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29 **ABSTRACT**

30 Studies on phytoplankton and in particular Harmful Algal Blooms (HABs) species in southern
31 Mediterranean waters are scarce. We performed from April 2008 to June 2009 weekly
32 investigations on microphytoplankton community structure and abundance in two contrasted
33 marine ecosystems located in the western Moroccan Mediterranean coast, M'diq Bay and Oued
34 Laou Estuary. Simultaneously, we measured the main physico-chemical parameters. Globally,
35 the two studied areas showed comparable values of the assessed abiotic environmental factors.
36 Temperature and salinity followed seasonal variation with values ranging from 13.5 °C to 21.4
37 °C and 31 to 36.8, respectively. Average nutrient values in surface water ranged from 0.7 to
38 45.76 µM for dissolved inorganic nitrogen, 0.02 to 2.10 µM for PO₄ and 0.23 to 17.46 µM for
39 SiO₄ in the study areas. A total of 92 taxa belonging to 8 taxonomic classes were found. The
40 highest number of microphytoplankton abundance reached 1.2 x 10⁶ cells L⁻¹ with diatoms being
41 the most abundant taxa. Factorial Discriminant Analysis (FDA) and Spearman correlation test
42 showed a significant seasonal discrimination of dominant microphytoplankton species. These
43 micro-organisms were associated with different environmental characteristics, in particular
44 temperature and salinity. Numerous HABs species were encountered regularly along the year.
45 Although *Dinophysis* species and *Prorocentrum lima* were present in both sites, no Lipophilic
46 Shellfish Poisoning was detected in the analyzed bivalve mollusks. Domoic acid (DA), produced
47 by toxic species of *Pseudo-nitzschia* was found with concentrations up to 18 µg DA g⁻¹ in the
48 sweet clam *Callista chione*. Data showed that the observed persistent and dramatic paralytic
49 shellfish poisoning (PSP) intoxication of mollusks resulted probably of *Gymnodinium catenatum*
50 proliferations in both studied areas. Contrary to sweet clam *C. chione*, the cockle
51 *Acanthocardia tuberculatum* showed a permanent and extremely high toxicity level during the
52 15 months survey with up to 7545 µg Equivalent Saxitoxin kg⁻¹ flesh (ten times higher than the
53 sanitary threshold of 800µg eqSTX Kg⁻¹flesh). The present work highlights for the first time the
54 dynamic of microphytoplankton including HABs species and their associated toxin accumulation

55 in the commercially exploited shellfish in the southern western Mediterranean waters of
56 Morocco. Furthermore, the acquired data will help us to improve the monitoring of HABs
57 species and related toxins in these coastal marine systems.

58 **Keywords:** SW Mediterranean, phytoplankton diversity, environmental factors, HABs, toxins.

59

60 **1. Introduction**

61 The microalgae community has been shown to respond rapidly to environmental changes
62 (Cloern, 2001; Carstensen et al., 2015). Phytoplankton species composition vary with several
63 environmental factors including physical (irradiance, temperature and turbulence), chemical
64 (inorganic and organic nutrients, oligo-elements, vitamins) and biological (competition and
65 predation) (Boyd et al., 2010; Bužančić et al., 2016). To understand the functioning of a marine
66 ecosystem, it is necessary to study its structure, composition and evolution at time and space scales.
67 Phytoplankton is an important trophic component participating to aquatic ecosystem functioning.
68 Phytoplankton community structure is controlled by various environmental conditions (Gailhard,
69 2003; Trombetta et al., 2019). Knowing which environmental factor control phytoplankton species
70 development helps to understand their evolution and dynamics. Southern coastal Mediterranean
71 marine ecosystems are knowing along the last decades' important perturbations due to massive
72 urbanization, aquaculture and recreative activities and are under the pressure of climate change
73 (Hallegraef 2010; Macias et al., 2015; 2018; Wells et al., 2015; 2018). These anthropogenic factors
74 could influence phytoplankton and thus ecosystem functioning (Bužančić et al., 2016). The studies
75 on the temporal variation of the community structure of phytoplankton in the Moroccan western
76 Mediterranean waters are scarce (El Madani et al., 2011; Daoudi et al., 2012; Rijal Leblad et al.,
77 2013). Two sites were selected for the present study, M'diq Bay and Oued Laou Estuary holding an
78 important socio-economic activity (fishing, shellfish harvesting and recreative activities). M'diq

79 Bay holds aquaculture activity (mussels and fish) and is characterized by an important shellfish
80 catching (559 tons' year⁻¹) particularly cockle and clam (Rijal Leblad 2012).

81 In the last decades, Harmful Algal Blooms (HABs) are increasing in frequency, intensity
82 and geographic distribution impacting human health and aquaculture (Hallegraeff, 1993; Vand
83 Dolah, 2000). Both northern and southern Mediterranean waters were exposed to these noxious
84 phenomena (Vila et al. 2001a; 2005; Laabir et al., 2011; Abdenadher et al., 2012; Fertouna-
85 Benlakhhal et al., 2015; Zmerli-Triki et al., 2016; Abadie et al.; 2019; Ben-Rejeb-Jenhani et al.,
86 2019). Moroccan marine waters are not spared by episodes of toxic microalgae blooms. Paralytic
87 Shellfish Poisoning (PSP) events have been recorded in Moroccan Atlantic waters since 1969
88 causing several intoxications leading some times to human death (Tber, 1983; Bourhilli, 1982;
89 Taleb et al., 2003). Morocco's Mediterranean waters have long known PSP events which caused
90 closure of shellfish harvesting for several years (Taleb et al., 2001). These PSP events were
91 associated to *Gymnodinium catenatum* development (Tahri, 1998; Taleb et al., 2001). *Gymnodinium*
92 *catenatum* was reported for first time in Spanish Mediterranean waters (Delgado, 1990; Bravo et al.,
93 1990; Gomez 2003). There were indications of the presence of this species in Tunisian (Dammak-
94 Zouari et al., 2009) and in Algerian coastal waters (Frehi et al., 2007). However information is still
95 lacking about the dynamic of this neurotoxic dinoflagellate in the Mediterranean marine coastal
96 waters. In the Western Mediterranean Sea, the occurrence of *Pseudo-nitzschia* was documented in
97 the coastal waters of Spain (Quijano-Scheggia et al., 2010; Busch et al., 2016), France (Quiroga
98 2006; Grzebyk et al., 2017), Italy (Cerino et al., 2005; Ruggiero et al., 2015), Tunisia (Sahraoui et
99 al., 2009; Melliti Ben Garali et al., 2019), Algerian (Illoul et al., 2008) and Morocco (Rijal leblad et
100 al., 2013). *Dinophysis* and its toxic compounds were reported only few times in Mediterranean
101 waters (Aissaoui et al. 2014; Garcia-Altres et al. 2016; Bazzoni et al., 2018).

102 The purpose of this study is to investigate the taxonomic composition and abundance of the
103 microphytoplankton community in two contrasted areas (M'diq Bay and Oued Laou Estuary) of the
104 western Mediterranean waters with a focus on HABs species. We also aim to analyze the effect of

105 the main abiotic environmental factors on microphytoplankton succession and in particular on the
106 potentially toxic microalgae. Another objective was to determine to which extent the observed
107 dramatic PSTs intoxications of the cockles and the clams are related to the presence of paralytic
108 toxin producing species in the water column of the studied ecosystems.

109

110 **2. Material and Methods**

111 *2.1. Study sites*

112 Oued Laou Estuary (Station 1; 35°27' 310 N - 05°05' 06 W) and M'diq Bay (Station 2;
113 35°41'646 N - 05°19'075 W) (Figure. 1), are located in the western Moroccan coast of
114 Mediterranean Sea adjacent to the Gibraltar Strait. M'diq Bay receives terrestrial inputs through
115 a temporal torrential stream (Rijal Leblad, 2012). The hinterland of this area is characterized by a
116 watershed in rocky mountain knowing a high urbanization. Oued Laou Estuary is less urbanized
117 and receives permanent inputs of fresh water through the Oued Laou River, rich in organic
118 matter. The hinterland of this marine coastal area is characterized by irrigated farming and
119 market gardening.

120 *2.2. Phytoplankton and physico-chemical parameters*

121 Sampling was performed weekly in the water column of S1 and S2 (depth of 7-10 m) from April
122 2008 to June 2009, except when weather conditions did not allowed sailing. Two seawater
123 samples were collected from each site at subsurface (- 0.5 m) of the water column. The first
124 seawater samples were preserved using Lugol's iodine acidic solution for the identification and
125 quantification of microphytoplankton species. Then samples were settled in 25 mL chamber,
126 stored in the dark at ambient temperature, the settling time was 24 hours and samples were
127 analyzed within the same week of sampling. The microphytoplankton species were identified
128 and quantified using a Leica DMIL inverted photonic microscope (Uthermol, 1958). The second

129 Sea water sample was stored at -20 °C until chemical analyses. Inorganic nutrient (ammonium
130 NH₄, nitrite NO₂, nitrate NO₃, phosphate PO₄ and silicate SiO₄) concentrations were measured
131 using the protocol of Aminot and Chaussepied (1983) by spectrophotometer (Unico SQ4802
132 UV/VIS double Beam spectrophotometer model). Temperature, practical salinity and pH were
133 measured *in situ* at each station during sampling period with a multi-parameter probe (WTW
134 197i. Practical salinity and pH calibrations were performed monthly (see the protocol in
135 [www.geotechenv.com / Manuals / WTW_Manuals / Multi_197i](http://www.geotechenv.com/Manuals/WTW_Manuals/Multi_197i)).

136

137 *2.3. Domoic acid analyses*

138 The concentrations of DA were determined by High-performance liquid chromatography
139 (HPLC) (Shimadzu 10vp type). This apparatus is composed of a SCL-10vp Controller, a LC-
140 10ADvp Quaternary Pump, a CTO-10vp Colonne Four, a SIL-10ADvp Autosampler, a SPD-
141 M10Avp Photodiode Array Detector, a Vydac C18 column (250 × 4.6 mm, with 5 mm) and the
142 Guard Cartridge (Vydac C18, 5 mm). DA was analyzed monthly and in periods of high *Pseudo-*
143 *nitzschia* abundance (> 10⁵ cells L⁻¹). It was measured in the whole meat of cockles and sweet
144 clams according to Quilliam's (1995) protocol. Analyses in triplicates were performed using
145 about 100 g of shellfish meat (ten to fifteen individuals were required to have such amount of
146 meat). After being shredded and homogenated, 4 g of meat were added to 16 mL of solvent
147 extraction (methanol-water, 1:1) and then homogenized (Ultra-Turrax for 3 minutes at about
148 10,000 rpm). The homogenate was centrifuged at least at 4,000 rpm for 10 min to obtain
149 supernatant. The later was analyzed using the following chromatographic conditions: mobile
150 phase flow rate of 1 mL min⁻¹, detector wave length of 242 nm, injection volume of 20 mL and
151 an oven temperature for the column of 40 °C. The determination of DA content in samples was
152 done with a detection limit of 0.3 mg g⁻¹

153

154 *2.4. Paralytic shellfish toxicity analyses using bioassay*

155 Commercially exploited bivalve mollusks as cockle (*Acanthocardia tuberculatum*) and the
156 sweet clam (*Callista chione*) were sampled every two weeks at M'diq Bay and Oued Laou
157 Estuary during 2008 and 2009. The seabed over 10-15 m was raked using a dredge and samples
158 brought back using a net. The samples were put in a cooler with ice blocks until they reach the
159 laboratory. PSP toxicity analysis was carried out by mouse bioassay according to the AOAC
160 method (1990). Briefly, 100 g homogenised tissues were mixed with 100 ml 0.1 M chlorydic
161 acid and boiled for 5 mn, pH adjusted to 2-3 and centrifuged for 15 min at 3000 rpm. One
162 milliliter of the supernatant was injected intraperitoneally to three 20 g Albinos mice. The values
163 reported are expressed in $\mu\text{g STXeq kg}^{-1}$ mollusk meat.

164

165 *2.5. Lipophilic shellfish poisoning analysis*

166 The analyses the lipophilic toxins (Okadaic Acid-group toxins, Pectenotoxin group and
167 Azaspiracid group) were based on the extraction of these toxins using acetone and
168 dichloromethane (DCM) from shellfish samples. The extracts were watched with
169 dichloromethane (DCM) and water. After evaporation of the DCM, the obtained residue was
170 solubilized with a solution of tween 60 at 1%. One milliliter of the extract was injected into the
171 intraperitoneal cavity of Suisse albino mice weighing between 19 and 21 g. A positive result
172 corresponds to the death of 2 or 3 mice injected during a 24-hour observation period. These
173 analyses were conducted according to the method of EURLMB (EU Harmonised SOP MBA
174 Lipophilic Version 4, Vigo, Spain).

175

176 *2.6. Statistics*

177 SStatistical analyses were performed using the software XLSTAT 2011. We used a Spearman
178 correlation and Factorial Discriminant Analysis (FDA) to highlight if there is any seasonal
179 dissimilarities and describe the main patterns of temporal variability of microphytoplakton taxa
180 in relation to environmental factors.

3. Results

3.1. Physico-chemical parameters

Results of physico-chemical parameters are summarized in Table 1 and shown in Figure 2. The lowest seasonal mean temperature values were recorded in winter (14.32 ± 0.67 °C at S1 and 16.07 ± 0.52 °C at S2) and the highest values in summer (20.12 ± 1.68 °C at S1 and 19.50 ± 1.29 °C at S2) (Fig. 2A). Temperature in M'diq Bay was slightly lower than that of Oued Laou Estuary from April to October 2008. The highest seasonal mean salinity values were recorded in summer (36.54 ± 0.09 at S1 and 36.51 ± 0.05 at S2) while the lowest values were observed in winter during rainfall periods with 33.83 ± 2.05 at S1 and 35.06 ± 0.35 at S2 (Fig. 2B, D). Spring and autumn seemed to be transitional periods. However, we observed an important decrease in salinity (up to 31 in November and December 2008 and up 32-33 in April 2009) in Oued Laou estuary (Fig. 2B). This decrease could be related to heavy rainfall events (Fig. 2D) and the subsequent lower salinity of S1 seawater due to freshwater input from Oued Laou river. Along the seasons, pH was slightly basic and oscillated around 8 for both sites. The lowest values were measured during autumn (7.93-7.86) and winter (7.8-7.97) while the maximum values were observed in spring (8.06-8.12) summer (8.11-8.14) in both stations (Fig. 2C). The rainfall episodes were registered during autumnal and winter periods whereas spring and summer were dry. Rainfall level at S2 was generally greater than that recorded at S1 with the highest value ($258 \text{ mm month}^{-1}$) recorded at S2 at October. Summer was characterized by low or no precipitation.

3.2. Nutrients

Data are summarized in Table 1 and shown in Figure 3. The nutrient concentrations fluctuated widely in function of the seasons and were quite similar between S1 and S2. The seasonal mean values of ammonium (NH_4^+) varied widely and ranged from 0.09 to $43.13 \mu\text{M}$. The highest mean values were observed in autumn with $24.53 \pm 10.19 \mu\text{M}$ at S1 and $17.74 \pm 14.63 \mu\text{M}$ at S2. The

206 lowest mean values were observed in spring with 0.09 μM at S1 and 0.12 μM at S2. Nitrite
207 concentrations at S1 and S2 station were relatively low. They ranged between 0 (not detected) to
208 0.19 μM . The highest values were recorded in autumn and winter with values up to 0.17 μM at
209 S1 and 0.19 μM at S2. Nitrate ranged between 0.17 to 7.52 μM with an average of 1.79 ± 1.66
210 μM at S1 and 0.89 ± 0.66 μM at S2. At S1, the highest values were recorded at the end of May
211 2008 (5.39 μM), mid-August 2008 (3.27 μM) and during the period of November 2008 - early
212 March 2009 (7.51 μM) end of April 2009 (4.63 μM) and early June 2009 (4.02 μM). During the
213 period of October 2008 - March 2009, we observed an increase in the concentration of nitrate at
214 S1. In early October 2008, nitrate concentration did not exceed 1 μM . Thereafter, it increased
215 gradually to reach the highest registered values (7.51 μM in March 2009). After, there was a
216 drop at the end of March 2009. At S2, the highest levels were recorded in autumn and winter
217 period. The maximum values, 2.59 and 2.37 μM , were recorded in November 2008 and the
218 second week of February 2009, respectively, with decreasing trend in spring (0.72 ± 0.34 μM)
219 and summer periods (0.32 ± 0.21 μM).

