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Contribution to the ecological study of *epigean Cladocera* and *Copepoda (Cyclopoida)* from groundwater in Northern Tunisia

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Abstract

The present study is a contribution to the biogeography and ecology of cladocera and copepoda Cyclopoida, from groundwater in Bizerte area (Northern Tunisia) throughout an annual cycle *(*November 2006 - October 2007). Eleven taxa of Crustacean were sampled and identified: four cladocera (*Ceriodaphnia reticulata*, *Ceriodaphnia laticaudata*, *Daphnia magna* and *Daphnia similis*) and seven copepoda cyclopoida (*Cyclops abyssorum mauritaniae*, *Eucyclops lilljeborgii*, *Thermocyclops dybowskii*, *Acanthocyclops rhenanus, Eucyclops macruroides*, *Macrocyclops albidus* and *Diacyclops* sp). *C. abyssorum mauritaniae* was sampled for the first time in Tunisia, in Rimel's well near the Rimel Sea in Bizerte. The relationships between taxa and the measured environmental and spatial variables (Temperature, Salinity, Conductivity, Turbidity, pH and Depth) were examined using both unconstrained (Principal Components Analysis, PCA) and constrained (MDS) ordination techniques. The MDS ordination according to biotic and physico-chemical parameters allowed grouping stations in four distinct habitats according to the ecological preferences of its species. *D. magna*, *D. similis* and *C. abyssorum mauritaniae* seems tolerate waters with high salinity and turbidity. *C. reticulata* colonize biotopes characterized by neutral pH and a low water temperature. In contrast, *M. albidus* shows a relatively tolerance to warmer water and *E. macriroides* colonizes deep ecosystems characterized by a low conductivity.

Keywords: biogeography; cladocera; copepoda cyclopoida; ecology; groundwater

1. Introduction

Groundwater are inhabited by species that are accidentally or passively transported to caves and wells and which not occur at the surface at all, or are only rarely thrown up by springs. This reserve was continuously supplying by underlying water sheet and temporarily from rainfall that has entered the earth by precipitation. Groundwater has a critical role as a source of irrigation and industrial purposes [13] but is also a vital part of the ecosystems that support wetlands and some animal's species. Hydraulic exchange occurs at various spatial and temporal scales [9], the infiltration of stream water during spates promotes faunal exchange, so upwelling of hyporheic water and downwelling of stream water are the mechanisms that enhance biological exchange between surface and groundwater [14]. The biological exchanges make this hydrosystem as an important biological reservoir of stygobite species such as crustacean zooplankton [3, 10, 19]. These ecosystems represent a real refuge [18] for many freshwater species where it's accomplishing a part or a total of their life cycle [8]. In Mediterranean basin, several researches were interested to cyclopoids and calanoids copepoda in groundwater [2, 26, 27, 28]. In Tunisia, many studies were interested to superficial freshwater zooplankton and allowed to establish complete inventories of the temporary pools and ponds of Algeria [30, 31] and North Tunisia [22]. Only some researches were interest to zooplankton in groundwater [33, 34, 35]. The fragmentary studies concerning taxonomy, biogeography and ecological aspects of the zooplankton in Tunisian groundwater, incited us to enhance this study to identify zooplankton colonizing these biotopes and determining the interactions between the various environmental parameters measured and species collected.

2. Materials and methods

2.1. Study area

Samples were carried out from November 2006 to October 2007 in six wells located in Bizerte region (Fig. 1). The wells prospected surrounding the most important hydrological system of Ichkeul Lake - Bizerta lagoon, they were chosen because of their diversity (Coleoptera, Ephemeroptera, Gasteropoda...) [33] and their accessibility.

During the study period the waters sampled are relatively fresh (T°C 20 °C ± 2), neutral to alkaline (7.38 < pH < 7.65), clear (15.33 < Tur < 106.41 NTU) and was qualified with excessive mineralization according to the classification established by [23] (CE >1300 µS/cm at 20°C). Rimel's well (ST1), near the Sea, shows a high degree of salinity (S = 3.14 psu), than the other stations (S < 1.07psu) (Tab. 1).

