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Seasonal patterns of viral, microbial and planktonic communities in Sidi Salem: a freshwater reservoir (North of Tunisia)

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Abstract – We investigated the distribution and dynamics of viruses, prokaryotes and small eukaryotic phytoplankton in Sidi Salem freshwater reservoir (Northern Tunisia). Samples were collected from the deepest station at different depths throughout the water column for 2 years (February 2009 to January 2011). The reservoir was characterized by seasonal alternations of thermal stratification and homothermy. Among the different microbial communities counted using flow cytometry (FCM), picocyanobacteria constituted an important autotrophic component since they were always present and their highest concentration reached 3.02 and 2.65×10^5 cells.mL^{-1} in March 2009 and June 2010, respectively. The heterotrophic prokaryotic communities (represented mainly by bacteria) were characterized by a clear separation between two subgroups referred to as high-DNA and low-DNA content populations, and the highest concentrations of heterotrophic bacteria (*i.e.*, 3.8×10^7 cells.mL^{-1}) were recorded in spring 2009. Several viral groups referred to as virus-like particles (VLP) groups 1, 2 and 3 could also be discriminated using FCM. VLP1 and VLP2 displayed a significant correlation with the heterotrophic bacteria ($r = 0.80$ and 0.78, $P < 0.001$) but seem to be independent from picocyanobacteria and/or chlorophyll *a*, suggesting these VLPs were mainly bacteriophages. At last, the virus to prokaryotic ratio could be high, especially in summer (mean = 22, max = 487), suggesting a strong coupling between bacteria and viruses, at least at certain periods of the year.

Key words: Flow cytometry / viruses / bacteria / picocyanobacteria / freshwater reservoir

Introduction

In aquatic microbial ecology, the term picoplankton traditionally refers to all cells that fall into the size class 0.2–3 μm; that includes picocyanobacteria, heterotrophic bacteria, archaea and small eukaryotic phototrophs referred to as picoeukaryotes (Li *et al.*, 1983; Whitman *et al.*, 1998; Worden and Not, 2008; Auguet *et al.*, 2010). These heterotrophs contribute largely to the cycling of carbon and nutrients in aquatic systems (Sarmiento and Gruber, 2006) and also form an important nutrient resource for higher trophic levels (*i.e.*, the heterotrophic nanoflagellates, ciliates, metazooplankton) (Parvathi *et al.*, 2014).

Autochthonous and allochthonous factors can impact both auto- and heterotrophic organisms, affecting their distribution, structure, diversity as well as interactions among the organisms. The dynamics of picoplankton in aquatic ecosystems are not only controlled by abiotic factors (temperature, light and nutrients), but also by biotic factors such as natural death, viral lysis, predation and parasitism (Parvathi *et al.*, 2014). Viruses play an important role in regulating carbon and nutrient fluxes, food-web dynamics and microbial diversity in aquatic systems (Suttle, 2005; Jacquet *et al.*, 2010; Breitbart, 2012). The factors that influence viral abundance and dynamics in aquatic environments are complex and are found to vary with aquatic ecosystems (Clasen *et al.*, 2008). Studies have shown that the viral abundance is influenced more by the bacterial abundance in marine environments and by chlorophyll *a* concentration in nutrient-rich lakes (Pradeep Ram *et al.*, 2010). A few studies in the lacustrine environments have included the influence of grazers on the picoplankton and bacterial abundances in studying viral dynamics (Personnic *et al.*, 2009; Berdjeb *et al.*, 2011).

The expansion of urban areas associated with agricultural pressure has promoted the progressive eutrophication of surface waters worldwide, contributing to the deterioration of water quality (Ben Rejab Jenhani

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et al., 2012). Such hydrosystem dystrophy leads generally, because of excessive nutrient enrichment, to symptomatic changes such as increasing production of aquatic macrophytes, the apparition of phytoplankton blooms (among which harmful cyanobacteria), the loss of biodiversity, the modification of transparency and of water coloration (Dauta and Feuillade, 1995).

Tunisia is a Mediterranean country characterized by aridity on the major part of its territory. To this aridity is added the variability of the Mediterranean climate, with erratic and unpredictable periods of large drought and violent floods, so that water is often limited and distributed unequally in time and space (Benzarti, 2003). Facing this shortage of water, Tunisia has developed a strategy of surface water resources mobilization. Consequently, the continuous increase of artificial water systems (reservoirs) has provided a privileged investigation field for limnological research (Ben Mammou and Louati, 2007). Being the first link in the food webs, phytoplankton plays a crucial role in aquatic ecosystems. All processes related to this compartment may affect directly or indirectly the environment. Thus, a monitoring of the environmental conditions and phytoplankton assemblages has been carried out in several reservoirs from which problems of eutrophication, especially due to phosphorus, have often lead to significant shifts in phytoplankton species' composition towards bloom-forming cyanobacteria and could result in fish mortality (Mouelhi *et al.*, 2000; Turki, 2002; Ben Rejab Jenhani *et al.*, 2006; Fathalli *et al.*, 2006; El Herry *et al.*, 2008). The Sidi Salem reservoir, investigated in the context of this study, is intensively used for irrigation and provides drinking water, fish breeding and electric power supply in the Northwest of Tunisia. It is thus a reservoir of major importance for multiple purposes and its environmental survey is critical.

So far, only a few studies have been conducted in such a reservoir dealing with plankton dynamics and nothing is known about some key microorganisms such as the pico- or the virioplankton. Indeed, researches carried out so far on this temperate reservoir have been limited and mainly concerned sedimentology, fish farming and zooplankton diversity (Ben Mammou, 1998; Mouelhi *et al.*, 2000; Djemali *et al.*, 2003). To the best of our knowledge, this is thus the first study of different viral and microbial communities for this reservoir that was sampled for such a goal during two consecutive years. Furthermore, it is appearing that study can currently evaluated the vertical distribution of micro-organisms in the deepest zone of the reservoir and to assess the relationships between these communities and their chemical and/or physical environment in the semi arid region.

Materials and methods

Sampling site

The Sidi Salem dam (36°35′20″N and 9°23′45″E) is located at 8 km in the North Western part of the town of Testour. It has been built on the Medjerda stream in 1981. It is considered as one of the largest dams in Tunisia with a 4300 km^2 area, and 57 m of maximum depth (Table 1). This dam is used for irrigation; drinkable water, fish farming and electric power supply (Fig. 1). For our purpose, water samples were collected monthly from February 2009 to January 2011, using a 1-L containing Niskin bottle at different depths (0, 5, 10, 15, 20 and 25 m) from the deepest station in the reservoir.

Table 1. Morphometric and hydrologic characteristics of the studied reservoir (Meteorology Service and Agricultural Minister of Tunisia, 2011).

	Sidi Salem Reservoir
Latitude	9°23′34″N
Longitude	36°35′40″E
Construction	1981
Water stream	Medjerda stream
Surface area (ha)	4710
Maximum depth (m)	57
Volume (mm^3)	485
Water temperature (°C)	18
Annual mean precipitation (mm)	450
Annual mean inputs (mm^3)	334.3
Annual mean evaporation (mm)	1493.5
Annual mean siltation (mm^3)	6.8

Environmental parameters

Water temperature, salinity, pH and dissolved oxygen concentrations were measured using a multi-parameter probe (WTW model). Transparency was measured using the Secchi disc. Dissolved inorganic nutrient (NO_3^--N, NO_2^--N, PO_4^{3-}-P, NH_4^+-N, $Si(OH)_4$) were measured using colorimetric methods with the autoanalyseur Bran-Luebbe III. The chlorophyll *a* concentration was determined from 300 mL of water filtered on Whatman GF/C filters (0.45 µm pore size filter and 25 mm-diameter), and the quantity of pigments was determined using a fluorometric method (Welschmeyer, 1994) after a methanol extraction (Herbland *et al.*, 1985).

