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# Copepod assemblages as a bioindicator of environmental quality in three coastal areas under contrasted anthropogenic inputs (Gulf of Gabes, Tunisia)

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*The study of copepod assemblages indicated the presence of 22 species and 12 families in the southern coast of Sfax, 20 species and 13 families in the northern coast and 14 species and 8 families in the Ghannouch area, with a dominance of Oithonidae (79, 51 and 43% in the southern, northern and Ghannouch coasts, respectively). The relative abundance and the richness diversity of Oithonidae were found to be the most relevant indicators of anthropogenic pollution. Oithona nana, Euterpina acutifrons and Acartia clausi differed significantly in abundance between these three areas under differing degrees of pollution. The study of the structure, composition and density of the copepod fauna showed that the southern coast was a pollution-resistant ecosystem ( $H' = 1.49 \pm 0.33 \text{ bits ind}^{-1}$ ; 22 species; density =  $51.375 \pm 4.340 \times 10^3 \text{ ind m}^{-3}$ ) followed by Ghannouch area ( $H' = 1.74 \pm 0.28 \text{ bits ind}^{-1}$ ; 15 species; density =  $11.979 \pm 5.651 \times 10^3 \text{ ind m}^{-3}$ ) and the northern coast, considered as a restored area ( $H' = 1.95 \pm 0.26 \text{ bits ind}^{-1}$ ; 21 species; density =  $6.516 \pm 4.304 \times 10^3 \text{ ind m}^{-3}$ ). The three ecosystems can thus be classified according to their degree of resistance to the anthropogenic inputs based on the results of the physico-chemical parameters and the species diversity as follows: southern coast > Ghannouch area > northern coast.*

**Keywords:** Sfax, Ghannouch,  $\text{PO}_4^{3-}$ , copepod, anthropogenic pressure, indicators

## INTRODUCTION

Marine coastal ecosystems include intertidal and nearshore systems that are influenced by atmospheric, terrestrial and autochthonous processes (Kennedy *et al.*, 2002; Carr *et al.*, 2003; Ruttenberg & Granek, 2011; Bahloul *et al.*, 2015). These ecosystems are generally sensitive to changes in upstream terrestrial systems and to direct inputs (Ruttenberg & Granek, 2011). Thus, they are undergoing significant and growing anthropogenic threats (Cloern, 2001; Newton *et al.*, 2003; Beaugrand *et al.*, 2010; Brandt, 2010; Burrows *et al.*, 2011; Bahri-Trabelsi *et al.*, 2013; Bahloul *et al.*, 2015; Serranito *et al.*, 2016).

Zooplankton plays a pivotal role in aquatic food webs by transferring carbon to higher trophic levels, consuming microorganisms (bacteria, protists) and serving as a prey for fish and invertebrates (De-Young *et al.*, 2004; Sampey *et al.*, 2007; Ziadi *et al.*, 2015). Zooplankton communities are known to quickly respond to fluctuations in environmental

factors particularly in coastal areas where the combination of land and marine influences drives strong spatiotemporal variability (Siokou-Frangou, 1996). Zooplankton can thus be considered as useful indicators of ecosystem health status (Hays *et al.*, 2005; Longhurst, 2007). The presence or absence of certain zooplankton species may indicate the relative influence of different water types on ecosystem structures and may serve as an early indication of a biological response to environmental and climatic changes (Hays *et al.*, 2005; Ziadi *et al.*, 2015). Zooplankton signatures may characterize specific hydrographic conditions in most of the world's ecosystems. Several studies have been undertaken in the Gulf of Gabes regarding the characterization of local zooplankton assemblages (Drira *et al.*, 2010a, b, 2014; Ben Ltaief *et al.*, 2015, 2017). In this area, the functioning of the coastal environments is highly complex due to the interaction of various factors, i.e. water movements, tide currents, anthropogenic inputs, marine traffic and fishing activities (Drira *et al.*, 2008, 2016; Feki *et al.*, 2013). The Gulf of Gabes is subject to a variety of human activities (Bejaoui *et al.*, 2004; Gargouri *et al.*, 2011, 2015). These activities include urban settlements, industrial areas and intense maritime traffic, resulting in the discharge of industrial and municipal effluents enriched in nutrients and pollutants which might negatively

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affect the water quality and the state of the ecosystem (Bejaoui *et al.*, 2004; Gargouri-Ben Ayed *et al.*, 2007; Gargouri *et al.*, 2011, 2015; Aloulou *et al.*, 2012; Ben Salem *et al.*, 2015; Ben Salem & Ayadi, 2016; Drira *et al.*, 2016, 2017).

The main goal of this study was to improve knowledge about the spatial distribution of zooplankton abundance and composition in a large spectrum of three coastal areas in the Gulf of Gabes characterized by different degrees of pollution: (1) the northern coast of Sfax city which is an area restored via the Taparura project (Callaert *et al.*, 2009); (2) the southern coast of Sfax city; and (3) Ghannouch coast, close to Gabes city, which is considered as highly polluted. The second aim was to relate the differences observed between the three sampled areas in respect of zooplankton abundance with physical (temperature, salinity and pH), chemical (ammonium ions ( $\text{NH}_4^+$ ), nitrates ( $\text{NO}_3^-$ ), nitrites ( $\text{NO}_2^-$ ), total nitrogen (T-N), orthophosphate ( $\text{PO}_4^{3-}$ ), total phosphorus (T-P) and silicon atoms ions  $\text{Si}(\text{OH})_4$ ) and biogeochemical (suspended particulate matter (SPM), particulate organic carbon and nitrogen (POC and PON), chlorophyll-*a* (chl-*a*) and phaeopigment-*a* (Phaeo-*a*)) water parameters characterizing the trophic and pollution status of each zone.

## MATERIALS AND METHODS

### Study area

The Gulf of Gabes (Eastern Mediterranean Sea, between  $35^\circ\text{N}$  and  $33^\circ\text{N}$ , Tunisia), is endowed with rich aquatic resources contributing to about 65% of the national fish production in Tunisia (DGPA, 2004). Sampling was carried out in the Gulf of Gabes during October and November 2014, in three coastal areas i.e. the southern and the northern coasts of Sfax and the Ghannouch area. Thirty stations were sampled in the Gulf of Gabes among which 10 sampling stations were chosen for each area (SC, NC and GA) taking into account the pollution gradient (Figure 1).

The coastline of Sfax concentrates a great number of industrial activities, mainly related to phosphates, salt works, tanneries, lead foundry, textiles, ceramics industry, soap factories and building materials (Barhoumi *et al.*, 2009).

The southern coast of Sfax (hereafter called SC) lies between the fishing harbour in the north and Gargour village in the south. This area is marked by the presence of the Société Industrielle d'Acide Phosphorique et d'Engrais

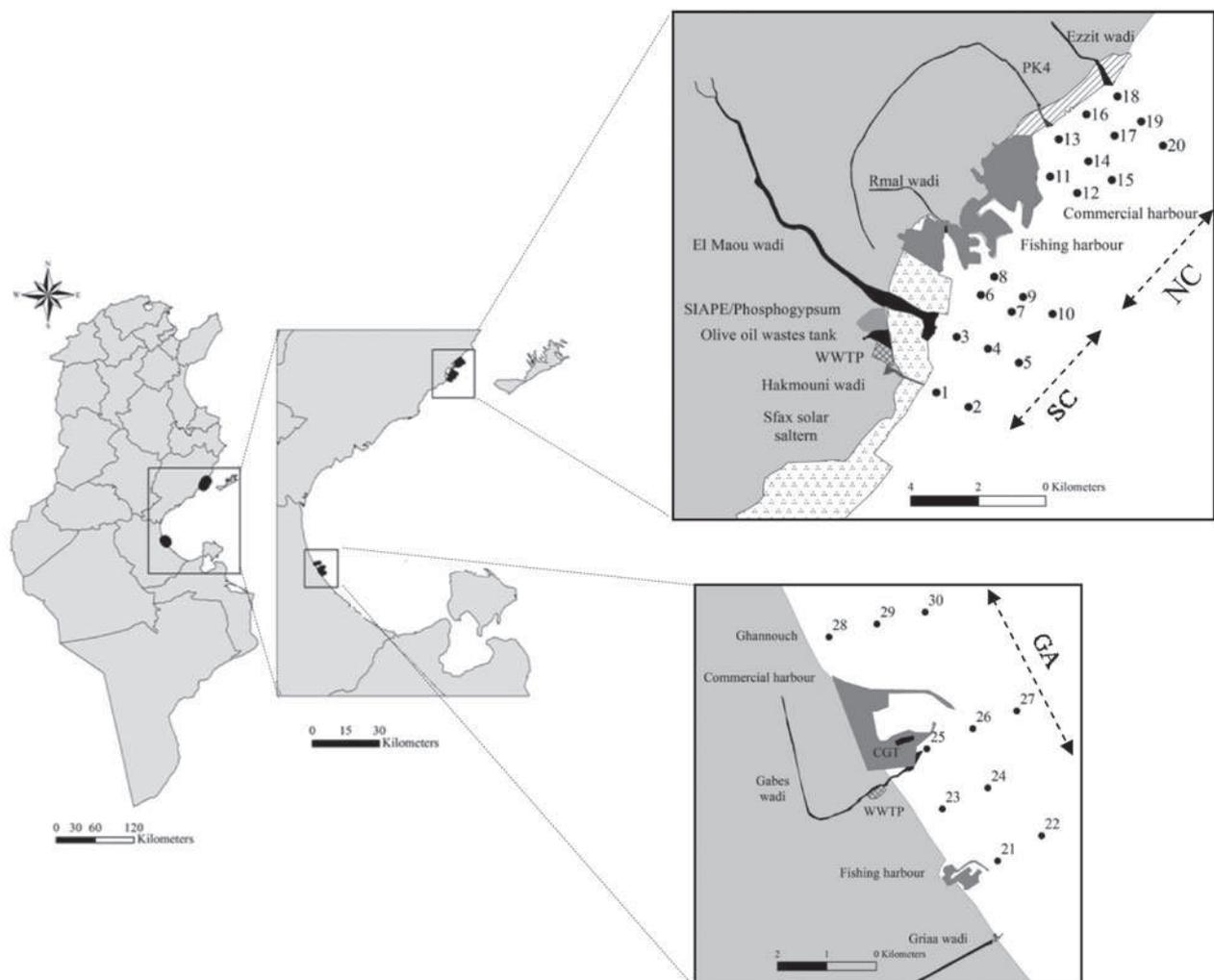


Fig. 1. Location of the studied stations in the southern (stations 1–10) and northern (stations 11–20) coastal areas of Sfax and the Ghannouch area (stations 21–30) sampled during autumn (October–November 2014).

