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# Impacts of restoration of an uncontrolled phosphogypsum dumpsite on the seasonal distribution of abiotic variables, phytoplankton, copepods, and ciliates in a man-made solar saltern

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**Abstract** The restoration of an uncontrolled phosphogypsum landfill was investigated for its effects on the seasonal distribution of phytoplankton, ciliates, and copepods. Sampling was carried out monthly from September 2007 to August 2008 at four ponds of increasing salinity (A1, 41 psu; A5, 46 psu; A16, 67 psu; and C31, 77 psu) in the Sfax solar saltern (southeastern Tunisia). Physicochemical and biological analyses were carried out using standard methods. Results showed drastic reduction of phosphate input and greater diversity of phytoplankton, ciliates, and copepods than before restoration. Pennate diatoms and new ciliates, considered bio-indicators of less-stressed marine ecosystems, proliferated in the A1 pond for the first time after restoration. Copepods appeared to feed on a wide range of prey. Economically, removal of the 1.7 million m<sup>3</sup> of phosphate improved the quality of

the site's salt production, enabling the salt company to receive the quality ISO 9001 accreditation.

**Keywords** Solar saltern · Phytoplankton · Ciliates · Copepods · Physicochemical variables · Phosphogypsum restoration

## Introduction

Coastal solar salterns are artificial and transitional ecosystems located between the marine environment and inland waters. They have been constructed in many areas worldwide and generally consist of a series of interconnected ponds featuring a salinity gradient ranging from near that of seawater level to a several-fold concentration ensuring a yield of halite used mainly as cooking salt and magnesium-rich bittern brines used to make road salt (Javor 1989, Khemakhem et al. 2010). Salterns are also biologically very rich, harboring birds, fish, plants and micro-organisms (Elevi-Bardavid et al. 2007; Abid et al. 2008). Despite their considerable landscape and economic value, and though studies clearly indicate that the salt-production process via evaporation and precipitation is closely related to biological processes (Davis 1990, 2000), salterns are now threatened biotopes, mainly due to human interference. This is particularly true of the Sfax saltern located on the southwestern Mediterranean coast and which suffered from pollution by phosphogypsum (Rekik et al. 2012)

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and petroleum products (Elloumi et al. 2008). Indeed, the anthropogenic nutrient input in the saltern's receiving pond may have impacted the development of the ponds biological communities and correlatively the quality of the salt produced (1990). It is therefore of interest to examine how algae, protozoans, and crustacean zooplankton, subjected to a long-term period of phosphogypsum-inducing stress, respond to improving ambient environmental conditions: persistence of the salt constraints in the saltern with the potential subsequent enhancement of the quality of the salt yield by approximately 300,000 tons of halite and 25,000 tons of bittern brine. In addition, the saltern had experienced the development of two green macroalgae (belonging especially to the genera *Enteromorpha* and *Cladophora*) and one red alga (*Chondria*) whose decomposition, along with that of the leaf waxes of land plants, had led to foul odors and an unpleasant appearance. The ponds, interconnected by pipes and channels along a 12-km stretch of seacoast, thus required significant expense and effort for maintenance and cleanliness (Elloumi et al. 2009). Consequently, Tunisian authorities undertook the Taparura project in 2006 to attack the phosphogypsum pollution at its source, to clean the ponds of an excess of macrophytes and to generally improve the regional environment. We (Abid et al. 2008; Elloumi et al. 2009; Khemakhem et al. 2010) and others (Joint et al. 2002; Oren 2005) have shown that salterns harbor a variety of organisms ranging from marine like to extreme halophiles and hypothesized that this restoration might lead to a change in the composition of the saltern's biological communities located near the phosphogypsum landfill. Salterns are, in some regards, well-studied systems (Oren 2005; Elloumi et al. 2009), yet aspects associated with anthropogenic impacts, especially from phosphogypsum residues on biological communities, have never before been reported. This lack of information may be explained by (1) the major cost and time constraints placed on this kind of study by the sorting and identification of the species present and (2) the hardship of discriminating between stress due to the salt gradient and that caused by phosphogypsum residues.

The present study is a part of an extensive research project undertaken to determine how the removal of nearly 1.7 million m<sup>3</sup> of phosphate plate surrounding the landfill toe, and the dredging of nearly 0.5 million contaminated underwater samples, have affected the environmental quality in coastal ecosystems. The solar saltern of Sfax is a good example of this type of

situation. From September 2007 to August 2008, we examined biological communities in four high-salinity lagoons, in order to detect how species richness and abundance compared along the salinity gradient (41–77 psu) during our post-restoration sampling period as opposed to pre-restoration data. Since the potential for niche partitioning based on the salinity gradient is greater for phytoplankton and ciliates than for copepods (Khemakhem et al. 2010), we chose this range of salinity so as to allow occurrence of all zooplankton, phytoplankton and ciliates as we have previously shown that within the 77 to below 120 salinity range, the copepod population, for example, would be marked by the presence of only two species namely *Bryocampus* sp. and *Mesochra lilljeborgi* (Khemakhem et al. 2010). Finally, and since very few saltern studies have simultaneously considered many compartments within the plankton community, we attempted to assess, after restoration, the relationships between copepods and their potential prey such as phytoplankton and ciliates, which are currently hypothesized to be advantaged in such systems (Khemakhem et al. 2010).

