

## Environmental controlling factors of copepod communities in the Gulf of Tunis (south western Mediterranean Sea)

Yosra BEN LAMINE<sup>1</sup>, Olivier PRINGAULT<sup>1,2</sup>, Mehdi AISSI<sup>1</sup>, Cherif ENSIBI<sup>1</sup>, Ezzeddine MAHMOUDI<sup>3</sup>,  
Ons DALY YAHIA KÉFI<sup>4</sup> and Mohamed Néjib DALY YAHIA<sup>1\*</sup>

(1) *UR Biologie Marine (FST/Université El Mannar). Laboratoire de Biodiversité et Fonctionnement des Systèmes Aquatiques, Université de Carthage, Faculté des Sciences de Bizerte, 7021 Zarzouna, Bizerte, Tunisie*

\* Corresponding author : nejib.daly@gmail.com

(2) *UMR 5119 CNRS-UM2-IFREMER- IRD-ECOSYM, Station Méditerranéenne de l'Environnement Littoral, 2, rue des chantiers, 34200 Sète, France*

(3) *Laboratoire de Biosurveillance de l'Environnement, Université de Carthage, Faculté des Sciences de Bizerte, 7021 Zarzouna, Bizerte, Tunisie*

(4) *UR Biologie Marine (FST/Université El Mannar). Groupe de Recherche en Océanographie et Ecologie du Plancton, Institut National d'Agronomie de Tunisie, 43 avenue Charles Nicolle, 1080 Tunis, Tunisie*

**Abstract:** The copepod community structure and the distribution of the main groups of zooplankton were studied along an inshore-offshore gradient in the Gulf of Tunis during the rainy and dry seasons of 2007-2008. Hydrological parameters were also measured to assess the potential role of abiotic and biotic factors in the distribution of copepod species. The copepod community in the Gulf of Tunis comprises 86 species dominated by *Paracalanus parvus*, *Clausocalanus lividus*, *Centropages kroyeri* and *Acartia clausi*. Time had a greater influence than space (horizontal and vertical gradients) in shaping the copepod community structure with a significant influence of the seasons; winter (cold and rainy) resulted in hydrological conditions that were strongly different from those observed in summer (warm and dry). These hydrological differences were concomitant with changes in the community structure, with a high copepod diversity observed in winter while the summer period was characterized by a low specific richness and the dominance of a few species, *Centropages kroyeri* and *Paracalanus parvus* along the inshore-offshore gradient and *Paracalanus aculeatus* along the vertical. Canonical correspondence analysis showed that temperature, salinity and to a lesser extent chlorophyll *a* were the most important environmental factors structuring the copepod community. Interestingly, temperature and salinity showed a negative significant correlation with copepod specific richness. Competition with grazers (cladoceran) as well as top down control by predators (chaetognaths and siphonophors) were also identified as key factors for the copepod community structure.

**Résumé :** *Facteurs environnementaux contrôlant les communautés de copépodes du Golfe de Tunis (sud-ouest de la Méditerranée).* La structure de la communauté des copépodes et la répartition des principaux groupes zooplanctoniques ont été étudiés le long d'un gradient côte-large dans le golfe de Tunis pendant les saisons sèches et pluvieuses de 2007 à 2008. Les paramètres hydrologiques ont également été mesurés afin d'évaluer le rôle potentiel de facteurs abiotiques et biotiques dans la répartition des espèces de copépodes. La communauté des copépodes du golfe de Tunis a été composée de 90 espèces et dominée par *Paracalanus parvus*, *Clausocalanus lividus*, *Centropages kroyeri* et *Acartia clausi*. La structure de

la communauté a été plutôt régie par les conditions hydrologiques saisonnières contrastées (hiver, froid et pluvieux, et été, chaud et sec), que par les gradients observés à l'échelle spatiale (gradients horizontaux et verticaux). Ces différences hydrologiques ont été concomitantes avec des changements dans la structure de la communauté, avec une grande diversité de copépodes observée en hiver alors que la période estivale a été caractérisée par une faible richesse spécifique et la dominance de quelques espèces, *Centropages kroyeri*, *Paracalanus parvus* le long du gradient côte-large et *Paracalanus aculeatus* le long du gradient vertical. L'analyse canonique de correspondance a montré que la température, la salinité et dans une moindre mesure la Chla étaient les facteurs abiotiques les plus importants dans la structuration de la communauté de copépodes. La température et la salinité ont montré une corrélation négative significative avec la richesse spécifique des copépodes. La compétition avec les brouteurs (cladocères) ainsi que le contrôle "Top-Down" par les prédateurs (chaetognathes et siphonophores) ont également été identifiés comme des facteurs clés dans la structuration de la communauté des copépodes.

*Keywords:* Copepod diversity • Multivariate analysis • Temperature • Salinity • Top-down control • Competition

## Introduction

Mesozooplankton are well suited for studying the ecosystem response to environmental and climate variability. They have a short generation time, high density and continuous reproduction. The populations of these small organisms have the potential to respond to and reflect event-scale and seasonal changes in environmental conditions (Richardson, 2008). Moreover, many mesozooplankton taxa are known to be indicator species whose presence or absence may represent the relative influence of different water types on ecosystem structure. Thus mesozooplankton may serve as sentinel taxa that reflect changes in marine ecosystems by providing early indications of a biological response to environment and climate variability (Hays et al., 2005).

Several studies in the Mediterranean Sea have attempted to examine the role played by mesozooplankton distribution in understanding regional ecosystem functioning: i) hydrographical conditions controlling zooplankton composition and standing stock (Youssara & Gaudy, 2001; Riandey et al., 2005; Licandro & Icardi, 2009), ii) climate forcing modified food web dynamics by changing biological trophic interactions (de Puellès et al., 2003; Molinero et al., 2008), iii) anthropogenic pressure engendering a multivorous food web based dominated by microbial web (Hlaili et al., 2008) and iv) nutrient dynamics controlling zooplankton size and diversity depending on phytoplankton communities (Townsend et al., 2010).

In aquatic ecosystems, copepods are generally the most important component of mesozooplankton in terms of abundance, biomass and diversity, and serve as an important link by transferring energy and organic materials between primary producers (phytoplankton) and animals of higher trophic levels such as planktivorous fish and carnivorous

invertebrates (Ara, 2004; Mitra & Davis, 2010). Spatial-temporal studies of copepod diversity and abundance are fundamental to assess their contribution to the production and flow of particulate and dissolved organic materials, as well as their role in estuary trophodynamics (Perissinotto et al., 2003). Studies on Copepods are quite scarce in the Tunisian waters. Indeed, Daly Yahia et al. (2004) supplied a series of data (from 1993 to 1995); their sampling strategy covered the whole area of the Bay of Tunis. Additionally, Drira et al. (2009) studied the summer spatial distribution of the copepod community in both the neritic and oceanic areas of the Gulf of Gabes (Tunisia, eastern Mediterranean Sea) coupled with environmental factors. However, the Gulf of Tunis which communicates and directly affects the Bay of Tunis has never been the subject of mesozooplankton investigation. Thus, it was important to examine spatiotemporal mesozooplankton dispatching in this area open to water exchanges between both Mediterranean sub-basins.

The aim of this study was to assess the role of abiotic and biotic factors in the structuring of copepod communities in the south western Mediterranean Sea. To that end, we performed a field survey that described the occurrence, abundance patterns, diversity and seasonal distribution of copepods in the Gulf of Tunis, from data collected along an inshore-offshore gradient in different seasons in 2007 and 2008.

## Materials and Methods

### *Description of the study and sampling area*

The Gulf of Tunis is located between 36°42'-37°10'N latitude and 10°11'-11°5'E longitude in the northern

Tunisian coast extending for approximately 1,500 Km<sup>2</sup>. It reaches a maximum depth of 150 m. A sandy bottom mainly covers the central zone of this retention area (Afli et al., 2008b). It is surrounded by an important wetland framework including major drainage lagoons, wadis and river systems with the most important being the Madjerda River (Fig. 1).