Biological parameters

Roughly 2 mL samples were fixed with paraformaldehyde (2% final concentration) for 30 min in the dark, flash frozen in liquid nitrogen and kept at −80 °C. Autotrophic small eukaryotes, picocyanobacteria, heterotrophic bacteria and viruses were counted using a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air cooled laser providing 15 mW at 488 nm. Before flow cytometry (FCM) analysis, samples were thawed at 37 °C and preceded immediately. For bacterial and VLP analysis, 5 µL of the sample was diluted in 495 µL of 0.02 µm filtered TE buffer (0.1 mM Tris–HCL and 1 mM EDTA, pH 8), and incubated with SYBR Green I (at a final 10^{-5} dilution of the commercial stock solution; Molecular Probes), for

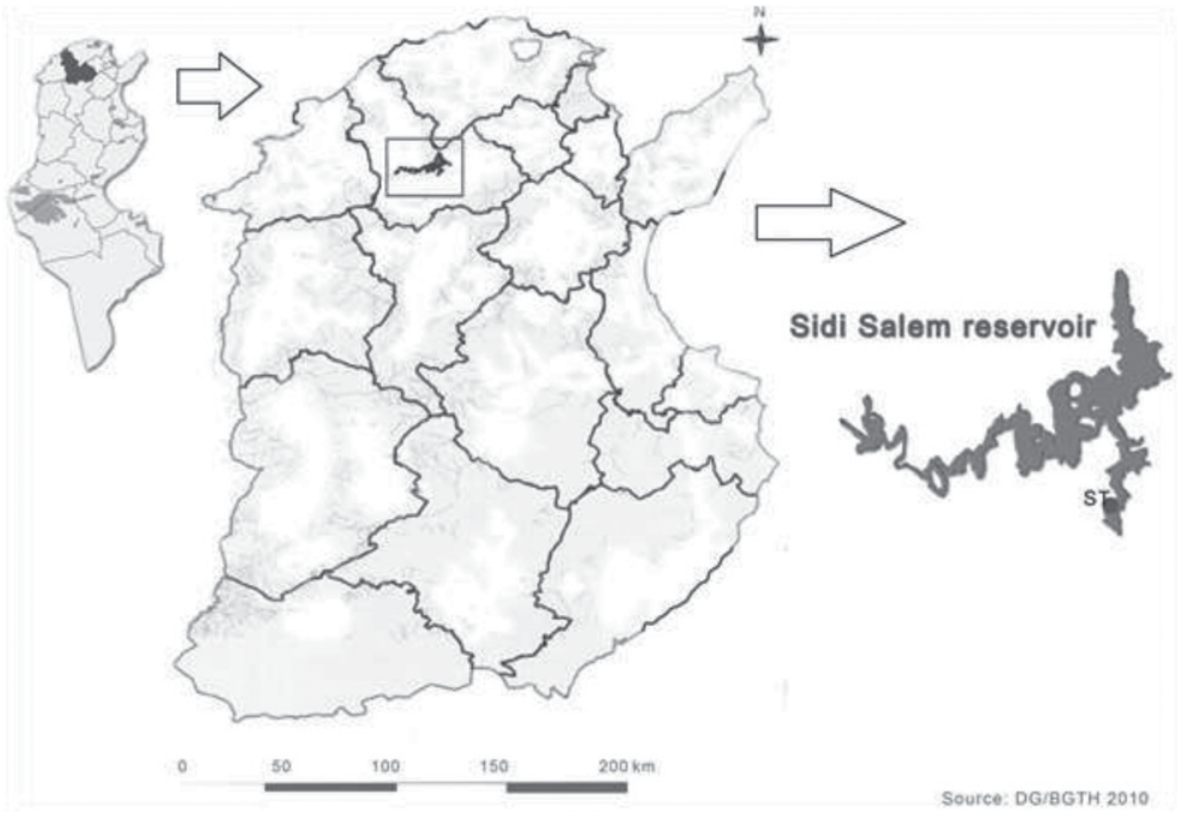

Fig. 1. Geographic location of the of Sidi Salem reservoir. ST• symbolizes the sampling station that corresponds to the deepest part of the reservoir.

5 min at ambient temperature, followed by 10 min at 75 °C, and 5 min after at room temperature, prior to FCM analysis (based on Brussaard (2004) and modified by Jacquet *et al.*, 2013). For photosynthetic cells (*i.e.*, the picocyanobacteria and small eukaryotes) no fluorochrome was added. Thus analysis was made on 1-mL fixed samples in which a suspension of 1-µm beads (molecular probes) previously diluted to be added. Two settings were used to analyse the autotrophic cells in order to discriminate all populations. Flow cytometer listmode files obtained were then transferred and analyzed on a PC using the custom-designed software CYTOWIN (Vaulot, 1989). More details about the FCM analysis and data treatment can be obtained elsewhere (Marie *et al.*, 1999, 2000).

Statistical analysis

The data recorded in this study were submitted to a normalized principal component analysis (PCA) (Dolédec and Chessel, 1989). A Pearson test performed with XL-stat was used to determine the correlations between the selected microorganisms groups and environmental variables. The data recorded for the viral, microbial and planktonic (Picocyanobacteria) communities' composition between depth and seasons were submitted to analysis of variance (ANOVA). Data were transformed in ln $(x+1)$ where necessary to meet the assumption of homogeneity of variances (homogeneity confirmed by non-significant Cochran's *C*-test). Studente–Newmane–Keuls (SNK) test was employed for *a posteriori* multiple comparisons of means between the season and depth.

Results

Hydrological characteristics

Water temperature was on average 18 ± 7.1 °C, with the highest temperatures (28 and 26.9 °C) recorded in summer (2009 and 2010) (Table 2). A thermal stratification developed during spring and summer. The spring brewing was mainly observed in May (2009 and 2010) between 10 and 15 m. A well-marked thermocline was observed between 10 and 15 m, in July 2009, while it started at 5 m depth in August 2010. Water temperature began to mix at the end of autumn and was mixed throughout the water column during winter months (Fig. 2(A)).The mean concentration of dissolved oxygen varied between 5.7 and 8.6 mg.L^{-1}, The highest values were recorded in the 0–10 m surface layer, and values decreased only slightly from summer to fall. No marked desoxygenation along the water column was recorded (Fig. 2(B)). The mean levels of salinity were between 0.8 and 1.2 g.L^{-1}, the highest level (1.1 and 1.3 g.L^{-1}) being observed in surface in summer and autumn 2010, with water level reduction and intense of evaporation (Fig. 2(C)). By contrast, the lowest values (0.9 g.L^{-1}) were

Table 2. Mean, minimum and maximum values (mean, min–max) for the biological and physicochemical variables measured in Sidi Salem reservoir in this study from February 2009 to January 2011. VLP1, VLP2: viruses abundances (particles mL^{-1}); Bact: heterotrophic bacteria abundances (cells mL^{-1}); Pico: picocyanobacteria abundances (cells.mL^{-1}); Chla: chlorophyll *a* ($\mu g.L^{-1}$); Temp: temperature (°C); Oxy: dissolved oxygen ($mg.L^{-1}$); Sal: salinity ($g.L^{-1}$), TP, $P\text{-}PO_4$, TN, $N\text{-}NO_3$, $N\text{-}NH_4$ concentrations ($\mu mol.L^{-1}$).