220 Silicate concentrations at S2 (3.39 ± 2.91 μM) exceeded those measured at S1 (2.16 ± 1.54 μM),
221 the highest values were observed during spring (4.10 ± 3.90 μM) and autumn (4.12 ± 2.05 μM) at
222 S2. Silicate content displayed several fluctuations, the values ranged between 0.23 to 17.46 μM ,
223 with alternation of enrichment and decreasing period. A gradual decrease in silicate was
224 observed from July to September 2008, and an increase from October until March 2009 reaching
225 a maximum value of 17.46 μM in March. Another decrease coincided with the massive
226 development of the diatoms genera *Pseudo-nitzschia* and *Leptocylindrus*. After this bloom,
227 silicate contents peaked during mid-April to June 2009, and showed a decline in June 2009.

228 Phosphate concentrations varied between 0 and 2.10 μM . Generally, phosphate concentrations at
229 S2 were slightly higher than those recorded at S1. The highest values were recorded during
230 autumn, with 0.94 ± 0.83 μM at S1 and 0.64 ± 0.65 μM at S2. Low values were measured during
231 spring for S1 0.16 ± 0.10 μM and winter for S2 0.25 ± 0.22 μM . The monthly value of the Si/P

232 ratio showed large temporal fluctuations (Fig. 4). The highest values (up to 20) of Si/P ratio were
 233 recorded at the M'diq bay during 2008 sampling period and thereafter this ratio remained
 234 relatively low. Figure 4 showed the correspondence of the peaks of Si/P ratio and the
 235 abundances of diatoms.

236 3.3. Phytoplankton structure

237 The phytoplankton community composition and abundance in S1 and S2 showed the presence of
 238 different classes: Bacillariophyceae, Dinophyceae, Euglenoidea, Dictyochophyceae,
 239 Chlorophyceae, Cyanophyceae, Cryptophyceae and Prymnesiophyceae. However, most recorded
 240 species belong to two major classes, Bacillariophyceae and Dinophyceae (Fig. 5 and Fig. 6). The
 241 total abundance of phytoplankton (cells L⁻¹) showed important seasonal variations (Fig 7.). The
 242 highest values were observed in spring (12.10⁵ cells L⁻¹) and summer (12.10⁵ cells L⁻¹). In
 243 contrast, low abundances were observed in autumn (19.10⁴ cells L⁻¹) and winter (71.10³ cells L⁻¹).
 244 The average phytoplankton cell abundance recorded in S2 (18.10⁴ ± 20.10⁴ cells L⁻¹) was
 245 slightly higher than that recorded in S1 (14.10⁴ ± 17.10⁴ cells L⁻¹). Bacillariophyceae was the
 246 dominant group followed by Dinophyceae in both sites (Fig 5, 6). At Oued Laou Estuary (Fig.8),
 247 the dominant taxa were: *Heterocapsa* spp., *Skeletonema* spp., *Thalassiosira* spp., *Noctiluca*
 248 *scintillans*, *Nitzschia* spp., *Scrippsiella* sp., *Pseudo-nitzschia* spp., *Guinardia striata*,
 249 *Leptocylindrus* spp. *Gymnodinium catenatum*, *Euglena* sp., *Plagioselmis* sp., *Chaetoceros* spp.
 250 and *Asterionella glacialis*. Except *Pseudo-nitzschia* spp., *Leptocylindrus* spp. and *Chaetoceros*
 251 spp. which showed an average relative densities of 29.5%, 24% and 14.5% respectively, the
 252 other taxa were poorly represented in this ecosystem; At M'diq Bay (Fig.9), the dominant taxa
 253 were: *Thalassiosira* spp., *Thalassionema nitzschioides*, *Prorocentrum triestinum*, *Nitzschia* spp.,
 254 *Gyrodinium* spp., *Skeletonema* spp., *Scrippsiella* sp., *Leptocylindrus* spp., *Pseudo-nitzschia* spp.,
 255 *Plagioselmis* sp., *Chaetoceros* spp., and *Asterionella glacialis*. The relative dominance values of
 256 these taxa varied from 0% to 90%. The average of the relative densities of *Leptocylindrus* spp.,

257 *Pseudo-nitzschia* spp. and *Chaetoceros* spp. were 24%, 23.5% and 20% respectively. These taxa
258 dominated the microphytoplankton community.

259 3.4. Harmful Algal Blooms species and mollusks intoxications

260 Results showed the presence of four *Dinophysis* species (*Dinophysis caudata*, *Dinophysis fortii*,
261 *Dinophysis acuminata* and *Dinophysis rotundata*) with the highest densities (up to 560 cells L⁻¹
262 when counting all *Dinophysis* species cells) registered between the end of May and mid-
263 September 2008. *Dinophysis* species were mostly present and abundant in Oued Laou Estuary
264 (Fig. 10). There was one important peak of DST producing species *Prorocentrum lima* in Oued
265 Laou (1280 cells L⁻¹) on August the 28th 2008. There was no intoxication of the mollusks with
266 LSP during the 15 months' study. The benthic dinoflagellate *Ostreopsis* was found at 4
267 occasions during the survey with densities not exceeding 80 cells L⁻¹.

268 Oued Laou Estuary and M'diq Bay showed the development of species belonging to *Pseudo-*
269 *nitzschia* genus in spring which is related to Amnesic Shellfish Syndrome (ASP). We showed the
270 presence of DA in two analyzed shellfish species, but the sweet clam (*C. chione*) was so far the
271 most contaminated with up to 15.94 µg DA g⁻¹ mollusk meat in M'diq Bay (April 6th 2009) and
272 16.88 in Oued Laou (September 10th 2008). The cockle contained low DA with concentrations
273 not exceeding 2.06 µg DA g⁻¹ mollusk meat (April 7th 2009) and 4.73 µg DA g⁻¹ mollusk meat
274 (April 6th 2009) in S1 and S2, respectively (Fig. 11).

275 *Alexandrium* spp. registered the highest densities in M'diq Bay with up to 5200 cells L⁻¹ in
276 March 3th 2008. This genus was less present at Oued Laou Estuary with concentrations not
277 exceeding 1480 cells L⁻¹ (March 13th 2008). *G. catenatum* which has been related to PSP
278 syndrome showed almost similar distributions in M'diq Bay (up to 2960 cells L⁻¹) and Oued Laou
279 Estuary (maximum of 3960 cells l⁻¹ in January 22st 2008 (Fig. 12). December 2009 registered
280 also an important development of *G. catenatum* with up to 2040 cells L⁻¹ in M'diq Bay.
281 Permanent and high levels of PSTs contamination were observed in Oued Laou Estuary in the
282 cockles (up to 7545 µg STX equiv Kg meat⁻¹, a value almost 10 times higher than sanitary

283 threshold). Cockles from M'diq Bay were also permanently contaminated but with concentration
284 up to 3720 $\mu\text{g STX equiv Kg meat}^{-1}$ (registered in January 31th 2008). The sweet clams were
285 less contaminated by PST in both sites with concentrations ranging from 0 to 1945 $\mu\text{g STX}$
286 $\text{equiv Kg meat}^{-1}$ and from 0 to 1230 $\mu\text{g STX equiv Kg meat}^{-1}$, in Oued Laou and M'diq Bay,
287 respectively.

288

289 *3.5. Correlation between phytoplankton and environmental factors*

290 Statistical analyses were applied to highlight any correlation between environmental parameters
291 and species richness and species diversity of phytoplankton in the studied areas (Table 2).
292 Globally, M'diq Bay and Oued Laou Estuary showed the same trend in their correlations with
293 environmental factors (Table 2). Temperature and salinity were correlated positively to species
294 diversity and species richness. This later, was negatively correlated with nitrate (Table 2). Only
295 salinity and pH showed positive correlation with total phytoplankton (Table 2). Three
296 microphytoplankton groups showed a positive correlation with salinity including
297 Prymnsiophyceae, Cryptophyceae and Dictyophyceae and Diatomophyceae (Table 2). Except
298 Raphidophyceae and Chlorophyceae, all the other the phytoplankton groups were positively
299 correlated with pH (Table 2). DIN, NH_4 and SiO_4 were not correlated with any of the examined
300 phytoplankton variables. Nitrate was negatively correlated with Diatomophyceae,
301 Prymnsiophyceae and Euglenoidea, (Table 2). Dictyophyceae was positively correlated with
302 nitrate and nitrite. Dinophyceae and Diatomophyceae were negatively correlated with nitrite.
303 Factorial analyses related to the season indicated that 92.43% (S1) and 95.22% (S2) of the
304 variance was explained by two axes (F1 and F2) (Fig. 13A and Fig. 13C). In relation to the
305 seasons, the variables showed four groups. For both stations, the temporal variability followed a
306 seasonal cycle with a seasonal transition (Fig. 13B and Fig. 13D). Data showed positive
307 correlations between environmental factors and microphytoplankton species on a seasonal basis.
308 For station 1, the correlations were as follows : in winter (NO_2 , NO_3 and *Thalassionema*

309 *nitzschioides*), in spring (SiO₄ and *Nitzschia* spp., *Noctiluca scintillans*, *Scrippsiella* sp., *Pseudo-*
310 *nitzschia* spp., *Thalassiosira* spp., *Euglena* sp., and *Asterionella glacialis*), in summer (pH,
311 temperature, salinity and *Dinophysis* spp., *Leptocylindrus* spp., *Chaetoceros* spp., *Heterocapsa*
312 spp. and *Skeletonema* spp.) and in autumn (PO₄, NH₄ and *Guinardia delicatula*, *Guinardia*
313 *striata*, *Ceratium* spp., *Pleurosigma* spp. and *Gymnodinium catenatum*). For station S2, the
314 obtained positive correlations were in winter (NO₂, NO₃ and *Thalassiosira* spp.), in spring (SiO₄,
315 pH, *Alexandrium* spp., *Nitzschia* spp., *Scrippsiella* sp., *Prorocentrum triestinum*, *Pseudo-*
316 *nitzschia* spp., *Chaetoceros* spp., *Thalassionema nitzschioides*, *Skeletonema* spp., *Eucampia*
317 *zodiacus*, *Gyrodinium* spp., and *Asterionella glacialis*), in summer (Temperature, Salinity,
318 *Leptocylindrus* spp., *Plagioselmis* sp., *Dinophysis* spp. and *Guinardia striata*) and in autumn
319 (PO₄, NH₄ and *Gymnodinium catenatum*). Factorial analyses according to the season indicated
320 that the discrimination was dominated by temperature and salinity during summer. Winter was
321 characterized by high levels of nitrate and nitrite. During summer, in both stations a gradual
322 decrease of nitrate was observed and coincided with an increase in water temperature and
323 salinity. Autumn was characterized by high values of ammonium and phosphate whereas winter
324 showed high values of nitrite and nitrate which could be explained by nitrification processes
325 during winter season. The highest phytoplankton biomass and diversity were recorded in spring
326 and summer. Spearman analyses were applied to highlight the correlation between the major
327 phytoplankton species and the environmental factors, this is shown in Tables 3 and 4.

328 **4. Discussion**

329 *Phytoplankton distribution and diversity in M'diq Bay and Oued Laou Estuary*

330 The inventory of phytoplankton species in Oued Laou Estuary and M'diq Bay showed the
331 dominance of two groups: Bacillariophyceae and Dinophyceae. This finding is in agreement with
332 previous studies showing that these groups are the main phytoplankton components in the
333 Mediterranean (Vilicic et al. 2002, El Madani 2011, Armi et al., 2010, Daoudi et al., 2012; Salhi
334 et al., 2018; Draredja et al., 2019). In our study, in term of cell abundance, the diatoms

335 dominated largely with *Pseudo-nitzschia*, *Leptocylindrus* and *Chaetoceros* being the major taxa.
336 Species belonging to Bacillariophyceae showed a high diversity in spring whereas
337 dinoflagellates were more diversified during summer season. This corresponds to previous works
338 on the phytoplankton in the Nador lagoon located in the South Western Mediterranean, Morocco
339 (El Madani et al., 2011; Daoudi et al., 2012). According to Smayda (1984), this seasonal
340 succession is typical of temperate ecosystems. The phytoplankton communities in Oued Laou
341 Estuary and M'diq Bay showed a similar seasonal trend. The phytoplankton mean cell
342 abundance was generally slightly higher in M'diq Bay (18×10^4 cells l^{-1}) when compared to that
343 of Oued Laou (14×10^4 cells l^{-1}). This could be the consequence of the relatively low levels of
344 silicate present in Oued Laou Estuary required by diatoms for their growth. The dominance of
345 diatoms in both ecosystems indicates that the Western Moroccan Mediterranean coast is a water
346 mixing area. Margalef *et al.*, (1979) and Gailhard (2003) suggested that the vertical mixing of
347 water column favors the development of diatoms. Hydrological factors such as mixing with
348 nutrient-rich freshwater during rainfall periods are known to impact the development of
349 phytoplankton in a given ecosystem. Physicochemical parameters of water masses together with
350 climatic conditions particularly air temperature fluctuate seasonally in the Mediterranean
351 temperate ecosystems (Dhib et al., 2013; Laanaia et al., 2013; Salhi et al., 2018; Trombetta et al.
352 2019). Also, the seasonal variability of phytoplankton communities structure and environmental
353 parameters have been demonstrated at weekly and biweekly time scales in various coastal and
354 estuarine marine waters (Jouenne et al., 2007; Lopes et al., 2007; Armi et al., 2010). In our study,
355 temperature seems to be the most important factor which affect the diversity and richness of
356 phytoplankton species in the two investigated areas. The highest phytoplankton abundance was
357 observed in spring and summer. Temperature is correlated to species richness and diversity
358 (table 2). This is in accordance with the recent work of Trombetta et al., (2019) suggesting that
359 water temperature drives phytoplankton blooms in coastal waters. Phytoplankton diversity and
360 dynamic could be impacted by the observed increase in temperature in the Mediterranean (Shen

361 et al., 2016; Wells et al., 2015; 2019; Kim et al., 2019). To verify this hypothesis, particularly for
362 HABs species, a monitoring of phytoplankton diversity and dynamics is under way in Oued
363 Laou estuary and Mdiq bay with the aim of comparing these data with those acquired 10 years
364 ago. Diatoms were significantly associated with DIN concentrations in winter and with silicates
365 in autumn. The decreasing concentrations of DIN and NO_3 measured in spring and summer
366 could be associated to the observed increase in dinoflagellates.

