



This is an Accepted Manuscript of an article published by Taylor & Francis in
Chemistry and Ecology on 17 October 2019, available online:

<https://doi.org/10.1080/02757540.2019.1676417>

Document downloaded from:



1 Factors driving the seasonal dynamics of *Pseudo-nitzschia* species and domoic acid at mussel
2 farming in the SW Mediterranean Sea

3 Sondes Melliti Ben Garali ^{a,b,*}, Inès Sahraoui^a, Pablo de la Iglesia ^c, Mohamed Chalghaf^b, Jorge
4 Diogène^c, Jamel Ksouri ^b, Asma Sakka Hlaili ^a

5 ^a Laboratoire de Phytoplanctonologie, Faculté des Sciences de Bizerte, Université de Carthage, Bizerte, Tunisia

6 ^b Institut Supérieur de Pêche et d'Aquaculture de Bizerte, Errimel, B.P.15.7080 Bizerte, Tunisia

7 ^c Institut de Recherche et Technologie Agroalimentaire (IRTA), Ctra. Poble Nou, Km 5.5, 43540, Sant Carles de
8 la Rapita, Tarragona, Spain

9

10 **ABSTRACT**

11 The seasonal variations in *Pseudo-nitzschia* species and domoic acid (DA) concentration were
12 investigated, at three shellfish farms in SW coastal Mediterranean. In parallel, the toxicity of
13 mussels was tested. Two distinct groups of species were enumerated according to morphology
14 and size (*Pseudo-nitzschia delicatissima* and *P. seriata* groups). DA was detected over a
15 nine-week period from July to October 2012 in the Lagoon, with a maximum concentration
16 recorded in July (12.71 ng DA l⁻¹). DA was positively correlated with the presence of *P.*
17 *seriata*-group and *P. delicatissima*-group and was mostly occurred during P limitation period
18 in seawater. No DA was found in mussels that were collected during the period of DA absence
19 in seawater. Our results suggest that temperature, salinity, inorganic and organic nutrients were
20 significant for the seasonal dynamics of *P. seriata* and *P. delicatissima* groups, but that the P
21 limitation was the most driving factor for DA production in these areas. The relative influence
22 of environmental factors should be further studied to better understand the recent surfacing of
23 massive blooms of toxigenic *Pseudo-nitzschia* in SW Mediterranean coast.

24 **Keywords:** Domoic acid, *Pseudo-nitzschia*, diatoms, Mediterranean lagoon.

25

26 **Introduction**

27 Domoic acid (DA) was firstly described in the red macroalga *Chondria armata* (Takemoto, *et*
28 *al.*, 1966), and later recorded in 1987 in Prince Edward Island, Eastern Canada, where it was
29 responsible for three deaths and the poisoning of over 100 people (Bates *et al.*, 1998). Since
30 this time, the toxin was discovered in many parts of the world, and become a threat in several
31 regions worldwide (Trainer *et al.*, 2008). Research into the risks associated with DA toxicity
32 has highlighted the importance of both the acute and chronic effects of DA on the health of
33 many marine organisms including mammals, seabirds and humans (Bates, 2000; Scholin *et al.*,
34 2000). The toxin can be transferred effectively to higher trophic levels *via* filter feeders such as
35 molluscan shellfish, copepods crustaceans and fishes (reviewed by Bargu *et al.*, 2008; Lelong
36 *et al.*, 2012; Trainer *et al.*, 2012). Once ingested, the toxin can provoke Domoic Acid Poisoning
37 (DAP) in birds and marine Mammals (Hallegraeff, 2003) and Amnesic Shellfish Poisoning
38 (ASP) in humans, which represents a serious threat for their health (Trainer *et al.*, 2008, 2012;
39 Lefebvre and Robertson, 2010).

40 The neurotoxin is naturally produced by some diatom species of genus *Pseudo-nitzschia*
41 H. Peragallo. Among the fifty one species of this genus, twenty six are known to be toxic,
42 although not always (Lelong *et al.*, 2012; Trainer *et al.*, 2012; Lundholm, 2018; Bates *et al.*,
43 2018). Two *Nitzschia* species (*N. navis-varingica* and *N. bizertensis*) have been also reported
44 to produce domoic acid (e.g. Bates, 2000; Kotaki *et al.*, 2000; Lundholm *et al.*, 2003;
45 Bouchouicha Smida *et al.*, 2014). The genus *Pseudo-nitzschia* constitutes a frequent component
46 of the planktonic diatom community and can reach bloom abundances that could impact the
47 fauna and the shellfish industry, and be a hazard to public health. In 1988, a bloom of
48 *Pseudo-nitzschia* resulted in closure of shellfish harvesting areas in the Bay of Fundy, eastern
49 Canada, where the blue mussels and clams have become contaminated with high levels of DA

50 (Martin *et al.*, 1990), In European Atlantic coasts, DA has affected shellfish production areas
51 of Spain since 1994 (Arévalo *et al.*, 1997) and in France since 1998 (Amzil *et al.*, 2001)

52 In the northern Mediterranean Sea, the occurrence of *Pseudo-nitzschia* was well
53 documented from French, Spanish, Italian and Greek coasts (Kaninou-Grigoriadou *et al.*, 2005,
54 Quiroga 2006, Amato *et al.*, 2007, Quijano-Scheggia *et al.*, 2008, Giménez *et al.*, 2013).
55 However, studies on *Pseudo-nitzschia* dynamics, diversity and toxicity are relatively scarce in
56 southwestern (SW) Mediterranean (Turki *et al.*, 2004; Andréa *et al.*, 2011; Giménez *et al.*,
57 2013), although their blooms are increasing in magnitude and frequency (Sahraoui *et al.*, 2009,
58 Bouchouicha Smida, 2014).

59 In the last decade, shellfish activity was intensively developed in several SW
60 Mediterranean waters, as in the Lagoon and the Bay of Bizerte. Unfortunately, blooms of
61 *Pseudo-nitzschia* were repeatedly observed in these areas during the last few years (Turki *et al.*,
62 2014, Sahraoui *et al.*, 2012, Bouchouicha Smida *et al.* 2014), exceeding in some cases the
63 warning threshold density of 10^5 cells l^{-1} (Auby, 2006). Recently, strains of three species of
64 *Pseudo-nitzschia*, isolated from Bizerte Lagoon, were identified as DA producers in culture [*P.*
65 *brasiliiana* (11.6 ng DA mL^{-1}) *P. delicatissima* (7.5–9.5 fg $cell^{-1}$ DA) and *P. calliantha* (13.4-
66 149.1 ng DA mL^{-1})] (Sahraoui *et al.*, 2009, 2011). Furthermore, some works have reported low
67 levels of DA (2 μg DA l^{-1}) in seawater of the Bizerte Lagoon and in local shellfish samples, as
68 *Mytilus galloprovincialis* (0.13 - 1.60 μg DA g^{-1} tissue) and oysters (*Crassostera gigas*) (0.42
69 – 2.50 μg DA g^{-1} tissue) (Turki *et al.*, 2014; Bouchouicha Smida *et al.*, 2014). However, these
70 reports were punctual in time and until now no long-term investigation of DA on shellfish was
71 done.

72 Managing *Pseudo-nitzschia* spp. blooms and DA occurrence in shellfish areas, as Lagoon
73 and Bay of Bizerte, requires long-term field investigations in order to understand species

74 dynamics and toxicity. The aim of the study is to analyze seasonal variation of *Pseudo-nitzschia*
75 species in relation to environmental factors at three mussel aquaculture sites, during an annual
76 cycle (from March 2012 to April 2013). Data provided here are concerning the main
77 *Pseudo-nitzschia* groups ("*P. delicatissima*" and "*P. seriata*"). The study also focuses on the
78 presence of DA in both seawater and local shellfish samples, based in more accurate technique
79 of DA detection (the LC-MS/MS and LC-UV) than previously used by De la Iglesia *et al.*
80 (2008).

81 **Materials and methods**

82 *Study site*

83 The study was carried out during 14 months at coastal waters, in three shellfish farming areas
84 located in the SW Mediterranean Sea. One is within the Bizerte Bay (station 1, 37°15'33'
85 N, 09°59'24" E) and the two others were inside the Bizerte Lagoon (station 2, 37°15'59" N,
86 09°52'22" E and, station 3, 37°13'55" N, 09°51'58" E) (Fig. 1). The lagoon is connected to the
87 Mediterranean Sea through a 7 Km long, 300 m with and 12 m deep channel. Marine inflows
88 are important in summer while freshwater is mainly supplied in winter (20 Mm³ yr⁻¹) from
89 several surrounding rivers and the Lake Ichkeul (Béjaoui *et al.*, 2008). Hence, water salinity
90 shows a seasonal pattern and varies throughout the year from 30 to 39 psu (Sakka Hlaili *et al.*,
91 2007; Béjaoui *et al.*, 2010) Tidal force is negligible compared to wind forcing, which is the
92 main factor controlling water circulation in the lagoon (Béjaoui *et al.*, 2008). The lagoon is a
93 very important shellfish aquaculture area, including approximately 330 ha divided into 10
94 shellfish production farms (Fig. 1). The production (20 – 223 tons yr⁻¹) is mainly composed of
95 mussels (*Mytilus galloprovincialis*) and Pacific oysters (*Crassostrea gigas*) (DGPA, 2013).
96 Additionally, clams (*Ruditapes decussata*) are also harvested in variable quantities.
97 The Bay of Bizerte is a wide opening ecosystem, (37°20'9"-37°29'9" N, 10°05'20"-9°52'00" E,
98 Fig. 1), with depth varying from 16 to 20 m and salinity from 36 to 38 psu (Addad *et al.*, 2008).

99 Hydrodynamics is mainly driven by the current flowing from west to east with a speed of 0.2–
100 0.5 m s⁻¹ and long-shore currents that steer northeast or east of the Bay (Béjaoui et al., 2008).
101 Within the bay, a recent shellfish farm is acting (Fig. 1), with mussels and oysters as main
102 produced species.