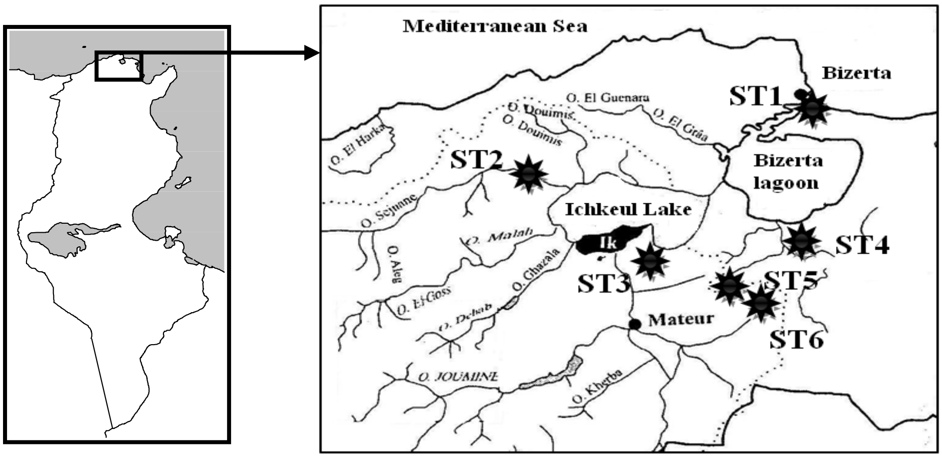


Fig. 1. Location of the six sampling wells sites in Bizerte region (Northern Tunisia) from November 2006 to October 2007. ST1: Well of Rimel; ST2: Well of North Ichkeul; ST3: Well of Essouden; ST4: Well of Gabsi; ST5: Ain Smara; ST6: Well of Ain Smara

Table 1

Geographical coordinates and mean values (± SE) of principle physicochemical parameters of waters from the six wells sampled in Bizerte region from November 2006 to October 2007. A: altitude (m a.s.l.); WT: water temperature (°C); S: salinity (PSU); C: Conductivity (µS/cm at 20°C); T: turbidity (NTU); D: Depth (m).

Sampling sites	GPS coordinates N	E	A	WT	pH	S	C	T
ST1	37°15.361	009°40.059	5	20,5 ±6.00	7,65 ±0.17	3,14 ±0.43	4202 ±638.71	96 ±91.42
ST2	37° 2.329	009°40.059	28	18,3 ±3.9	7,62 ±0.19	0,85 ±0.06	2027 ±100.59	17,58 ±9.31
ST3	37°05.450	009°46'.682''	94	19,5 ±4.3	7,51 ±0.12	0,58 ±0.04	1445 ±51.44	15,33 ±11.94
ST4	37°06.315	009°51'.381''	67	20,3 ±5.09	7,46 ±0.05	0,48 ±0.03	1316 ±89.74	27,41 ±13.96
ST5	37°03.046	009°49'.645''	88	21,2 ±4.95	7,38 ±0.13	1,07 ±0.09	2391 ±180.08	106,41 ±62.79
ST6	37°03.057	009°49'.638''	88	21,2 ±4.87	7,55 ±0.10	0,81 ±0.05	1996 ±304.36	46,75 ±24.29

2.2. sampling, conservation and methods:

In each sampling site, physico-chemical parameters were measured with each correspondent materiel. At each biotope the zooplankton was collected along the water column of the well, with a cylindrical net (30 cm in diameter) provided by a collector (85µm mesh size). The four replicate collected from the well were pooled and preserved in 5% formalin. The organisms were sorted from representative subsamples in the laboratory, examined and identified under a stereomicroscope (Leica MZ 16) and a binocular microscope (Leica DME). All taxa were identified according to [21] and [1] for Cladocera and [11] for cyclopoid copepoda.

