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Changes in Copepod Community Between Two Contrasting Samplings in a Highly Polluted Mediterranean Coastal Zone (Sfax Bay, Tunisia)

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Abstract

Changes of taxonomic composition, morphology (total length) and reproductive mode of copepod assemblages between the cold and warm periods were studied in the Southern coast of Sfax, in relation with environmental factors and pollution degree (heavy metal concentration). Salinity, temperature and heavy metal concentration seem to be the most important factors influencing the total length during both sampling periods. A total of 23 different copepod species were identified during the survey period. The copepod abundance was higher in cold (74%; 9.78 ± 10.04 × 103 ind m-3) than in warm (67%; 7.11 ± 8.81 × 10^3 ind m^{-3}) periods. This result could be attributed to an increase of temperature and salinity in August, which favors the development of smaller copepod species. During the cold season, larger adult broadcaster of calanoids species (total length between 0.98 and 1.22 mm) such as *Acartia (Acartia) danae* (Giesbrecht) (Tl = 1.22 mm), *Paracartia grani* (Sars) (Tl = 1.12 mm) and *Paracartia latisetosa* (Krichagin) (Tl = 1.06 mm) were associated to low temperature and salinity. During the warm period, smaller egg-carrying species of harpacticoids (total length between 0.18 and 0.3 mm) such as *Harpacticus littoralis* (Sars), *Tisbe furcata* (Baird) and *Tigriopus sp.* (Norman) were associated with high temperature and heavy metal pollution. H. *littoralis* displayed a very high abundance of ovigerous females (Nfo = 12.8 × 10^3 ind m^{-3}, representing 87.2% of total number of females) with high number of eggs per sac (E = 26 ± 2 egg sac^{-1}).

Keywords: Southern coast of Sfax; Salinity; Total length; Heavy metals; Copepod; Warm and cold periods

Introduction

Most zooplankton organisms are very sensitive to physical, chemical and biological factors [1-4]. Their population dynamics in aquatic ecosystems is closely related to seasonal temperature variation [5-7]. Copepods are the most abundant zooplankton assemblages in marine ecosystems [8-10]. They play a pivotal role in aquatic food webs by transferring nutrient and energy from primary (e.g. phytoplankton) to tertiary (e.g. planktivorous fish) producers [11-15]. As they are highly dependent on environmental conditions (e.g. chemical and organic contaminants, temperature and salinity), copepod species have long been used as bioindicators of environmental quality and water mass origin [16,17]. Marine copepods can thus be an excellent tool for evaluating the impact of marine pollution throughout coastal regions because they quickly respond to different types of stress in different ways (e.g. decreased fecundity, mortality) [18-22].

The Southern coast of Sfax is a transitional system under high anthropogenic pressure [23-25]. Pollution by organic and chemical wastes in this area is mainly due to industrial and fishing activities [26]. Moreover, additional anthropogenic inputs of heavy metals in the Gulf of Gabes from the phosphogypsum waste released from the GCT-Gabes' and the SIAPE-Sfax phosphoric acid industrial complexes might be critical to the coastal marine ecosystems. This situation could lead to serious human health risks and chronic intoxication caused by their potential bioaccumulation in some aquatic fauna such as shrimp, fish, crab, shellfish, mollusk, copepods and cephalopods [27-34]. Several studies have already highlighted the importance of copepod assemblages as bioindicators of water quality changes in the coasts of Sfax [35,36]. Recent studies have evaluated the impact of pollutants on the survival, as well as genetic transcription and expression in several copepod species (Kmiha et al. submitted). In this work, we focused on the copepod community composition

and on the morphology (total length) and reproduction traits (broadcasters, vs. sac spawners) in relation with temperature, salinity, and heavy metal concentration in two contrasting periods.

Materials and Methods

Study Site

The Southern coastline of Sfax is in the Southeast of Tunisia in the Northern part of the Gulf of Gabes (Southern Mediterranean Sea). Heavy metal inputs (Zn, Ni, Pb, Co, Cr, Cd, Mn and Cu) from the waste waters of more than 100 industrial plants including the phosphoric acid and fertilizer plant (SIAPE), the wastewater treatment station and the excessive marine traffic impacts this area.

