

# COUPLING OF PHYTOPLANKTON COMMUNITY STRUCTURE TO NUTRIENTS, CILIATE AND ZOOPLANKTON AROUND THE BESSILA ISLANDS (KNEISS ISLANDS, CENTRAL MEDITERRANEAN SEA)

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Research

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## CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

## ABSTRACT

**Background:** To investigate the structural diversity of the planktonic phytoplankton through summer 2009 and summer 2010, to identify the role of the main abiotic factors that determine the phytoplankton community structure and functioning and to study the summer spatial distribution of phytoplankton in relation to its potential predators such as ciliate and zooplankton.

**Methods:** Phytoplankton and ciliate samples were analysed under an inverted microscope after 24 to 48 h settling using the Utermöhl method. Zooplankton enumeration was performed under a vertically mounted deep-focus dissecting microscope.

**Results:** Our results demonstrated a striking difference among years is seen in suspended matter concentrations with lower values in summer 2010 ( $47.37 \pm 23.12 \text{ mg l}^{-1}$ ), contrasting with the higher suspended matter values in summer 2009 ( $92.88 \pm 7.15 \text{ mg l}^{-1}$ ). A total of 50 phytoplankton taxa identified during this study of three different algal classes (cyanobacteriae, diatoms and dinoflagellate). We found: first, in summer 2010, the phytoplankton abundance was about 3 times that in summer 2009, second, a high abundance of cyanobacteriae (43 % of total phytoplankton) and diatoms (54 % of total phytoplankton) in summer 2009 and 2010 respectively. In summer 2009, we observed a cyanobacteriae growth with dominant opportunistic and nitrogen-fixing *Oscillatoria* sp. Dominance of the benthic and large diatoms genus such as *Navicula* and *Nitzschia* has been reported in summer 2010. Concerning dinoflagellates, they were strongly dominated by mixotrophic and heterotrophic dinoflagellates species, like *Polykrikos kofoidii*, *Ceratium lineatum*, *Protoperidinium steinii*.

**Conclusions:** This study indicates that the abiotic factors of the coast area around Kneiss Islands are associated with the spatial and inter-annual variations of the phytoplankton.

## 1. INTRODUCTION

The coast of Sfax is a part of the south-western Mediterranean sea and is located in southeast of Tunisia (Rekik et al., 2012, 2016). It is bounded by the Kneiss islands in the southeast, which is composed of four little islands with Bessila is the main island, Hajer island in the north, Laboua island in the centre and Gharbia island in the south (Gueddari and Oueslati 2002). These islands are uninhabited by human populations (Mosbahi et al., 2015). The Kneiss Islands represent the most important coastal wetlands in Mediterranean Sea, and make up a very important intertidal area exploited for clam harvesting by the local population (Mosbahi et al., 2016a). The

archipelago of Kneiss, represent an important site in terms of fish resources and ornithological diversity (Mosbahi et al., 2016b). This ecosystem has long been recognized as one of the most important areas for migratory water birds during winter via the Mediterranean (Hamza et al., 2015). The islands are colonized by the seagrass *Zostera noltei*, protected species listed in the "IUCN Red List" of threatened species in Mediterranean Sea, as characterizing a diversified habitat requiring monitoring and protection (Mosbahi et al., 2016a). The Kneiss Islands were declared as a "Nature Reserve" in 1993, then as a "Specially Protected Area of Mediterranean Importance" (SPAM) in 2001, an "Important Bird Area" (IBA) in 2003 and a "RAMSAR Site" in 2007 (Mosbahi et al., 2016a). However, this coast is subject to degradation and loss of biodiversity caused by bottom-trawling fishing (M'Rabet, 1995) and pollution due to the important industrial development linked to the phosphate treatment (Rekik et al., 2012), as well as maritime discharges rendering the harbour environment hostile for native species and opening a window for the proliferation of opportunistic exotic species and impacting marine systems and changing the structure and functioning of communities (Galil, 2000).

Mosbahi et al. (2015, 2016ab) presented research investigate the seasonal and spatial structure of the intertidal macrozoobenthic communities of the area around the Kneiss Islands. However, data concerning the spatial and seasonal distribution of phytoplankton assemblages in the Kneiss Island coast were scarce. Our objective was 1) to investigate the structural diversity of the planktonic phytoplankton through summer 2009 and summer 2010; 2) to identify the role of the main abiotic factors that determine the phytoplankton community structure and functioning; and 3) to study the summer spatial distribution of phytoplankton in relation to its potential predators such as ciliate and zooplankton. To the best of our knowledge, there have been no previous comprehensive field studies that include all of the same parameters found in our study.