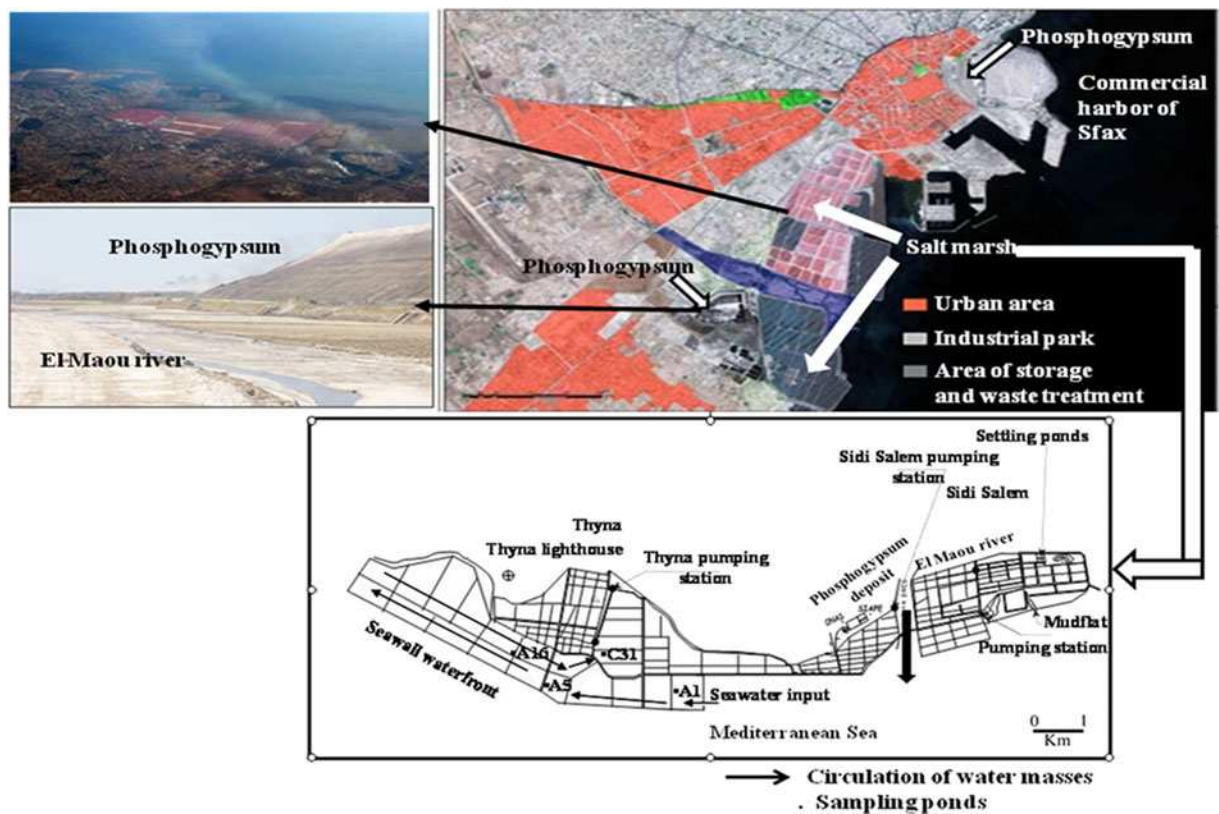
## Materials and methods

### Study site

The solar saltern under study is located in the southern part of the city of Sfax along the east-central coast of Tunisia (between 34°39'0.1" N and 10°42'35" E) extending over an area of nearly 1,500 ha. These man-made systems are formed by ponds (20–70 cm depth and 37–400 psu) connected by pipes and channels along a 12-km section of seacoast. Of increasing salinity, ranging from that of seawater to that of crystallizers, the ponds attain a salt concentration rarely seen in natural environments in which halite (used mainly as cooking salt) is produced and where magnesium-rich bittern brines are collected (Elloumi et al. 2008) (Fig. 1). The saltern is separated from the sea by an artificial red silt seawall (height, 4 m). Depending on meteorological conditions, seawater influx and circulation between the ponds are entirely controlled in order to ensure an optimal yield of halite and bittern brine.

### Sampling and laboratory analyses

Sampling was carried out monthly from September 2007 to August 2008 in the four ponds whose salinity



**Fig. 1** Circulation of water masses and location of the sampling ponds A1, A5, A16, and C31 and phosphogypsum deposited near the Sfax solar saltern

increased as follows: A1, 41 psu; A5, 46 psu; A16, 67 psu; and C31, 77 psu. The seasons were defined as (1) autumn, September to November 2007; (2) winter, December 2007 and January–February 2008, (3) spring, March to May 2008, and (4) summer, June to August 2008. Water samples were collected at 10–20 cm depth with a 1-l Van Dorn bottle.

Temperature and pH were measured in situ using a mercury glass thermometer graduated in  $0.1\text{ }^{\circ}\text{C}$  and a Metrohm® type pH meter. Salinity, as totally dissolved salts, were estimated by the dry residue method, which consists of evaporating a 50 ml sample (24 h,  $180^{\circ}\text{C}$ ) in a previously sterilized crystallizing dish (by heating at  $550^{\circ}\text{C}$  for 1 h), and calculating the salt content from the difference in weight before and after evaporation (Elloumi et al. 2009). Concentration of suspended matter was determined by measuring the dry weight of the residue after water filtration through a Whatman GF/C membrane. Total nitrogen and total phosphate (T-P) concentrations were assessed (after transformation into  $\text{NH}_4^+$  and  $\text{PO}_4^{3-}$ , with nitrogen persulfate

and potassium persulfate, respectively at  $120^{\circ}\text{C}$ ) by spectrophotometry (APHA 2012). Nitrates were assayed by a Shimadzu ion chromatograph HIC-6A, equipped with an ion-exchange resin (i.e., the stationary phase) that is extremely stable at a wide range of salt concentrations and adapts well to a pH range of 2–12 (detection limit for  $\text{NO}_3^-$ ,  $0.07\text{ }\mu\text{mol l}^{-1}$ ).

Water samples (200 ml), taken in duplicate for phytoplankton and ciliates, were fixed with acid Lugol's iodine (1 % final concentration) and stored in the dark at  $4\text{ }^{\circ}\text{C}$  until laboratory analysis. Phytoplankton and ciliates were counted under an inverted microscope ( $\times 400$ ) using the Uthermöl (1958) method. Chlorophyll *a* was extracted using 10 ml of acetone and determined spectrophotometrically after filtering a 0.15-l sample through a Whatman GF/C filter. Concentrations were then calculated according to Strickland and Parsons (1968) equations. Crustacean zooplankton, collected by filtering 50 l of water through a  $50\text{-}\mu\text{m}$  mesh net, were transferred to a 125-ml flask and fixed with formaldehyde (5 %). For each sample, the community structure was determined by

the Shannon–Weaver diversity index ( $H'$ ) (Shannon and Weaver 1949).

Statistics

Mean and standard deviation were reported when appropriate. One-way ANOVA analysis, followed by a post-hoc comparison using Tukey’s test, was applied to identify significant differences in physicochemical and biological parameters among the ponds. ANOVA tests were undertaken using Excel-Stat software. Prior to the ANOVA tests, the normality of all variables was checked by means of the Kolmogorov–Smirnov test and no departure from normality was detected for any variable. Pearson’s correlation analysis was performed to evaluate potential relationships between copepod abundance and abiotic and biotic variables. The data recorded in this study were examined with principal component analysis (PCA) in order to relate the distribution of copepods to the variables under study.

Results

Physicochemical parameters

Salinity, temperature, suspended matter and nutrient ranges are given in Table 1. Due to the shallowness of the four sampled ponds, no thermal stratification occurred. Water temperature differed slightly from pond to pond, with seasonal variations similar in all ponds (Table 1), varying from 11.5 (A1, January 2008) to

30°C (A16, September 2007) and correlating negatively with salinity, especially from April to September. This paradox is explained by the opening of water gates by the saltern’s managers to feed the ponds with seawater. The annual average of N/P, expressed as DIN/DIP ratio where DIN is dissolved inorganic nitrogen ( $DIN=NO_2^-+NO_3^-+NH_4^+$ ) and DIP is dissolved inorganic phosphorus ( $DIP=PO_4^{3-}$ ), which is the indicator of nutrient limitation for phytoplankton, ranged from 0.51 at A1 to 0.80 at A16. These averages were less than the Redfield ratio (16), suggesting a potential N limitation.

Phytoplankton

In this study, we identified a total of 72 phytoplankton taxa including diatoms, dinoflagellates, Cyanobacteriae, Euglenophyceae, and silicoflagellates. The mean values of phytoplankton abundance at each pond did not exceed  $10^5$  cells  $\Gamma^{-1}$ , varying from  $57.99\pm 29.98 \times 10^3$  (A16) to  $85.44\pm 26.58 \times 10^3$  cells  $\Gamma^{-1}$  (A5) (Fig. 2; Table 2). Diatoms dominated phytoplankton abundances in ponds A1 and A5 (salinity, <50 psu) while dinoflagellates dominated in ponds with salinity of >50 psu (Fig. 2). Abundance of both diatoms and dinoflagellates were significantly different from pond to pond (ANOVA,  $p<0.001$ ). During the sampling period, phytoplankton diversity was greater in pond A1 due to a highly diverse diatom community (Table 2).

Chlorophyll *a* concentrations ranged from 0.055 (A1, June 2008) to 0.187 mg  $\Gamma^{-1}$  (C31, June 2008). Their temporal variation showed a maximum in March