For constrained MDS ordination methods (*non-metric multidimensional scaling*), we incorporated into our analysis several environmental variables considered to be potentially important in structuring zooplankton communities. The data are transformed with log (x+1) and realized by the software PRIMER 6 (*Plymouth Routines In Multivariate Ecological Research*) [6]. We used PCA, an unconstrained ordination method, to summarize the distribution of zooplankton taxa across our study sites and examine its tolerance to environmental parameters with the software of STATBOX.

3. Results

3.1. Taxonomic data

Eleven taxa were identified (Tab. 2), four species of cladocera and seven cyclopoids copepoda.

Table 2

Species collected in six wells of groundwater in Bizerte region (Northern Tunisia) from November 2006 to October 2007.

Species	Sites	Total Numbers (ind-m-^{3})
Cladocera		
Ceriodaphnia reticulata (Jurine 1820)	W1, W2, W3, W4, W5 et W6	9969
Ceriodaphnia laticaudata (Müller 1867*)*	W2	234
Daphnia magna (Straus 1820)	W1	934
Daphnia similis (Claus 1876)	W1	91
Copepods		
Cyclops abyssorum mauritaniae (Lindberg 1950)	W1	12575
Thermocyclops dybowskii (Lande1890)	W6	13
Acanthocyclops rhenanus (Kiefer 1936)	W2	872
Eucyclops macruroides (Lilljeborg 1901)	W4	12071
Eucyclops lilljeborgi (Sars 1914)	W6	523
Macrocyclops albidus (Jurine 1820)	W5	761
Diacyclops sp (Kiefer 1927)	W3	18

C. abyssorum mauritaniae (Lindberg, 1950) is sampled for the first time in Tunisia (Fig. 2). In the North African area, this species was only sampled in Lakes of the Moroccan middle Atlas [1, 22] and in several aquatic ecosystems in the north of Algeria [15]. This cosmopolitan species is typically of freshwater [29]. *C. abyssorum mauritaniae* was sampled only in the well of Rimel (ST1). Specimens captured have a relatively big size (female: 1.6-2.2 mm ; male : 1.4-1.6 mm) (Figs 2-A), with transparent or red body, first antenna with 17 articles exceeding the half of the second thoracic segment. The fifth thoracic segment developed well laterally with points. The terminal article of the fifth thoracic leg carries only two silks or spines. The spine of the second article is short and insert in the middle of the internal edge, it's very developed, but shorter than the article which carries it (Figs 2-B).

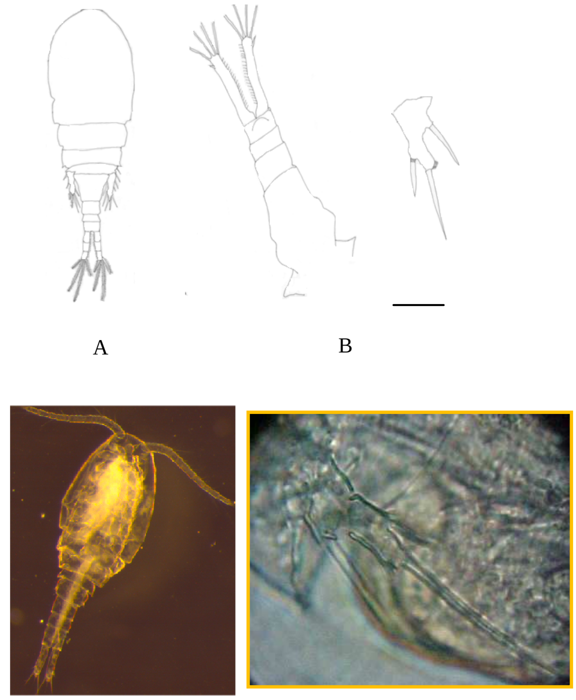


Fig. 2. *Cyclops abyssorum mauritaniae* sampled in Rimel well from November 2006 to October 2007, A: general habitus of copepodite 5, B: The fifth pair of thoracic leg.

