Seasonal variations of phytoplankton assemblages in relation to environmental factors in Mediterranean coastal waters of Morocco, a focus on HABs species

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ABSTRACT

Studies on phytoplankton and in particular Harmful Algal Blooms (HABs) species in southern Mediterranean waters are scarce. We performed from April 2008 to June 2009 weekly investigations on microphytoplankton community structure and abundance in two contrasted marine ecosystems located in the western Moroccan Mediterranean coast, M'diq Bay and Oued Laou Estuary. Simultaneously, we measured the main physico-chemical parameters. Globally, the two studied areas showed comparable values of the assessed abiotic environmental factors. Temperature and salinity followed seasonal variation with values ranging from 13.5 °C to 21.4 °C and 31 to 36.8, respectively. Average nutrient values in surface water ranged from 0.7 to 45.76 µM for dissolved inorganic nitrogen, 0.02 to 2.10 µM for PO_4 and 0.23 to 17.46 µM for SiO_4 in the study areas. A total of 92 taxa belonging to 8 taxonomic classes were found. The highest number of microphytoplankton abundance reached 1.2 x 10^6 cells L^{-1} with diatoms being the most abundant taxa. Factorial Discriminant Analysis (FDA) and Spearman correlation test showed a significant seasonal discrimination of dominant microphytoplankton species. These micro-organisms were associated with different environmental characteristics, in particular temperature and salinity. Numerous HABs species were encountered regularly along the year. Although *Dinophysis* species and *Prorocentrum lima* were present in both sites, no Lipophilic Shellfish Poisoning was detected in the analyzed bivalve mollusks. Domoic acid (DA), produced by toxic species of *Pseudo-nitzschia* was found with concentrations up to 18 µg DA g^{-1} in the sweet clam *Callista chione*. Data showed that the observed persistent and dramatic paralytic shellfish poisoning (PSP) intoxication of mollusks resulted probably of *Gymnodinium catenatum* proliferations in both studied areas. Contrary to sweet clam *C. chione*, the cockle *Achanthocardia tuberculatum* showed a permanent and extremely high toxicity level during the 15 months survey with up to 7545 µg Equivalent Saxitoxin kg^{-1} flesh (ten times higher than the sanitary threshold of 800µg eqSTX Kg^{-1}flesh). The present work highlights for the first time the dynamic of microphytoplankton including HABs species and their associated toxin accumulation

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

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

1. Introduction

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

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

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

The purpose of this study is to investigate the taxonomic composition and abundance of the microphytoplankton community in two contrasted areas (M'diq Bay and Oued Laou Estuary) of the western Mediterranean waters with a focus on HABs species. We also aim to analyze the effect of the main abiotic environmental factors on microphytoplankton succession and in particular on the potentially toxic microalgae. Another objective was to determine to which extent the observed dramatic PSTs intoxications of the cockles and the clams are related to the presence of paralytic toxin producing species in the water column of the studied ecosystems.

2. Material and Methods

2.1. Study sites

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

2.2. Phytoplankton and physico-chemical parameters

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

Sea water sample was stored at -20 °C until chemical analyses. Inorganic nutrient (ammonium NH_4, nitrite NO_2, nitrate NO_3, phosphate PO_4 and silicate SiO_4) concentrations were measured using the protocol of Aminot and Chaussepied (1983) by spectrophotometer (Unico SQ4802 UV/VIS double Beam spectrophotometer model). Temperature, practical salinity and pH were measured *in situ* at each station during sampling period with a multi-parameter probe (WTW) 197i. Practical salinity and pH calibrations were performed monthly (see the protocol in www.geotechenv.com / Manuals / WTW_Manuals / Multi_197i).

2.3. Domoic acid analyses

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

2.4. Paralytic shellfish toxicity analyses using bioassay

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

2.5. Lipophilic shellfish poisoning analysis

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

2.6. Statistics

SStatistical analyses were performed using the software XLSTAT 2011. We used a Spearmen correlation and Factorial Discriminant Analysis (FDA) to highlight if there is any seasonal dissimilarities and describe the main patterns of temporal variability of microphytoplakton taxa 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 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 µM. The highest mean values were observed in autumn with 24.53±10.19 µM at S1 and 17.74±14.63 µM at S2. The lowest mean values were observed in spring with 0.09 µM at S1 and 0.12 µM at S2. Nitrite concentrations at S1 and S2 station were relatively low. They ranged between 0 (not detected) to 0.19 µM. The highest values were recorded in autumn and winter with values up to 0.17 µM at S1 and 0.19 µM at S2. Nitrate ranged between 0.17 to 7.52 µM with an average of 1.79± 1.66 µM at S1 and 0.89± 0.66 µM at S2. At S1, the highest values were recorded at the end of May 2008 (5.39 µM), mid-August 2008 (3.27 µM) and during the period of November 2008 - early March 2009 (7.51 µM) end of April 2009 (4.63 µM) and early June 2009 (4.02 µM). During the period of October 2008 - March 2009, we observed an increase in the concentration of nitrate at S1. In early October 2008, nitrate concentration did not exceed 1 µM. Thereafter, it increased gradually to reach the highest registered values (7.51 µM in March 2009). After, there was a drop at the end of March 2009. At S2, the highest levels were recorded in autumn and winter period. The maximum values, 2.59 and 2.37 µM, were recorded in November 2008 and the second week of February 2009, respectively, with decreasing trend in spring (0.72± 0.34 µM) and summer periods (0.32±0.21 µM).

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

Phosphate concentrations varied between 0 and 2.10 µM. Generally, phosphate concentrations at S2 were slightly higher than those recorded at S1. The highest values were recorded during autumn, with 0.94± 0.83 µM at S1 and 0.64± 0.65 µM at S2. Low values were measured during spring for S1 0.16 ± 0.10 µM and winter for S2 0.25± 0.22 µM. The monthly value of the Si/P ratio showed large temporal fluctuations (Fig. 4). The highest values (up to 20) of Si/P ratio were recorded at the M'diq bay during 2008 sampling period and thereafter this ratio remained relatively low. Figure 4 showed the correspondence of the peaks of Si/P ratio and the abundances of diatoms.