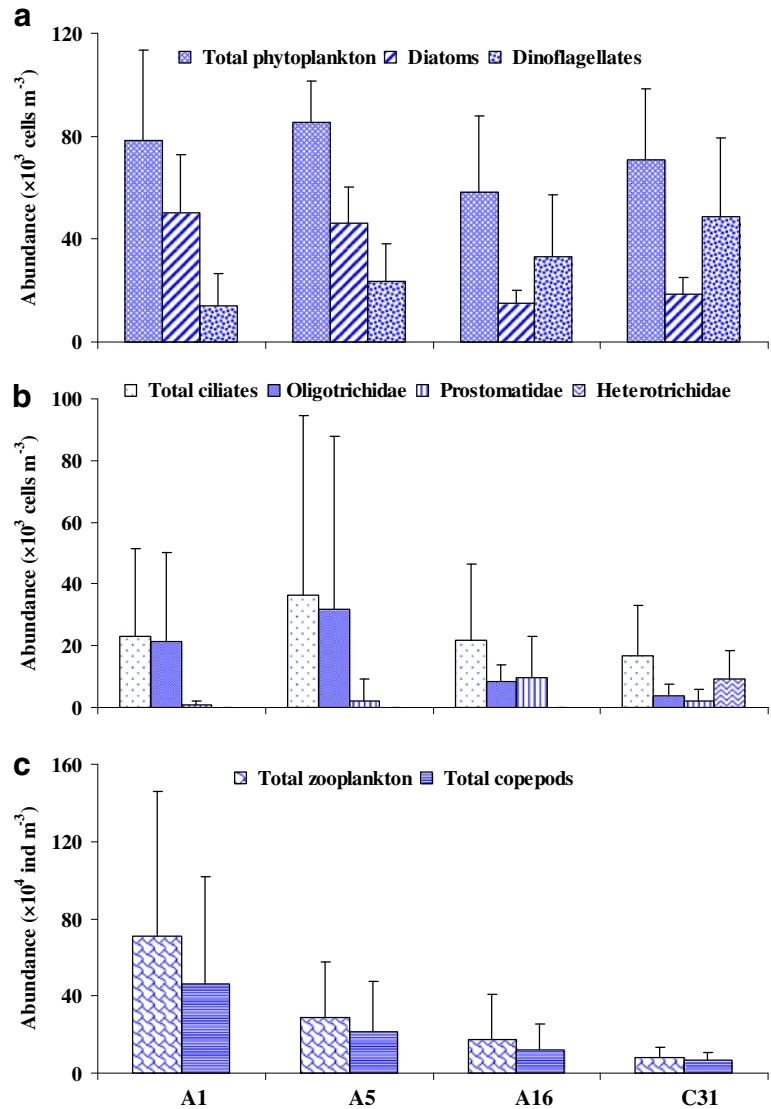
**Table 1** Annual mean±SD of physicochemical variables at ponds A1, A5, A16, and C31

Physicochemical parameters (mean±SD)	Ponds				F value (df)
	A1	A5	A16	C31	
<b>Physical parameters</b>					
Salinity (psu)	41.5±2.3	46.4±5.2	67.6±7.2	77.0±4.3	129.62 (44)***
Temperature (°C)	20.7±5.5	21.1±5.5	20.9±5.8	21.2±6.1	0.02 (44)
SM ( $\times 10^3$ mg $\Gamma^{-1}$ )	1.1±0.5	1.2±1.1	1.1±0.9	1.3±0.5	0.20 (44)
<b>Chemical parameters</b>					
$NO_2^-$ ( $\mu\text{mol } \Gamma^{-1}$ )	1.3±2.2	0.2±0.2	0.3±0.1	0.6±0.3	2.14 (44)
$NO_3^-$ ( $\mu\text{mol } \Gamma^{-1}$ )	2.3±1.2	6.5±3.7	7.1±5.4	6.8±6.7	2.63 (44)
$NH_4^+$ ( $\mu\text{mol } \Gamma^{-1}$ )	6.3±5.7	5.2±5.8	5.3±4.5	5.7±7.8	0.08 (44)
$PO_4^{3-}$ ( $\mu\text{mol } \Gamma^{-1}$ )	19.1±11.9	15.3±5	15.8±11.5	17.5±13.8	0.29 (44)
T-N ( $\mu\text{mol } \Gamma^{-1}$ )	16.3±7.7	6.65 ±11.6	13.2±15.9	17.0±11.8	1.82 (44)
T-P ( $\mu\text{mol } \Gamma^{-1}$ )	60.5±57.3	9.0±4.78	8.6±7.0	14.5±11.2	8.62 (44)***

In the last column, results of one-way ANOVA analysis. F values—between groups mean square/within-groups mean square

\* $p<0.05$ ; \*\* $p<0.01$ ; \*\*\* $p<0.001$ , significant difference between sampled ponds

**Fig. 2** Temporal and spatial variation of average phytoplankton abundances (a), ciliates (b), the total zooplankton, and total copepods (c) in ponds A1, A5, A16, and C31



2008 in the first three ponds while the highest value was recorded in June 2008 at pond C31, (Fig. 3).

Ciliates

The ciliate community was composed of nine groups dominated by Oligotrichidae, Prostomatidae, and Heterotrichidae, the latter observed only in pond C31 (Fig. 2). Other groups, namely Hypotrichidae, Gymnostomatidae, Pleuronematina, Colpodea, Hymenostomatidae, and Peritrichidae were numerically inferior. Average total ciliate abundances varied from  $16.65 \pm 16.31 \times 10^3$  (C31) to  $36.26 \pm 58.25 \times 10^3$  (A5) cells  $l^{-1}$  (Fig. 2; Table 3). Oligotrichidae dominated the ciliate

abundance at ponds with less than 50 psu (A1 and A5). Annual mean values of  $H'$  showed a general decrease according to salinity, the highest being 1.79 bits  $cell^{-1}$  at A1 and the lowest, 1.36 bits  $cell^{-1}$ , at C31 (Table 3).

Copepod community composition and dynamics

A total of 17 taxa belonging to four orders (Calanoida, Cyclopoida, Harpacticoida, and Poecilostomatida) were distinguished. Ten taxa were found at all ponds. Harpacticoida dominated the copepod assemblage at C31 on all sampling dates. Cyclopoida dominated during winter, spring and summer at A5 and during autumn, winter and summer at A16. Calanoida

**Table 2** Quantitative and structural characteristics of the phytoplankton community sampled in ponds A1, A5, A16, and C31

	Total phytoplankton abundance ( $\times 10^3$ cell $\Gamma^{-1}$ )	Diversity index (bits cell $^{-1}$ )	Evenness	Abundance of dominant species ( $\times 10^3$ cell $\Gamma^{-1}$ )	Occurrence frequency of dominant species (%)	Dominant species
<b>A1</b>						
13/09/2007	143.2	2.71	0.15	49.53	41.66	<i>Thalassiosira</i> sp.
29/10/2007	99.5	3.64	0.21	21	8.33	<i>Oscillatoria</i> sp.
27/11/2007	104	3.21	0.19	33	33.33	<i>Eutreptia globulifera</i>
12/12/2007	66.5	3.67	0.22	16.5		<i>E. globulifera</i>
28/01/2011	29	2.1	0.14	14	83.33	<i>Nitzschia longissima</i>
02/02/2011	33	3.73	0.24	12.2		<i>N. longissima</i>
04/03/2011	37	2.67	0.17	11.2	41.66	<i>Prorocentrum triestinum</i>
04/04/2011	89	2.65	0.16	35		<i>E. globulifera</i>
05/05/2011	92.5	3.51	0.21	17.5		<i>E. globulifera</i>
06/06/2011	96	2.66	0.16	26	25	<i>Euglena</i> sp.
23/07/2011	54.8	1.59	0.1	37.6		<i>N. longissima</i>
08/08/2011	99	2.88	0.17	32.77		<i>N. longissima</i>
<b>A5</b>						
13/09/2007	82.3	1.62	0.32	48.6	100	<i>N. longissima</i>
29/10/2007	88.4	2.29	0.46	42	66.66	<i>Prorocentrum micans</i>
27/11/2007	96	3.1	0.62	21		<i>P. micans</i>
12/12/2007	97.55	0.91	0.18	26.25	58.33	<i>E. globulifera</i>
28/01/2011	99.1	0.72	0.14	35		<i>E. globulifera</i>
02/02/2011	71.95	0.85	0.17	19	91.66	<i>Navicula</i> sp.
04/03/2011	44.8	0.66	0.14	18		<i>Navicula</i> sp.
04/04/2011	97	0.72	0.14	47	16.66	<i>Euglena</i> sp.
05/05/2011	103.5	0.99	0.19	29		<i>N. longissima</i>
06/06/2011	110	0.79	0.15	53		<i>N. longissima</i>
23/07/2011	76	0.49	0.10	55.2		<i>N. longissima</i>
08/08/2011	79.15	0.58	0.11	51.9		<i>N. longissima</i>
<b>A16</b>						
13/09/2007	27.2	0.69	0.14	13	100	<i>Nitzschia</i> sp.
29/10/2007	38.14	1	0.2	10		<i>Nitzschia</i> sp.
27/11/2007	43.95	0.82	0.16	19	50	<i>Oxyrrhis marina</i>
12/12/2007	57.37	0.78	0.15	25		<i>O. marina</i>
28/01/2011	70.8	0.69	0.13	31		<i>O. marina</i>
02/02/2011	75.42	0.72	0.13	40.25		<i>O. marina</i>
04/03/2011	80.05	0.61	0.11	49.5		<i>O. marina</i>
04/04/2011	77.2	0.71	0.13	31	66.66	<i>Euglena</i> sp.
05/05/2011	88.15	0.95	0.18	18.5		<i>Nitzschia</i> sp.
06/06/2011	99.1	0.77	0.14	36	16.66	<i>Oscillatoria</i> sp.
23/07/2011	9.5	0.81	0.18	4		<i>Nitzschia</i> sp.
08/08/2011	18.35	0.84	0.18	8.5		<i>Nitzschia</i> sp.
<b>C31</b>						
13/09/2007	129.5	0.92	0.17	36.5	91.66	<i>P. micans</i>
29/10/2007	71.47	0.97	0.19	15.6	66.66	<i>Oxyrrhis marina</i>
27/11/2007	76	1.03	0.21	21		<i>O. marina</i>