367 In our study, the highest phytoplankton densities were recorded in spring and summer
368 corresponding to the increase of temperature and to high nutrient concentrations which were
369 registered in winter. Less important phytoplankton biomass occurred in winter and coincided
370 with rainfall but with low temperature and light intensity levels. The spring and summer seem to
371 be the most favorable periods for phytoplankton development and diversity. In winter, heavy
372 rainfalls allowed nutrients enrichment both ecosystems, particularly ammonium. This was shown
373 in another Mediterranean ecosystem (Alexandria, Egypt) (Abdel-Halim and Khairy 2007).
374 Ammonium turns into nitrite then into nitrate due to bacterial nitrification. Boutaib et al., (2011)
375 showed an increase in bacterial activity during the wet season (autumn and winter) in Moroccan
376 Western Mediterranean coasts. The decrease in nutrient levels was observed during spring and
377 summer. This reduction was probably related to microalgae development during spring. A
378 significant negative correlation between nutrients and microalgae was observed (Tables 2-4). In
379 Oued Laou Estuary, mineral phosphorus concentration is low and very close to the values
380 provided by Redfield et al. (1963) for Mediterranean waters. In contrast, the phosphorus
381 concentrations in M'diq Bay were relatively important probably due to aquaculture activities and
382 to effluent discharges from the three major cities: Ceuta, F'nideq and M'diq with more than
383 220000 inhabitants. The silicate nutrient is an essential element for the development of
384 Bacillariophyceae and Dictyochophyceae (Richard, 1987). In both ecosystems, silicates were
385 generally abundant in comparison with other nutrients. Generally inorganic N:P ratios were
386 higher than 16, they were $47.61 (\pm 39.87)$ in S1 and $43.34 (\pm 28.25)$ in S2. Inorganic Si:P ratios

387 in S1 were lower than 20 (12.81 ± 10.65), however in S2 they were slightly higher than 20
388 (25.35 ± 28.25). Our results were similar to those of Berland et al. (1973) and Daoudi et al.
389 (2012) who suggested that the phosphorus could be considered as the limiting nutrient in
390 Mediterranean Sea.

391
392 *HABs species and related toxicity in M'diq Bay and Oued Laou Estuary*

393 This is the first study on the dynamics of several HABs species developing in South Western
394 Mediterranean waters with measurements of environmental factors. Data showed that *Pseudo-*
395 *nitzschia* genera developed in M'diq Bay and Oued Laou with up to 35×10^4 cells.L⁻¹ in March
396 30th 2009 with toxic species resulting of DA intoxication with up to $15.94 \mu\text{g DA g}^{-1}$ mollusk
397 meat In M'diq Bay (April 6th 2009) and $16.88 \mu\text{g DA g}^{-1}$ in Oued Laou (September 10th 2008).
398 The first human intoxication with DA after ingestion of mussels was in 1978 in Al-Hoceima
399 Bay, 190 km away from Oued Laou (Mediterranean coast), where patients who had eaten
400 mussels (*Mytilus galloprovincialis*) suffered from loss of memory and disorientation. *Pseudo-*
401 *nitzschia* blooms have been documented in Moroccan Mediterranean waters by Rijal Leblad et
402 al. (2013) who showed the presence in M'diq Bay waters of eight toxic species: *P. multistriata*,
403 *P. cuspidata*, *P. galaxiae*, *P. multiseris*, *P. pseudodelicatissima*, *P. pungens var. aveirensis*, *P.*
404 *calliantha* and *P. fraudulenta*.

405 In our study, the blooms of *Pseudo-nitzschia* followed the increase of the Si/P ratio. Silicates
406 were proven to increase *Pseudo-nitzschia* abundance (Thorel et al., 2017). The correlation
407 between DA production and the nutritional status seems to be complex in natural populations,
408 since they are frequently composed of numerous species (Trainer et al. 2009). Studies on
409 *Pseudo-nitzschia* dynamics, diversity and toxicity remain relatively scarce in the South
410 Mediterranean (Sahraoui et al. 2009; Rijal leblad et al., 2013; Melliti Ben Garali et al., 2019).
411 Here, *Pseudo-nitzschia* correlated to salinity and temperature but negatively to nitrate. In Bizerte
412 Bay, (Mediterranea,Tunisia), Melliti Ben Garali et al., 2019 showed a positive correlation

413 between *Pseudo-nitzschia* abundance and salinity, silicate, phosphorus and urea. Studies are
414 under way to on the biology and ecology of Pseudo-nitzshia species responsible for ASP in these
415 fragile marine ecosystems.

416 *G. catenatum* in M'diq Bay and Oued Laou showed moderate abundances of up to 3960 cells l⁻¹.
417 However, extremely high levels of PSTs contamination were observed permanently in both
418 investigated ecosystems in the cockles reaching 7545 µg STX equiv Kg meat⁻¹, a value almost
419 10 times higher than sanitary threshold. The sweet clams were less contaminated by PSTs in
420 both sites with concentrations ranging from 0 to 1945 µg STX equiv Kg meat⁻¹. A peak of *G.*
421 *catenatum* abundance (>4000 cells L⁻¹) observed in January 2008 corresponded to a maximum
422 level of PSP in mollusks which was not the case of *Alexandrium* cells. This suggest that *G.*
423 *catenatum* was the main responsible of the mollusks PSP intoxications. This finding based on a
424 bi-weekly survey during two years corroborated the finding of Tahri (1998) and Taleb et al.
425 (2001) who showed that since 1994 (corresponding to the implementation of toxic species and
426 intoxications monitoring in Morocco, RSSL network), PSP outbreaks have been frequently
427 reported and the causative organism have been identified as *G. catenatum*. This dinoflagellate
428 species have been reported to proliferate in Spanish (Fraga, 1996, Busch et al., 2016), Portuguese
429 (Moita et al., 1998, Silva et al., 2015), Algerian (Frehi et al., 2007) and Tunisian (Dammak-
430 Zouari et al., 2009) marine coastal waters. In our study, *G. catenatum* abundance was correlated
431 with ammonium in Oued Laou Estuary and with nitrate and nitrite in M'diq Bay. According
432 Band-Schmidt et al., (2014) toxin production of *G. catenatum* changed with temperature. The
433 growth rate of this neurotoxic species varies with temperature; the highest growth was obtained
434 between 21 and 24 °C (Band-Schmidt et al., 2010; 2014). In our study, *G. catenatum* was
435 abundant in autumn with temperatures of 18-19 °C. However, it was demonstrated that *G.*
436 *catenatum* is rather an eurythermal and cosmopolitan species tolerating temperatures from 16 to
437 33 °C (Band-Schmidt et al., 2014) evolving in Pacific, Atlantic and Indian Oceans but also in
438 Mediterranean Sea.

439 Our data showed the persistence of high level of PSP toxins during the 15 months' survey in the
440 cockles in M'diq Bay and Oued Laou with very high concentrations ($> 3000 \mu\text{g STX equiv Kg}$
441 meat^{-1}) even when *G. catenatum* densities were low (fig 10). The sweet clam presented high PSP
442 levels only in January and March 2008 corresponding to the highest *G. catenatum* densities in
443 the two studied ecosystems. These results reinforce the hypothesis that *G. catenatum* is
444 responsible for the observed PSP intoxication of the mollusks. Taleb et al. (2001) showed the
445 cockle presents PSP toxicity throughout the year while other mollusks like the sweet clam
446 accumulate it seasonally. Sagou et al. (2005) showed that the cockle sequester PSP toxins
447 preferably in non-visceral organs contrary to sweet clam that sequester them in visceral tissues
448 (digestive gland) which could explain the depuration/retention fate of PSTs. Taleb et al., (2001)
449 attributed the high toxicity levels of cockles to the biotransformation of C-toxins (with low
450 specific toxicity) to dc-carbamoylsaxitoxin (dcSTX) with relatively high toxicity. Rijal Leblad et
451 al., (2017) showed throughout laboratory experiments a partial and progressive elimination of
452 PSP toxins in two investigated mollusks, with a slower elimination kinetic in the tuberculate
453 cockle when compared with the sweet clam, needing 120 and 3 days to reach levels of $80 \mu\text{g}$
454 $\text{SXTeq } 100\text{g}^{-1}$ of meat, respectively.

455 In our study, *Dinophysis caudata* showed the highest abundance, followed by *Dinophysis fortii*,
456 *Dinophysis acuminata* and *Dinophysis rotundata*. Even though *Prorocentrum lima* is known to
457 be a benthic species, it showed abundances of up to $1300 \text{ cells.L}^{-1}$ in the water column of Oued
458 Laou Estuary during August 2008. The other recorded benthic dinoflagellate *Ostreopsis* showed
459 low abundance not exceeding 90 cells.L^{-1} . The occurrence of benthic HABs species have been
460 well documented in Western Mediterranean (Vila et al., 2001; Dhib et al., 2015; Ben Gharbia et
461 al. 2016; 2019) but not yet in Algerian and Moroccan coastal waters. *D. caudata* was shown to
462 produce Dinophysistoxins, PTX-2 and OA responsible for DSP intoxication (Trainer et al.,
463 2013). DSTs are associated to many species of *Dinophysis* species and *P. lima* (Bazzoni et al.,
464 2018). In our study, using the biological method we noted the absence the detection of DST

465 intoxication in S1 and S2. However, DSTs related to *Dinophysis* species developing have been
466 shown in NW Mediterranean Sea by Bazzoni et al. (2018) and García-Altare et al. (2016) in
467 some in shellfish farming areas of Italian and Spanish waters, respectively. In our study,
468 *Dinophysis* correlated to pH in both sites. Moreover, correlated to salinity and phosphorus in S1
469 and S2 respectively.

470 **5. Conclusion**

471 For years, PSP events threat the consumer's health and cause dramatic economic lose due to
472 permanent closure of shellfish harvesting areas of the investigated Mediterranean waters, M'diq
473 Bay and Oued Laou Estuary. Our data clearly showed that *G. catenatum* was responsible of PSTs
474 contamination in the study areas and better explained its relationships with environmental factors.
475 The next step should be to isolate several strains of *G. catenatum* from Moroccan waters, to
476 characterize them at the genetic and toxic levels. Ecophysiological studies should help us to better
477 understand how the environmental factors influence the growth and toxin production of this
478 dinoflagellate. ASP was observed but DA did not exceed 18 $\mu\text{g.g}^{-1}$ meat which remains below the
479 sanitary threshold. However, *Pseudonitzschia* species have to be isolated and their identity
480 determined genetically in addition to the cellular toxin characterization. Concerning DST, the used
481 biological method have to be completed by direct chemical analyses of lipophilic toxins using
482 HPLC-MS/MS technique which have to be implemented in the monitoring program of toxins.
483 Finally, increasing sampling frequency and long term monitoring is important to better known the
484 driving environmental parameters and among them temperature of the dynamic of phytoplankton
485 including HABs species developing in these fragile and exploited coastal marine ecosystems.

486

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 490 collaboration in HABs studies.

491

492 **Figures and Tables Legend**

493 **Fig. 1.** Location of the sampled stations (S1 : Oued Laou Estuaty and S2 : M'diq Bay) in the Moroccan
 494 Western Mediterranean sea.

495

496 **Fig. 2.** Temporal variation of Rainfall (mm.month⁻¹), pH, temperature (°C) and Salinity in M'diq Bay and
 497 Oued Laou Esturay stations located in the Moroccan Western Mediterranean sea.

498

499 **Fig. 3.** Temporal variation of phosphorus (PO₄), Silicate (SiO₄) and Dissolved Inorganic Nitrogen (DIN)
 500 expressed in μmole.l⁻¹ at M'diq Bay and Oued Laou Estuary

501

502 **Fig. 4.** Temporal variations of Diatom abundance (Cells.L⁻¹), Si/P Ratio and N/P Ratio at M'diq Bay and
 503 Oued Laou Estuary

504 **Fig. 5.** Contribution in percentage of different taxa to total phytoplankton abundance at the Oued Laou
 505 Estuary (A) and M'diq Bay (B)

506

507 **Fig. 6.** Temporal variation of cell densities of the different phytoplankton groups in Oued Laou Estuary
 508 (A) and M'diq Bay (B)

509

510 **Fig. 7.** Temporal succession of the phytoplankton abundance, species richness and species diversity
 511 values (H') in Oued Laou Estuary and M'diq Bay.

512

513 **Fig. 8.** Annual succession of the dominant Taxa at Oued Laou Estuary from April 2008 to June 2009. The
 514 values correspond to relative densities. Astg. : *Asterionella glacialis*, Chae : *Chaetoceros* spp, Plag :
 515 *Plagioselmis* sp., Eugl : *Euglena* sp., Gy.ca : *Gymnodinium catenatum*, Lept : *Leptocylindrus* spp, Nitz :
 516 *Nitzschia* spp, No.sc. : *Noctiluca scintillans*, Psz. : *Pseudo-nitzschia* spp, Gu.de. : *Guinardia delicatula*,
 517 Gu.st. : *Guinardia striata*, Scri : *Scrippsiella* sp, Skel : *Skeletonema* spp, Thal : *Thalassiosira* spp, Thnu :
 518 *Thalassionema nitzschioides*).

519

520 **Fig. 9.** Annual succession of the dominant Taxa at M'diq bay from April 2008 to June 2009. The values
 521 correspond to relative densities. Astg : *Asterionella glacialis*, Chae : *Chaetoceros* spp, Plag :
 522 *Plagioselmis* sp., Gyr : *Gyrodinium* spp., Lept : *Leptocylindrus* spp, Nitz : *Nitzschia* spp, Prrt :

523 *Prorocentrum triestinum*, Psz : *Pseudo-nitzschia* spp, Scri : *Scrippsiella* sp, Skel : *Skeletonema* spp,
 524 Thal : *Thalassiosira* spp, Th.nu. : *Thalassionema nitzschioides*).

525

526 **Fig. 10.** Temporal variation in cells L⁻¹ of (A) *Dinophysis*. spp., (B) *Prorocentrum lima* and (C)
 527 *Ostreopsis* spp. at Oued Laou Estuary and M'diq Bay.

528

529 **Fig. 11.** Temporal variation of (A) *Pseudo-nitzschia* spp. (cells L⁻¹) and domoic acid (µgDA g⁻¹ shellfish
 530 flesh) in the cockle and sweet clam at Oued Laou Estuary (B) and M'diq Bay (C).

531

532 **Fig. 12.** Temporal variations of (A) *Alexandrium* spp., (B) *Gymnodinium catenatum*, paralytic shellfish
 533 poisoning amount (µg STXeq Kg⁻¹ shellfish flesh) in the cockle and sweet clam at Oued Laou Estuary (C)
 534 and M'diq Bay (D).

535

536 **Fig. 13.** Factorial Discriminant Analysis (FDA) for Oued Laou Estuary (A) and M'diq Bay (C). Astg. :
 537 *Asterionella glacialis*, Chae : *Chaetoceros* spp, Plag : *Plagioselmis* sp., Eugl : *Euglena* sp., Gy.ca :
 538 *Gymnodinium catenatum*, Lept : *Leptocylindrus* spp, Nitz : *Nitzschia* spp, No.sc : *Noctiluca scintillans*,
 539 Psz : *Pseudo-nitzschia* spp, Gu.de : *Guinardia delicatula*, Gu.st : *Guinardia striata*, Scri : *Scrippsiella* sp,
 540 Skel : *Skeletonema* spp, Thal : *Thalassiosira* spp, Thnu : *Thalassionema nitzschioides*, Din : *Dinophysis*
 541 spp, Het : *Heterocapsa* sp., Cer : *Ceratium* spp., Pleu: *Pleurosigma* spp). T°: Temperature, NO₃: nitrate,
 542 NO₂ : nitrite, NH₄⁺: ammonium and PO₄: phosphorus.

543 FDA sample ordination plot considering the seasons; Oued Laou Estuary (B) and M'diq Bay (D).

544

545 **Table 1.** Mean values of physico-chemical environmental parameters at M'diq Bay (Station 2), Mean and
 546 Standard Error (SE); n = number of samples; Min = minimum value; Max = maximum value. NH₄:
 547 ammonium, NO₂: nitrite, NO₃: nitrate, PO₄: phosphorus and SiO₄: silicate.

548

549 **Table 2.** Spearman correlations linking phytoplankton groups and environmental factors in Mdiq Bay (S2)
 550 and Oued Laou Estuary(S1). * Raphidophyceae and Chlorophyceae species were not observed in Mdiq bay.