**KEYWORDS:** Central Mediterranean Sea, Kneiss Islands, Nutrients, Phytoplankton, Summer

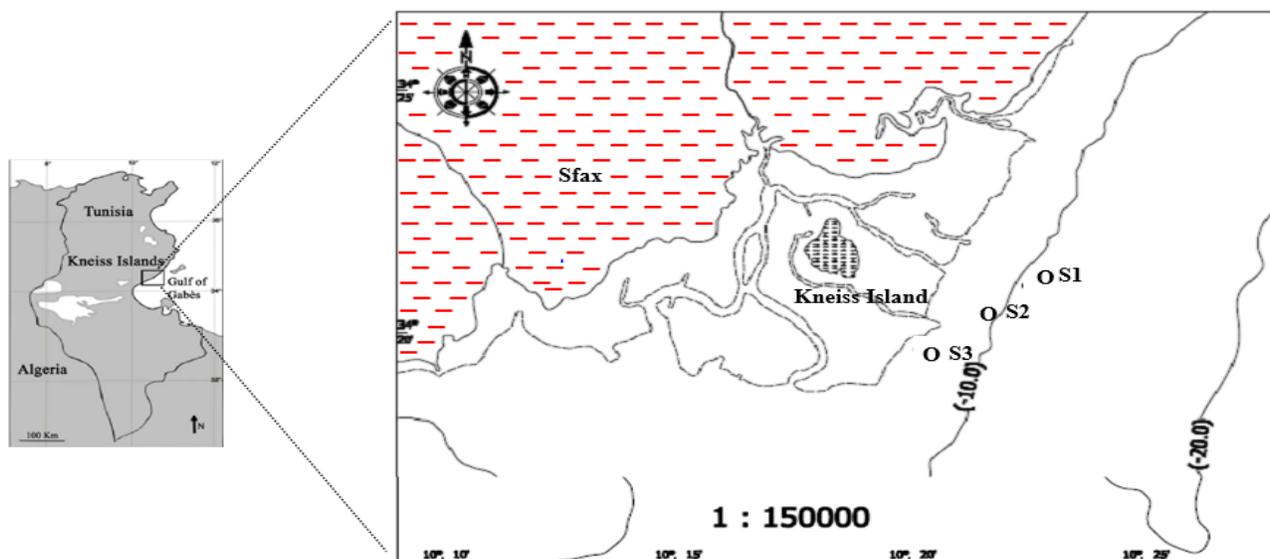
## 2. MATERIAL AND METHODS

### 2.1. Study site

The Kneiss Islands, located in the south-eastern Tunisia, between latitudes 34°10'-34°30'N and longitudes 10°-10°30'E (Mosbahi et al., 2015). The total surface-area of the archipelago (22.027 ha) can be divided into two main sectors: the subtidal channels and the intertidal areas (Bali and Gueddari 2011). The mudflats of the Kneiss Islands are composed by muddy to sandy muddy sediments (Bali and Gueddari 2011). The tides are semidiurnal, attaining a range of 2.3 m during spring tides (Sammari et al., 2006). At low tide, the Kneiss Islands are surrounded by vast mud and sand flats (Abdennadher et al., 2010). The Kneiss archipelago is composed of four islands (Bessila island, Hajer island, Laboua island and Gharbia island). The three sampling stations are close to Bessila island and far from the others. The distance between the stations: S1- S2 and S2- S3 was 2 Km and between S1- S3 was 4 Km. The stations S1 (< 10 m), S2 (< 10 m) and S3 (> 10 m) were under different depths due to different distances of the coast.

### 2.2. Field sampling

Nutrients, phytoplankton, ciliate and zooplankton samples were collected in July 2009 and July 2010. Water samples were collected on 3 stations around the Kneiss Islands coast (Figure 1). Seawater samples for physico-chemical analyses, phytoplankton and ciliate examination were collected from the surface water with a Van Dorn-type closing bottle at each station. Zooplankton was collected using a cylindro-conical net (30 cm aperture, 100 cm high, and 100 µm mesh size). Nutrient samples (120 mL) were stored immediately in the dark at 20 °C. Phytoplankton samples (1 L) were preserved with Lugol iodine solution (4%) for enumeration (Bourrelly, 1985). Zooplankton samples were preserved in 2% buffered formaldehyde solution and were stained with rose Bengal to facilitate dissection. Plankton samples were kept at low temperature (4 °C) in the dark until analysis.



**Figure 1.** Location of sampling stations on the Kneiss islands.

### 2.3. Physico-chemical variables

Physical parameters (temperature, salinity, and pH) were measured immediately after sampling using a multi-parameter kit (Multi 340 i/SET). Suspended matter concentrations were measured using the dry weight of the residue after filtration of 0.5 L of seawater onto Whatman GF/C membrane filters. Chemical parameters (nitrite, nitrate, ammonium, orthophosphate, silicate, total nitrogen and total phosphate) were analyzed with a Bran and Luebbe type 3 autoanalyzer. The N/P ratio was calculated from DIN ( $\text{DIN} = \text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$ ) to DIP ( $\text{DIP} = \text{PO}_4^{3-}$ ).

### 2.4. Phytoplankton enumeration

For phytoplankton, ciliate and zooplankton analyses, there were three samples for each sampling station. Phytoplankton and ciliate samples (50 mL) were analysed under an inverted microscope after 24 to 48 h settling using the Utermöhl method (Utermöhl, 1958). Zooplankton enumeration was performed under a vertically mounted deep-focus dissecting microscope (Olympus TL 2). Plankton species identification was made according to various keys (Rekik et al., 2012; 2013ab; 2015abc).

### 2.5. Statistical analysis

The data recorded in this study were submitted to a normalized principal component analysis (PCA) (Dolédéc and Chessel 1989). Simple log (x+1) transformation was applied to data in order to correctly stabilize variance (Frontier, 1973). Means and standard deviations (SD) were reported when appropriate. The potential relationships between the biological (phytoplankton, ciliates and zooplankton) and the physicochemical parameters were tested with Pearson's correlation coefficient. Also, Pearson's rank correlation was performed to determine the correlations between the biotic variables. The results were illustrated by a dendrogram showing the steps in the hierarchical clustering solution and the values of the squared Euclidean distances between clusters.