		VLP1 ($\times 10^7$)	VLP2 ($\times 10^6$)	Bact ($\times 10^6$)	Pico ($\times 10^5$)	Chla	Temp	Oxy	Sal	TP	PO_4	TN	NO_3	NH_4
Spring	2009	6.1 (1.2–14)	3.7 (1–8.5)	7.7 (1.2–20.2)	0.9 (0.4–1.1)	5 (4.3–5.5)	16.1 (14.3–18.1)	8.6 (8.2–8.8)	0.8 (0.7–0.9)	2.6 (2–3.2)	17 (13–23)	21 (19–24)	5.6 (3.8–7)	1.6 (1.2–2.4)
Summer	2009	2.6 (1.2–5.2)	2.2 (0.3–5)	1.9 (0.3–3)	0.3 (0.2–0.7)	6.3 (5.2–7.2)	23.9 (19.2–28.1)	5.7 (5.3–5.9)	0.8 (0.8–0.8)	3.8 (3.6–4)	34 (56–74)	27 (25–29)	7.7 (6.6–8.3)	3.5 (2.7–4.5)
Autumn	2009	2.2 (1.1–3.2)	1.7 (0.9–2.9)	1.8 (1.4–2)	0.5 (0.2–0.9)	4.3 (3.8–4.8)	19.1 (18–20.3)	7 (6.3–7.4)	1 (1–1.03)	3.7 (3.3–4.4)	42 (31–53)	18 (17–20)	3.6 (3.2–4.5)	1.6 (2.5–8.6)
Winter	2010	2.3 (1.5–3.6)	1.4 (0.8–2.1)	1.9 (1.2–2.3)	1.4 (1.3–1.6)	4.4 (3.7–5.1)	12.6 (12.1–13.4)	8.1 (7.8–8.3)	0.9 (0.8–0.9)	3.5 (3.2–3.8)	29 (21–37)	22 (20–24)	3.8 (2.6–4.83)	(1.4–8)
Spring	2010	1.3 (0.8–2.2)	0.8 (0.4–1.2)	1.1 (0.7–1.3)	0.6 (0.4–0.9)	3.7 (2.9–4.2)	17.2 (15.5–19.4)	5.7 (5.4–6)	1 (1–1.1)	3.6 (2.8–4.6)	22 (14–34)	20 (20–22)	3.5 (2.9–3.7)	2.6 (2.2–3.1)
Summer	2010	1.6 (1.2–2.3)	2.1 (0.9–7)	1.3 (1–1.8)	0.9 (0.6–1.1)	4.2 (3.9–4.7)	23.3 (20.6–26.9)	6.9 (6.7–7.1)	1 (1–1.1)	1.8 (1.4–2.3)	86 (13–97)	19 (18–20)	3.8 (3.3–4.4)	2.2 (1.4–3.7)
Autumn	2010	3.1 (1.2–10.4)	2.6 (1–8.6)	3.7 (1.5–12)	0.8 (0.7–0.9)	3.5 (2.8–4)	20.4 (20.1–20.9)	6.6 (6.4–6.8)	1.3 (1.2–1.3)	2.4 (1.9–2.8)	23 (21–25)	24 (22–25)	4.5 (3.3–6.5)	1.8 (1.4–2.2)
Winter	2011	1.7 (1.4–2)	1.8 (1.3–2.2)	1.8 (1.6–2)	0.2 (0.1–0.3)	7 (5.3–10.1)	11.7 (11.3–12)	8.2 (7.7–8.8)	1.2 (1.2–1.25)	3.1 (2.1–4.2)	31 (20–69)	35 (23–50)	2.2 (1.3–3.7)	1.6 (1–1.9)

recorded in April 2009, coinciding with a previous rainy period. Transparency oscillated in a small range, between 0.75 and 3 m, the lowest value being recorded in spring and the largest in winter. During the study period, NO_3^- and TN displayed high concentrations with 3.74×10^1 and 5.06×10^1 $\mu mol.L^{-1}$, respectively in January 2011, with two peaks marked at 10 and 20 m. Along the water column, low nutrient concentrations were recorded in the epilimnion during spring and summer 2010 (Fig. 2(D) and (H)). PO_4^{3-} higher concentrations (5.65×10^1 and 6.95×10^1 $\mu mol.L^{-1}$) were observed at 10 m in spring 2009 and deeper in January 2011, while low concentrations (1.32×10^1 $\mu mol.L^{-1}$) were recorded during summer 2010 (Fig. 2(E)). NH_4^+ oscillated between 0.8 and 8.03 $\mu mol.L^{-1}$ (Fig. 2(H)). N/P was relatively high (14.63) in January 2011, along the water column and showed fluctuations closely related to the seasonal cycle, in association with rainy periods or biological assimilation. $Si(OH)_4$ ranged from 1.08 to 9.27×10^1 $\mu mol.L^{-1}$ along the water column, increasing gradually with depth. $Si(OH)_4$ patterns followed the seasonal water stratification, but a decrease occurred throughout the stratification processes. Chlorophyll *a* displayed almost identical temporal succession in the water column, with a high level (7.75 $mg.m^{-3}$) marked in January 2011 in the different layers. The minima levels were registered during the autumn with the lowest concentration (2.80 $mg.m^{-3}$) marked in 25 m (Fig. 3(A)).

Biological characteristics

Picocyanobacterial abundance ranged from 1.42×10^4 to 1.62×10^5 cells.mL^{-1} (Table 2). The highest concentrations (1.62×10^5 cells.mL^{-1}) were observed in winter 2010 along the water column, and the peaks were detected at surface and 5 m, respectively. The vertical distributions of picocyanobacteria displayed a similar profile each year, and abundances tended to decrease with depth. Concentrations of picocyanobacteria showed smaller vertical variations in November, December and January in 2009 and in 2010 than during the other months, in association with the absence of thermal stratification at these periods (Fig. 3(B)). The mean concentrations of heterotrophic bacteria reached 1.11×10^5 and 7.65×10^6 cells mL^{-1} in spring 2009 and 2010, respectively. The lowest abundance was detected in summer 2009 (3.33×10^5 cells mL^{-1}). In spring 2009; a peak (with 2.02×10^7 cells mL^{-1}) was detected at 5 m. The vertical distributions of heterotrophic bacteria showed important fluctuations during the period of study (Fig. 3(C)). This community was characterized by a clear separation between high-DNA (HDNA) and low-DNA (LDNA) content populations. LDNA and HDNA abundances ranged between 1.32×10^5 and 4.03×10^7 cells.mL^{-1} and between 5.92×10^4 and 2.13×10^7 cells.mL^{-1}, respectively. The proportion fluctuated between 4.5 and 69.4% and the highest and lowest percentages for HDNA and LDNA were observed in summer and spring 2010,