3.2. density and diversity of zooplankton communities:

From November 2006 to October 2007, zooplankton populations are generally low in winter (271.37 ind m-^{3}), increase in spring (1050.39 ind m-^{3}) and decline in autumn (362.77 ind m-^{3}) (Fig. 3).

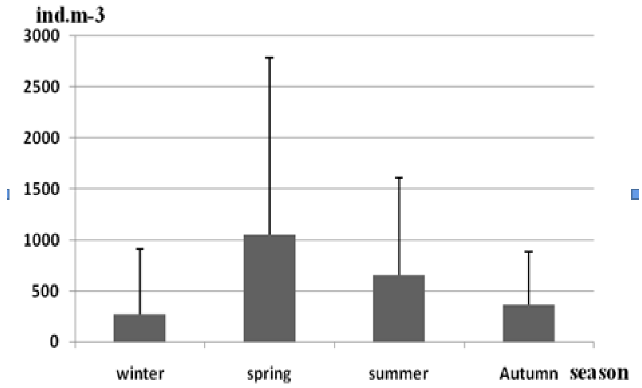
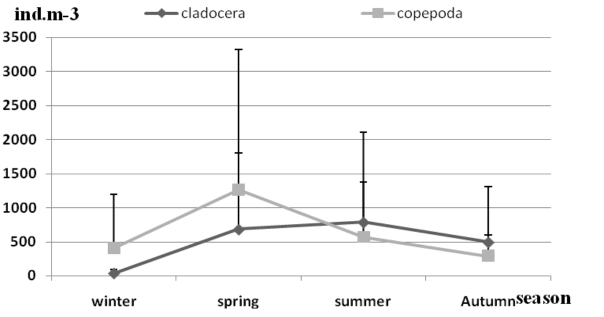


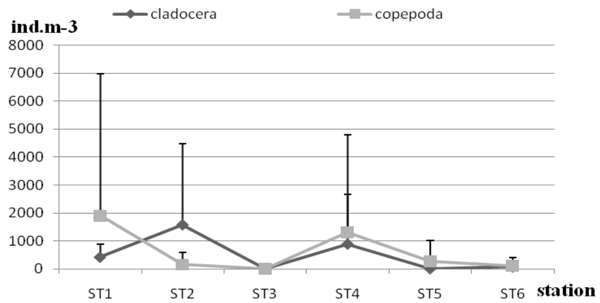
Fig. 3. Seasonal fluctuation of mean densities (± SE) of the total community of zooplankton collected in Bizerte region from November 2006 to October 2007.

The cladoceran species densities varied from 10.22 ind.m-^{3} (well of Essouden) to 1571.98 ind.m-^{3} (well of north Ichkeul) for the zones evaluated (Fig. 4a) and from

33.84 ind.m-^{3} (winter) to 785.12 ind.m-^{3} (summer) throughout the year (Fig. 4b). However copepods varied from 4.41 ind m-^{3} (well of Essouden) to 1912.42 ind m-^{3} (well of Rimel) for the zones evaluated (Fig. 4a) and from 289.60 ind m-^{3} (autumn) to 1252.16 ind m-^{3} (spring) throughout the year (Fig. 4b).



A



B

Fig. 4. temporal (A) and spatial (B) fluctuations of mean densities (± SE) of Cladocera and Copepods in Bizerte region from November 2006 to October 2007.

The spatial diversity shows a large fluctuation in the several prospected sites (Fig 5). The north Ichkeul well (ST2) is colonized by three species, *C. reticulata* (Pi = 79.82%), *C. laticaudata* (Pi = 5.07 %) and *A. rhenanus* (Pi = 15 %) (Fig 6). The well is the most diversified (H' = 0.56 bits) and equilibrated (J' = 0.69). The Gabsi well (ST4), is colonized by *C. reticulata* (Pi = 27.80%) and *E. macruroides* (72.17%), this biotope is less diversified (H' = 0.41bits) and equilibrated (J' = 0.59) than the last one. The species shows an unequal distribution in their biotopes, but the wells become most diversified and equilibrated probably because of equal division of food resources between the diverse species colonizing these hydrosystems.