(SIAPE) industry, which has released large amounts of phosphogypsum wastes for 40 years. These phosphogypsum wastes are a significant source of phosphates ( $\text{PO}_4^{3-}$ ), chloride ( $\text{Cl}^-$ ) and sulphates ( $\text{SO}_4^{2-}$ ) for seawater and may explain the high chemical oxygen demand (COD) in the SC surface waters (Bahloul *et al.*, 2015; Drira *et al.*, 2016). Besides the SIAPE industry and its phosphogypsum wastes, the SC comprises several industrial areas related to textiles, tanneries, salt, olive oil, food processing, construction materials, ceramics and glass. Hence, several industrial effluents are released to the sea in this area. All these anthropogenic inputs have been shown to alter the marine environment and biodiversity in SC (Zaghden *et al.*, 2005; Gargouri, 2006; Aloulou *et al.*, 2012; Rekik *et al.*, 2013; Bahloul *et al.*, 2015).

The northern coast of Sfax (hereafter called NC), extending from the commercial harbour to wadi Ezzit and beyond, also suffers from the pressure of human activities (Hamza-Chaffai *et al.*, 1997; Tayibi *et al.*, 2009) and is subjected to increasing eutrophication with both red (Louati *et al.*, 2001) and green tides caused by coastal *Ulva rigida* replacing the *Posidonia oceanica* seagrass beds (Ben Brahim *et al.*, 2010). Previous studies in NC have focused on the sources and distribution of hydrocarbons in sediments (Louati *et al.*, 2001; Zaghden *et al.*, 2005) and marine bivalves (Hamza-Chaffai *et al.*, 2003).

This area was recently restored through the *Taparura* project (2006–present), which aimed at remediating this part of Sfax city's coast. The project included the rehabilitation of a former complex industrial site, the reclamation of beaches and restoration of the area (Callaert *et al.*, 2009). It led to significant improvement of plankton communities and water quality (Rekik *et al.*, 2013, 2015). Indeed, this zone was strongly polluted by the phosphogypsum wastes from the NPK phosphoric acid industry, situated near the commercial harbour. The NPK was closed in 1992 and the *Taparura* project allowed the burial and confinement of the phosphogypsum wastes and the rehabilitation of the area between the commercial harbour and Sidi Mansour. In the NC, there are also the outlet of the rainwater drainage channel ('PK4'), which crosses the city from south-west to north-east, the outlet of the wadi Ezzit, which receives untreated domestic and industrial effluents.

Ghannouch area (hereafter called GA) includes a chemical industry complex as well as a commercial harbour, located 3 km north of Gabes city (Bejaoui *et al.*, 2004). This complex houses the 'GCT-Gabes' phosphoric acid industry. Contrary to the SIAPE which stores its phosphogypsum wastes on land in an unprotected dome, the GCT-Gabes directly discharges its phosphogypsum wastes into the sea via an open channel. Organic pollution and drastic pollution by phosphate coming from the discharged sewage waters of the chemical plants of Ghannouch (Zaouali, 1993) favoured the emergence of green tides and *Valonia* to *Ulva* and the red tide or phytoplankton bloom (Hamza-Chaffai *et al.*, 1995). This pollution also caused the disappearance of *Caulerpa* meadows, regression of *Posidonia* seagrass beds and decreasing diversity of the benthic fauna (Zaouali, 1993). Besides chemical industries, trawling practices (shrimps fishing) contribute to the deterioration of the Gabes ecosystem as well (Zaouali, 1993).

## Sampling and on board measurements

Sampling was performed on board the vessel 'Taparura' between 10:30 am and 3:30 pm (18 and 23 October 2014;

SC and NC, respectively), 8:30 am and 12:30 am (13 November 2014; GA) around high tide and under conditions of calm sea and sunny weather. Seawater samples were collected at  $\sim 0.1$  m depth using 4 l Nalgene<sup>®</sup> polycarbonate bottles. The bottles were opened below the water surface to avoid sampling of the surface microlayer. They were extensively washed with 1 M hydrochloric acid (HCl) and Milli-Q water before use, rinsed three times with the respective sample before filling and placed in the cold and in the dark after collection.

Zooplankton was collected using a cylindro-conical net (30 cm aperture, 100 cm height, 100  $\mu\text{m}$  mesh size) equipped with an Hydro-Bios flowmeter. The net was towed obliquely from near bottom to surface at each station at a mean speed of  $1 \text{ m s}^{-1}$  during 4 mins. After collection, zooplankton samples (200 ml) were rapidly preserved in a buffered formaldehyde solution (2%). They were stained with Rose Bengal to identify the internal tissues of the different zooplankton species and also to facilitate copepod dissection. *In situ* measurements of temperature, salinity and pH were carried out with measuring cells type TetraCon<sup>®</sup> 4-electrode system and a refractometer.

## Filtration, chemical and biogeochemical analyses and zooplankton identification

Back in the laboratory, samples were immediately filtered under a low vacuum ( $< 50$  mm Hg) through pre-combusted ( $500^\circ\text{C}$ , 4 h) GF/F ( $\sim 0.7 \mu\text{m}$ ) glass fibre filters (25 or 47 mm diameter, Whatman) using glassware filtration systems. Nutrients, i.e.  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ ,  $\text{Si}(\text{OH})_4$ , T-N and T-P, were analysed with a BRAN and LUEBBE type 3 autoanalyser and their concentrations were determined colorimetrically using a UV-visible 6400/6405 spectrophotometer according to the 'Standard Methods for the Examination of Water and Wastewater' (APHA, 1992).

For Chl-*a* and Phaeo-*a* analyses, 250–300 ml of samples were filtered. Filters were then extracted with methanol (RP prolabo) according to Raimbault *et al.* (2004). After 30 min of extraction in the dark at  $4^\circ\text{C}$ , a fluorescence measurement was performed with a fluorometer model 10 Turner Designs (Sunnyvale, USA) at  $\lambda_{\text{Ex}}/\lambda_{\text{Em}}$  of 450/660 nm. The acidification method was applied to determine Phaeo-*a* concentrations. The fluorometer was calibrated with solutions of methanol (96%) and Chl-*a* (Sigma C5753). For SPM, POC and PON between 250 and 1100 ml of sample were filtered with pre-weighted GF/F filters (the same filter was used for SPM, POC and PON analyses). After filtration, filters were dried at  $60^\circ\text{C}$  for 24 h and reweighed on the same balance. SPM concentration was calculated as the difference between filter weight before and after sample filtration, normalized to the filtration volume (Neukermans *et al.*, 2012). POC and PON quantification were performed simultaneously with an autoanalyser II Technicon (New York, USA), using the wet-oxidation procedure according to Raimbault *et al.* (1999). POC and PON had a detection limit of 0.50 and 0.10  $\mu\text{m}$ , respectively.

Zooplankton samples were identified according to Rose (1933), Bradford-Grieve (1999) and Costanzo *et al.* (2007). The different copepod species were sorted into four demographic classes (nauplii, copepodids, adult males and adult females). Miscellaneous zooplankton were also counted

according to Tregouboff & Rose (1978a, b). Enumeration was performed under a vertically mounted deep-focus dissecting microscope (Olympus TL 2) and numerical density was expressed in individual  $m^{-3}$ . Total length of body size for the adult copepod was measured for each species in each sampled station (10 individuals for each species in each sampling set).

## Data processing and statistical analysis

We applied the Geographic Information Systems (GIS) tools using ArcGIS 10.2 version software to make contour plots. Kriging was the method used to build maps relative to spatial distribution for all dataset parameters. Mesozooplankton diversity was measured using a range of univariate and multivariate diversity measurements. Species diversities were assessed using the Shannon diversity index  $H'$  (Shannon & Weaver, 1949) and using the formula proposed by  $J'$  Pielou's evenness index (1966):

$$H' = - \sum_{ni} \frac{ni}{N} \log_2 \frac{ni}{N},$$

$$J' = H' / \log_2 S;$$

where  $n_i$  is the number of individuals belonging to the species  $i$  and  $N$  is the total number of individuals in each station.

To identify the suitable environmental health indicator of these three coastal marine areas under contrasting anthropogenic inputs, we calculated the Indicator Value (IndVal) for each taxa as per Dufrene & Legendre (1997) as used recently in Hemraj *et al.* (2017). Indicator species of each station was extracted by an indicator species analysis (Dufrene & Legendre, 1997). The highest indicator value for given species was saved as a summary of the overall indicator value of that species. The IndVal of each species was computed as follows:

$$\text{IndVal} = RA_{kj} \times RF_{kj} \times 100$$

where  $RA_{kj}$  is the relative abundance of species  $j$  in group  $k$ , and  $RF_{kj}$  is the relative frequency (presence/absence) of species  $j$  in group  $k$ .

All statistical analyses were conducted using the XLStat 2014 software. ANOVA was applied to identify significant differences between these three sampled areas for physico-chemical and biogeochemical variables. The spatial variability of copepod communities in relation to environmental variables was assessed using multivariate analysis after data transformation [ $\log_{10}(x + 1)$ ] (Sokal & Rohlf, 1981). Moreover, to explain the relationship between physico-chemical (depth, temperature, salinity and pH), chemical ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , T-N, T-P, N/P ratio and  $\text{Si}(\text{OH})_4$ ) and biogeochemical (copepods, chl- $a$  and SPM) parameters, we used a canonical correspondence analysis (CCA) (Ter-Braak, 1986) assessed by over 30 observations (30 stations). Pearson's rank correlations were used to determine the potential correlations between the copepod community and the physico-biogeochemical variables.

## RESULTS

### Physico-chemical and biogeochemical parameters

Mean values  $\pm$  standard deviation (SD) of physico-chemical and biogeochemical parameters recorded in surface waters of the three studied areas are given in Table 1. Surface water temperature was warmer in SC ( $26.8 \pm 0.23^\circ\text{C}$ ) than in NC ( $21.91 \pm 0.7^\circ\text{C}$ ) and GA ( $19.8 \pm 1.68^\circ\text{C}$ ) (Table 1; Figure 2A) and the difference was significant (ANOVA,  $P < 0.0001$ ). The highest temperature ( $27.3^\circ\text{C}$ ) was recorded at station 10 from the SC and the lowest one ( $18^\circ\text{C}$ ) at stations 21–24 from the GA (Table 1; Figure 2A). Salinity averaged at  $38.4 \pm 3.4$  psu, varying from 32 psu at stations 3 (SC), 25 and 30 (GA) to 45 psu at stations 16 (NC). It was significantly higher in the NC than in the two other areas (ANOVA,  $P < 0.0001$ ) (Table 1; Figure 2B). pH (mean value of  $8.05 \pm 0.08$ ) was higher in the NC ( $8.11 \pm 0.06$ ) than in the GA ( $8.04 \pm 0.07$ ) and the SC ( $7.99 \pm 0.07$ ) (ANOVA,  $P < 0.0001$ ) (Table 1; Figure 2C).