**Table 2** (continued)

	Total phytoplankton abundance ( $\times 10^3$ cell $\Gamma^{-1}$ )	Diversity index (bits cell $^{-1}$ )	Evenness	Abundance of dominant species ( $\times 10^3$ cell $\Gamma^{-1}$ )	Occurrence frequency of dominant species (%)	Dominant species
12/12/2007	55	1.19	0.25	12.75		<i>O. marina</i>
28/01/2011	34	1.05	0.23	6.5	25	<i>Gymnodinium nagasakiens</i>
02/02/2011	72.69	0.99	0.20	25.4		<i>O. marina</i>
04/03/2011	111.39	0.79	0.15	46.3		<i>O. marina</i>
04/04/2011	60.1	0.92	0.19	20	16.66	<i>Prorocentrum lima</i>
05/05/2011	54.97	1.06	0.22	10		<i>P. lima</i>
06/06/2011	49.84	0.86	0.18	12	66.66	<i>Gymnodinium</i> sp.
23/07/2011	44.7	1.06	0.22	9	16.66	<i>Amphiporora</i> sp.
08/08/2011	87.1	1.07	0.21	21.25		<i>P. micans</i>

dominated the copepod assemblage at A1, in March 2008 and at A5 in September 2007 and March 2008 (Figs. 2 and 4). *Oithona nana* (Cyclopoida) was the most abundant copepod at ponds A1, A5 and A16 (Table 4). *Bryocamptus* sp. (Harpacticoida) was the most abundant copepod at C31. The seasonal distribution of the copepod is presented in Fig. 4 and Table 4. Seasonal averages of  $H'$  showed a general decrease as salinity increased from A1 to C31.  $H'$  varied from 1.4 (winter, C31) to 3.2 bits  $\text{ind}^{-1}$  (winter, A1) (Fig. 5).

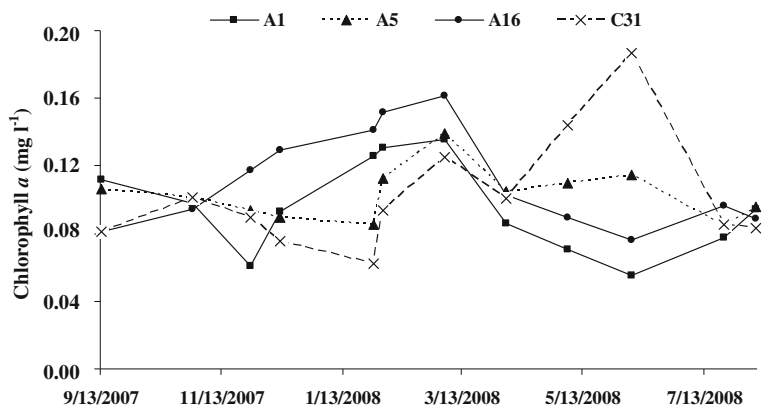
Average annual abundance of total zooplankton decreased from  $75.50 \times 10^4$   $\text{ind m}^{-3}$  at A1 to  $5.46 \times 10^4$   $\text{ind m}^{-3}$  at C31 parallel with the increase in pond salinity. Copepods were the dominant zooplankton (Fig. 2) accounting for 66.59, 67.51, 73.27, and 82.05 % of total zooplankton abundance in A1, A5, A16, and C31, respectively. The monthly variation of copepod abundance clearly differed among the ponds. However, two peaks were observed during the same

period (October 2007 and July 2008) for ponds A5 and C31 (Fig. 4). Only one major peak of copepod abundance was observed in A1 ( $1.8 \times 10^6$   $\text{ind m}^{-3}$ , January 2008) (Fig. 4).

Principal component analysis

Component axes  $F1$  and  $F2$  explained 84.29 % of the variance (Fig. 6), with the former explaining 55.87 %. The  $F1$  axis shows negative correlation of salinity to total phytoplankton, diatoms, oligotrochida, total zooplankton, total copepods and thalassophylic copepods. Total copepods and total zooplankton also showed no negative correlation with  $\text{NO}_3^-$ . The three species from marine contamination and *Tisbe battagliai* correlated positively with T-P,  $\text{NO}_2^-$ , and  $\text{NH}_4^+$ . However, halophylic copepods, dinoflagellates, Heterotrichidae, Hymenostomatidae, Pleuronematina, suspended matter, and salinity are found in the positive part of the analysis.

**Fig. 3** Temporal and spatial variation of chlorophyll *a* concentration in ponds A1, A5, A16, and C31





**Table 3** Quantitative and structural characteristics of the ciliate community sampled in ponds A1, A5, A16, and C31

	Total ciliate abundance ( $\times 10^3$ cell $l^{-3}$ )	Diversity index (bits cell $^{-1}$ )	Evenness	Abundance of dominant species ( $\times 10^3$ cell $l^{-3}$ )	Occurrence frequency of dominant species (%)	Dominant species
<b>A1</b>						
13/09/2007	11	1.78	0.13	5	75	<i>Strombidium capitatum</i>
29/10/2007	2	1	0.09	1	8.33	<i>Mesodinium</i> sp.
				1		<i>S. capitatum</i>
27/11/2007	3	0.91	0.07	2	33.33	<i>Urotricha</i> sp.
12/12/2007	8	1.45	0.11	3	66.66	<i>Lohmaniella oviformis</i>
28/01/2011	13	1.98	0.14	6		<i>L. oviformis</i>
02/02/2011	6.75	2.15	0.18	3		<i>L. oviformis</i>
04/03/2011	0.5	2.32	0.25	0.1	50	<i>Strombidium conicum</i>
				0	25	<i>Halteria</i> sp.
				0	25	<i>Leegardiella sol</i>
				0	25	<i>Colpoda</i> sp.
				0	8.33	<i>Pleuronema</i> sp.
04/04/2011	61	2	0.12	24	16.66	<i>Strombidium acutum</i>
05/05/2011	69.5	2.15	0.13	16		<i>L. oviformis</i>
06/06/2011	78	2.31	0.14	24	16.66	<i>Tintinnopsis beroidea</i>
23/07/2011	11	1.67	0.12	5		<i>Leegardiella sol</i>
08/08/2011	11	1.73	0.12	3		<i>S. capitatum</i>
<b>A5</b>						
13/09/2007	6	1.25	0.09	4	75	<i>S. capitatum</i>
29/10/2007	27	0.5	0.04	24	41.66	<i>Urotricha</i> sp.
27/11/2007	3	1.58	0.09	1		<i>S. capitatum</i>
				1	33.33	<i>Strombidium neptuni</i>
				1		<i>Urotricha</i> sp.
12/12/2007	94.5	1.59	0.09	66	25	<i>S. conicum</i>
28/01/2011	186	1.59	0.09	132	83.33	<i>L. oviformis</i>
				132		<i>S. capitatum</i>
02/02/2011	93.1	1.29	0.09	66		<i>S. conicum</i>
04/03/2011	2	1	0.09	1		<i>L. oviformis</i>
				1		<i>S. capitatum</i>
04/04/2011	1	0	0	1		<i>L. oviformis</i>
05/05/2011	3.5	0.62	0.05	2		<i>S. capitatum</i>
06/06/2011	6	1.25	0.09	4		<i>S. capitatum</i>
23/07/2011	6	1.79	0.142	3	16.66	<i>Tintinnidium balechi</i>
08/08/2011	7	1.52	0.12	2		<i>S. capitatum</i>
<b>A16</b>						
13/09/2007	5.7	0.78	0.06	4.9	75	<i>L. oviformis</i>
29/10/2007	12.5	1.85	0.13	6.5	66.66	<i>Urotricha</i> sp.
27/11/2007	4	2	0.16	1	75	<i>L. oviformis</i>
				1	25	<i>S. capitatum</i>
				1	41.66	<i>T. beroidea</i>
				1	66	<i>Urotricha</i> sp.
12/12/2007	11	1	0.07	9.5		<i>Urotricha</i> sp.

