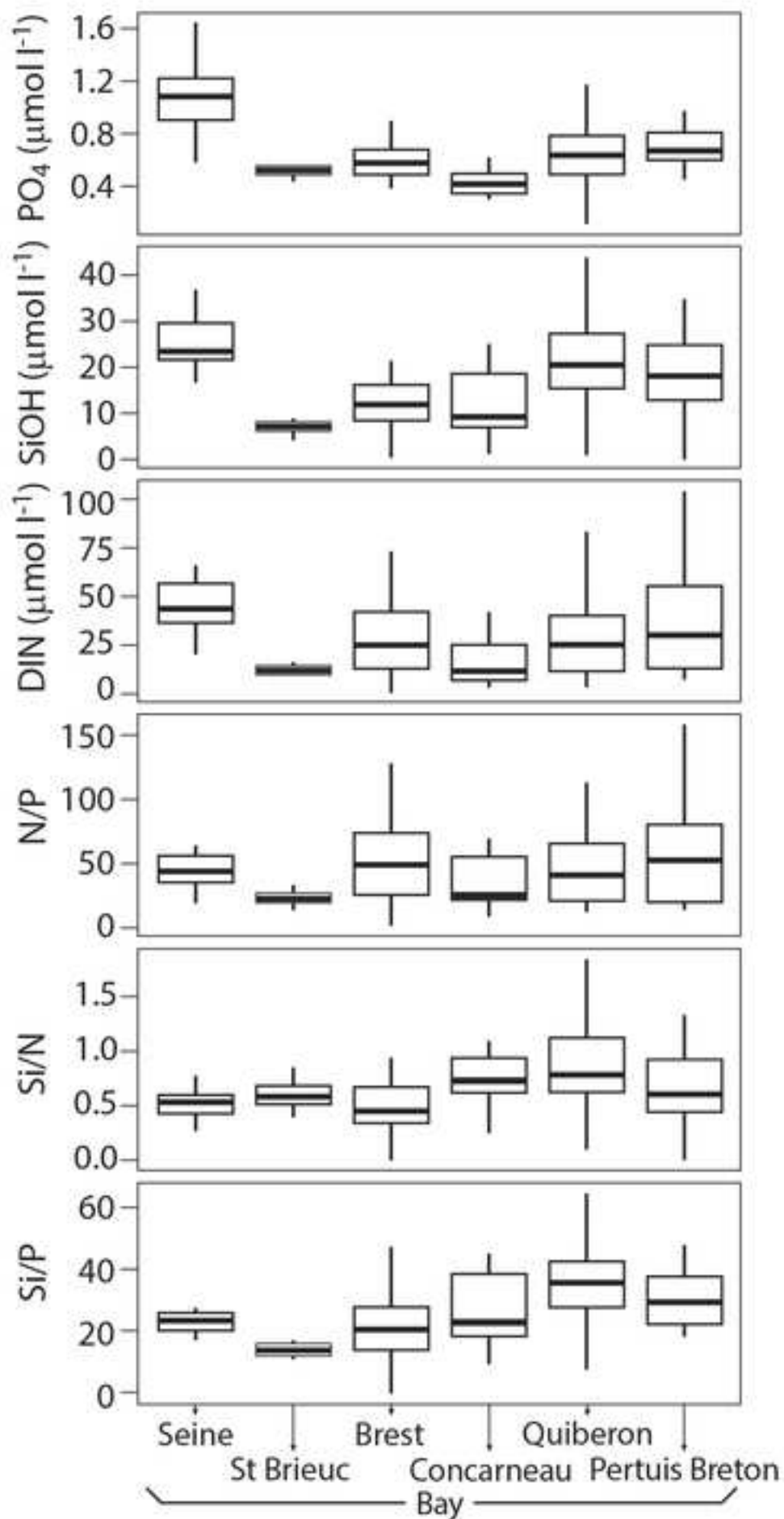