3.3. Phytoplankton structure

The phytoplankton community composition and abundance in S1 and S2 showed the presence of different classes: Bacillariophyceae, Dinophyceae, Euglenoidea, Dictyochophyceae, Chlorophyceae, Cyanophyceae, Cryptophyceae and Prymnesiophyceae. However, most recorded species belong to two major classes, Bacillariophyceae and Dinophyceae (Fig. 5 and Fig. 6). The total abundance of phytoplankton (cells L^{-1}) showed important seasonal variations (Fig 7.). The highest values were observed in spring (12.10^5 cells L^{-1}) and summer (12.10^5 cells L^{-1}). In contrast, low abundances were observed in autumn (19.10^4 cells L^{-1}) and winter (71.10^3 cells L^{-1}). The average phytoplankton cell abundance recorded in S2 ($18.10^4 \pm 20.10^4$ cells L^{-1}) was slightly higher than that recorded in S1 ($14.10^4 \pm 17.10^4$ cells L^{-1}). Bacillariophyceae was the dominant group followed by Dinophycea in both sites (Fig 5, 6). At Oued Laou Estuary (Fig.8), the dominant taxa were: *Heterocapsa* spp.*, Skeletonema* spp.*, Thalassiosira* spp.*, Noctiluca scintillans, Nitzschia* spp.*, Scrippsiella* sp.*, Pseudo-nitzschia* spp.*, Guinardia striata, Leptocylindrus* spp. *Gymnodinium catenatum, Euglena* sp.*, Plagioselmis* sp.*, Chaetoceros* spp. and *Asterionella glacialis.* Except *Pseudo-nitzschia* spp., *Leptocylindrus* spp. and *Chaetoceros* spp. which showed an average relative densities of 29.5%, 24% and 14.5% respectively, the other taxa were poorly represented in this ecosystem; At M'diq Bay (Fig.9), the dominant taxa were: *Thalassiosira* spp.*, Thalassionema nitzschioides*, *Prorocentrum triestinum, Nitzschia* spp.*, Gyrodinium* spp.*, Skeletonema* spp.*, Scrippsiella* sp.*, Leptocylindrus* spp.*, Pseudo-nitzschia* spp.*, Plagioselmis* sp., *Chaetoceros* spp.*,* and *Asterionella glacialis.* The relative dominance values of these taxa varied from 0% to 90%. The average of the relative densities of *Leptocylindrus* spp.*,* *Pseudo-nitzschia* spp. and *Chaetoceros* spp. were 24%, 23.5% and 20% respectively. These taxa dominated the microphytoplankton community.

3.4. Harmful Algal Blooms species and mollusks intoxications

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

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

Alexandrium spp. registered the highest densities in M'diq Bay with up to 5200 cells L^{-1} in March 3th 2008. This genus was less present at Oued Laou Estuary with concentrations not exceeding 1480 cells L^{-1} (March 13th 2008). *G. catenatum* which has been related to PSP syndrome showed almost similar distributions in M'diq Bay (up to 2960 cells L^{1}) and Oued Laou Estuary (maximum of 3960 cells l^{-1} in January 22st 2008 (Fig. 12). December 2009 registered also an important development of *G. catenatum* with up to 2040 cells L^{-1} in M'diq Bay. Permanent and high levels of PSTs contamination were observed in Oued Laou Estuary in the cockles (up to 7545 µg STX equiv Kg meat $^{-1}$, a value almost 10 times higher than sanitary threshold). Cockles from M'diq Bay were also permanently contaminated but with concentration up to 3720 µg STX equiv Kg meat $^{-1}$ (registered in January 31th 2008). The sweet clams were less contaminated by PST in both sites with concentrations ranging from 0 to 1945 µg STX equiv Kg meat $^{-1}$and from 0 to 1230 µg STX equiv Kg meat $^{-1}$,in Oued Laou and M'diq Bay, respectively.

3.5. Correlation between phytoplankton and environmental factors

Statistical analyses were applied to highlight any correlation between environmental parameters and species richness and species diversity of phytoplankton in the studied areas (Table 2). Globally, M'diq Bay and Oued Laou Estuary showed the same trend in their correlations with environmental factors (Table 2). Temperature and salinity were correlated positively to species diversity and species richness. This later, was negatively correlated with nitrate (Table 2). Only salinity and pH showed positive correlation with total phytoplankton (Table 2). Three microphytoplankton groups showed a positive correlation with salinity including Prymnosiophyceae, Cryptophyceae and Dictyophycea and Diatomophyceae (Table 2). Except Raphidophycea and Chlorphycea, all the other the phytoplankton groups were positively correlated with pH (Table 2). DIN, NH_4 and SiO_4 were not correlated with any of the examined phytoplankton variables. Nitrate was negatively correlated with Diatomophyceae, Prymnosiophyceae and Euglenoidea, (Table 2). Dictyophyceae was positively correlated with nitrate and nitrite. Dinophyceae and Diatomophyceae were negatively correlated with nitrite.

