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## Space-time variation of ciliates related to environmental factors in 15 nearshore stations of the Gulf of Gabes (Tunisia, Eastern Mediterranean Sea)

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### Abstract

Diversity and structure of ciliate communities in the Gulf of Gabes (Tunisia) were investigated based on a survey of 15 nearshore stations along 237 Km, by monthly sampling over a year. Ciliated protozoa were identified to genus and/or species level and enumerated. Statistical tools were used to explain ciliate assemblage. High ciliate species richness from 63 taxa was recorded, including new records of several species. This study showed a longitudinal distribution of ciliate communities, which are organized at northern (from Tabia to Port of Gabes) and southern stations (from Zarrat to Jabiat Haj Ali). The number of taxa increased significantly at northern stations but decreased at the southern ones. This distribution was mainly influenced by salinity and phytoplankton abundance. Ciliate taxa were grouped into five size-classes: 15-30 µm, 30-50 µm, 50-100 µm, 100-200 µm and >200 µm. In terms of abundance, most abundant size groups were small ciliates (15-30 µm), accounting for 15 to 79 %, while the greatest biomass contribution came from the 50-100 µm size classes. Thus, we conclude that there is a high diversity of ciliate communities with a geographical distribution that is influenced by abiotic and biotic factors along the coast of the Gulf of Gabes.

**Keywords:** Gulf of Gabes, Tunisia, Eastern Mediterranean Sea, size classes, planktonic ciliates, abundance, biomass.

### Introduction

The coastal area of the Gulf of Gabes, which is located along the South-East coast of Tunisia (Eastern Mediterranean Sea), is the consequence of floristic and faunistic disturbance since the end of the 19<sup>th</sup> century (Kchaou *et al.*, 2009). Only a few studies have been conducted in the Gulf of Gabes focussing on plankton communities such as phytoplankton (Bel Hassen *et al.*, 2008, 2009a, b; Drira *et al.*, 2008, 2009, 2010a), copepods (Drira *et al.*, 2010a) and spring ciliate distribution along the coast and in the open sea (Hannachi *et al.*, 2009). The seasonal distribution of the ciliate community, coupled with environmental factors along the coast of the Gulf of Gabes, is investigated at three sampling stations (Tabia, Karboub and the Port of Gabes) (Kchaou *et al.*, 2009). However, data on large scale distribution of ciliate assemblages are lacking, except, for a study on a man-made solar saltern receiving water inputs from the Gulf of Gabes (Elloumi *et al.*, 2006, 2008, 2009a, b).

Marine planktonic ciliates are a major, ubiquitous and diverse group of protozoa and they are divided into tintinnids and non-loricate ciliates, which belong to subclass Oligotrichia and Choreotrichia in class Spirotrichea (Lynn, 2008). Much attention has been given to their role as pri-

mary consumers of pico- and nano-sized producers, as well as nutrient regenerators and an important food source of metazoan zooplankton and fish larvae (Gomez, 2007). It is also important to evaluate the role of physical and chemical processes with respect to the biological responses of the ecosystem (Bel Hassen *et al.*, 2008). Ciliated protozoa (ciliates) play important ecological roles in coastal waters, especially regarding their interaction with environmental parameters (Jiang *et al.*, 2013). Recent studies suggest that ciliates may also be useful indicators of marine water quality (Kchaou *et al.*, 2009; Jiang *et al.*, 2013; Zhang *et al.*, 2013). Therefore, we investigated the species composition, abundance and biomass of ciliates together with abiotic parameters (water temperature, pH, salinity and nutrients) and biotic parameters (phytoplankton abundance) at 15 stations serving for collecting seashells along the coast of the Gulf of Gabes. We hypothesized that: (a) the ciliate community in the Gulf of Gabes would change according to environmental factors, from the northern to the southern stations of the Gulf of Gabes, (b) Spirotrichea would be dominant in the 15 nearshore stations of the Gulf of Gabes, as reported in other Mediterranean area, and (c) species composition, abundance biomass and size group of ciliates would change between the stations. To test these

hypotheses, we analysed the dynamics of ciliate species composition and biomass and their relationships with environmental factors using common statistical methods such as Canonical Correspondence Analysis (CCA), Cluster Analysis (CA), ANOVA and Pearson's correlation to analyse the samples from 15 coast stations during one year (March 2006 to February 2007) in the Gulf of Gabes.

## Material and Methods

### The study area

The Gulf of Gabes is located in the southern Mediterranean Sea and covers the second-widest continental shelf area (35.900 km<sup>2</sup>). The Gulf of Gabes (Between 35°N and 33°N) extends from "Ras Kapoudia" (35°N) to the Tunisian-Libyan border, and shelters various islands (Kerkennah and Djerba) and lagoons (Bougrara and El Bibane) (Fig. 1) (Ben Brahim *et al.*, 2010; Drira *et al.*, 2010a). More detailed characteristics are given in Hattab *et al.* (2013).

### Sampling

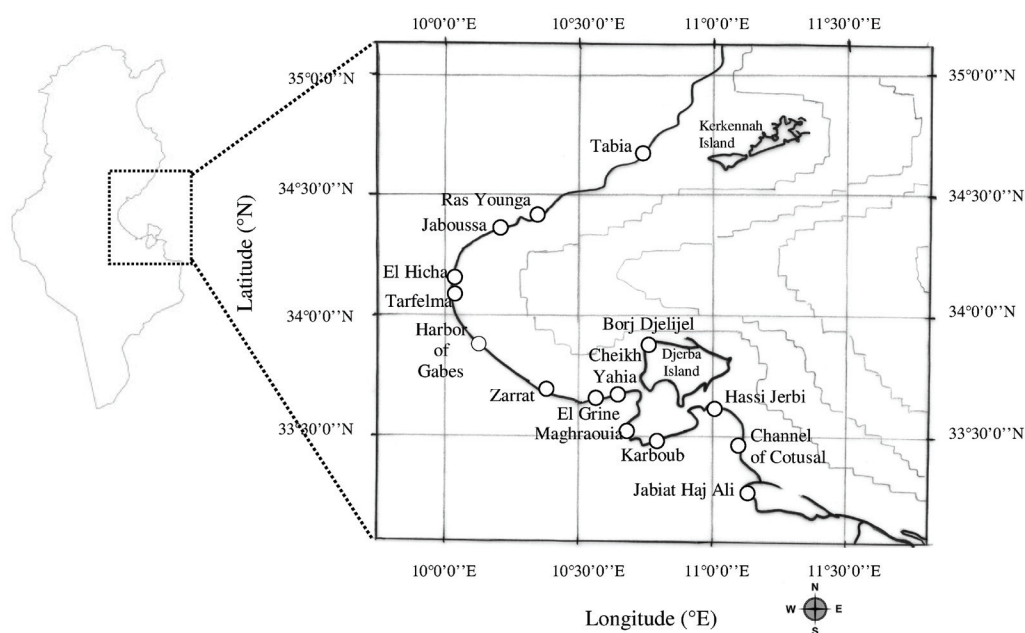
Samples from 15 very shallow (< 1 m) stations were taken monthly, between March 2006 and February 2007 (Fig. 1). Water samples were collected between 10 and 20 cm depth with a Van Dorm bottle. Samples for nutrient analyses were preserved immediately upon collection (-20 °C, in the dark), and those for ciliate and phytoplankton enumeration (200 ml) were preserved with Lugol's iodine solution (2 % final concentration) and stored in the dark at low temperature (4 °C) until analysis. Water samples for chlorophyll-*a* analysis were filtered by vacuum filtration through Whatman GF/F glass fibre filters, and the filters were immediately stored at -20 °C.

### Physico-chemical variables

Temperature and salinity were measured immediately after sampling using a multi-parameter kit (Multi 340 i/SET). Nutrients (NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup>), Total-nitrogen and Total-phosphate (after transformation into NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup>, with nitrogen persulfate and potassium persulfate, respectively at 120 °C) were analysed with a BRAN and LUEBBE type 3 auto-analyser and concentrations were determined colorimetrically using a UV-visible (6400/6405) spectrophotometer (APHA, 1992).

### Phytoplankton and ciliate enumeration

Sub-samples (50 ml) were counted under an inverted microscope after fixation with a Lugol (4%) iodine solution (Bourrelly, 1985) and settling for 24 to 48 hours (Drira *et al.*, 2009) using the Utermöhl (1958) method. At least 200 ciliates were counted for each sample and were identified to genus or species level by consulting the works of Alder (1999), Petz (1999) and Strüder-Kypke & Montagnes (2002). Tintinnids were identified using lorica morphology and species description according to Kofoid & Campbell (1929, 1939), Balech (1959) and Abboud-Abi Saab (2008). The dimensions of 20-40 individual cells for each taxa were measured at × 1250 magnification by image analysis. Mean biovolume of each taxa was estimated from appropriate geometric shapes. Ciliate biovolumes were estimated by geometric approximation and biomass values were calculated using the conversion factor of 0.19 pgC mm<sup>-3</sup> of biovolume (Putt & Stoecker, 1989), which was corrected for shrinkage of Lugol's iodine-fixed cells (Gifford & Caron, 2000). The plasma of tintinnids was assumed to constitute 30 % of the lorica volume (Gilron & Lynn, 1989). Ciliates were divided



**Fig. 1:** Geographical map focussing on the monitoring network ciliates and phytoplankton sampling 15 nearshore stations in Gulf of Gabes. Map from Google earth.

into five size classes: CI (15-30  $\mu\text{m}$ ), CII (30-50  $\mu\text{m}$ ), CIII (50-100  $\mu\text{m}$ ), CIV (100-200  $\mu\text{m}$ ) and CV (> 200  $\mu\text{m}$ ).

### Ciliate community structure

#### Diversity index

The ciliate community structure was studied by calculating the species diversity index  $H'$  (bits cell<sup>-1</sup>) (Shannon & Weaver, 1949).

$$H' = -\sum_{i=1}^{ni} \frac{ni}{N} \log_2 \frac{ni}{N}$$

$ni/N$ : the frequency of species  $i$  in the sample

$N$ : the number of community species

#### Dominance index

The dominance index for the ciliates was calculated according to the formula  $\delta = (n1 + n2)/N$ , where  $\delta$  is the dominance index that was equal to the percentage contribution of the two most important species ( $n1 + n2$ ) of the total standing stock and  $N$  was total individual abundance.

### Chlorophyll-*a* analysis

Sub-samples (0.5 l) for quantification of chlorophyll-*a* concentration, were filtered using Whatman GF/C filters (1.2  $\mu\text{m}$  pore size filter and 25 mm- diameter) and pigment extraction was performed with 90% acetone (Lorenzen, 1967). The concentrations were determined by spectrophotometry, based on the absorbance at 750 and 663 nm. The concentrations were then estimated using the equations of Scor-Unesco (1966).