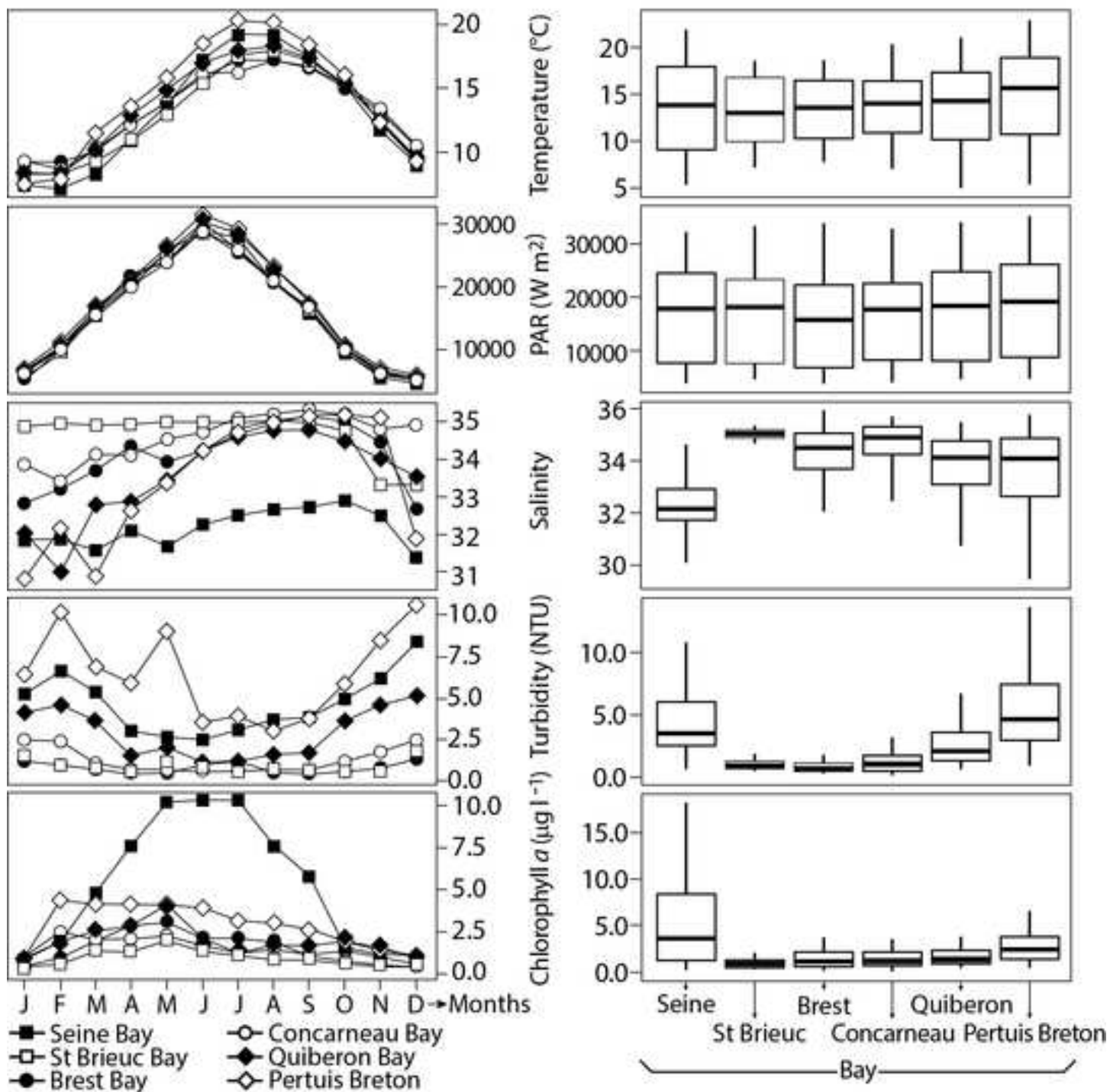