Sampling strategy and in situ measurements

Eighteen stations were sampled in summer (15th 94 of August, from 12:15 p.m. to 7:30 p.m.) and winter (16th 95 of January, from 8:30 a.m. to 1:30 p.m.) in 2013 (Figure 1). The depths of the stations varied from 3 to 6.2 m in August and from 4.2 and 7.4 m in January. At each station, salinity and temperature were measured using a multi-parameter kit (multi 340i/SET). Water transparency was determined with a Secchi disk. Seawater samples were taken at ~0.1-m depth using PVC Van Dorn bottles (1.5 L) deployed horizontally to determine the suspended particulate matter (SPM), pH, nutrients and chlorophyll-a. Samples for nutrient analyses (120 mL) were preserved immediately upon collection (-20°C, in the dark). For heavy metal analyses, seawater samples were collected at ~0.1-m depth using 4 L Nalgene polycarbonate bottles. The bottles were opened below the water surface to avoid sampling of the surface microlayer. Before use, they were extensively washed with 1 M hydrochloric acid (HCl) and Milli-Q water, rinsed three times with the respective sample before filling. After collection, they were placed in cold and dark conditions. Zooplankton was collected using a cylindro conical net (30 cm aperture, 100 cm height, 100 µm mesh size) equipped with a Hydro-Bios flowmeter. In order to collect representative samples of the whole water column, the net was towed obliquely from near the bottom to the surface at a mean speed of 1 m s^{-1}. The sample volume was calculated by multiplying the net aperture area (0.071 m^2) by the distance travelled by the net (difference in flowmeter readings before and after sampling (using a conversion coefficient of 1.33). Zooplankton samples were rapidly preserved in a buffered formaldehyde solution (2%). After then, they were stained with Rose Bengal to identify the internal tissues of the different zooplankton species and to facilitate copepod dissection.

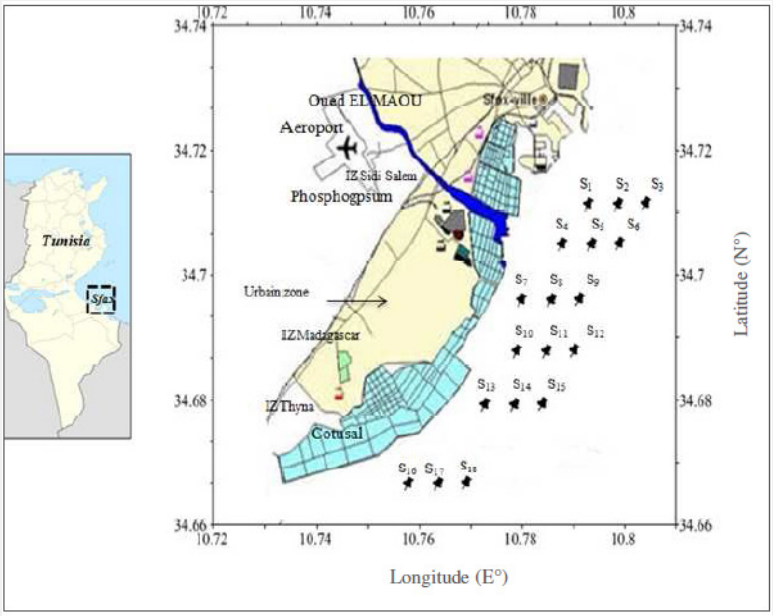

Figure 1: Zooplankton sampling stations in the southern coast of Sfax among season.

Sample Analyses

Nutrients (NO_2-, NO_3-, NH_4+, PO4^{3}-, $Si(OH)_4$, T-N and T-P) were analyzed with a BRAN and LUEBBE type 3 auto-analyzer and their concentrations were determined calorimetrically using a UV-visible (6400/6405) spectrophotometer (APHA, 2012). We also calculated the N/P: DIN (DIN = NO_2- + NO_3- + NH_4+) to DIP (DIP = PO43-) ratio. The SPM concentrations were determined by measuring the dry weight of the residue after filtration of 1 L of seawater onto Whatman GF/C filters. Chl-a analyses were carried out by spectrophotometry, after extraction of the pigments in 10 mL acetone (90%) for 24 h, in the dark at 4°C [37] and the concentrations then estimated using the SCOR-UNESCO (1966) equations. Trace metals, i.e. cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lead

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Pb), manganese (Mn), and zinc (Zn) were analyzed by means of flame 125 atomic absorption spectrophotometry (AAS) (Perkin Elmer A-Analyst 200 instrument copyright @ 2007, version 6 model). Seawater samples were filtered under vacuum filtration using a 0.2 µm filter and the liquid phase underwent acid attack.

Zooplankton samples were identified according to Rose [38], Bradford-Grieve [39] and Costanzon et al. [40] for copepods and according to Tregouboff & Rose [41,42] for non-copepod zooplankton. The names of species were checked using WORMS (http://www.marinespecies.org/). The different copepod species were sorted into four stage development classes (nauplii, copepodids, adult males and adult females). For cyclopoids, poecilostomatoids and harpacticoids fecundity parameters (number of ovigerous females the number of ovigerous females (*Nfo*) and number of eggs per sac (*N*)), were estimated. Enumeration was performed under a vertically mounted deep-focus dissecting microscope (Olympus TL 2) and numerical density (individual $m^{-3)}$ was calculated using the sampling volume. Total length (Tl in mm) of adults of each species was also measured. Copepod dominance index (Y) was calucalted using the following formula: $Y = \frac{ni}{N} \times fi$ where n_i is the number of individuals of species *i* and *fi* is the frequency of species *i* occurring in a sample and *N* is the total number of species. Species with a *Y* value of more than or equal to (20%) were defined as dominant species. Species diversity was assessed by using the Shannondiversity index *H'* (Shannon & Weaver, 1949): $H' = -\sum_{ni}^{S=1} \frac{ni}{N} \log_2 \frac{ni}{N}$ where ni is the number in the sample. We also calculated the Pielou's evenness index $J' = \frac{H'}{H' \max}$ [43] and the Margalef species diversity index [44], $DMg = (S-1) / log_2 N$ where N is the total number of individuals and S is the number of species