### Statistical analyses

Mean and standard deviation (Mean  $\pm$  SD) were reported when appropriate. Non-linear data were logarithmically ( $\log_{10}$ ) transformed where it was necessary to satisfy homogeneity of variance (Sokal & Rohlf, 1981). The potential relationships between variables were tested by Pearson's correlation coefficient. ANOVA analysis was applied to identify significant differences in physico-chemical and biological parameters between the 15 sampling stations and probability values were adjusted for the number of simultaneous tests using the sequential Bonferroni method (Rice, 1989).

Cluster analysis (CA) was performed using XLStat to identify the difference between study stations in the distribution of ciliate classes, clustering the samples with similar species composition. Results were illustrated in a dendrogram where steps in the hierarchical clustering solution and values of the squared Euclidean distances between clusters were shown.

The data recorded in this study were submitted to Normalized Canonical Correspondence Analysis (CCA), which was applied to physical (temperature and salinity), chemical ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , T-N, T-P, N/P ratio) and biological parameters (chlorophyll-*a*,  $H'$ , total

phytoplankton abundance, diatoms, dinoflagellates, total ciliate abundance, total ciliate biomass, size classes of ciliate) assessed at 15 stations (Ter-Braak, 1986).

## Results

### Physico-chemical parameters

The annual mean values of physico-chemical parameters recorded at these 15 stations are summarized in Table 1. Average water temperature ranged between 19.9 and 23.4  $^{\circ}\text{C}$  with means showing no significant difference between stations (ANOVA;  $F_{(14,165)} = 0.47$ ;  $p = 0.944$ ) (Table 1). The lowest temperature ( $19.9 \pm 4.0$   $^{\circ}\text{C}$ ,  $n = 12$ ) was recorded at Tarfelma station, while the highest ( $23.4 \pm 5.0$   $^{\circ}\text{C}$ ,  $n = 12$ ) at Tabia station. However, the salinity values differed significantly between stations during the study (ANOVA;  $F_{(14,164)} = 11.71$ ,  $p < 0.001$ ). Water salinity ranged from  $37.2 \pm 1.3$ ,  $n = 12$  at Jaboussa to  $44.7 \pm 2.1$ ,  $n = 12$  at Karboub station. The stations located in the southern part of the Gulf of Gabes showed the highest salinities. Total average nitrogen (T-N) concentrations varied between  $18.4 \pm 6.1$   $\mu\text{M}$ ,  $n = 12$  at the Tabia station and  $25.5 \pm 10.7$   $\mu\text{M}$ ,  $n = 12$  in the Port of Gabes station (Table 1). Nitrate concentrations ( $\text{NO}_3^-$ ) did not differ significantly between stations (ANOVA;  $F_{(14,165)} = 1.41$ ;  $p = 0.151$ ) and were extremely high, with a highest concentration of  $3.6 \pm 2.8$   $\mu\text{mol L}^{-1}$ ,  $n = 12$  at Hassi Jerbi.  $\text{NO}_2^-$  concentrations were rather low (ANOVA;  $F_{(14,164)} = 1.83$ ;  $p = 0.038$ ) and did not exceed 0.9  $\mu\text{M}$  (Table 1). Ammonium concentrations were high at all stations, reaching  $5.7 \pm 5.4$   $\mu\text{M}$ ,  $n = 12$  in the channel of Cotusal and no significant difference in their mean between stations was recorded (ANOVA;  $F_{(14,164)} = 1.62$ ;  $p = 0.080$ ). Total phosphorus and orthophosphate concentrations showed a statistically significant difference ( $P < 0.001$ ) in their mean value between stations (ANOVA;  $F_{(14,164)} = 3.66$  and  $F_{(14,164)} = 2.93$ , respectively). The highest values of total phosphorus were recorded at Zarrat and the Port of Gabes, reaching  $7.3 \pm 5.5$  and  $11.5 \pm 11.2$   $\mu\text{M}$ ,  $n = 12$  (Table 1). However, the highest values of orthophosphate, which did not exceed  $2.2 \pm \mu\text{M}$ ,  $n = 12$ , were recorded in the Port of Gabes (Table 1). The N/P ratio calculated in this study ( $\text{DIN} = \text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+ / \text{DIP} = \text{PO}_4^{3-}$ ) varied from 3.5 at Jaboussa station to 24.5 at Karboub station. For a majority of stations, the N/P ratio was lower than the Redfield ratio (16), except at Karboub, Hassi Jerbi and the channel of Cotusal, where it was 24.5, 19.3 and 19.7 respectively, which suggests a potential P limitation at these stations.

### Composition and structure of the ciliates community

A summary of the ciliate taxa observed during the entire study period at the 15 sampling stations is given in Table 2. During the entire survey period, a total of 63 taxa belonging to 8 classes, namely Spirotrichea, Prostomatea, Colpodea, Litostomatea, Oligohymenophorea, Karyorelectea, Heterotichea and other ciliates were identified. The Spirotrichea was the most dominant group. The mean length and biovol-



**Table 1.** Annual mean  $\pm$  S.D. ( $n=12$ ) of physico-chemical and biological parameters at the 15 nearshore sampling stations along the Gulf of Gabes. In the last row, results of one-way ANOVA analysis. F value: between-groups mean square/within-groups mean square. d.f.: degree freedom \* Significant difference between sampled stations: (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ).

	Physico-chemical parameters										Biological parameters				
	Temperature (°C)	Salinity	NH <sub>4</sub> <sup>+</sup> (µM)	NO <sub>2</sub> <sup>-</sup> (µM)	NO <sub>3</sub> <sup>-</sup> (µM)	PO <sub>4</sub> <sup>3-</sup> (µM)	T-N (µM)	T-P (µM)	N/P ratio	Total ciliate abundance ( $\times 10^3$ cells l <sup>-1</sup> )	Total ciliate biomass (µgC l <sup>-1</sup> )	Total phytoplankton abundance ( $\times 10^3$ cells l <sup>-1</sup> )	Diatoms ( $\times 10^3$ cells l <sup>-1</sup> )	Dinoflagellates ( $\times 10^3$ cells l <sup>-1</sup> )	
Tabia	23.4 $\pm$ 5.0	37.6 $\pm$ 0.6	2.1 $\pm$ 0.9	0.3 $\pm$ 0.1	2.3 $\pm$ 1.0	0.8 $\pm$ 0.4	18.4 $\pm$ 6.1	6.7 $\pm$ 5.3	6.4	1.3 $\pm$ 1.7	0.9 $\pm$ 1.6	3.8 $\pm$ 3.7	2.6 $\pm$ 3.9	1.1 $\pm$ 1.0	
Ras Younga	22.0 $\pm$ 5.2	38.6 $\pm$ 1.1	3.3 $\pm$ 2.6	0.4 $\pm$ 0.4	1.7 $\pm$ 1.1	0.7 $\pm$ 0.3	19.4 $\pm$ 5.0	5.0 $\pm$ 2.9	7.8	0.3 $\pm$ 0.2	0.7 $\pm$ 0.0006	4.7 $\pm$ 9.2	4.1 $\pm$ 9.1	0.5 $\pm$ 0.5	
Jaboussa	21.1 $\pm$ 4.9	37.2 $\pm$ 1.3	2.9 $\pm$ 2.3	0.2 $\pm$ 0.2	1.5 $\pm$ 1.2	1.3 $\pm$ 1.3	21.5 $\pm$ 6.9	5.3 $\pm$ 5.2	3.5	0.3 $\pm$ 0.2	0.6 $\pm$ 0.0004	2.2 $\pm$ 2.1	1.5 $\pm$ 1.2	0.7 $\pm$ 1.2	
El Hicha	20.0 $\pm$ 4.2	37.8 $\pm$ 2.9	3.7 $\pm$ 1.9	0.5 $\pm$ 0.7	3.0 $\pm$ 0.9	1.0 $\pm$ 1.1	20.1 $\pm$ 5.6	4.1 $\pm$ 3	7.4	0.6 $\pm$ 0.8	1.2 $\pm$ 0.002	3.0 $\pm$ 3.0	2.3 $\pm$ 2.3	0.5 $\pm$ 0.6	
Tarfelma	19.9 $\pm$ 4.0	37.6 $\pm$ 3.0	3.5 $\pm$ 2.3	0.6 $\pm$ 0.5	2.8 $\pm$ 1.9	0.6 $\pm$ 0.7	22.7 $\pm$ 4.9	3.7 $\pm$ 2.2	10.8	1.0 $\pm$ 3.6	1.5 $\pm$ 0.007	2.7 $\pm$ 2.8	1.5 $\pm$ 1.7	0.6 $\pm$ 0.7	
Harbor of Gabes	20.9 $\pm$ 4.0	36.7 $\pm$ 1.0	4.0 $\pm$ 2.4	0.6 $\pm$ 0.5	2.6 $\pm$ 2.6	2.2 $\pm$ 2.7	25.5 $\pm$ 10.7	11.5 $\pm$ 11.2	15.8	0.9 $\pm$ 0.7	1.9 $\pm$ 3.7	8.4 $\pm$ 4.2	7.1 $\pm$ 4.0	1.2 $\pm$ 0.6	
Zarrat	20 $\pm$ 7.2	37.5 $\pm$ 3.4	3.3 $\pm$ 1.9	0.4 $\pm$ 0.3	2.2 $\pm$ 1.1	1.3 $\pm$ 0.9	20.1 $\pm$ 6.1	7.3 $\pm$ 5.5	4.5	0.5 $\pm$ 0.5	1.8 $\pm$ 0.002	6.0 $\pm$ 11.5	5.0 $\pm$ 11.6	0.6 $\pm$ 0.5	
El Grine	20.5 $\pm$ 4.4	39.1 $\pm$ 1.7	3.8 $\pm$ 3.3	0.4 $\pm$ 0.3	1.7 $\pm$ 0.9	0.9 $\pm$ 1.0	19.1 $\pm$ 5.7	3.8 $\pm$ 1.7	6.3	0.6 $\pm$ 4.2	0.9 $\pm$ 0.0008	3.2 $\pm$ 3.7	1.4 $\pm$ 1.8	1.8 $\pm$ 3.5	
Cheikh Yahia	20.7 $\pm$ 4.4	38.3 $\pm$ 2.0	3.4 $\pm$ 2.0	0.9 $\pm$ 1.6	2.6 $\pm$ 1.3	1.1 $\pm$ 0.9	21.2 $\pm$ 9.1	5.5 $\pm$ 3.3	6.2	0.7 $\pm$ 0.3	2.2 $\pm$ 0.001	2.0 $\pm$ 2.0	1.2 $\pm$ 1.4	0.8 $\pm$ 0.8	
Maghraouia	20.4 $\pm$ 4.3	38.8 $\pm$ 1.5	3.8 $\pm$ 2.6	0.2 $\pm$ 0.1	2.3 $\pm$ 2.8	1.0 $\pm$ 0.9	20.6 $\pm$ 9.7	4.8 $\pm$ 1.9	6.2	0.6 $\pm$ 0.5	1.9 $\pm$ 0.002	13.1 $\pm$ 21.9	1.3 $\pm$ 1.7	10.7 $\pm$ 21.2	
Karboub	21.0 $\pm$ 4.5	44.7 $\pm$ 2.1	5.6 $\pm$ 2.7	0.8 $\pm$ 0.7	3.2 $\pm$ 0.7	0.5 $\pm$ 0.3	23.1 $\pm$ 6.1	4.2 $\pm$ 1.1	24.5	6.3 $\pm$ 14.2	8.7 $\pm$ 19.8	31.6 $\pm$ 40.1	0.1 $\pm$ 0.4	31.5 $\pm$ 40.1	
Borj Djeljel	20.5 $\pm$ 4.4	38.7 $\pm$ 1.8	2.6 $\pm$ 1.7	0.4 $\pm$ 0.4	3.4 $\pm$ 3.4	0.6 $\pm$ 0.4	22.0 $\pm$ 6.0	4.2 $\pm$ 3.3	10.1	0.3 $\pm$ 0.3	0.9 $\pm$ 0.001	4.2 $\pm$ 8.5	1.2 $\pm$ 1.5	3.0 $\pm$ 7.7	
Hassi Jerbi	21.3 $\pm$ 4.4	42.5 $\pm$ 4.4	5.0 $\pm$ 3.7	0.7 $\pm$ 0.8	3.6 $\pm$ 2.8	0.5 $\pm$ 0.4	25.5 $\pm$ 7.7	2.5 $\pm$ 1.8	19.3	0.5 $\pm$ 0.7	1.0 $\pm$ 0.003	1.9 $\pm$ 2.3	1.0 $\pm$ 1.4	0.9 $\pm$ 1.2	
Channel of Cotalal	20.3 $\pm$ 4.4	42.0 $\pm$ 5.2	5.7 $\pm$ 5.4	0.5 $\pm$ 0.5	2.9 $\pm$ 2.1	0.5 $\pm$ 0.3	23.7 $\pm$ 7.9	3.0 $\pm$ 2.1	19.7	0.4 $\pm$ 0.3	0.6 $\pm$ 0.005	4.3 $\pm$ 5	1.0 $\pm$ 0.9	3.3 $\pm$ 4.7	
Jabiat Haj Ali	20.4 $\pm$ 4.5	42.1 $\pm$ 4.4	3.9 $\pm$ 3.2	0.5 $\pm$ 0.5	2.2 $\pm$ 1.9	0.8 $\pm$ 0.7	20.4 $\pm$ 7.4	3.3 $\pm$ 1.8	8.4	1.6 $\pm$ 1.5	2.7 $\pm$ 0.003	1.8 $\pm$ 1.8	0.9 $\pm$ 0.9	0.9 $\pm$ 1.0	
F-values (d.f)	0.47(165)	11.70***(164)	1.61(164)	1.82*(164)	1.41(164)	2.93*** (164)	1.65(164)	3.66*** (164)	2.56***(164)	1.63 (162)	1.02 (162)	1.82*(163)	1.2(163)	2.25***(162)	