The concentration of total nitrogen (T-N) averaged  $15.8 \pm 4 \mu\text{m}$  and varied from  $11.6$  (station 25, GA) to  $28.9 \mu\text{m}$  (station 13, NC) with no significant difference between sites (ANOVA,  $P > 0.6$ ) (Table 1; Figure 3A). The relatively important T-N concentrations were due to the high contribution of  $\text{NH}_4^+$ , close to 66% of T-N, which displayed a mean concentration of  $5.2 \pm 1.6 \mu\text{m}$  and showed highest value in the SC (ANOVA,  $P < 0.001$ ) (Table 1; Figure 3B).  $\text{NO}_3^-$  concentration was also quite high, ranging from  $1.3$  (station 10; SC) to  $11.4 \mu\text{m}$  (station 13; NC), while  $\text{NO}_2^-$  concentration was much lower ( $0.03$ – $2.6 \mu\text{m}$ , stations 25, GA and 13, NC) (Table 1; Figure 3C, D). Both oxidized nitrogen forms did not vary significantly between the three areas ( $P > 0.4$ ). The concentration of total phosphorus (T-P) was on average  $13.1 \pm 5.7 \mu\text{m}$ , ranging from  $5$  (station 2) to  $25.1$  (station 24)  $\mu\text{m}$  (Table 1; Figure 3E) with highest values in GA ( $P = 0.05$ ).  $\text{PO}_4^{3-}$  concentration was on average  $3.2 \pm 2.4 \mu\text{m}$  with minimal and maximal values  $0.5$ – $9.6 \mu\text{m}$  at stations 2 and 24, respectively and no significant difference between sites ( $P > 0.05$ ) (Table 1; Figure 3F). The N/P ratio varied between 2.5 (station 7) and 30.3 (station 1) and was significantly higher in the SC than in the two other sites ( $P < 0.05$ ) (Table 1; Figure 3G).  $\text{Si}(\text{OH})_4$  concentration was on average  $5.2 \pm 4.1 \mu\text{m}$  with minimal and maximal values  $1.5$ – $19.5 \mu\text{m}$  at stations 1 and 26, respectively ( $P > 0.9$ ) (Table 1; Figure 3H). SPM showed a mean value of  $19.8 \pm 11 \text{ mg l}^{-1}$  and varied between  $8.5$  and  $59.5 \text{ mg l}^{-1}$  at stations 5 and 16, respectively with no significant difference between sites ( $P > 0.1$ ) (Table 1). Chl- $a$  concentration was higher in the SC ( $11.7 \pm 13 \mu\text{g l}^{-1}$ ) than in the GA ( $6.5 \pm 1.7 \mu\text{g l}^{-1}$ ) and the NC ( $5.1 \pm 4.2 \mu\text{g l}^{-1}$ ) (Table 1) but the differences were not significant ( $P > 0.1$ ). Phaeo- $a$  concentration was higher in the SC ( $3.3 \pm 3 \mu\text{g l}^{-1}$ ) than in the NC ( $1.6 \pm 1 \mu\text{g l}^{-1}$ ) and the GA ( $1.4 \pm 0.4 \mu\text{g l}^{-1}$ ) with no significant difference between zones ( $P > 0.5$ ) (Table 1). POC and PON showed a similar trend, with minimal and maximal concentrations in GA and NC, respectively; but with no significant difference between zones ( $P > 0.2$ ). C/N ratio, averaged  $5.9 \pm 0.9 \mu\text{g l}^{-1}$ , with higher mean values in GA ( $6.4 \pm 0.5 \mu\text{g l}^{-1}$ ) than in NC ( $6.1 \pm 1 \mu\text{g l}^{-1}$ ) and in SC ( $5.1 \pm 0.6 \mu\text{g l}^{-1}$ ) ( $P < 0.001$ ) (Table 1).

**Table 1.** Mean values and standard deviation (SD) of physico-chemical and biogeochemical parameters of 30 stations sampled in the northern and southern coastal areas of Sfax and the Ghannouch area sampled in October–November 2014. In the last column, results of ANOVA test for the comparison between these three sampled areas. Asterisks denote significant differences between different sampled areas: \* $P < 0.05$ ; \*\* $P < 0.001$ ; \*\*\* $P < 0.0001$ .

Parameters	Southern coastal area			Northern coastal area			Ghannouch area			Stations with min value	Stations with max value	F (P values)
	Min	Max	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD			
Physical and chemical parameters												
Depth (m)	1	9	4.9 $\pm$ 2.7	0.9	2	1.6 $\pm$ 0.5	2.8	10.5	8.0 $\pm$ 2.4	18	22	23.778 (0.0001)***
Temperature ( $^{\circ}$ C)	26.5	27.3	26.8 $\pm$ 0.23	21	23.2	21.91 $\pm$ 0.7	18	22	19.8 $\pm$ 1.68	21–24	10	8.617 (0.001)***
Salinity (psu)	32	40	38.0 $\pm$ 2.7	38	45	41.5 $\pm$ 2.07	32	40	35.7 $\pm$ 2.7	3, 25, 30	16	12.781 (0.0001)***
Ph	7.9	8.11	7.99 $\pm$ 0.07	8	8.21	8.11 $\pm$ 0.06	7.97	8.14	8.04 $\pm$ 0.07	1	17	8.645 (0.001)***
Biogeochemical parameters												
NO <sub>3</sub> <sup>-</sup> ( $\mu$ m)	1.31	7.51	3.15 $\pm$ 1.92	1.71	11.44	3.07 $\pm$ 2.95	1.33	3.1	2.13 $\pm$ 0.48	10	13	0.905 (0.417)
NO <sub>2</sub> <sup>-</sup> ( $\mu$ m)	0.03	0.88	0.25 $\pm$ 0.3	0.04	2.64	0.37 $\pm$ 0.8	0.04	0.35	0.15 $\pm$ 0.1	25	13	0.315 (0.733)
NH <sub>4</sub> <sup>+</sup> ( $\mu$ m)	5.48	10.22	6.42 $\pm$ 1.36	2.99	7.57	5.01 $\pm$ 1.71	3.27	5.21	4.14 $\pm$ 0.7	19	1	8.297 (0.002)***
T-N ( $\mu$ m)	12.98	26.31	16.41 $\pm$ 3.96	11.99	28.98	16.16 $\pm$ 5.4	11.61	18.28	14.72 $\pm$ 2.35	25	13	0.472 (0.629)
PO <sub>4</sub> <sup>3-</sup> ( $\mu$ m)	0.45	7.92	3.11 $\pm$ 2.82	1.25	2.97	2.07 $\pm$ 0.62	1.3	9.56	4.46 $\pm$ 2.6	2	24	2.661 (0.088)
T-P ( $\mu$ m)	4.95	24.14	13.49 $\pm$ 7.25	8.02	11.26	9.75 $\pm$ 1.26	9.95	25.13	16 $\pm$ 5.42	2	24	3.347 (0.050)*
Si(OH) <sub>4</sub> ( $\mu$ m)	1.56	9.41	4.38 $\pm$ 3.2	3.58	17.48	5.92 $\pm$ 4.18	2.48	19.48	5.37 $\pm$ 5	1	26	0.926 (0.408)
N/P ratio	2.50	30.29	9.57 $\pm$ 8.50	3.30	16.74	6.07 $\pm$ 3.95	2.57	7.45	3.66 $\pm$ 1.50	7	1	<b>3.889 (0.033)*</b>
SPM (mg l <sup>-1</sup> )	8.31	32.17	15.4 $\pm$ 8.42	9.66	59.38	24.14 $\pm$ 15.56	16.98	22.66	20.12 $\pm$ 1.87	5	16	1.970 (0.158)
Chlorophyll- <i>a</i> ( $\mu$ g l <sup>-1</sup> )	2	40.7	11.7 $\pm$ 13	1.7	16.1	5.1 $\pm$ 4.2	2.95	8.7	6.5 $\pm$ 1.7	14	8	1.935 (0.164)
Phaeopigment- <i>a</i> ( $\mu$ g l <sup>-1</sup> )	0.43	9.70	3.32 $\pm$ 3.07	0.57	3.53	1.62 $\pm$ 1.02	0.65	2.09	1.44 $\pm$ 0.40	10	8	3.043 (0.064)
POC ( $\mu$ g l <sup>-1</sup> )	178.43	2129.44	764.73 $\pm$ 722.88	283.49	2937.04	1013.80 $\pm$ 942.12	294.37	657.88	487.84 $\pm$ 113.94	10	18	1.459 (0.25)
PON ( $\mu$ g l <sup>-1</sup> )	31.18	483.85	160.32 $\pm$ 167.73	58.19	522.32	163.22 $\pm$ 152.86	41.61	107.48	76.76 $\pm$ 18.39	10	18	1.395 (0.265)
C/N ratio	4.40	6.32	5.17 $\pm$ 0.60	4.70	7.69	6.08 $\pm$ 1.08	5.85	7.07	6.39 $\pm$ 0.46	6	13	6.840 (0.004)**
Zooplankton												
Total zooplankton ( $\times 10^3$ ind m <sup>-3</sup> )	6	157.32	61.73 $\pm$ 51.73	2.57	20.85	7.73 $\pm$ 5.5	4.82	28.36	15.77 $\pm$ 6.64	16	4	11.170 (0.000)***
Non-copepod zooplankton ( $\times 10^3$ ind m <sup>-3</sup> )	0.8	29.5	10.35 $\pm$ 9.04	0.18	4.1	1.21 $\pm$ 1.28	1.11	7.2	3.79 $\pm$ 1.79	16	4	12.604 (0.000)***
Total copepods ( $\times 10^3$ ind m <sup>-3</sup> )	5.2	127.82	51.37 $\pm$ 43.41	2.39	16.73	6.51 $\pm$ 4.3	3.33	24.18	11.98 $\pm$ 5.65	16	4	10.675 (0.000)***
Calanoids ( $\times 10^3$ ind m <sup>-3</sup> )	0.41	21.9	9.13 $\pm$ 8.5	1.27	4.48	2.07 $\pm$ 0.94	1.28	13.73	5.8 $\pm$ 3.65	7	2	2.421 (0.108)
Harpacticoids ( $\times 10^3$ ind m <sup>-3</sup> )	0	1.4	0.45 $\pm$ 0.52	0	0.65	0.25 $\pm$ 0.25	0.14	1.81	0.72 $\pm$ 0.6	8, 9, 13	22	2.047 (0.149)
Cyclopoids ( $\times 10^3$ ind m <sup>-3</sup> )	3.14	107.75	37.41 $\pm$ 35.85	0.78	5.08	2.54 $\pm$ 1.49	1.72	10	4.92 $\pm$ 2.49	16	4	17.203 (0.0001)***
Adult male ( $\times 10^3$ ind m <sup>-3</sup> )	0.69	27.983	6.98 $\pm$ 8.44	0.5	1.88	1.18 $\pm$ 0.5	0.13	5.19	1.18 $\pm$ 1.48	24	4	9.994 (0.001)***
Adult female ( $\times 10^3$ ind m <sup>-3</sup> )	0.95	60.05	22.27 $\pm$ 19.97	0.49	4.24	1.75 $\pm$ 1.09	0.6	8.63	6.07 $\pm$ 2.84	16	4	9.343 (0.001)***
Copepodit ( $\times 10^3$ ind m <sup>-3</sup> )	1.04	30.24	13.32 $\pm$ 11.22	0	0.003	0.001 $\pm$ 0	0.85	3.58	2.41 $\pm$ 1.01	16	4	257.789 (0.0001)***
Nauplii ( $\times 10^3$ ind m <sup>-3</sup> )	0.45	8.7	4.36 $\pm$ 3.03	0	0.006	0.001 $\pm$ 0.002	0	1.14	0.52 $\pm$ 0.43	16, 19–20, 27, 30	4	45.899 (0.0001)***
Length of copepod species (mm)	0.70	0.72	0.71 $\pm$ 0.004	0.68	0.69	0.68 $\pm$ 0.001	0.82	0.83	0.82 $\pm$ 0.001	15	21	6816.340 (0.000)***
Sex ratio	0.13	2.35	0.63 $\pm$ 0.81	0.36	2.45	0.86 $\pm$ 0.6	0.01	0.83	0.27 $\pm$ 0.28	24	16	3.646 (0.040)*
Number of copepod species	9	18	12.6 $\pm$ 2.59	8	15	11.9 $\pm$ 2.33	5	12	8.1 $\pm$ 2.18	30	3	11.775 (0.000)***
Shannon index (bits ind <sup>-1</sup> )	0.96	1.94	1.49 $\pm$ 0.33	1.63	2.4	1.95 $\pm$ 0.26	1.24	2.21	1.74 $\pm$ 0.28	9	19	6.160 (0.006)**