Factorial analyses related to the season indicated that 92.43% (S1) and 95.22% (S2) of the variance was explained by two axes (F1 and F2) (Fig. 13A and Fig. 13C). In relation to the seasons, the variables showed four groups. For both stations, the temporal variability followed a seasonal cycle with a seasonal transition (Fig. 13B and Fig. 13D). Data showed positive correlations between environmental factors and microphytoplankton species on a seasonal basis. For station 1, the correlations were as follows : in winter (NO_2, NO_3 and *Thalassionema* *nitzschioides*), in spring (SiO_4 and *Nitzschia* spp., *Noctiluca scintillans, Scrippsiella* sp., *Pseudo-nitzschia* spp., *Thalassiosira* spp., *Euglena* sp., and *Asterionella glacialis*), in summer (pH, temperature, salinity and *Dinophysis* spp., *Leptocylindrus* spp., *Chaetoceros* spp., *Heterocapsa* spp. and *Skeletonema* spp.) and in autumn (PO_4, NH_4 and *Guinardia delicatula, Guinardia striata, Ceratium* spp., *Pleurosigma* spp. and *Gymnodinium catenatum*). For station S2, the obtained positive correlations were in winter (NO_2, NO_3 and *Thalassiosira* spp.), in spring (SiO_4, pH, *Alexandrium* spp., *Nitzschia* spp., *Scrippsiella* sp., *Prorocentrum triestinum, Pseudo-nitzschia* spp., *Chaetoceros* spp., *Thalassionema nitzschioides, Skeletonema* spp., *Eucampia zodiacus, Gyrodinium* spp., and *Asterionella glacialis*), in summer (Temperature, Salinity, *Leptocylindrus* spp., *Plagioselmis* sp., *Dinophysis* spp. and *Guinardia striata*) and in autumn (PO_4, NH_4 and *Gymnodinium catenatum).* Factorial analyses according to the season indicated that the discrimination was dominated by temperature and salinity during summer. Winter was characterized by high levels of nitrate and nitrite. During summer, in both stations a gradual decrease of nitrate was observed and coincided with an increase in water temperature and salinity. Autumn was characterized by high values of ammonium and phosphate whereas winter showed high values of nitrite and nitrate which could be explained by nitrification processes during winter season. The highest phytoplankton biomass and diversity were recorded in spring and summer. Spearman analyses were applied to highlight the correlation between the major phytoplankton species and the environmental factors, this is shown in Tables 3 and 4.

4. Discussion

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

The inventory of phytoplankton species in Oued Laou Estuary and M'diq Bay showed the dominance of two groups: Bacillariophycae and Dinophyceae. This finding is in agreement with previous studies showing that these groups are the main phytoplankton components in the Mediterranean (Vilicic et al. 2002, El Madani 2011, Armi et al., 2010, Daoudi et al., 2012; Salhi et al., 2018; Draredja et al., 2019). In our study, in term of cell abundance, the diatoms dominated largely with *Pseudo-nitzschia, Leptocylindrus* and *Chaetoceros* being the major taxa. Species belonging to Bacillariophyceae showed a high diversity in spring whereas dinoflagellates were more diversified during summer season. This corresponds to previous works on the phytoplankton in the Nador lagoon located in the South Western Mediterranean, Morocco (El Madani et al., 2011; Daoudi et al., 2012). According to Smayda (1984), this seasonal succession is typical of temperate ecosystems. The phytoplankton communities in Oued Laou Estuary and M’diq Bay showed a similar seasonal trend. The phytoplankton mean cell abundance was generally slightly higher in M’diq Bay ($18x10^4$ cells l^{-1}) when compared to that of Oued Laou ($14x10^4$ cells l^{-1}). This could be the consequence of the relatively low levels of silicate present in Oued Laou Estuary required by diatoms for their growth. The dominance of diatoms in both ecosystems indicates that the Western Moroccan Mediterranean coast is a water mixing area. Margalef *et al.*, (1979) and Gailhard (2003) suggested that the vertical mixing of water column favors the development of diatoms. Hydrological factors such as mixing with nutrient-rich freshwater during rainfall periods are known to impact the development of phytoplankton in a given ecosystem. Physicochemical parameters of water masses together with climatic conditions particularly air temperature fluctuate seasonally in the Mediterranean temperate ecosystems (Dhib et al., 2013; Laanaia et al., 2013; Salhi et al., 2018; Trombetta et al. 2019). Also, the seasonal variability of phytoplankton communities structure and environmental parameters have been demonstrated at weekly and biweekly time scales in various coastal and estuarine marine waters (Jouenne et al., 2007; Lopes et al., 2007; Armi et al., 2010). In our study, temperature seems to be the most important factor which affect the diversity and richness of phytoplankton species in the two investigated areas. The highest phytoplankton abundance was observed in spring and summer. Temperature is correlated to species richness and diversity (table 2). This is in accordance with the recent work of Trombetta et al., (2019) suggesting that water temperature drives phytoplankton blooms in coastal waters. Phytoplankton diversity and dynamic could be impacted by the observed increase in temperature in the Mediterranean (Shen et al., 2016; Wells et al., 2015; 2019; Kim et al., 2019). To verify this hypothesis, particularly for HABs species, a monitoring of phytoplankton diversity and dynamics is under way in Oued Laou estuary and Mdiq bay with the aim of comparing these data with those acquired 10 years ago. Diatoms were significantly associated with DIN concentrations in winter and with silicates in autumn. The decreasing concentrations of DIN and NO_3 measured in spring and summer could be associated to the observed increase in dinoflagellates.

In our study, the highest phytoplankton densities were recorded in spring and summer corresponding to the increase of temperature and to high nutrient concentrations which were registered in winter. Less important phytoplankton biomass occurred in winter and coincided with rainfall but with low temperature and light intensity levels. The spring and summer seem to be the most favorable periods for phytoplankton development and diversity. In winter, heavy rainfalls allowed nutrients enrichment both ecosystems, particularly ammonium. This was shown in another Mediterranean ecosystem (Alexandria, Egypt) (Abdel-Halim and Khairy 2007). Ammonium turns into nitrite then into nitrate due to bacterial nitrification. Boutaib et al., (2011) showed an increase in bacterial activity during the wet season (autumn and winter) in Moroccan Western Mediterranean coasts. The decrease in nutrient levels was observed during spring and summer. This reduction was probably related to microalgae development during spring. A significant negative correlation between nutrients and microalgae was observed (Tables 2-4). In Oued Laou Estuary, mineral phosphorus concentration is low and very close to the values provided by Redfield et al. (1963) for Mediterranean waters. In contrast, the phosphorus concentrations in M'diq Bay were relatively important probably due to aquaculture activities and to effluent discharges from the three major cities: Ceuta, F'nideq and M'diq with more than 220000 inhabitants. The silicate nutrient is an essential element for the development of Bacillariophyceae and Dictyochophyceae (Richard, 1987). In both ecosystems, silicates were generally abundant in comparison with other nutrients. Generally inorganic N:P ratios were higher than 16, they were 47.61 (± 39.87) in S1 and 43.34 (± 28.25) in S2. Inorganic Si:P ratios in S1 were lower than 20 (12.81 ± 10.65), however in S2 they were slightly higher than 20 (25.35 ± 28.25). Our results were similar to those of Berland et al. (1973) and Daoudi et al. (2012) who suggested that the phosphorus could be considered as the limiting nutrient in Mediterranean Sea.