**Table 3** (continued)

	Total ciliate abundance ( $\times 10^3$ cell $\Gamma^{-3}$ )	Diversity index (bits cell $^{-1}$ )	Evenness	Abundance of dominant species ( $\times 10^3$ cell $\Gamma^{-3}$ )	Occurrence frequency of dominant species (%)	Dominant species
28/01/2011	18	0	0	18		<i>Urotricha</i> sp.
02/02/2011	53.5	1.01	0.06	27.62		<i>Urotricha</i> sp.
04/03/2011	89	2.02	0.12	37.25		<i>Urotricha</i> sp.
04/04/2011	15	2.28	0.16	4		<i>T. beroidea</i>
05/05/2011	13.5	2.23	0.16	3	41.66	<i>S. conicum</i>
				3		<i>L. oviformis</i>
06/06/2011	12	2.18	0.16	4		<i>S. conicum</i>
23/07/2011	15	1.58	0.11	5		<i>S. conicum</i>
				5	33.33	<i>Strombidium chlorophilum</i>
				5		<i>Urotricha</i> sp.
08/08/2011	10.35	1.18	0.08	2.5		<i>S. conicum</i>
						<i>S. chlorophilum</i>
						<i>Urotricha</i> sp.
C31						
13/09/2007	39.33	1.48	0.11	4	91.66	<i>Fabrea salina</i>
29/10/2007	39.38	2.34	0.2	1.4		<i>F. salina</i>
27/11/2007	39.41	1.97	0.12	14		<i>F. salina</i>
12/12/2007	39.42	1.73	0.13	7		<i>F. salina</i>
28/01/2011	39.47	1.5	0.11	8	25	<i>Aspidisca</i> sp.
02/02/2011	39.48	1.4	0.12	4		<i>Aspidisca</i> sp.
04/03/2011	39.51	1.3	0.11	1.7		<i>F. salina</i>
04/04/2011	39.54	0.79	0.054	19		<i>F. salina</i>
05/05/2011	39.57	1.1	0.073	23		<i>F. salina</i>
06/06/2011	39.60	1.4	0.09	27		<i>F. salina</i>
23/07/2011	39.65	0.59	0.04	6		<i>F. salina</i>
08/08/2011	39.66	1.04	0.08	5		<i>F. salina</i>

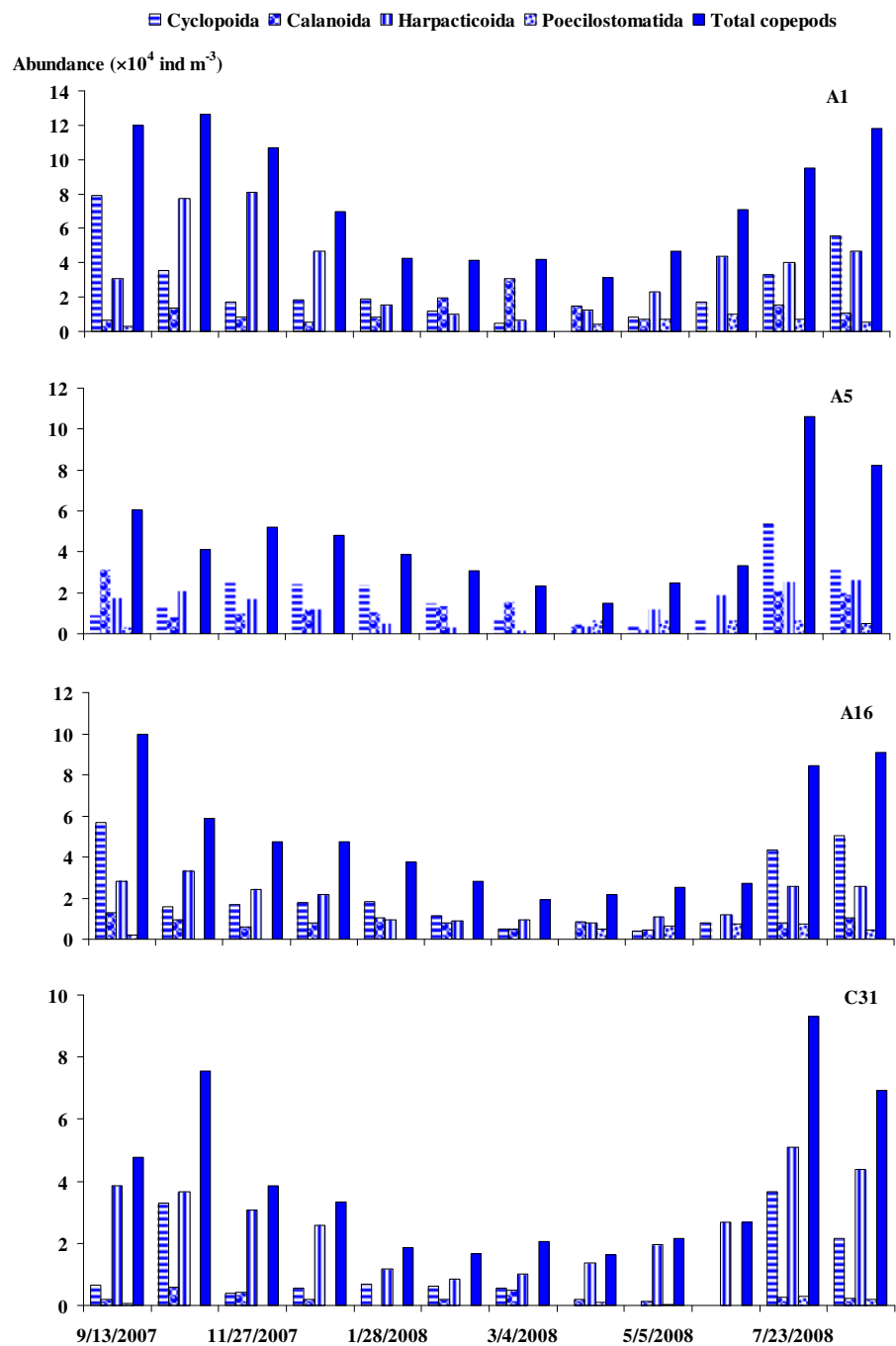
In summary, the PCA shows that copepod distribution is more susceptible to the influence of salinity, suspended matter, phytoplankton (diatoms and dinoflagellates) and nutrients than to that of temperature, chlorophyll *a* and total ciliates. The sample score distribution (Fig. 6) indicated clear differences from one pond to another.