This region forms a junction with three major subsets in

the Mediterranean: the Algerian-Provencal basin in the west, the Tyrrhenian Sea in the north and the Ionian Sea in the east. The geographic position of the Gulf of Tunis plays a key role in surface water exchange between the western and eastern Mediterranean as it opens up to the Tunisian-Sicilian and Tunisian-Sardinian straits. The Gulf of Tunis is under the influence of the Modified Atlantic Waters (Astraldi et al., 1999; Beranger et al., 2004).

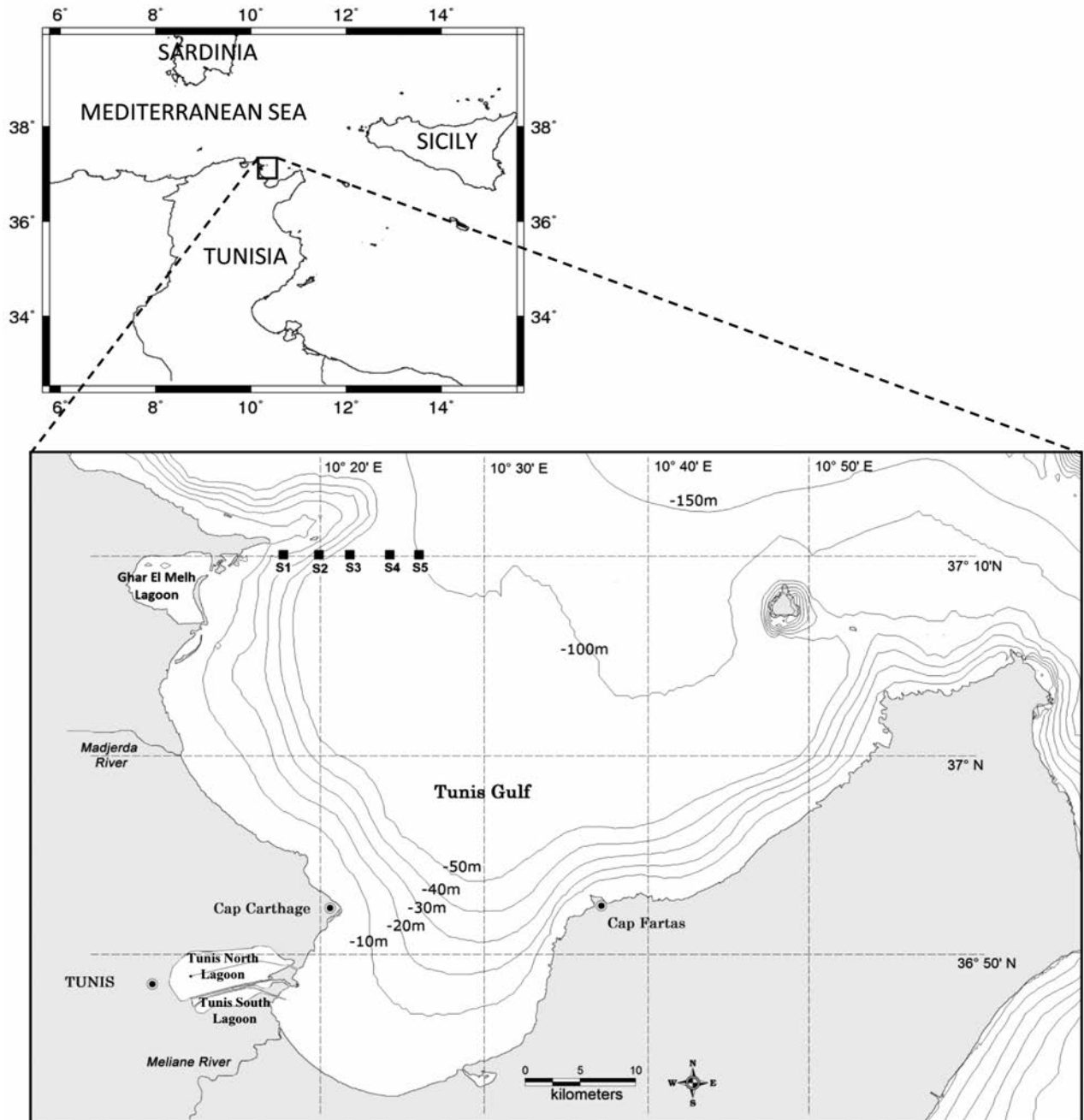


Figure 1. Map of the Gulf of Tunis with the 5 sampling stations.

### Analytical methods

Five seasonal campaigns (05-12-2007, 07-08-2007, 11-23-2007, 02-01-2008 and 04-30-2008) were conducted in the Gulf of Tunis with sampling along a transect from inshore to offshore of five stations (Fig. 1). Two sampling strategies were employed to assess the horizontal and the vertical distribution of copepods. Stations from 1 to 5 were sampled at the surface (0-20 m) and additional samplings were performed in the most oceanic station (station 5) along the water column (20-40 m; 40-60 m; 60-80 m and 80-100 m).

Mesozooplankton samples were collected during a series of vertical hauls (0-20 m; 20-40 m; 40-60 m; 60-80 m and 80-100 m) using a Nansen closing net (mesh size 220  $\mu\text{m}$ ). Mesozooplankton samples were preserved immediately after collection with buffered formaldehyde (4%). Copepod species and other mesozooplankton taxa were counted and identified according to Rose (1933), Tregouboff & Rose (1957), Huys & Boxshall (1991), and Boxshall & Halsey (2004) using a binocular and a microscope at species level for copepods.

For copepods, we only presented the variation of the relative abundance (more than 0.1%) of the main copepod species. To distinguish between copepod species characters (Atlantic, oceanic, neritic...) different references were used including Seguin et al. (1993), Belmonte & Polenza (2001), Beaugrand et al. (2002), Daly Yahia et al. (2004), Khelifi-Touhami et al. (2007).

For each station, temperature and salinity were measured using a multiparameter WTW Multi 340i set at different depths (surface, -2.5 m, -5 m, -7.5 m, -10 m, -15 m, -25 m, -50 m, -75 m and -100 m). Nitrite, nitrate, phosphates and chlorophyll *a* were carried out using spectrophotometric methods according to Aminot & Chaussepied (1983). Water transparency was measured using a Secchi disc.

### Statistical analysis

Univariate indices, number of copepods species (*S*) and diversity index (Shannon-Wiener,  $H'$ ) were calculated for each sample. Principal component analyses (PCA) using the Gower similarity index were performed with the abiotic factors to assess the role of time and space in shaping the environmental conditions. Canonical correspondence analyses (CCA) using the relative abundances of copepods following the recommendations of Ramette (2007) were then performed to estimate the role of abiotic and biotic factors on the copepod community structure as a function of sampling time and space. This multivariate analysis is appropriate when species display unimodal (bell shaped or Gaussian) relationships with environmental gradients (Ramette, 2007). In addition CCA was used rather than modeling relative abundances and it accommodates well the presence of many zeros in the species table (Ramette, 2007). Relative

abundances were transformed with arcsin ( $x^{0.5}$ ) to obtain a normal distribution of data (Legendre & Legendre, 1998). Multivariate analyses were performed using MVSP v3.12d software (Kovach Computing Service, Anglesey Wales).