**Table 2.** Species composition (Lynn and Small, 1997), mean length ( $\mu\text{m}$ ) and biovolume ( $\mu\text{m}^3$ ) of the ciliate community at the 15 nearshore sampling stations along the Gulf of Gabes.

Class	Subclass	Order	Species	Length ( $\mu\text{m}$ )	Biovolume ( $\times 10^3 \mu\text{m}^3$ )
Spirotrichea	Oligotrichia	Strombidiida	<i>Strombidium</i> spp. (Claparède & Lachmann, 1859)	33	4.5
	Choreotrichia	Choreotrichida	<i>Strobilidium</i> spp. (Schewiakoff, 1892)	27	3.2
			<i>Strombidinopsis acuminatum</i> (Fauré-Fremiet, 1924)	74	28.5
			<i>Strombidinopsis</i> sp. (Schewiakoff, 1892)	40	7.9
			<i>Pelagostrobilidium</i> sp. (Petz, Song & Wilbert, 1995)	59	48.6
			<i>Halteria</i> sp. (Dujardin, 1841)	20	1.3
			<i>Leegaardiella</i> spp. (Lynn & Montagnes, 1988)	20	2
			<i>Lohmanniella oviformis</i> (Leegaard, 1915)	17	1.1
			<i>Tontonia gracillima</i> (Fauré-Fremiet, 1924)	33	3.4
		Tintinnida	<i>Tintinnopsis</i> spp. (Stein, 1867)	81	16.2
			<i>Tintinnidium</i> spp. (Kent, 1881)	78	28.5
			<i>Ascampbelliella</i> spp. (Corliss, 1960)	61	30.7
			<i>Acanthostomella</i> sp. (Jørgensen, 1927)	91	51.4
			<i>Favella</i> spp. (Brandt, 1906)	114	116.4
			<i>Helicostomella</i> spp. (Jørgensen, 1924)	159	26.5
			<i>Codonella</i> spp. (Haeckel, 1873)	59	36.8
			<i>Codonellopsis</i> spp. (Jørgensen, 1924)	93	57.4
			<i>Codonaria</i> sp. (Kofoid & Campbell, 1939)	69	52
			<i>Clevea melchersi</i> (Balech, 1948)	150	3.9
			<i>Stenosemella avellana</i> (Meunier, 1919)	93	94.5
			<i>Salpingella</i> spp. (Jørgensen, 1924)	112.5	2.2
			<i>Salpingacantha</i> sp. (Kofoid & Campbell, 1929)	245	10
			<i>Undella columbiana</i> (Wailes, 1925)	40	6.5
			<i>Rhabdonella spiralis</i> (Fol, 1881)	300	306.6
			<i>Dadayiella ganymedes</i> (Entz Sr., 1884)	98	23
			<i>Eutintinnus fraknoi</i> (Daday, 1887)	101	9.3
			<i>Epiplocylis undella</i> (Ostenfeld and Schmidt, 1901)	125	149
			<i>Metacylis jørgenseni</i> (Cleve, 1902)	50	32.1
			<i>Ormosella acantharus</i> (Kofoid and Campbell, 1929)	150	24.5
			<i>Poroecus apiculatus</i> (Cleve, 1899) Cleve, 1902	100	41.9
			<i>Xystonellopsis</i> sp. (Jørgensen, 1924)	78	28.5
			<i>Luminella parvicollis</i> (Marshall, 1934)	25	4.1
	Hypotrichia	Euplodida	<i>Euplote charon</i> (Bory, 1824) Berlin, 1830	60	52.7
Prostomatea		Prostomatida	<i>Urotricha</i> sp. (Claparède & Lachmann, 1858)	19	1.6
			<i>Balanion comatum</i> (Wulff, 1919)	23	2.2
			<i>Bursellopsis</i> sp. (Corliss, 1960)	88	100.5
		Prorodontida	<i>Coleps</i> sp. (Nitzsch, 1827)	38	9.9
Colpodea		Colpodida	<i>Colpoda</i> sp. (Müller, 1773)	27	3.3
Litostomatea	Haptoria	Haptorida	<i>Mesodinium rubrum</i> (Lohmann, 1908)	25	1.9

(continued)

Table 2 (continued)

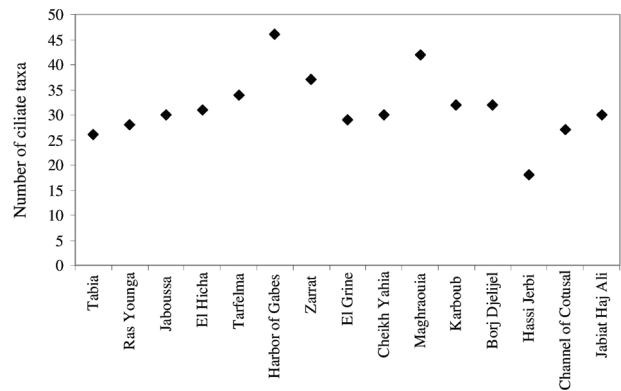
Class	Subclass	Order	Species	Length ( $\mu\text{m}$ )	Biovolume ( $\times 10^3 \mu\text{m}^3$ )
			<i>Enchelyodon laevis</i> (Quennerstedt, 1867)	59	3.5
			<i>Didinium</i> sp. (Stein, 1859)	36	13.3
			<i>Monodinium balbianii</i> (Fabre-Domergue, 1888)	38	6.1
Oligohymenophorea	Scuticociliatia	Philasterida	<i>Uronema marinum</i> (Dujardin, 1841)	25	2.1
			<i>Philaster hiatti</i> (Thompson, 1969)	48	12.8
		Scuticociliatida	<i>Cyclidium glaucoma</i> (Müller, 1773)	30	5.2
			<i>Pleuronema</i> sp. (Dujardin, 1836)	25	2
			<i>Scuticociliate</i> sp.	18	1.4
	Peritrichia	Sessilida	<i>Phascolodon vorticella</i> (Stein, 1859)	43	15
			<i>Vorticella fromenteli</i> (Kahl, 1935)	180	233.6
	Peniculia	Peniculida	<i>Urocentrum</i> sp. (Nitzsch, 1827)	25	6.6
			<i>Paramecium</i> sp. (Müller, 1773)	62	20.7
Karyorelectea		Loxodida	<i>Aspidisca lynceus</i> (Ehrenberg, 1830)	48	13.6
			<i>Loxode</i> sp. (Ehrenberg, 1833)	74	6.8
Heterotrichea		Heterotrichida	<i>Fabrea salina</i> (Henneguy, 1889)	75	44.3
			<i>Blepharisma japonicum</i> (Suzuki, 1954)	116	25.1
			<i>Spirostomum teres</i> (Claparède & Lachmann, 1859)	235	7.1
Phyllopharyngea		Cyrtophorida	<i>Dysteria</i> spp. (Faria et al., 1922)	53	21.9
Other ciliates			<i>Acineta tuberosa</i> (Pallas, 1766)	75	18.4
			<i>Chlamydonella</i> sp. (Deroux, 1970)	28	3.6
			<i>Meseres</i> sp. (Schewiakoff, 1893)	25	1.5
			<i>Tiarina fusus</i> (Claparède & Lachmann, 1858) Bergh, 1880	33	2.7
			<i>Tracheloraphis phoenicopterus</i> (Cohn, 1866)	698	57
			<i>Urozona buetschlii</i> (Schewiakoff, 1889).	38	14.8

ume values for each species of ciliate recorded during the entire study period at the 15 sampling stations are summarized in Table 2. Ciliates ranged in length from 17  $\mu\text{m}$  (one Choreotrichida species *Lohmanniella oviformis*) to 698  $\mu\text{m}$  (*Tracheloraphis phoenicopterus*). In terms of biovolume, ciliate species ranged from  $0.8 \times 10^3 \mu\text{m}^3$  (one Strombidiida *Strombidium dalum*) to  $701.2 \times 10^3 \mu\text{m}^3$  (one species undetermined) (Table 2). Figure 2 shows that the number of ciliate taxa ranged between 18 and 46 at Hassi Jerbi and the Port of Gabes, respectively. Moreover, the number of taxa increased significantly at the northern stations from Tabia to the Port of Gabes (Linear regression;  $r^2 = 0.79$ ). However, from the Port of Gabes to southern station Jabiat Haj Ali the number of taxa decreased (Linear regression;  $r^2 = 0.44$ )