HABs species and related toxicity in Mdiq Bay and Oued Laou Estuary

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

In our study, the blooms of *Pseudo-nitzschia* followed the increase of the Si/P ratio. Silicates were proven to increase *Pseudo-nitzschia* abundance (Thorel et al., 2017). The correlation between DA production and the nutritional status seems to be complex in natural populations, since they are frequently composed of numerous species (Trainer et al. 2009). Studies on *Pseudo-nitzschia* dynamics, diversity and toxicity remain relatively scarce in the South Mediterranean (Sahraoui et al. 2009; Rijal leblad et al., 2013; Melliti Ben Garali et al., 2019). Here, *Pseudo-nitzschia* correlated to salinity and temperature but negatively to nitrate. In Bizerte Bay, (Mediterranea,Tunisia), Melliti Ben Garali et al., 2019 showed a positive correlation between *Pseudo-nitzschia* abundance and salinity, silicate, phosphorus and urea. Studies are under way to on the biology and ecology of Pseudo-nitzshia species responsible for ASP in these fragile marine ecosystems.

G. catenatum in M'diq Bay and Oued Laou showed moderate abundances of up to 3960 cells l^{-1}. However, extremely high levels of PSTs contamination were observed permanently in both investigated ecosystems in the cockles reaching 7545 µg STX equiv Kg meat $^{-1}$, a value almost 10 times higher than sanitary threshold. The sweet clams were less contaminated by PSTs in both sites with concentrations ranging from 0 to 1945 µg STX equiv Kg meat $^{-1}$. A peak of *G. catenatum* abundance (>4000 cells L^{-1}) observed in January 2008 corresponded to a maximum level of PSP in mollusks which was not the case of *Alexandrium* cells. This suggest that *G. catenatum* was the main responsible of the mollusks PSP intoxications. This finding based on a bi-weekly survey during two years corroborated the finding of Tahri (1998) and Taleb et al. (2001) who showed that since 1994 (corresponding to the implementation of toxic species and intoxications monitoring in Morocco, RSSL network), PSP outbreaks have been frequently reported and the causative organism have been identified as *G. catenatum*. This dinoflagellate species have been reported to proliferate in Spanish (Fraga, 1996, Busch et al., 2016), Portuguese (Moita et al., 1998, Silva et al., 2015), Algerian (Frehi et al., 2007) and Tunisian (Dammak-Zouari et al., 2009) marine coastal waters. In our study, *G. catenatum* abundance was correlated with ammonium in Oued Laou Estuary and with nitrate and nitrite in M'diq Bay. According Band-Schmidt et al., (2014) toxin production of *G. catenatum* changed with temperature. The growth rate of this neurotoxic species varies with temperature; the highest growth was obtained between 21 and 24 ºC (Band-Schmidt et al., 2010; 2014). In our study, *G. catenatum* was abundant in autumn with temperatures of 18-19 °C. However, it was demonstrated that *G. catenatum* is rather an eurythermal and cosmopolitan species tolerating temperatures from16 to 33 °C (Band-Schmidt et al., 2014) evolving in Pacific, Atlantic and Indian Oceans but also in Mediterranean Sea.

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

In our study, *Dinophysis caudata* showed the highest abundance, followed by *Dinophysis fortii, Dinophysis acuminata and Dinophysis rotundata*. Even though *Prorocentrum lima* is known to be a benthic species, it showed abundances of up to 1300 cells.L^{-1} in the water column of Oued Laou Estuary during August 2008. The other recorded benthic dinoflagellate Ostreopsis showed low abundance not exceeding 90 cells.L^{-1}. The occurrence of benthic HABs species have been well documented in Western Mediterranean (Vila et al., 2001; Dhib et al., 2015; Ben Gharbia et al. 2016; 2019) but not yet in Algerian and Moroccan coastal waters. *D. caudata* was shown to produce Dinophysistoxins, PTX-2 and OA responsible for DSP intoxication (Trainer et al., 2013). DSTs are associated to many species of *Dinophysis* species and *P. lima* (Bazzoni et al., 2018). In our study, using the biological method we noted the absence the detection of DST intoxication in S1 and S2. However, DSTs related to Dinophysis species developing have been shown in NW Mediterranean Sea by Bazzoni et al. (2018) and García-Altares et al. (2016) in some in shellfish farming areas of Italian and Spanish waters, respectively. In our study, *Dinophysis* correlated to pH in both sites. Moreover, correlated to salinity and phosphorus in S1 and S2 respectively.