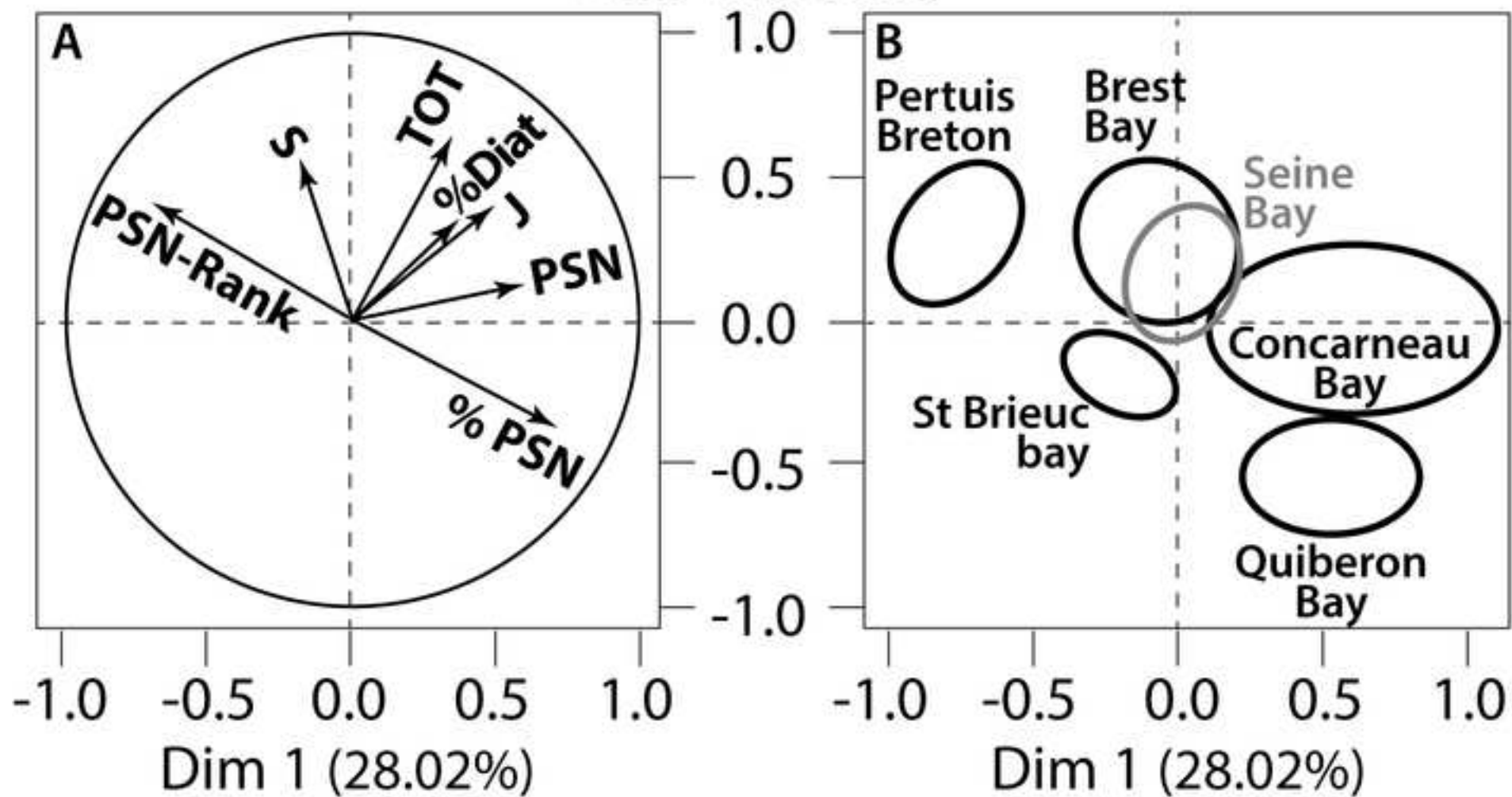