(Fig. 2). Figure 3 shows that the spatial distribution of the percentage of each protozooplankton size class is expressed according to abundance and biomass. For the size classes expressed according to abundance, the largest species ( $> 200 \mu\text{m}$ ) prevailed at Maghraouia station while the smallest ones (15-30  $\mu\text{m}$ ) were concentrated at Karboub station, reaching 13 % of total size class (Fig. 3a). At most stations, the ciliate community was dominated by the 15-30  $\mu\text{m}$  size class, which accounted for 15 to 79 % of the total size class. It was followed by the 30-50  $\mu\text{m}$  and 50-100  $\mu\text{m}$  size class, which accounted for 8 to 51 % and 12 to 48 % of total size class, respectively. The contribution of the largest species (larger than 100  $\mu\text{m}$ ) did not exceed 14 % of total ciliate abundance (Fig. 3a). However, at most stations, the great-

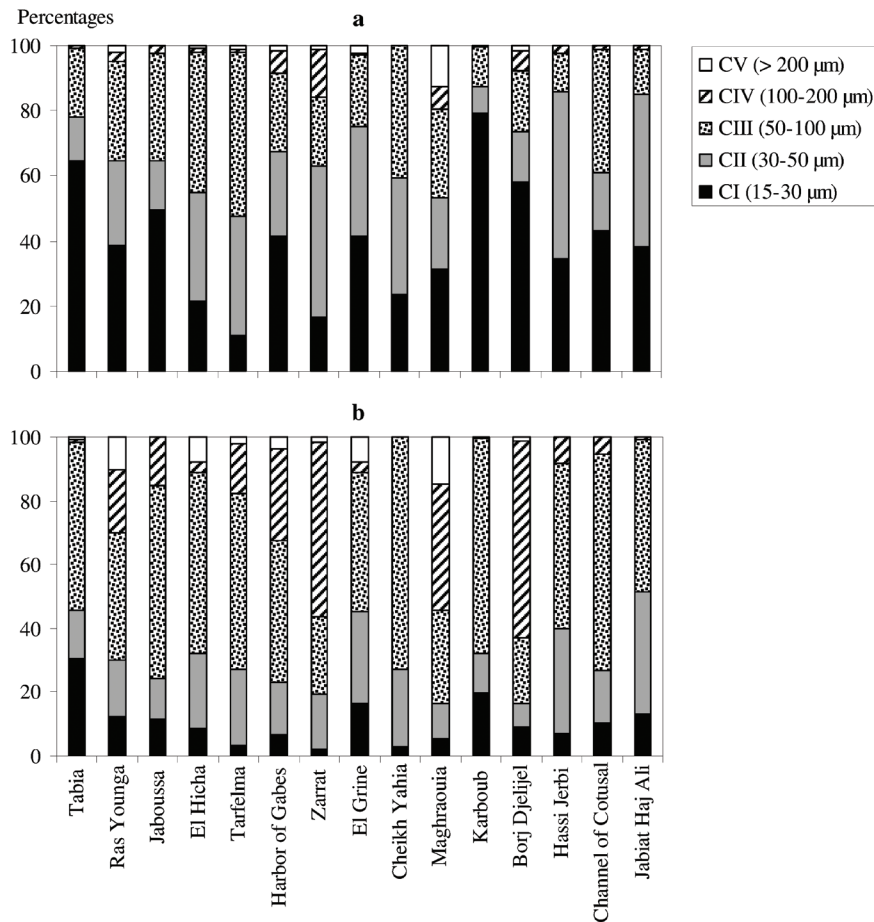
est biomass contribution came from the 50-100  $\mu\text{m}$  size class, which accounted for 21 to 73 % of total biomass production (Fig. 3b). It was followed by the 100-200  $\mu\text{m}$  and 30-50  $\mu\text{m}$  size classes, which accounted for 1 to 62 % and 7 to 38 % of total biomass production, respectively. The contribution of the smallest species from 15- 30  $\mu\text{m}$  was the lowest, varying from 2 to 30% of total biomass production. The largest species ( $> 200 \mu\text{m}$ ) prevailed at Maghraouia and Ras Younga stations, accounting for 15 and 10 % of total biomass production, respectively (Fig. 3b). The ANOVA test to which the Bonferroni correction was applied, showed that for both abundance and biomass of the five studied size classes of ciliates differed significantly among the sampled stations (For abundance,  $F_{(4,70)} = 30.98$ ;  $p = 0.000$ ; For Biomass,  $F_{(4,70)} = 28.11$ ;  $p = 0.000$ ).

The means of the Shannon Weaver biodiversity index ranged from 0.80 (at Hassi Jerbi) to 2.43 (Port of Gabes) (Fig. 4a). Changes of this index showed a difference in species diversity between northern (Tabia - Port of Gabes) and southern stations (Zarrat - channel of Cotusal) of the Gulf of Gabes. Results of one way ANOVA indicate that the mean diversity for northern stations is significantly higher than that of the southern stations (ANOVA;  $F_{(1,178)} = 15.1$ ;  $p = 0.000$ ). The highest values of index diversity were recorded at the Port of Gabes



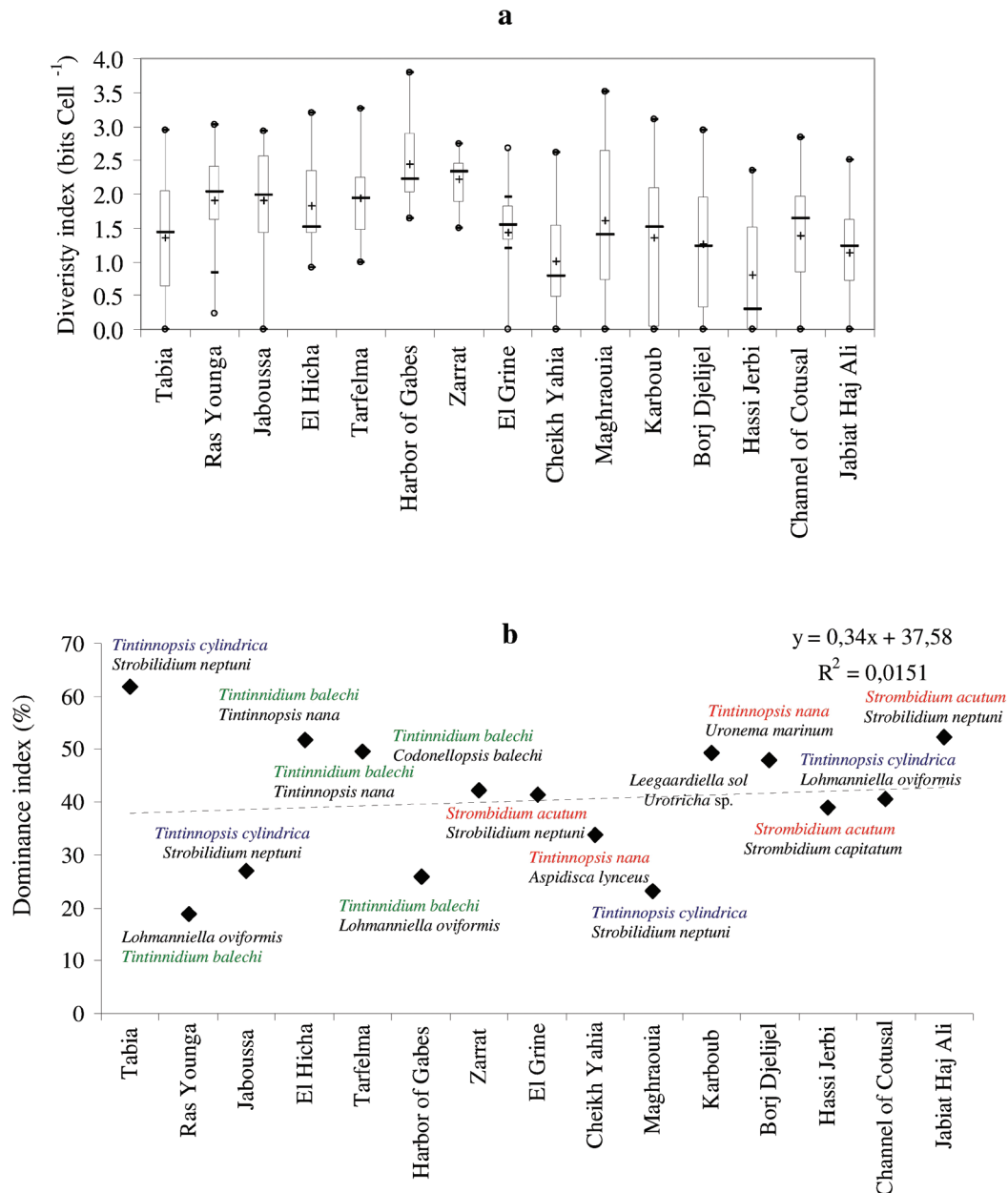
**Fig. 2:** Distribution of the number of ciliate taxa along the coast of the Gulf of Gabes.

station and varied from 1.6 to 3.8 bits  $\text{cell}^{-1}$ , while the lowest were recorded at Hassi Jerbi and varied from 0 to 2.35 bits  $\text{cell}^{-1}$  (Fig. 4a). During spring, the ciliate community had a high biodiversity value, with the lowest index characterizing summer and autumn. Figure 4b shows that *Tintinnidium balechi* dominated in the northern area, while *Strombidium acutum* proliferated in the southern region. We also recorded that *Tintinnopsis cylindrica* was distributed homogenously in both the northern and southern area along the coast of the Gulf of Gabes (Fig. 4b).



**Fig. 3:** Spatial variation of percentage abundance (a) and biomass (b) of five different size classes of ciliates in 15 nearshore stations in Gulf of Gabes. Size classes of ciliates: CI (15-30  $\mu\text{m}$ ), CII (30-50  $\mu\text{m}$ ), CIII (50-100  $\mu\text{m}$ ), CIV (100-200  $\mu\text{m}$ ) and CV ( $> 200 \mu\text{m}$ ).





**Fig. 4:** (a) Box plots showing the mean difference of diversity index of ciliate community in 15 nearshore stations in Gulf of Gabes. (b) Relationships between the dominance index and station. Dashed line represent the linear regressions of the dominant ciliate in 15 nearshore stations in Gulf of Gabes. Y: equation of linear regression, R<sup>2</sup>: coefficient of determination.