103 *Sampling*

104 The sampling was carried out bi-monthly, from March 2012 to April 2013, in the stations 2 and
105 3 (Fig. 1). Station 1, was monitored during the same period, except on 14 March, 10 May, 18
106 September, 21 October and 12 December 2012 and 19 February 2013, due to rough weather.
107 Characteristics of the three stations are reported in Table 1. At each station, water temperature
108 and salinity were recorded *in situ*, using a multi-parameter (WTW, Multi1970i). At each station,
109 water samples (three replicates) were collected, using a Hydrobios water sampler, from the
110 chlorophyll *a* maximum depth. The later was distinguished from the Chl *a* vertical profile
111 determined before each period of sampling. The samples were stored in isothermal containers
112 and processed within a few hours (2-4 h) after sampling. These samples served for analyses of
113 nutrients, domoic acid, Chl *a* and for identification and enumeration of phytoplankton. Each
114 analysis was done in triplicate.

115 The shellfish sampling was performed every 15 days, from March 2012 to June 2012, at
116 the three farms. At least 2 kg of mussels (mean length: 6 cm ± 0.77; width: 3 cm ± 0.38) were
117 taken per sample and served for analysis of domoic acid in their edible tissues.

118 *Analyses*

119 *Nutrient analyses*

120 Water samples (1000 ml) were filtered through 0.2 µm polycarbonate filters (Millipore).
121 The filtrates were collected in acid-washed vials and stored frozen (-20°C) until analyses.
122 Nutrient concentrations were determined by spectrophotometric methods. Nitrite and nitrate
123 were analyzed according to Wood *et al.* (1967), and ammonia following the procedure of

124 Aminot and Chaussepied (1983). Phosphorous levels were determined as described in Murphy
125 and Riley (1962), whereas, reactive dissolved silicate was analyzed according to Aminot and
126 Chaussepied, 1983 Urea was analyzed using the diacetyl monoxime thiosemicarbizide
127 technique (Price and Harrison, 1987), modified to account for a longer time period (72 h) and
128 lower digestion temperature (22°C). Detection limits of the analytic methods were 0.01, 0.02
129 and 0.1 µM, for nitrite, phosphate and silicate, respectively, 0.05 µM for nitrate and ammonia,
130 and 0.002 µM for urea.

131 *Phytoplankton analyses*

132 For chlorophyll *a* (Chl *a*), samples (1000 ml) were filtered through Whatman GF/F filters.
133 Pigment concentration was determined using the standard spectrophotometric method (Parsons
134 *et al.*, 1984), following extraction with 10 ml of 90% acetone overnight at 4°C in the dark.

135 For phytoplankton identification and enumeration, samples (150 ml) were fixed with
136 acidic Lugol's solution (3% final concentration). The count of cells was carried out, after
137 settling for 24 h, under an inverted microscope (100x oil immersion objective) (CETI)
138 (Utermöhl 1931; Lund *et al.*, 1958).

139 Potentially toxic diatoms cannot be accurately distinguished at species level by the light
140 microscopy (Trainer *et al.*, 2008). Therefore, *Pseudo-nitzschia* cells were assigned to one of
141 two groups based on their transapical axis and morphology: the *Pseudo-nitzschia delicatissima*
142 group (width < 3 mm) and the *Pseudo-nitzschia seriata* group (width > 3 mm) (Hasle and
143 Syvertsen, 1997).

144 *Domoic acid analysis*

145 According to the analysis of DA in shellfish and seawater (particulate), certificate calibration
146 solution of DA (CRM-DA-f, 101.8 ± 2.1 µg ml⁻¹) was obtained from the Measurement Science
147 and Standards, National Research Council of Canada. HPLC gradient grade and LC-MS
148 hypergrade acetonitrile, methanol and formic acid were purchased from Merck (Darmstadt,

149 Germany). Milli-Q water was obtained from a Millipore water purification system (Bedford,
150 MA, USA).

151 *Domoic acid analysis in shellfish by liquid chromatography-UV absorbance detection (LC-UV)*

152 A minimum of 100 g per sample of whole tissue was drained in a sieve before homogenization
153 of the pooled individuals. Then, aliquots of 4.0 ± 0.1 g of the homogenate tissue were accurately
154 weighed into a 50 ml Falcon centrifuge tube and vortex-mixed in a digital multi-tube vortexer
155 DVX-2500 (VWR Int., West Chest, PA, USA), with 16 ml of methanol/water (1:1, v/v). After
156 extraction, samples were centrifuged at $2.795 \times g$ for 20 min (MR 22i Centrifuge, Joan, France)
157 and the supernatant was filtered through a $0.45 \mu\text{m}$ cut-of nylon syringe filter (Whatman).
158 Conventional chromatographic separations (Quilliam *et al.*, 1995; CEN 2008) were performed
159 on an HPLC Alliance 2659 (Waters, Milford, MA, USA) equipped with reversed phase column
160 Zorbax C18 ($4.6 \times 250 \text{ mm}^2$, $5\mu\text{m}$ particle size) purchased from Agilent Technologies (Santa
161 Clara, CA, USA). A photodiode array detector 2996 (Waters, Milford, MA, USA) operated at
162 242 ± 10 nm wavelength. Isocratic elution was carried out with a mobile phase consisting of
163 acetonitrile: water (1:9) with 0.1% formic acid at 1.2 ml min^{-1} flow rate. The column oven was
164 set at 40°C and injection volume was $20 \mu\text{l}$. The limit of quantification for DA and its isomer
165 epidomoic acid was 0.5 mg kg^{-1} . The method is accredited under ISO 17025 by the Spanish
166 National Accreditation Body (ENAC) and applied for official control analysis of DA in
167 shellfish from shellfish harvesting areas (accreditation 900 LE/1797).

168

169 *Domoic acid analysis in phytoplankton by rapid resolution liquid chromatography-tandem*
170 *mass spectrometry (LC-MS/MS)*

171 For analysis of particulate DA in seawater samples, 1000 ml of seawater were filtered
172 through GF/F filters (Whatman) and processed according to the method by rapid resolution

173 liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Melliti Ben Garali *et*
174 *al.*,2016).

175 ***Statistical analyses***

176 Statistical analyses were performed in SPSS software.11.0 for Windows. An analysis of
177 variance (ANOVA) was used to test the significance of the temporal variations of abiotic and
178 biotic variables. The conditions of normality of data distribution (Kolmogorov-Smimov test)
179 and homogeneity of variance (Bartlett-Box test) were respected. The Sperman's correlation was
180 calculated to test the linear relationship between the diatoms abundance (total and
181 *Pseudo-nitzschia* groups) and environmental factors. Spearman's rank analysis was also
182 conducted to determine whether DA was correlated with the presence of *Pseudo-nitzschia* (total
183 and groups) and environmental factors (temperature, salinity and nutrient levels and ratios).

184 **Results**

185 ***Hydrological and chemical environment***

186 Water temperature was similar among the three stations ($P > 0.05$) and varied between 11
187 (April 2013) and 30.4 °C (July 2013) (Fig. 2a). No spatial variation was found in salinity, which
188 was around 38.2 PSU, except from 14 March to 3 May 2012, when it dramatically fell to 14–
189 24 PSU (Fig. 2b). This was associated with frequent rains and considerable fresh water influx
190 from the surrounding rivers.

191 The water of the three stations was characterized by high levels of inorganic nitrogen
192 ($\text{NO}_2 + \text{NO}_3 + \text{NH}_4$: 5 – 50 μM ; Fig. 2f). Organic nitrogen (i.e. urea) reached also relatively high
193 concentrations ranging from 0.29 (March 2012) to 6 μM (September 2012) (Fig. 2c).
194 Concentrations of PO_4^{3-} fluctuated between 0.14 (March 2012) and 6.1 μM (July 2012),
195 whereas those of $\text{Si}(\text{OH})_4$ varied from 0.2 (December 2012) to 5.3 μM (February 2013)
196 (Fig. 2d).

197 As nutrient levels, the N:P, Si:P and Si:N ratios significantly varied over time ($P < 0.01$).
198 At the three stations, there was severe limitation on Si (Si:P= 0.11 – 7.16; Si:N= 0.14 – 0.47)
199 during the sampling period. The N-nutrients were also limiting in station 1 (N:P = 0.5 – 10)
200 over all sampling period and in station 2 (N:P = 2.6 – 14) during most dates. In contrast, P
201 limitation (N: P = 23 – 87) was detected in station 3 during two periods, from 01 March to 29
202 May 2012 and from 06 July to 03 October 2012.

203 *Phytoplankton communities*

204 From June to the end of the sampling period, Chl *a* was relatively similar among all
205 stations, ranging from 0.35 to 6.905 $\mu\text{g l}^{-1}$. Some biomass peaks (6.41-6.82 $\mu\text{g l}^{-1}$) were observed
206 from November to September 2012, but they were less pronounced than those of the first
207 sampling period.

208 Phytoplankton abundances were always higher at station 3 ($2.8 \times 10^6 - 5.7 \times 10^7$ cells l^{-1})
209 compared to those at stations 1 and 2 ($1.1 - 7.0 \times 10^6$ cells l^{-1}) ($P < 0.05$), particularly from 01
210 March to 10 May 2012. This period was characterized by the most pronounced bloom in most
211 stations.

212 *Dynamics of diatoms and Pseudo-nitzschia*

213 As observed for total phytoplankton (Fig. 3), diatoms reached very high densities during
214 the first sampling period (01 March - 10 May 2012), particularly at station 3 ($2.6 \times 10^7 - 35 \times 10^7$
215 cells l^{-1}) and then at station 2 ($0.5 - 5.5 \times 10^7$ cells l^{-1}) (Fig. 4b, c). At marine station 1, the diatoms
216 bloom was less pronounced ($0.6 - 1.4 \times 10^7$ cells l^{-1}). The observed blooms were mainly
217 composed by species of *Chaetoceros* in all stations. Subsequently, diatoms were less abundant
218 in the three stations ($2.5 \times 10^5 - 4.3 \times 10^6$ cells l^{-1}), but showed some peak density (Fig. 4).