## 3. RESULTS

### 3.1. Hydrological features

The mean values of physical variables recorded at the three sampled stations are summarised in Table 1. Temperature varied among stations and seasons (Table 1). The temperature was in the range 31 - 38 °C, the lowest values being observed at station 3 in summer 2009 and stations 1 and 3 in summer 2010. Salinity varied from 37.50 psu in summer 2010 at station 2 to 39 psu in summer 2009 at station 1. The mean salinity values showed a slight difference between the two-seasons (Table 1). The mean pH values ranged from  $8.03 \pm 0.06$  (summer 2010) to  $8.28 \pm 0.13$  (summer 2009) (Table 1). The mean pH values were usually alkaline, suggesting a more pronounced photosynthetic activity. Suspended matter concentration in summer 2010 (28.00-73.00 mg l<sup>-1</sup>) was lower than in summer 2009 (84.66-97.00 mg l<sup>-1</sup>) (Table 1).

Variables	Summer 2009			Summer 2010		
	Minimum	Maximum	Mean $\pm$ SD	Minimum	Maximum	Mean $\pm$ SD
<b>Physical variables</b>						
T ( $^{\circ}$ C)	31.00	38.00	35.33 $\pm$ 3.79	31.00	32.00	31.33 $\pm$ 0.58
S (p.s.u.)	38.50	39.00	38.73 $\pm$ 0.25	37.50	38.00	37.77 $\pm$ 0.25
pH	8.15	8.40	8.28 $\pm$ 0.13	8.00	8.10	8.03 $\pm$ 0.06
Suspended matter (mg l <sup>-1</sup> )	84.66	97.66	92.88 $\pm$ 7.15	28.00	73.00	47.37 $\pm$ 23.12
<b>Chemical variables</b>						
NO <sub>3</sub> <sup>-</sup> ( $\mu$ M)	1.38	2.53	2.12 $\pm$ 0.64	2.42	2.77	2.65 $\pm$ 0.20
NO <sub>2</sub> <sup>-</sup> ( $\mu$ M)	0.22	1.03	0.59 $\pm$ 0.41	0.18	0.63	0.35 $\pm$ 0.24
NH <sub>4</sub> <sup>+</sup> ( $\mu$ M)	0.78	1.60	1.10 $\pm$ 0.44	1.82	8.04	4.02 $\pm$ 3.49
Total-N ( $\mu$ M)	12.35	15.20	13.54 $\pm$ 1.48	11.11	17.19	13.78 $\pm$ 3.11
PO <sub>4</sub> <sup>3-</sup> ( $\mu$ M)	0.16	0.48	0.29 $\pm$ 0.17	0.25	0.33	0.29 $\pm$ 0.04
Total-P ( $\mu$ M)	0.98	3.38	2.31 $\pm$ 1.22	4.42	7.54	6.31 $\pm$ 1.66
N/P ratio	6.94	21.63	16.55 $\pm$ 8.33	15.60	36.76	23.95 $\pm$ 11.26
Si(OH) <sub>4</sub> ( $\mu$ M)	2.46	7.41	4.95 $\pm$ 2.48	11.11	13.45	12.05 $\pm$ 1.23
<b>Biological variables</b>						
Phytoplankton ( $\times 10^2$ cells l <sup>-1</sup> )	11.00	33.00	20.00 $\pm$ 11.53	30.00	128.00	63.00 $\pm$ 56.29
Cyanobacteriae ( $\times 10^2$ cells l <sup>-1</sup> )	1.00	21.00	8.67 $\pm$ 10.78	5.00	44.00	18.33 $\pm$ 22.23
Diatoms ( $\times 10^2$ cells l <sup>-1</sup> )	3.50	5.00	4.16 $\pm$ 0.76	14.00	74.00	34.00 $\pm$ 34.64
Dinoflagellates ( $\times 10^2$ cells l <sup>-1</sup> )	6.50	8.00	7.17 $\pm$ 0.76	10.00	12.00	10.67 $\pm$ 1.15
Ciliates ( $\times 10^2$ cells l <sup>-1</sup> )	6.00	15.00	9.33 $\pm$ 4.93	2.00	4.00	3.00 $\pm$ 1.00
Zooplankton ( $\times 10^2$ ind m <sup>-3</sup> )	21.50	123.50	66.74 $\pm$ 51.86	53.44	83.47	67.64 $\pm$ 15.08

**Table 1.** Minimum, maximum and mean  $\pm$  SD of physical-chemical and biological variables on the Kneiss islands during summer 2009 and summer 2010