## Results

### Environmental conditions

The hydroclimatic condition in this area during the sampling period was similar to that generally observed in the south western Mediterranean Sea, with an average air temperature around 25-30°C during summer concomitant with low precipitation and south eastern dominant winds. In contrast, fall and winter seasons were characterized by intense precipitations, low temperature (average 10-15°C) and north western dominant winds. Sea surface temperature (SST) was high in the summer season in July 2007 reaching  $24.7 \pm 0.1^\circ\text{C}$  and low during winter in February 2008 ( $15.4 \pm 0.16^\circ\text{C}$ ). Sea surface salinity (SSS) reached  $36.84 \pm 0.15$  in April 2008 and fell to  $35.92 \pm 0.12$  in February 2008 (Table 1a). Water transparency reached its maximum value in May ( $13.8 \pm 4.3$  m) while a low value was observed in April 2008 ( $9.6 \pm 4.3$  m) (Table 1a). For nutrients, the waters were concentrated with N ions ( $0.58 \pm 0.17$   $\mu\text{mol}$ ) in May 2007. The lowest concentrations were recorded in April 2008 ( $0.14 \pm 0.1$   $\mu\text{mol}$ ). Phosphate concentrations were low in April ( $0.01$   $\mu\text{mol} \pm 0.01$ ) and reached a maximum value in July ( $0.36 \pm 0.27$   $\mu\text{mol}$ ). Chlorophyll *a* presented a maximum average concentration in July 2007 ( $4.46 \pm 3.46$   $\text{mg}\cdot\text{m}^{-3}$ ), for the other four campaigns, it ranged from  $1.04 \pm 0.31$   $\text{mg}\cdot\text{m}^{-3}$  in November 2007 and  $1.63 \pm 1.6$   $\text{mg}\cdot\text{m}^{-3}$  in May 2007. Environmental conditions along the vertical at Station 5 showed the same variability with time as observed for the surface waters along the inshore-offshore gradient (Table 1b). Temperature and salinity were minimal in February 2008 while the highest values were observed in July 2007. For all seasons, Chlorophyll *a* was maximal at the surface waters.

Mesozooplankton groups showed different distributions according to time and space (Table 1). Copepods were the dominant group with maximum abundance of 2275  $\text{ind}\cdot\text{m}^{-3}$  in July 2007 for station 4 while the minimum was observed at station 5 in November 2007 (104  $\text{ind}\cdot\text{m}^{-3}$ ). In terms of total abundance, they were followed by appendicularians and cladocerans. Relative abundance of the copepod group ranged from 46 to 90% (with an average of 72%) of the total mesozooplankton collected between May 2007 and April 2008 in the Gulf of Tunis. Covariances of relative abundances calculated between stations for the same campaign were very low, suggesting a strong spatial homogeneity along the horizontal and vertical gradient.



**Table 1a.** Environmental conditions observed at the surface (0-20 m) for stations 1 to 5 during the 5 sampling periods. Transp: Transparency; Clado: Cladocera; Hydro: Hydromedusae; Sipho: Siphonophores; Chaeto: Chaetognaths; Appendi: Appendicularians; Dolio: Doliolids; Copep: Copepods. These data were used for the multivariate analysis (CCA). Discrete data of hydrological parameters have been averaged.

Date	Stations	T°C	Salinity	Transp	NO <sub>2</sub> +NO <sub>3</sub> μM	PO <sub>4</sub> μM	Chla mg.m <sup>-3</sup>	Clado Ind.m <sup>-3</sup>	Hydro Ind.m <sup>-3</sup>	Sipho Ind.m <sup>-3</sup>	Chaeto Ind.m <sup>-3</sup>	Appendi Ind.m <sup>-3</sup>	Dolio Ind.m <sup>-3</sup>	Copep Ind.m <sup>-3</sup>
5/12/07	1	18.3	36.5	6.5	0.63	0.01	4.5	44.51	1.48	0	7.59	19.41	0.42	339.36
	2	18.3	36.6	15.6	0.75	0.01	1.2	49.79	1.21	0.95	10.99	186.13	6.75	531.71
	3	18.5	36.4	14.8	0.34	0.02	0.85	61.29	0.37	0.21	11.29	18.46	5.17	758.34
	4	18.7	36.4	14	0.49	0.23	0.5	99.26	3.48	1.9	5.06	74.31	3.27	557.86
	5	18.7	36.4	18	0.71	0.91	1.1	3.38	8.86	6.75	8.91	402.53	3.38	379.99
7/8/07	1	24.7	36.7	4	0.10	0.19	9.2	54.22	1.27	1.9	15.19	236.92	9.49	426.36
	2	24.8	36.4	6	0.32	0.13	7	55.27	0.47	0.32	13.82	14.98	0.42	1045.28
	3	24.8	36.7	10	0.10	0.34	2.9	214.87	2.74	0.53	12.87	32.33	32.07	491.09
	4	24.7	36.6	22.5	0.08	0.82	1.3	4.22	6.75	12.24	6.42	323.21	4.22	2274.77
	5	24.6	36.8	17.5	0.41	0.31	1.9	19.83	7.59	12.24	30.57	337.13	3.8	376.35
11/23/07	1	18.5	36.3	8	0.19	0.01	1.2	78.9	0.42	0.42	27.64	19.62	0.42	570.00
	2	19.4	36.5	4.5	0.20	0.01	1.1	218.3	2.95	0.74	16.32	37.24	38.66	453.39
	3	19.1	36.3	13	0.28	0.03	1.3	5.91	2.53	13.08	8.96	127.43	1.69	880.56
	4	19.6	36.6	14	1.45	0.01	0.5	16.88	5.06	8.44	28.69	329.11	3.38	1300.21
	5	18.5	36.5	15	0.76	1.08	1.1	2.43	3.06	9.28	11.31	225.74	32.17	104.18
2/1/08	1	15.2	36	6	0.12	0.01	0.7	205.38	2.32	0.84	6.9	11.6	19.73	678.80
	2	15.3	36	8	0.33	0.01	1.1	11.39	4.64	7.59	7.59	97.89	2.95	337.83
	3	15.5	35.9	10	0.16	0.01	1	4.64	2.95	1.69	1.19	197.05	1.69	1710.44
	4	15.5	35.9	13	0.79	0.68	1.1	0.84	1.48	5.49	3.3	101.69	9.7	222.35
	5	15.6	35.7	15	1.01	0.01	1.5	13.5	0.42	2.74	1.69	13.08	0	134.28
4/30/08	1	19	36.9	5	0.10	0.01	1.5	16.03	5.91	1.69	0	37.13	5.91	519.08
	2	19.7	36.9	6	0.10	0.01	1.7	0.42	8.02	5.06	6.25	222.36	6.75	278.06
	3	19.3	36.8	9	0.10	0.01	1.5	3.06	3.06	6.33	8.86	63.71	4.54	861.05
	4	18.6	37	13	0.32	0.01	1	17.3	0.84	1.69	4.01	15.19	0	1398.59
	5	18	36.6	15	0.10	0.01	1.4	3.16	0	5.7	14.84	27.43	0.84	112.84

The PCA (Fig. 2a) confirmed that the environmental conditions of this part of the Gulf of Tunis followed a seasonal pattern since the analysis separated significantly for summer, winter and spring seasons; the campaigns of May and November 2007 appeared to be similar environmentally. However, the two most distant stations from the coast (4 and 5) appeared to have different abiotic characteristics relative to the coastal ones. As observed for surface data, the second PCA (Fig. 2b), focused on the vertical distribution of hydrological conditions in station 5, also showed a clear effect of the sampling period. Clusters of the different sampling depths (0-20 m, 20-40 m, 40-60 m, 60-80 m and 80-100 m) for the same campaign separated all seasons. The influence of depth was not obvious suggesting a relative homogeneity of the water column at the different sampling periods.