The well of Rimel, regroups *C. reticulata* (Pi = 3.55 %), *D. magna* (Pi = 6.92%), *D. similis* (Pi = 0.74%) and *C. abyssorum mauritanea* (Pi = 88.77%). This well is less diversified (H'= 0.40 bits) and less equilibrated (J'=0.45) than the two previous biotopes. This less diversity can be explained by the high abundance and dominance of *Thermocyclops dybowskii* in the well.

Essouden (ST3) and well of Ain Smara (ST6) show also a less diversity (respectively H' = 0.13 and 0.14 bits) (respectively J' = 0.19 and 0.16) due to the dominance and abundance of *C. reticulata* (Pi = 56.94 ind.m^{-3}) in Essouden and *E. lilljeborgii* (Pi = 66.49 ind.m^{-3}) in the well of Ain Smara.

Ain Smara (ST5) is colonized by two species, *M. albidus* and *C. reticulata*, the abundance and dominance of *M. albidus* (Pi = 97.15 %) explain the very low diversity (H' = 0.075 bits, J' = 0.10) registered in this well.

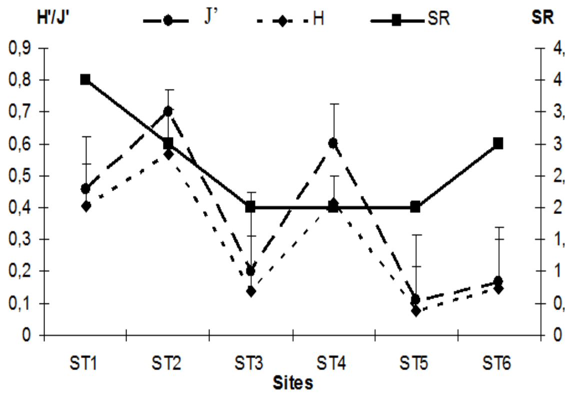


Fig. 5. Spatial fluctuation of diversity index (H', J') and species richness (SR) in sampling ground water in Bizerte region from November 2006 to October 2007.

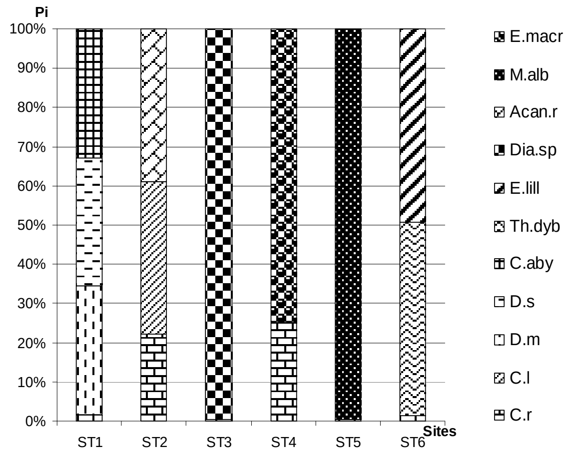


Fig. 6. Spatial variation of abundance in Bizerte region ground waters from November 2006 to October 2007

C.r: *Ceriodaphnia reticulata*,C.l: *C. laticaudata*,D.m: *Daphnia magna*, D.s: *D. similis*, M..alb: *Macrocyclops albidus*, C.aby: *Cyclops abyssorum mauritaniae* E.lill: *Eucyclops lilljeborgii*, E.macr: *E. macriroides*, Th.dy: *Thermocyclops dybowskii*, Dia.sp: *Diacyclops sp*, Acan.r: *Acanthocyclops rhenanus*.