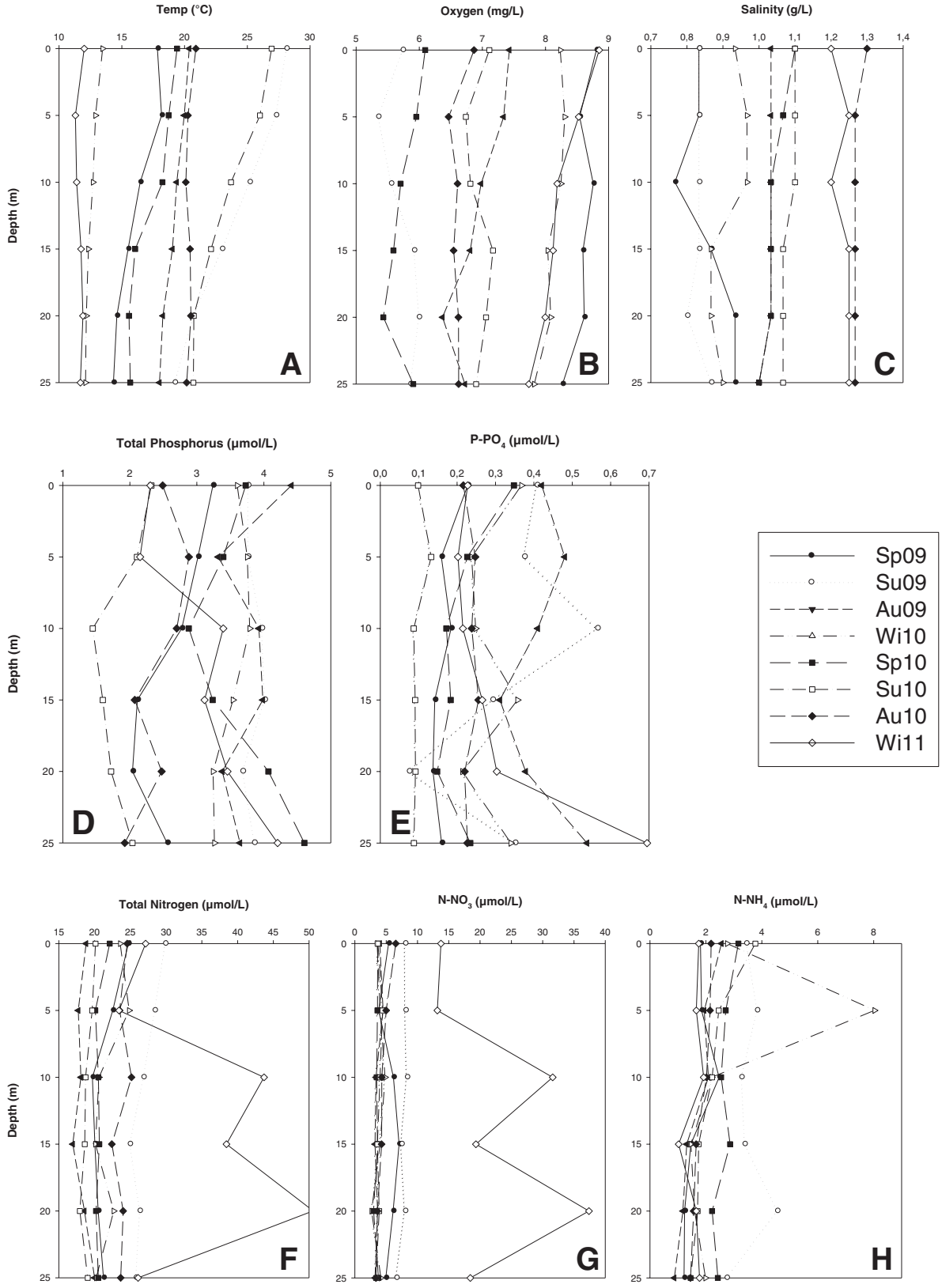

Fig. 2. Vertical profiles of the different hydrological parameters measured in the Sidi Salem reservoir throughout the period of the study. Sp, spring; Su, summer; Au, autumn; Wi, winter.

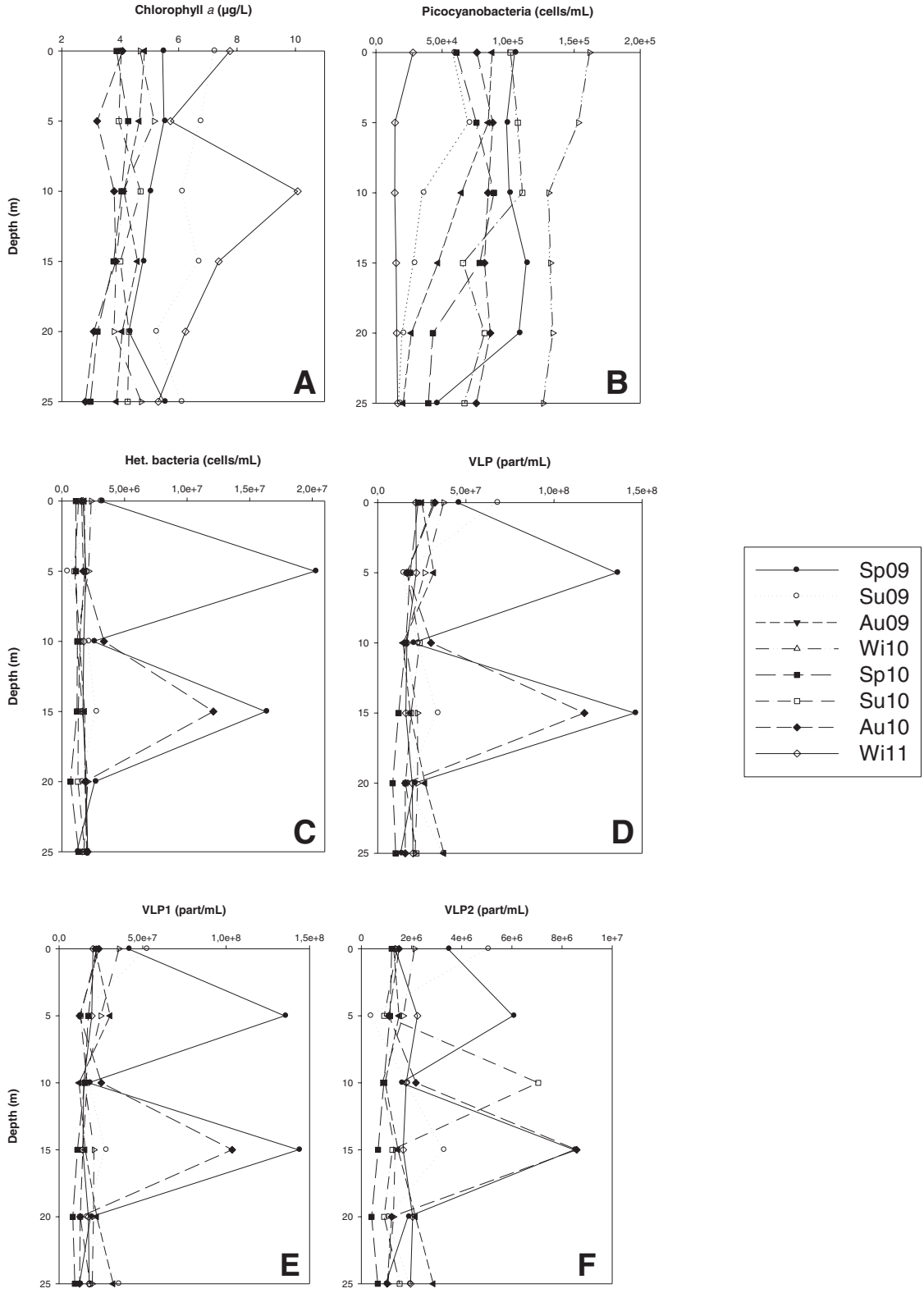

Fig. 3. Vertical profiles of the different biological parameters measured in the Sidi Salem reservoir throughout the period of the study. Sp, spring; Su, summer; Au, autumn; Wi, winter.