219 Within the Bizerte Lagoon (i.e. stations 2 and 3), *Pseudo-nitzschia* cells exhibited
220 permanent presence from April 2012 (at station 2) or June 2012 (at station 3), until the end of
221 the sampling period (Fig. 4b, c). At both stations, cell densities, varying from 2.5×10^4 to $1.5 \times$

222 10^6 cells l^{-1} , rapidly increased during September - October 2012. In contrast, at the Bizerte Bay
223 (i.e. station 1), *Pseudo-nitzschia* species were sporadically observed at few dates, with a density
224 of $7 - 30 \times 10^4$ cells l^{-1} (Fig. 4a). When observed, *Pseudo-nitzschia* contributed 1.5 - 24.07 % of
225 total phytoplankton and 5.2- 40% of diatoms. The highest contributions were obviously found
226 at lagoonal stations.

227 The two groups (*P. seriata* group and *P. delicatissima*-group) were observed during most
228 sampling period (Fig. 5). At station 1, the *P. seriata*-group was more present contributing 70 –
229 100 % of total *Pseudo-nitzschia* abundance. The second group, *P. delicatissima*-group, was
230 found only during four dates (14 Jun and 20 July 2012; 19 February and 13 April 2013) and
231 was contributed 50 to 100% of total *Pseudo-nitzschia*. At stations 2 and 3, these groups have
232 almost similar allocations to the total *Pseudo-nitzschia* (20-80%).(Fig. 5b, c). In some dates,
233 *Pseudo-nitzschia* communities were exclusively composed by *P. seriata* group (as at station 3
234 in 07 March 2012 and 14 June 2013) or by *P. delicatissima* group (as at station 2 in 03
235 September and 12, 20 December 2012, and 07 March 2013).

236 ***Relating environmental conditions to Pseudo-nitzschia occurrence***

237 The relationship between environmental data and *Pseudo-nitzschia* assemblage data were
238 tested in order to determine which variables best explained/matched the species group data
239 (Table 2). The results showed that the two identified groups were ecologically similar. The *P.*
240 *delicatissima*-group significantly ($P < 0.01$) correlated to salinity, silicate, phosphate, urea and
241 Chl *a*. With the exceptions of total inorganic N, all correlations were positive. The *P. seriata*-
242 group positively correlated to salinity, urea, water temperature, phosphate and Chl *a*, but
243 negatively to total inorganic N. When considered both *Pseudo-nitzschia* groups, they positively
244 ($P < 0.01$) correlated to salinity, silicate, phosphate, urea and Chl *a*, with the exceptions of total
245 inorganic N. Total diatoms were positively related to phosphate, total inorganic N and Chl *a*,
246 but total diatoms negatively correlated to salinity ($P < 0.01$).

247 *Domoic acid levels*

248 LC-MS analysis showed that particulate domoic acid was present in seawater from July
249 to October 2012 only at lagoonal stations (i.e. stations 2 and 3), but it was more prevailing at
250 station 3 (6 dates) than at station 2 (3 dates) (Fig. 6). Levels of DA ranged from 0.85 to
251 12.71 ng l⁻¹, with the highest value observed on 06 July 2012 at station 3. In this station, the
252 DA presence was associated with a period of P limitation (N: P = 26.43 to 62). Effectively, DA
253 was negatively correlated to PO₄³⁻ levels (-0.543; P<0.01) but positively related to N:P ratio
254 (0.420). However, there was a positive correlation between DA and inorganic N-nutrients at
255 station 3 (0.721; P<0.01). The pronounced DA concentration was measured when *Pseudo-*
256 *nitzschia* community in station 3 was composed by *P. seriata* (70%) and *delicatissima* (30%)
257 groups (Fig. 5c). Furthermore, the DA occurrence was significantly positively correlated to the
258 presence of *P. seriata*-group (0.745; P<0.01) and *P. delicatissima*-groups (0.740; P<0.01). In
259 station 2, a significant linear relationship was also found between DA and the occurrence of
260 both *Pseudo-nitzschia* groups (0,530). In both stations, DA exhibited a significant positive
261 relationship with temperature and salinity, as observed for *Pseudo-nitzschia* groups (Table 2).

262 Although *Pseudo-nitzschia* species and DA was found in seawater, no DA was detected
263 in tissues of mussels collected from March to June 2012 in the three stations.

264 **Discussion**

265 **Diversity of *Pseudo-nitzschia* community**

266 Results revealed that the nutrient-enriched waters of the Bizerte Lagoon and Bay are
267 suitable for proliferation of potentially toxic diatoms. The same trend has been previously
268 reported in the Lagoon by Bouchouicha *et al.* (2014), and in other eutrophic environments, e.g.
269 NW Adriatic Sea (Penna *et al.*, 2006). In the lagoon, *Pseudo-nitzschia* composed a large
270 fraction of total phytoplankton and total diatoms. These contributions were in the range of those
271 previously reported in the Bizerte lagoon (68% of total phytoplankton Sahraoui *et al.*, 2011).

272 Similarly, in other coastal environments, such as Santa Monica Bay, California and NE of the
273 Adriatic, the genus *Pseudo-nitzschia* have often contributed to potentially harmful
274 phytoplankton (92 - 100%) (Penna *et al.*, 2006; Shipe *et al.*, 2008).

275 During the study, abundance of *Pseudo nitzschia* were similar as those reported by
276 previous studies in the lagoons of Bizerte (Bouchouicha Smida *et al.*, 2014) and of Nadoor
277 (Daoudi *et al.*, 2009). As observed in previous studies (Sahraoui *et al.*, 2009; Downes-Tettmar
278 *et al.*, 2013), *Pseudo-nitzschia* was positively correlated with temperature and salinity, during
279 our sampling. Both factors were reported to be important in controlling *Pseudo-nitzschia*
280 growth as well in laboratory as in field (Bates, 1998; Doucette *et al.*, 2008).

281 High levels of inorganic nutrient in seawater may also stimulate the *Pseudo-nitzschia*
282 proliferation (Downes-Tettmar *et al.*, 2013). This was supported by positive correlations
283 observed, during our study, between *Pseudo-nitzschia* cell density, silicate and phosphate. In
284 other ecosystems (as Western English Channel), negative correlation was rather observed
285 between these diatoms, phosphate and silicate (Downes-Tettmar *et al.*, 2013). Beside the
286 inorganic nutrients, organic material, including urea, may stimulate the *Pseudo-nitzschia*
287 growth (Loureiro *et al.*, 2009). Furthermore, Hillebrand and Sommer (1996), showed that
288 *Pseudo-nitzschia multiseries* grew equally well on glutamine and urea as on nitrate. Similar
289 result was observed during our study, since these diatoms were significantly related to urea.
290 Moreover, the most contribution of *Pseudo-nitzschia* was excited by the high concentration of
291 urea. In fact, there is clear evidence that *Pseudo-nitzschia* can utilize multiple sources of
292 nitrogen especially urea. This preference preferably in urea increased under the conditions
293 limiting of the N:P ratio. The study of Kudela *et al.* (2008) showed that *Pseudo-nitzschia*
294 *australis* exhibits the highest affinity for nitrate followed by ammonium then urea. In our recent
295 work, we reported that Melliti Ben Garali *et al.*, 2016. Nitrogen is also a necessary component
296 for synthesis of domoic acid

297 During one year sampling, the *Pseudo nitzschia* communities were composed by species
298 belonging to two groups of *Pseudo-nitzschia*. Several works have also reported that several
299 groups frequently contributed to *Pseudo nitzschia* assemblages in the Lagoon and Bay of
300 Bizerte (Sahraoui et al., 2012) and in the Northwestern Mediterranean Sea (Andree et al., 2011;
301 Loureiro et al., 2009).

302 However, there is not a strong degree of seasonal separation between the two groups, as
303 observed in other waters (Fehling *et al.*, 2006; Kaczmarska *et al.*, 2007; Downes Tettermar *et*
304 *al.*, 2013). Effectively, both groups prevailed and peaked at the same periods. The only
305 difference was found between the beginning of *P. seriata*-group and *P. delicatissima*-group
306 occurrences. It seemed that the first group appeared earlier than the second.

307 The *P. delicatissima*-group exhibited a pronounced occurrence in the Bizerte Lagoon,
308 while in the marine station their presence was sporadic. The dominance of *P. delicatissima* -
309 group was previously reported for other Mediterranean areas (Sahraoui *et al.*, 2009). During
310 our study, some environmental factors (temperature, salinity, phosphate, total nutrient and urea)
311 enhanced the proliferation of *P. delicatissima*-group. In contrast, there was a negative
312 relationship between this group and total inorganic N This result agrees with previous
313 suggestions that species belong to *P. delicatissima*-group are effective scavengers in low
314 nutrient conditions (Fehling *et al.*, 2006). In contrast to *P. delicatissima* -group, the *P. seriata*-
315 group was more prevalent throughout the year in the Lagoon as in the Bay. This result suggests
316 that the *P. seriata*-group was eury-halin and hence could have a large temporal and spatial
317 distribution. The occurrence of *P. seriata*-group was positively related to temperature,
318 phosphate, and urea. This agrees with results of Fehling *et al.* (2006) in Scottish waters. As
319 observed for *P. delicatissima*-group, negative relationship was found between the *P. seriata*-
320 group and total N-nutrient. Conversely to our finding, a previous study, in the sampled sites,
321 showed that *P. seriata*-group was uncorrelated with environmental factors and exhibited a

322 narrow spatio-temporal dispersion (Sahraoui *et al.*, 2009). The patterns in occurrence of
323 *Pseudo-nitzschia* groups in Bizerte Lagoon suggest a degree of annual variation. Furthermore,
324 several *Pseudo nitzschia* species that belong to the *P. seriata*-group may show different
325 adaptation strategies to environmental conditions, as salinity and N nutrients.