MDS ordination according to physico-chemical parameters and specific densities, with the use of Bray Curtis Similarity distance on 39%, groups the explored sites on four biotopes (Fig 7). This ordination gives a perfect representation with stress "0" (Clarke & Warwick, 2001). The first biotope (Rimel well) wish is characterized by a high mineralization (CE = 4202.7 µS/cm; S = 3.14 psu) and a high turbidity (Tur = 96 NTU) is colonized by three species *C. abyssorum mauritaniae D. magna* and *D. similis*, these species probably tolerate high mineralisation and turbidity. The second biotope regroups North ichkeul and Gabsi well, with similarity percentage of 41.98%. The high density of *C. reticulata* in the both biotope contributes at this similarity. The third biotope regroups Essouden and Ain Smara with similarity percentage of 39.62%. However, in the two previous biotopes, the low density registered of *C. reticulata*, respectively 40.89 ind.m-3 and 57.45ind.m-3, reproaches these two biotopes. The last well (ST6) forms a separate biotope because of its species characteristic (*T. dybowskii* and *E. lilljeborgi*).

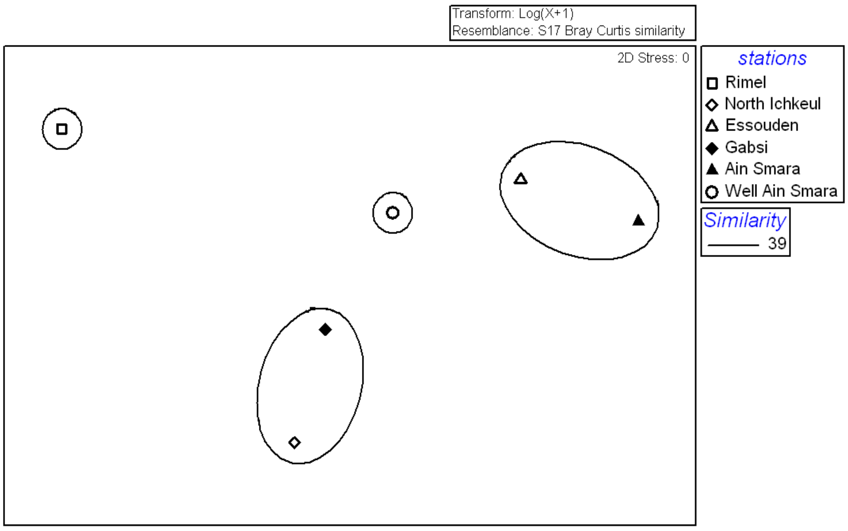


Fig. 7. MDS Ordination and site's grouping according to the similarity of BRAY-CURTIS in 39 % (November 2006-October 2007).