### Ciliate community dynamics

Total ciliate abundance varied between  $0.32 \pm 0.2 \times 10^3$  ind l<sup>-1</sup>,  $n = 12$  at Ras Younga, Jaboussa and Borj Djeljel stations and  $6.3 \pm 14.2 \times 10^3$  ind l<sup>-1</sup>,  $n = 12$  at Karboub station (Mean  $\pm$  SD =  $1.1 \pm 1.5 \times 10^3$  ind l<sup>-1</sup>;  $n = 15$ ) (Table 1). Total ciliate biomass ranged between  $0.6 \pm 0.005$   $\mu$ gC l<sup>-1</sup>,  $n = 12$  at Jaboussa and the channel of Cotusal stations and  $8.7 \pm 19.8$   $\mu$ gC l<sup>-1</sup>,  $n = 12$  at Karboub station (Mean  $\pm$  SD =  $1.8 \pm 2.0$   $\mu$ gC l<sup>-1</sup>;  $n = 15$ ) (Table 1). No significant difference in the means of both total abundance and biomass of ciliate taxa was found between stations (ANOVA; for abundance  $F_{(14,162)} = 1.64$ ;  $p = 0.074$ ; for biomass  $F_{(14,162)} = 1.02$ ;  $p = 0.433$ ) (Table

1). But a positive significant correlation was recorded between ciliate abundance and biomass (Pearson's correlation,  $r_p = 0.96$ ;  $N = 15$ ;  $p < 0.001$ ) (Table 3). Figure 4 shows that ciliate abundance and biomass fluctuated markedly at a seasonal scale as well as a spatial scale. There were several peaks, mainly during the warm-dry season (May-August, September-November) while both the lowest cell density and biomass occurred during the cool-wet season (December to February). The southern station, Jabiat Haj Ali, showed the highest ciliate densities during the four seasons, ranging from 2000 to 4200 cells l<sup>-1</sup>. Moreover, the highest concentrations appeared at Karboub Station, in the south of the Gulf of Gabes, which

**Table 3.** Correlation matrix for the physical, chemical and biological parameters under study at the 15 nearshore sampling stations along the Gulf of Gabes. Number of variables:  $n = 15$ . Chl *a*: Chlorophyll-*a*, H': Diversity index (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ).

	T (°C)	Salinity	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup>	T-N	T-P	N/P	Total ciliate abundance	Total ciliate biomass	Total phytoplankton abundance	Dinoflagellates	Ciliate length	H'	Chl <i>a</i>
Temperature	1															
Salinity	-0.041	1														
NH <sub>4</sub> <sup>+</sup>	-0.325	<b>0.782***</b>	1													
NO <sub>2</sub> <sup>-</sup>	-0.168	0.429	0.505	1												
NO <sub>3</sub> <sup>-</sup>	-0.196	0.438	0.436	<b>0.570*</b>	1											
PO <sub>4</sub> <sup>3-</sup>	-0.071	<b>-0.615*</b>	-0.259	-0.110	-0.351	1										
T-N	-0.232	0.332	<b>0.612*</b>	0.505	<b>0.623*</b>	0.113	1									
T-P	0.217	<b>-0.583*</b>	-0.325	-0.067	-0.244	<b>0.881***</b>	0.087	1								
N/P	-0.018	<b>0.734**</b>	<b>0.827***</b>	<b>0.577*</b>	<b>0.681**</b>	-0.290	<b>0.762***</b>	-0.152	1							
Total ciliate abundance	0.095	<b>0.628*</b>	0.453	0.441	0.272	-0.234	0.151	-0.064	<b>0.581*</b>	1						
Total ciliate biomass	-0.063	<b>0.604*</b>	0.479	0.504	0.283	-0.137	0.190	-0.016	<b>0.539*</b>	<b>0.967***</b>	1					
Total phytoplankton abundance	0.017	0.501	0.473	0.243	0.234	-0.091	0.194	0.082	<b>0.552*</b>	<b>0.879***</b>	<b>0.898***</b>	1				
Diatoms	0.118	0.357	0.382	0.363	0.172	0.106	0.221	0.319	<b>0.542*</b>	<b>0.855***</b>	<b>0.885***</b>	<b>0.885***</b>	1			
Dinoflagellates	-0.004	<b>0.622*</b>	<b>0.515*</b>	<b>0.275</b>	0.287	-0.265	0.178	-0.124	<b>0.574*</b>	<b>0.910***</b>	<b>0.972***</b>	<b>0.972***</b>	<b>0.802***</b>	1		
Ciliate length	-0.040	-0.497	-0.235	<b>-0.548*</b>	-0.253	0.194	-0.202	0.255	-0.275	-0.312	0.036	0.036	-0.101	1		
H'	-0.172	<b>-0.630*</b>	-0.232	-0.340	-0.398	<b>0.658**</b>	-0.033	<b>0.665**</b>	-0.246	-0.163	-0.130	0.038	-0.153	<b>0.670**</b>	1	
Chl <i>a</i>	-0.120	-0.241	0.006	0.004	-0.220	<b>0.741**</b>	0.342	<b>0.624*</b>	0.068	-0.002	0.028	0.028	-0.088	-0.003	0.442	1

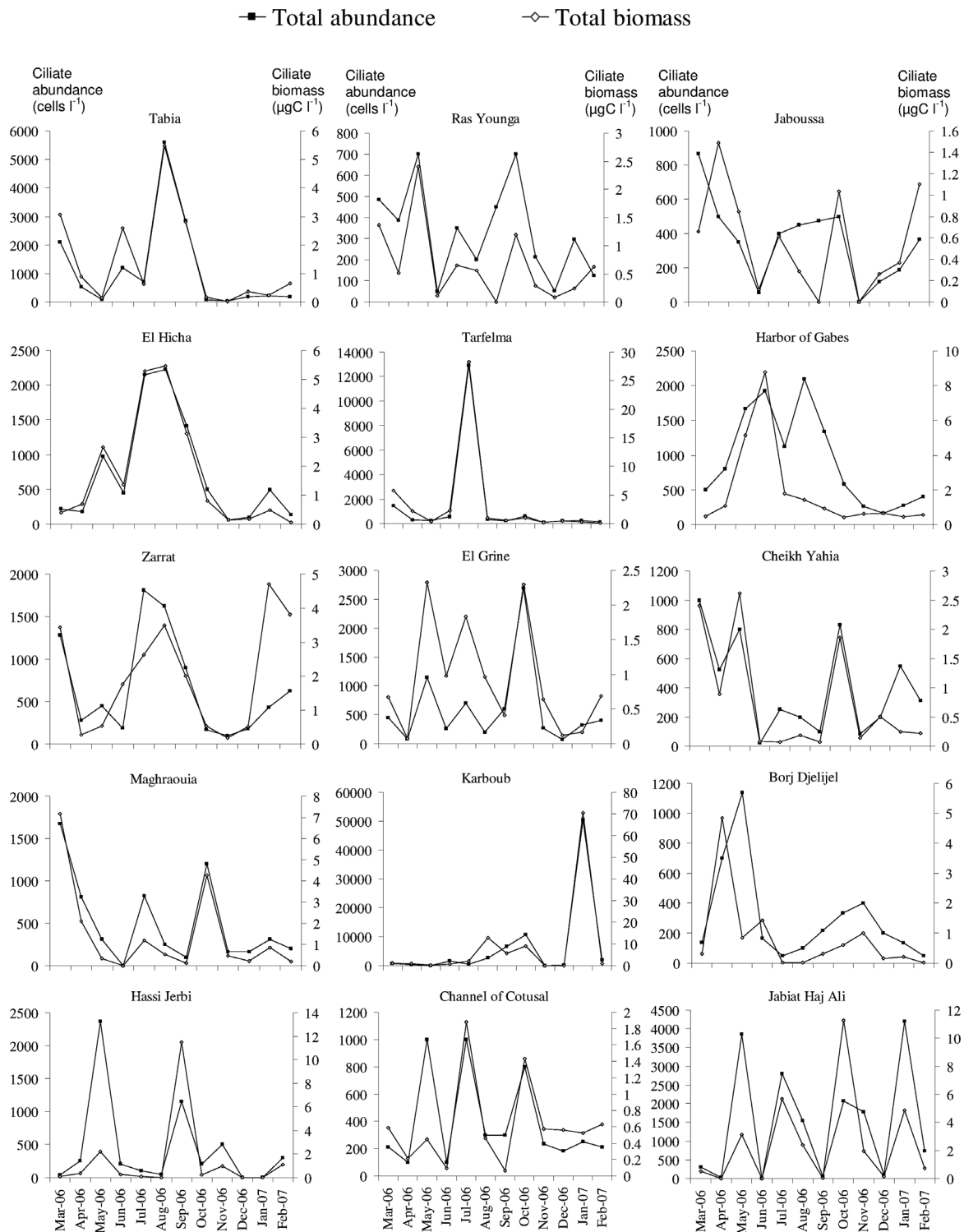
reached  $5 \times 10^4$  cells l<sup>-1</sup> (Fig. 5). The ciliate density in Ras Younga and Jaboussa did not exceed 900 cells l<sup>-1</sup>.

Figure 6 showed the percentage contribution of the most abundant taxa to the total ciliate abundance and biomass. Spirotrichea were the most numerous ciliates at all stations (> 60 % of total abundance) except Karboub where they represented 36.6 % and 24.6 % of total abundance and biomass, respectively (Fig. 6a, 6b). Moreover, the contribution of the other classes at the southern stations (from Cheikh Yahia to Borj Djeljel) was higher than at the northern ones (from Tabia to El Grine). Tintinnida, which represented the loricate Spirotrichea, were the most abundant order, composed by 58 genera/species (Table 2). They represented 2.6 to 30 % of total Spirotrichea at Karboub and Jabiat Haj Ali, respectively. However, the Tintinnida accounted for 2.0 to 83 % of total ciliate at Karboub and Tarfelma, respectively. At these stations, the naked ciliates dominated and represented 98 and 84 % of total ciliates, respectively. At the other stations, the relative contribution of naked ciliates was low and varied from 17.02 and 73.79 % of total ciliate abundance.

The Spirotrichea class was followed by the Oligohy-  
menophorea, with the highest percentage (30 %) of total abundance recorded at Borj Djeljel. The Heterotrichea class, composed mainly of *Fabrea salina*, was only recorded at Karboub, contributing to 10 % and 50 % of total abundance and biomass, respectively (Fig. 6a, 6b). Karyolectea and Colpodea were the least abundant classes; they did not exceed 10 % of the total ciliate abundance and biomass (Fig. 6a, 6b).