#### Copepod community structure

Copepod community consisted of 86 species of different origins and ecological affinities (Table 2). Four main

groups could be distinguished: the indicator species of the Atlantic influence in the Mediterranean, the neritic Mediterranean species, the oceanic Mediterranean species and species of mixed character (Table 2). A community of permanent species was distinguished: it consisted of species such as *Acartia clausi* (Giesbrecht, 1889), *Centropages typicus* (Kröyer, 1849), *Temora stylifera* (Dana, 1849), *Mesocalanus tenuicornis* (Dana, 1849), *Paracalanus parvus* (Claus, 1863), *Clausocalanus jobei* (Frost & Fleminger, 1968), *Clausocalanus lividus* (Frost & Fleminger, 1968) and *Oncaea mediterranea* (Claus, 1863). However, some species were observed in only one campaign such as *Eucalanus* sp. (May 2007), *Euchaeta spinosa* (Giesbrecht, 1892) (November 2007), *Candacia armata* (Boeck, 1872) (February 2008) and *Diaixis pigmaea* (T. Scott, 1896) (April 2008).

Interestingly, the diversity index of Shannon-Wiener (H') and species richness (Fig. 3 left panels) showed similar temporal trends in all stations. Both diversity estimates showed a decrease in May 2007 and July 2007

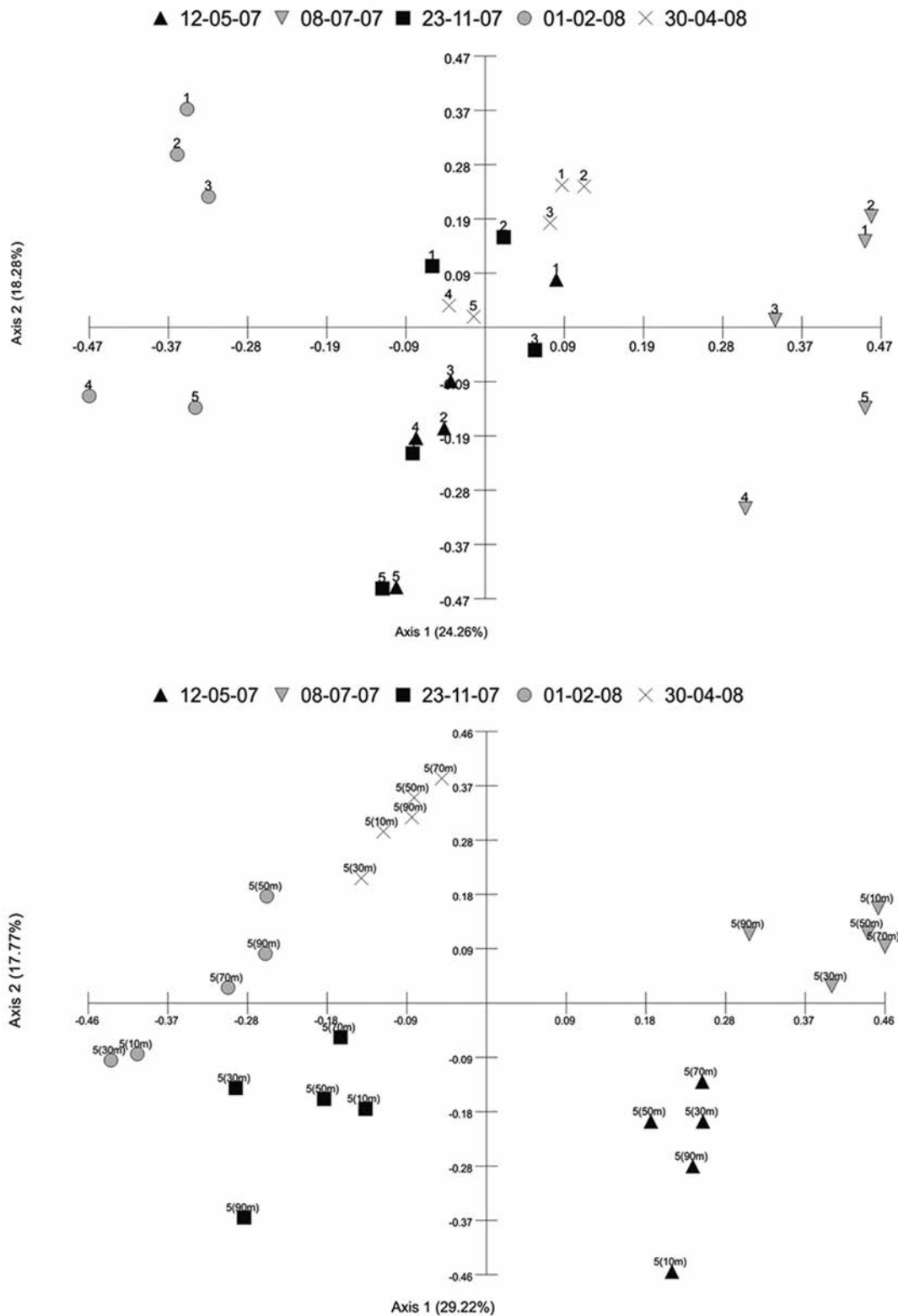
**Table 1b.** Environmental conditions observed along the vertical (0-100 m) for station 5 during the 5 sampling periods. Transp: Transparency; Clado: Cladocoeurans; Hydro: Hydromedusae; Sipho: Siphonophores; Chaeto: Chaetognaths; Appendi: Appendicularians; Dolio: Doliolids; Copep: Copepods. These data were used for the multivariate analysis (CCA). Discrete data of hydrological parameters have been averaged.

Date	Depth m	T°C	Salinity	Transp	NO <sub>2</sub> +NO <sub>3</sub> µM	PO <sub>4</sub> µM	Chla mg.m <sup>-3</sup>	Clado Ind.m <sup>-3</sup>	Hydro Ind.m <sup>-3</sup>	Sipho Ind.m <sup>-3</sup>	Chaeto Ind.m <sup>-3</sup>	Appendi Ind.m <sup>-3</sup>	Dolio Ind.m <sup>-3</sup>	Copep Ind.m <sup>-3</sup>
5/12/07	0-20	18.2	36.5	18	0.91	0.46	2.136	27.22	0.63	4.01	19.62	140.3	20.46	519.08
	20-40	18.4	36.3	18	0.65	0.15	1.602	9.39	0.21	0	1.55	25.11	6.33	326.72
	40-60	17.8	36.5	18	0.85	0.01	1.068	9.74	0.42	2.04	5.43	27.22	4.57	331.43
	60-80	17.6	36.5	18	0.89	0.36	0.534	2.6	0	0.44	4	5.72	4.5	211.12
	80-100	17.5	36.5	18	0.89	0.13	1.602	4.48	1.39	0.64	5.57	27.81	5.7	111.76
7/8/07	0-20	24.3	36.6	17.5	0.46	0.171	6.47	231.22	3.59	0.63	25.74	62.87	57.59	278.06
	20-40	24.4	36.7	17.5	0.32	0.112	2.67	198.52	1.9	0.42	0	1.79	6.54	152.23
	40-60	24.4	36.5	17.5	0.08	0.464	0.5	308.44	1.27	3.31	27.67	49.12	55.49	312.02
	60-80	24.1	37.7	17.5	0.02	0.1	0.5	329.92	3.21	0.7	0	48.2	45.48	332.60
	80-100	18	37	17.5	0.60	0.01	0.5	53.83	2.18	1.46	21.38	29.56	9.72	187.89
11/23/07	0-20	18.4	36.5	15	0.65	0.15	2.67	34.6	2.53	2.53	1.69	59.92	19.41	861.05
	20-40	18.4	35.9	15	1.50	0.77	0.89	16.46	3.8	4.64	2.11	29.96	4.64	598.83
	40-60	18.4	36.8	15	1.56	0.11	1.78	6.89	4.92	2.95	0	29.11	238.4	506.10
	60-80	18.4	36.9	15	1.37	0.711	0.89	29.2	1.41	3.76	0.06	62.29	238.14	545.37
	80-100	18.4	36.2	15	4.85	0.28	1.78	47.68	1.96	2.42	1.94	36.21	5.19	306.57
2/1/08	0-20	15.3	35.8	15	1.63	0.01	0.89	0	11.81	6.75	11.81	264.98	10.13	1398.59
	20-40	15.5	35.8	15	2.53	0.01	0.89	0.84	4.22	3.38	0.69	179.75	3.38	1147.98
	40-60	15.6	35.5	15	0.10	0.01	0.5	0.28	0.84	1.13	10.97	7.03	1.69	647.01
	60-80	15.6	35.8	15	0.10	0.01	3.56	1.13	1.12	3.95	0.76	21.87	0.92	488.60
	80-100	15.6	35.9	15	0.52	0.01	0.89	0.43	4.35	3.75	0	40.55	7.09	585.55
4/30/08	0-20	17.9	36.7	15	0.10	0.01	0.89	3.38	5.06	10.13	21.94	318.99	50.63	112.84
	20-40	18.1	36.4	15	0.10	0.01	1.78	0.84	2.53	3.38	0.42	175.53	16.88	166.79
	40-60	18.1	36.3	15	0.43	0.01	0.5	0.28	1.41	3.94	0.86	10.69	3.38	175.86
	60-80	18.3	36.9	15	0.17	0.01	0.5	0.01	1.59	2.52	9.09	58.34	2.2	141.30
	80-100	17.9	37.4	15	0.59	0.01	0.5	0.68	3.64	1.94	4.22	6.86	7.22	117.38