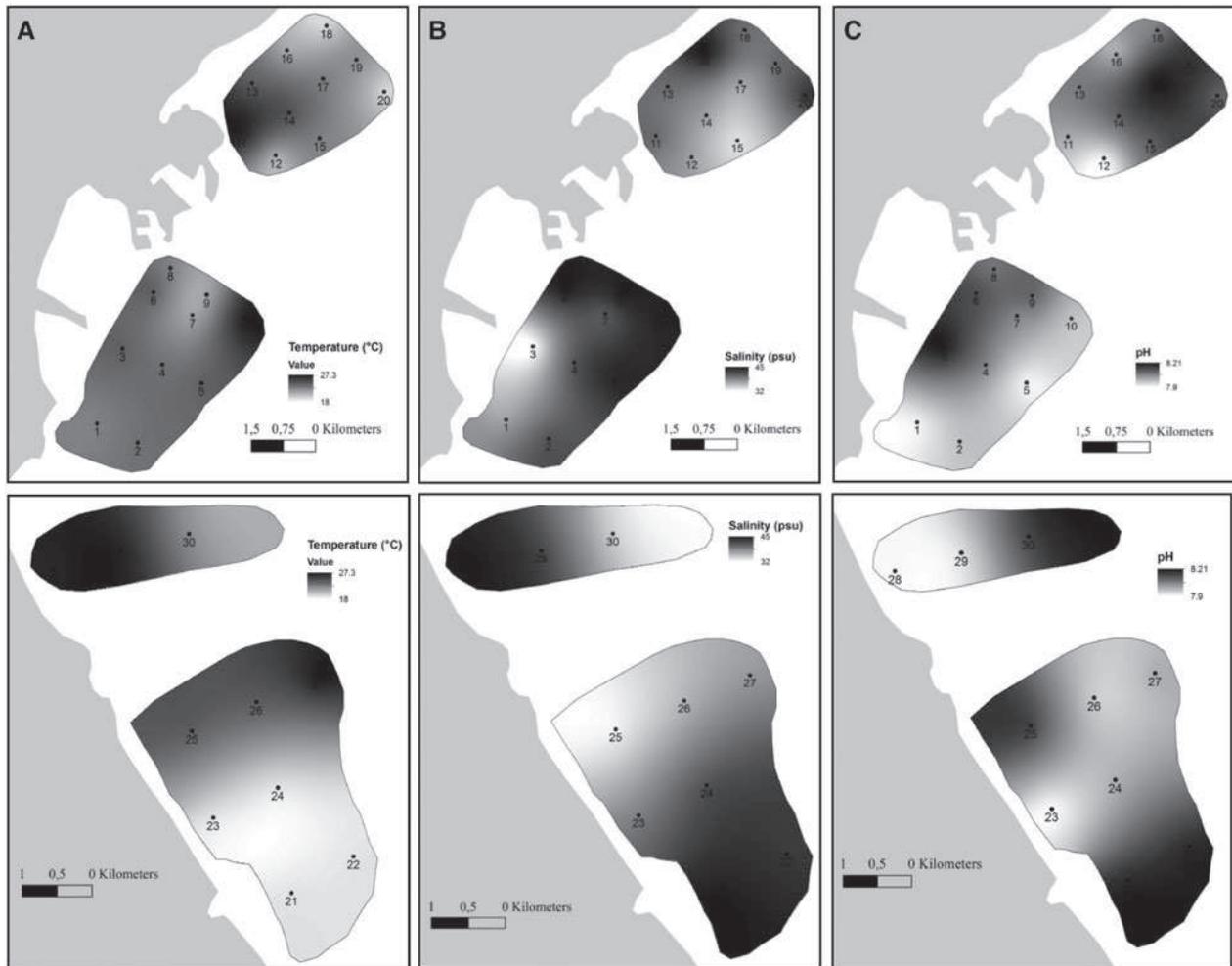


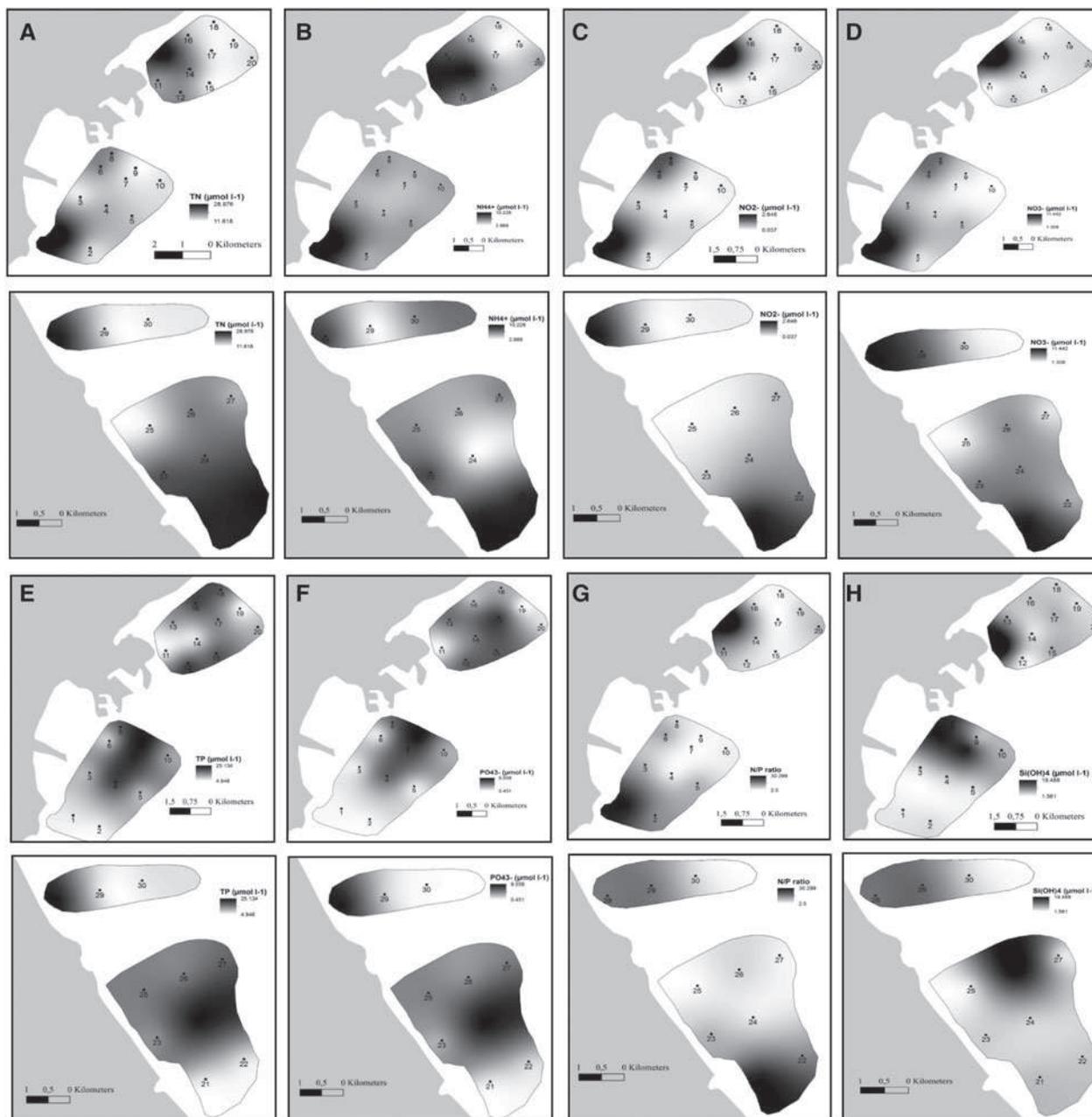
Fig. 2. Spatial variation of physical parameters, i.e. temperature (A), salinity (B) and pH (C) in stations sampled in the northern and southern coastal areas of Sfax and the Ghannouch area during autumn (October–November 2014).

## Zooplankton

The total zooplankton abundance varied from  $2.57 \times 10^3$  (station 16) to  $157.32 \times 10^3 \text{ ind m}^{-3}$  (station 4) (Table 1; Figure 4A). Zooplankton assemblages were dominated by copepods which represented 82, 80 and 75% of total zooplankton abundance, in SC, NC and GA, respectively (Table 2). The density of non-copepod zooplankton varied from  $0.18 \times 10^3$  (station 16) to  $29.5 \times 10^3 \text{ ind m}^{-3}$  (station 4) (Table 1; Figure 4B). Polychaete larvae, cirriped larvae, ostracods, jellyfish, zoea, fish eggs and gastropods were permanent components of meroplankton contributing to 90, 67 and 85% of the non-copepod abundance, in SC, NC and GA, respectively (Table 2). On the other hand, appendicularians, cladocerans, foraminifera and amphipods were also permanent components of the holoplankton but did not exceed 33% of non-copepod abundance (Table 2). Total copepods varied from 2.39 (station 16) to  $127.82 \times 10^3 \text{ ind m}^{-3}$  (station 4) (Table 1; Figure 4C).