### Phytoplankton composition

The phytoplankton community of the Gulf of Gabes was dominated by diatoms and dinoflagellates (Fig. 7). Total phytoplankton abundance varied from  $1.8 \pm 1.8 \times 10^3$  cells l<sup>-1</sup>,  $n = 12$  at Jabiat Haj Ali station to  $31.6 \pm 40.1 \times 10^3$  cells l<sup>-1</sup>,  $n = 12$  at Karboub station (Table 2). Statistically, total phytoplankton abundance shows a significant difference in the mean value between stations (ANOVA;  $F_{(14,163)} = 1.82$ ;  $p = 0.040$ ). At the most stations, diatoms dominated the phytoplankton community, while Karboub, Maghraouia, the Channel of Cotusal and Borj Djeljel stations were dominated by dinoflagellates (99.51, 81.72, 75.82 and 71.54 % of total phytoplankton abundance) (Fig. 7). Dinoflagellate abundance showed a significant difference between stations (ANOVA;  $F_{(14,162)} = 2.25$ ;  $p = 0.008$ ). Chlorophyll-*a* concentration was slightly more important at the northern stations ( $3.0 \pm 2.2$  mg m<sup>-3</sup>;  $n = 6$ ) than at the southern ones ( $2.4 \pm 0.8$  mg m<sup>-3</sup>;  $n = 9$ ). However, ANOVA shows that there was no significant difference between the chlorophyll-*a* concentration at northern and southern stations (ANOVA;  $F_{(1,13)} = 0.47$ ;  $p = 0.503$ ). A significant positive correlation was recorded between the total phytoplankton abundance and Chlorophyll-*a* concentration at the northern stations (Pearson's correlation;  $r_p = 0.77$ ;  $N = 6$ ;  $p = 0.069$ ), while the total phytoplankton abundance and the Chlorophyll-*a*

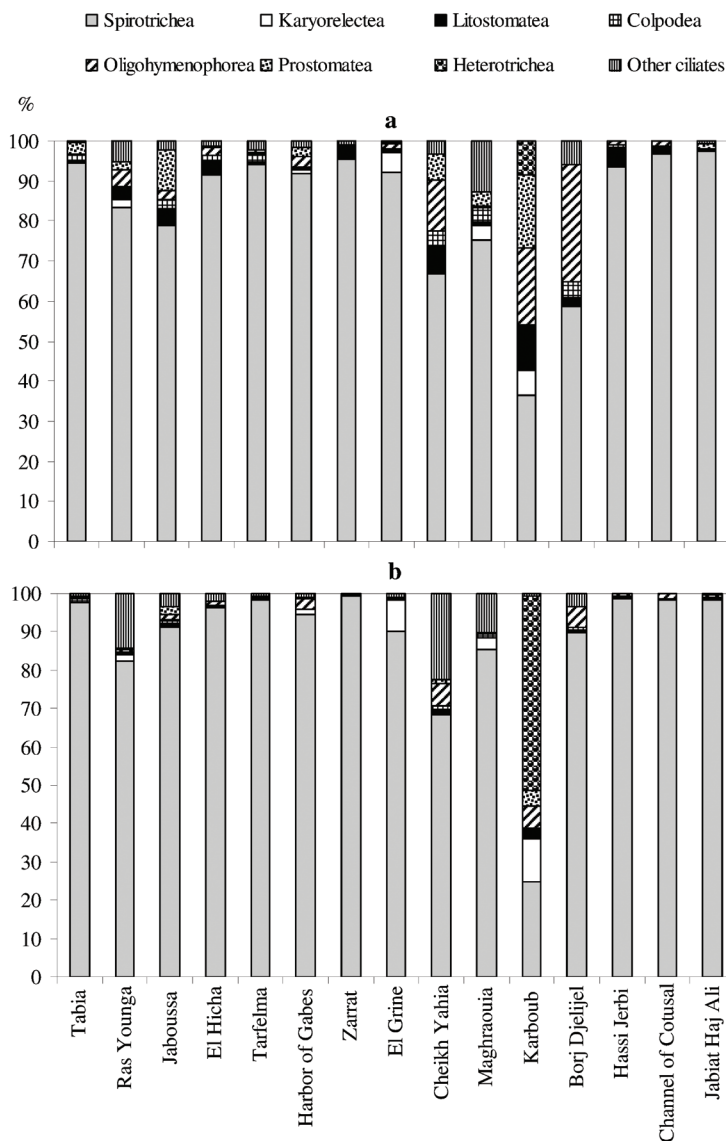


**Fig. 5:** Spatial and temporal distribution of the total abundance (cells l<sup>-1</sup>) and biomass (µgC l<sup>-1</sup>) ciliates in 15 nearshore stations in Gulf of Gabes.

concentration at the southern stations was negatively correlated (Pearson's correlation;  $r_p = -0.12$ ;  $N = 9$ ;  $p = 0.760$ ).

A summary of the dinoflagellate taxa observed during the entire study period at the 15 sampling stations is given in Table 4. During the entire survey period, a total of 37 taxa were identified. The mean length and biovolume values for each ciliate species recorded during the

entire study period at the 15 sampling areas are summarized in Table 4. Dinoflagellates ranged in length from 22.5 µm (*Protoperdinium* sp.) to 450.62 µm (*Neoceratium macroceros*). In terms of biovolume, dinoflagellate species varied from  $1.04 \times 10^3 \mu\text{m}^3$  (*Podolampas* sp.) to  $621.54 \times 10^3 \mu\text{m}^3$  (*Neoceratium macroceros*), representing (5%) autotrophic dinoflagellate taxa (Table 4).



**Fig. 6:** Relative contribution (%) of ciliates groups to total ciliates abundance (a) and biomass (b) in 15 nearshore stations in Gulf of Gabes.

### Statistical analysis

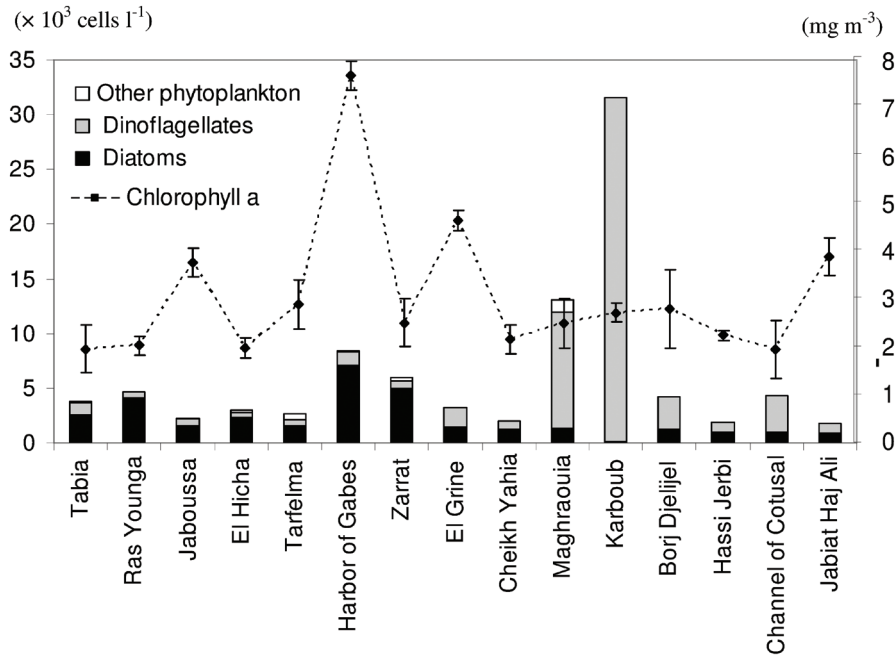
The correlation matrix for the physical, chemical and biological variables recorded during this study is summarized in Table 3. Canonical correspondence analysis (CCA) was performed for total ciliate abundance and biomass, total phytoplankton abundance, ciliate size class, environmental variables and different stations (Fig. 8a). A positive relationship was shown between 4 size classes of ciliates (CII, CIII, CIV and CV), T-P,  $PO_4^{3-}$  and 14 stations in the Gulf of Gabes on the first CCA axis. However, a negative relationship was shown between total ciliate abundance and biomass, CI (15-30  $\mu\text{m}$ ), total phytoplankton;  $N-NH_4^+$ ,  $N-NO_2^-$ ,  $N-NO_3^-$ , T-N, N/P, temperature, salinity and Karboub station (Fig. 8a).

the distribution of sample scores indicates clearly identical characteristics between stations, with the exception of Karboub, which exhibit clearly different characteristics. The cluster analysis dendrogram illustrates 3 groups (Fig. 8b).

### Discussion

#### Physico-chemical variables

Our results agree with and complement other studies performed in arid to semi-arid Mediterranean areas, which showed higher values for both temperature and salinity (Bel Hassen *et al.*, 2009a,b; Kchaou *et al.*, 2009). The mean water temperature and salinity recorded at the 15 sampling stations were  $20.8 \pm 0.9$  °C and  $39.3 \pm 2.3$ , respectively. The values of salinity recorded at the southern stations (from Zarrat to Jabiat Haj Ali: Average  $40.41 \pm 2.45$ ,  $n = 9$ ) were significantly higher than those recorded at the northern stations (from Tabia to the Port of Gabes: Average  $37.58 \pm 0.63$ ,  $n = 6$ ) (ANOVA;  $F_{(1,13)} = 7.44$ ;  $p = 0.017$ ). However, no significant difference was observed in water temperature between northern and southern stations (ANOVA;  $F_{(1,13)} = 1.99$ ;  $p = 0.181$ ). The low concentrations of ( $N-NO_2^-$ ,  $N-NO_3^-$ ,  $N-NH_4^+$ ) as well



**Fig. 7:** Mean relative contribution of phytoplankton groups to total phytoplankton abundance and chlorophyll-*a* concentration in 15 nearshore stations in Gulf of Gabes. Bars represent standard deviation. Number of variables:  $n = 12$ .

as their low magnitude relative to total nitrogen (Table 1) and the resulting low N/P ratios ( $< 16$  Redfield ratio) at 11 stations, suggest that N availability was the limiting element for algal growth (Table 1). The N/P ratios was positively correlated with total nitrogen (Pearson's correlation;  $r_p = 0.762$ ;  $N = 15$ ;  $p = 0.009$ ) (Table 3). On the other hand, the Port of Gabes, Karboub, Hassi Jerbi and the Channel of Cotusal were characterized by high N/P ratios ( $> 16$  Redfield ratio), suggesting that the waters at these stations were P limited. The assumption that nitrogen is the key limiting nutrient in euphotic layers has prevailed for most marine pelagic systems (Dugdale & Goering, 1967). However, a number of other studies carried out in the eastern basin, suggest that the factors limiting primary productivity in the waters were sometimes solely P and sometimes both P and N (Becos-Kontos, 1977; Krom *et al.*, 1991, 2004; Tüfekçi *et al.*, 2013). The heterogeneity of the limiting factors at the 15 stations may be due to the anthropogenic activities along the coastline of the Gulf of Gabes. In addition, the chlorophyll-*a* concentrations were low and did not exceed  $7.5 \text{ mg m}^{-3}$ . We inferred that the 15 stations along the coastline of the Gulf of Gabes were characterised by oligotrophic waters, confirming the observations reported for the surface waters of the Mediterranean Sea (Thingstad & Rassoulzadegan, 1999; Thingstad *et al.*, 2005; Bel Hassen *et al.*, 2009 a,b; Drira *et al.*, 2010 a,b).