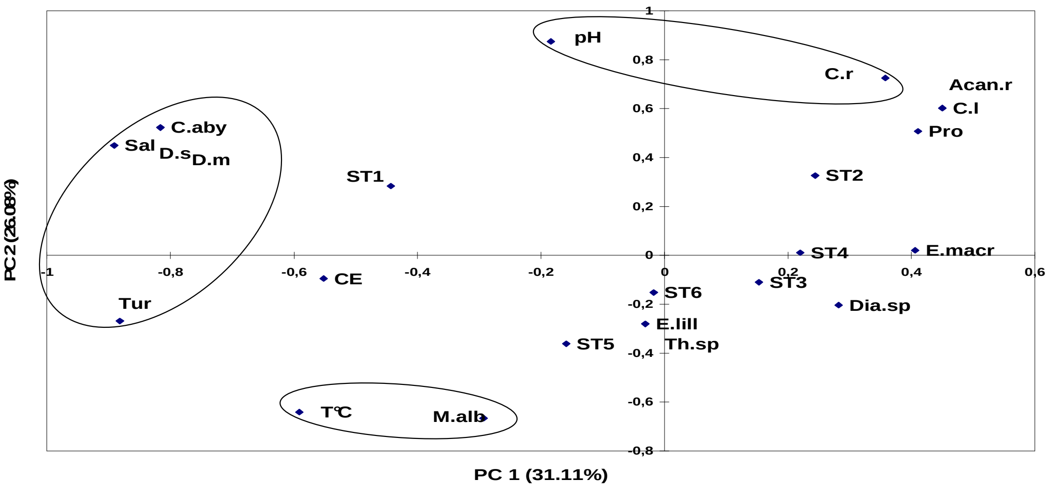


Fig. 8. Two-dimensional PCA ordination of the ground water samples of Bizerte area in the two first axes. Physical, chemical, and biological parameters are indicated as vectors.

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The PCA allowed establishing a degree of affinity between biotic and abiotic parameters (Figs 8 and 9).The first three axes with the largest eigenvalues accounted for 31.11%, 26.08 % and 16.91% of the variance in the data. When considering the variables that weighted most heavily in PC1 and PC2 axes, it can be seen that well of Rimel (ST1) which is colonized by *D. magna*, *D. similis* and *C. abyssorum mauritanea* (respectively CTR = 0.66%) is characterized by a high salinity and turbidity (CTR = 0.77%), its nearness with Sea offers such conditions. The PC1 axis defines only an increasing gradient in its negative side of salinity and turbidity, so *D.*

magna, *D. similis* and *C. abyssorum mauritaniae* of Rimel well (ST1) tolerate waters with high salinity and turbidity. The PC2 axis is defined by density of *C. reticulata* (CTR=0.76%) in North Ichkeul well (ST2), which is characterized by neutral pH (CTR = 0.76%) and a low water temperature. In contrast, *M. albidus* from Ain Smara, shows a relatively tolerance to warmer water. The PC3 axis defines one gradient in its negative side by the density of *E. macriroides* which colonizes biotopes characterized by a high depth (CTR = 0.50%) and low conductivity (CTR = 0.45%).

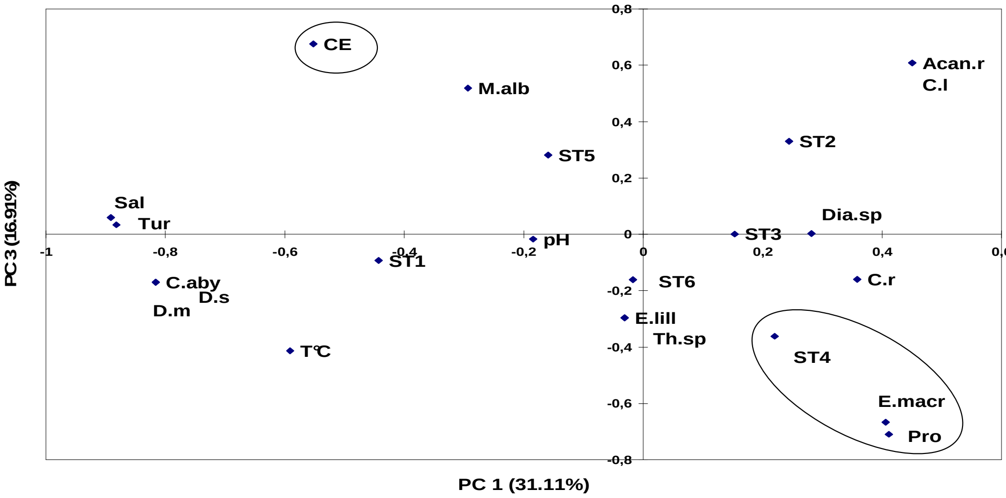

Fig. 9. Two-dimensional PCA ordination of the ground water samples of Bizerte area from November 2006 to October 2007 in the third axis