A total of 25 different copepod species were identified throughout the study period belonging to three different orders: Calanoida, Cyclopoida and Harpacticoida (Table 2; Figure 5D–F). Calanoida was the most diverse order (12 species) followed by Cyclopoida (seven species) and

Harpacticoida (six species), which contributed to 19, 80 and 1%, respectively to the total zooplankton abundance in SC, 43, 52 and 5% in NC and 51, 43 and 6% in GA (Table 2). Among calanoid copepods, *Paracalanus parvus* (Claus, 1863) and *Paracartia grani* (Sars, 1904) were the most abundant species in SC (6 and 3.5%) and NC (10 and 16% total zooplankton abundance), whereas *Paracalanus parvus* (22.5% total zooplankton abundance) prevailed in GA. Among cyclopoid copepods, *Oithona nana* (Giesbrecht, 1892) and *Oithona similis* (Claus, 1866) were the most abundant species representing 59, 33 and 24% of total zooplankton abundance, in SC, NC and GA, respectively. *Oithona similis* was the only ubiquitous and cosmopolitan species in this study period (100% occurrence frequency) (Table 2). Conversely, *Oithona setigera* (Crisafi, 1959), *Acamthocyclops* sp. (Kiefer, 1927) and *Paracartia latisetosa* (Kritchagin, 1873) were specific to SC and totally absent in the two other areas, whereas *Tisbe battagliai* (Volkmann-Rocco, 1972) and *Tigriopus* sp. (Mori, 1932) were recorded only in NC. The main differences in copepod between the two sampled areas nearest in time (NC and SC) were found to be influenced by the water column depth. In fact, the noticeable presence of meiobenthic copepods such as *Tigriopus* and *Tisbe* were observed only in the shallower area (NC mean depth: 1.6 m). However, the



**Fig. 3.** Spatial variation of nutrient compounds, i.e. total nitrogen (T-N) (A), ammonium ( $\text{NH}_4^+$ ) (B), nitrite ( $\text{NO}_2^-$ ) (C), nitrate ( $\text{NO}_3^-$ ) (D), total phosphate (T-P) (E), orthophosphate ( $\text{PO}_4^{3-}$ ) (F), N/P ratio (G) and silicate ( $\text{Si}(\text{OH})_4$ ) (H) in stations sampled in the northern and southern coastal areas of Sfax and the Ghannouch area during autumn (October–November 2014).

highest abundance of all main copepod taxa was recorded in the deeper area (SC mean depth: 4.9 m) which corroborates this fact (Tables 1 & 2, and CCA). All these species did not exceed 40% of occurrence frequency. Harpacticoids did not exceed 6% of total zooplankton abundance during the survey period and the highest abundance was observed with *Euterpina acutifrons* (Dana, 1847) in GA which represented 4.7% of total zooplankton (Table 2).

The abundance peak of copepods recorded at station 4 was associated with a high density of cyclopoids, adult male and female, copepodid and nauplii (Tables 1 & 2; Figures 4C & 5C–E, G–H). During this period, low percentages of larval stages (copepodids: 29, 1 and 25%, nauplii: 9, 22 and 6% of total copepod abundance) and high numbers of adults (62,

47 and 69% of total copepod abundance) were recorded at SC, NC and GA, respectively. The sex-ratio (adult male/adult female) did not exceed 0.89 in most stations and was  $>1$  (male dominance) only at stations 3, 7, 16 and 20 where it varied from 1.06 to 2.45 (Table 1; Figure 5F). Shannon–Weaver diversity index ( $H'$ ) for copepods was relatively low with values ranging between 0.96 (10 species, station 9 in SC) and 2.4 bits  $\text{ind}^{-1}$  (15 species, station 19 in NC) (Table 1; Figure 5A). Evenness index ( $J$ ) was higher in the GA ( $0.6 \pm 0.1$ ) and in NC ( $0.6 \pm 0.08$ ) than in SC ( $0.5 \pm 0.1$ ) (Figure 5B).

Most of the zooplankton parameters displayed significant differences between NC, SC and GA, except for calanoid and harpacticoid (ANOVA,  $P < 0.05$ ). Total zooplankton,

**Table 2.** Quantitative aspects (D, Density; RA, Relative abundance; FO, Frequency of occurrence; TL, Total length) of the zooplankton taxa sampled from 30 stations of the northern and southern coastal areas of Sfax and the Ghannouch area sampled in October–November 2014 (Abbr: Abbreviation, \*: Large copepods with TL > 1.45 mm).

Zooplankton	Abbr	Southern coastal area				Northern coastal area				Ghannouch area			
		D (ind m <sup>-3</sup> )	RA (%)	FO (%)	TL (mm)	D (ind m <sup>-3</sup> )	RA (%)	FO (%)	TL (mm)	D (ind m <sup>-3</sup> )	RA (%)	FO (%)	TL (mm)
Copepods			82				80				75		
Calanoids			19				43				51		
<i>Acartia clausi</i> (Giesbrecht, 1889)	<i>Acl</i>	347.992 ± 661.81	0.61	80	1.02	19.626 ± 31.962	0.32	40	1.06	–	–	–	–
<i>Acartia discaudata</i> (Giesbrecht, 1882)	<i>Adi</i>	1.2717 ± 4.021	0.002	10	1.20	–	–	–	–	15.543 ± 49.151	0.10	10	1.30
<i>Acartia</i> sp. (Dana, 1846)	<i>Aca</i>	131.585 ± 188.125	0.23	70	1.14	20.799 ± 42.937	0.34	30	1.15	63.302 ± 151.765	0.42	20	1.16
<i>Paracalanus parvus</i> (Claus, 1863)	<i>Ppa</i>	3296.7355 ± 3667.654	5.75	90	0.81	609.529 ± 550.77	10	100	0.84	3403.281 ± 1743.181	22.31	100	0.81
<i>Paracartia grani</i> (Sars, 1904)	<i>Pgr</i>	1938.0378 ± 2850.466	3.38	90	1.13	953.156 ± 245.02	15.64	100	1.15	485.082 ± 592.992	3.18	60	1.14
<i>Paracartia latisetosa</i> (Kritchagin, 1873)	<i>Pla</i>	1.526 ± 4.825	0.003	10	1.13	–	–	–	–	–	–	–	–
<i>Temora longicornis</i> (Müller, 1792)	<i>Tlo</i>	125.851 ± 356.164	0.22	40	0.86	76.277 ± 96.902	1.25	60	0.88	510.234 ± 849.184	3.35	40	0.82
<i>Temora stylifera</i> (Dana, 1849)	<i>Tst</i>	–	–	–	–	20.135 ± 43.807	0.33	20	0.85	641.855 ± 1081.192	4.21	60	0.85
<i>Temora</i> sp. (Baird, 1850)	<i>Tsp</i>	346.019 ± 494.655	0.60	90	0.80	–	–	–	–	141.3 ± 446.829	0.93	10	0.82
<i>Centropages krøyeri</i> (Giesbrecht, 1893)	<i>Ckr</i>	2010.816 ± 2756.877	3.51	90	0.78	277.133 ± 283.274	4.55	80	0.78	226.503 ± 573.476	1.49	30	0.76
<i>Agladiaptomus leptopus</i> (Forbes, 1882)	<i>Ale</i>	0.1 ± 0.316	0.0002	10	0.86	8.901 ± 19.337	0.15	20	1.63*	269.883 ± 411.052	1.77	40	1.60*
<i>Eucalanus</i> sp. (Dana, 1852)	<i>Esp</i>	933.814 ± 1657.699	1.63	100	0.69	90.752 ± 85.856	1.49	100	0.71	49.666 ± 157.06	0.33	10	0.68
Harpacticoids			1				5				6		
<i>Clytemnestra scutellata</i> (Dana, 1847)	<i>Csc</i>	45.165 ± 110.298	0.08	40	0.16	30.697 ± 42.916	0.50	70	0.18	–	–	–	–
<i>Euterpina acutifrons</i> (Dana, 1847)	<i>Eac</i>	349.566 ± 490.107	0.61	80	0.25	52.832 ± 69.231	0.87	60	0.29	724.812 ± 601.595	4.75	100	0.27
<i>Harpacticus littoralis</i> (Sars, 1910)	<i>Hli</i>	52.945 ± 70.244	0.09	50	0.33	97.97 ± 137.835	1.61	60	0.35	–	–	–	–
<i>Tisbe battagliai</i> (Volkman-Rocco, 1972)	<i>Tba</i>	–	–	–	–	59.035 ± 94.881	0.97	40	0.23	–	–	–	–
<i>Microsetella norvegica</i> (Boeck, 1865)	<i>Mno</i>	10.061 ± 27.981	0.02	20	0.21	6.994 ± 22.118	0.11	10	0.26	–	–	–	–
<i>Tigriopus</i> sp. (Mori, 1932)	<i>Tsp</i>	–	–	–	–	4.38 ± 9.253	0.07	20	0.23	–	–	–	–
Cyclopoids			80				52				43		
<i>Oithona nana</i> (Giesbrecht, 1892)	<i>Ona</i>	20,593.312 ± 21,463.701	35.90	100	0.57	777.948 ± 597.39	12.77	80	0.61	974.489 ± 644.897	6.39	100	0.56
<i>Oithona plumifera</i> (Baird, 1843)	<i>Opl</i>	2829.25 ± 4368.842	4.93	100	0.63	367.42 ± 224.239	6.03	100	0.66	831.479 ± 880.519	5.45	80	0.63
<i>Oithona similis</i> (Claus, 1866)	<i>Osi</i>	13,191.252 ± 13,569.645	22.99	100	0.48	1309.853 ± 1014.211	20.86	100	0.57	3120.144 ± 1791.003	17.27	100	0.52
<i>Oithona</i> sp. (Baird, 1843)	<i>Osp</i>	637.283 ± 1310.432	1.11	40	0.48	24.031 ± 49.854	0.39	30	0.49	–	–	–	–
<i>Oithona setigera</i> (Crisafi, 1959)	<i>Ose</i>	21.035 ± 66.519	0.04	10	0.51	–	–	–	–	–	–	–	–
<i>Acanthocyclops vernalis</i> (Fischer, 1853)	<i>Ave</i>	144.291 ± 442.027	0.25	30	0.90	66.538 ± 76.68	1.09	50	1.01	–	–	–	–
<i>Acanthocyclops</i> sp. (Kiefer, 1927)	<i>Asp</i>	2.289 ± 7.238	0.004	10	0.68	–	–	–	–	–	–	–	–
Non-copepod zooplankton			18				20				25		
Meroplankton			90				67				85		
Polychaeta larvae	<i>Pla</i>	585.594 ± 771.279	1.0	100	–	82.276 ± 75.335	1.4	90	–	259.073 ± 182.159	1.7	80	–
Cirripedia larvae	<i>Cla</i>	1227.195 ± 1283.605	2.1	100	–	245.18 ± 321.581	4.0	90	–	420.155 ± 632.759	2.8	60	–