5. Conclusion

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

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Figures and Tables Legend

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

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

Fig. 3. Temporal variation of phosphorus (PO_4), Silicate (SiO_4) and Dissolved Inorganic Nitrogen (DIN) expressed in µmole.l^{-1} at M'diq Bay and Oued Laou Estuary

Fig. 4. Temporal variations of Diatom abundance (Cells.L^{-1}), Si/P Ratio and N/P Ratio at M'diq Bay and Oued Laou Estuary

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

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

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

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

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

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

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

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

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

Fig. 13. Factorial Discriminant Analysis (FDA) for Oued Laou Estuary (A) and M'diq Bay (C). Astg. *: Asterionella glacialis,* Chae *: Chaetoceros* spp, Plag : *Plagioselmis* sp.*,* Eugl : *Euglena* sp.*,* Gy.ca *: Gymnodinium catenatum,* Lept : *Leptocylindrus* spp*,* Nitz : *Nitzschia* spp*,* No.sc : *Noctiluca scintillans,* Psz : *Pseudo-nitzschia* spp*,* Gu.de *: Guinardia delicatula,* Gu.st : *Guinardia striata, Scri : Scrippsiella* sp*,* Skel : *Skeletonema* spp*,* Thal : *Thalassiosira* spp*,* Thnu : *Thalassionema nitzschioides,* Din : *Dinophysis* spp*,* Het *: Heterocapsa* sp.*,* Cer *: Ceratium* spp.*,* Pleu: *Pleurosigma* spp). T°: Temperature, NO_3: nitrate, NO_2 : nitrite, NH_4^+: ammonium and PO_4: phosphorus.
FDA sample ordination plot considering the seasons; Oued Laou Estuary (B) and M'diq Bay (D).

Table 1. Mean values of physico-chemical environmental parameters at M'diq Bay (Station 2), Mean and Standard Error (SE); n = number of samples; Min = minimum value; Max = maximum value. NH_4: ammonium, NO_2: nitrite, NO_3: nitrate, PO_4: phosphorus and SiO_4: silicate.

Table 2. Spearman correlations liking phytoplankton groups and environmental factors in Mdiq Bay (S2) and Oued Laou Estuary(S1). * Raphidophycea and Chorophycea species were not observed in Mdiq bay.

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

spp, Pleu: *Pleurosigma* spp). Si: silicate, PO_4: phosphorus, NH_4: ammonium, NO_2: nitrite and NO_3: nitrate.

Table 4. Values of Spearman rank correlation coefficient between dominant species and environmental parameters in M'diq bay (* significant). Astg : *Asterionella glacialis*, Chae : *Chaetoceros* spp, Plag : *Plagioselmis* sp., Gyr : *Gyrodinium* spp., Lept : *Leptocylindrus* spp, Nitz : *Nitzschia* spp, Prrt : *Prorocentrum triestinum*, Psz : *Pseudo-nitzschia* spp, Scri : *Scrippsiella* sp, Skel : *Skeletonema* spp, Thal : *Thalassiosira* spp, Th.nu : *Thalassionema nitzschioides*, Alex: *Alexandrium* spp, Gy. ca: *Gymnodinium catenatum*, Din: *Dinophysis* spp, Euc: *Eucampia zodiacus*, Gu.st : *Guinardia striata*). Si: silicate, PO_4: phosphorus, NH_4: ammonium, NO_2: nitrite and NO_3: nitrate.

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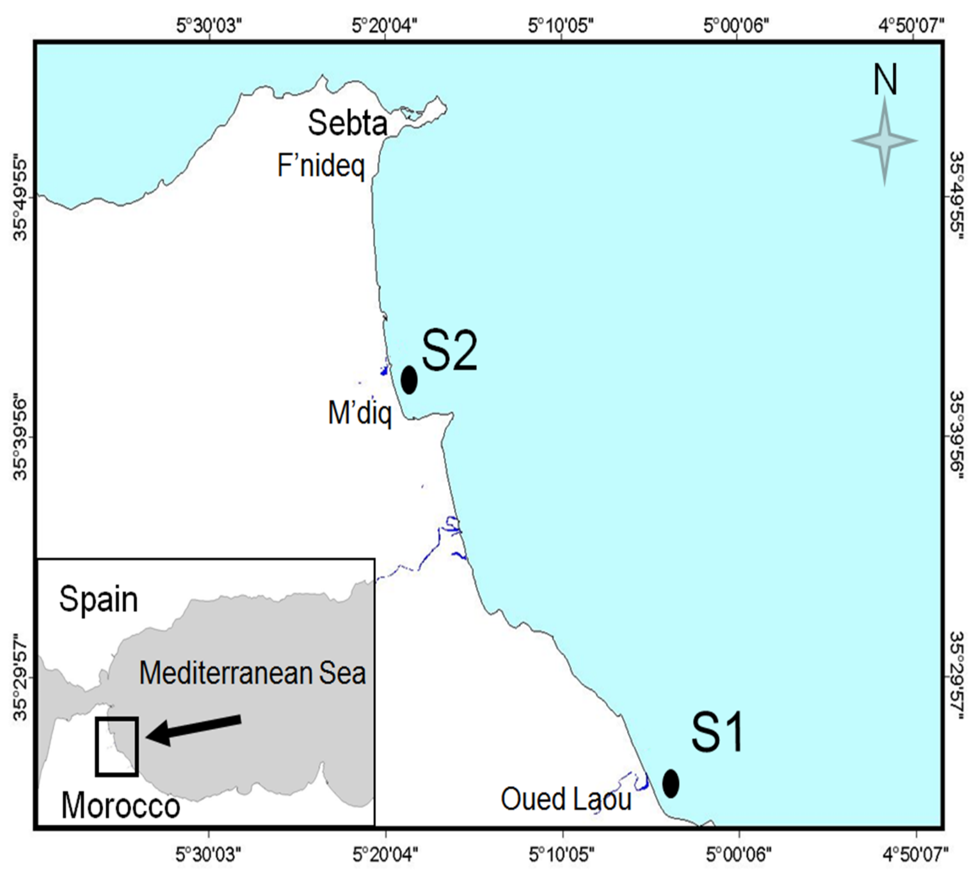
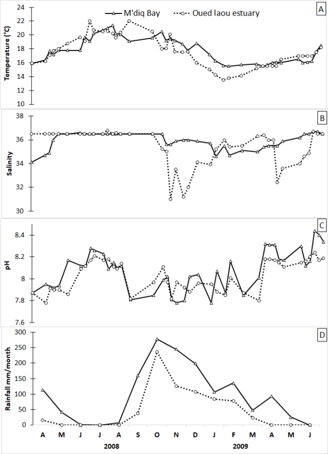
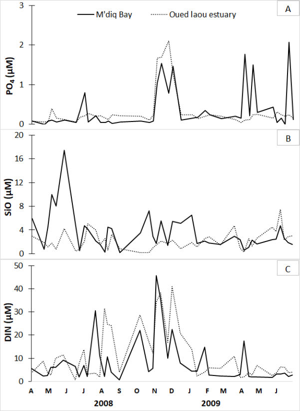
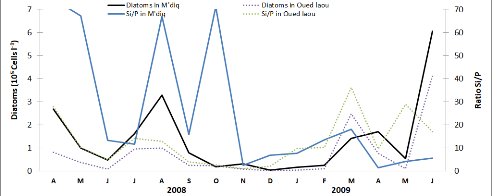