Data processing and statistical 148 analysis

Correlations between the different biological and physico-chemical parameters was tested using Pearson's rank correlation (XL-Stat software) and differences between the two sampling periods for the different parameters analyzed (ANOVA tests). Moreover, a canonical correspondence analysis (CCA) [45] was applied to explain the relationships between physical (temperature, salinity, turbidity, and pH, biogeochemical (orthophosphate, Zn, Ni, Pb, Co, Cr, Cd, Mn and Cu and suspended particulate matter) and biological (chlorophyll-a; harpacticoids, calanoids, cyclopoids and dominant copepod species abundance and mean body length of adult copepods) parameters. Variables that did not reach the normality assumption were logarithmically transformed to ensure homogeneity of variance [46].

Results

Mean values ± SD of the different physical (water temperature, salinity, pH, and transparency), biogeochemical (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, T-N, T-P, N/P ratio, $Si(OH)_4$), trace metals (SPM, Zn, Ni, Pb, Co, Cr, Cd, Mn and Cu) and biological (Chl-a, copepod abundance, total length of copepod species and diversity index) parameters are summarized in Table1.

Table 1: Mean values (± standard deviation, SD) of physical and biogeochemical parameters and of diversity indexes for copepods (H': Shannon and Wiener diversity index, *Y*: dominance index, *S*: Species number and D_{Mg}: Margalef species diversity index) in the southern coast of Sfax among sampling periods (January–August 2013). In the last column are shown results of one-way ANOVA (F values and p significance) to test differences between sampling periods. Significant degrees for p are: *p < 0.05; ** p < 0.001; ***p < 0.0001; ns = non-significant (p > 0.05)

Variables	January 2013			August 2013			ANOVA between sampling (p levels)
Physical Parameters	**Min**	**Max**	**Mean ± SD**	**Min**	**Max**	**Mean ± SD**	
Depth (m)	4.2	7.4	5.84 ± 1.15	3	6.2	4.64 ± 1.15	**
Temperature (°C)	12.5	15	14.05 ± 0.85	23	25.5	24.54 ± 0.89	***
Salinity (p s u)	30	35	32.5 ± 0.77	32	42	39.38 ± 2.03	***
pH	7.1	8.2	7.66 ± 0.32	7.67	7.92	7.83 ± 0.10	***
Transparency (m)	0.8	4	2.40 ± 1.16	3.8	7	5.39 ± 1.11	***
Suspended particulate matter (mg L^{-1})	22	72	45.89 ± 11.57	51.2	87.2	64.51 ± 11.02	***
Biogeochemical parameters							
NO_3^- (µM)	0.98	2.47	1.52 ± 0.48	1.31	6.05	2.93 ± 1.19	***
NO_2^- (µM)	0.097	0.4	0.22 ± 0.07	0.1	1.57	0.31 ± 0.34	ns
NH_4^+ (µM)	0.13	0.5	0.29 ± 0.11	0.7	12	3.15 ± 2.80	***
T-N (µM)	10.02	19.99	16.86 ± 2.86	12.59	46.89	17.74 ± 7.85	***
$PO4_3^-$(µM)	0.35	3.9	1.15 ± 0.85	1.44	14.14	3.30 ± 3.21	***
T-P (µM)	1.52	5.18	3.40 ± 1.21	4.42	49.3	12.62 ± 10.10	***
$Si(OH)_4$ (µM)	1.36	5.3	3.01 ± 1.08	11.12	113.13	22.50 ± 23.37	***
N/P ratio	4.24	32.08	11.52 ± 7.30	0.68	7.33	2.99 ± 2.11	***
Lead (Pb) (µg L^{-1})	0	410	620 ± 89	1	561	750 ± 120	**