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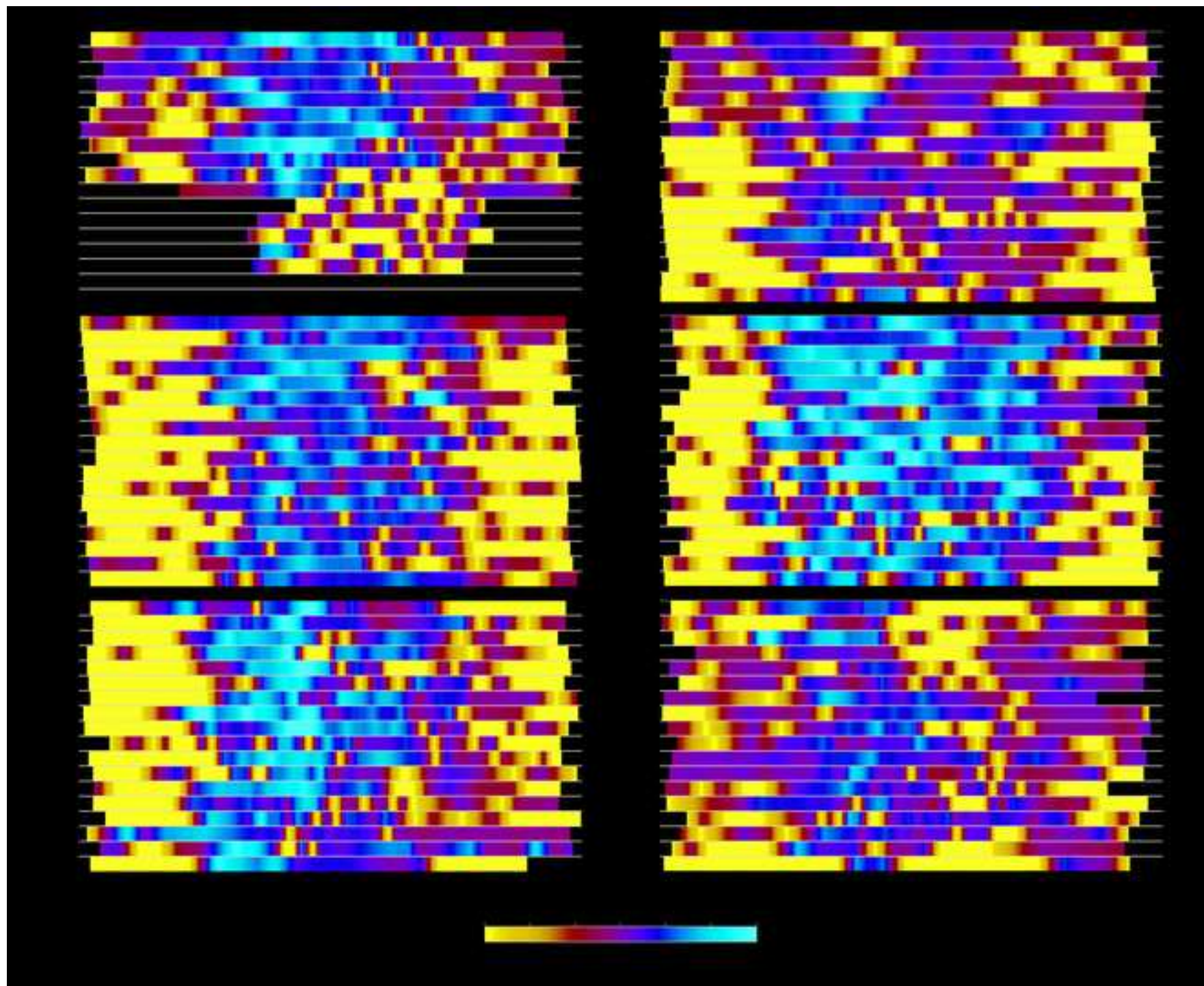
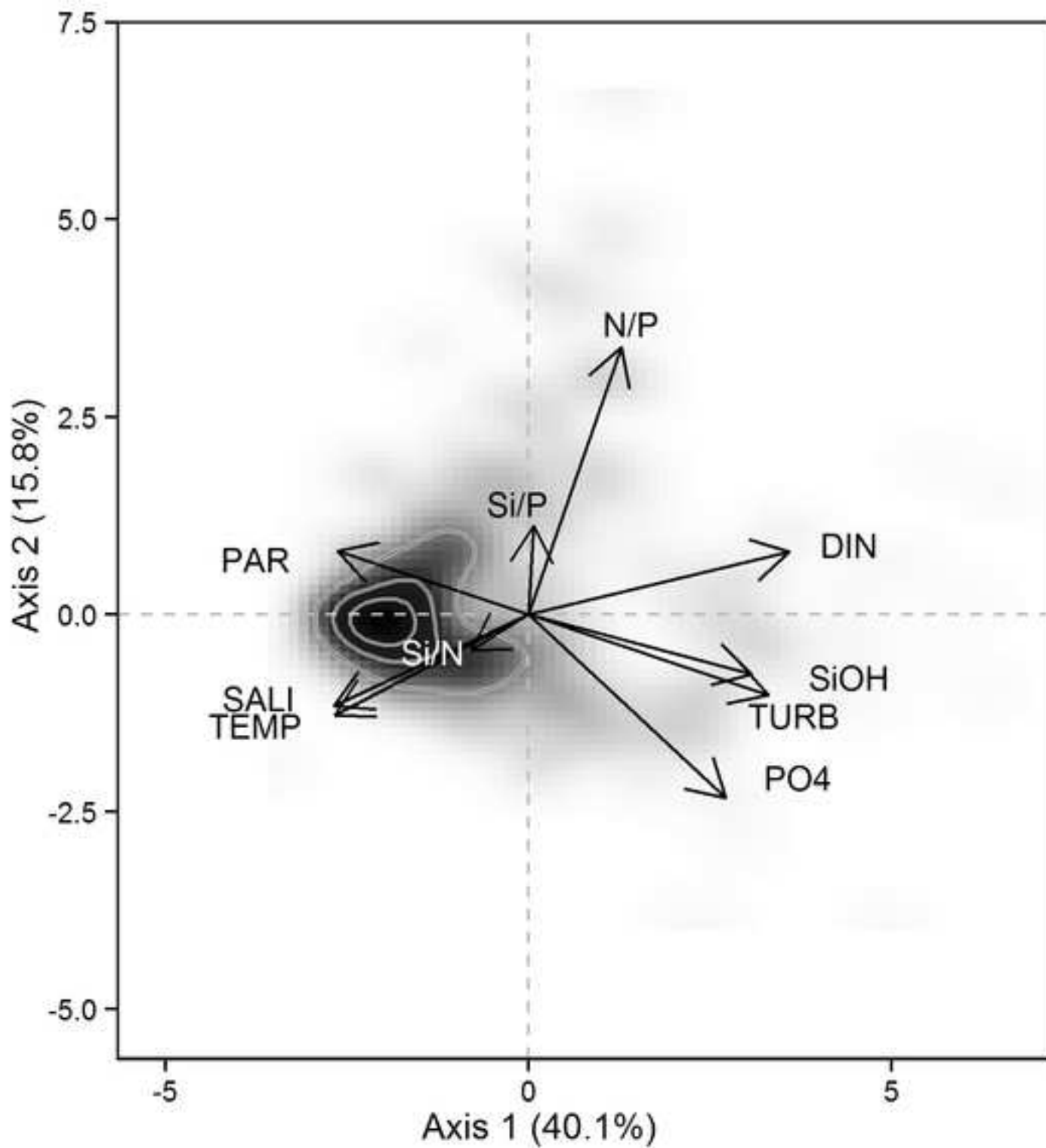