5°30'03"
5°20'04"
5°10'05"
5°00'06"
4°50'07"
35°49'55"
35°39'56"
35°29'57"
N
Sebta
F'nideq
S2
M'diq
S1
Oued Laou
Spain
Mediterranean Sea
Morocco

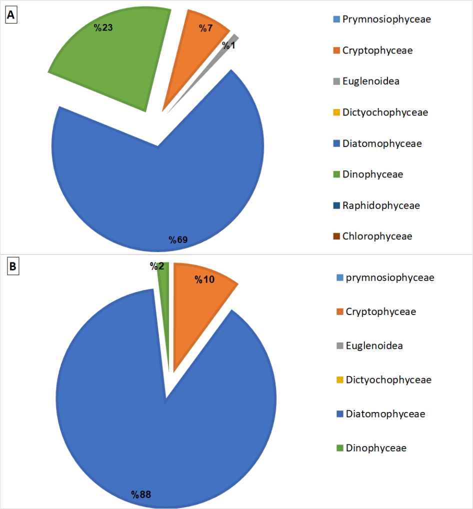


M'diq Bay
Oued laou estuary
A
B
C
D
Temperature (°C)
Salinity
pH
Rainfall mm/month
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
2008
2009

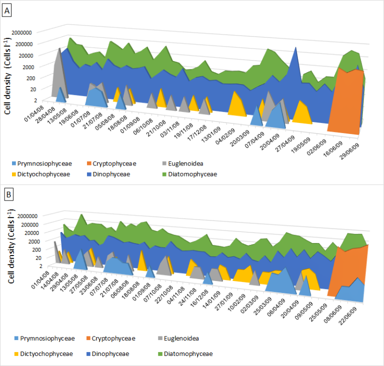


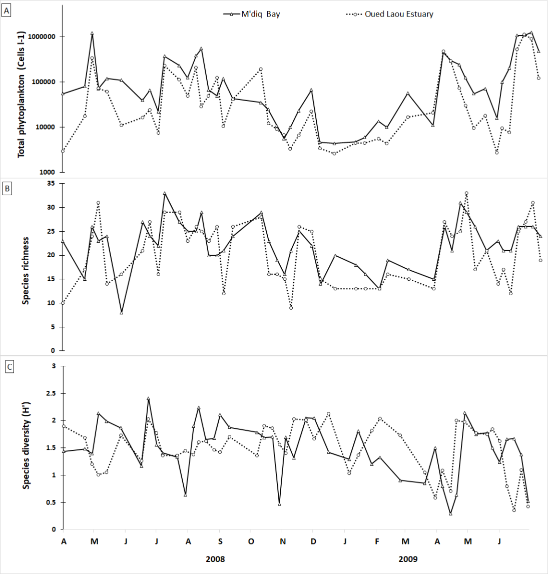
M'diq Bay
Oued laou estuary
A
B
C
PO_4 (µM)
SiO (µM)
DIN (µM)
A M J J A S O N D J F M A M J
2008
2009


Diatoms in M'diq
Si/P in M'diq
Diatoms in Oued laou
Si/P in Oued laou
Diatoms (10^5 Cells l^{-1})
Ratio Si/P
0
1
2
3
4
5
6
7
10
20
30
40
50
60
70
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
2008
2009

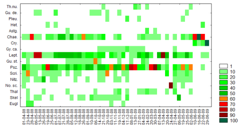


A
%23
%7
%1
%69
Prymnosiophyceae
Cryptophyceae
Euglenoidea
Dictyochophyceae
Diatomophyceae
Dinophyceae
Raphidophyceae
Chlorophyceae
B
%2
%10
%88
prymnosiophyceae
Cryptophyceae
Euglenoidea
Dictyochophyceae
Diatomophyceae
Dinophyceae

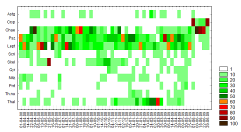

A
Cell density (Cells l-1)
2000000
200000
20000
2000
200
20
2
01/04/08
28/04/08
13/05/08
19/06/08
01/07/08
21/07/08
05/08/08
18/08/08
01/09/08
06/10/08
21/10/08
03/11/08
19/11/08
17/12/08
13/01/09
04/02/09
20/03/09
07/04/09
20/04/09
27/04/09
19/05/09
02/06/09
16/06/09
29/06/09
Prymnosiophyceae
Cryptophyceae
Euglenoidea
Dictyochophyceae
Dinophyceae
Diatomophyceae
B
Cell density (Cells l-1)
2000000
200000
20000
2000
200
20
2
01/04/08
14/04/08
29/04/08
13/05/08
27/05/08
23/06/08
07/07/08
21/07/08
06/08/08
18/08/08
01/09/08
07/10/08
22/10/08
04/11/08
24/11/08
16/12/08
14/01/09
27/01/09
10/02/09
02/03/09
25/03/09
06/04/09
20/04/09
09/05/09
25/05/09
08/06/09
22/06/09
Prymnosiophyceae
Cryptophyceae
Euglenoidea
Dictyochophyceae
Dinophyceae
Diatomophyceae