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Iron (Fe) (µg L^{-1})	80	330	210 ± 71	190	510	340 ± 93	**
Copper (Cu) (µg L^{-1})	6	62	30 ± 15	8	80	41 ± 20	*
Zinc (Zn) (µg L^{-1})	0	150	54 ± 22	0	300	111 ± 75	**
Manganese (Mn)(µg L^{-1})	13	150	71 ± 31	35	180	109 ± 51	**
Cadmium (Cd) (µg L^{-1})	10	35	24 ± 8.4	22	70	49 ± 10	**
BiologicalParameters							
Chlorophyll-a concentration (×10^3 µg L^{-1})	2.65	3.76	2.43	0	2.46	1.11 ± 0.81	***
Zooplankton abundance (×10^3 ind $m^{-3)}$	4.31	72.19	13.15 ± 15.51	1.93	18.56	10.31±16.35	ns
Copepod abundance							
(× 10 ind m)	3.1	46.62	9.78 ± 10.04	1.51	12.81	7.11 ± 8.81	**
Calanoids abundance							
(× 10 ind m)	0.26	13	2.39 ± 2.93	0.17	35.09	1.94 ± 0.94	ns
Cyclopoids abundance							
(× 10 ind m)	0.29	17.63	4.09 ± 4.03	0.09	30.75	1.70 ± 1.55	ns
Harpacticoids abundance (× 10 ind m)	0.11	5.73	1.59 ± 1.48	0.25	41.97	2.3 ± 3.47	ns
Poecilostomatoids abundance (× 10 ind m)	0.01	0.21	0.06 ± 0.05	0	0.44	0.02 ± 0.03	***
Other zooplankton abundance (× 10 ind m)	0.53	25.57	3.36 ± 5.62	0.06	37.75	2.09 ± 5.49	ns
Total length of all copepod species (mm)	0.18	1.22	0.6 ± 0.36	0.22	1.11	0.59 ± 0.28	ns
Diversity index							
Y	0.34	0.83	0.57 ± 0.14	0.19	0.94	0.54 ± 0.14	ns
S	8	22	15 ± 3.82	18	23	20 ± 2.28	ns
DMg	1.28	3.79	4.11 ± 0.94	2.73	5.05	4.01 ± 0.74	ns
H'	1.02	2.64	1.75 ± 0.51	1.4	3.14	2.53 ± 0.42	**

Physical and biogeochemical parameters

Water temperature and salinity were significantly higher in August (24.54 ± 0.89 °C; 39.38 ± 2.03 psu) than in January (14.05 ± 0.85 °C; 32.5 ± 0.77 psu). pH was significantly higher in August (7.83 ± 0.10) (Table 1). All biogeochemical parameters displayed higher values in August than in January except for the SPM concentrations. These parameters displayed significant differences between the two periods, except for NO_2^-.

Biological parameters and 173 diversity indexes

Chl-a concentration as well as abundances of copepods and poecilostomatoids were significantly higher in January than August ($p < 0.0001$). Calanoids, harpacticoids and cyclopoids as well as total zooplankton did not vary significantly between the two periods. The copepod diversity indexes did not vary significantly between sampling periods ($p > 0.05$) except for the Shannon index which was significantly higher in August than January ($p < 0.001$). A total of 23 different copepod species were identified throughout the survey period; their abundance and their morphological (total length) and reproductive (broadcasters, sac spawners) traits varied between the cold and the warm periods (Table 2). The species richness ranged from 8 to 23 species per sample (mean = 15 in January and 20 in August). Moreover, the copepod community was more diversified in August (H'= 1.4 - 3.14 bits $ind^{-1)}$ than in January (H'= 1.02 – 2.64 bits ind^{-1}) ($p < 0.001$).

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Table 2: Taxonomic composition of the copepod community: mean values per season and per species of total length (TL mm); abundance (A, × 10^3 ind m^{-3}); relative abundance (RA, %); dominance index (Y); number of females (Nf, × 10^3 ind m^{-3}); number of ovigerous females (Nfo × 10^3 ind m^{-3}); number of eggs per sac (E); indication of the spawning mode: S = egg-carrier; B: broadcast spawners. * = dominant species.