Ostracoda	Ost	41.92 ± 55.043	0.1	50	-	45.808 ± 45.036	0.8	90	-	30.52 ± 68.324	0.2	20	-
Medusa	Med	-	-	-	-	1.865 ± 5.898	0.0	10	-	8.407 ± 26.586	0.1	10	-
Zoea	Zoe	112.884 ± 215.171	0.2	80	-	27.782 ± 22.984	0.5	90	-	170.407 ± 226.864	1.1	50	-
Fish eggs	Egg	6451.115 ± 6078.932	11.2	100	-	253.472 ± 400.921	4.2	90	-	2014.726 ± 1439.617	13.2	90	-
Gastropod viliger	Gvi	842.95 ± 776.626	1.5	100	-	166.31 ± 179.514	2.7	80	-	320.468 ± 555.849	2.1	40	-
Holoplankton			10				33				15		
Appendicularia	App	228.195 ± 301.928	0.4	90	-	203.045 ± 281.079	3.3	100	-	369.711 ± 261.316	2.4	100	-
Cladocera	Clad	138.73 ± 255.875	0.2	80	-	34.901 ± 54.504	0.6	50	-	98.288 ± 107.32	0.6	70	-
Foraminifera	For	137.803 ± 206.7608	0.2	90	-	62.457 ± 62.394	1.0	90	-	36.243 ± 114.611	0.2	10	-
Amphipods	Amp	0.254 ± 0.804	0.0	10	-	2.758 ± 6.648	0.0	20	-	0	0.0	-	-
Nematoda	Nem	37.896 ± 85.007	0.1	30	-	0	0.0	-	-	0	0.0	-	-
Mysidacea	Mys	-	-	-	-	24.345 ± 52.245	0.4	20	-	27.2 ± 86.014	0.2	10	-
Euphausiacea	Eup	236.864 ± 269.975	0.4	80	-	44.972 ± 40.795	0.7	80	-	22.819 ± 72.163	0.1	10	-
Bivalvia	Biv	314.621 ± 622.31	0.5	70	-	24.111 ± 31.278	0.4	50	-	16.108 ± 50.938	0.1	10	-

non-copepod zooplankton, total copepods, cyclopoid, adult males, adult females, copepodit, nauplii and number of copepod species were more abundant in SC than in NC and GA (ANOVA,  $P < 0.0001$ ) (Table 1). The lowest abundances were always recorded at NC with total zooplankton being 8-fold and 2-fold less abundant than at SC and GA respectively and particularly low values for nauplii and copepodites almost absent in this area. The Shannon and Weaver diversity index ( $H'$ ) for copepods was significantly higher at NC than at SC and GA. The length of copepod species was significantly higher in GA ( $0.82 \pm 0.001$  mm) and in SC ( $0.71 \pm 0.004$  mm) than in NC ( $0.68 \pm 0.001$  mm) (Table 1) ( $P < 0.0001$ ). Small planktonic copepods ( $< 1.45$  mm) contributed to 100, 99.9 and 98% of total copepod abundance in SC, NC and GA, respectively, while the largest copepods (1.45–2.5 mm) represented mostly by one species *Aglaodiaptomus leptopus* (Forbes, 1882) (1.6 mm) did not exceed 2% at NC and GA (Table 2).

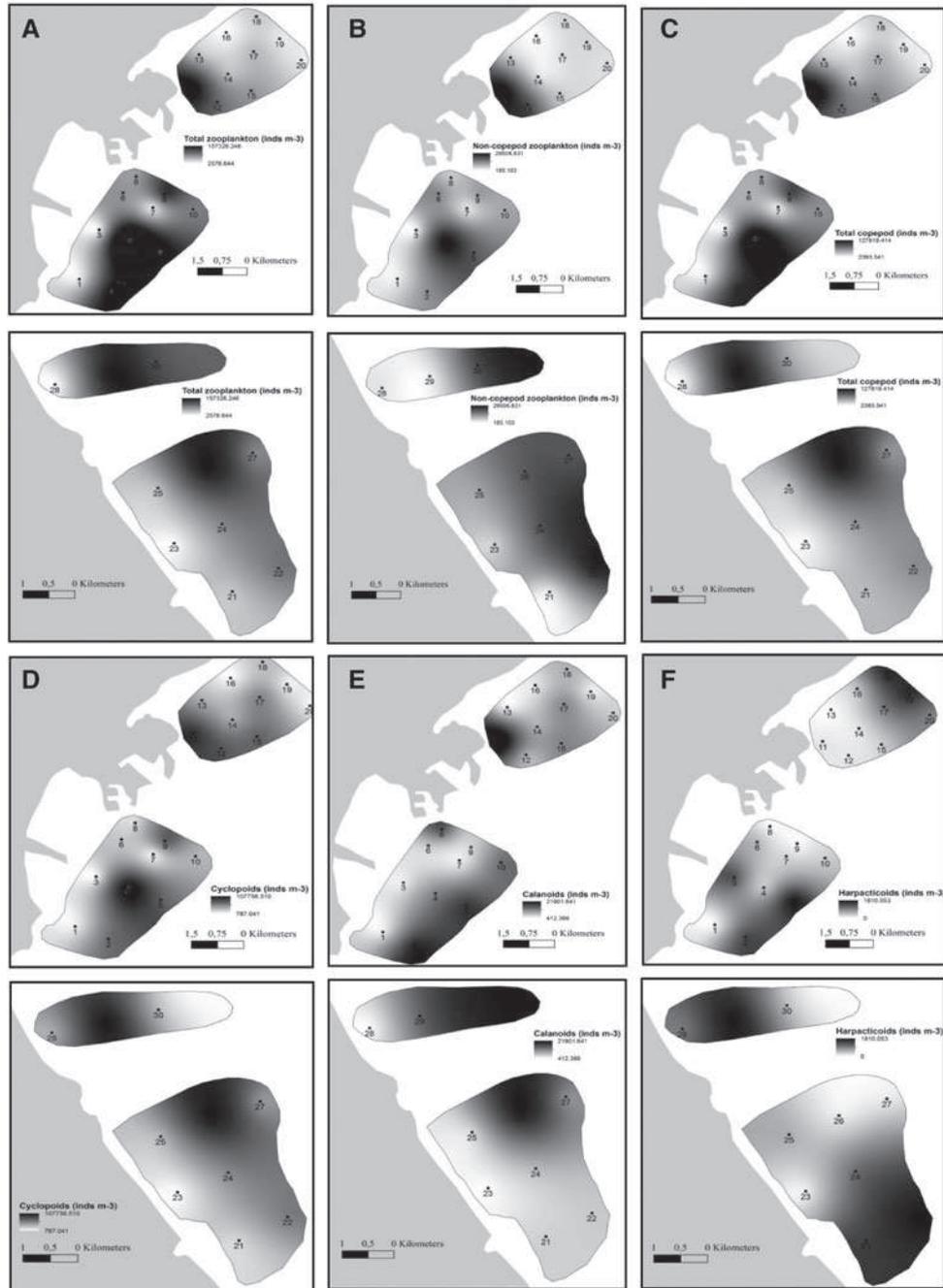
The classification of copepod species according to their Indicator Value (IndVal) in these three coastal marine ecosystems under contrasting anthropogenic inputs showed that each zone was characterized by a suitable and specific environmental health indicator. In fact, in the SC, several species have a high IndVal ( $> 40\%$ ). They were composed of 10 copepod species which represented 45% of the copepod species' richness in this area and in particular *Oithona nana* (Indval = 92%) which was the most indicative of water quality in this zone, followed by *Eucalanus* sp. (Dana, 1852) (87%) and *Oithona similis* (77%) (Table 3). Concerning the NC, IndVal was very low and only *Tisbe battagliai* (40%) and *Harpacticus littoralis* (Sars, 1910) (39%) could be good indicators. However, in GA the best indicator species are *Euterpina acutifrons* (64%), *Temora stylifera* (Dana, 1849) (58%) and *Paracalanus parvus* (47%) (Table 3).

## Multivariate analysis

The Canonical Correspondence Analysis (CCA) on the zooplankton parameters and various physico-chemical and biogeochemical factors explained 46.5% for the F1 and F2 axes (Figure 6). The F1 axis (26.9%), selected positively SC stations (1–10) with calanoid, cyclopoid, total copepod, non-copepod-zooplankton, total zooplankton and temperature. We note that temperature displayed significant positive correlations with total zooplankton ( $r = 0.59$ ,  $P < 0.05$ ), total copepods ( $r = 0.60$ ,  $P < 0.05$ ) and cyclopoids ( $r = 0.57$ ,  $P < 0.05$ ). F1 axis selected negatively NC stations (11–20) with Evenness index ( $J$ ), the diversity index ( $H'$ ), pH, sex ratio and salinity. The F2 axis (19.6%) selected negatively the GA stations (21–30) with harpacticoid, T-P,  $PO_4^{3-}$  and SPM (Figure 6A). The plots of the copepod species confirmed our observation with SC characterized by *Oithona nana* and *Oithona similis* among cyclopoid copepods, and GA characterized by *Paracalanus parvus* among calanoid copepods and *Euterpina acutifrons* among harpacticoid copepods (Figure 6B).

## DISCUSSION

Zooplankton is recognized among the best indicators for investigating and documenting environmental changes (Siokou-Frangou & Papathanassiou, 1991; Sipkay et al.,



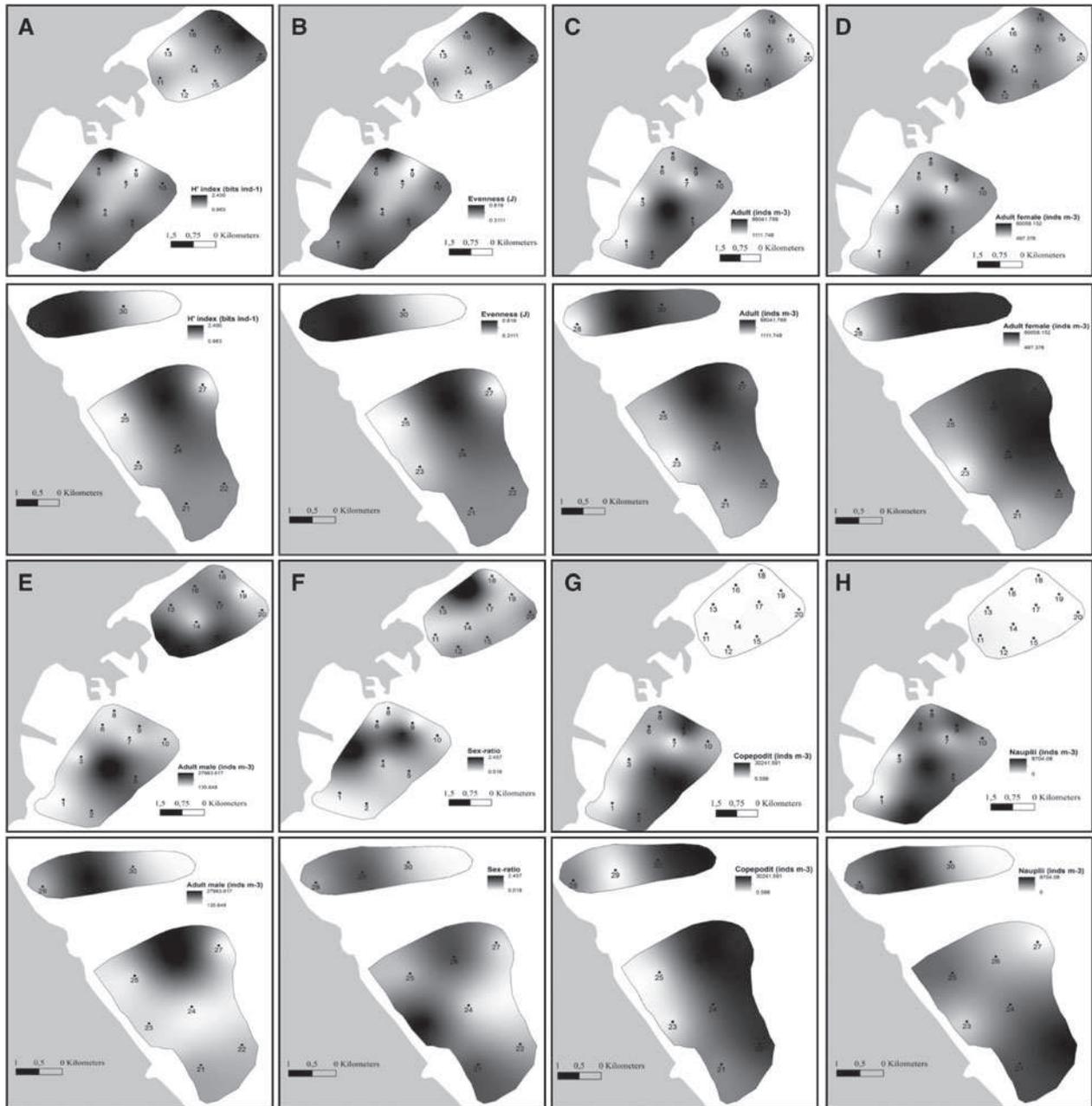
**Fig. 4.** Spatial variation of zooplankton parameters, i.e. abundance of total zooplankton (A), non-copepod zooplankton (B), total copepods (C), cyclopoids (D), calanoids (E) and harpacticoids (F) in stations sampled in the northern and southern coastal areas of Sfax and the Ghannouch area during autumn (October–November 2014).