326 **Domoic acid in seawater and shellfish.**

327 Most studies focused on DA levels in bivalves, but they are scarcer in seawater. In the
328 SW Mediterranean Lagoon and Bay of Bizerte, the study of Sahraoui *et al.* (2012) was the first
329 to detect DA in seawater (0.5 to 2 $\mu\text{g l}^{-1}$) during one occasion characterized by a bloom of *P.*
330 *brasiliiana*, observed at one station of the Lagoon far from shellfish areas. To accurate our
331 knowledge about the impact of *Pseudo-nitzschia* and DA presence on the shellfishing activity,
332 our study have assessed DA occurrence during one year monitoring at three areas of mussel
333 production, by using a more sensible methods, as LC-MS/MS and LC-UV for analyses of DA
334 in seawater and shellfish, respectively.

335 DA was detected when the *Pseudo nitzschia* community was almost shared between *P.*
336 *seriata* and *P. delicatissima*-groups. These observations highlight the potential of *P. seriata*-
337 group and *P. delicatissima*-group to produce DA in these mytiliculture areas. Some species
338 belonging to the *P. delicatissima* group (*P. calliantha*, *P. brasiliiana* and *P. delicatissima*) were
339 previously isolated from the Bizerte Lagoon and were confirmed to be toxin producer (Sahraoui
340 *et al.*, 2012). So, these species may also be the causative diatoms of DA measured during this
341 study.

342 Before our study, the *P. seriata*-group was found to be scarce in the Bizerte Bay and
343 Lagoon (Sahraoui *et al.*, 2012). However, during our sampling, species within this complex
344 exhibited an important proliferation and may contribute to the DA presence in the lagoonal
345 seawater. Effectively, some species in *P. seriata*-group (as *P. seriata* and *P. australis*) have
346 been found to be toxic in seawaters for other ecosystems (Bates *et al.*, 2004; Fehling *et al.*,

2004; Howard *et al.*, 2007). In station 1, no DA was found while *Pseudo nitzschia* cells were present. Therefore, the samples which displayed an absence of DA may have been composed predominantly by non-toxin producer *Pseudo nitzschia* species. This suggested that there was a change in species composition within *Pseudo-nitzschia* groups between lagoonal and marine waters. Furthermore, the absence of DA in marine waters, when *Pseudo nitzschia*. groups were prevailing, may be related to the environmental conditions, which could be not favorable to toxin production in these waters.

DA was more observed when phosphate was limiting. Moreover, DA was positively and negatively correlated to N: P ratio and phosphate levels, respectively. Nutrient stress was previously reported as stimulated factor for DA production in natural water and culture (Howard *et al.*, 2007). However, silicate limitation, rather than phosphate limitation, was reported as the main factor controlling DA in other areas (Fehling *et al.*, 2004, Downes-Tettmar *et al.*, 2013). Hence, there was evidence that phosphate limitation seemed to be the most driving factor for DA production in the lagoon of Bizerte. In contrast to phosphate, inorganic N-nutrients were available when DA was measured and even DA showed positive correlation to these nutrients. The N-nutrients were reported to be necessary for toxin production, as DA is a nitrogen containing molecule (Bates, 1992). DA occurrence was also correlated positively to water temperature and salinity, as was shown previously in the Bizerte Lagoon (Sahraoui *et al.*, 2012) and other waters (Fehling *et al.*, 2004).

DA was measured when *Pseudo-nitzschia* reached high abundances. These densities compared well with other studies (Sahraoui *et al.*, 2012, Downes Tettmar *et al.*, 2013) and even exceeded the threshold which triggers a requirement for DA analysis in shellfish (Turki *et al.*, 2014). However, DA concentrations found during our study were lower than those reported for other coastal waters, as southern Californian waters (7.3 mg l⁻¹, Trainer *et al.*, 2000; 2.33 mg l⁻¹, Busse *et al.*, 2006); Mobile Bay (8 mg l⁻¹, Macintyre *et al.*, 2011); Luand Bay (14.01 ng

372 l⁻¹, Blanco *et al.*, 2010) and Gulf of Mexico (8000 ng l⁻¹, Macintyre *et al.*, 2011). This may
373 indicate that low intensity toxin-producing *Pseudo-nitzschia* species were present at lagoonal
374 stations during our sampling.

375 The bivalves were sampled during March to June 2012, when no DA was found in the
376 seawater, indicating the absence of toxic producer *Pseudo nitzschia* species. Obviously, no DA
377 was also measured in the sampled mussels. During the period characterized by DA occurrence
378 in seawater, we were unable to collect mussels because of high shellfish mortality following an
379 anoxic incident (DGPA, 2013). Although this lack, DA levels in seawater were very low, so if
380 mussels could be contaminated, their toxicity would have likely been below the value of 20 mg
381 kg⁻¹ (CODEX STAN 292-2008; Regulation (CE) No. 853/2004), as reported by Bouchouicha
382 Smida *et al.* (2014). This was contrary to what was observed in other Mediterranean sites
383 (Amzil *et al.*, 2001; Kaniou-Grigoriadou *et al.*, 2005). In Europe Atlantic coasts, DA has
384 affected shellfish production areas of Spain, Portugal and France (Arévalo *et al.*, 1997; Amzil
385 *et al.*, 2001; Vale and Sampavo, 2001).

386 **Conclusion**

387 Results revealed that the nutrient-enriched waters of the Bizerte Lagoon and Bay are
388 suitable for proliferation of potentially toxic diatoms Although the *Pseudo-nitzschia* spp.
389 produced only low concentrations of DA there is a theoretical potential for toxic events to occur
390 at these sites.

391 These findings indicate that during periods and conditions such as these, toxin production
392 is could occur in this region. Species within this (*P. seriata* group) complex exhibited an
393 important proliferation and may contribute to the DA presence in the Lagoon. However, further
394 investigation is needed to gain an improved understanding of the different *Pseudo-nitzschia*
395 spp. at the study sites and to establish which species are toxin producers. In summary, this study,
396 conducted over 14 months shows how important detailed sampling of the environment is to the

397 understanding of *Pseudo-nitzschia* dynamics and toxin production. The information obtained
398 is useful for phytoplankton monitoring programmes and the eventual inclusion into for
399 forecasting toxic events.

400 **Acknowledgments**

401 We thank the owners of shellfish farms Bizerte lagoon which we give access to their shellfish
402 farms. We also wish to thank the entire team of the IRTA for the DA analyses. English text
403 was edited by native speaker. The authors thank the two anonymous reviewers for their
404 constructive and helpful comments.

405

406

407

408

409

410

411

412

413

414

415

416

417

418 **References**

419 Amato, A., Kooistra, W., Ghiron, J. H.L., Mann, D.G., Proschold, T., 2007. Reproductive
420 isolation among sympatric cryptic species in marine diatoms. *Protist*. 158, 193-207.

- 421 Aminot, A., Chausspiéd, M., 1983. Manuel des analyses chimiques en milieu marin. Centre
422 national pour l'exploitation des océans. Brest, ISBN: 2.902721.10.2.
- 423 Amzil Z., *et al.*, 2001. Domoic acid accumulation in French shellfish in relation to toxic species
424 of *Pseudonitzschia multiseries* and *P. Pseudodelicatissima*. *Toxicon*, 37, 1711-1719.
- 425 Andree, K., Fernández-Tejedor, M., Elandaloussi, L. M., Quijano-Scheggia, S., Sampedro, N,
426 *et al.*, 2011. Quantitative PCR coupled with melt curve analysis for detection of selected
427 *Pseudo-nitzschia* spp. (Bacillariophyceae) from the Northern Mediterranean Sea. *Applied*
428 *and Environmental Microbiology*, 77, 1651–1659.
- 429 Arévalo., 1997. Seguimiento de biotoxinas marinas en las Rías Gallegas: control y evaluación
430 durante los años 1995–1996. In *J. Vieites, F.Leira (Eds.), V Reunión Ibérica de*
431 *Fitoplancton Tóxico y Biotoxinas*, Vigo, Spain; 90–101.
- 432 Auby, I., 2006. Phytoplancton et phycotoxines. Bilan des connaissances générales. La
433 surveillance dans le bassin d'Arcachon. Laboratoire Environnement-Ressources
434 d'Arcachon, IFREMER.
- 435 Bargu S., 2008. Note on the occurrence of *Pseudo-nitzschia australis* and domoic acid in squid
436 from Monterey Bay, CA (USA). *Harmful Algae*, 7, 45-51.
- 437 Bates, S.S., Léger, C., 1992. Response of *Nitzschia pungens* f. *multiseries* to irradiance: growth
438 and domoic acid production. In: Therriault, J.C., Levasseur, M. (Ed.). Proceedings of the
439 third Canadian workshop on *harmful marine algae*, 9-10.
- 440 Bates S.S., Garrison, D.L., Horner, R.A., 1998. Bloom dynamics and physiology of domoic
441 acid- producing *Pseudo-nitzschia* species. In: Anderson, D.M., Cembella, A.D.,
442 Hallegraeff, G.M. (Eds.). Physiological ecology of harmful algal blooms. *Springer-*
443 *Verlag, Heidelberg*, 267-292 pp.
- 444 Bates S.S., Richard D.J.A., 2000. Shellfish harvest area closure due to domoic acid – Mill river,
445 Prince Edward Island. *H A N* 21, 6-7.