Order	Species	Jan-13						Aug-13						Spawning Mode
		TL (mm)	A	RA (%)	Y	Nf	NfoE	TL (mm)	A	RA (%)	Y	Nf	NfoE	
Calanoids (0.98-1.22 mm)	Acartia (Acartia) danae	1.22	11.7	12.84	0.033	3.69		1.11	3.16	1.73	0.011	2.11		B
	(Giesbrecht,1889) *													
	Paracartia grani	1.12	9.57	11.18	0.051	1.57		1.02	11.81	16.41	0.01	9.5		B
	(Sars GO, 1904) *													
	Paracartia latisetosa (Krichagin, 1873) *	1.06	2.21	10.47	0.025	1.85		1.01	6.11	1.52	0	3.5		B
	Acartia (Acartiura) longiremis (Lilljeborg, 1853)	1.05	0.37	6.2	0.001	0.12		0.98	5.7	1.72	0.017	1.8		B
	Acartia sp.	1.03	0.27	5.2	0.042	0.11		0.93	0.45	1	0.01	0.2		B
	Acartia (Acartiura) clausi (Giesbrecht, 1889)	1.02	4	3.74	0.012	2.45		0.97	3.16	1.92	0.014	2.7		B
	Temora longicornis (Müller OF, 1785)	0.99	0.13	2.29	0.001	0.1		0.81	1.2	1.11	0.01	0.98		S
	Paracalanus parvus parvus (Claus, 1863)	0.98	0.8	1.03	0.002	0.42		0.76	0.65	0.6	0.006	0.12		S
Cyclopoids (0.42-0.6 mm)	Oithona plumifera plumifera (Baird, 1843)	0.6	4	3.59	0.036	2.8	1.96 16	0.62	2.17	2.21	0.012	1.4	1.2 16	S
	Oithona setigera (Dana, 1849)	0.55	3.36	6	0.007	3.05	2.01 13	0.45	1.99	1.15	0.017	1.02	0.98 15	S
	Oithona sp.	0.54	7	0.72	0.003	5.63	3.5 19	0.39	2.01	1.04	0.011	1.74	1.66 20	S
	Oithona nana (Giesbrecht, 1893)	0.52	19.6	7.6	0.196	12.8	9.8 18	0.53	11.19	8.64	0.049	8.7	7.9 19	S
	Oithona similis (Claus, 1866)	0.51	22	8.11	0.268	18.21	13.5 16	0.49	10.61	7.04	0.039	7.8	6.3 18	S
Poecilostomatoids (0.19-0.42 mm)	Corycaeus clausi (Dahl F., 1894)	0.42	1.23	0.16	0.02	0.08	0.03 6	0.43	0.1	0.77	0.009	0.003	0 5	S
	Corycaeus specious (Dana, 1849)	0.41	0.23	0.11	0.01	0.52	0.01 7	0.42	0.03	0.51	0.013	0.01	0.01 6	S
	Triconia minuta (Giesbrecht, 1893)	0.32	1.03	0.33	0.01	0.2	0.10 8	0.33	0.05	0.26	0.011	0.01	0.01 5	S
	Oncaea mediterranea (Claus, 1863)	0.19	0.07	0.11	0.01	0.1	0.08 4	0.29	0.11	0.23	0.014	0.44	0.216	S
	Triconia conifera (Giesbrecht, 1891)	0.21	0.48	0.31	0	0.19	0.12 6	0.22	0.05	0.15	0.011	0.01	0.01 6	S
Harpacticoids (0.18-0.3 mm)	Harpacticus littoralis (Sars G.O., 1910)*	0.3	3.44	6.04	0.01	3.12	2.10 10	0.5	15.35	29.02	0.026	14.5	12.8 26	S
	Tisbe furcata furcata (Baird, 1837)*	0.29	2.4	2.28	0.018	2	1.02 9	0.39	9.07	11.81	0.021	8.1	7.9 24	S
	Tigriopus sp. (Norman, 1869) *	0.22	1.87	1.85	0.004	1.02	0.63 8	0.32	8.1	10.61	0.002	7.07	6.3 20	S
	Euterpina acutifrons (Dana, 1847)	0.21	4.86	9.3	0.084	3.85	3.09 18	0.22	6.56	0.46	0.002	5.8	3.01 19	S
	Clytemnestra scutellata (Dana, 1852)	0.18	0.49	0.55	0.001	0.12	0.00 0	0.38	0.12	0.12	0.001	0.01	0.01 10	S

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August, small egg carrying harpacticoids (total length between 0.18 and 0.3 mm) and more specifically *Harpacticus littoralis* (29.02%), *Tisbe furcata* (11.81%) and *Tigriopus* sp. (10.61%) were the most abundant species. *H. littoralis* showed a very high abundance of ovigerous females (*Nfo* = 12.8 × 10^3 ind m^{-3}, representing 87.2% of total female number) with the highest number of eggs per sac (E = 26 ± 2 egg sac^{-1}) compared to the other species (Table 2). However, in January, large broadcaster calanoids (total length between 0.98 and 1.22 mm) mainly *Acartia (Acartia) danae* (12.84%), *Paracartia grani* (11.18%) and *Paracartia latisetosa* (10.74%) were dominant. Other species as *Euterpina acutifrons* (Dana), a more ubiquitous harpacticoid, displayed no marked difference in fecundity parameters and prosome length between periods ($p > 0.05$). All calanoid species displayed a larger adult body size in January (0.98-1.22 mm) compared to August (0.76-1.11 mm) whereas poecilostomatoids ($p < 0.05$) and harpacticoids ($p < 0.001$) showed an opposite trend (0.19-0.42 and 0.18-0.3, in January and 0.22-0.43 and 0.22-050 mm in August).