551

552 **Table 3.** Values of Spearman rank correlation coefficient between dominant species and environmental
 553 parameters in Oued Laou Estuary (*: significant). Astg. : *Asterionella glacialis*, Chae : *Chaetoceros* spp,
 554 Plag : *Plagioselmis* sp., Eugl : *Euglena* sp., Gy.ca : *Gymnodinium catenatum*, Lept : *Leptocylindrus* spp,
 555 Nitz : *Nitzschia* spp, No.sc : *Noctiluca scintillans*, Psz : *Pseudo-nitzschia* spp, Gu.de : *Guinardia*
 556 *delicatula*, Gu.st : *Guinardia striata*, Scri : *Scrippsiella* sp, Skel : *Skeletonema* spp, Thal : *Thalassiosira*
 557 spp, Th.nu : *Thalassionema nitzschioides*, Din : *Dinophysis* spp, Het : *Heterocapsa* spp, Cer : *Ceratium*

558 spp, Pleu: *Pleurosigma* spp). Si: silicate, PO₄: phosphorus, NH₄: ammonium, NO₂: nitrite and NO₃:
559 nitrate.

560

561 **Table 4.** Values of Spearman rank correlation coefficient between dominant species and environmental
562 parameters in M'diq bay (* significant). Astg : *Asterionella glacialis*, Chae : *Chaetoceros* spp, Plag :
563 *Plagioselmis* sp., Gyr : *Gyrodinium* spp., Lept : *Leptocylindrus* spp, Nitz : *Nitzschia* spp, Prtt :
564 *Prorocentrum triestinum*, Psz : *Pseudo-nitzschia* spp, Scri : *Scrippsiella* sp, Skel : *Skeletonema* spp,
565 Thal : *Thalassiosira* spp, Th.nu : *Thalassionema nitzschioides*, Alex: *Alexandrium* spp, Gy. ca:
566 *Gymnodinium catenatum*, Din: *Dinophysis* spp, Euc: *Eucampia zodiacus*, Gu.st : *Guinardia striata*). Si:
567 silicate, PO₄: phosphorus, NH₄: ammonium, NO₂: nitrite and NO₃: nitrate.

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573 **References**

574

575 Abadie, E., Rhodes, L., Chomérat, N., Chiantella, C., Crottier, A., Laabir, M. 2018. Occurrence of the
576 neurotoxic dinoflagellate *Vulcanodinium rugosum* in Mediterranean: What are the driving
577 environmental factors of its dynamic ?.Harmful Algae. 75, 75-86.

578 Abdel-Halim, A.M., Khairy, H.M., 2007. Potential impact of some abiotic parameters on a
579 phytoplankton community in a confined bay of the Eastern Mediterranean Sea: Eastern
580 Harbour of Alexandria, Egypt. Med. Mar. Sc. 8, 49-64.

581 Abdenadher, M., Hamza, A., Fekih, W., Hannachi, I., Zouari Bellaaj, A., Bradai, M.N., Aleya, L.,
582 2012. Factors determining the dynamics of toxic blooms of *Alexandrium minutum* during a
583 10-year study along the shallow south western Mediterranean coasts. Estuar. Coast. Shelf Sci.
584 106, 102–111.

- 585 Aissaoui, A., Dhib, A., Reguera, B., Ben Hassine, O.K., Turki, S., Aleya, L., 2014. First evidence
586 of cell deformation occurrence during a *Dinophysis* bloom along the shores of the Gulf of
587 Tunis (SW Mediterranean Sea). *Harmful Algae*. 39, 191-201.
- 588
- 589 Aminot, A., Chaussepied, M., 1983. *Manuel des analyses chimiques en milieu marin*. Brest,
590 CNEXO, 395p.
- 591 AOAC, 1990. Paralytic Shellfish Poisoning, Biological method, final action. In AOAC (Eds.)
592 official methods of analysis. 15th ed. Method n° 959.08 JAOAC, Arlington, VA.881-882.
- 593 Armi, Z, Trabelsi, E., Turki., S., Béjaoui, B., Maïz Naceur, B., 2010. Seasonal phytoplankton
594 responses to environmental factors in a shallow Mediterranean lagoon. *J. Mar. Sc. Tech.* 15,
595 417-426.
- 596 Band-Schmidt, C.J., Bustillos-Guzman, J.J., Hernandez-Sandoval, F.E., Núñez-Vazquez, E.J.,
597 Lopez-Cortes D.J., 2014. Effect of temperature on growth and paralytic toxin profiles in
598 isolates of *Gymnodinium catenatum* (Dinophyceae) from the Pacific coast of Mexico.
599 *Toxicon*. 90, 199-212.
- 600 Band-Schmidt, C.J., Bustillos-Guzman, J.J., Lopez-Cortes, D.J., Garate-Lizarraga, I., Núñez-
601 Vazquez, E.J., Hernandez-Sandoval, F.E., 2010. Ecological and physiological studies of
602 *Gymnodinium catenatum* in the Mexican Pacific: a review. *Mar. Drugs*. 8, 1935-1961.
- 603 Bazzoni, A.M., Mudadu, A.G., Lorenzoni, G., Soro, B., Bardino, N., Arras, I., Sanna, G., Vodret,
604 B., Bazzardi, R., Marongiu, E., 2018. Detection of *Dinophysis* species and associated okadaic
605 acid in farmed shellfish: a two-year study from the western Mediterranean area. *J. Vet. Res.*
606 62, 137-144.
- 607 Ben Gharbia, H., Kéfi-Daly Yahia, O., Amzil, Z., Chomérat, N., Abadie, E., Masseret, E., Sibat, M.,
608 Zmerli Triki, H., Nouri, H. and Laabir, M. 2016. Toxicity and Growth Assessments of Three
609 Thermophilic Benthic Dinoflagellates (*Ostreopsis cf. ovata*, *Prorocentrum lima* and *Coolia*
610 *monotis*) Developing in the Southern Mediterranean Basin. *Toxins*. 8, 297-335.
- 611 Ben Gharbia, H., Laabir, M., Ben Mhamed, A., Gueround, S., Daly Yahia, N., Nouri, H., M'Rabeta,
612 C., Shilig, A., Kéfi-Daly Yahia, O. 2019. Occurrence of epibenthic dinoflagellates in relation to
613 biotic substrates and to environmental factors in Southern Mediterranean (Bizerte Bay and Lagoon,

- 614 Tunisia): an emphasis on the harmful *Ostreopsis spp.*, *Prorocentrum lima* and *Coolia monotis*.
 615 Harmful Algae, in press.
- 616 Ben Rejeb Jenhani, A., Fathalli, A., Ben Naceur, H., Hayouni, D., Aouani, J., Romdhane, M.S.,
 617 2019. Screening for alien and harmful planktonic species in the Gulf of Gabes (Tunisia,
 618 Southeastern Mediterranean Sea). Reg. Stud. Mar. Sci, 27
- 619 Berland, B.R., Bonin, D.j., Maestrini, S.Y., Pointier, J.P., 1973. Etude de la fertilité des eaux
 620 marines au moyen de tests biologiques effectués avec des cultures d’algues. II Limitation
 621 nutritionnelle et viabilité de l’inoculum. Int. Revue. Gesanten. Hydrobiol. 58, 203-220.
- 622 Bourhilli, E.H., 1982. Intoxication alimentaire par les moules (51 cas), Agadir, octobre et
 623 novembre 1982. Thèse n°33. Faculté de Médecine et de Pharmacie, Rabat 84 pp.
- 624 Boutaib, R., Marhraoui, M., Oulad Abdellah, M.K., Bouchrif, B., 2011. Comparative study on
 625 faecal contamination and occurrence of *Salmonella spp.* and *Vibrio parahaemolyticus* in two
 626 species of shellfish in Morocco. Open. Environmental. Sciences. 5, 30-37.
- 627 Boyd, P.W., Strzepek, R., Fu, F., Hutchins, D.A., 2010. Environmental control of open-ocean
 628 phytoplankton groups: Now and in the future. Limnol. Ocean. 55(3), 1353–1376.
- 629 Bravo, I., Reguera, B., Martínez, A., Fraga, S., 1990. First report of *Gymnodinium catenatum*
 630 Graham on the Spanish Mediterranean coast. In: Granéli, E., Sundtröm, B., Edler, L.,
 631 Anderson, D.M. (Eds.), Toxic Marine Phytoplankton. Elsevier, New York, pp. 449–452.
- 632 Busch, J.A., Andree K.B., Diogene, J., Fernandez-Tejedor, M., Toebe, K., John, U., Krock, B.,
 633 Urban Tillmann, U., Cembella, A.D., 2016. Toxigenic algae and associated phycotoxins in
 634 two coastal embayments in the Ebro Delta (NW Mediterranean). Harmful Algae. 55, 191–
 635 201.
- 636 Bužančić, M., Gladan, Z.N., Marasović, I., Kušpilić, G., Grbec, B., 2016. Eutrophication influence
 637 on phytoplankton community composition in three bays on the eastern Adriatic coast.
 638 Oceanologia. 58, 302-316.
- 639 Carstensen, J., Klais, R., Cloern, J.E., 2015. Phytoplankton blooms in estuarine and coastal waters:
 640 Seasonal patterns and key species. Estuar Coast Shelf Sci. 162, 98–109.

- 641 Cerino, F., Orsini, L., Sarno, D., Carmela, D.A., Tartaglione L., Zingone, A., 2005. The
642 alternation of different morphotypes in the seasonal cycle of the toxic diatom *Pseudo-*
643 *nitzschia galaxiae*. Harmful Algae. 4, 33–48.
- 644 Cloern, J.E., 2001. Our evolving conceptual model of the coastal eutrophication problem. Mar.
645 Ecol. Progr. Ser. 210, 223 –253.
- 646 Dammak-Zouari, H., Hamza, A., Bouain, A., 2009. Gymnodiniales in the Gulf of Gabes (Tunisia).
647 Cah. Biol. Mar. 50, 153-170.
- 648 Daoudi, M., Serve, L., Rharbi, N., El Madani, F., Vouvé, F., 2012. Phytoplankton distribution in
649 the Nador lagoon (Morocco) and possible risks for harmful algal blooms. Trans. Waters. Bull.
650 6(1), 4-19.
- 651 Delgado, M., 1990. Phytoplankton distribution along the Spanish coast of the Alborán Sea. Sci.
652 Mar. 54, 169–178.
- 653 Dhib, A., Frossard, V., Turki, S., Aleya, L. 2013. Dynamics of harmful dinoflagellates driven by
654 temperature and salinity in a northeastern Mediterranean lagoon. Environ. Monit. Asses. 185,
655 3369–3382.
- 656 Dhib, A., Fertouna-Bellakhal, M., Turki, S., Aleya, L., 2015. Harmful planktonic and epiphytic
657 microalgae in a Mediterranean Lagoon: the contribution of the macrophyte *Ruppia cirrhosa* to
658 microalgae dissemination. Harmful Algae 45, 1–13.
- 659 Draredja, MA ., Frihi, H. Boualleg, C., Gofart, A., Abadie, E., Laabir, M. 2019. Seasonal variations of
660 phytoplankton community in relation to environmental factors in a protected meso-oligotrophic
661 southern Mediterranean marine ecosystem (Mellah lagoon, Algeria) with an emphasis of HAB
662 species. Environ. Monit. Assess. 191(10).
- 663 EL Madani, F., Chiaâr, A., Chafi, A., 2011. Phytoplankton composition and abundance
664 assessment in the Nador lagoon (Mediterranean coast of Morocco). Acta. Botanica. Croatia.
665 70(2), 269-288.
- 666 Fertouna-Bellakhal, M., Dhib, A., Fathalli, A., Bellakhal, M., Chome, N., Masseret, E., Laabir, M.,
667 Turki, S., Aleya, L., 2015. *Alexandrium pacificum* Litaker sp. nov (Group IV): Resting cyst

- 668 distribution and toxin profile of vegetative cells in Bizerte Lagoon (Tunisia, Southern Mediterranean
669 Sea). *Harmful Algae*. 48, 69-82.
- 670 Fraga, S., 1996. Wintering of *Gymnodinium catenatum* Graham in Iberian waters. In. Yasumoto,
671 T., Oshima, Y., Fukuyo, Y. (Eds.), *Harmful and Toxic Algal Blooms*. IOC of UNESCO,
672 Paris, pp. 211-214.
- 673 Frehi, H., Coute, A., Mascarell, G., Perrette-Gallet, C., Ayada, M., Kara, MH., 2007. Harmful and
674 red-tide dinoflagellates in the Annaba bay (Algeria). *Comptes Rend Biol*. 330, 615-628.
- 675 Gailhard, I., 2003. Analyse de la variabilité spatio-temporelle des populations microalgales
676 côtières observées par le « Réseau de surveillance du phytoplancton et des phycotoxines »
677 (REPHY). Thèse de Doctorat d'université. Université d'Aix-Marseille II, Aix-Marseille,
678 France. 187p.
- 679 García-Altare, M., Casanova, A., Fernández-Tejedor, M., Diogène, J., De la Iglesia, P., 2016.
680 Bloom of *Dinophysis* spp. dominated by *D. sacculus* and its related diarrhetic shellfish
681 poisoning (DSP) outbreak in Alfacs Bay (Catalonia, NW Mediterranean Sea): Identification
682 of DSP toxins in phytoplankton, shellfish and passive samplers. *Reg. Stud. Mar. Sc.* 6, 19–28.
- 683 Gobler, C.J., Doherty, O.M., Owen, M., Hattenrath-Lehmann, T.K., Theresa, K., Griffith, A.W.,
684 Kang, Y., Litaker, R.W., 2017. Ocean warming since 1982 has expanded the niche of toxic
685 algal blooms in the North Atlantic and North Pacific oceans. *Proc. National. Acad. Sci. USA*.
686 14(19), 4975-4980.
- 687 Gomez, F., 2003. The toxic dinoflagellate *Gymnodinium catenatum*: an invader in the
688 Mediterranean sea. *Acta. Bot. Croat.* 62, 65–72.
- 689 Grzebyk, D., Audic, S., Lasserre, B., Abadie, E., de Vargas, C., Bec, B., 2017. Insights into the
690 harmful algal flora in northwestern Mediterranean coastal lagoons revealed by
691 pyrosequencing metabarcodes of the 28S rRNA gene. *Harmful Algae*. 68, 1–16.
- 692 Hallegraeff, G.M., 1993. A review of harmful algal blooms and their apparent global increase.
693 *Phycologia* 32, 79–99.