- 446 Bates S.S., 2004. Interaction between bacteria and the domoic-acid-producing diatom *Pseudo-*
447 *nitzschia multiseriis* (Hasle) Hasle; can bacteria produce domoic acid autonomously?
448 *Harm. Algae*, 3, 11-20.
- 449 Béjaoui, B., Harzallah, A., Moussa, M., Chapelle, A., Solidoro, C., 2008. Analysis of
450 hydrobiological pattern in the Bizerte lagoon (Tunisia). *Estuar. Coast. Shelf Science*, 80
451 (1), 121–129.
- 452 Blanco, J., Livramento, F., Rangel, I.M., 2010. Amnesic shellfish poisoning (ASP) toxins in
453 plankton and molluscs from Luanda Bay, Angola. *Toxicon*, 55, 541-546.
- 454 Bogan, Y. M Kennedy D.J., Harkin A.L., Gillespie J., Vause B.J., Beukers-Seukers-Stewart
455 B.D., Hess P., Slater J.W., 2007. 2007. Variation in domoic acid concentration in king
456 scallop (*Pecten maximus*) from fishing grounds around the Isle of Man. *Harmful Algae*,
457 6, 81–92.
- 458 Bouchouicha, D., Lundholm, N., Sahraoui, I., Lambert, C., Hadj,
459 Mabrouk, H., Sakka Hlaili, A., 2014. First detection of domoic acid in *Mytilus*
460 *galloprovincialis* and *Ostrea edulis* linked to the presence of *Nitzschia bizertensis* in the
461 Bizerte lagoon (SW Mediterranean). *Harmful Algae*, 23, 49-63.
- 462 Busse, L.B., Venrick, E.L., Antrobus, R., Miller, P.E., Vigilant, V., 2006. Domoic acid in
463 phytoplankton and fish in San Diego, CA, USA. *Harmful Algae*, 5, 91-101.
- 464 CODEX STAN 292-2008. Standard for live and raw bivalve mollusks.
- 465 Daoudi, M., Voué, F., Serve, L. El Madani F., 2009. Spatio-temporal dynamics of toxic
466 phytoplankton in Nador lagoon (Marocco). *European Conference on Coastal Lagoon*
467 *Resarch*. Monpolier, France. December. 208: 14-18.
- 468 De la Iglesia, P., Giménez, G., Diogène, J., 2008. Determination of dissolved domoic acid in
469 seawater with reversed phase extraction disks and rapid resolution liquid chromatography

- 470 tandem mass spectrometry with head column trapping. *Journal of Chromatography. A*,
471 1215(1–2), 116–124.
- 472 DGPA (Direction Générale de la pêche et de l'aquaculture), Ministère de l'agriculture,
473 Tunisie, *annuaire statistique*, 2012-2013.
- 474 Dortch, Q., Robichaux, R., Pool, S., Milsted, D., Mire, G., 1997. Abundance and vertical flux
475 of *Pseudo-nitzschia* in the northern Gulf of Mexico. *Marine Ecology Progress Series*.
476 146: 249-264.
- 477 Doucette, T. A., Tasker, R. A., 2008. Domoic acid: Detection methods, pharmacology, and
478 toxicology. In L. M. Botana (Ed.), *Seafood and freshwater toxins. pharmacology,*
479 *physiology and detection (2nd Ed.)*. Boca Raton: CRC.
- 480 Downes-Tettmar, N., Rowland, S., Widdicombe, C., Woodward, M., Llewellyn, C., 2013.
481 Seasonal variation in *Pseudo-nitzschia* spp. and domoic acid in the Western English
482 Channel. *Continental Shelf Research* 53. 40–49pp.
- 483 Fehling J., Davidson, K., Bolch, C. J. S., Bates, S.S., 2004. Growth dynamics of non –toxic
484 *Pseudo nitzschia delicatissima* and toxic *P. seriata* (Bacillariophyceae) under simulated
485 spring and summer photoperiods. *Harmful Algae*, 4, 763-769.
- 486 Fehling, J., Davidson, K., Bolch, C. J. S., Tett, P., 2006. Seasonality of *Pseudo-nitzschia* spp.
487 (Bacillariophyceae) in Western Scottish waters. *Marine Ecology Progress Series* 323,
488 91–105.
- 489 Giménez, G., Casanova, A, Fernández- Tejedor, M de la Iglesia, P Jorge Diogène., 2013.
490 Management of domoic acid monitoring in shellfish from the Catalan Coast.
491 *Environmental Monitoring and Assessment*, 85(8), 6653-66.
- 492 Hasle, G.R., Syvertsen, E.E., 1997. Marine diatoms. In: Tomas, C.R. (Ed.). *Identifying marine*
493 *diatoms and dinoflagellates*. *Academic Press*, 5-385.

- 494 Hallegraeff, G.M., 2003. Harmful algal blooms: a global overview. In: Hallegraeff, G.M.,
495 Anderson, D.M., Cembella, A.D. (Eds.). *Manual on harmful marine microalgae*.
496 UNESCO. Paris, 25-49.
- 497 Hillebrand, H., Sommer, U., 1996. Nitrogenous nutrition of the potentially toxic diatom
498 *Pseudo-nitzschia pungens f. multiseriata* Hasle. *Journal of Plankton Research*, 18, 295–
499 301.
- 500 Howard, M.D.A; Cochlan, P., Ladizinsky, N., Kudela, R., 2007. Nitrogenous preference of
501 toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory
502 experiments. *Harmful Algae*, 6(2), 206-217.
- 503 James, K. D., Gillman, M., Fernández-Amandi, M., López- Rivera, A., Fernández P,
504 2005. Amnesic shellfish poisoning toxins in bivalve mollusks in Ireland. *Toxicon*,
505 46, 852-858.
- 506 Kaczmarek, I, Martin, J.L., Ehrman, J.M., Le Gresley, M.M.,; 2007. *Pseudo-nitzschia* species
507 dynamics in the Quoddy Region, Bay of Fundy. *Harmful Algae*, 6, 861-874.
- 508 Kaniou-Grigoriadou, I., 2005. Investigation on the presence of domoic acid in Greek shellfish.
509 *Harmful Algae*, 4, 717-723.
- 510 Kudela, R., Jenny, Q., Lane, A., Cochlan. W, 2008. The potential role of anthropogenically
511 derived nitrogen in the growth of harmful algae in California, USA. *Harmful algae*, 8,
512 103–110.
- 513 Kotaki, Y., Yoshida, M., Thuoc, C.V., Huyen, N.T.M., Hoi N.C., 2000. Domoic acid production
514 in *Nitzschia* sp. Nov. (Bacillariophyceae), isolated from a shrimp culture pond in Do Son,
515 Vietnam. *Journal of Phycology*. 36, 1057-1060.
- 516 Lefebvre, K.A., Robertson, A., 2010. Domoic acid and human exposure risks: a
517 *Review*. *Toxicon*, 56, 218-230.

- 518 Lelong, A., Garet, H., Soudant, P., Bates, S., 2012. *Pseudo-nitzschia* (Bacillariophyceae)
519 species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms.
520 *Phycologia*, 51, 168-216.
- 521 Loureiro, S., Garcés, E., Fernández-Tejedor, M., Vaqué D., Camp J., 2009. *Pseudonitzschia*
522 spp. (Bacillariophyceae) and dissolved organic matter (DOM) dynamics in the Ebro Delta
523 (Alfacs Bay, NW Mediterranean Sea). *Estuarine, Coastal and Shelf Science*, 83,539-549.
- 524 Lund, J.W.G., 1958. The inverted microscope method of estimation algal numbers, and the
525 statistical basis of estimating by counting. *Hydrobiol*, 11, 143-170.
- 526 Lundholm N., Moestrup O, Hasle G.R., Hoff-Emden K. 2003. A study of the *Pseudonitzschia*
527 *pseudodelicatissima/ cuspidata* complex (Bacillariophyceae): what is *P.*
528 *pseudodelicatissima*. *Journal of Phycology*, 39, 797–813.
- 529 Mamán, L., Fernández, L., Ocaña, A., Marco, J., Morales, J., 1999. Seguimiento de
530 fitoplancton tóxico en la costa de Andalucía. Incidencias durante los años 1997 y 1998.
531 In I. Márquez (Ed.), *Actas de la VI Reunión Ibérica sobre fitoplancton tóxico y*
532 *biotoxinas*, Sevilla Spain, 41–49.
- 533 Maranda, L., Wang, R., Masuda K., Shimizu,Y., 1990. Investigation of the source of domoic
534 acid in mussels. In : Granéli, E., Sundström, B., Edler, L., Anderson, D.M. (Eds.). *Toxic*
535 *marine phytoplankton*. Elsevier. New York, 300-304 pp.
- 536 Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of
537 phosphate in natural waters. *Anal. Chem. Acta*, 27,31-36.
- 538 Parsons, E.R., et al ., 1984. *Biological oceanographic processes*. Pergamon Press. Oxford. 3rd.
539 330 pp.
- 540 Pérez, L., Takahashi, M., Hargrave, B., 2001. Episodios tóxicos en las costas andaluzas durante
541 los años 1999 y2000. In: C. Gomis, et al. (Eds.), *Actas de la VII Reunión Ibérica sobre*
542 *fitoplancton tóxico y biotoxinas*, Alicante, Spain. 189–200.