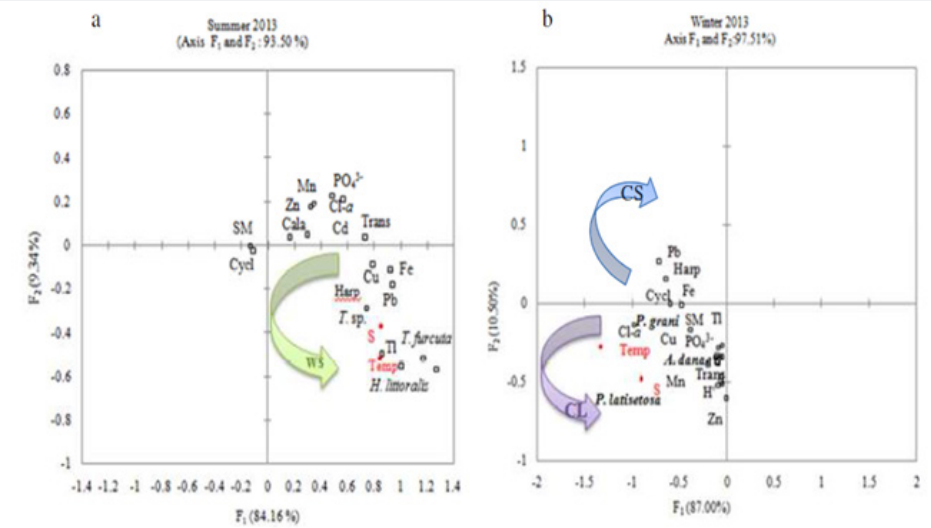

Figure 2: Canonical correspondence ordination (CCA) of the copepod species coupled with environmental parameters in the Southern coast of Sfax during warm (A) and cold (B) periods. Biplots of the species (frequency of occurrence > 71.43%) and selected physical, biogeochemical and biological parameters. Tem: Water temperature (°C); S: salinity (psu); SM: Suspended particulate matter (mg l-1); Tur: Turbiity (NTU); PO43- :Orthophosphate (µmol l-1); major element: Fe: Iron (µg l-1) and Mn: Manganese (µg l-1), trace metals: Zn: Zinc (µg l-1), Pb: Lead (µg l-1); Cu: Copper (µg l-1); Cd: Cadmium (µg l-1): Chl-a: Chlorophyll-a); Harp: Harpacticoids; Cal: Calanoids; Cyc: Cyclopids; Tl: Total length; cold-water small (CS); Cold-water large (CL): P. grani: Paracartia grani, A. danae: Acartia danae and P. latisetosa: Paracartia latisetosa (with reproduction mode: Broadcast spawner) and Warm-water small (WS): H. littoralis: Harpacticus littoralis; T. furca: Tisbe furca (with reproduction mode: Sac spawner)

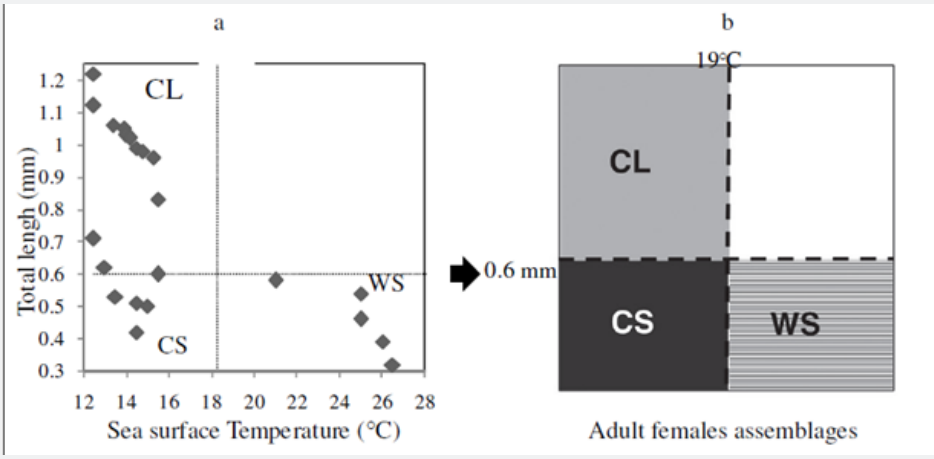

Figure 3: (A) Relationship of mean total length of adult females at the period of optimal abundance and corresponding surface seawater temperature (during August or January 2013) of the 23 copepod taxa. (B) Classification of copepod taxa based on the temperature and total length (mm) criteria: cold-water large (CL), cold-water small (CS), and warm-water small (WS) in the Southern coast of Sfax during August and January 2013.