respectively (Fig. 4). The concentration of the most abundant VLP group, i.*e.,* VLP1, ranged between 8.13×10^6 and 1.43×10^8 part.mL^{-1}. The concentration of VLP2 was always lower, and ranged between 3.28×10^5 and 8.59×10^6 part.mL^{-1}. The values of VLP3 were even lower than VLP2 and ranged between 7.9×10^3 and 1.89×10^6 part mL^{-1}. Total VLP concentrations varied from 8.43×10^6 to 1.46×10^8.part.mL^{-1}. The abundance

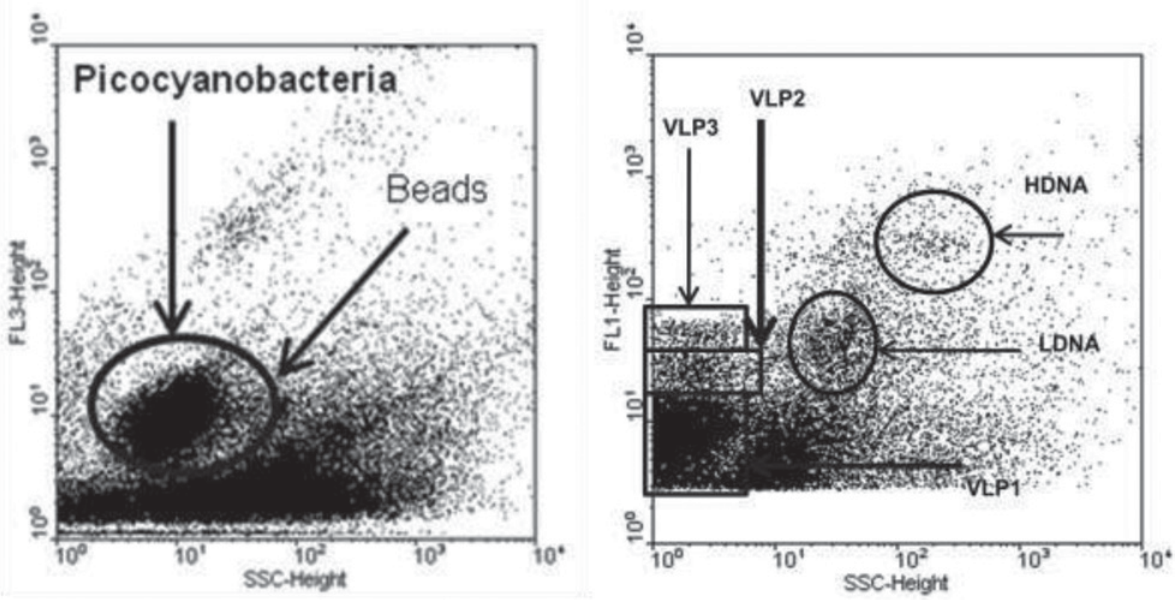

Fig. 4. FCM Cytograms showing the different microbial communities.

of viruses, showed an increase in April 2009, with a peak (1.46×10^8 part.mL^{-1}) at 5 m (Fig. 3(D)–(F)). Like the heterotrophic bacteria, the VLP community was highly dynamic. The virus-to-prokaryotic ratio used as a proxy of virus–prokaryote interactions varied between 56.3 and 99.3. The highest values were observed during February 2009 at 25 m. On average the VPR was 22 and the mean values calculated for each month ranged varied between 8 and 109.

Relationships between variables

The various environmental and biological factors showed significant seasonal variations. ANOVA analysis showed that the abundances of viruses, bacteria and picocyanobacteria changed markedly with season and depths. The picocyanobacteria distribution through the water column was not homogeneous; their abundance decrease with depth in spring 2009, summer and autumn 2009. The high abundance of bacteria and virus was detected in the euphotic zone in the summer, spring and autumn, respectively (Table 3). The seasonal succession of the selected microorganisms showed a significant variation observed in the layer 20 m in all season. However, the abundance of picocyanobacteria was important in the summer 2009 and autumn 2010 in 5 m depth. There was any significant distribution of bacteria and virus in the surface and 5 m depth in all season, but a significant abundance of bacteria marked in summer 2009 at 10 m.Viral abundance was significant in winter and autumn at 20 m (Table 4a and 4b).

To describe the relationships between the selected microorganisms and others parameters we used Pearson linear correlations based on spatial analyses (Table 5). A significant correlation was found between the bacteria and the viruses (VLP) considered all together ($r = 0.94$, $P < 0.0001$) but also with VLP1 and VLP2 considered thus separately ($r = 0.80$, $P < 0.001$; $r = 0.78$, $P < 0.001$). An inverse relationship between the VPR and bacterial abundance was recorded with the highest VPR values recorded at times of relatively low bacterial abundance. A weak correlation was observed between pH and VLP2 ($r = 0.56$, $P < 0.05$). The picocyanobacteria were highly correlated with temperature, oxygen, ammonium and chlorophyll *a* ($r = 0.94$, $P < 0.0001$; $r = 0.85$, $P < 0.001$; $r = 0.87$, $P < 0.001$; $r = 0.83$, $P < 0.001$). At last, a positive relationship was observed between chlorophyll *a*, temperature and oxygen ($r = 0.88$, $P < 0.001$; $r = 0.84$, $P < 0.001$). There was no relationship between the viruses and the autotrophs.

The PCA discriminated two groups around the F1 and F2 axes. F1 explained 36.9% of the variability and positively selected the group G1, mainly composed by the picocyanobacteria, chlorophyll *a*, dissolved oxygen, temperature and pH. F2 represented 29.8% of the variability and negatively selected the group G2, constituted by bacterial and viral groups. In addition the statistical analysis revealed that microbial community abundance was more important in the upper lit layers (Fig. 5).

Discussion

This study showed that the Sidi Salem's reservoir is characterized by a thermal stratification which associated with dissolved oxygen one; the hypolimnion is relatively well oxygenated and has no total anoxia period, even in summer, although the gradient is most pronounced when the stratification is stable. The amount of chlorophyll *a*, reveals two algal development periods (spring and autumn). The evolution of physicochemical parameters is

Table 3. ANOVA results of depth variability for Picocyanobacteria (Pico), bacteria and virus abundances in the Sidi Salem reservoir.