- 694 Hallegraeff, G.M., 2010. Ocean climate change, phytoplankton community responses, and
695 harmful algal blooms: a formidable predictive challenge. *J. Phycol.* 46, 220–235.
- 696 Illoul, H., Maso, M., Fortuno, J.M., Cros, L., Morales-Blake, A., Séridji, R., 2008. Potentially
697 harmful microalgae in coastal waters of the Algiers area (Southern Mediterranean Sea).
698 *Cryptogamie Algol.* 29, 261-278.
- 699 Jouenne, F., Lefebvre, S., Véron, B., Lagadeuc, Y., 2007. Phytoplankton community structure and
700 primary production in small intertidal estuarine-bay ecosystem (eastern English Channel,
701 France). *Mar. Biol.* 151, 805–825.
- 702 Kim, G.U., Seo, K.H., Chen, D.L., 2019. Climate change over the Mediterranean and current
703 destruction of marine ecosystem. *Scientific Reports*. Vol 9. DOI: 10.1038/s41598-019-55303.
- 704 Laabir, M., Jauzein, C., Genovesi, B., Masseret, E., Grzebyk, D., Cecchi, P., Vaquer, A., Perrin, Y.,
705 Collos, Y., 2011. Influence of temperature, salinity and irradiance on the growth and cell yield of the
706 harmful red tide dinoflagellate *Alexandrium catenella* colonising Mediterranean waters. *J. Plankton*
707 *Res.* 33, 1550-1563.
- 708 Laanaia, N., Vaquer, A., Fiandrino, A., Genovesi, B., Pastoureaud, A., Cecchi, P., Collos, Y. ; 2013.
709 Wind and temperature controls on *Alexandrium* blooms (2000-2007) in Thau lagoon (Western
710 Mediterranean). *Harmful Algae.* 28, 31-36.
- 711 Lopes, C.B., Lillebo, A.I., Dias, J.M., Pereira, E., Vale, C., Duarte, A.C., 2007. Nutrient dynamics
712 and seasonal succession of phytoplankton assemblages in a Southern European Estuary: Ria
713 de Aveiro, Portugal. *Estuar. Coast. Shelf. Sci.* 71, 480–490.
- 714 Macias, D., García-Gorríz, E., Stips, A., 2018. Major fertilization sources and mechanisms for
715 Mediterranean Sea coastal ecosystems. *Limnol. Oceanogr.* 63, 897–914.
- 716 Macias, D.M., Garcia-Gorriz, E., Stips, A., 2015. Productivity changes in the Mediterranean Sea
717 for the twenty-first century in response to changes in the regional atmospheric forcing. *Front.*
718 *Mar. Sci.* 2, 79.
- 719 Margalef, R., Estrada, M., Blasco, D., 1979. Functional morphology of organisms involved in Red
720 Tides, as adapted to decaying turbulence. In Taylor DL, Seliger H.H., (eds) *Toxic Dinoflagel-*

- 721 late Blooms, Proc. 2nd Int. Conf., Key Biscayne, Florida, USA, Oct. 31-Nov. 15, 1978: 89-
722 94.
- 723 Melliti Ben Garali, S., Sahraoui, I., De la Iglesia, P., Chalghaf, M., Diogène, J., Jamel Ksouri, J.,
724 Sakka Hlaili, A., 2019. Factors driving the seasonal dynamics of *Pseudo-nitzschia* species and
725 domoic acid at mussel farming in the SW Mediterranean Sea. *Chemistry Ecology*. DOI:
726 10.1080/02757540.2019.1676417
- 727 Moita, M.T., Vilarinha, M.G., Palma, A.S., 1998. On the variability of *G. catenatum* Graham
728 blooms in Portuguese waters. In : Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.),
729 Harmful Algae : Xunta de Galicia and IOC of UNESCO, Spain, pp. 118-121.
- 730 Quilliam, M.A., 1995. Chemical methods for domoic acid, the amnesic shellfish poisoning (ASP)
731 toxin. In: G.M. Hallegraeff, D.M. Anderson & A.D. Cembella (Eds.), *Manual on Harmful*
732 *Marine Microalgae, Monographs on Oceanographic Methodology, Vol. 11, Chapter 9.*
733 *Intergovernmental Oceanographic Commission (UNESCO), Paris, 247-266 (2003*
- 734 Quiroga, I., 2006. *Pseudo-nitzschia* blooms in the bay of Banyuls-sur-mer, northwestern
735 Mediterranean Sea. *Diatom Res.* 21, 91–104.
- 736 Redfield, A.C., Ketchum, B.H., Richards, F.A., 1963. The influence of organisms on the
737 composition of seawater. In M. N. Hill (Eds.). *The sea, Vol. 2. Interscience.* p. 26– 77
- 738 Richard, M., 1987. *Atlas de phytoplancton marin. Vol. 2, Diatomophycées.* CNRS, Paris
- 739 Rijal Leblad, B., Lundholm, N., Goux, D., Veron, B., Sagou, R., Taleb, H., Nhhala, H., Er-
740 Raioui, H., 2013. *Pseudo-nitzschia* Peragallo (Bacillariophyceae) diversity and domoic acid
741 accumulation in tuberculate cockles and sweet clams in M'diq Bay, Morocco. *Acta. Botanica.*
742 *Croatia.* 72 (1), 35–47.
- 743 Rijal leblad, B., 2012. Variabilité de la contamination des bivalves (*Callista chione* &
744 *Acanthocardia tuberculata*) par les phycotoxines paralysantes (PSP) et amnésiantes (ASP)
745 dans la frange littorale méditerranéenne marocaine: cas de la baie de M'Diq et de l'estuaire de
746 Oued Laou. MSc Thesis N° 57. Université Abdelmalek Assaadi, Morocco. 176p.

- 747 Rijal Leblad, B., Nhhala1, H., Daoudi, M., Marhraoui, M., Ouelad Abdellah, N.K., Veron, B., Er-
748 Raioui, H., 2017. Contamination and depuration of Paralytic Shellfish Poisoning by
749 *Acanthocardia tuberculata* cockles and *Callista chione* clams in Moroccan waters. J. Mater.
750 Environ. Sci. 8(S), 4634-4641.
- 751 Ruggiero, M.V., Sarno, D., Barra, L., Kooistra, W.H.C.F., Montresor, M., Zingone, A., 2015.
752 Diversity and temporal pattern of *Pseudo-nitzschia* species (Bacillariophyceae) through the
753 molecular lens. Harmful Algae. 42, 15–24.
- 754 Sagou, R., Amanhir, R., Taleb, H., Vale, P., Blaghen, M., Loutfi, M. 2005. Comparative study on
755 differential accumulation of PSP toxins between cockle (*Acanthocardia tuberculatum*) and
756 sweet clam (*Callista chione*). Toxicon. 46, 612-618.
- 757 Sahraoui, I., Sakka Hlaili, A., Hadj Mabrouk, H., 2009. Blooms of the diatom genus *Pseudo-*
758 *nitzschia* H. Peragallo in Bizerte Lagoon (Tunisia. SW Mediterranean). Diatom Res. 24,175–
759 190.
- 760 Salhi, N., Zmerli Triki, H., Molinero, J.C., Laabir, M., Sehli, E., Bellaaj-Zouari, A., Daly Yahia,
761 N., Kefi Daly Yahia, O., 2018. Seasonal variability of picophytoplankton under contrasting
762 environments in northern Tunisian coasts, southwestern Mediterranean Sea Mar. Pol. Bul.
763 129, 866–874.
- 764 Shen, A., Ma, Z., Jiang, K., Li, D., 2016. Effects of temperature on growth, photophysiology,
765 Rubisco gene expression in *Prorocentrum donghaiense* and *Karenia mikimotoi*. Ocean Sci. J.
766 51 (4), 581–589.
- 767 Silva, T., Caeiro, M.F., Reis Costa, P., Amorim, A., 2015. *Gymnodinium catenatum* Graham
768 isolated from the Portuguese coast: Toxin content and genetic characterization. Harmful
769 Algae. 48, 94-104.
- 770 Smayda, T.J., 1984. Variations and long-term changes in Narragansett Bay, a phytoplankton-
771 based coastal marine ecosystem: relevance to field monitoring for pollution assessment. In

- 772 Concept in marine pollution, pp. 663–679. Ed. By H. H. White. Maryland Seagrass College,
773 University of Maryland
- 774 Tahri, L.J., 1998. *Gymnodinium catenatum* Graham blooms on Moroccan waters. In Reguera, B.,
775 Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), Harmful Algae. Xunta de Galicia and IOC of
776 UNESCO, Spain, pp. 66-67.
- 777 Taleb, H., Vale, P., Blaghen, M., 2003. Spatial and temporal evolution of PSP toxins along the
778 Atlantic shore of Morocco. *Toxicon*. 41, 199-205.
- 779 Taleb, H., Vale, P., Jaime, E., Blaghen, M., 2001. Study of paralytic shellfish poisoning toxin
780 profile in shellfish from the Mediterranean shore of Morocco. *Toxicon*, 39, 1855-1861.
- 781 Tber, A., 1983. Personal communication. Laboratoire d'analyses des services vétérinaires,
782 Casablanca, Maroc 5p.
- 783 Thorel, M., Claquin P., Schapira, M., Le Gendre, R., Riou, P., Goux, D., LeRoy,
784 B., Raimbault, V., Deton-Cabanillas, A.F., Bazin, P., Kientz-Bouchart, V., Fauchot, J.,
785 2017. Nutrient ratios influence variability in *Pseudo-nitzschia* species diversity and particulate
786 domoic acid production in the Bay of Seine (France). *Harmful Algae*. 68, 192-205.
- 787 Trainer, V.L., Hickey, B.M., Lessard, E.J., Cochlan, W.P., Trick, C.G., Wells, M.L., MacFadyen,
788 A., Moore, S.K., 2009. Variability of *Pseudo-nitzschia* and domoic acid in the Juan de Furca
789 eddy region and its adjacent shelves. *Limnol. Oceanog.* 51, 289-308.
- 790 Trainer, V.L., Moore, L., Bill, B.D., Adams, N.G., Harrington, N., Borchert, J., da Silva, D.A.M.,
791 and Bich-Thuy, L., Eberhart, B.T., 2013. Diarrhetic Shellfish Toxins and Other Lipophilic
792 Toxins of Human Health Concern in Washington State. *Mar. Drugs*. 11, 1815-1835.
- 793 Trombetta, T., Vidussi, F., Mas, S., Parin, D., Simier, M., Mostajir, B., 2019. Water temperature
794 drives phytoplankton blooms in coastal waters. *Plos One*. DOI: 10.1080/02757540.2019.1676417
- 795 Uthermol, H., 1958. Zur vervollkommnung der quantitaven phyto-plankton-methodik. *Mitt. Int.*
796 *Ver. Ther. Angew. Limnol.*, 9

- 797 Van Dolah, F.M., 2000. Marine algal toxins: origins, health effects, and their increased
798 occurrence. *Environ. Health Perspect.* 108, 133–141.
- 799 Vila, M.; Garcés, E.; Masó, M.; Camp, J. 2001a. Is the distribution of the toxic dinoflagellate
800 *Alexandrium catenella* expanding along the NW Mediterranean coast? *Mar. Ecol. Prog. Ser.*
801 222, 73–83.
- 802 Vila, M., Garcés, E., Masó, M., 2001b. Potentially toxic epiphytic dinoflagellate assemblages on
803 macroalgae in the NW Mediterranean. *Aquat. Microb. Ecol.* 26, 51–60.
- 804 Vila, M., Giacobbe, M.G., Masó, M., Gangemib, E., Sampedroa, N., Pennam, A., Azzarob, F.,
805 Campa, J., Galluzzic, L., 2005. A comparative study on recurrent blooms of *Alexandrium*
806 *minutum* in two Mediterranean coastal areas. *Harmful Algae.* 4, 673–695.
- 807 Vilicic, D., Marasovi, I., Miokovi, D., 2002. Checklist of phytoplankton in the eastern Adriatic Sea.
808 *Acta. Botanica. Croatica.* 61, 57–91.
- 809 Wells, M.L., Karlson, B., Wulff, A., Kudela, R., Trick, C., Asnaghi, V., Berdalet, E., Cochlan, W.,
810 Davidson, K., De Rijcke, M., Dutkiewicz, S., Hallegraeff, G., Flynn, K., Legrand, C., Paerl,
811 H., Silke, J., Suikkanen, S., Thompson, P., Trainer, V., 2019. Future HAB Science: Directions
812 and Challenges in a Changing Climate. *Harmful Algae.* in press. .
- 813 Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S., Trick, C.G., Kudela, R.M., Ishikawa, A.,
814 Bernard, S., Wulff, A., Anderson, D.M., 2015. Harmful algal blooms and climate change:
815 Learning from the past and present to forecast the future. *Harmful Algae.* 49, 68–93.
- 816 Zmerli-Triki, H., Laabir, M., Moeller, P., Chomérat, N., Kéfi Daly-Yahia, O., 2016. Production of
817 goniodomin A by the dinoflagellate *Alexandrium pseudogonyaulax* developing in southern
818 Mediterranean (Bizerte Lagoon, Tunisia). *Toxicon.* 111, 91-99.