2009; Bagheri *et al.*, 2013). The variations of zooplankton populations are closely related to environmental parameters such as temperature, pH and salinity (Pascual & Guichard, 2005; Rossi & Jamet, 2009; Srichandan *et al.*, 2013). Main zooplankton taxa have short life cycles and their community structure is able to reflect real-time scenarios as it is less enforced by the stability of individuals from previous years (Richardson, 2008; Bagheri *et al.*, 2013). Thus, hypoxic/anoxic conditions related to organic enrichment are found to be associated with the decrease of zooplankton abundance in eutrophic and/or organically polluted systems (Stalder & Marcus, 1997; Park & Marshall, 2000; Gordina *et al.*, 2001)

and high turbidity can increase the death rate of copepods (e.g. Castel & Feurtet, 1992).

### Anthropogenic inputs status of the coastal zone

We found quite high  $\text{PO}_4^{3-}$  concentrations, very probably related to inputs from the phosphate processing industries (SIAPE-Sfax and GCT-Gabes), revealing different anthropogenic input degrees. In this context, we may say that GA is the most affected by  $\text{PO}_4^{3-}$  followed by SC and NC. The



**Fig. 5.** Spatial variation of zooplankton parameters, i.e. Shannon index (E) (A), Evenness (J) (B), abundance of adult (C), adult female (D), adult male (E), sex-ratio (F), copepodit (G) and nauplii (H) in stations sampled in the northern and southern coastal areas of Sfax and the Ghannouch area during autumn (October–November 2014).

high concentration of  $\text{PO}_4^{3-}$  ( $4.46 \pm 2.60 \mu\text{m}$ ) in GA was in the range of values previously reported by Baccar (2014) in the Gabes area ( $3.73 \pm 1.57 \mu\text{m}$ ), while the values we found at SC ( $3.11 \pm 2.81 \mu\text{m}$ ) and NC ( $2.07 \pm 0.62 \mu\text{m}$ ) were far higher than the ones noted by Rekik *et al.* (2012) ( $0.28 \pm 0.05 \mu\text{m}$ ) in SC or by Drira *et al.* (2009) in the offshore waters of the Gulf of Gabes ( $0.06 \pm 0.03 \mu\text{m}$ ). The highest levels of  $\text{PO}_4^{3-}$  were always found near the potential sources, i.e. the SIAPE-Sfax plant (station 4), wadi El Maou (station 7), the Sfax fishing harbour (station 9) and the commercial harbour (stations 25 and 27) and the GCT-Gabes phosphoric acid industry (station 28) for GA. Therefore, the high apparent availability of inorganic phosphates in the SC and GA areas were related to the release of phosphate residues from

the SIAPE and GCT-Gabes industries (Bejaoui *et al.*, 2004; Ben Brahim *et al.*, 2010; Rekik *et al.*, 2012; Ben Salem *et al.*, 2015; Drira *et al.*, 2016). Ammonium, which was the chemical factor with the most significant differences between areas, showed the highest value in the SC (ANOVA,  $P < 0.001$ ). The importance of ammonium compared with nitrite and nitrate was a typical finding of coastal eutrophic waters due to anthropogenic pollution, mainly represented by untreated discharges (Nuccio *et al.*, 2003; Bouchouicha-Smida *et al.*, 2012). Our result also showed that NC was slightly alkaline ( $\text{pH} 8.12 \pm 0.06$ ) compared with GA ( $\text{pH} = 8.05 \pm 0.07$ ), and SC ( $\text{pH} = 7.99 \pm 0.07$ ), which is in agreement with results reported by Rekik *et al.* (2013) at the same season ( $8.16 \pm 0.3$ ) for this area.

**Table 3.** Classification of copepod species according to their Indicator Value (IndVal) in each zone i.e. the northern and southern coastal areas of Sfax and the Ghannouch area sampled in October–November 2014.

Southern coastal area		Northern coastal area		Ghannouch area	
Copepod species	IndVal (%)	Copepod species	IndVal (%)	Copepod species	IndVal (%)
<i>Oithona nana</i> (Giesbrecht, 1892)	92.2	<i>Tisbe battagliai</i> (Volkman-Rocco, 1972)	40.0	<i>Euterpina acutifrons</i> (Dana, 1847)	64.3
<i>Eucalanus</i> sp. (Dana, 1852)	86.9	<i>Harpacticus littoralis</i> (Sars, 1910)	39.0	<i>Temora stylifera</i> (Dana, 1849)	58.2
<i>Oithona similis</i> (Claus, 1866)	77.2	<i>Clytemnestra scutellata</i> (Dana, 1847)	28.3	<i>Paracalanus parvus</i> (Claus, 1863)	46.6
<i>Acartia clausi</i> (Giesbrecht, 1889)	75.7	<i>Paracartia grani</i> (Sars, 1904)	28.2	<i>Aglaodiaptomus leptopus</i> (Forbes, 1882)	38.7
<i>Centropages krøyeri</i> (Giesbrecht, 1893)	72.0	<i>Tigriopus</i> sp. (Mori, 1932)	20.0	<i>Temora longicornis</i> (Müller, 1792)	28.7
<i>Oithona plumifera</i> (Baird, 1843)	70.2	<i>Acanthocyclops vernalis</i> (Fischer, 1853)	15.8	<i>Oithona plumifera</i> (Baird, 1843)	16.5
<i>Temora</i> sp. (Baird, 1850)	63.9	<i>Oithona plumifera</i> (Baird, 1843)	9.1	<i>Oithona similis</i> (Claus, 1866)	15.4
<i>Paracartia grani</i> (Sars, 1904)	51.7	<i>Centropages krøyeri</i> (Giesbrecht, 1893)	8.8	<i>Acartia discaudata</i> (Giesbrecht, 1882)	9.2
<i>Acartia</i> sp. (Dana, 1846)	42.7	<i>Eucalanus</i> sp. (Dana, 1852)	8.4	<i>Paracartia grani</i> (Sars, 1904)	8.6
<i>Paracalanus parvus</i> (Claus, 1863)	40.6	<i>Paracalanus parvus</i> (Claus, 1863)	8.3	<i>Acartia</i> sp. (Dana, 1846)	5.9
<i>Oithona</i> sp. (Baird, 1843)	38.5	<i>Oithona similis</i> (Claus, 1866)	7.4	<i>Oithona nana</i> (Giesbrecht, 1892)	4.4
<i>Euterpina acutifrons</i> (Dana, 1847)	24.8	<i>Temora longicornis</i> (Müller, 1792)	6.4	<i>Temora</i> sp. (Baird, 1850)	2.9
<i>Clytemnestra scutellata</i> (Dana, 1847)	23.8	<i>Microsetella norvegica</i> (Boeck, 1865)	4.1	<i>Centropages krøyeri</i> (Giesbrecht, 1893)	2.7
<i>Acanthocyclops vernalis</i> (Fischer, 1853)	20.5	<i>Acartia</i> sp. (Dana, 1846)	2.9	<i>Eucalanus</i> sp. (Dana, 1852)	0.5
<i>Harpacticus littoralis</i> (Sars, 1910)	17.5	<i>Euterpina acutifrons</i> (Dana, 1847)	2.8		
<i>Microsetella norvegica</i> (Boeck, 1865)	11.8	<i>Oithona nana</i> (Giesbrecht, 1892)	2.8		
<i>Acanthocyclops</i> sp. (Kiefer, 1927)	10.0	<i>Acartia clausi</i> (Giesbrecht, 1889)	2.1		
<i>Oithona setigera</i> (Crisafi, 1959)	10.0	<i>Oithona</i> sp. (Baird, 1843)	1.1		
<i>Paracartia latisetosa</i> (Kritchagin, 1873)	10.0	<i>Aglaodiaptomus leptopus</i> (Forbes, 1882)	0.6		
<i>Temora longicornis</i> (Müller, 1792)	7.1	<i>Temora stylifera</i> (Dana, 1849)	0.6		
<i>Acartia discaudata</i> (Giesbrecht, 1882)	0.8				
<i>Aglaodiaptomus leptopus</i> (Forbes, 1882)	0.0				

## Copepod assemblages as a bioindicator of environmental quality

Investigations on zooplankton community in relation with anthropogenic inputs have already been conducted in the Mediterranean Sea (Siokou-Frangou, 1996; Jamet *et al.*, 2001; Isinibilir *et al.*, 2008; Papantoniou *et al.*, 2015). One previous study has been performed so far in the coastal waters of the Gulf of Gabes, i.e. in the SC (Drira *et al.*, submitted). In this study, we tried to determine the effects of anthropogenic inputs on planktonic copepods by comparing the abundance and spatial distribution of the main species in three coastal marine areas with different anthropogenic input levels. Our results showed a clear dominance of copepods (nauplii stage to the adult stage) in the three areas, representing 76, 83 and 84% of total zooplankton in GA, SC and NC, respectively. The dominance of copepods has already been reported in several studies in the Gulf of Gabes: in SC (5–50%; Ben Salem *et al.*, 2015), NC (82%, Rekik *et al.*, 2012), in the city of Gabes: Ghannouch and Zarrat (46–83%; Baccar, 2014) and in offshore waters of the Gulf of Gabes (83%; Drira *et al.*, 2014; Ben Ltaief *et al.*, 2015).