- 543 Penna, A., Ingarao, C., Ercolessi, M., Rocch, M., Penna, N., 2006. Potentially harmful
544 microalgal distribution in an area of the NW Adriatic coastline: Sampling procedure and
545 correlations with environmental factors. *Est, Coastal and Shelf Sci.* 70: 307-316.
- 546 Price, N.M., Harrison, P.J., 1987. A comparison of methods for the measurement of dissolved
547 urea concentration in seawater. *Mar. Biol.* 92, 307–319. *pseudodelicatissima*. *Toxicon*, 39,
548 1245-1251.
- 549 Quijano-Scheggia, S., Garces, E., Flo, E., Fernandez-Tejedor, M., Diogene, J.,
550 2008. Bloom dynamics of the genus *Pseudo-nitzschia* (Bacillariophyceae) in two coastal
551 bays, NE Spain (Mediterranean Sea). *Scientia Marina*, 72, 577–590.
- 552 Quilliam, M.A., Xie M., Hardstaff W.R., 1995. Rapid extraction and cleanup for liquid
553 chromatographic determination of domoic acid in unsalted seafood. *J. AOAC. Int.* 78:
554 543-554.
- 555 Quiroga I., 2006. *Pseudo-nitzschia* bloom in the bay of Banyuls-sur-mer, Northwestern
556 Mediterranean sea. *diatom research.* 21: 91-104.
- 557 Sahraoui I., Sakka Hlaili, A., Hadj Mabrouk, H., Claude Leger, L., Bates, S.S., 2009. Blooms
558 of the Diatom Genus *Pseudo-nitzschia* H. Peragallo in Bizerte Lagoon (Tunisia, SW
559 Mediterranean). *Diatom research.* 24, 175-190.
- 560 Sahraoui, I. Bates, S.S., Bouchouicha, D., Hadj Mabrouk, H., Sakka Hlaili, A., 2011. Toxic
561 and potentially toxic *Pseudo-nitzschia* populations in Bizerte Lagoon (Tunisia, SW
562 Mediterranean) during 2006-2007, and first report of domoic acid production by *Pseudo-*
563 *nitzschia brasiliiana* Lundholm, Hasle, Fryxell, *diatom research..* 26 : 293-303.
- 564 Sahraoui I., Grami B., Bates S.S., Bouchouicha D., Chikaoui M.A., Hadj Mabrouk., 2012.
565 Response of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) populations and
566 domoic acid to environmental conditions in a eutrophied, SW Mediterranean coastal
567 lagoon (Tunisia). *Estuarine, Coastal and Shelf Science*, 102-103, 95-104 pp

- 568 Sakka Hlaili, A., Grami, B., Hadj Mabrouk, H., Gosselin, M., Hamel, D., 2007. Phytoplankton
569 growth and microzooplankton grazing rates in a restricted Mediterranean lagoon (Bizerte
570 Lagoon, Tunisia). *marine biology*. 151: 767-783.
- 571 Scholin, C.A., M Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordaro
572 J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T.,
573 Loscutoff, S., Lowenstine, L.J., Marin, R. I.I.I., Miller, P.E., McLellan, W.A., Moeller,
574 P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V., Van
575 Dolah F.M., 2000. Mortality of sea lions along the central California coast linked to a
576 toxic diatom bloom. *Nature*, 403, 80-84.
- 577 Shipe R.F., Leinweber, A., Gruber, N., 2008. Abiotic controls of potentially harmful algal
578 blooms in Santa Monica Bay, California. *continental shelf research*, 28, 2584-2593.
- 579 Takemoto, T., 1966. Studies on the constituents of *Chondria armata*. 8. On the structure of
580 domoic acid. *Yakugaku. Zasshi, Journal of the Pharmaceutical Society of Japan*. 86, 874-
581 877
- 582 Thessen, A.E., Bowers, H.A., Stoecker D.K., 2009. Intra- and interspecies differences in growth
583 and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. *Harm. Algae*, 8,
584 792-810.
- 585 Trainer, V.L., Adams N.G., Bill, B.D., Anulacion, F., Wekell, J.C., 1998. Concentration and
586 dispersal of a *Pseudo-nitzschia* bloom in Penn Cove, Washington, USA. *Natural. Toxin*,
587 6, 113-126
- 588 Trainer, V.L., Adams N.G., Bill, B.D., Stehr, C.M., Wekell, J.C., *et al.*, 2000. Domoic acid
589 production near California coastal upwelling zones, June 1998. *limnology. oceanography*.
590 45(8): 1818-1833.

- 591 Trainer, V.L., Hickey, B.M., Bates,S.S., 2008. Toxic diatoms. In : Walsh, P.J., Smith, S.L.,
592 Fleming, L.E., Solo-Gabrielle, H., Gerwick, W.H. (Eds.) Oceans and human health : risks
593 and remedies from the sea). *Elsevier Science Publishers*, New York, 219-237 pp.
- 594 Trainer, V. L., Bates, SS., Lundholm, N., Thessen, A. E., Cochlan, W.P., 2012. *Pseudo-*
595 *nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on
596 ecosystem health. *Harmful Algae* 14: 271–300.
- 597 Turki S., 2004. Suivi des microalgues planctoniques toxiques dans les zones de production,
598 d'elevage des molluques bivalves et d'exploitation des oursins du Nord de la Tunisie.
599 *Bull. Inst. Natn. Scien. Tech. Mer de Salammbô*, 31, 83-96.
- 600 Turki, S., Dhib, A., Belakhal, M.F., Frossard, V., Balti, N., 2014. Harmful algal blooms (HABs)
601 associated with phycotoxins in shellfish: What can be learned from five years of
602 monitoring in Bizerte Lagoon (Southern Mediterranean Sea) *Ecological Engineering*. 67,
603 39–47.
- 604 Utermöhl, H., 1931. Neue Wege in der quantitativen Erfassung des Planktons (mit besonderer
605 Berücksichtigung des Ultraplanktons). *Verh. Int. Ver. Theor. angewandte limnologie*, 5,
606 567- 596.
- 607 Vale, P., Sampayo, M. A. M., 2001. Domoic acid in Portuguese shellfish and fish. *Toxicon*,
608 39, 893–904.
- 609 Wood E.D., Armstong F.A.J., Richards F.A., 1967. Determination of nitrate in sea water by
610 cadmium-copper reduction to nitrite. *J. Mar. Biol. Ass. U.K.*,47, 23-31.
- 611
- 612
- 613
- 614
- 615

616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631

Figure captions

632
633
634
635
636
637
638
639
640
641

Fig. 1. Study sites: location of sampling stations

Fig.2. Bi-monthly variation of the physico-chemical factors at the sampling stations

Fig.3. Temporal variation in Chl *a* concentrations and phytoplankton abundance at the sampling stations (Averages \pm SD)

Fig. 4. Temporal variation in abundance of total diatoms and *Pseudo-nitzschia* spp. at the sampling stations (Averages \pm SD) (// period without of sampling)

642 Fig. 5. Temporal variation in relative abundance of the two *Pseudo-*
643 *nitzschia* groups (*P.serriata* group and *P. delicatissima* group) at
644 the sampling stations (// period without of sampling)