### Composition of the ciliate community

The present study is an attempt to analyse ciliate species composition and its seasonal distribution along the

nearshore zone of the Gulf of Gabes. This is the first investigation of the planktonic ciliates along the northern eastern coast of North Africa. Our study indicates that two distinct areas can be differentiated along the coastline of the Gulf of Gabes, the northern and southern zones. In the present study, ciliates showed a significant positive correlation with salinity, which indicates that salinity may play an important role in ciliate distribution, similar to that in other waters (Elloumi *et al.*, 2009a; Yu *et al.*, 2013).

The total number of ciliate taxa recorded in the present study (63 species) was higher than observed in other studies conducted in the Gulf of Gabes (Elloumi *et al.*, 2009a; Hannachi *et al.*, 2009; Kchaou *et al.*, 2009), which recorded between 30 and 61 ciliate species in plankton samples. Pitta *et al.* (2005) recorded 84 ciliate taxa in the eastern Mediterranean Sea, SE Levantine Basin, after enrichment of the oceanic surface water with orthophosphate. Jiang *et al.* (2011) found a total of 64 ciliate taxa in Jiaozhou Bay, northern China. The high ciliate species richness found in this study may be due to the conditions prevailing along the coastline of the Gulf of Gabes, which differ from those in the oceanic region (Chiang *et al.*, 2003). The ciliate taxa increased significantly at the northern station but decreased at the southern one (Fig. 2). This was mainly due to the higher salinity recorded at the southern stations and the oligotrophic state. Salinity was significantly positively correlated with both ciliate abundance and biomass (Pearson's correlation;  $r_p = 0.6$ ,  $N = 15$ ;  $p < 0.05$ ) and negatively with the diversity index  $H'$  (Pearson's correlation,  $r_p = -0.63$ ;  $N = 15$ ;  $p < 0.05$ ) (Table 3). The highest values of total phosphorus were recorded at the Port of Gabes and Zarrat stations, coinciding with the high number of



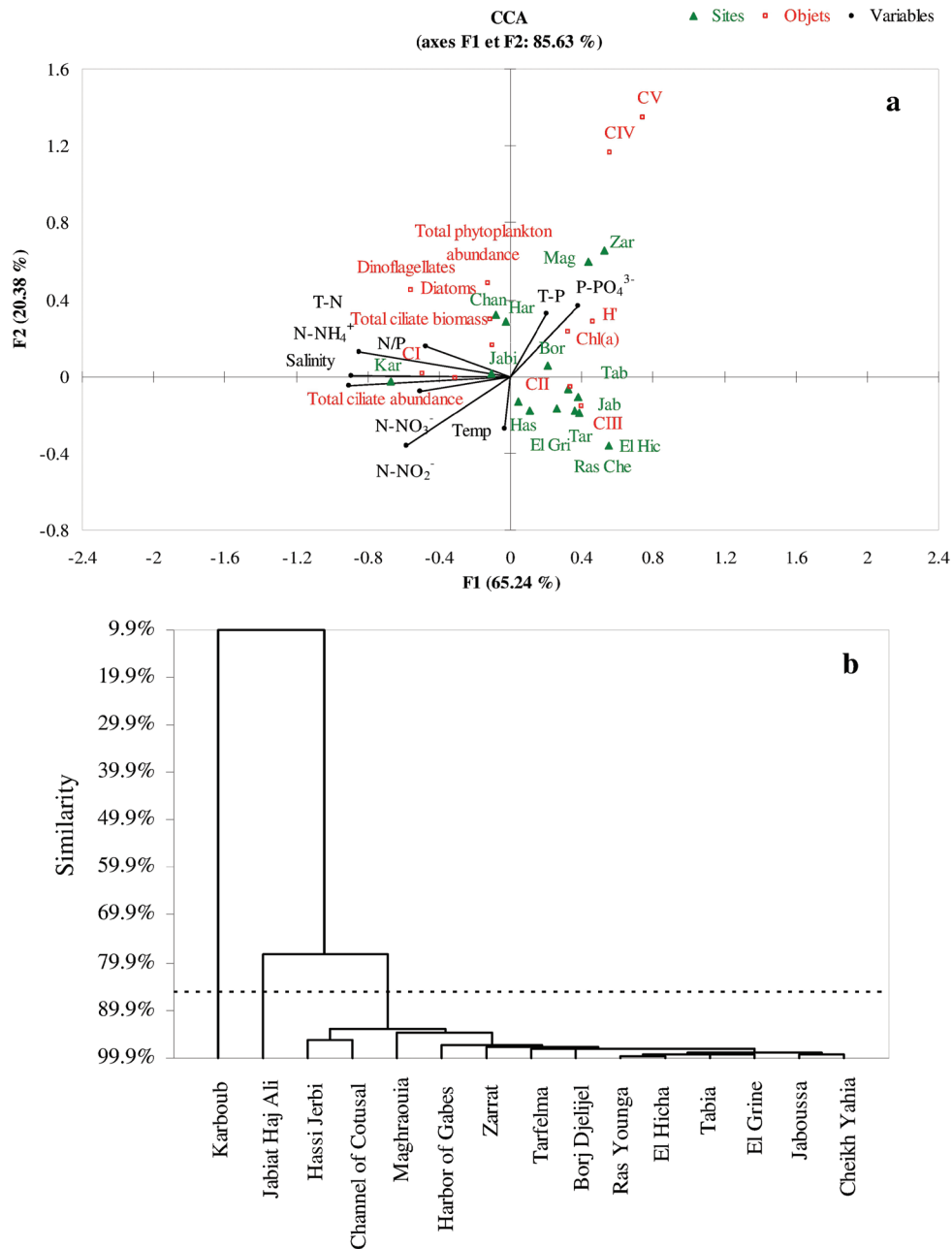
**Table 4.** The main dinoflagellate taxa and their mean length ( $\mu\text{m}$ ) and biovolume ( $\mu\text{m}^3$ ) at the 15 nearshore sampling stations along the Gulf of Gabes.

Dinoflagellates species	Length ( $\mu\text{m}$ )	Biovolume ( $\times 10^3 \mu\text{m}^3$ )	Taxa criteria
<i>Cochlodinium</i> sp. (Schütt, 1896)	70	44.87	Mixotrophe
<i>Dinophysis acuminata</i> (Claparède & Lachmann 1859)	36.53	25.64	Mixotrophe
<i>Dinophysis rotundata</i> (Claparède & Lachmann 1859)	36.07	19.93	Mixotrophe
<i>Gonyaulax polygramma</i> (Stein, 1883)	70	91.58	Autotrophe
<i>Gonyaulax</i> sp. (Diesing, 1866)	38.33	10.05	Autotrophe
<i>Gonyaulax spinifera</i> (Diesing, 1866)	35.83	15.95	Mixotrophe
<i>Gymnodinium catenatum</i> (Graham, 1943)	160	253.293	Mixotrophe
<i>Gymnodinium</i> sp. (Stein, 1878)	23.88	4.17	Mixotrophe
<i>Gyrodinium</i> sp. (Kofoid & Swezy, 1921)	25	1.73	Mixotrophe
<i>Gyrodinium spirale</i> (Kofoid & Swezy, 1921)	73.75	34.73	Mixotrophe
<i>Gyrodinium fusiforme</i> (Kofoid & Swezy, 1921)	65	7.65	Mixotrophe
<i>Karenia selliformis</i> (Haywood <i>et al.</i> 2004)	40	28.14	Toxic
<i>Karlodinium</i> sp. (Larsen, 2000)	17.5	2.06	Mixotrophe
<i>Neoceratium candelabrum</i> (Gómez Moreira & López-Garcia, 2010)	233.33	521.48	Mixotrophe
<i>Neoceratium falcatum</i> (Gómez Moreira & Lopez-Garcia, 2010)	270	62.05	Mixotrophe
<i>Neoceratium furca</i> (Gómez Moreira & López-Garcia, 2010)	185	131.85	Mixotrophe
<i>Neoceratium fusus</i> (Gómez Moreira & Lopez-Garcia, 2010)	315	52.63	Mixotrophe
<i>Neoceratium lineatum</i> (Gómez Moreira & Lopez-Garcia, 2010)	240	346.03	Mixotrophe
<i>Neoceratium macroceros</i> (Gómez Moreira & Lopez-Garcia, 2010)	450.62	621.54	Mixotrophe
<i>Neoceratium teres</i> (Gómez Moreira & Lopez-Garcia, 2010)	128.33	91.54	Mixotrophe
<i>Neoceratium tripos</i> (Gómez Moreira & Lopez-Garcia, 2010)	197.5	173.01	Mixotrophe
<i>Podolampas</i> sp. (Kofoid, 1907)	100	1.04	Heterotrophe
<i>Polykrikos</i> sp. (Bütschli, 1873)	75.58	22.45	Heterotrophe
<i>Prorocentrum compressum</i> (Dodge, 1975)	35	1.38	Mixotrophe
<i>Prorocentrum concavum</i> (Fukuyo, 1981)	29.16	8.13	Mixotrophe
<i>Prorocentrum gracile</i> (Schütt, 1895)	55	17.98	Mixotrophe
<i>Prorocentrum lima</i> (Stein, 1878)	33.57	6.53	Toxic
<i>Prorocentrum micans</i> (Ehrenberg, 1834)	40	20.72	Mixotrophe
<i>Prorocentrum minimum</i> (Schiller, 1933)	18.33	2.15	Mixotrophe
<i>Prorocentrum rathymum</i> (Ehrenberg, 1834)	35	16.48	Mixotrophe
<i>Prorocentrum triestinum</i> (Schiller, 1918)	55	11.51	Mixotrophe
<i>Protoperidinium depressum</i> (Balech, 1974)	101	65.88	Mixotrophe
<i>Protoperidinium diabolium</i> (Balech, 1974)	80	35.46	Autotrophe
<i>Protoperidinium globulum</i> (Balech, 1974)	50	12.45	Autotrophe
<i>Protoperidinium ovum</i> (Balech, 1974)	72.66	18.18	Autotrophe
<i>Protoperidinium</i> sp. (Balech, 1974)	22.5	2.38	Not defined
<i>Scrippsiella trochoidea</i> (Loeblich III, 1965)	30	6.28	Mixotrophe

ciliate taxa of 46 and 37, respectively. Total phosphorus was strongly correlated with  $H'$  (Pearson's correlation;  $r_p = 0.66$ ;  $N = 14$ ;  $p < 0.01$ ) (Table 3). Madonni & Baghiroli (2007) have stated that the structure of ciliate communities is strongly affected by physical, chemical and geomorphological characteristics. It seems that the trophic state is necessary to determinate the pattern of variation in the spatial and temporal distribution of planktonic ciliates as reported by Velho *et al.* (2005). The Pearson test shows a significant correlation between ciliate density and the N/P ratio (Pearson's correlation;  $r_p = 0.58$ ;  $N = 15$ ;  $p < 0.05$ ) (Table 3). Recent investigations in the Mediterranean Sea (Dolan 2000; Pitta *et al.*, 2001; Van Wambeke *et al.*, 2002) demonstrated a distinct longitudinal gradient of increas-

ing oligotrophy from west to east in terms of biomass and production of bacteria, autotrophic picoplankton and nanoplankton as well as the standing stocks of ciliate communities. During the study period, the ciliate assemblage was characterised by the dominance of Spirotrichea (Table 2, Fig. 6) known to be typical of the oligotrophic conditions of the Gulf of Gabes nearshore zone, as reported by Hannachi *et al.* (2009) and Kchaou *et al.* (2009).