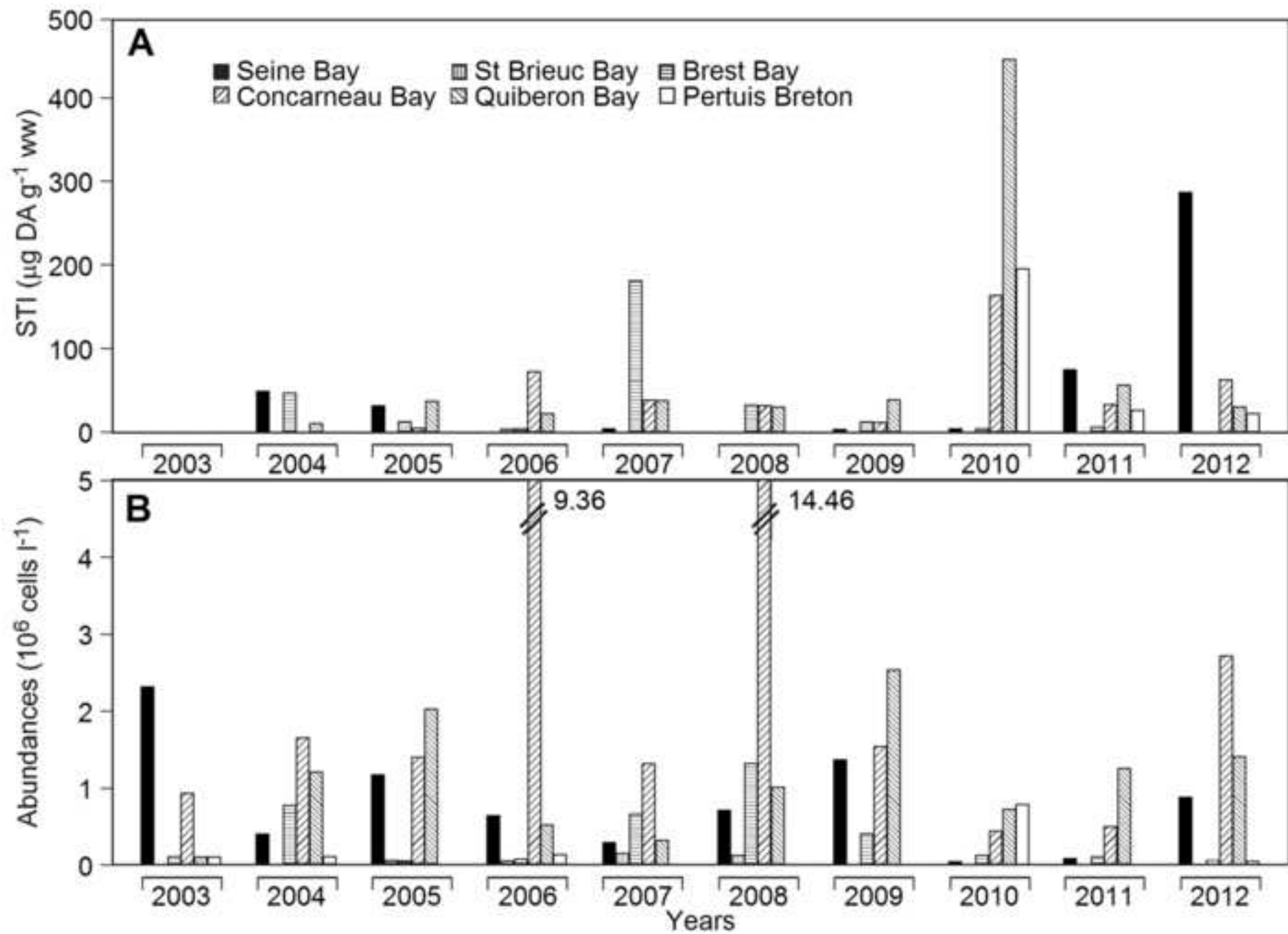