645 Fig. 6. Temporal variation of particulate domoic acid levels at the
646 sampling stations (Average \pm SD)

647

648

649

650

651

652

653

654

655

656

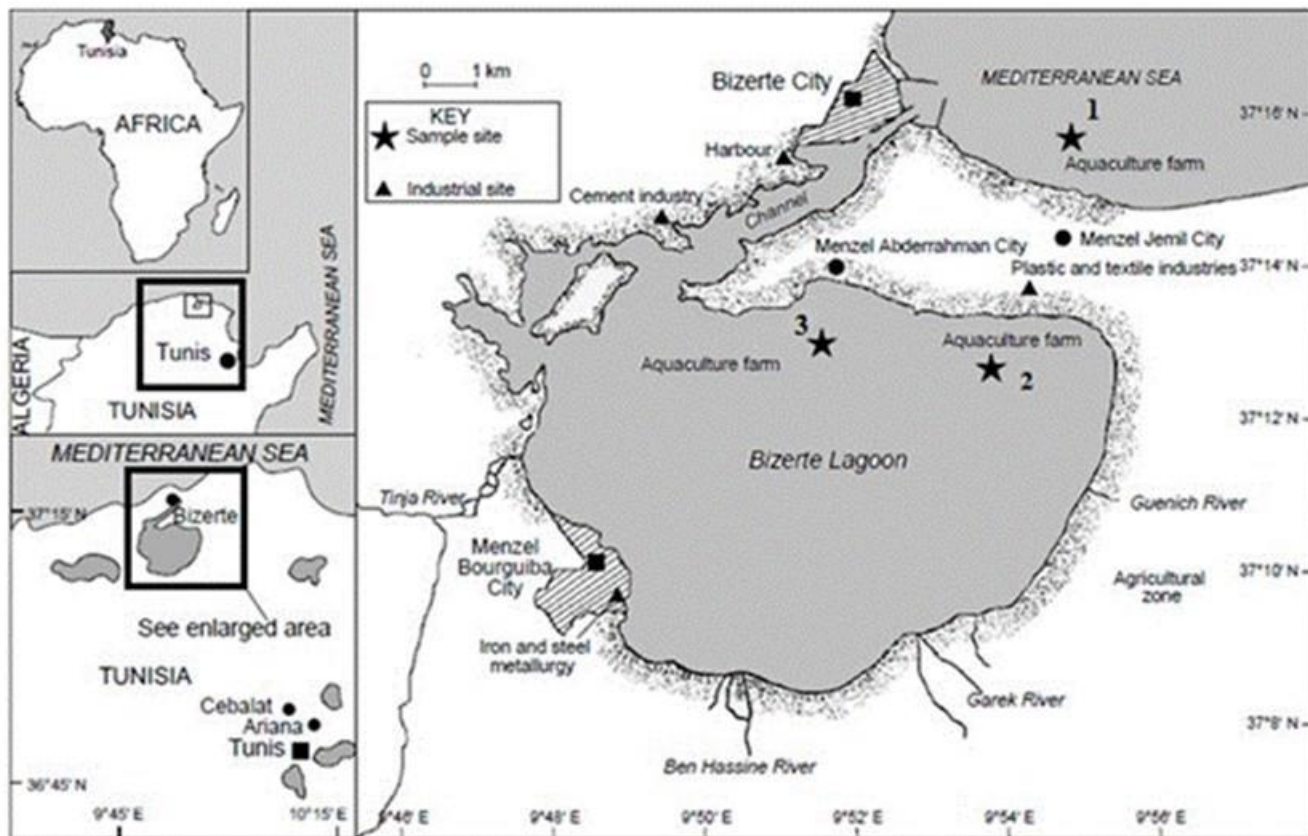
657

658

659

660

661



662
663
664
665
666
667
668
669
670
671
672
673
674
675

Fig. 1

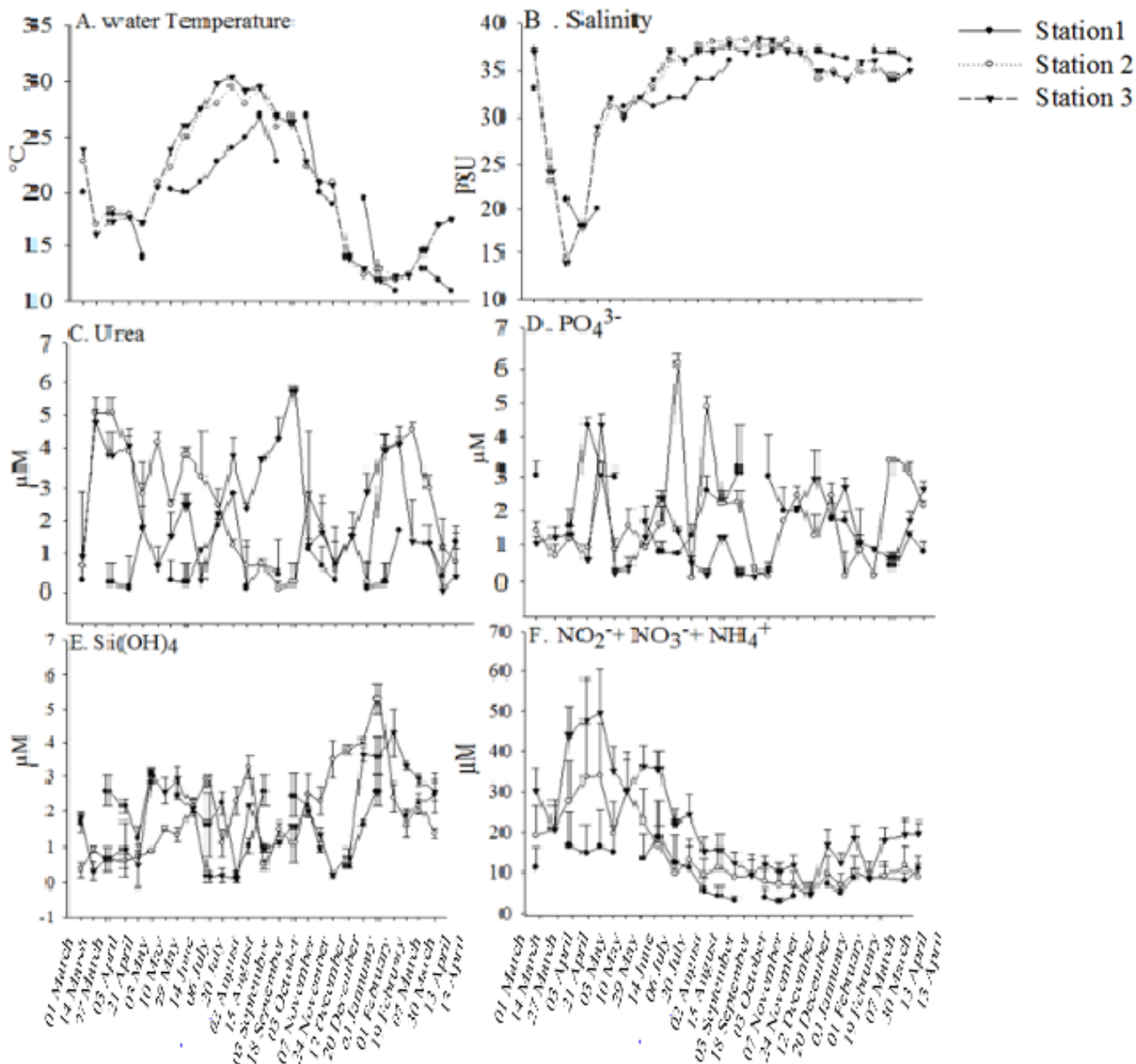


Fig.2

676

677

678

679

680

681

682

683

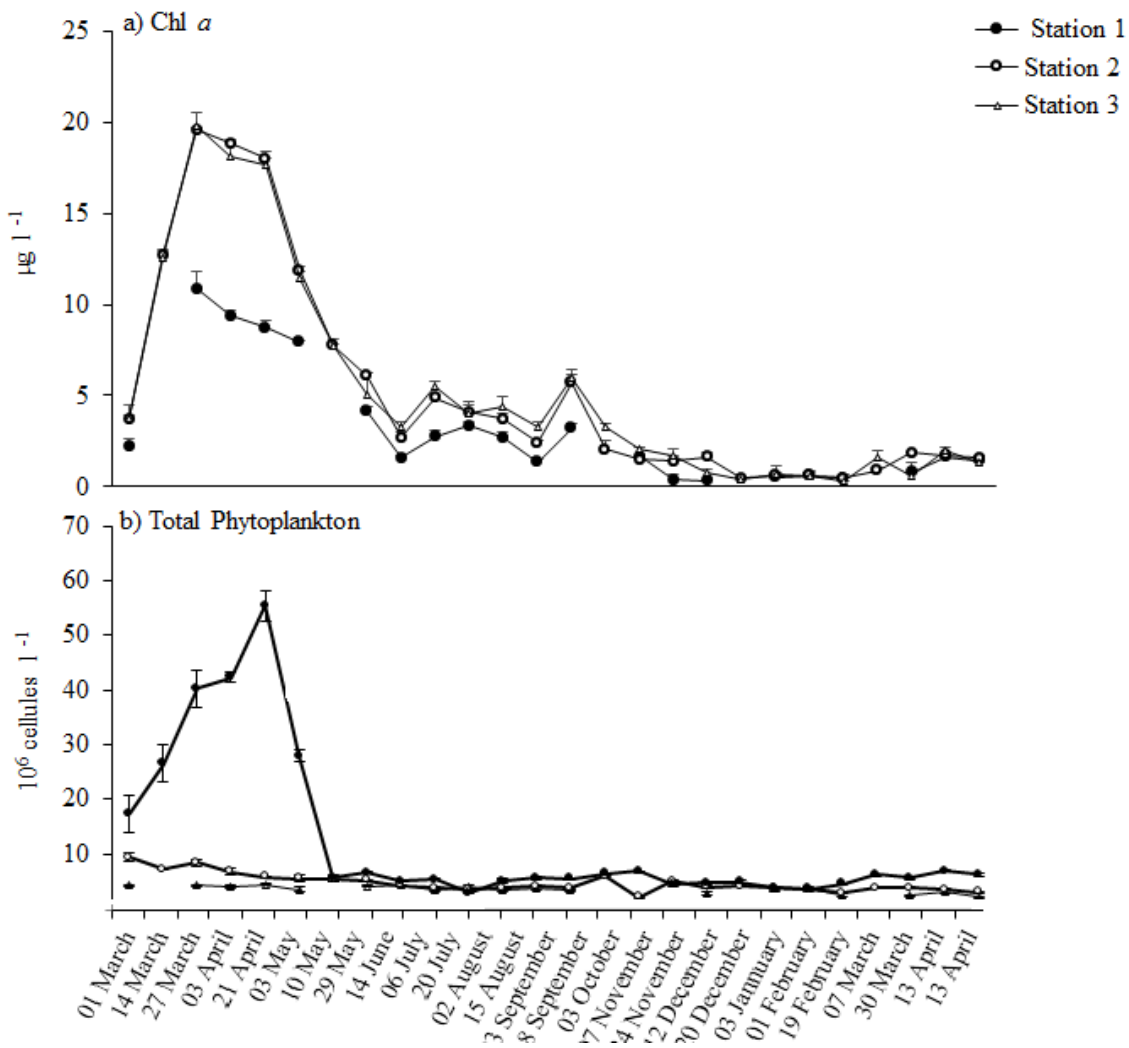


Fig.3

684

685

686

687

688

689

690

691

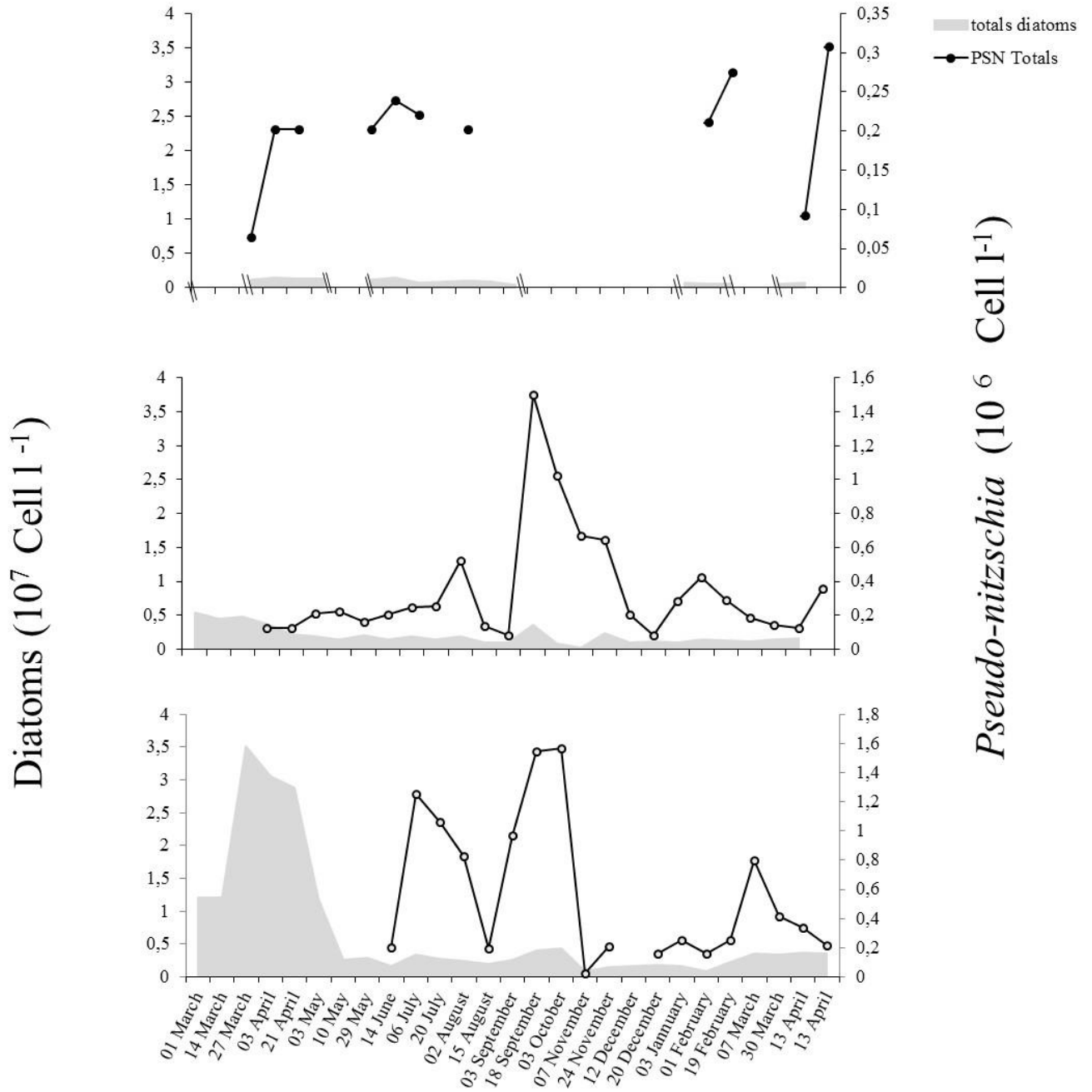
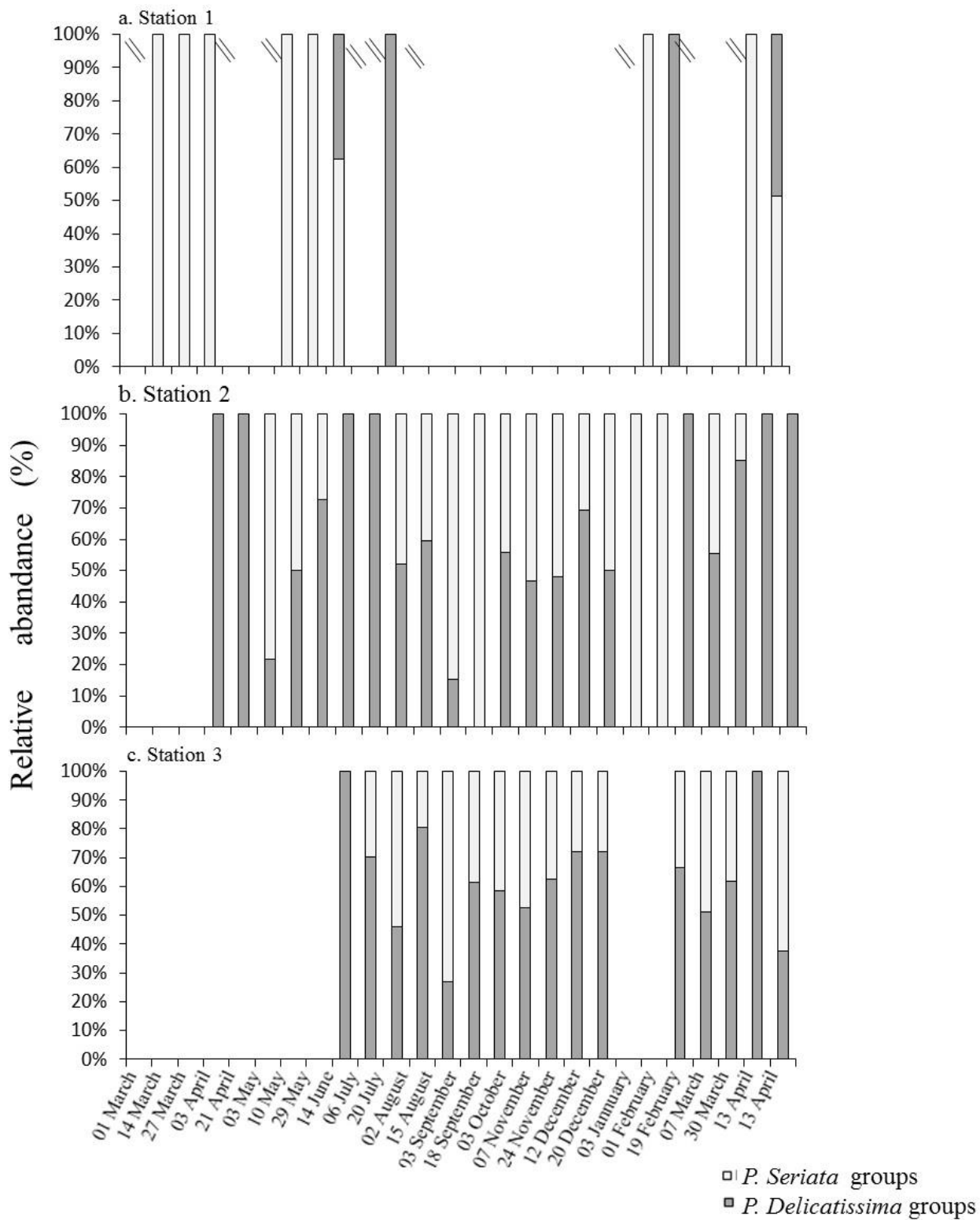


Fig. 4

692

693



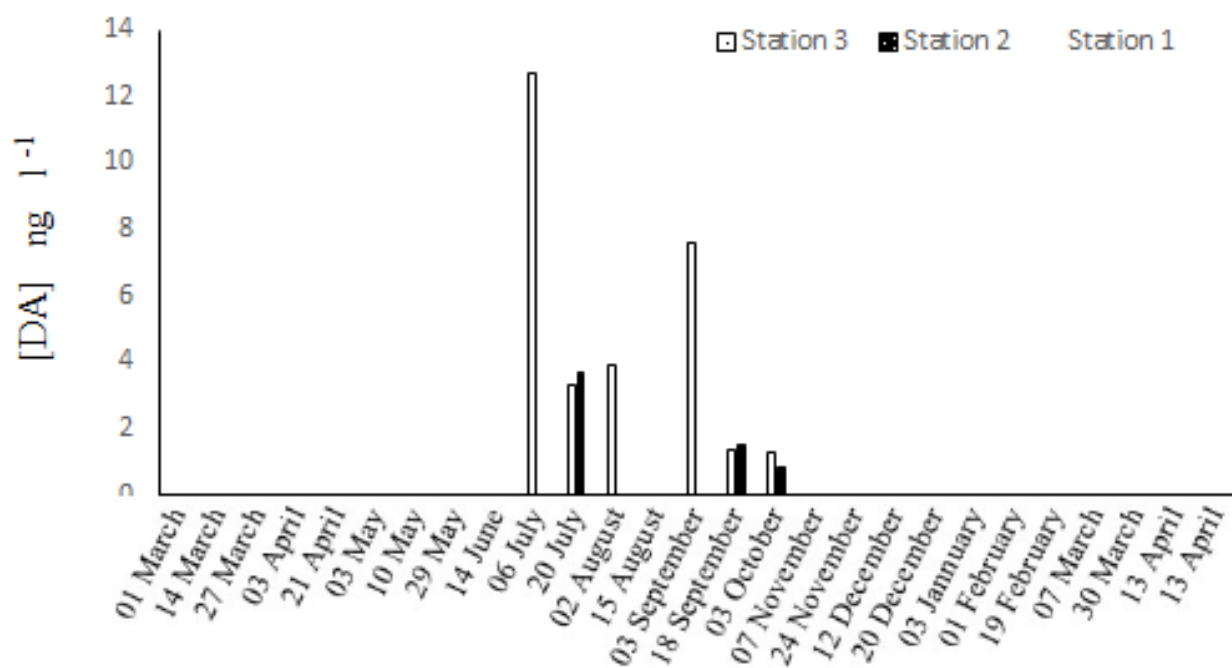
694

695

Fig. 5

696

697



698

699

Fig. 6

700

701

702

703

704

705

706

707

708

709

710

711

712

713 Table 1. Characteristics of the study stations located in three shellfish farms (geographical location, maximal depth and depth of euphotic zone

714 (Z_{eu})

715

Stations	Location		Depth (m)		Area (ha)	Production capacity (tons)	Reacing technic	Creation	Species
	Latitude (°N)	Longitude (°E)	Max	Z_{eu}					