In this study, we reported the presence of 25 species belonging to 13 families and three orders, namely calanoid, cyclopoid and harpacticoid, while poecilostomatoid were virtually absent. We found a preponderance of cyclopoids, particularly in SC (with 80% of total copepod abundance), while calanoids were also important in GA (51%) and NC (43%). This is consistent with previous works showing that cyclopoids are numerically the most important group in the Gulf of Gabes (Drira *et al.*, 2009, 2014; Ben Ltaief *et al.*, 2015). This predominance of cyclopoids in such polluted areas agrees with their cosmopolitan and less demanding character in terms of environmental conditions compared

with other groups (Sarkka *et al.*, 1998). Adult copepods dominated other developmental stages of copepods in the three study sites with adult females being predominant (>58% of adults). The dominance of females was already reported in the Gulf of Gabes (2005–2007) (Drira *et al.*, 2010a, b, 2014). Dominance of females compared with males, which reduces the sex ratio (Kiorboe, 2006), may be due to the higher mortality of males because of their increased vulnerability to predation during their search for mates (Mendes-Gusmão *et al.*, 2013). In addition, environmental factors such as pollution have strong effects on copepod sex ratio, and suggest that differential physiological longevity of males and females may be more important in determining the sex ratio (Mendes-Gusmão *et al.*, 2013). Oithonids were numerically very important with *O. nana* (36% of zooplankton abundance in SC, 12.7% in NC and 6.4% in GA) and *O. similis* (23% in SC, 21% in NC and 17% in GA) as the main species. In previous studies, *O. nana* were also reported at a very high abundance in SC (Drira *et al.*, submitted) as well as in NC before (2007; Rekik *et al.*, 2012) and after (2009–2010; Rekik *et al.*, 2013) the *Taparura* restoration process. In the CCA analysis, Oithonids (and *O. similis*) were negatively correlated with the NC stations corresponding to the less disturbed area and positively correlated with the polluted SC stations, which clearly indicates an affinity of these copepods for anthropogenic inputs. In general, oithonidae may survive in a wide range of habitats and maintain their populations under adverse conditions because they are morphologically less specialized than calanoids (Paffenhöfer, 1993). In agreement with our study, Oithonidae, and more specifically *O. nana*, are often associated with a high degree of anthropogenic inputs and regarded as a bio-indicator species of anthropogenic pollution (Annabi-Trabelsi *et al.*, 2005; Drira *et al.*, 2014; Serranito *et al.*, 2016). The good adaptation of

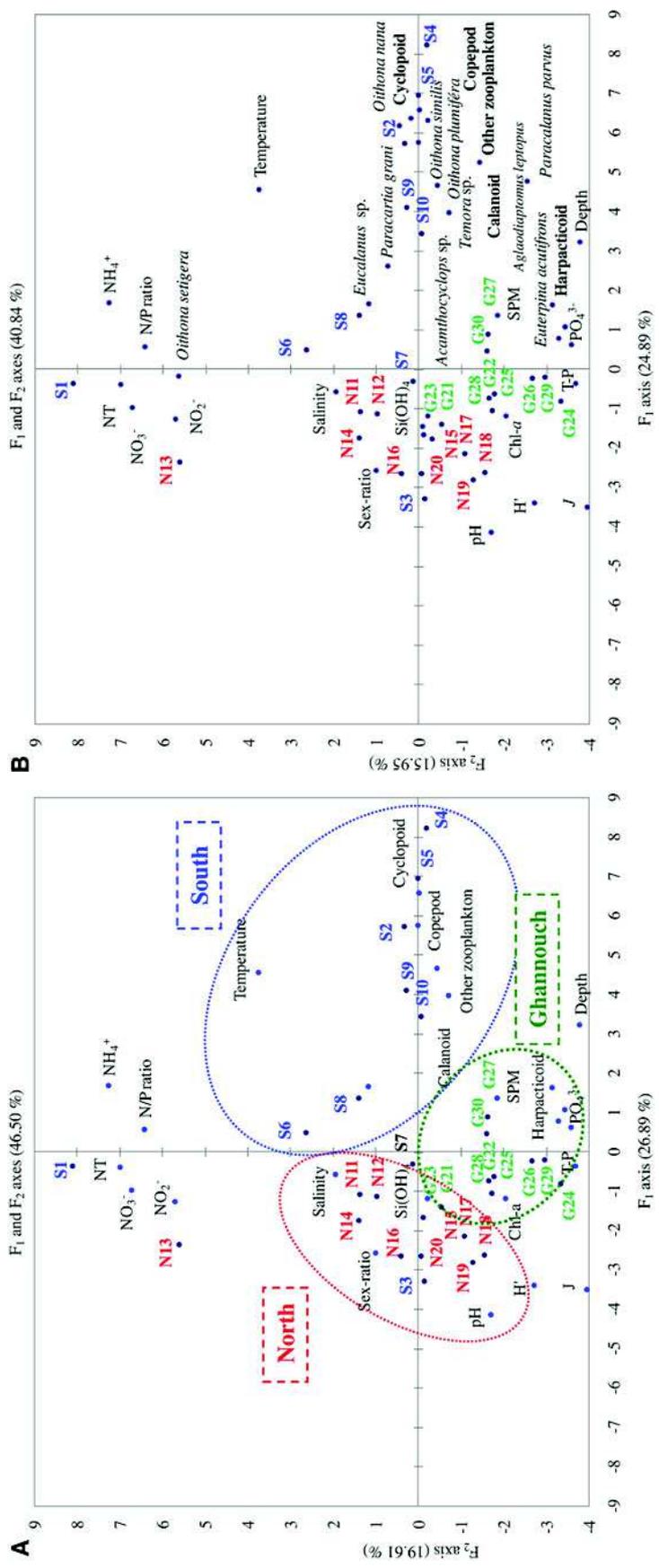


Fig. 6. Canonical correspondence analysis (CCA) (Axis I and II) on mean values of several physical and chemical parameters with (A) zooplankton group and (B) copepod species in stations sampled in the northern and southern coastal areas of Sfax and the Ghannouch area sampled during autumn (October–November 2014).

*O. nana* to anthropized areas can be partly explained by its feeding habits (Serranito *et al.*, 2016). It has been reported to be more flexible in its diet compared with other copepods, thus able to adapt to a wide range of food resources (Moraitou-Apostolopoulou, 1976; Lampitt & Gamble, 1982; Rekik *et al.*, 2012; Serranito *et al.*, 2016) and having a mixotrophic diet by incorporating faecal matter, ciliates protozoa and dinoflagellates (Williams & Muxagata, 2006). This small euryoecious species is characterized by a high tolerance to various environmental parameters (Riccardi & Mariotto, 2000). Its shorter life cycle and higher reproduction rate compared with larger copepods could also partly explain its higher success in adapting to new conditions (Gallienne & Robins, 2001). *Oithona similis* is a ubiquitous and abundant cyclopoid not only in our study site, but also in the Algerian basin (Riandey *et al.*, 2005), in the Bay of Tunis (Daly-Yahia *et al.*, 2004), in the lagoon of Tunis (Annabi-Trabelsi *et al.*, 2005) and in the offshore waters of the Gulf of Gabes (Drira *et al.*, 2009). In our study, harpacticoid and more specifically *Euterpina acutifrons*, were clearly associated to highly polluted conditions as they were correlated to GA stations characterized by the highest SPM and  $\text{PO}_4^{3-}$  concentrations. *Euterpina acutifrons*, known as eurythermic and euryhaline, lives in marine coastal areas (Furnestin, 1960; Moreira *et al.*, 1982; Delia Vinas *et al.*, 2010). The plasticity of this species which occupies several coastal habitats is often due to its broad trophic spectrum (phytoplankton, microplankton and detritus) (Goswami, 1976; Moreira *et al.*, 1983).

The taxonomic diversity is also strongly influenced by anthropogenic inputs (Danilov & Ekelund, 1999). In the present work, we showed that NC, considered as a restored environment in term of phosphogypsum contamination, was characterized by a taxonomic diversity ( $H' = 1.95 \text{ bits ind}^{-1}$ ,  $J' = 0.6$ ) higher than in GA ( $H' = 1.74 \text{ bits ind}^{-1}$ ,  $J' = 0.6$ ) and SC ( $H' = 1.49 \text{ bits ind}^{-1}$ ,  $J' = 0.5$ ). Taxonomic diversity (as Pielou's evenness) as well as Oithonidae relative abundance were singled out as the most pertinent indicators of anthropogenic pollution in the case study of the Bay of Toulon (Mediterranean Sea) (Serranito *et al.*, 2016). In our study, based on the same indicators we could classify the three study sites according to the significance of pollution impact on copepods as follows:  $\text{NC} < \text{GA} < \text{SC}$  with the diversity ( $H' = 1.95, 1.74$  and  $1.49 \text{ bits ind}^{-1}$ , respectively), and  $\text{GA} < \text{NC} < \text{SC}$  with the percentage of oithonidae (43, 51 and 79%, respectively). We can also note that the mean Indicator Values for Oithonidae are also higher for the SC area (48%) than for NC and GA (3.7 and 12.2% respectively), which is consistent with the indicator based on this copepod family. In this context, we may assume that GA, although the most affected by orthophosphates ( $4.46 \pm 2.60 \mu\text{m}$ ) is a more pollution-resistant ecosystem than SC ( $3.11 \pm 2.81 \mu\text{m}$ ) compared with NC ( $2.07 \pm 0.62 \mu\text{m}$ ).

## CONCLUSION

This study was undertaken to assess the zooplankton communities in accordance with anthropogenic inputs in the Sfax northern and southern coasts and in the Ghannouch area during October and November 2014. These three areas were characterized by different degrees of anthropogenic inputs characterized by levels of  $\text{PO}_4^{3-}$  with highest values at GA (stations 25 and 27 near the commercial harbour and station 28 in

front of GCT-Gabes) and lowest at NC. The most abundant species in the three environments were *O. nana*, *O. similis* and *Paracalanus parvus* while two species were reported for the first time in the Gulf of Gabes (*Agladiaptomus leptopus* and *Eucalanus* sp.). *Oithona nana* and *O. similis* could be used as an indicator of anthropogenic inputs in the Gulf of Gabes. Our results indicate that the fluctuation of copepod abundances may be a useful tool to evaluate the ecosystem health status. The present work shows that the Northern coast, considered as a restored and reclaimed environment, is characterized by slightly higher species diversity, while the Ghannouch area, although the most affected by orthophosphates, was found to be more pollution-resistant than the southern coast. Meanwhile, our study can be useful in the management of this ecosystem for planning the best disposal options for treating urban and industrial wastes in the gulf's coastal waters.

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