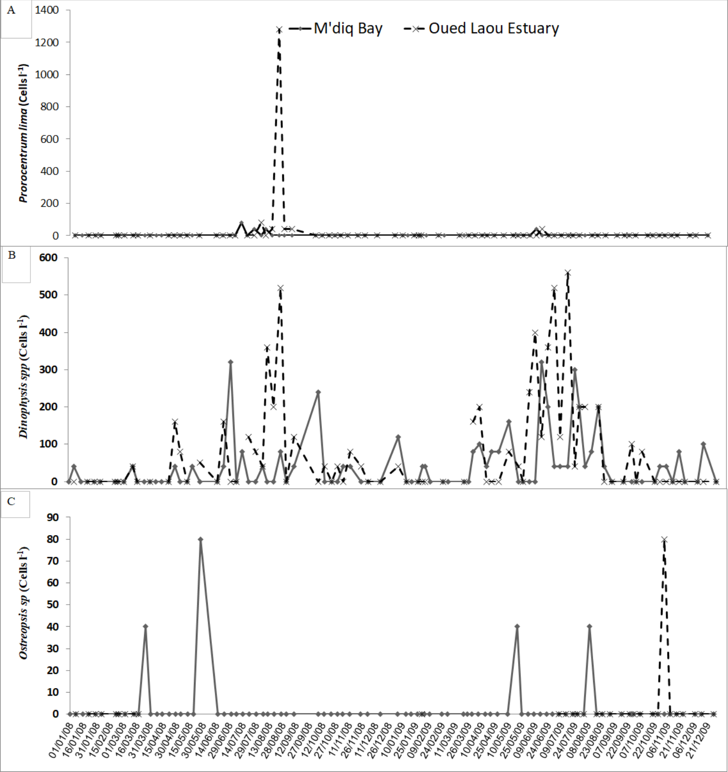


A
M'diq Bay
Oued Laou Estuary
Total phytoplankton (Cells l-1)
1000000
100000
10000
1000
B
Species richness
35
30
25
20
15
10
5
0
C
Species diversity (H')
3
2.5
2
1.5
1
0.5
0
A
M
J
J
A
S
O
N
D
J
F
M
A
M
J
2008
2009



Th.nu.
Gu. de.
Pleu.
Het.
Astg.
Chae.
Cry.
Gy. ca.
Lept.
Gu. st.
Psz.
Scri.
Nitz.
No. sc.
Thal
Skel
Eugl
01-04-08
23-04-08
28-04-08
06-05-08
13-05-08
26-05-08
19-06-08
25-06-08
01-07-08
07-07-08
21-07-08
28-07-08
05-08-08
12-08-08
18-08-08
26-08-08
01-09-08
10-09-08
06-10-08
14-10-08
21-10-08
29-10-08
03-11-08
11-11-08
19-11-08
04-12-08
17-12-08
08-01-09
13-01-09
27-01-09
04-02-09
24-02-09
20-03-09
30-03-09
07-04-09
13-04-09
20-04-09
24-04-09
27-04-09
06-05-09
19-05-09
25-05-09
02-06-09
10-06-09
16-06-09
22-06-09
29-06-09
1
10
20
30
40
50
60
70
80
90
100


Astg
Cryp
Chae
Psz
Lept
Scri
Skel
Gyr
Nitz
Prrl
Th.nu
Thal
1
10
20
30
40
50
60
70
80
90
100



A
B
C
M'diq Bay
Oued Laou Estuary
Prorocentrum lima (Cells l-1)
Dinophysis spp (Cells l-1)
Ostreopsis sp (Cells l-1)
01/01/08
16/01/08
31/01/08
15/02/08
01/03/08
16/03/08
31/03/08
15/04/08
30/04/08
15/05/08
30/05/08
14/06/08
29/06/08
14/07/08
29/07/08
13/08/08
28/08/08
12/09/08
27/09/08
12/10/08
27/10/08
11/11/08
26/11/08
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26/12/08
10/01/09
25/01/09
09/02/09
24/02/09
11/03/09
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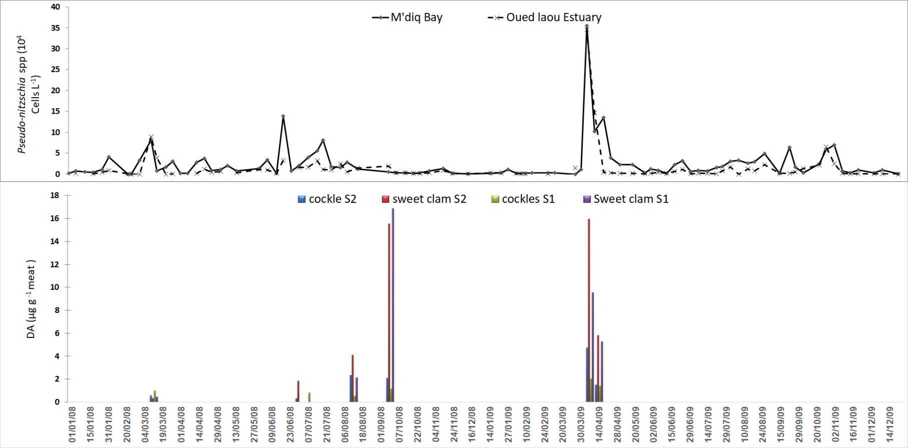
06/12/09
21/12/09