### 3.2. Nutrients

NO<sub>3</sub><sup>-</sup> concentration varied between 1.38 and 2.77  $\mu$ M in the study area, with the lowest concentration (1.38  $\mu$ M) observed at station 3 in summer 2009, whereas the highest concentration (2.77  $\mu$ M) value was observed in summer 2010 at station 3. The average values of NO<sub>3</sub><sup>-</sup> concentration at both summer 2009 and 2010 were relatively close: 2.12  $\pm$  0.64 and 2.65  $\pm$  0.20  $\mu$ M respectively (Student test,  $t=1.34$ ;  $p=0.24$ ; no significant difference) (Table 1). NO<sub>2</sub><sup>-</sup> concentration was very low over the seasons and its mean values (0.59  $\pm$  0.41 and 0.35  $\pm$  0.24  $\mu$ M) were detected in summer 2009 and 2010 respectively (Table 1). NH<sub>4</sub><sup>+</sup> concentrations were more available during summer 2010 (4.02  $\mu$ M) than during summer 2009 (1.10  $\mu$ M) (Table 1). NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were larger in summer 2010 than in summer 2009, whereas NO<sub>2</sub><sup>-</sup> concentrations showed the opposite trend (Table 1). Total nitrogen (T-N) values were about 13  $\mu$ M at both summer 2009 and 2010. Nitrogen appeared mainly in its dissolved organic form (71.86% (summer 2009) – 49.07% (summer 2010)) with the dissolved inorganic form (DIN = NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) representing only 28.14% in summer 2009 but 50.93% of the total nitrogen during summer 2010. The mean value of orthophosphate concentration was 0.29  $\mu$ M, showed similar variations in both summer 2009 and 2010 (Table 1). When considering total phosphate concentrations, values were, 8 times that of orthophosphate concentration in the summer 2009 and up to 21 times in the summer 2010 (Table 1). The N/P: ratio mean value was about 17 (summer 2009) and 24 (summer 2010). This average was more important than the Redfield ratio (16), suggesting a potential phosphate limitation. Silica concentration was more important in summer 2010 (12.05  $\pm$  1.23  $\mu$ M) than in summer 2009 (4.95  $\pm$  2.48  $\mu$ M) (Table 1). The highest concentration of Si(OH)<sub>4</sub> was recorded at station 3 during summer 2010 (13,45  $\mu$ M), and the lowest (2,46  $\mu$ M) at station 1 in summer 2009.

### 3.3. Phytoplankton community structure and inter-annual variations

In the present study, 50 phytoplankton taxa were observed, 24 identified to the species level. Diatoms were the

richest group with 24 taxa, followed by dinoflagellate with 23 taxa. The genus *Protoperidinium* (8 species) was the most diverse among dinoflagellates and the genus *Grammatophora* and *Nitzschia* (2 species) among diatoms (Table 2). During the investigated period, the important phytoplankton abundance was recorded in summer 2010 (Student test,  $t=1.29$ ;  $p=0.26$ ; no significant difference). The highest ( $128 \times 10^2$  cells  $l^{-1}$ ) abundance value was observed during summer 2010, while the lowest ( $11 \times 10^2$  cells  $l^{-1}$ ) during summer 2009 at station 1 (Table 1). Cyanobacteriae abundance varied from  $8.67$  to  $18.33 \times 10^2$  cells  $l^{-1}$ , exhibiting their highest abundance in summer 2010, and the lowest in summer 2009. Overall, cyanobacteriae showed high abundance distributions in station 2 (summer 2009) and 1 (summer 2010) (Fig. 2). Diatoms abundance ranged from  $3.50$  to  $74 \times 10^2$  cells  $l^{-1}$  (mean  $\pm$  SD =  $19.08 \pm 27.33 \times 10^2$  cells  $l^{-1}$  for the study period), showing a remarkable increase in summer 2010 (mean  $\pm$  SD =  $34.00 \pm 34.64 \times 10^2$  cells  $l^{-1}$ ) ascribed to the high abundance of different diatoms species including *Navicula* sp. (maximal abundance =  $28 \times 10^2$  cells  $l^{-1}$  at station 1, summer 2010), *Fragilaria* sp. (maximal abundance =  $10^3$  cells  $l^{-1}$  at station 1, summer 2010), *Nitzschia longissima* and *Rhabdonema* sp. (maximal abundance =  $6 \times 10^2$  cells  $l^{-1}$  at station 1, summer 2010) respectively. The highest ( $12 \times 10^2$  cells  $l^{-1}$ ) dinoflagellates abundance was observed in summer 2010 at station 3 and the lowest ( $6.50 \times 10^2$  cells  $l^{-1}$ ) in summer 2009 at station 1. Dinoflagellates mean abundance per season increased in summer 2010 ( $10.67 \pm 1.15 \times 10^2$  cells  $l^{-1}$ ) and reached its maximum in summer 2009 ( $7.17 \pm 0.76 \times 10^2$  cells  $l^{-1}$ ), varying significantly from year to year, and among sampling sites (Fig. 2). Dinoflagellates diversity varied significantly with respect to seasons, decreasing in summer 2010 (10 species) and exhibiting a remarkable increase in summer 2009 (16 species) (Table 2). Small dinoflagellates (6 species) consisted of different *Prorocentrum* such as *P. gracile*, *P. lima*, *P. micans*, *P. rathymum* and *P. triestinum* and the genera of *Peridinium*. Medium-size dinoflagellate (14 species) was essentially represented by different *Protoperidinium* (*P. curvipes*, *P. depressum*, *P. divergens*, *P. ovatum*, *P. ovum*, *P. pyriforme* and *P. steinii*), *Scrippsiella trochoidea*, *Amphidinium* sp. and *Gymnodinium* sp., although other taxa such as *Pyrophacus* sp., *Akashiwa sanguineum* and *Polykrikos kofoidii* were also observed. Large dinoflagellates (3 species) comprised the genera *Ceratium* (Table 3).