**Discussion**

In this study, the nutrient concentrations recorded in pond A1 were much lower than those reported before restoration in the same pond (Abid et al. 2008; Khemakhem et al. 2010) which is characteristic of eutrophic systems and can be attributed to anthropogenic

interference in the Sfax coastal waters (Khemakhem et al. 2010). The improvement of the A1 trophic state (Table 5) is attributed to the restoration efforts undertaken during 2007 along the part of the city’s shoreline in direct contact with the first of the local solar saltern ponds. We noticed a drastic decrease in T-P and phosphates from 185.8 and 145.9  $\mu\text{mol } \Gamma^{-1}$ , respectively (Abid et al. 2008), to 60.46 and 19.1  $\mu\text{mol } \Gamma^{-1}$  as is the case in the present study. In contrast, the phosphorous concentrations in the other ponds were comparable to those of previous studies (Table 5), confirming that the phosphorus there depends more on the internal recycling processes (e.g., release from sediment, mineralization of organic matter) which are a common feature in most coastal lagoons (Abdennadher et al. 2012)

**Fig. 4** Temporal and spatial variation of copepod groups and total copepods in ponds A1, A5, A16, and C31



than it does on seawater inflow. In pond A1, we also noted a slight decrease in the relative dinoflagellate contribution from 22–43 (Khemakhem et al. 2010) to 18 % as found in the present study, while that of diatoms did not change. However, within these diatoms, an increase in the diversification of the pennate species was observed after restoration (14 versus

8 species) (Abid et al. 2008) while that of centric diatoms did not change (8 versus 8 species). The shifts in dominance of pennate over fast-growing centric diatoms indicate a phytoplankton community under less stress than prior to restoration. Moreover, while small diatoms, especially *Chaetoceros* sp. and *Nitzschia longissima* invaded pond A1 before restoration,

**Table 4** Quantitative and structural characteristics of the copepod community sampled in ponds A1, A5, A16, and C31

	Copepod total abundance ( $\times 10^4$ ind $m^{-3}$ )	Diversity index (bits ind $^{-1}$ )	Evenness	Abundance of dominant species ( $\times 10^4$ ind $m^{-3}$ )	Occurrence frequency of dominant species (%)	Dominant species
<b>A1</b>						
13/09/2007	19.17	2.23	0.13	6.00	66.66	<i>Oithona similis</i>
29/10/2007	27.72	2.98	0.18	4.05	91.66	<i>Clytemnestra scutellata</i>
27/11/2007	52.00	2.84	0.17	2.96		<i>C. scutellata</i>
12/12/2007	116.11	2.82	0.18	1.89		<i>C. scutellata</i>
28/01/2011	180.55	2.50	0.16	1.33	91.66	<i>Oithona nana</i>
02/02/2011	94.50	4.38	0.29	1.46	50	<i>Paracartia grani</i>
04/03/2011	8.59	2.20	0.14	2.10		<i>P. grani</i>
04/04/2011	5.97	1.82	0.12	1.47	66.66	<i>Acartia clause</i>
05/05/2011	8.59	2.73	0.18	0.95		<i>C. scutellata</i>
06/06/2011	12.13	2.57	0.16	1.73	83.33	<i>Canuella perplexa</i>
23/07/2011	14.29	3.30	0.20	1.80	66.66	<i>O. similis</i>
08/08/2011	17.82	2.99	0.18	3.90		<i>O. similis</i>
<b>A5</b>						
13/09/2007	10.50	2.77	0.17	1.44	91.66	<i>Bryocamptus</i> sp.
29/10/2007	41.89	2.82	0.18	1.31		<i>Bryocamptus</i> sp.
27/11/2007	21.35	2.70	0.17	1.52	66.66	<i>O. similis</i>
12/12/2007	14.44	2.59	0.17	1.25		<i>O. similis</i>
28/01/2011	7.32	1.99	0.13	1.38	91.66	<i>Oithona nana</i>
02/02/2011	5.36	2.22	0.15	1.03	91.66	<i>P. grani</i>
04/03/2011	3.50	1.88	0.13	1.08		<i>P. grani</i>
04/04/2011	6.63	1.78	0.13	0.66	50	<i>Oncaea conifera</i>
05/05/2011	3.74	2.76	0.19	0.65	66.66	<i>C. perplexa</i>
06/06/2011	6.00	2.44	0.16	0.93		<i>C. perplexa</i>
23/07/2011	87.48	2.70	0.16	3.75		<i>O. nana</i>
08/08/2011	49.49	2.99	0.18	2.13		<i>O. nana</i>
<b>A16</b>						
13/09/2007	43.50	1.87	0.11	5.20	91.66	<i>O. nana</i>
29/10/2007	15.70	1.96	0.12	2.37	100	<i>Bryocamptus</i> sp.
27/11/2007	7.96	1.62	0.10	2.04		<i>Bryocamptus</i> sp.
12/12/2007	5.21	2.59	0.17	1.82		<i>Bryocamptus</i> sp.
28/01/2011	1.18	2.15	0.17	1.04	66.66	<i>O. similis</i>
02/02/2011	3.59	2.90	0.20	0.64	91.66	<i>O. nana</i>
04/03/2011	4.62	2.38	0.17	0.68	83.33	<i>C. perplexa</i>
04/04/2011	2.33	2.29	0.16	0.69	91.66	<i>P. grani</i>
05/05/2011	2.90	3.19	0.22	0.46	50	<i>O. conifer</i>
06/06/2011	3.32	2.47	0.17	0.80		<i>O. nana</i>
23/07/2011	20.30	3.16	0.19	2.60		<i>O. nana</i>
08/08/2011	31.81	2.55	0.15	3.90		<i>O. nana</i>
<b>C31</b>						
13/09/2007	4.88	1.24	0.08	3.65	100	<i>Bryocamptus</i> sp.
29/10/2007	11.39	2.05	0.13	3.34		<i>Bryocamptus</i> sp.
27/11/2007	6.24	1.31	0.09	2.88		<i>Bryocamptus</i> sp.

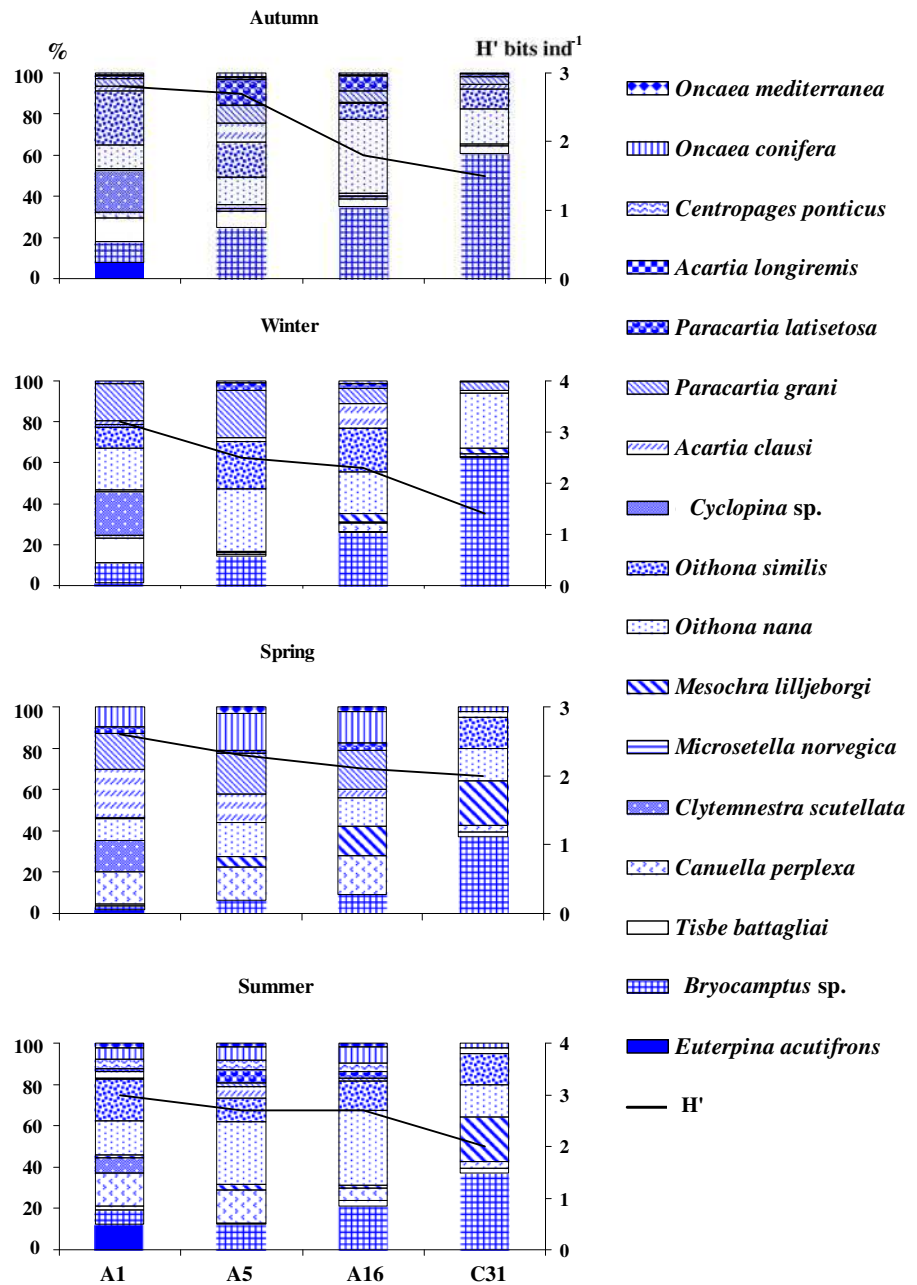
**Table 4** (continued)