A clear dominance of Spirotrichea has been recorded by several studies of marine ecosystems (Rekik *et al.*, 2012). Spirotrichea, generally large-sized, dominate in different Mediterranean area (Dolan *et al.*, 2002; Hannachi *et al.*, 2009). According to Balkis (2004) and Hannachi *et al.* (2009), the quantitative dominance of



**Fig. 8:** (a) Canonical correspondence analysis (CCA) (Axis I and II) on mean values of environmental, biological variables (CI: size class1; CII: size class2; CIII: size class3; CIV: size class4; CV: size class5) in the 15 nearshore stations in Gulf of Gabes (Tab: Tabia; Ras: Ras Younga; Jab: Jaboussa; Tar: Tarfelma; El Hic: El Hicha; Har: Harbor of Gabes; Zar: Zarrat; El Gri: El Grine; Mag: Maghraouia; Kar: Karboub; Has: Hassi Jerbi; Che: Cheikh Yahia; Bor: Borj Djeljel; Jabi: Jabiat Haj Ali; Chan: Channel of Cotusal). (b) Dendrogram between the 15 nearshore sampling stations in Gulf of Gabes.

oligotrichs found during their study was related to the oligotrophic status of the Gulf of Gabes (Bel Hassen *et al.*, 2008; Drira *et al.*, 2010a). The quantitative dominance of tintinnids during their study was related to pollution; the Gulf of Gabes is the subject of pollution from anthropogenic and industrial activities (Drira *et al.*, 2008; Hannachi *et al.*, 2009). The differences in the community composition of tintinnid ciliates suggest that ciliate communities in the east and central Mediterranean are more diverse in terms of numbers of endemic species, numbers of species, and species evenness (Dolan *et al.*, 1999). The

study on the spatial succession of ciliate size classes at each station in the Gulf of Gabes is therefore important for understanding their significance in the trophic organization and community structure of the pelagic ecosystem. This study shows that 5 major size-classes of the ciliate assemblage are the overwhelmingly commonest sizes found (15-30  $\mu\text{m}$ , 30-50  $\mu\text{m}$ , 50-100  $\mu\text{m}$ , 100-200  $\mu\text{m}$ , > 200  $\mu\text{m}$ ). The size distribution of biomass did not vary significantly between stations (ANOVA;  $F_{(14,447)} = 0.95$ ;  $p = 0.503$ ). The largest species (> 200  $\mu\text{m}$ ) prevailed at the Maghraouia station (*Helicostomella subulata*, *Spirost-*

*mum teres* and *Tracheloraphis phoenicopterus*). This may be explained by the abundance of phytoplankton as food for ciliates, which reached a density of  $13.1 \times 10^3$  cells  $l^{-1}$  (Table 1). Most of the ciliate biomass was represented by 50-100  $\mu m$  size classes (Fig. 3b), which was mainly dominated by the order tintinnida (Table 2). Fifty-four tintinnid species were identified, 18 of which were of the genus *Tintinnopsis* (Table 2). Tintinnid species richness was greater in the Gulf of Gabes than in other sea areas (Yu *et al.*, 2013). The lower contributions of the smallest species, from 15-30  $\mu m$ , which accounted for 2 to 30 % of total biomass may be due to the grazing pressure of mesozooplankton, such as copepods (Kchaou *et al.*, 2009). Pérez *et al.* (1997) noted that copepods preferentially consume micro-sized ciliates. Quevedo & Anadón (2000) found that small ciliates, < 20  $\mu m$ , were dominant in a coastal area of the southern Bay of Biscay, contributing about 63 % of total ciliates. Nano-ciliates were present in all samples and were numerically the dominant size group of the ciliate assemblage, representing between 51 and 70 % of oligotrich abundance. In terms of biomass, their contribution was less important (Pérez *et al.*, 2000). The size of the ciliate assemblages has been related to the trophic status of the water, with large forms, mainly phytophagous, dominating in oligotrophic waters (Gismervik *et al.*, 2002). Indeed, these large forms are capable of exploiting lower minimum food concentrations (Pérez *et al.*, 1997; Modigh & Castaldo, 2002).

In the present study, ciliate abundance and biomass showed a strong seasonal variation throughout the year. The peaks of ciliate abundance and biomass correspond completely. Contrarily, the observation of Yu *et al.* (2013) in the sea area around Zhangzi Island, Northern Yellow Sea, showed that the ciliate abundance was lower, while ciliate biomass was higher. Total ciliate abundance varied between  $0.32 \pm 0.2 \times 10^3$  cells  $l^{-1}$  and  $6.3 \pm 14.2 \times 10^3$  cells  $l^{-1}$ ,  $n = 12$  (Mean  $\pm$  SD =  $1.1 \pm 1.5 \times 10^3$  cells  $l^{-1}$ ;  $n = 15$ ), higher than that recorded by Hannachi *et al.* (2009). The latter authors found that ciliate abundance ranged from 0 to  $20.51 \times 10^2$  cells  $l^{-1}$  (Mean  $\pm$  SD =  $19.7 \pm 26.2$  cells  $l^{-1}$ ), showing a gradual decrease from the coastal area (Mean  $\pm$  SD =  $28.48 \pm 32.95$  cells  $l^{-1}$ ) to the open sea (Mean  $\pm$  SD =  $10.96 \pm 11.92$  cells  $l^{-1}$ ). The highest ciliate density recorded at the southern stations may be induced by the salinity on the one hand and abundance of phytoplankton on the other hand. We found a significant correlation between both density and biomass of ciliates and salinity (Pearson's correlation;  $r_p = 0.6$ ;  $N = 15$ ;  $p < 0.05$ ) (Table 3). Moreover, the density and the biomass of ciliates were strongly correlated with total phytoplankton, Diatom and Dinoflagellate abundance (Table 3). Dinoflagellates were not well correlated with chlorophyll-*a* concentration (Pearson's correlation,  $r = -0.088$ ;  $N = 15$ ;  $p < 0.001$ ) (Table 4). This aspect could be explained by the heterotrophic dinoflagellate taxa such as *Podolampas* sp. and *Polykrikos* sp. (Table 4).

However, there is no correlation between chlorophyll-*a* concentration and total ciliate abundance (Table 3). The lower chlorophyll-*a* concentration of the eastern basin stations corresponded with a relatively high stock of ciliates (Dolan *et al.*, 1999). Significant relationships were found between chlorophyll-*a* concentration and total ciliates (Dolan *et al.*, 1999; Modigh, 2001). Considering the seasonal succession of the plankton community, the ciliates are efficient predators of phytoplankton (Schweizer, 1997; Pauleto *et al.*, 2009). Ciliates are the main grazers of phytoplankton since mesozooplankton is unable to consume small-sized cells belonging to the pico- and nano- fractions, which dominate in oligotrophic environments (Siokou- Frangou *et al.*, 2002; Tsagarakis *et al.*, 2010). Ciliates also serve as a link between the microbial loop and the classical food-web, and contribute significantly to the transfer of energy to higher predators (Pitta *et al.*, 2009).

CA and CCA show clearly identical characteristics between stations, with the exception of Karboub. The highest ciliate density and biomass recorded at Karboub can be attributed to the dominance of *Fabrea salina*. It is likely that the Karboub station represents a complex ecosystem, and our study underlines the importance of examining the planktonic communities, which are strongly influenced by the sabkha environments, for management practices to maintain and improve seashell farm income in this location (Kchaou *et al.*, 2009). The high salt concentration found in water samples throughout the study is the clearest evidence of the Sabkha El Meleh influence on the Karboub ecosystem (Kchaou *et al.*, 2009). This is reflected by the presence of substantial amounts of halotolerant Heterotrichea *F. salina* (9.2 % of the ciliate total abundance), in Karboub samples, a species that was also found in high abundance in the primary ponds of Sfax saltern (Elloumi *et al.*, 2008), yet in higher salinities (range 80-200). This change in ambient salinity resulted in smaller *F. salina* at the Karboub station (length = 75  $\mu m$ ; biovolume =  $44.3 \mu m^3$ ) than in the Sfax saltern (length = 111  $\mu m$ ; biovolume =  $108.1 \mu m^3$ ) (Elloumi *et al.*, 2006, 2009b). We assume that the size of *F. salina* may have been indirectly rather than directly related to salinity. In our study, *Fabrea salina* peaked in January while phytoplankton declined. We suspect that the January flooding in the sabkha was probably the greatest supplier of *F. salina* to the Karboub station. Our assumption is supported by at least two observations. Firstly, the phytoplankton decline in January and secondly the ratio  $NO_3^-/NH_4^+$ , the indicator of bacterial mineralization did not change from November to January, excluding the possibility of invoking a predation of *F. salina* on bacteria. This may help to explain the small size of *F. salina* found at the Karboub *Dunaliella*-free station. Our findings suggest that factors other than temperature (e.g. salinity, prey availability) were involved in the environmental forcing of ciliate species composition in the

nearshore zone of typical arid to semi-arid coastal environments (Kchaou *et al.*, 2009). Our findings suggest that ciliates living along the coastline could serve as good bioindicators for detecting the salt input coming from areas such as the coastal waters. Obviously, this approach is more resource-intensive than the use of an electronic salinity meter. While the tintinnid community found in many marine areas was highly diversified, only two tintinnids (*Tintinnopsis* sp. and *Tintinnidium* sp.) were found. This result suggests that these species performed better than other tintinnids probably owing to more flexible adaptation strategies. The ciliate species found at the Karboub station seem to be an important component of the food web in the nearshore zone of the Gulf. Halotolerant species such as *Heterotricha F. salina* appeared because of the Sabkha interference.

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