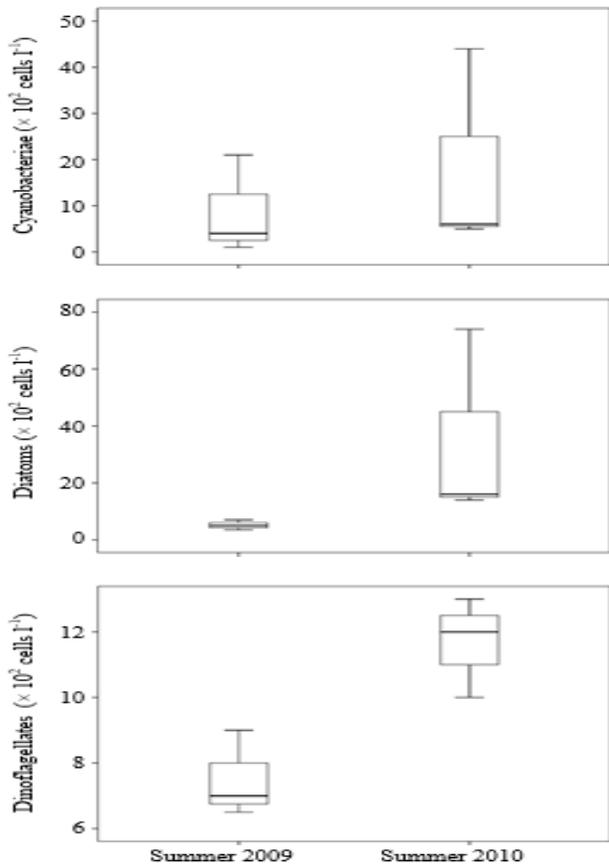
The spatial and annual distribution of phytoplankton abundance with the prevailing potential predators (total ciliate and zooplankton) is illustrated in Figure 3. The abundance of ciliate (dominated by the Tintinnid which accounted for 45 - 75% of total ciliate abundance) showed significant correlations between abundance of ciliate and dinoflagellates for both years ( $r = 0.97$ ,  $n = 18$ ,  $p = 0.05$ , summer 2009) and ( $r = 0.87$ ,  $n = 18$ ,  $p = 0.05$ , summer 2010). The abundance of phytoplankton was correlated with that of zooplankton (dominated by copepod with percentages varying between 45 – 84%). Cyanobacteriae were positively correlated to zooplankton abundance, ( $r=0.89$ ,  $n=18$ ,  $p<0.05$ ) and ( $r=0.90$ ,  $n=18$ ,  $p<0.05$ ), during summer 2009 and 2010 respectively. Highly significant correlations were found between diatoms and zooplankton abundance ( $r=0.79$ ,  $n=18$ ,  $p<0.05$ , summer 2009) and ( $r=0.91$ ,  $n=18$ ,  $p<0.05$ , summer 2010) (Fig. 3).

### 3.4. Statistical analysis

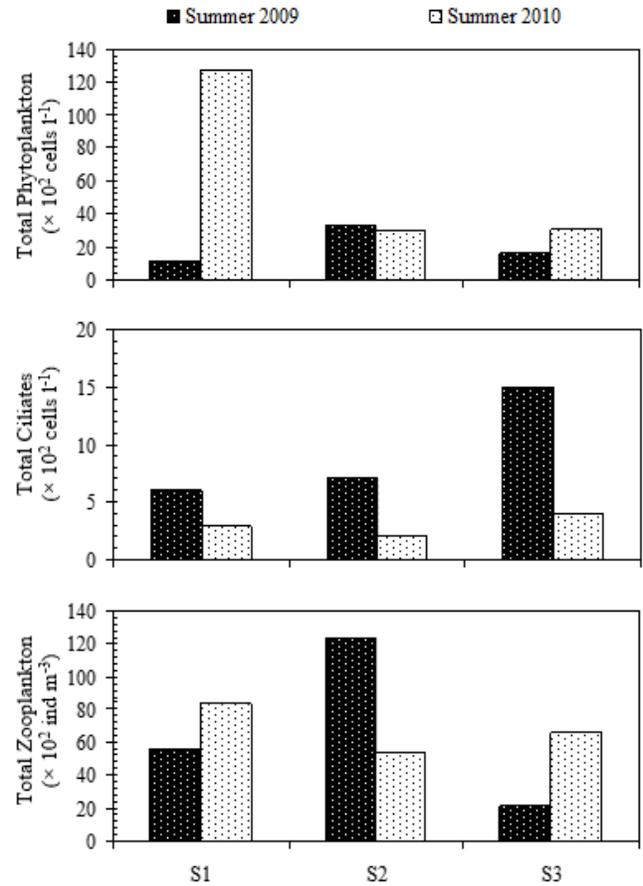
PCA allowed discrimination of four groups around the components of the F1 and F2 axes (Fig. 4), explaining 100 % of the variance. The F1 axis, explaining 65.60 % of the variability of abiotic and biotic parameters, positively selected group G1 composed of pH, suspended matter,  $NO_2^-$ , T-P, Si(OH)<sub>4</sub>, diatoms and dinoflagellates. The F2 axis, representing 34.40 % of the variability, positively selected group G2 comprising chemical factors such as  $PO_4^{3-}$  and T-N correlated with ciliate concentrations. G3 comprised salinity. The group G4 was formed by phytoplankton, cyanobacteriae and zooplankton together with temperature,  $NO_3^-$ ,  $NH_4^+$  and N/P ratio (Fig. 4). This combination was selected in summer 2009.