Source of variation		Spring 2009		Summer 2009		Autumn 2009		Winter 2009		Spring 2010		Summer 2010		Autumn 2010		Winter 2011	
	d.f.	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Pico																	
Depth	5	11.84	18.60*	12.63	37.61*	8.32	12.31*	4.41	3.18	1.49	7.22*	5.89	23.70*	1.743	2.69	1.42	0.99
Residual	12	0.637		0.336		0.67		1.38		0.21		0.25		0.65		1.42	
Cochran's *C*-test		$C = 0.335$ ns		$C = 0.306$ ns		$C = 0.612$ ns		$C = 0.284$ ns		$C = 0.383$ ns		$C = 0.405$ ns		$C = 0.293$ ns		$C = 0.280$ ns	
Transformation		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)	
SNK test		0 m = 5 m 0 m > 10 m 10 m = 15 m 15 m = 20 m 10 m > 20 m 20 m > 25 m		0 m = 5 m > 10 m = 15 m > 20 m = 25 m		0 m = 5 m = 10 m = 15 m > 20 m = 25 m		–		0 m 5 m = 10 m = 15 m = 20 m = 25 m		0 m > 5 m = 10 m = 15 m = 20 m > 25 m		–		–	
Bacteria																	
Depth	5	1.92	8.07*	4.12	14.35*	1.28	0.83	1.21	0.67	2.72	3.16*	6.95	16.8*	1.26	1.08	0.16	0.18
Residual	12	0.24		0.31		1.54		1.79		0.86		0.41		1.17		0.08	
Cochran's *C*-test		$C = 0.333$ ns		$C = 0.32$ ns		$C = 0.87$ ns		$C = 0.899$ ns		$C = 0.810$ ns		$C = 0.41$*		$C = 0.310$*		$C = 0.279$*	
Transformation		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		None		None		None	
SNK test		0 m = 5 m = 10 m = 15 m = 20 m > 25 m		0 m = 5 m = 10 m = 15 m > 20 m = 25 m		–		–		0 m = 5 m = 10 m = 15 m > 20 m = 25 m		0 m = 5 m = 10 m > 15 m = 20 m = 25 m		–		–	
Virus																	
Depth	5	9.49	7.33*	4.44	17.68*	7.38	18.09*	0.57	0.91	6.44	9.55*	6.16	14.23*	5.53	26.68*	0.06	0.11
Residual	12	1.29		0.25		0.41		0.62		0.67		0.43		0.21		0.53	
Cochran's *C*-test		$C = 0.304$ ns		$C = 0.465$ ns		$C = 0.583$*		$C = 0.518$ ns		$C = 0.528$ ns		$C = 0.410$ ns		$C = 0.306$ ns		$C = 0.273$ ns	
Transformation		ln ($x + 1$)		ln ($x + 1$)		None		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)		ln ($x + 1$)	
SNK test		0 m = 5 m = 10 m > 15 m = 20 m = 25 m		0 m = 5 m = 10 m = 15 m > 20 m = 25 m		0 m = 5 m = 10 m = 15 m > 20 m = 25 m		–		0 m = 5 m = 10 m > 15 m = 20 m = 25 m		0 m = 5 m = 10 m > 15 m = 20 m = 25 m		0 m = 5 m = 10 m = 15 m > 20 m = 25 m		–	

MS, mean square; *F*, Fischer statistic; * = significant effect ($P < 0.05$); ns, not significant.

Table 4. ANOVA results for seasonal variation in each depth of bacteria, virus and picocyanobacteria in Sidi Salem reservoir.

Depth			Bacteria		Virus		Picocyanobacteria	
(a)								
0 m	Source of variation	d.f.	*MS*	*F*	*MS*	*F*	*MS*	*F*
	Season	7	0.947	1.93 ns	5.98105	1.42 ns	1.66	1.67 ns
	Residual	16	0.49		4.26105		0.995	
	Cochran's *C*-test		$C = 0.532$ ns		$C = 0.415$*		$C = 0.311$ ns	
	Transformation		ln $(x + 1)$		None		ln $(x + 1)$	
0 m	SNK test				–		–	
	Source of variation	d.f.	*MS*	*F*	*MS*	*F*	*MS*	*F*
	Season	7	2.09	1.58 ns	0.917	0.83 ns	1.625	5.41*
	Residual	16	1.29		1.1		0.3	
	Cochran's *C*-test		$C = 0.781$ ns		$C = 0.444$ ns		$C = 0.448$ ns	
	Transformation		ln $(x + 1)$		ln $(x + 1)$		ln $(x + 1)$	
5 m	SNK test						Sp 2010 = Su 2010 = Win 2011 = At 2009 Sp 2010 < At 2010 = Sp 2009 Win 2010 = Su 2009 > Su 2010	
	Source of variation	d.f.	*MS*	*F*	*MS*	*F*	*MS*	*F*
	Season	7	0.77	3.27*	0.885	0.994 ns	3.185	5.99*
	Residual	16	0.235		0.89		0.532	
	Cochran's *C*-test		$C = 0.354$*		$C = 0.445$ ns		$C = 0.435$ ns	
	Transformation		None		ln $(x + 1)$		ln $(x + 1)$	
10 m	SNK test		Win 2010 = Sp 2010 < Su 2009 Win 2011 = Su 2010 = At 2009 = At 2010 = Sp 2009		–		Win 2011 = At 2009 < Win 2010 At 2010 = Win 2010	
(b)								
	Source of variation	d.f.	*MS*	*F*	*MS*	*F*	*MS*	*F*
	Season	7	2.53	4.91*	25.8	1.503 ns	1.83	2.06 ns
	Residual	16	0.514		24.5		0.889	
	Cochran's *C*-test		$C = 0.531$ ns		$C = 0.969$ ns		$C = 0.331$*	
	Transformation		ln $(x + 1)$		ln $(x + 1)$		None	
15 m	SNK test		Su 2010 < all other seasons				–	
	Source of variation	d.f.	*MS*	*F*	*MS*	*F*	*MS*	*F*
	Season	7	3.124	3.99*	3.375	7.65*	4.707	6.003*
	Residual	16	0.783		0.44		0.784	
	Cochran's *C*-test		$C = 0.669$ ns		$C = 0.249$*		$C = 0.331$ ns	
	Transformation		ln $(x + 1)$		None		ln $(x + 1)$	
20 m	SNK test		Su 2009 = Sp 2010 = Su 2010 > Sp 2009 = At 2009 = Win 2010 = At 2010 = Win 2011		Sp 2010 = Su 2010 = Su 2009 = Sp 2009 < Wi 2011 = At 2009 = At 2010 = Win 2010		Su 2009 = At 2009 < Su 2010 Sp 2010 = At 2010 > Su 2009 = At 2009	
	Source of variation	d.f.	*MS*	*F*	*MS*	*F*	*MS*	*F*
	Season	7	5.67	8.51*	2.7	1.82 ns	2.3	2.93*
	Residual	16	0.67				0.78	
	Cochran's *C*-test		$C = 0.812$ ns		$C = 0.309$*		$C = 0.353$*	
	Transformation		ln $(x + 1)$		None		None	
25 m	SNK test		Sp 2009 < win 2011 At 2009 = Su 2009 = Su 2010 Win 2010 = Sp 2010 = At 2010 > At 2009 = Su 2009 = Su 2010		–		At 2009 = Win 2011 > Other season	

MS, mean square, *F*, Fischer statistic; * = significant effect ($P < 0.05$); ns, not significant; Sp, spring; At, autumn; Win, winter; Su, summer.

Table 5. Correlation matrix (Pearson test) for physical, chemical and biological variables sampled/analysed in the reservoir of Sidi Salem during 2 years of monitoring (*$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$).

	T °C	pH	O_2	Salinity	PO_4	NH_4^+	NO_3^-	SIO_2	chla	Bacteria	Picocyano	VLP1	VLP2
T °C	1	0.444	**0.900*** **	0.247	−0.045	**0.918*** **	−0.287	−0.166	**0.888****	0.129	**0.944*** **	0.067	0.174
pH		1	**0.743****	−0.342	−0.443	0.152	0.231	−**0.594***	**0.454***	0.221	**0.548***	0.111	**0.566***
O_2			1	0.133	−0.075	**0.681****	−0.161	−0.430	**0.842****	0.044	**0.853****	0.065	0.293
Salinity				1	**0.454***	0.391	−**0.680***	0.260	−0.027	−0.285	−0.027	0.088	−**0.580***
PO_4^-					1	−0.129	−**0.642***	−0.311	0.172	−0.277	−0.332	0.265	−0.120
NH_4^+						1	−0.295	0.199	**0.687****	0.119	**0.872****	−0.027	−0.076
NO_3^-							1	0.308	−0.256	−0.333	−0.013	−**0.737****	−0.126
SIO_2^-								1	−0.459	−0.370	−0.099	−**0.628****	−**0.799****
Chla									1	0.156	**0.834****	0.161	**0.422***
Bacteria										1	0.215	**0.804****	**0.779****
Picocyano											1	−0.038	0.265
VLP1												1	**0.689****
VLP2													1