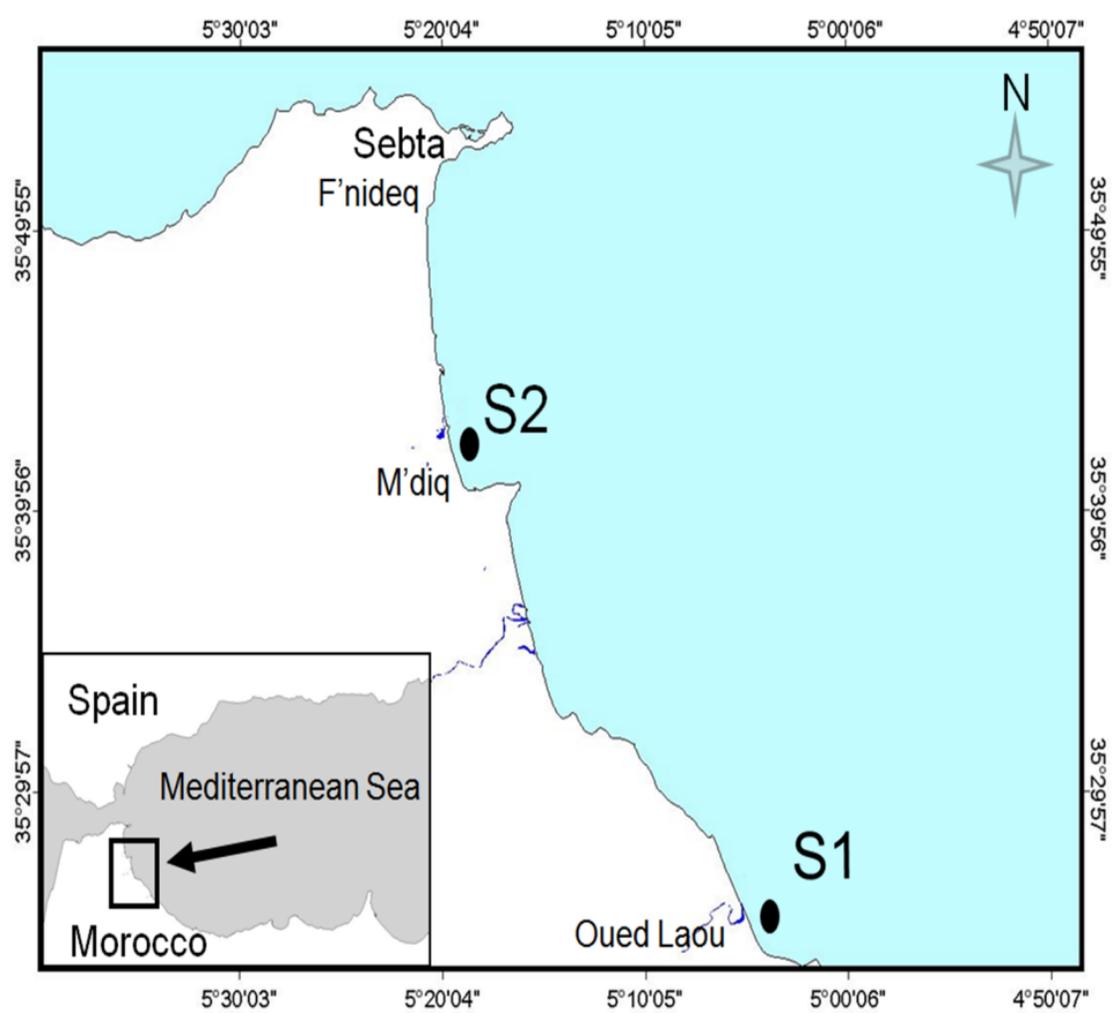
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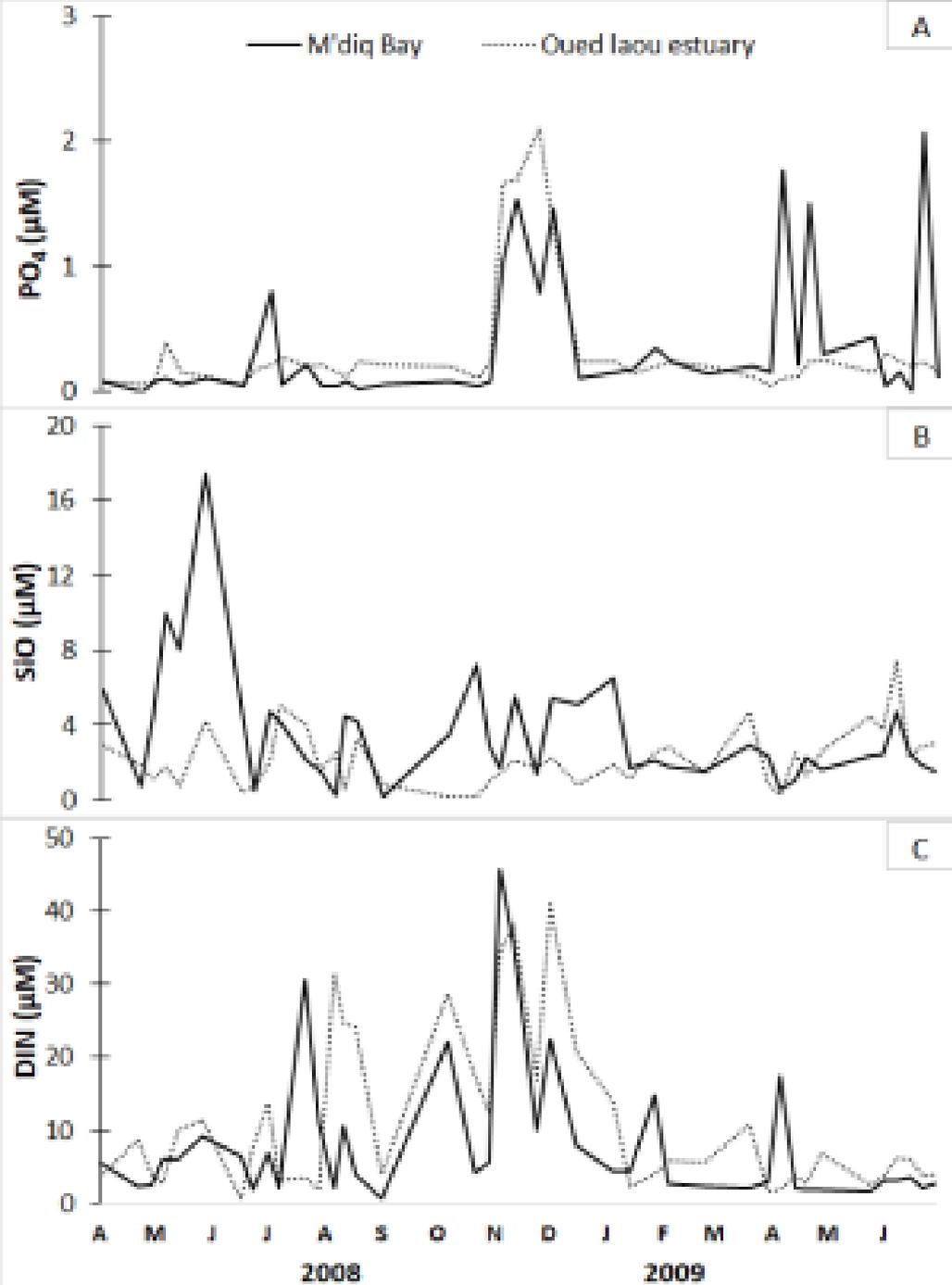
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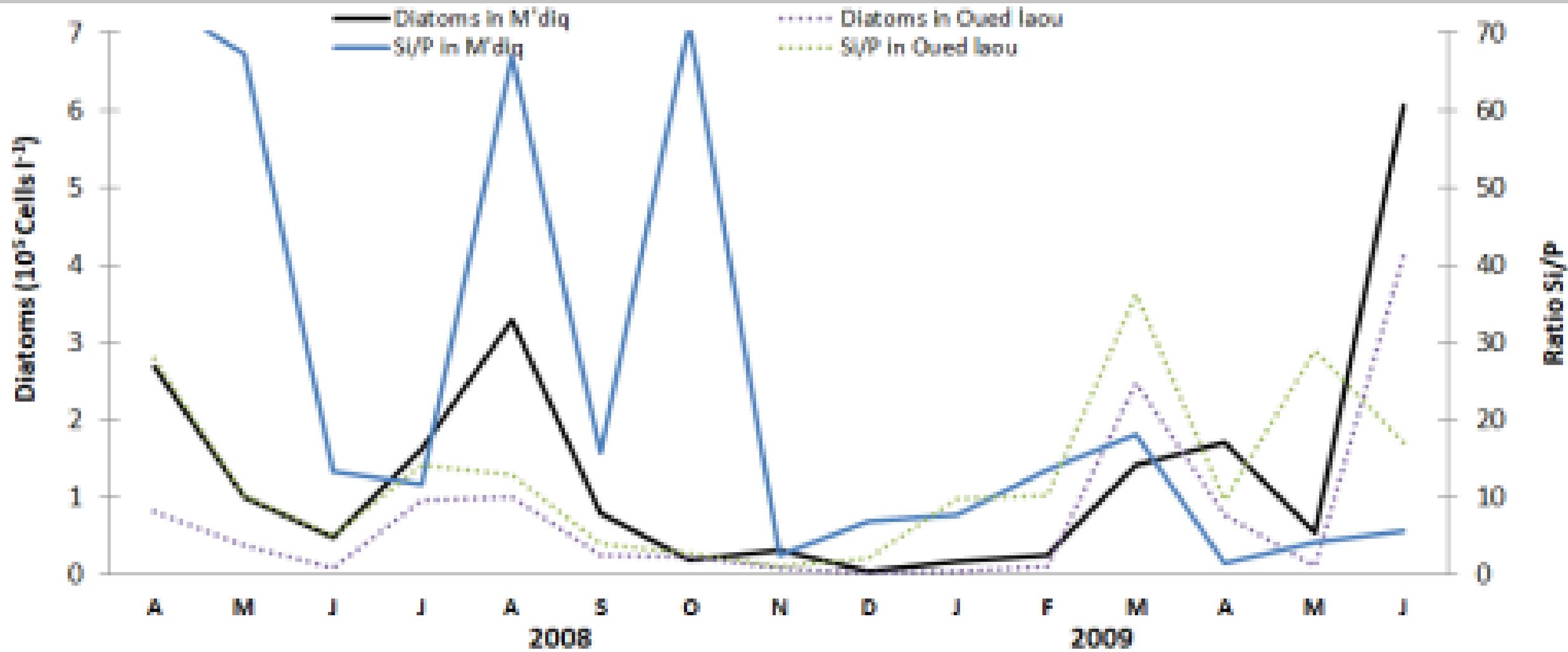
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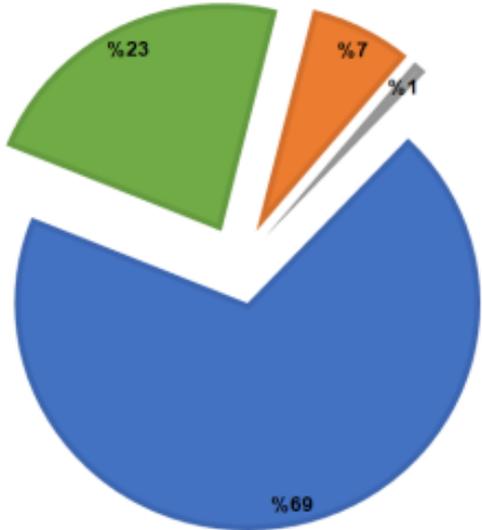
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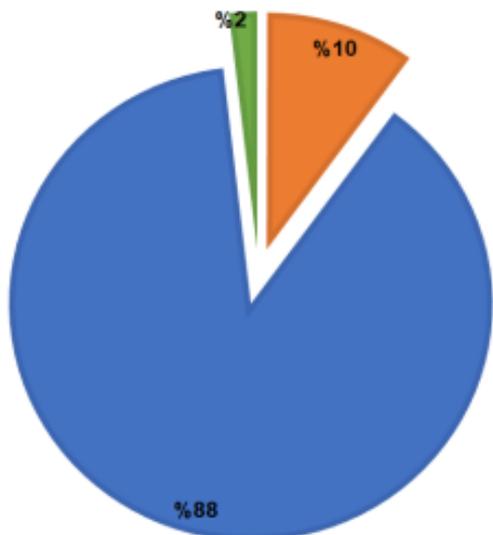






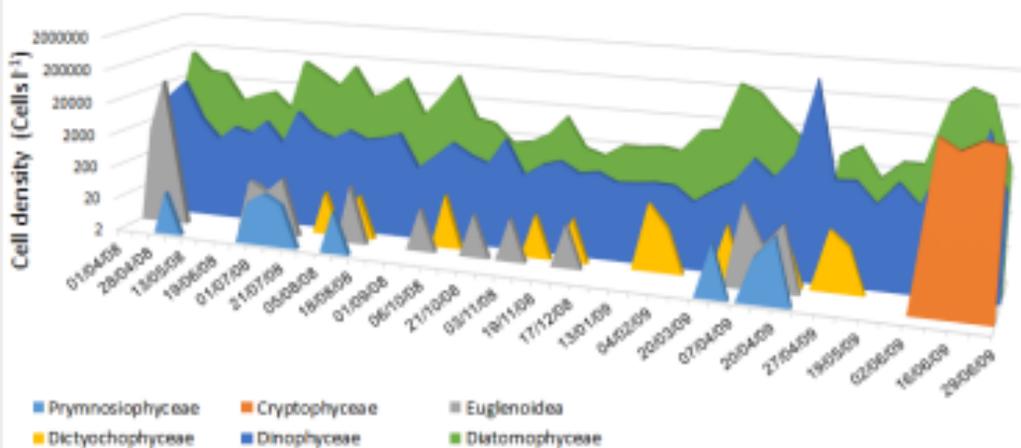
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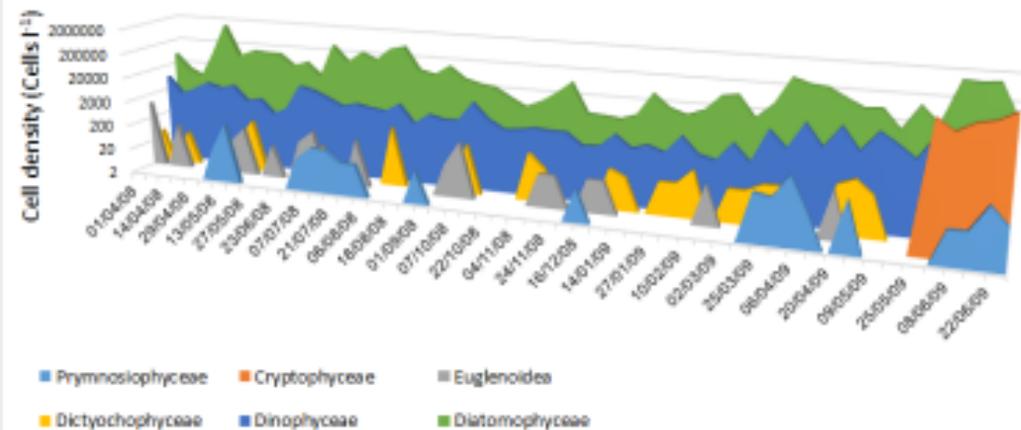
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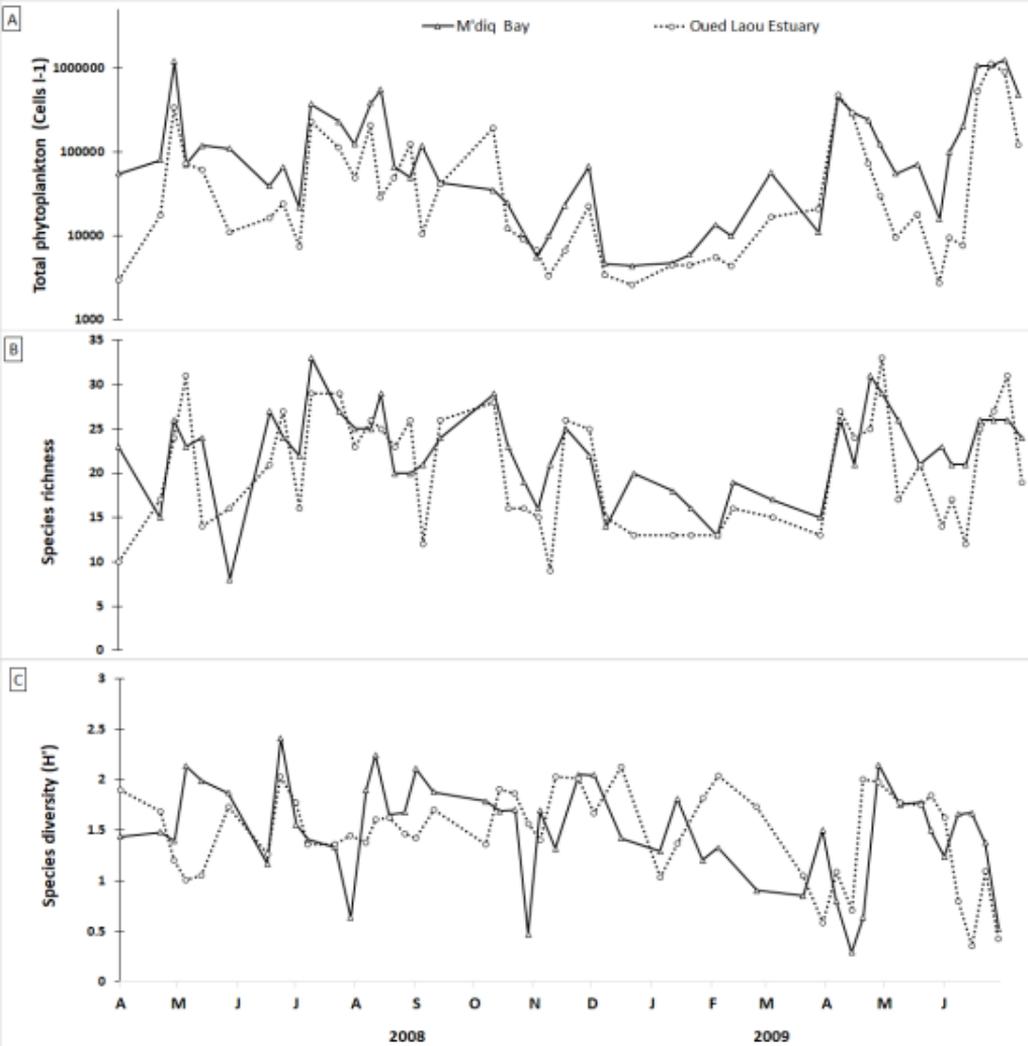
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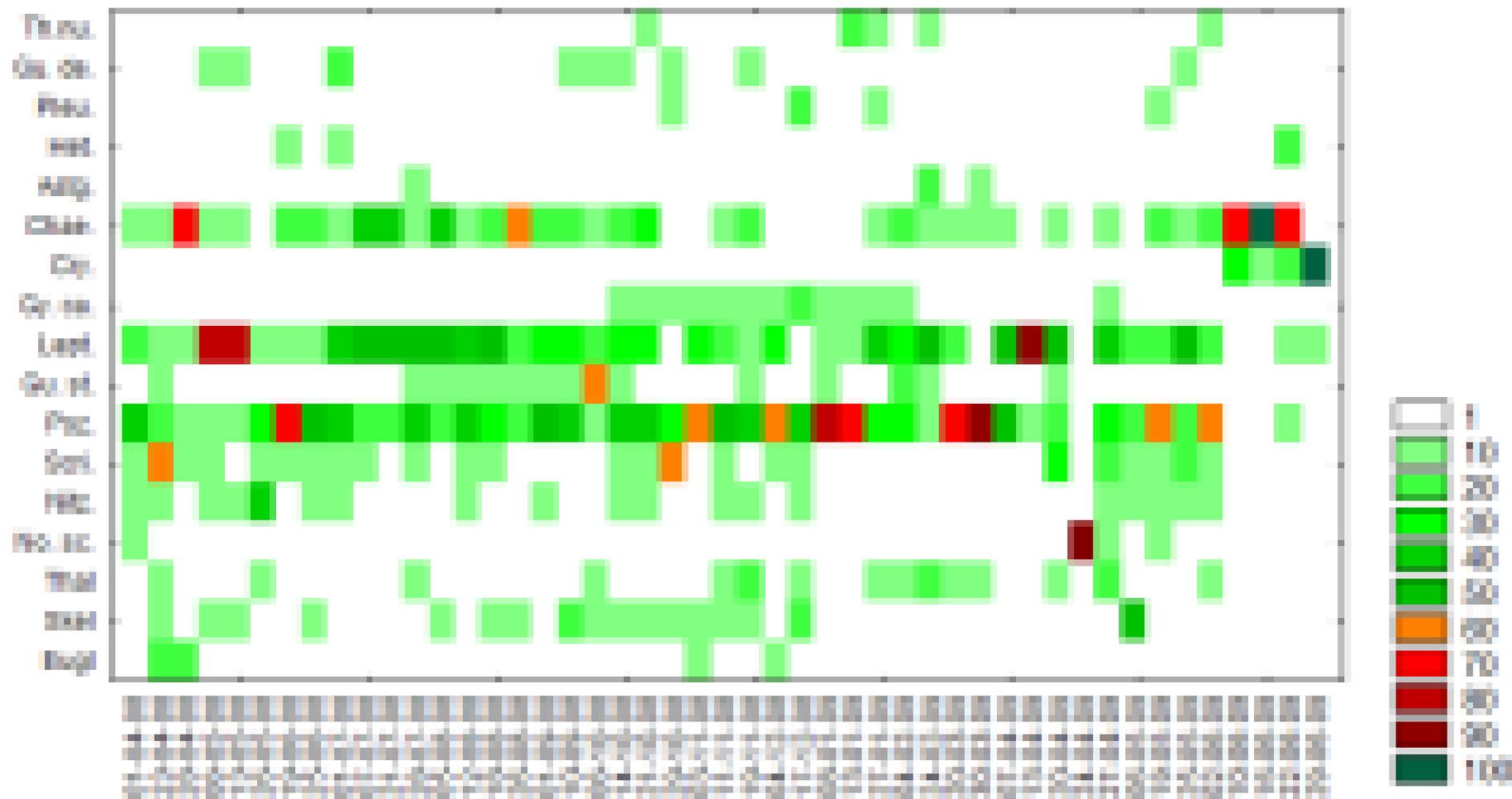
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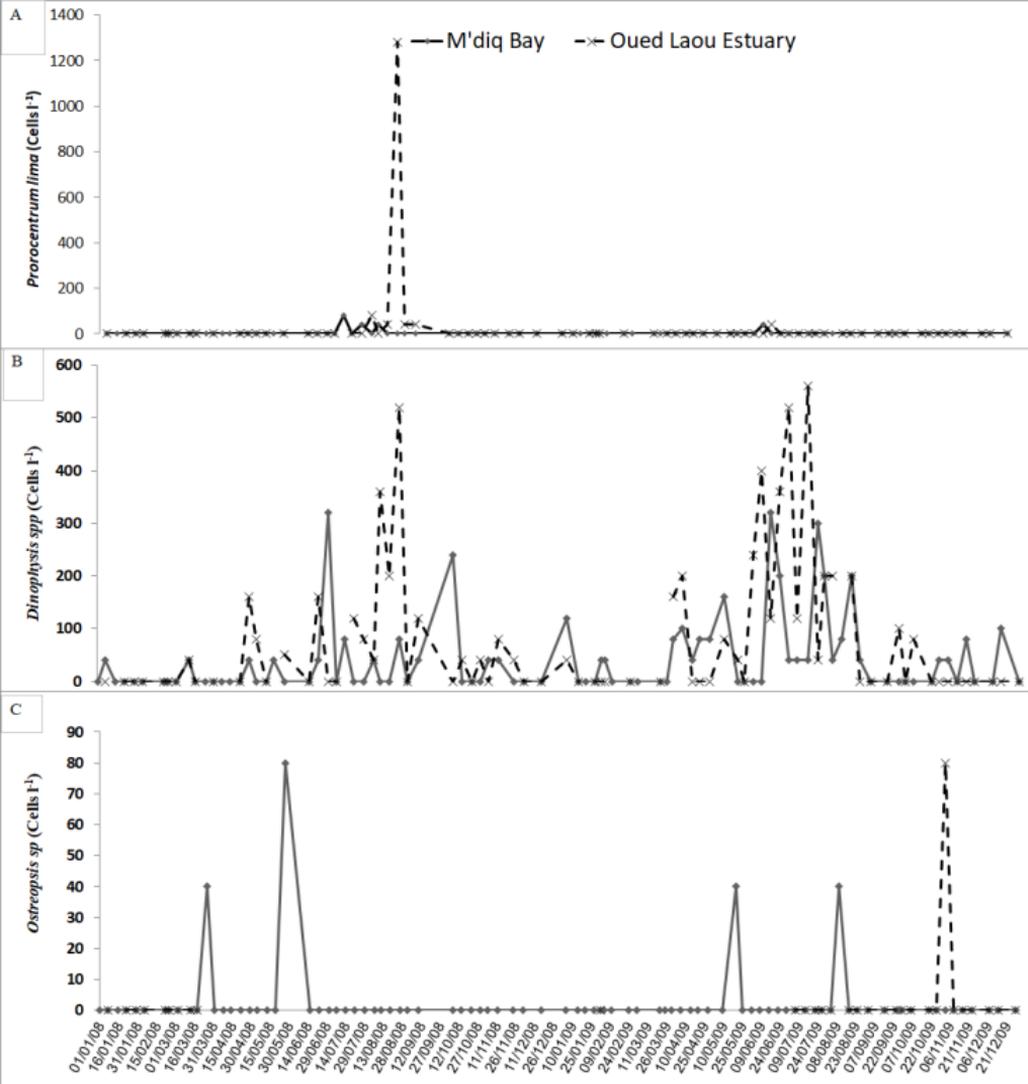