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Multivariate analysis

In August 2013, the CCA allowed the discrimination of two groups around the F1 and F2 axes explaining 93.50% of the variance (Figure 2). The first axis F1 (84.16%) selected positively egg-carrying copepod species such as *H. littoralis, T. furcata* and *Tigriopus* sp. associated to high values of total length, temperature, salinity, Cu, Pb and Fe. This group was opposed to cyclopoids and calanoids associated to SPM, Zn and Mn. In January, abundances of almost all copepod species and groups were correlated to temperature, salinity and chl-a (Figure 2). On the second axis, larger species such as *A. danae, P. grani* and *P. latisetosa* were associated with temperature, salinity and heavy Cu, Mn and Zn concentrations. This group was opposed to small cyclopoids and harpacticoids which were associated with pH and Pb concentration. Besides, *H. littoralis* was positively correlated with water temperature (r = 0.50cold-water large (CL: size > 0.6 mm, temperature < 19°C), cold-water small (CS: size < 0.6 mm, temperature < 19°C) and warm-water small (WS: size < 0.6 mm, sea surface temperature > 19°C) copepod species (Figure 3).

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Discussion

In the present study, we focused on the variability of copepod assemblages in relation to physical and biogeochemical parameters in the Southern coast of Sfax in January and August 2013. The lowest pH values (7.1 in January and 7.67 in August, below the current seawater value 8) may reflect the influence of the SIAPE industrial effluent released in seawater. Indeed, it has been shown that this effluent was strongly acidic (pH = 1.54) (Masri, 2005; Drira et al., 2016). The highest PO43- concentration (14.14 µM) was observed in August at station 7 located in front of the SIAPE manufactory, which may be related to release through of phosphogypsum wastes. In August, the mean value of PO43- concentration (3.3 ± 3.21 µM) was in the range of values previously reported by Baccar [47] in the Gabes area (3.73 ± 1.57 µM). The highest levels of PO43- were always found near the potential sources, i.e. the SIAPE and wadi El Maou. Previous studies have shown high heavy metal concentrations in the sediment close to the GCT-Gabes' and the SIAPE-Sfax phosphoric acid industrial complexes [48,49].

Our results showed higher amounts of Zn, Cd, Mn, Cr and Pb in August than January 2013. The SIAPE plant is a source of high amounts of heavy metals (e.g. Cd, Zn, Cu, Co, Ni and Pb). Drira et al., [25] reported mean concentrations of metals analyzed in the surface water of 26-77, 9-236, 217-318, 0-310, 446- 919, 383-1015 µg l-1 for Cd, Zn, Cu, Co, Ni and Pb µg l-1 respectively. However, industrial activities mainly tanneries and paper industry located near the coast are responsible for heavy metal pollution i.e. Cr (500 µg l-1) and Hg (1 µg l-1), respectively [50]. Another source of trace metal pollution in this area is the fishing harbour (primarily Cd, Mn and Pb which represented 5, 1000 and 500 µg l-1, respectively). All analyzed metals (except for Fe and Mn) are considered as moderate to extreme pollutants [51]. In the southern region of the Gulf of Gabes the major factor behind this pollution by heavy metals is phosphogypsum wastes, as shown in several previous works [48,49,52].

In this study, we found that copepod was the dominant group representing 67 to 74% of total zooplankton. Similar values were previously reported in the Gulf of Gabes (2005-2007; 69- 83%; [53,54] and in the Southern coast of Sfax (2008; 66%). The copepod community was more diversified in August (H'= 1.4 - 3.14 bits ind-^{1}) than in January (H'= 1.02 – 2.64 bits ind-^{1}), contrarily to results reported by Daly-Yahia et al. [55] in the Bay of Tunis (using the same copepod taxa level) with the highest diversity index in January (up to 3.83 bits ind-1), and the lowest in August (down to 0.24 bits ind-^{1}). Similarly, Siokou-Frangou [56] showed that in the Saronikos Gulf (Ionian Sea), the maximum diversity index (calculated for the whole zooplankton) was observed in January while the minimum values occurred in August. The copepod abundance was higher in cold (74%; 9.78 ± 10.04 × 103 ind m-^{3}) than in warm (67%; 7.11 ± 8.81 × 10^{3} ind m-^{3}) period.

Salinity and temperature have been found to control many copepod species distributions in marine coastal ecosystems [57-59]. It is well established that temperature is an important controlling factor for the biological cycles of copepod species [60,61]. In the present study, mean temperature values varied from 14.05 ± 0.85°C in January to 24.54 ± 0.89°C in August, in agreement with previous observations in the Gulf of Gabes (23.07 ± 2.47°C in summer; [62] and in the Southern coast of Sfax (15.33 ± 0.64°C in winter). This highwater temperature in August induced a decrease in total copepod abundance but an important increase of the percentage of harpacticoids (39% of total copepod abundance; r = 0.542, p < 0.05). Positive effects of temperature on harpacticoid abundance were shown in laboratory conditions, in which the highest density was recorded

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at an optimum of 28°C [63] as well as in Mediterranean aquatic environments such as in Nasser lake (Egypt) [64] and the Sicily Channel (Central Mediterranean) [65]. In other Mediterranean regions, in agreement with our study, temperature was negatively correlated with copepod density [66]. Our results are also congruent with other previous studies which demonstrated that temperature strongly affects the copepod body length [67-69] through effects on their productivity, growth and development time [70-72].