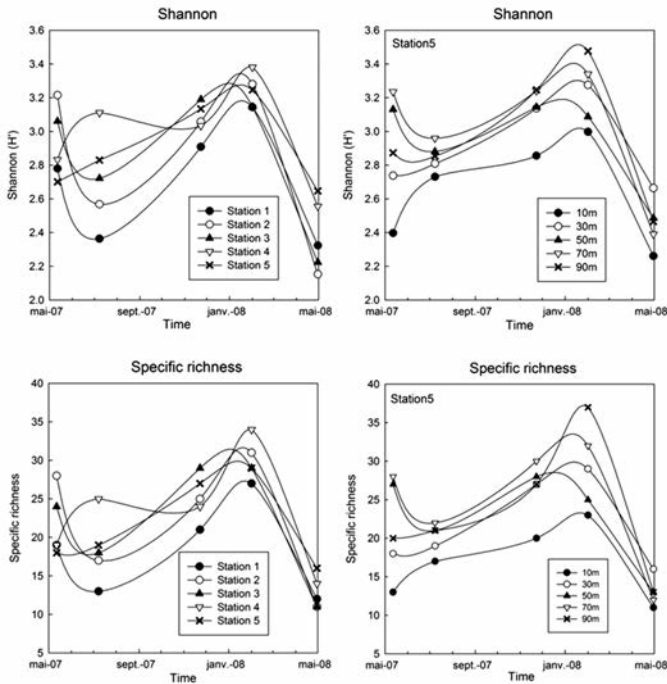
and increased to reach a maximum value in February 2008 and then decreased to reach minimum values during the month of April 2008. Minimum values were observed for station 1 in July 2007 ( $S = 13$  and  $H' = 2.36$ ) whereas maximum values were measured at station 4 in February 2008 ( $S = 34$  and  $H' = 3.38$ ). A similar temporal pattern was observed for the vertical at station 5. Both diversity estimates peaked in February 2008, maximum values were observed for the deepest samples (80-100 m,  $S = 37$  and  $H' = 3.48$ ) and minimum values were observed for all depths (except for 40-60 m and 60-80 m) in May 2007. Surface waters (0-20 m) showed minimum values during all sampling periods.

The species *Paracalanus parvus* dominated (more than  $23 \pm 2.78\%$ ) the copepod community during the study period ranging from  $14.04 \pm 3.62\%$  in February 2008 to  $30.74 \pm 4.55\%$  in April 2008, followed by *Clausocalanus lividus* in May 2007 ( $8.68 \pm 2.93\%$ ), November 2007 ( $13.37 \pm 3.28\%$ ) and February 2008 ( $12.46 \pm 3.52\%$ ). *Centropages kroyeri* (Giesbrecht, 1892) in July 2007 ( $17.36 \pm 4.88\%$ ) and *Acartia clausi* (Giesbrecht, 1889) in April 2008 ( $13.48 \pm 7.22\%$ ). For

the five campaigns, the temporal evolution of the k-dominance (data not shown) along the horizontal scale showed a consistency with the diversity indices trends. In February 2008, 80% of the community were represented by 9 to 10 species (depending on station), whereas in April 2008, only 3 to 4 species accounted for 80% of the total community. At station 5, the dominance distribution followed the same temporal trends as observed for diversity indices. In February 2008, the deepest sampling point (90 m) showed the most homogenous distribution with 80% of the total community represented by 16 species (over a total of 37) whereas during April 2008 only 4 to 6 species (depending on the water depth) accounted for 80% of the total community. Surface water was dominated by *Paracalanus parvus* and *Clausocalanus parapergens* (Frost & Fleminger, 1968) with a value up to 32% for *P. parvus* (July 2007) whereas in deep waters *Clausocalanus lividus*, *C. furcatus* (Brady, 1883) and *Centropages typicus* were the dominant species with up to 26% for *C. typicus* (80-100 m April 2008)



**Figure 2.** Principal component analysis performed with the environmental data for the 5 sampling campaigns. Upper panel: surface data (0-20 m) for the 5 stations. Lower panel: values at the different sampling depths (0-20 m, 20-40 m, 40-60 m, 60-80 m and 80-100 m) for station 5.

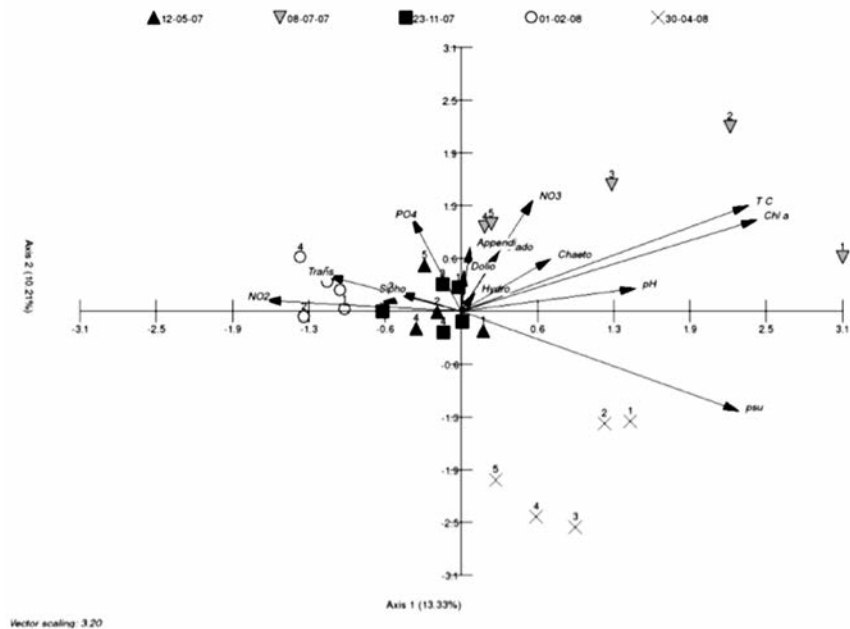


**Figure 3.** Diversity indices (specific richness and Shannon index) for the copepod community as a function of time for the 5 stations (surface data) and along the vertical for station 5.