	Copepod total abundance ( $\times 10^4$ ind $m^{-3}$ )	Diversity index (bits $ind^{-1}$ )	Evenness	Abundance of dominant species ( $\times 10^4$ ind $m^{-3}$ )	Occurrence frequency of dominant species (%)	Dominant species
12/12/2007	4.89	1.21	0.08	2.49		<i>Bryocamptus</i> sp.
28/01/2011	2.60	0.95	0.07	1.18		<i>Bryocamptus</i> sp.
02/02/2011	2.72	1.98	0.14	0.63		<i>Bryocamptus</i> sp.
04/03/2011	3.31	2.47	0.17	0.56		<i>Bryocamptus</i> sp.
04/04/2011	4.52	1.67	0.12	1.10	58.33	<i>Mesochra lilljeborgi</i>
05/05/2011	5.00	1.78	0.12	0.98		<i>M. lilljeborgi</i>
06/06/2011	5.60	1.25	0.08	1.60		<i>Bryocamptus</i> sp.
23/07/2011	16.20	2.48	0.15	2.50		<i>Bryocamptus</i> sp.
08/08/2011	10.12	2.33	0.14	2.96		<i>Bryocamptus</i> sp.

they now appeared to share the total diatom biomass with larger diatom species namely *Thalassiosira* sp. and *Navicula* sp. Since the hydrographic conditions such as water turbulence remained nearly the same in pond A1 over the years, the appearance of large diatoms may be attributed to decreased phosphorus inputs resulting from restoration. Indeed, it is known that as phosphorus concentration decreases large diatoms develop a greater competitive advantage for P assimilation (Reynolds 1997). This confirms the findings of previous investigations in coastal waters showing that nutrients are the main resource, with temperature as the main seasonal signal for phytoplankton growth since the salinity level is still near that of seawater. The restoration of the coastal waters therefore broadens the spectrum of existing conditions, enabling the coexistence of a great number of species. The present findings provided information on the direct impact of restoration on the phytoplankton community and demonstrated the suitability of using phytoplankton as bioindicators of environmental stress (Aleya et al. 2011). This is also confirmed by the diversification observed within the ciliate community, namely Oligotrichida, with 19 new taxa recorded during this study though they were missing in the previous study by Elloumi et al. (2009). Among these new Oligotrichida taxa, we found *Strombidium capitatumis* to be a bioindicator of less stressed marine ecosystems (Jiang et al. 2011). High species ciliate diversity is commonly observed in marine systems of low organic pollution (Ismael and Dorgham 2003) and in less stressed marine sites (Jiang et al. 2011). The copepod composition in the Sfax saltern is comparable to other Mediterranean coastal environments, with, however, an evident prevalence of

halophylic species in adaptation to the rising salt concentration (Abdennadher et al. 2012; Badosa et al. 2006; Hannachi et al. 2011). During this study, we found ten species in pond A1 cited for the first time in the Sfax solar saltern, compared with the findings of Toumi et al. (2005) in their previous study period of 1999–2000. Among them were large species such as *Centropages kroyeri* and *Clytemnestra scutellata*, known to indicate a good ecosystem status (Kambursha and Fonda-Umani 2006) and/or a modification in the prey spectrum (Calbet et al. 2001). We infer, with regard to food resources represented by phytoplankton and available data on ciliates, that the increase in the size of potential prey as seen in our study may also be an important driver of zooplankton community structure in the saltern. Previous studies along the coast of Sfax (Drira et al. 2010; Rekik et al. 2012) and in other marine systems (Verity and Smetacek 1996; Rollwagen-Bollens et al. 2011) indicate that the quantity and quality of specific types of food would be expected to affect variation in zooplankton composition. We also previously found in laboratory experiments that copepods preferred large ciliates when these were abundant (Hartmann et al. 1993). Copepods in ponds A1 and A5 (salinity, <50) were mainly thalassophylic. A slight increase in salinity in A1 from adjacent seawater positively influenced total copepod abundance. Christou (1998) also reported the importance of salinity as the most significant factor affecting the inter-annual variability of copepod abundances in Mediterranean coastal areas with a salinity of 39 psu. In addition, the salinity of A1 may be optimal for the egg production of dominant copepods and the hatching success of their remaining eggs (Peck and Holste

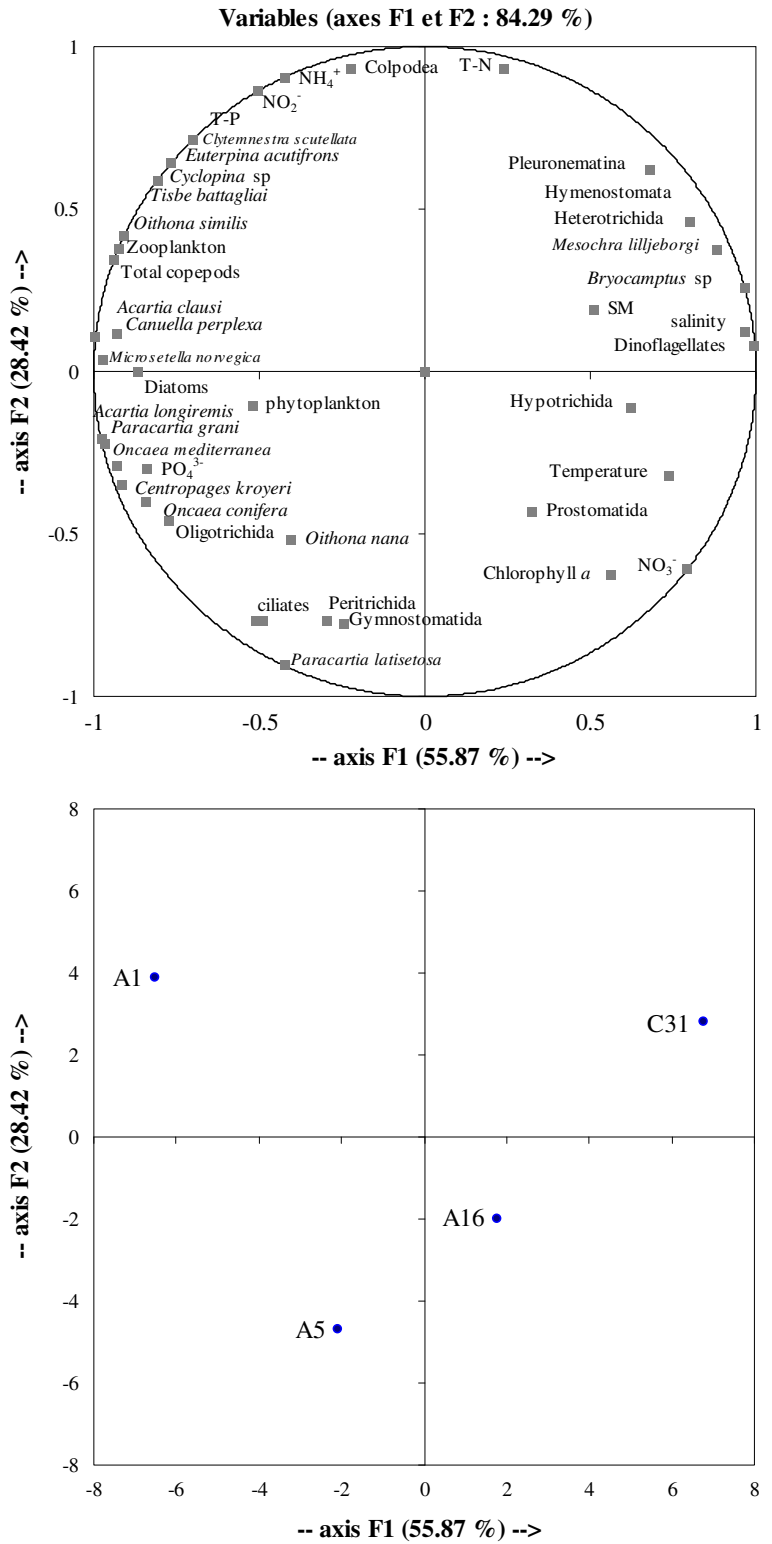
**Fig. 5** Seasonal variation of species abundances and H' in ponds A1, A5, A16, and C31