In summer 2010, the PCA distinguished between four groups surrounding the F1 and F2 component axes thus explaining 100 % of the variance. The axes selected a group G1 comprising the biological parameters (Dinoflagellates and ciliates) and several physico-chemical variables (salinity,  $NO_3^-$ ,  $PO_4^{3-}$ , T-P and Si(OH)<sub>4</sub>). F1 component axis, which extracted 62.07 % of the variability, selected positively the group G2, with phytoplankton, cyanobacteriae, diatoms and zooplankton correlating to abiotic parameters (pH,  $NH_4^+$  and T-N). The gradient along the F1 axis is mainly due to temperature,  $NO_2^-$  and T-N (selected negatively in G3) and to suspended matter (selected negatively in G4) (Fig. 4).

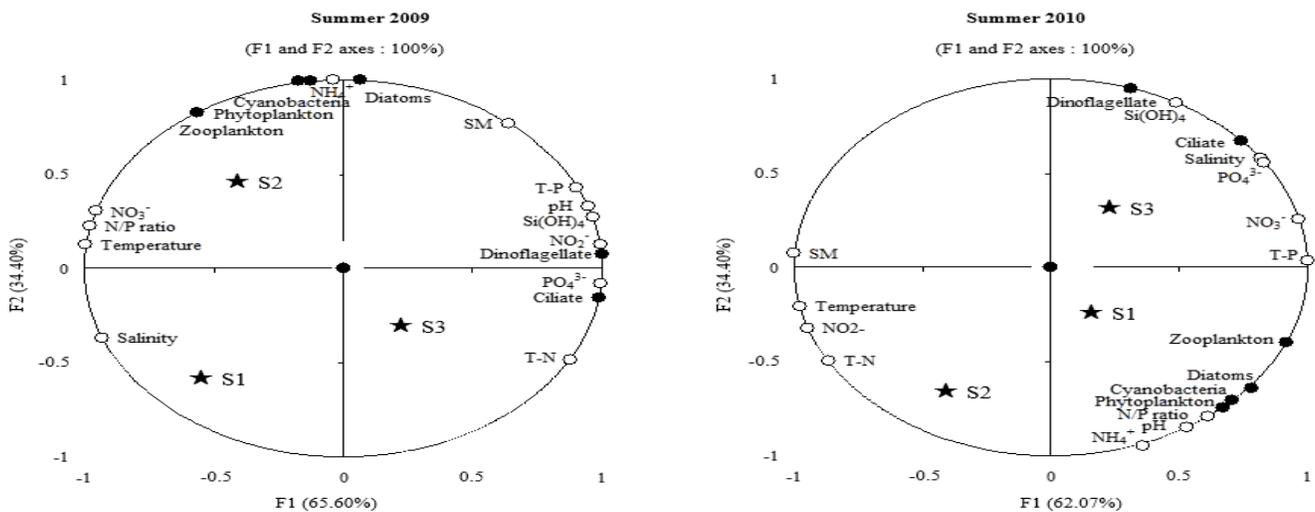
Figure 5 displays results of hierarchical clustering and the resulting dendrogram. The dendrogram from the cluster analysis emphasizes 2 natural groupings. It was possible to define the locations into two groups determined at 35 % dissimilarity of the physicochemical parameters during both summer 2009 and 2010. Cluster 1 groups the stations 1 and 2. This combination was selected in summer 2009. During summer 2010, another cluster with a group formed by stations 1 and 3. The dendrogram of similarity determined two groups for biotic variables in summer 2009 and 2010. Cluster formed by stations 1 and 2 for both summer 2009 and 2010.



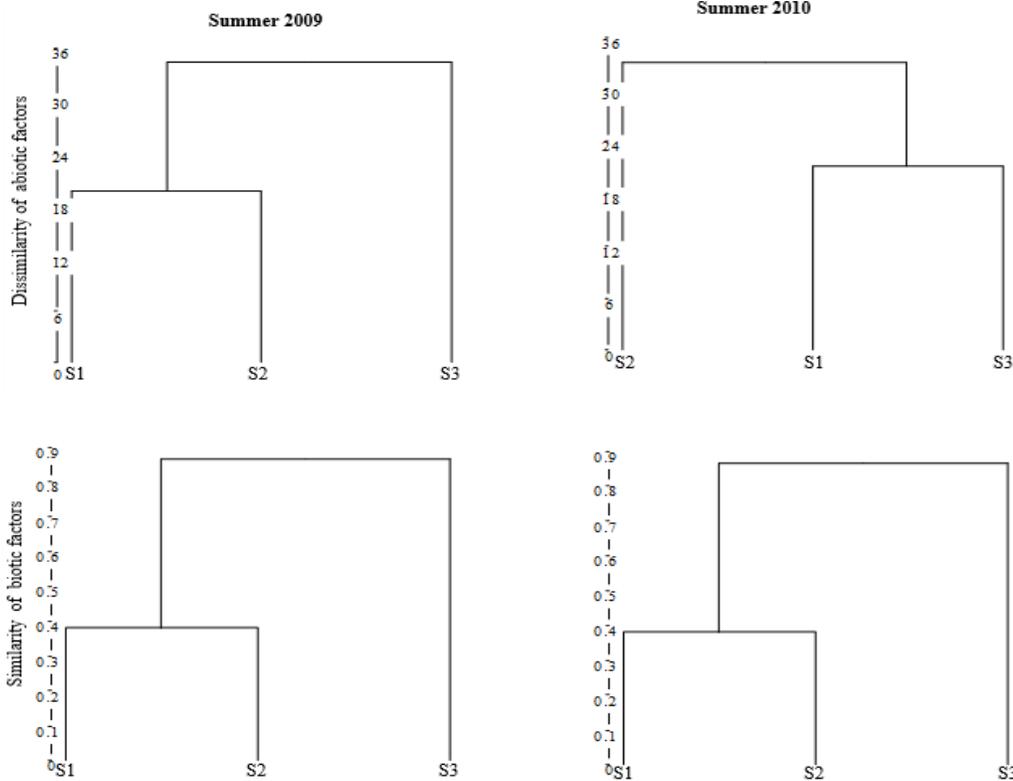
**Figure 2.** Spatial and annual variations of phytoplankton abundance groups: cyanobacteria, diatoms and dinoflagellates in the Kneiss islands.