#### *Copepod response to environmental factors*

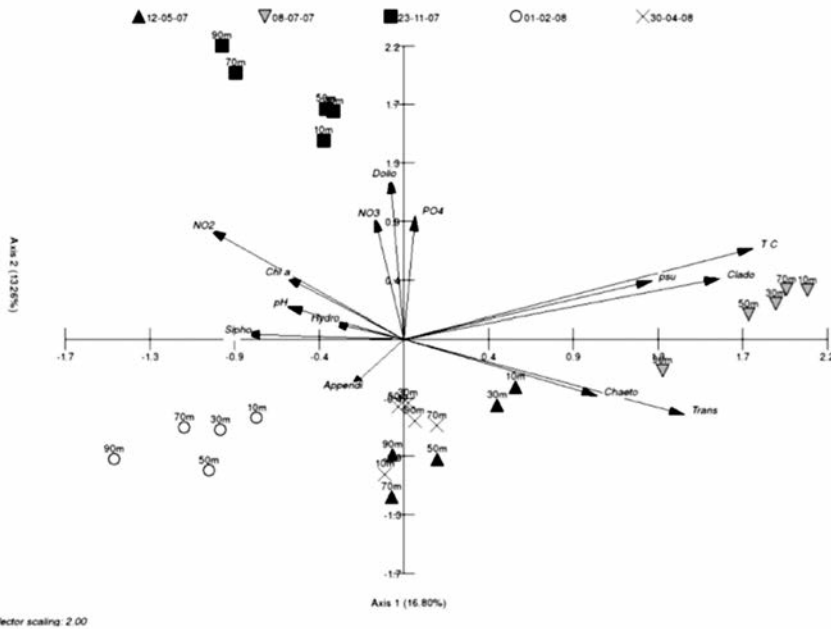
In order to assess the potential roles of abiotic and biotic factors (mesozooplankton abundances) on the structure of the copepod community, CCA was performed using the surface dataset of each sampling station (Fig. 4) and each sampling depth for station 5 (Fig. 5). CCA showed that, as observed for the environmental conditions (Fig. 2), time had a greater effect than space in structuring the copepod community. Stations were clustered as a function of sampling period. Salinity had a positive effect on the copepod community structure observed in April 2008 whereas  $T^{\circ}C$  and Chlorophyll  $a$ , nitrate concentration as well as the abundance of chaetognaths, cladocerans and appendicularians had positive effects on the community observed during July

2007. Winter community (February 2008) was positively explained by the nitrite concentration, transparency and siphonophores abundance. The roles of abiotic and biotic factors in the structuring of the copepod community were also observed with the sampling depth for the station under oceanic influence (station 5, Fig. 6). As observed along the inshore-offshore gradient, the season had a greater impact on the copepod community structure composition than the vertical gradient: sampling depths were clustered as a function of the season. Interestingly, summer (July 2007) copepod communities along the water depth were structured by the same abiotic (Temperature) and biotic (cladocerans and chaetognaths) factors as observed for the inshore-offshore gradient. Nutrients, doliolids abundances as well as Chlorophyll  $a$  concentration positively structured the fall community, whereas winter (February 2007) community structures were explained by the abundance of appendicularians.



**Figure 4.** Canonical correspondence analysis of the copepod community as a function of the sampling campaigns with the surface data for the 5 stations. Appendi: appendicularians; Chaeto: chaetognaths; Clado: cladocerans; Dolio: doliolids; Hydro: hydromedusae; Spho: siphonophores. NO<sub>2</sub>: nitrites; NO<sub>3</sub>: nitrates; PO<sub>4</sub>: phosphates; Chla: chlorophyll  $a$ ;  $T^{\circ}C$ : water temperature; Trans: transparency; pH: pH.





**Figure 5.** Canonical correspondence analysis of the copepods community as a function of the sampling campaigns with the vertical data for station 5. Appendi: appendicularians; Chaeto: chaetognaths; Clado: cladocerans; Dolio: doliolids; Hydro: hydromedusae; Siph: siphonophores. NO<sub>2</sub>: nitrites; NO<sub>3</sub>: nitrates; PO<sub>4</sub>: phosphates; Chla: chlorophyll a; T°C: water temperature; Trans: transparency; pH: pH.

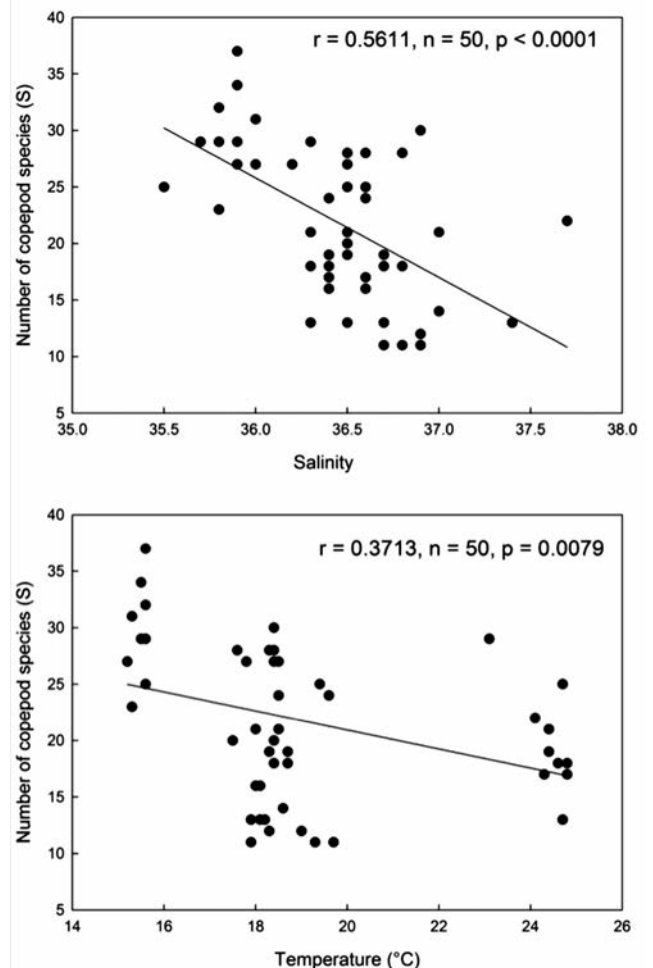
### Discussion

#### *Gulf of Tunis copepod assemblages compared to other coastal zones.*

This study showed that pelagic copepods hold a key position in the Gulf of Tunis, qualitatively with a community reaching 86 species and quantitatively as the main secondary producers (more than 70% of the total zooplankton). The copepod community showed different ecological and hydrological affinities with permanent species like *Acartia clausi*, *Centropages typicus*, *Temora stylifera*, *Mesocalanus tenuicornis*, *Paracalanus parvus*, *Clausocalanus jobei* and *Oncaea mediterranea*. The Atlantic species are regularly observed in the north Tunisian coastal plankton (Daly Yahia et al., 2004) and the community is materialized by the presence of indicator species like *Acartia danae* (Giesbrecht, 1889), *Centropages kroyeri*, *Mecynocera clausi* (Thompson, 1888) and *Mesocalanus tenuicornis*. The neritic community was the most important one in term of species richness, with species like *Acartia latisetosa* (Kricagin, 1873), *Centropages hamatus* (Lilljeborg, 1853), *Isias clavipes* (Boeck, 1864), *Labidocera wollastoni* (Lubbock, 1857) and small copepods



**Figure 6.** Relation between salinity and specific richness (S) of copepod community (upper panel) and between T°C and specific richness (S) of copepod community (lower panel). For both relationships, data from the horizontal gradient (station 1 to 5) and the vertical gradient have been pooled.



like *Nannocalanus minor* (Claus, 1863), *Clausocalanus furcatus* (Brady, 1883), *Clausocalanus jobei*, *Oncaea mediterranea*, *Coryceus speciosus* (Dana, 1849), *Oithona setigera* (Dana, 1849), *Oithona tenuis* (Rosendorn, 1917) and *Euterpina acutifrons* (Dana, 1848). An oceanic copepod community was also identified, with species like *Candacia longimana* (Claus, 1863), *Calanus helgolandicus* (Claus, 1863), *Euchirella rostrata* (Claus, 1866), *Euchaeta marina* (Prestandrea, 1833), *Copilia mediterranea* (Claus, 1863), and *Oithona plumifera* (Baird, 1843).