In this study, the high abundance of large calanoid copepods (CL = cold-water large copepods; < 19°C) in January may be linked to their good adaptation to cold conditions. Besides the larger adult body size of these calanoids in January compared to August may result from a longer development times and correlative lower growth rates in cold condition as currently observed for calanoids [73,74]. However, the small harpacticoid copepods were more abundant (WS warm water short copepods; >19°C) and displayed higher adult body size during August Increase in adult body size of harpacticoid in August may be linked to a salinity effect as observed by Miliou [75] for *Tisbe holothuriae.* Besides, in August abundance of small egg carrying species such as *H. littoralis* (Tl = 0.5 mm), *T. furcata* (Tl = 0.39 mm) and *Tigriopus* sp. (Tl = 0.32 mm) also suggests that these species are favoured by the warm temperature conditions at this period (23-25 °C). *Tigriopus* sp. is characterized by its high resistance to changes in temperature and environmental disturbances [76-79]. In January, high abundance of large broadcaster species such as A. danae (Tl = 1.22 mm 12%), *P. grani* (Tl = 1.12 mm, 11.8%) and *P. latisetosa* (Tl = 1.06 mm, 10.47%) were associated to low temperature (12.5–15°C) and salinity (30–35 psu). Besides, species of the genus *Acartia* frequently dominate the pelagic environment of costal marine waters [80-82].

Salinity may have also impacted the observed copepod distribution. Indeed, large costal species (i.e. *A. danae, P. grani* and *A. latisetosa*) had their highest abundance at the lowest salinities in the cold season whereas, small copepods (*H. littoralis T. furcata* and *Tigriopus sp*.) dominated at highest salinities in August. In January, due to increased rainfall and continental water runoff, in such an ultra-coastal zone, salinity drops down to very low values (S < 35). Such condition may have favored the more euryhaline species and Acartidae known to be well adapted to salinity variations (e.g [83,84] for *A. clausi* and *A. tonsa* respectively; [85] for *A. longiremis*). Finally, in addition to temperature and salinity effects, changes in food availability (namely biomass of phytoplankton) and pollution levels may also explain seasonal differences in copepod abundance and community composition. Indeed, lower copepod abundance, as well as higher percentage of small egg-carrying copepods in August, were related to the decrease of phytoplankton biomass (Chl-a concentration) and increase of heavy metal contamination Dependence of these omnivorous copepods (namely calanoid species) to phytoplankton abundance is well documented [86], whereas harpacticoids are less phytoplankton-dependant [87].

In January, high relative abundance of harpacticoids (in situation of low phytoplankton abundance) could have been linked to their wide prey composition. In fact, harpacticoids have been shown to manage to feed on detritus [88] as well as on microalgae [89-91]. It is known that heavy metals have potential negative effects on the hatching success of copepods [92]. Therefore, contamination by heavy metal could inhibit recruitment of nauplii, leading to decrease of copepod abundance. The reproductive success of copepods is vulnerable to contamination through all stages of their life cycle [93]. However, copepod resting eggs are much more sensitive to metals than adult copepod. In that case, it is important to consider the frequently ignored effect of these metals on the viability of some copepod species [92] since copepods are characterized by a great accumulation of metals [94]. Other impacts of heavy metal contamination on copepods have also been documented.

For example, damage effects (oxidative stress) of trace metals (as Cd or Ni) have been demonstrated on calanoid (870 and 12310 µg l^{-1}; for Cd and Ni, respectively; [95]) or harpacticoid (100 µg l^{-1} for Cd; [96]) copepods. However, harpacticoids have been shown to be less sensitive to metal contamination, compared to other contaminants as endocrine disrupting chemicals and biocides [97]. This lower sensitivity to metal contamination may partly explain the higher relative abundance of harpacticoid in August when metal contamination was high. On another hand Acartidae have been shown to strongly decrease their reproduction rates when exposed to metal concentration which exceeded the permissible limits in coastal waters [98], which could explain their rarefaction in August in the southern coast of Sfax [99-104].

Conclusion

The present study revealed that copepods are vulnerable to temperature, salinity and heavy metal pollution. In fact, small-sized harpacticoid species are more abundant in the warm season, i.e. *H. littoralis T. furcata* and *Tigriopus* sp. coupled with high salinity, temperature and high metal concentration. During the cold season, larger adult broadcaster species of calanoids such as *Acartia (Acartia) danae, Paracartia grani* and *Paracartia latisetosa* were associated with low temperature, salinity and low heavy metal pollution levels. These findings support the assessment of the ecological effects on marine copepods in environments polluted by heavy metals. More toxicity studies at the population level should be performed to provide a comprehensive overview on how copepod populations respond to heavy metal pollution Some heavy metals analyzed in the present study are required as a chemical marker under a future project of monitoring and management programs, for reliable estimates of water quality.

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