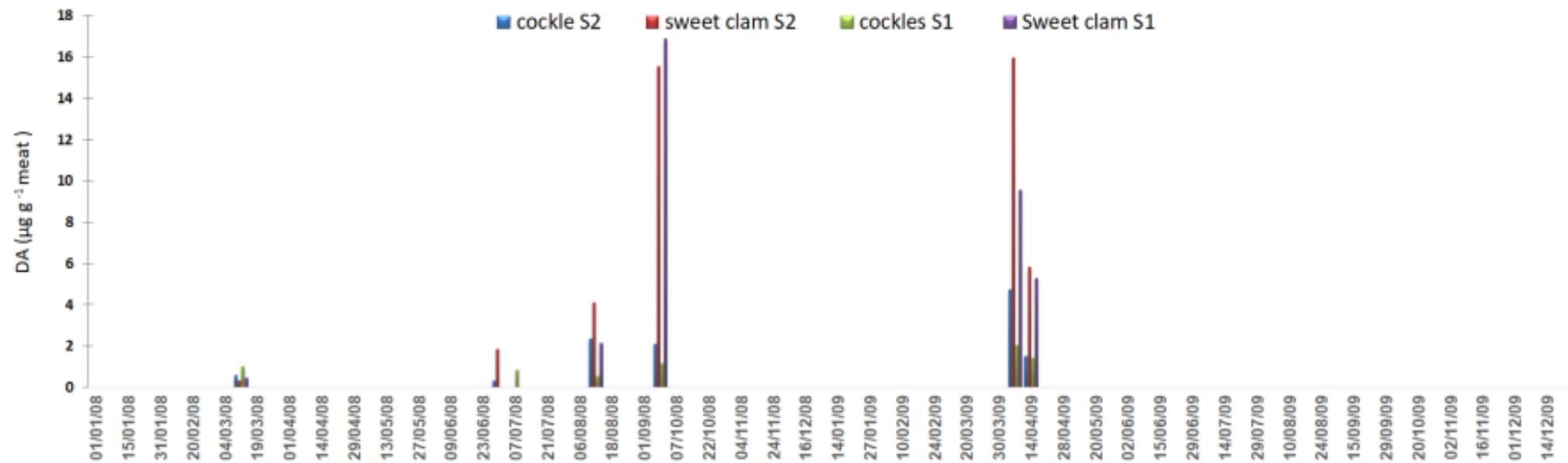
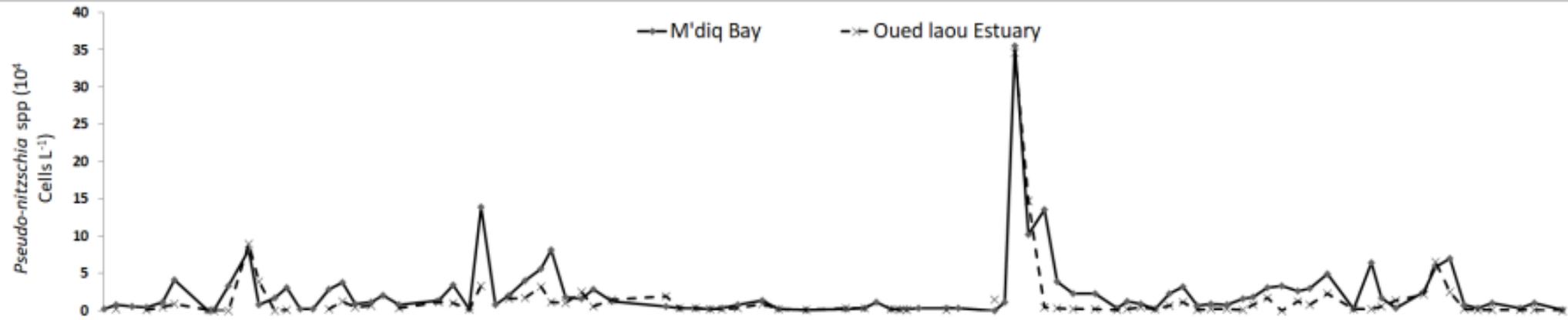
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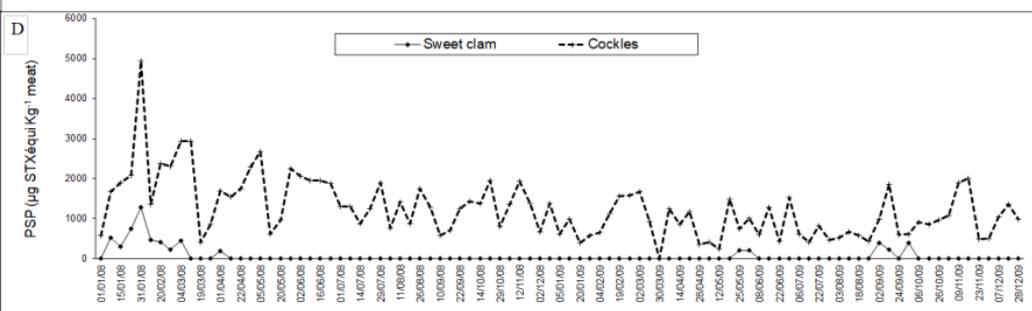
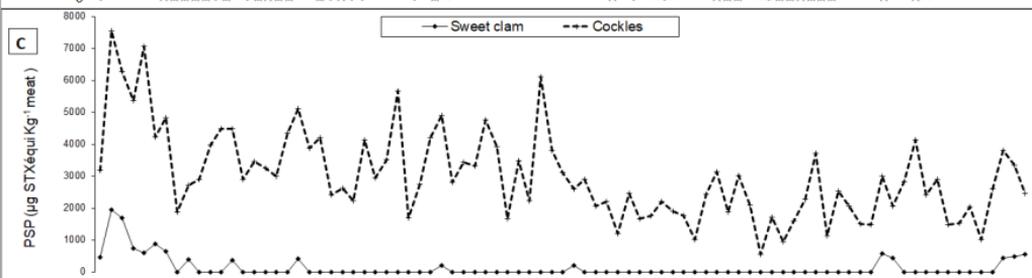
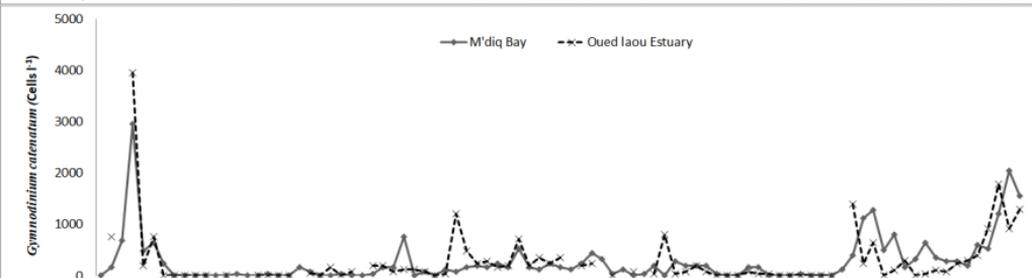
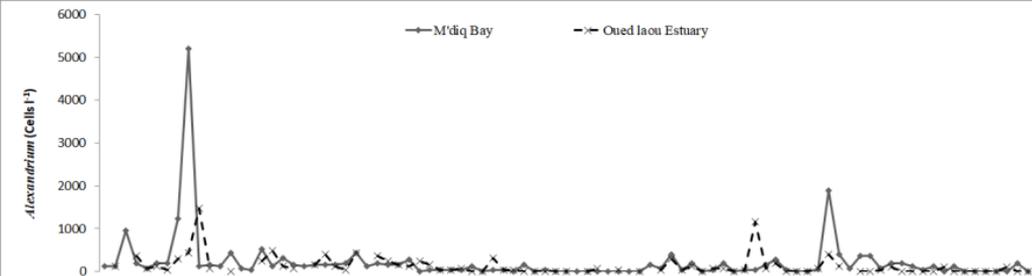