Compared to other Mediterranean coastal zones, the Gulf of Tunis copepod community showed a high specific richness. In the Bay of Tunis, Daly Yahia et al. (2004) identified 52 copepod species dominated by the family of *Acartiidae*, *Paracalanidae*, *Centropagidae*, *Oithonidae* and *Corycaeidae*, while Drira et al. (2009 & 2014) found respectively 31 and 47 copepod species in the Gulf of Gabes. Nevertheless *Acartiidae*, *Centropagidae*, *Oithonidae* and *Corycaeidae* were also the most diversified and abundant copepod families in the gulf of Gabes. Khelifi-Touhami et al. (2007) in the gulf of Annaba and El Kala (east coast of Algeria) showed that the diversity and ecological affinities of pelagic copepods in this area of the south western Mediterranean Sea was very high with a specific richness reaching 143 copepod species comprising 103 oceanic species and 20 Atlantic species, highlighting the role of the Modified Atlantic Waters on the diversity of the copepod community in this part of the Mediterranean Sea. Siokou-Frangou et al. (1996), working on the fall pelagic copepod oceanic community from the Tunisia-Sicily Strait to Cyprus showed the existence of an epipelagic (0-100 m) and a mesopelagic (100-300 m) community comprising 141 species of which 126 species were found in the Tunisia-Sicily Strait, 104 in the Ionian Sea and 95 in the Central Levantine Sea near Cyprus. The most abundant species of the epipelagic layer were *Clausocalanus furcatus* and *Oithona plumifera*. In the Bay of Villefranche-sur-Mer, Seguin (1981) showed that pelagic copepods specific richness reached 95 species dominated by common species belonging to the family of *Acartidae*, *Clausocalanidae*, *Trachydiidae*, *Temoridae*, *Centropagidae*, *Oithonidae*, *Paracalanidae*, *Oncaeidae* and *Corycaeidae*. More recently in the central part of the Ligurian Sea, during the summer-fall period, Raybaud et al. (2008) found that the oceanic pelagic copepod community comprised 60 species dominated at 90% by five genera, *Clausocalanus*, *Oithona*, *Pleuromamma*, *Calocalanus* and *Neocalanus*.

The copepod community of the Gulf of Tunis was more diversified during winter conditions (February 2008) with Shannon-Wiener diversity indexes reaching 3.48 bits/individual compared to the relative lowest values observed in July 2007 ( $H' = 2.36$ ) and April 2008 ( $H' =$

2.15). Nevertheless these values are considered to be “well-diversified species composition” for Mediterranean copepod community (Siokou-Frangou et al., 1995). Daly Yahia et al. (2004) observed the same pattern in the Bay of Tunis with the highest value in February 1995 reaching 3.83 bits.individual<sup>-1</sup>, while the lowest one occurred during summer ( $H' = 0.24$  in July 1995). Similarly, in the Ionian Sea, Siokou-Frangou et al. (1995) showed that in the Saronikos Gulf (Metopi station) the maximum Shannon-Wiener diversity was observed in winter (February 1985) and fall season (December 1984 and November 1985), while the minimum values occurred in June, July and September 1984 and 1985. In the Ligurian Sea, Raybaud et al. (2008) showed that the maximum Shannon-Wiener diversity of copepods was reached during the night sampling on September 20<sup>th</sup>, 2004 ( $H' = 2.9$ ) and on October 5<sup>th</sup>, 2004 ( $H' = 3.0$ ).

#### *Influence of the seasons on the local copepod assemblages.*

PCA with hydrological parameters (Fig. 2) clearly indicated that the influence of time was greater than space in shaping the environmental conditions of the water column sampled for the analysis of the copepod communities. Interestingly, the same observation could be made along the vertical for the most oceanic station where the different sampled depths were grouped as a function of the sampling time. This clearly indicated a relative homogeneity of the water masses on a sampling occasion for both the vertical and the horizontal horizon even though coastal stations (stations 1 & 2) in July 2007 and February 2008 exhibited hydrologic conditions slightly different than the oceanic stations. A similar clustering was observed for the copepod communities (Figs 4 & 5) along the horizontal and the vertical, suggesting that the hydrological parameters measured can be used to explain the distribution of copepods along an inshore-offshore gradient and along the water column for the most oceanic station. The results showed that the copepod community was strongly influenced by the season and to a lesser extent by the inshore-off shore gradient. Similarly, in the Gulf of Gabes, Drira et al. (2013) observed an important role of season in the zooplankton abundance in relation with the stratification of the water column controlled by changes in temperature and salinity. The greater role of time over space in shaping the copepod community has also been observed in the Pacific region where the El Nino event is a key parameter for the distribution of the zooplankton community (Keister & Peterson, 2003). In contrast, in estuaries where a strong environmental gradient can be observed, the role of time was weaker than space; community patterns observed along the horizontal hydrological gradient were consistent throughout the seasons (Park & Marshall, 2000). The Gulf of Tunis is

subject to high anthropic pressure which results in contamination by organic pollutants (Mzoughi et al., 2010; Mzoughi & Chouba, 2011) and heavy metals (Ennouri et al., 2010) along an inshore-offshore gradient; the stations on the bottom of the bay (nearby Tunis city) were the most contaminated. Within the Bay of Tunis, this anthropic pressure results in a spatial organization of the benthic communities (Afli et al., 2008a & b) as well as the pelagic communities (Souissi et al., 2000; Daly Yahia et al., 2004; Yahia-Kefi et al., 2005), suggesting the important role of pollutants in structuring the communities in this ecosystem. The weak role of space relative to time observed in the present study suggests that the sampled water masses and their inhabiting communities are probably more influenced by oceanic waters (i.e. the Main Atlantic Water, MAW) than by local coastal waters from the Gulf of Tunis as suggested by the hydrodynamic circulation observed in the sampling area showing a clear influence of the season (Ciappa, 2009; Ferjani & Gana, 2010).

*Role of abiotic and biotic factors on the copepod community structure.*

The similarities observed between the hydrological parameter clustering and the copepod community structure distribution clearly indicated that the abiotic factors measured can potentially be used in order to explain the distribution and structuration of the copepod community. CCA (Figs 4 & 5) suggested that despite the low range observed (35.5-37.6), salinity was an important structural factor along the inshore-offshore gradient for surface waters but also along the depth of the station under the oceanic influence (station 5). The role of salinity in shaping aquatic community structure is well known. For bacterial communities, salinity has been identified at the global scale as the major determinant rather than extremes of temperature or pH (Lozupone & Knight, 2007). On the regional scale where salinity gradients are well marked, in the Baltic Sea (Mollmann et al., 2000) or in estuaries (Soetaert & Vanrijswijk, 1993; Mouny & Dauvin, 2002), the role of salinity as a key factor for the copepod distribution has been clearly identified. Temperature has also been identified as a key component in the copepod community distribution in agreement with the temporal changes observed for the season with a dry and warm period in summer and relatively cold and wet period in winter. The role of temperature in shaping copepod community structure is not really new since it has been observed in different oceanic regions: the Atlantic (Beaugrand et al., 2002), the Pacific (Peterson & Keister, 2003) as well as in the north part of the Mediterranean basin (Siokou-Frangou et al., 1998). Interestingly, negative correlations between salinity and copepod diversity (number of species) and between temperature and copepod diversity were observed considering the whole dataset (Fig. 6).