T°C: water temperature, CE: Conductivity (µS/cm at 20°C), Tur: turbidity (NTU), S: salinity (PSU), C.r: *Ceriodaphnia reticulata*, C.l: *C. laticaudata*,D.m: *Daphnia magna*, D.s: *D. similis*, M..alb: *Macrocyclops albidus*, C.aby: *Cyclops abyssorum mauritanea*, E.lill:*Eucyclops lilljeborgii*, E.macr: *E. macriroides*, Th.dy*: Thermocyclops dybowskii,* Dia.sp: *Diacyclops sp,* Acan.r: *Acanthocyclops rhenanus*

However, we haven't determine the ecological preferences of some species as *D. similis* in Essouden well (ST3), *E. lilljeborgi* and *T. dybowskii* in well of Ain Smara (ST6), *A. rhenanus* and *C. laticaudata* in North Ichkeul. This can be explained by their low density during the sampling period, or their accidental infiltration in ground waters to carry out their life cycle. So this previous species can't adopt the biotope as a real refuge.

4. Discussion

Most species of zooplankton we recorded in wells were widely distributed across the study region in superficial water [34]. But they were sampled in ground water [33] probably of their infiltration in these biotopes to carry out their life cycle [2] or to escape predation [18]. In the present study, as it have been showed by [4] in Lake Bracciano in Italy, zooplankton densities are generally low in winter (271.37 ind m-^{3}), increase in spring (1050.39 ind m-3) and decline in autumn (362.77 ind m-^{3}). So, as in superficial water, the proliferation of zooplankton in ground water coincides with the bloom of phytoplankton and decrease in winter. [25] claimed that in limnetic plankton communities, only one to three copepods and two to four cladocerans were normally predominant. In prospected wells, *C. abyssorum mauritaniae* (Lindberg, 1950) and *C. reticulata* are predominant and show the highest densities (respectively 2231.16 ind.m^{-3} and 1734.20 ind.m^{-3}), because of their proliferation throughout all the year. In limnic superficial waters [16] according the fluctuation of species richness and diversity of plankton, to many factors mainly the size of the lake, its productivity and its water quality. Some authors attribute this variation to the levels of fish's predation and the geographic location of the lake in superficial water [7, 24]. However, in groundwater species richness and diversity of the biotopes depends essentially on species migration to carry out their life cycle [2] or to escape predation [18]. Several studies in recent years have revealed that a newly established planktivorous fish species can greatly change the zooplankton populations of a lake in superficial water [18, 32]. So evidence is strong that the changes in zooplankton populations, from predominantly large species to small ones, throughout the year have been attributed to the selective predation by Fishes, Coleoptera, and Ephemeroptera in our prospected wells. Besides these explanations, it seems also that the impoverishment of Essouden and well of Ain Smara is due to their overexploitation by the habitant of this region.

Species tolerance to environmental parameters has been frequently invoked to explain also the composition, density and diversity of zooplankton. Such important environmental gradients for zooplankton composition include temperature [32] and pH [20]. In our study spatial and temporal variations of zooplankton show that conductivity, turbidity and salinity are also important parameters to explain the total variance of density and diversity of zooplankton in groundwater. Values of Shannon-Weaver index of diversity and values of Pielou evenness index ranging respectively from 0.075 to 0.56 bits and 0.10 to 0.69 and that confirm that our wells prospected is home to a zooplanktonic community characteristic of instable physicochemical environments and an unequal zooplankton distribution in each biotope. *D. magna*, *D. similis* and *C. abyssorum mauritanea* were sampled in Rimel well, near the Rimel Sea, seems tolerate biotopes with high salinity and turbidity. [11] and [12], mention that *M. albidus* proliferate or survive in warmer waters as it have been revealed in our study and *E. macriroides* proliferate in waters characterized by a high depth and low conductivity.

5. Conclusion

This study allowed us to draw up a list of 11 species of cladocera and copepoda cyclopoida collected in Tunisian groundwater. *C abyssorum mauritaniae* was signalized for the first time in Tunisia; a brief description of our species was approached. The ecological study allowed us to determine the ecological preferences of the diverse species collected towards the physico-chemical parameters recorded.

Acknowledgments

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