1	37°15'33'	09°59'24'	20.0	12.0	8	20	Spinneret	2009	Mussels and oysters
2	37°15'59'	09°52'22'	4.0	19.2	150	100	Table	1963	Mussels and oysters
3	37°13'55'	09°51'58'	5.0	40.0	46	200	Spinneret	2002	Mussels and oysters

716

717

718

719

720

721

722

723
724

Table 2. Spearman's correlation coefficients between diatoms or *Pseudo-nitzschia* (total and two groups) and environmental factors recorded during the sampling period. (**: correlation is significant at the 0.01 level; *: correlation is significant at the 0.05 level)

Environmental Variables	Total diatoms	<i>P. delicatissima</i> -group	<i>P. seriata</i> -group	<i>Pseudo-nitzschia</i> (two groups)	Domoic acid
Water temperature	- 0.059	0.200*	0.234*	0.213*	0.345*
Salinity	- 0.274**	0.459**	0.535**	0.531**	0.326*
Silicate	-0.186*	0.244**	0.154	0.253**	
Phosphate	0.316**	0.354**	0.193*	0.338**	-0.543**
Total inorganic N	0.634**	-0.406**	-0.370**	-0.423**	
Urea	0.091	0.415**	0.336**	0.435**	0.721**
Chl <i>a</i>	0.522**	0.320**	0.243*	0.330**	

725