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1 **Two decades of *Pseudo-nitzschia* spp. blooms and king scallop (*Pecten***
 2 ***maximus*) contamination by domoic acid along the French Atlantic and**
 3 **English Channel coasts: seasonal dynamics, spatial heterogeneity and**
 4 **interannual variability**

5
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 7 Annie Chapelle^{e,*}

8
 9 **Tables**

10

11 **Table 1**

12 Geographic coordinates and lowest astronomical tide (LAT) of the 6 sampling stations in each
 13 studied bays

	Sampling station	Latitude	Longitude	LAT* (m)
Seine bay	Cabourg	49°18.50'N	0°07.54'W	5.0
St Brieuc bay	Loguivy	48°49.72'N	3°02.51'W	3.5
Brest bay	Lanveoc	48°18.56'N	4°26.91'W	18.5
Concarneau bay	Concarneau	47°50.00'N	3°56.99'W	20.0
Quiberon bay	Men erRoue	47°32.08'N	3°05.62'W	9.5
Pertuis Breton	L'éperon	46°16.38'N	1°14.03'W	1.0

14 * Lowest Astronomical Tide

15

16 **Table 2.**

17 Environmental characteristics of the 6 studied bays. Median values and lower (Q1) and upper
 18 quartile (Q3) calculated over the 1995 to 2012 period. For the Seine bay, data were not
 19 available before 1997. [Chl*a*]: chlorophyll *a* concentrations ($\mu\text{g l}^{-1}$); T: temperature ($^{\circ}\text{C}$);
 20 Turb.: turbidity (NTU); PAR: Photosynthetic Active Radiation (10^3 W m^{-2}). Chl*a*
 21 concentration, salinity, temperature and turbidity were measured in the field from sub-surface.
 22 Photosynthetic Active Radiation (PAR) were obtained with the ARPEGE model (Météo
 23 France) and the daily PAR was cumulated over the five days preceding samplings.

		[Chl <i>a</i>] ($\mu\text{g l}^{-1}$)	Salinity	Temp ($^{\circ}\text{C}$)	Turbidity (NTU)	PAR (10^3 W m^{-2})
Seine bay	median	4.1	32.5	15.8	3.5	17.5
	<i>Q1</i>	1.4	31.9	10.0	2.4	7.7
	<i>Q3</i>	8.1	33.0	18.5	5.4	24.4
St Brieuc bay	median	0.7	35.0	13.0	1.4	18.0
	<i>Q1</i>	0.5	34.8	9.5	0.9	7.5
	<i>Q3</i>	1.1	35.1	16.9	2.0	26.0
Brest bay	median	1.0	34.5	14.4	1.0	15.5
	<i>Q1</i>	0.6	33.8	0.8	0.6	6.7
	<i>Q3</i>	1.8	35.0	16.7	1.8	22.1
Concarneau bay	median	1.1	34.8	14.2	1.0	17.7
	<i>Q1</i>	0.7	34.1	11.0	0.5	8.1
	<i>Q3</i>	1.9	35.2	16.6	1.7	22.3
Quiberon bay	median	1.3	34.2	15.3	1.8	18.1
	<i>Q1</i>	0.9	33.2	11.4	1.2	8.0
	<i>Q3</i>	2.2	34.7	17.5	3.2	24.4
Pertuis Breton	median	2.8	33.8	16.1	9.1	19.0
	<i>Q1</i>	1.7	32.3	10.8	5.3	8.7
	<i>Q3</i>	4.3	34.6	19.3	16.5	26.0

24 Q1: lower quartile; Q3: upper quartile; NTU: Nephelometric Turbidity Unit

25

26 **Table 3**

27 First and third quantile values for environmental parameters corresponding to the 5% most
 28 abundant *Pseudo-nitzschia* data in Figure 7. SALI: salinity; TEMP: temperature (°C); TURB:
 29 turbidity (NTU); PO4 : phosphate ($\mu\text{mol l}^{-1}$); SIOH: silicate ($\mu\text{mol l}^{-1}$) ; DIN : dissolved
 30 inorganic nitrogen ($\mu\text{mol l}^{-1}$) ; N/P ratio ; Si/N ratio ; Si/P ratio ;PAR: Photosynthetic Active
 31 Radiation (W m^{-2}).

	Minimum	Maximum
SALI	34.02	35.2
TEMP	15.91	17.95
TURB	0.44	1.35
PO4	0.08	0.14
SIOH	1.175	4.62
DIN	0.34	1.11
N/P	2.63	11
Si/N	1.84	5.71
Si/P	11	43.04
PAR	21791	28227

44