M'diq Bay
Oued laou Estuary
Pseudo-nitzschia spp (10^4 Cells L^{-1})
cockle S2
sweet clam S2
cockles S1
Sweet clam S1
DA (µg g^{-1} meat)
01/01/08
15/01/08
31/01/08
20/02/08
04/03/08
19/03/08
01/04/08
14/04/08
29/04/08
13/05/08
27/05/08
09/06/08
23/06/08
07/07/08
21/07/08
06/08/08
18/08/08
01/09/08
07/10/08
22/10/08
04/11/08
24/11/08
16/12/08
14/01/09
27/01/09
10/02/09
24/02/09
20/03/09
30/03/09
14/04/09
28/04/09
20/05/09
02/06/09
15/06/09
29/06/09
14/07/09
29/07/09
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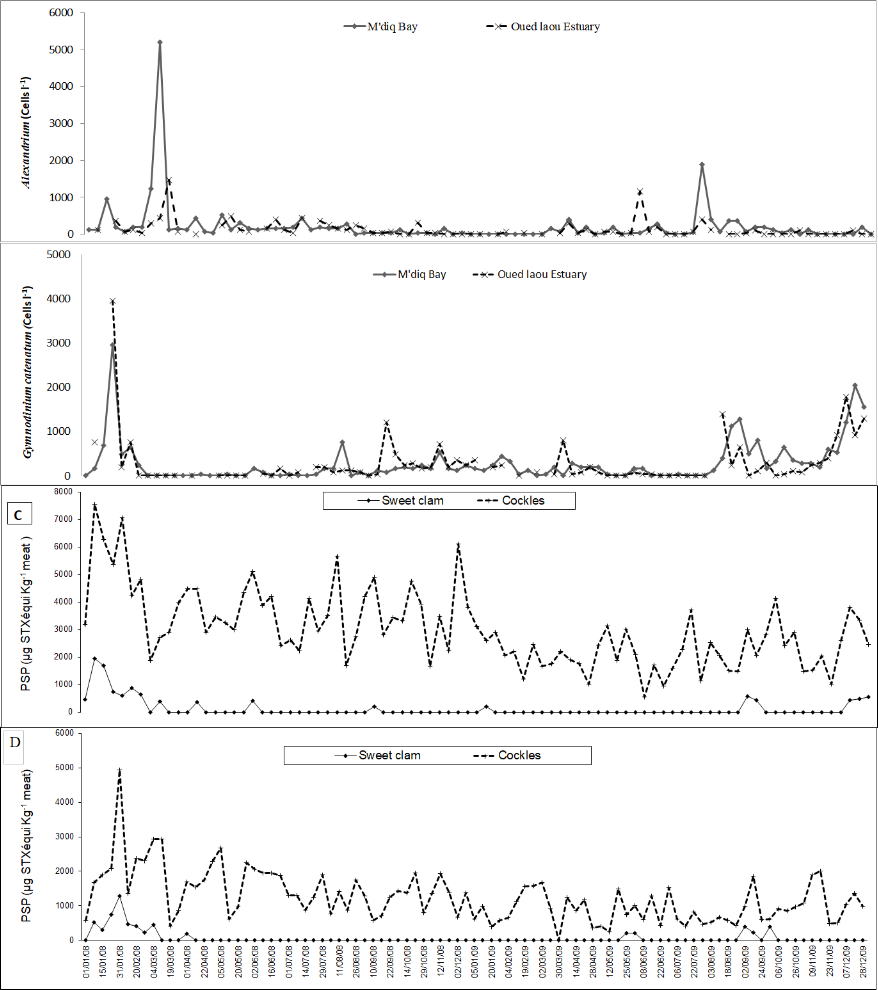
16/11/09
01/12/09
14/12/09


M'diq Bay
Oued laou Estuary
Alexandrium (Cells l-1)
Gymnodinium catenatum (Cells l-1)
C
Sweet clam
Cockles
PSP (µg STXéqui Kg-1 meat)
D
PSP (µg STXéqui Kg-1 meat)
01/01/08 15/01/08 31/01/08 20/02/08 04/03/08 19/03/08 01/04/08 22/04/08 05/05/08 20/05/08 02/06/08 16/06/08 01/07/08 14/07/08 29/07/08 11/08/08 26/08/08 10/09/08 22/09/08 14/10/08 29/10/08 12/11/08 02/12/08 05/01/09 20/01/09 04/02/09 19/02/09 02/03/09 30/03/09 14/04/09 28/04/09 12/05/09 25/05/09 08/06/09 22/06/09 06/07/09 22/07/09 03/08/09 18/08/09 02/09/09 24/09/09 06/10/09 26/10/09 09/11/09 23/11/09 07/12/09 28/12/09

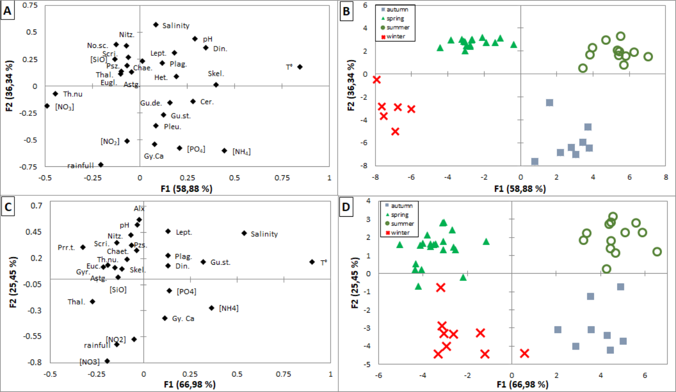

A
B
C
D
Salinity
Nitz.
pH
Din.
No.sc.
Scri.
[SiO]
Lept.
Plag.
Psz.
Chae.
Thal.
Eugl.
Astg.
Het.
Skel.
T°
Th.nu
[NO3]
Gu.de.
Cer.
Gu.st.
Pleu.
[NO2]
Gy.Ca
[PO4]
[NH4]
rainfull
F1 (58,88 %)
F2 (36,34 %)
autumn
spring
summer
winter
Alx
Prr.t.
Chaet.
Pzs.
Th.nu.
Euc.
Gyr.
Astg.
F1 (66,98 %)
F2 (25,45 %)

Table 1.

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	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
(n=19)	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	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
(n=22)	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	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
(n=13)	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	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
(n=14)	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	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
(n=9)	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	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
(n=9)	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	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
(n=6)	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	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
(n=9)	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

***Table 2.* :**

Variables	Site Number	Rainfall	T (°C)	Salinity	pH	[PO_4]	[NO_2]	[NH_4]	[NO_3]	DIN	[SiO_4]
Species Richess	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
Prymnosiophyceae	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

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*

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