**Figure 3.** Spatial and annual variations of the abundance of phytoplankton, ciliates and zooplankton in the Kneiss islands.



**Figure 4.** Principal component analysis (Axis I and II) of biological parameter abundance and selected environmental variables at sampled stations in the Kneiss islands.



**Figure 5.** Cluster analysis showing the dissimilarity among stations of abiotic and biological variables in the Kneiss islands.

Phytoplankton species	Summer 2009			Summer 2010		
	1	2	3	1	2	3
<b>Cyanobacteriae</b>						
<i>Anabeana</i> sp. (Bornet and Flahault, 1886)	-	■	■	■	■	■
<i>Oscillatoria</i> sp. (Gomont, 1822)	-	■	■	■	■	■
<i>Spirulina</i> sp. (Gomont, 1892)	-	■	■	■	■	■
<b>Diatoms</b>						
<i>Amphiprora ornata</i> (Stearn, 1973)	-	-	-	■	■	■
<i>Amphora</i> sp. (Ehrenberg, 1844)	-	-	-	■	■	■
<i>Chaetoceros</i> sp. (Ehrenberg, 1844)	■	-	-	■	■	■
<i>Climacosphenia</i> sp. (Ehrenberg, 1843)	-	-	-	■	■	■
<i>Cocconeis</i> sp. (Ehrenberg, 1836)	-	-	-	■	■	■
Large diatoms	-	-	-	■	■	■
<i>Coscinodiscus</i> sp. (Ehrenberg, 1839)	-	■	-	■	■	■
<i>Fragilaria</i> sp. (Lyngbye, 1819)	-	-	-	■	■	■
<i>Grammatophora marina</i> (Kützing, 1844)	-	-	-	■	■	■
<i>Grammatophora</i> sp. (Ehrenberg, 1840)	■	-	-	■	■	■
<i>Gyrosigma</i> sp. (Hassal, 1845)	-	-	-	■	■	■
Large diatoms	-	-	-	■	■	■
<i>Hemiaulus</i> sp. (Heiberg, 1863)	-	-	-	■	■	■
<i>Licmophora</i> sp. (Agardh, 1827)	-	■	-	■	■	■
<i>Melosira</i> sp. (Agardh, 1827)	-	-	-	■	■	■
<i>Navicula</i> sp. (Bory de St Vincent, 1822)	-	-	-	■	■	■
Large diatoms	-	-	-	■	■	■
<i>Nitzschia longissima</i> (Ralf, 1861)	■	-	-	■	■	■
Large diatoms	■	-	-	■	■	■
<i>Nitzschia</i> sp. (Hassall, 1845)	-	-	-	■	■	■
Large diatoms	-	-	-	■	■	■
<i>Pinnularia</i> sp. (Ehrenberg, 1843)	-	-	-	■	■	■
-	-	-	-	■	■	■
<i>Pleurosigma</i> sp. (Smith, 1852)	-	-	-	■	■	■
Large diatoms	-	-	-	■	■	■
<i>Rhabdonema</i> sp. (Mann, 1925)	-	-	-	■	■	■
Large diatoms	-	-	-	■	■	■
<i>Rhizosolenia</i> sp. (Brightwell, 1858)	-	-	-	■	■	■
-	-	-	-	■	■	■
<i>Skeletonema costatum</i> (Cleve, 1873)	■	-	-	■	■	■
-	■	-	-	■	■	■
<i>Striatella unipunctata</i> (Agardh, 1832)	-	■	-	■	■	■
Large diatoms	-	■	-	■	■	■
<i>Thalassionema nitzschoides</i> (Mereschkowsky, 1902)	-	-	-	■	■	■
-	-	-	-	■	■	■
<i>Thalassiosira</i> sp. (Hasle, 1960)	-	-	-	■	■	■