Although such relationships must be interpreted with caution due to the relatively small dataset gained from a one year survey, nevertheless this decrease of diversity should be viewed in the perspective of the salinity and temperature increase observed in the Mediterranean basin since the early 60's due to freshwater input and climate changes (Bethoux & Gentili, 1996 & 1999).

Chlorophyll *a* has also been identified as a structural factor of the copepod community especially for the populations observed in July 2007. The summer community was dominated by *Centropages kroyeri* (23% on average), and by *Paracalanus aculeatus* (Giesbrecht, 1888) (25% on average); both species are herbivorous. Summer conditions leading to the appearance of a phytoplankton bloom (up to 9.2 µg.L<sup>-1</sup> for station 1) are optimal for the dominance of herbivorous species. A correlation between phytoplankton bloom and copepod has already been observed in the Mediterranean sea (Siokou-Frangou et al., 1998) but also along a latitudinal gradient from 86.5°N to 46.5°S (Rombouts et al., 2010). Interestingly, as observed by Rombouts et al. (2010), high copepod diversity were concomitant with low Chlorophyll *a* content. Correspondence analysis indicated that biotic factors such as abundance of other zooplankton groups can also partly explain the copepod composition as a function of seasons. Cladocerans positively structured the copepod community along the depth (station 5) whereas along the inshore-offshore gradient the role of chaetognathes and siphonophores was more obvious. Therefore, copepod distribution was influenced both by competition with other herbivorous (cladocerans) but also by top down control by carnivorous organisms (chaetognathes and siphonophores). The top down control was nevertheless season dependant, with a dominance of chaetognathes in summer and siphonophores in winter. However, while the role of biotic factors was obvious in shaping the copepod community structure, this role has to be taken into account with the abiotic factors since the latter might also influence the presence of prey (phytoplankton), competitors (herbivore) or predators (Arnott & Vanni, 1993).

## Conclusion

This study revealed that copepod community structure in the Gulf of Tunis was influenced by abiotic and biotic factors. Nevertheless this influence was season dependant with temperature, salinity, cladocerans and chaetognathes as the main structuring factors in summer, whereas nutrients and doliolids positively structured the community during fall. In winter, water turbidity, appendicularians and siphonophores were the main structuring factors while the contrasted environmental conditions observed in spring did not allow for discriminating the main structuring factors.



The community was dominated by calanoid copepods (50% on average). These species could be used as environmental bioindicator models for future ecological and ecotoxicological studies considering the strong anthropic pressure in this area.

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Table 2b. Following

DATE	Station 5																
	12/05/07		08/07/07		23/11/07		01/02/08		30/04/08								
DEPTH (m)	0-20	20-40	40-60	60-80	80-100	0-20	20-40	40-60	60-80	80-100	0-20	20-40	40-60	60-80	80-100		
<i>Eucalanus monachus</i> <sup>c</sup> Giesbrecht, 1888																1.0	
<i>Eucalanus</i> sp																	
<i>Euchaeta acuta</i> <sup>c</sup> Giesbrecht, 1892																	1.0
<i>Euchaeta hebes</i> <sup>c</sup> Giesbrecht, 1888																	0.9
<i>Euchaeta marina</i> <sup>c</sup> (Prestandrea, 1833)																	1.1
<i>Euchaeta spinosa</i> <sup>d</sup> Giesbrecht, 1892																	1.0
<i>Euchirella rostrata</i> <sup>c</sup> (Claus, 1866)																	1.0
<i>Euterpina acutifrons</i> <sup>b</sup> (Dana, 1848)																	1.0
<i>Farranula rostrata</i> <sup>c</sup> (Claus, 1863)	4.5	5.0	3.7	6.9	4.1	1.9	2.3	3.5	3.8	1.8	7.3	5.3	5.1	1.4	4.0	2.6	3.2
<i>Heterorhabdus papilliger</i> Claus, 1863										1.8							
<i>Isias clavipes</i> <sup>b</sup> Boeck, 1864			0.5	0.8		1.9	2.3	3.5	3.8				0.2	0.2			
<i>Labidocera wollastoni</i> <sup>d</sup> (Lubbock, 1857)																	
<i>Labidocera brunescens</i> <sup>b</sup> (Czerniawski, 1868)																	
<i>Labidocera koyeri</i> <sup>d</sup> (Brady, 1883)																	
<i>Lucicutia flavicornis</i> <sup>d</sup> (Claus, 1863)			1.6	2.5													
<i>Mecynocera clausi</i> <sup>a</sup> Thompson, 1888			0.5	0.8													
<i>Mesocalanus tenuicornis</i> <sup>a</sup> (Dana, 1849)																	
<i>Nannocalanus minor</i> <sup>b</sup> (Claus, 1863)			7.8	7.2	2.2	2.6											
<i>Oithona decipiens</i> <sup>d</sup> Farran, 1913			1.1	1.9	5.3	1.7											
<i>Oithona plumifera</i> <sup>c</sup> Baird, 1843																	
<i>Oithona setigera</i> <sup>b</sup> (Dana, 1849)																	
<i>Oithona similis</i> <sup>d</sup> Claus, 1866																	
<i>Oithona tenuis</i> <sup>a</sup> Rosendorn, 1917			2.2	1.6	4.8	4.4	1.9	1.2	3.1	0.9	5.5	1.0	1.2				
<i>Oncaea confiera</i> <sup>d</sup> Giesbrecht, 1891			1.1	1.1	1.8	4.8	5.2	1.7									
<i>Oncaea media</i> <sup>b</sup> Giesbrecht, 1891																	
<i>Oncaea mediterranea</i> <sup>b</sup> (Claus, 1863)			1.1	1.8	3.2	5.1	1.9	4.6	0.6	2.1	1.9	1.0	1.2	2.7	4.8	7.6	4.4
<i>Oncaea minuta</i> <sup>d</sup> Giesbrecht, 1892			1.1	1.8	3.2	1.8	2.4	2.9	0.9	0.8	0.1	4.2	0.8	5.1	7.2	6.7	3.1
<i>Oncaea subtilis</i> <sup>c</sup> Giesbrecht, 1892																	
<i>Paracalanus aculeatus</i> <sup>d</sup> Giesbrecht, 1888			1.1	0.3	0.9	21.9	31.8	18.9	11.1	7.6	1.0	0.6	2.2	1.6	2.4		
<i>Paracalanus denudatus</i> <sup>b</sup> Sewell, 1929																	
<i>Paracalanus nanus</i> <sup>a</sup> Sars, 1907																	
<i>Paracalanus parvus</i> <sup>d</sup> (Claus, 1863)																	
<i>Pleuromma abdominale</i> <sup>c</sup> (Lubbock, 1856)																	
<i>Pleuromma gracilis</i> <sup>c</sup> (Claus, 1863)																	
<i>Pleuromma xiphias</i> <sup>a</sup> (Giesbrecht, 1889)																	
<i>Pontella</i> sp. <sup>d</sup>																	
<i>Pontella brevicornis</i> <sup>d</sup> (Lubbock, 1857)																	
<i>Pontella mediterranea</i> <sup>b</sup> (Claus, 1863)																	
<i>Sapphirina sinuicoda</i> <sup>d</sup> Brady, 1883																	
<i>Scolecithrix danae</i> <sup>c</sup> (Lubbock, 1856)																	
<i>Temora stylifera</i> <sup>a</sup> Dana, 1849																	
<i>Xanthocalanus mixtus</i> <sup>c</sup> Sars, 1920																	
<i>Xanthocalanus</i> sp																	
<b>TOTAL</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>