2006). However, this was not the case for pond A5 because the increase in salt concentration negatively affected copepod abundance leading to complete absence of *C. scutellata*, *Euterpina acutifrons*, and *Cyclopina sp.* These three taxa have been shown to persist in both coastal marine waters (Annabi-Trabelsi 2006; Drira et al. 2010) and within the polyhaline zone (37 > surface salinity > 18 psu) of estuaries (Hoffmeyer and Barria de Cao 2007; Chertoprud et al. 2009). In ponds A1 and A5,

thalassophylic copepods are forced to face salinity stress and an important seasonal temperature variation (the difference between the hot and the cold season is greater than 11°C) implying a trade-off between fighting osmotic stress and improvement of food availability. With the increase in salinity to >50 psu in A16 and C21, temperature positively affected total copepod abundances, thus *O. nana* dominated at A5 and A16 as it is an euryhaline and eurythermal species. The halophylic

**Fig. 6** PCA performed on biological parameters and different environmental factors in four ponds during 1 year of study



taxa *Bryocampus* sp. and *M. lilljeborgi* increased gradually in A16 and peaked in pond C31, their

demography showing positive correlations with salinity.

**Table 5** Min, max and mean±SD of physicochemical and biological variables at the first pond A1 before and after restoration

Physicochemical and biological parameters	Before restoration			Present work (2007–2008)			F values (df)
	Min	Max	Mean±SD	Min	Max	Mean±SD	
<b>Physical parameters</b>							
Temperature (°C)	12.6	32.3	24.5±6.3	11.5	29	20.7±5.57	0.91 (23)
Salinity (psu)	40.2	60.4	45.0±5.4	38	45.2	41.54±2.38	4.64 (23)*
pH	6.11	8.18	7.2±0.7	7.01	8.93	7.71±0.52	2.13 (23)
Suspended matter (µg l <sup>-1</sup> )	32	285.75	130.9±96.6	105	825	399.37±217.83	42.64 (23)***
<b>Chemical parameters</b>							
NO <sub>3</sub> <sup>-</sup> (µmol l <sup>-1</sup> )	1.79	74.24	45.6±32.8	1.79	15.41	6.52±3.73	3.02 (23)
NO <sub>2</sub> <sup>-</sup> (µmol l <sup>-1</sup> )	0.02	38.98	9.29±12.42	0.02	0.72	0.25±0.21	6.36 (23)*
NH <sub>4</sub> <sup>+</sup> (µmol l <sup>-1</sup> )	0.47	43.33	32.8±6.3	0.47	15.01	5.21±5.81	5.29 (23)*
Total nitrogen (µmol l <sup>-1</sup> )	12.09	771.43	322.0±205.8	2.78	26.75	16.36±7.73	13.21 (23)**
PO <sub>4</sub> <sup>3-</sup> (µmol l <sup>-1</sup> )	1.06	285.11	145.9±110.0	2.82	39.96	19.19±11.95	5.59 (23)*
Total phosphate (µmol l <sup>-1</sup> )	5.33	363.83	185.8±132.8	13.25	201.48	60.46±53.46	7.67 (23)**
Si(OH) <sub>4</sub> (µmol l <sup>-1</sup> )	3.63	43.63	12.71±11.29	2.46	43.64	12.65±13.84	0.0001 (23)
N/P ratio			0.8	0.139	7.87	1.92±2.89	0.71 (23)
<b>Biological parameters</b>							
Chlorophyll <i>a</i> concentration (mg l <sup>-1</sup> )	0.002	0.19	0.032±0.051	0.05	0.13	0.09±0.02	10.04 (23)**
Total phytoplankton density (×10 <sup>3</sup> cells l <sup>-1</sup> )	34.4	2,096	600±800	29	142.7	78.31±34.96	8.71 (23)**
Cyanobacteriae density (×10 <sup>3</sup> cells l <sup>-1</sup> )				0	21	1.85±6.03	
Diatoms density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	1,988	302.80±603.09	22.8	102.4	50.2±22.54	2.10 (23)
Dinophyceae density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	2,072	444.48±702.03	0	40.3	14.05±12.77	4.5 (23)*
Euglenophyceae density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	96	13.59±29.49	0	35	12.20±15.08	0.02 (23)
Silicoflagellate density (×10 <sup>3</sup> cells l <sup>-1</sup> )	–	–	–	0	14	1.75±4.35	
Total ciliate density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0.9	63.8	16.075±19.3	0.5	78	22.89±28.6	0.46 (23)
Oligotrichidae density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0.6	63.4	11.41±18.44	0.3	76	21.66±28.67	1.08 (23)
Colpodea density (×10 <sup>3</sup> cells l <sup>-1</sup> )	–	–	–	0	1	0.13±0.3	
Gymnostomatidae density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	0.8	0.15±0.31	0	1	0.08±0.28	0.18 (23)
Prostomatidae density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	31.6	3.99±9.51	0	4	0.75±1.28	3.19 (23)
Pleuronematina density (×10 <sup>3</sup> cells l <sup>-1</sup> )	–	–	–	0	0.1	0.012±0.03	
Heterotrichida density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	0.1	0.008±0.02	–	–	–	
Hymenostomatida density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	0.4	0.09±0.16	–	–	–	
Hypotrichida density (×10 <sup>3</sup> cells l <sup>-1</sup> )	0	0.4	0.13±0.18	0	2	0.25±0.62	0.18 (23)
Total zooplankton density (×10 <sup>3</sup> ind m <sup>-3</sup> )			160±270	52.46	2001.75	707.62±754.98	10.10 (23)**
Total copepod density (×10 <sup>3</sup> ind m <sup>-3</sup> )			106±193	59.65	1805.54	464.52±554.39	
Cyclopoid density (×10 <sup>3</sup> ind m <sup>-3</sup> )			9±1.8	0	79.2	24.94±22.86	
Calanoid density (×10 <sup>3</sup> ind m <sup>-3</sup> )			8.2±3.1	0	30.82	11.64±7.99	
Harpacticoid density (×10 <sup>3</sup> ind m <sup>-3</sup> )			1.6±3.8	6.52	81.19	36.13±24.77	
Poecilostomatoid density (×10 <sup>3</sup> ind m <sup>-3</sup> )			–	0	10	3.05±3.58	
Other zooplankton density (×10 <sup>3</sup> ind m <sup>-3</sup> )			51±48	15.29	1252.48	243.09±369.02	

In the last column, results of one-way ANOVA analysis

\**p*<0.05; \*\**p*<0.01; \*\*\**p*<0.001, significant difference between sampled stations



## Conclusions

This study presents an original survey of a planktonic community at a site that offers a unique environment with high annual reproducibility of seasonal dynamics in water ponds. The choice of the fine range of salinity (41–77 psu), added to the semi-arid climate and the strict operating conditions imposed by the salt producing company, combine to render the physicochemical variables of each pond highly stable from one year to the next. This, in turn, enhances our ability to uniquely identify the influence of phosphogypsum residue reduction on the seasonal distribution, composition and dynamics of planktonic communities, chiefly at four ponds featuring a salinity gradient. The restoration of the phosphogypsum landfill not only positively influenced the biological communities under study but also the quality of the salt produced as reported by the Franco-Tunisian salt company COTUSAL.

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