south coast of Sfax with 78 phytoplankton taxa (Rekik *et al.*, 2015c). A comparison with previous work (Rekik *et al.*, 2013a, 2015c) revealed a similar phytoplanktonic composition with the three dominant classes (cyanobacteriae, diatoms and dinoflagellates) but in different relative abundances. Our study explained two key results: (i) in summer 2010, the phytoplankton abundance was about 3 times that in summer 2009, (ii) a high abundance of cyanobacteriae (43 % of total phytoplankton) and diatoms (54 % of total phytoplankton) in summer 2009 and 2010 respectively. In summer 2009, we observed a cyanobacteriae growth with dominant opportunistic and nitrogen-fixing *Oscillatoria* sp. which, at first sight, might be an eutrophication indicator (Table 1 and Table 2) (Ye *et al.*, 2012). But, in other studies, authors have found the same pattern with the cyanobacteriae dominating the phytoplankton community forming colonies during summer in the oligotrophic Baltic Sea (Nausch *et al.*, 2008). Cyanobacteriae proliferation started in summer 2009 when dissolved inorganic phosphate was low. This result is in agreement with that of Rekik *et al.* (2013a) in north coast of Sfax after restoration. Cyanobacteriae abundance may have also been favored by the presence of a high concentration of suspended matter ( $r=0.69$ ,  $n=18$ ,  $p<0.05$ ) inducing low water transparency (Wang *et al.*, 2007) and the capacity of *Oscillatoria* sp. blooms to collect dust particles (Rubin *et al.*, 2011) essential for the high iron demand in *Oscillatoria* sp. to ensure important photosynthesis (Roe *et al.*, 2012). Diatoms are valuable indicators of ecological quality as they react directly and sensitively to many nutrients concentrations variations in marine environment (Davies, 2013). Our results showed an increase of diatoms abundance in summer 2010 with the maximum ( $74 \times 10^2$  cells  $l^{-1}$ ) was reached in station 1. In particular, nitrogen seemed to be the most important factors in the coastal environments which may influence the spatial distribution of diatoms. Diatoms abundance was correlated with  $NH_4^+$  concentrations ( $r=0.99$ ,  $n=18$ ,  $p<0.05$ ). Ammonia is a source of nitrogen and participates to the fertility of water since nitrogen is an essential plant nutrient (Feki *et al.*, 2016). The fact that nutrients have the greatest influence on diatoms abundance in our study area is clear from the strong positive correlation between their abundance and total phosphate ( $r=0.64$ ,  $n=18$ ,  $p<0.05$ ) and N/P ratio ( $r=0.98$ ,  $n=18$ ,  $p<0.05$ ). Dominance of the benthic and large diatoms genus such as *Navicula* and *Nitzschia* (Table 2) (Welker *et al.*, 2002) has been already reported in other studies (Feki-Sahnoun *et al.*, 2014). These diatoms species are also found in Kerkennah islands (Ben Brahim *et al.*, 2015a), in the north (Rekik *et al.*, 2013a, 2015a) and south (Rekik *et al.*, 2015c) coast of Sfax and the Gulf of Gabes (Feki-Sahnoun *et al.*, 2014). Benthic and large diatoms are identified by its opportunistic strategy as far as taking advantage of the nutrient accessibility is concerned (Aleya 1992). Concerning dinoflagellates, they were strongly abundant in summer 2009 (36 % of total phytoplankton abundance) than summer 2010 (17 % of total phytoplankton abundance), as repeatedly reported in the Gulf of Gabes (Feki-Sahnoun *et al.*, 2013) and in other Mediterranean marine ecosystems (Anderson *et al.*, 2012). Dinoflagellates are ubiquitous in marine environments. They are also very abundant in southern Tunisia as found by Bel Hassen *et al.* (2008) who proved that the small phytoplankton was the major contributors to the autotrophic abundance. An important number of dinoflagellates species in marine phytoplankton are deprived of chloroplasts (Sherr and Sherr 2007). The percentage of Mixotrophic and heterotrophic dinoflagellates in total dinoflagellates abundance was higher 49 % (summer 2009) - 56 % (summer 2010). Some mixotrophic and heterotrophic dinoflagellates species, like *Polykrikos kofoidii*, *Ceratium lineatum*, *Protoberidinium steinii*, *Protoberidinium depressum*, *Protoberidinium curvipes*, *Protoberidinium divergens*, attained high numbers in our coastal area. Similar observation was shown by Ltaief *et al.* (2015) in the Gulf of Gabes where important proliferation of heterotrophic and mixotrophic dinoflagellates was the distinctive characteristic of the summer cruise. Mixotrophic and heterotrophic *Protoberidinium* species had the potential to consume 30% – 80% (Gribblel *et al.*, 2007) of autotrophic dinoflagellates (Jeong *et al.*, 2010) and diatoms (Sherr and Sherr 2007). In general, food availability may be the most essential factor regulating seasonal dynamics of mixotrophic and heterotrophic dinoflagellates abundance (Ben Brahim *et al.*, 2015b). Mixotrophic and heterotrophic dinoflagellates species may be in competition with zooplankton to feed on autotrophic dinoflagellates and diatoms.

Our investigation showed that ciliate abundance was low in summer 2010, revealing a probable predation by mixotrophic and heterotrophic dinoflagellates and zooplankton (Jeong *et al.*, 2010). Zooplankton seems to be dependent probably on their capacity to exploit a wide range of food resources including phytoplankton and ciliate (Rekik *et al.*, 2015c). Zooplankton is able to complete a top-down control on phytoplankton and ciliate communities (Zervoudaki *et al.*, 2007). On the other hand, the genus *Protoberidinium* is known by feeding exclusively on diatoms (Sherr and Sherr 2007). Thus mixotrophic and heterotrophic dinoflagellates is in direct feeding competition with ciliate and zooplankton (Sherr and Sherr 2007). This competition may constitute another hypothesis explaining the simultaneous presence of mixotrophic and heterotrophic dinoflagellates, ciliate and zooplankton and the correlations recorded between them.

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