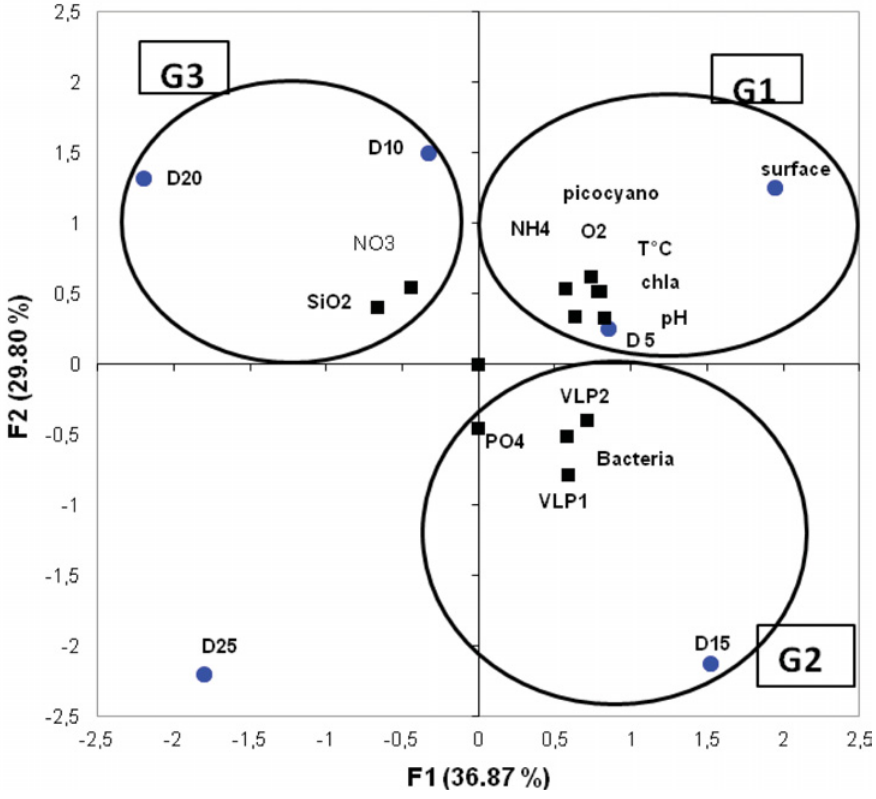

Fig. 5. PCA of the microbial communities with environmental variables throughout the water column in the Sidi Salem dam. D, depth; G, group.

mainly regulated by the hydrodynamic character of the reservoir's waters which leads to a relatively frequent mixing.

According to Ryding and Rast (1994), the values of TP, chlorophyll *a* and transparency indicate that Sidi Salem reservoir oscillated between a mesotrophic and eutrophic status. It was clear; that the deduction of dam Mediterranean area is subjected to high climate and anthropic pressures, and that the diagnosis of their trophic state will have imperatively gone through a model that will be more adapted. The same results were found by Mouelhi *et al.* (2000). This does not correlate with the study of Sellami *et al.* (2012) concluding that the reservoir of Sidi Saâd oscillated between oligotrophic and mesotrophic levels. As well, our result does not corroborate with the result of Ben Rejeb Jenhani *et al.* (2012) showing that the reservoir of Bir M'cherga can be considered as hypertrophic.

In our survey, the ratio N/P was higher, which reflects an excess of nitrogen to phosphorus. The significant finding of this ratio shows that, in addition to the apparent limitation of phosphorus added the nitrogen, phosphorus

is the most frequently limiting factor in freshwater and in various eutrophic lakes. Phosphorus limitation is gradually replaced by a nitrogen limitation in late summer. The same results were obtained in many studies of reservoirs in Tunisia (Ben Rejeb Jenhani *et al.*, 2006; El Herry *et al.*, 2008; Sellemi *et al.*, 2009).

Researcher have documented that the picocyanobacteria group presented the lowest abundance in winter and showed positive correlation between cell number and water temperature, which indicated the influence of thermal stratification on their growth (Wang *et al.*, 2009). In addition, picocyanobacteria distribution increased in the metalimnion of the reservoir. The complex variability of community structure is related to the spectrum of environmental variability, through the modulation of intrinsic factors (basin morphometry, thermal stratification and wind mixing) and such external influences as fluctuating supply of nutrients (Harris, 1980). Nevertheless, abiotic factors (temperature, precipitation) provide the primary force that drives temporal variability in species abundance, before even competitive interactions (Houlahan *et al.*, 2007). Our result was corroborated with several researches' concluding that the thermal conditions were significant not only for the ambient water temperature but for the maintenance of a density gradient resisting settlement and extension of the picophytoplankton peak. Elsewhere, the peaks of abundance in vertical distribution of picophytoplankton have been observed, variously in the lower metalimnion and upper hypolimnion of lakes Huron and Michigan (Fahnenstiel and Carrick, 1992) and also of lake Stechlin (Padisák *et al.*, 1998, 2003); in the metalimnion, beneath the steepest part of the thermocline in lake Constance and lake Maggiore (Weisse and Schweizer, 1991; Callieri and Pinolini, 1995); in the metalimnion of lake Baikal (Nagata *et al.*, 1994). Picocyanobacteria showed a negative correlation with depth, this may reflect the importance of light quality on their growth (Voros *et al.*, 1998). Moreover, light is known to be an important factor in niche differentiation of picocyanobacteria. As well, water column depth, which is roughly inversely related to the trophic state of the lake, is an important indicator of the presence of picophytoplankton and/or of its abundance relative to large species of phytoplankton (Callieri, 2007).

Nutrient supply is an important factor affecting the picophytoplankton distributions (Stockner and Shortreed, 1994; Vrede *et al.*, 1999). Picocyanobacteria exhibited a negative correlation with PO_4^{3-} in this reservoir, revealing that high phosphate concentration decreases picocyanobacterial growth rates (Schallenberg and Burns, 2001). However, high phosphate cannot constrain the picocyanobacteria growth at higher water temperature mainly in summer, suggesting that there are multi-factors in controlling picocyanobacteria abundance. Other factors such as grazing by ciliates (Chang *et al.*, 2003) or other microzooplankton, viral infections and co-sedimentation with organic particles (Fuhrman, 1999; Waite *et al.*, 2000) may also affect the picophytoplankton distributions. In our study, the presence of picocyanobacteria was important and abundant mainly in spring and early summer, when the thermal stratification and clear water phase took place. This corroborates the results found by several authors (Anneville and Leboulanger, 2001; Jacquet *et al.*, 2005; Cellamare *et al.*, 2010) in their research. Recently, it is well known that nutrient availability, sedimentation, and grazing were considered the major driving forces of microbial and algal communities in aquatic environments (Tijdens *et al.*, 2008). The picophytoplankton abundance and biomass increase and its relative importance decreases with the increase of trophic state in the freshwater system (Szelag-Wasielewaska, 1999; Stockner *et al.*, 2000; Bell and Kalff, 2001; Callieri and Stockner, 2002). Light and water temperature are also two important factors controlling picophytoplankton growth (Wehr, 1993; Agawin *et al.*, 2000; Wakabayashi and Ichise, 2004). As well the viral population dynamics have been associated with environmental factors such as seasonality, location, water depth, degree of stratification, tide height, salinity and chlorophyll *a* concentration (Wommack *et al.*, 1999; Frederickson *et al.*, 2003; Short and Suttle, 2003; Dorigo *et al.*, 2004). Moreover, the picocyanobacteria, presented a maximum abundance with thermal stratification in March 2009 and June 2010 in our meso-eutrophic lake. In agreement with the literature, picocyanobacteria are present in lakes of different trophies, but they are likely to be the dominant group all year long in the oligotrophic system while they took advantage of the low nutrient concentrations found in surface waters in spring and summer in mesotrophic lakes (Weisse, 1993; Callieri, 2007). Compared to marine, there is less information available on freshwater picocyanobacterial biodiversity, and the factors controlling the growth, distribution and productivity (Stockner and Antia, 1986; Callieri and Stockner, 2002). Similar to marine, freshwater picocyanobacteria show high diversity with specific niche adaptation and the abundance of freshwater *Synechococcus* follows a seasonal pattern with a peak in spring after winter mixing and a peak late summer (Weisse, 1993; Stockner *et al.*, 2000; Callieri and Stockner, 2002).