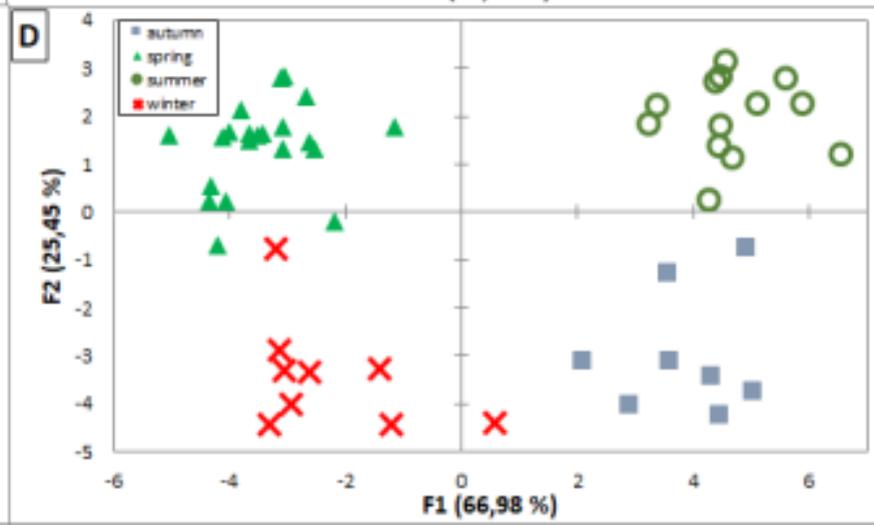
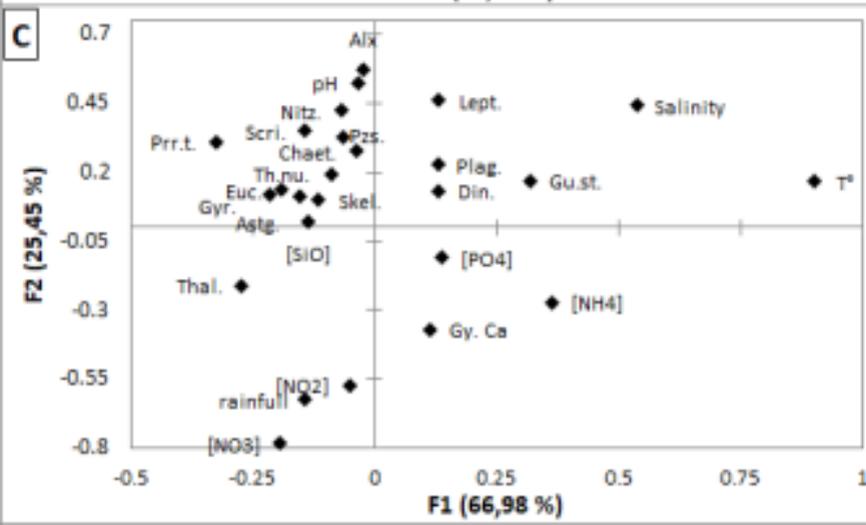
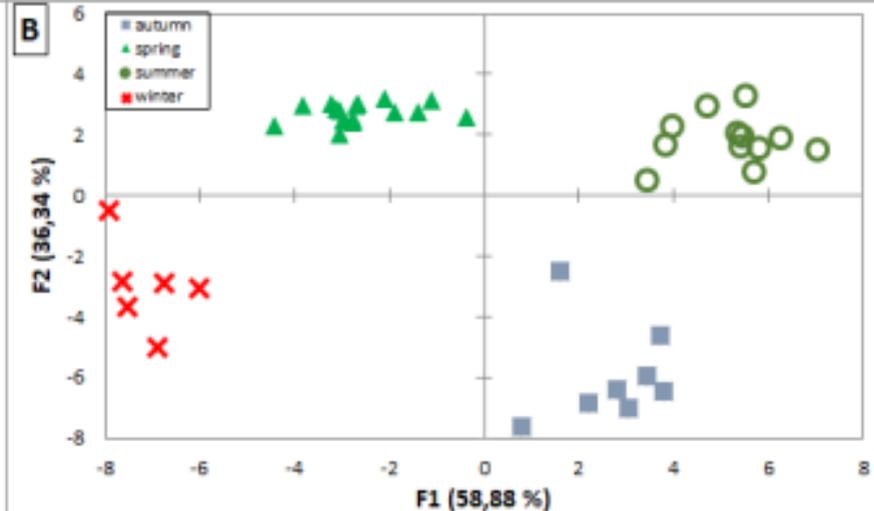
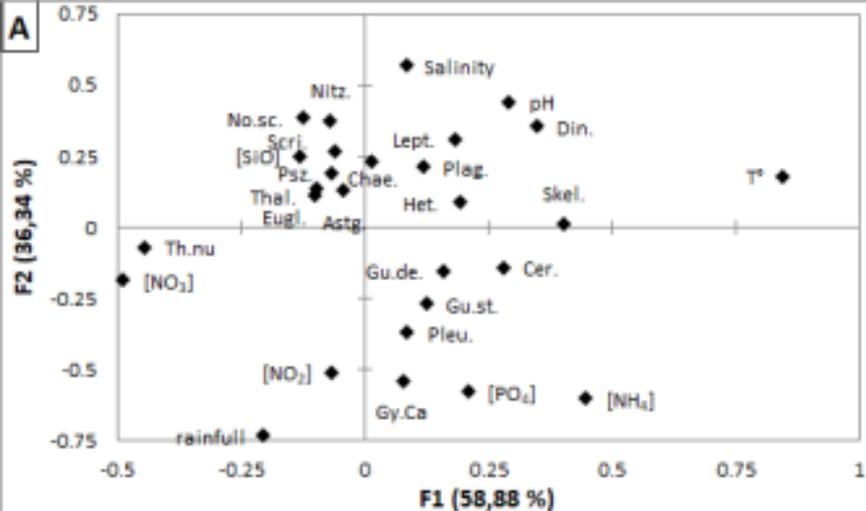












1 **Table 1.**

2

Station		T° (°C)	Salinity	pH	[NH ₄] (μM)	[NO ₂] (μM)	[NO ₃] (μM)	[PO ₄] (μM)	[SiO ₄] (μM)	Rainfall (mm/month)
Spring										
S1 (n=19)	Maen (SE)	16.85 (± 1.17)	35.18 (± 1.64)	8.06 (± 0.15)	2.77 (± 2.72)	0.03 (± 0.02)	1.89 (± 1.64)	0.16 (± 0.10)	2.44 (± 1.93)	5.33
	Min – Max	15.5 - 19.7	31 – 36.7	7.78 – 8.24	0.09 – 9.20	0 – 0.06	0.23 – 5.39	0.04 – 0.39	0.28 – 7.46	0 - 16
S2 (n=22)	Maen (SE)	16.46 (± 0.78)	35.73 (± 0.78)	8.12 (± 0.18)	3.75 (± 3.76)	0.03 (± 0.02)	0.72 (± 0.34)	0.30 (± 0.48)	4.1 (± 3.90)	47.58
	Min – Max	15.5 - 18.5	34.1 – 36.7	7.87 – 8.44	0.12 - 17.03	0.01 - 0.12	0.22 - 1.49	0 - 1.77	0.62 - 17.46	1 – 114.4
Summer										
S1 (n=13)	Maen (SE)	20.12 (± 1.68)	36.54 (± 0.09)	8.11 (± 0.13)	9.07 (± 9.85)	0.03 (± 0.03)	0.92 (± 0.82)	0.19 (± 0.06)	2.27 (± 1.39)	0
	Min -Max	17.5 – 22	36.5 – 36.8	7.81 – 8.21	0.86 – 29.97	0.01 – 0.12	0.23 – 3.27	0.04 - 0.27	0.56 – 5.08	
S2 (n=14)	Maen (SE)	19.50 (± 1.29)	36.51 (± 0.05)	8.14 (± 0.17)	6.01 (± 7.80)	0.04 (± 0.02)	0.32 (± 0.21)	0.32 (± 0.56)	2.39 (± 1.69)	3.16
	Min – Max	19.1 – 21.4	36.5 - 36.7	7.81 – 8.40	0.49 – 29.51	0.01 – 0.09	0.17 – 0.45	0.02 - 2.06	0.23 – 4.75	0 – 7.5
Autumn										
S1 (n=9)	Maen (SE)	18.39 (± 1.51)	33.83 (± 2.05)	7.96 (± 0.08)	24.53 (± 10.19)	0.07 (± 0.05)	1.60 (± 1.02)	0.94 (± 0.83)	1.24 (± 0.78)	148.5
	Min - Max	16 – 20.5	31 - 36.5	7.82 – 8.11	11.56 – 37.91	0.01 – 0.17	0.25 – 2.99	0.10 – 2.10	0.22 – 2.26	38 – 236.5
S2 (n=9)	Maen (SE)	19.43 (± 1.09)	36.06 (± 0.36)	7.93 (± 0.11)	17.74 (± 14.63)	0.05 (± 0.02)	1.38 (± 0.87)	0.64 (± 0.65)	4.12 (± 2.05)	258.83
	Min – Max	17.8 - 21.4	35.6 - 36.5	7.78 – 8.04	3.74 - 43.13	0.03 – 0.09	0.37 – 2.59	0.04 – 1.54	1.41 – 7.32	160.5 – 277.5
Winter										
S1 (n=6)	Maen (SE)	14.32 (± 0.67)	35.38 (± 0.83)	7.89 (± 0.08)	3.32 (± 4.27)	0.07 (± 0.05)	3.72 (± 2.37)	0.19 (± 0.05)	2.44 (± 1.29)	83.8
	Min - Max	13.5 – 15.2	33.90 – 36.30	7.80 – 8.01	0.22 – 11.68	0.02 – 0.13	1.02 – 7.52	0.12 – 0.23	1.13 – 4.75	24 – 84.9
S2 (n=9)	Maen (SE)	16.07 (± 0.52)	35.06 (± 0.35)	7.97 (± 0.14)	3.19 (± 4.02)	0.09 (± 0.05)	1.65 (± 0.43)	0.25 (± 0.22)	2.49 (± 1.61)	127.76
	Min – Max	15.5 – 17.2	34.6 – 35.7	7.78 – 8.17	0.22 – 13.26	0.05 – 0.19	1.05 – 2.38	0.10 – 0.82	1.30 – 6.50	48 – 135.8

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7**Table 2. :**

Variables	Site Number	Rainfall	T (°C)	Salinity	pH	[PO ₄]	[NO ₂]	[NH ₄]	[NO ₃]	DIN	[SiO ₄]
Species Richness	S1	-0.231	0.296	0.388	0.420	-0.042	-0.217	-0.095	-0.333	-0.094	-0.056
	S2	-0.713	0.339	0.497	0.353	0.049	-0.392	0.125	-0.435	-0.040	0.036
Species Diversity (H')	S1	0.143	-0.014	-0.304	-0.242	0.189	-0.056	0.098	0.184	0.173	0.040
	S2	-0.154	0.325	0.298	-0.193	-0.078	-0.092	0.142	-0.145	0.001	0.055
Prymnsiophyceae	S1	-0.031	0.080	0.170	0.327	0.001	-0.176	-0.126	-0.292	-0.138	0.005
	S2	-0.246	0.005	0.324	0.448	0.088	-0.074	-0.023	-0.274	-0.039	-0.142
Cryptophyceae	S1	-0.277	-0.032	0.186	0.420	0.074	0.042	-0.035	0.018	0.000	0.276
	S2	-0.218	-0.107	0.378	0.371	-0.067	-0.228	-0.115	-0.018	-0.115	-0.029
Euglenophyceae	S1	-0.011	0.059	0.095	0.149	-0.079	-0.176	0.116	-0.313	0.101	-0.173
	S2	-0.084	0.079	0.138	-0.269	-0.138	0.002	0.184	-0.122	0.237	0.205
Dictyochophyceae	S1	0.137	-0.147	-0.107	0.034	0.101	0.229	-0.027	0.035	0.099	-0.130
	S2	0.032	-0.224	-0.291	-0.203	0.177	0.334	0.034	0.298	0.095	0.154
Dinophyceae	S1	-0.378	0.181	0.170	0.386	-0.092	-0.047	-0.025	-0.196	-0.177	0.050
	S2	-0.573	-0.109	0.127	0.433	0.067	-0.388	-0.114	-0.237	-0.228	-0.068
Diatomophyceae	S1	-0.310	0.254	0.544	0.349	-0.263	-0.244	-0.194	-0.261	-0.130	-0.070
	S2	-0.713	0.101	0.362	0.400	-0.062	-0.377	-0.102	-0.445	-0.162	-0.164
Raphidophyceae	S1	-0.134	0.120	0.124	0.005	-0.224	-0.181	-0.250	-0.141	-0.196	-0.217
	S2	*	*	*	*	*	*	*	*	*	*
Chlorophyceae	S1	-0.065	0.174	0.177	0.079	0.273	-0.156	-0.147	-0.068	-0.155	0.152
	S2	*	*	*	*	*	*	*	*	*	*
Total phytoplankton	S1	-0.402	0.196	0.448	0.432	-0.237	-0.068	-0.120	-0.243	-0.244	-0.040
	S2	-0.692	0.064	0.397	0.469	-0.080	-0.455	-0.126	-0.409	-0.181	-0.179

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9 **Table 3.**

Variable	Gy.Ca	Din.	Het.	Cer.	No.sc.	Scri.	Chae.	Psz.	Lept.	Nitz.	Gu.de	Gu.st.	Skel.	Th.nu	Astg.	Thal.	Plag.	Eugl.	Pleu.
[SiO ₄]	-0.21	0.06	0.26	-0.09	0.12	0.06	-0.05	-0.21	0.00	0.01	-0.00	-0.13	-0.18	-0.12	0.23	0.00	0.32*	-0.13	-0.12
[PO ₄]	0.20	-0.02	0.03	0.18	-0.08	-0.23	-0.20	-0.27	-0.19	-0.31*	0.10	0.06	0.12	-0.26	-0.08	-0.09	0.01	-0.11	0.03
[NH ₄]	0.32*	0.00	-0.18	0.29*	-0.26	-0.09	-0.14	-0.05	-0.18	-0.17	0.20	0.06	0.36*	-0.37*	-0.19	-0.21	-0.09	0.08	-0.13
[NO ₂]	0.26	-0.15	-0.34*	0.01	-0.16	-0.32*	-0.25	-0.19	-0.21	-0.42*	-0.09	0.02	0.10	-0.19	0.05	0.03	0.02	-0.21	0.18
[NO ₃]	0.00	-0.24	-0.17	-0.15	-0.02	-0.26	-0.38*	-0.43*	-0.32*	-0.31*	-0.32*	-0.13	-0.15	-0.16	-0.06	-0.05	0.00	-0.27	0.06
T°	-0.03	0.25	0.29*	0.13	-0.06	0.19	0.36*	0.41*	0.32*	0.23	0.55*	0.40*	0.42*	-0.37*	0.16	-0.21	-0.03	0.05	-0.28
Salinity	-0.28	0.32*	0.27	0.01	-0.00	0.31*	0.56*	0.52*	0.52*	0.43*	0.46*	0.31*	0.25	-0.06	0.33*	0.08	0.18	0.09	-0.26
pH	-0.08	0.37*	0.30*	-0.03	0.04	0.30*	0.36*	0.15	0.27	0.13	0.14	-0.05	-0.05	0.12	0.36*	0.09	0.42*	0.14	-0.06
Rainfull	0.46*	-0.32*	-0.47*	0.15	-0.20	-0.32*	-0.37*	-0.19	-0.33*	-0.43*	-0.36*	-0.10	-0.12	0.17	-0.18	0.16	-0.27	-0.01	0.30*

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11 **Table 4**

Variable	Alex	Gy. Ca	Din.	Gyr.	Prr.t.	Scri.	Chaet.	Pzs.	Lept.	Euc.	Nitz.	Gu.st.	Skel.	Th.nu.	Astg.	Plag.	Thal.
T°	0.18	-0.03	-0.00	-0.26	-0.14	-0.13	0.34*	0.16	0.11	-0.17	-0.05	0.43*	0.42*	-0.14	-0.18	-0.10	-0.22
Salinity	0.27*	-0.14	0.20	-0.07	0.04	0.07	0.53*	0.26	0.25	-0.09	0.18	0.03	0.23	0.11	-0.05	0.37*	-0.31*
pH	0.28*	-0.13	0.29*	0.50*	0.35*	0.30*	0.39*	0.34*	0.27*	0.27*	0.14	-0.39*	-0.16	0.19	0.03	0.37*	0.01
[NO ₂]	-0.46*	0.34*	-0.22	-0.03	-0.44*	-0.35*	-0.39*	-0.38*	-0.29*	-0.10	-0.28*	0.00	-0.09	0.04	-0.10	-0.23	0.04
[PO ₄]	-0.07	0.21	0.33*	0.27*	-0.03	-0.04	-0.05	-0.04	0.02	0.37*	-0.09	-0.26	-0.10	0.30*	-0.23	-0.07	0.10
[NH ₄]	0.17	0.23	0.01	-0.16	-0.15	-0.13	0.00	-0.01	-0.06	-0.06	-0.1	-0.00	0.31*	0.13	-0.34*	-0.13	-0.11
[NO ₃]	-0.42*	0.39*	-0.16	0.08	-0.25	-0.27*	-0.56*	-0.44*	-0.44*	-0.01	-0.18	-0.22	-0.27*	0.03	-0.06	-0.03	0.13
[SiO ₄]	0.04	-0.01	-0.09	-0.24	0.07	-0.07	-0.17	-0.25	-0.21	-0.24	-0.08	-0.14	0.21	-0.05	-0.01	-0.03	-0.13
Rainfull	-0.27*	0.28*	-0.15	0.14	-0.12	-0.20	-0.50*	-0.31*	-0.40*	0.11	-0.16	-0.35*	-0.14	0.22	-0.06	-0.10	0.098

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