The flow cytometer (FC) allowed us to easily differentiate different groups within these microbial components, using scatter plots of either natural fluorescence (in the case of phytoplankton), or an added chemical dye such as SYBER Green I that binds to nucleic acid (in the case of bacteria and viruses) versus side scatter. Particles with similar fluorescence and side scatter properties form an individual group on a scatter plot, allowing different groups of microorganisms to be distinguished (Chisholm *et al.*, 1986; Marie *et al.*, 1999). In the present study, two distinct groups of bacteria marked by their high and low DNA fluorescence upon staining with SYBER Green I. The HDNA bacteria have often been considered to represent the active fraction (Vaque *et al.*, 2001; Lebaron *et al.*, 2002). The distribution of the two groups in this study was very similar; our results are very similar to data shown by Li and Dickie (2001), which enumerated bacteria from the surface of coastal waters over successive annual cycles and found that the patterns of seasonal

Table 6. Comparison of viral, bacterial and picocyanobacteria abundance reported in some freshwater lake with different trophic status.

Lake and location	Trophic status	Picocyanobacteria (10^4 cells.mL^{-1})	Heterotrophic bacteria (10^6 cells.mL^{-1})	Virus (10^6 part.mL^{-1})	References
Alte Donau, Austria	Eutrophic	–	–	17–117	Fisher and Velmirov (2002)
Rimov reservoir, Czech Republic	Meso-eutrophic	–	–	8–47	Simek *et al.* (2001)
Constance, Germany	Mesotrophic	9.5	–	10–40	Hennes and Simon (1995)
Pavin, France	Oligo-mesotrophic	10.7	–	10–54	Carrias *et al.* (1996) Bettarel *et al.* (2003, 2004)
Geneva, France	Oligo-mesotrophic	5.7	2.7	77	Parvathi *et al.* (2014)
Lemam, France	Mesotrophic	2.9	2.6	52	Personnic *et al.* (2009)
Bourget, France	Mesotrophic	2.7	2.7	64	Personnic *et al.* (2009)
Allal El Fassi reservoir, Maroc	Mesotrophic	1.7	5.4	–	Rachiq *et al.* (2002)
Sidi Salem Reservoir, Tunisia	Meso-eutrophic	8.9	7.6	67	Ben Romdhane *et al.*, present study

abundance were very similar for both groups. Bacterial abundance in Sidi Salem's reservoir was high, with concentration reaching (5.26 and 3.84×10^7 cells.mL^{-1}). In our surveys, the Heterotrophic bacteria showed a negative correlation with chlorophyll *a*, their biomass is a well-known characteristic of the eutrophic system; the same result was obtained by Zhang *et al.* (2013) in the Pearl River Estuary. However, many investigators have observed significant correlations between bacteria and chlorophyll *a* in various ecosystems, including the Chesapeake Bay (Ducklow *et al.*, 1999), the Mississippi River Plume (Liu *et al.*, 2004), the Danshui Estuary (Tsai *et al.*, 2011), the East China Sea (Pan *et al.*, 2005) and the northern South China Sea (Yuan *et al.*, 2011), indicating that primary production provides most of the carbon needed for heterotrophic bacteria production in these settings. In many estuarine environments, however, the relationship between heterotrophic bacteria and primary production is weak or non-existent (Shiah and Ducklow, 1994; Kelley *et al.*,1998) because dissolved organic carbon is abundant or bacterial growth depends mostly on other non-phytoplanktonic sources of carbon (Fouilland and Mostajir, 2010). Bacterial production and chlorophyll *a* measurements were shown to be significantly higher in the eutrophic basin of Lake Erie, Ohio, USA, than in the less productive basins (DeBruyn *et al.*, 2004). The fluctuation of vertical distributions of heterotrophic bacteria during the study period, explained that the bacterial community composition varied in response to environmental parameters' changing, also with their different adaptations proliferating under a range of light conditions (Casamayor *et al.*, 2000; Vila and Abella, 2001). Furthermore, it is generally accepted that phytoplankton, especially picofractioned phytoplankton, are the principal source of organic carbon for bacteria (Baines and Pace, 1991).

As regards virioplankton, the highest abundances were observed in surface waters. This viral peak followed the increase in bacterial abundance at the same period as reported in other ecosystems. It was mentioned before by several authors, in Alpine lakes (Personnic *et al.*, 2009), in seawater (Bergh *et al.*, 1989), the Chesapeake Bay (Wommack *et al.*, 1992), the Tampa Bay (Cochlan *et al.*, 1993), the Northern Adriatic Sea (Weinbauer and Peduzzi, 1995), the backwater system of the Danube River (Mathias *et al.*, 1995), the alkaline and hypersaline Mono lake (Brum *et al.*, 2005) and in Norwegian coastal waters (Bratbak *et al.*, 1996). In addition, such high values, especially for VLP1, recorded throughout the water column, were clearly associated with water column mixing, probably because bacteria were stimulated by both organic and inorganic nutrients (Personnic *et al.*, 2009). The highest ratios (VPR) that were found in summer and in deeper waters could also suggest a higher impact of viruses in bacteria when compared to surface waters, previously reported by several authors (Weinbauer and Hofle, 1998; Colombet *et al.*, 2006). Seasons in which a high VBR occurred could be expected to display greater virioplankton production and more bacterioplankton lysis. It is clear that VBR is only an indicator used to investigate possible relationships between viruses and their bacterial or phytoplankton hosts, so that it is clearly insufficient to infer the importance of the role (lytic effects) played by the viruses; as described by many authors (Wilson and Mann, 1997; Weinbauer and Rassoulzadegan, 2004; Abedon, 2006). In addition, our meso-eutrophic reservoir presented the higher abundances of picocyanobacteria, bacteria and viruses which represented (8.910^4, 7.6×10^6 and 67×10^6 part.mL^{-1}), respectively; compared with mesotrophic lake Bourget and Lemam (Personnic *et al.*, 2009) and mesotrophic reservoir Allal El Fassi (Rachiq *et al.*, 2002). However, our study reported the lower abundances of picocyanobacteria than showed by Hennes and Simon (1995). In their study, they have reported in mesotrophic Lake Constance that the abundances of viruses ranged from (10^{-4} 7×10^6 part.mL^{-1}), the similar results were also reported by Simek *et al.* (2001) in meso-eutrophic Rimov reservoir (Table 6).

Based on side scatter and green DNA dye complex fluorescence, FCM data allowed us to distinguish three

groups of viruses. Other studies also reported such discrimination (Castberg *et al.*, 2001; Chen *et al.*, 2001; Jacquet *et al.*, 2002; Goddard *et al.*, 2005; Duhamel and Jacquet, 2006). The abundance of VLP1 was higher than the abundance of VLP2 and also than the abundance of VLP3. Published works by different authors (Marie *et al.*, 1999; Wommack and Colwell, 2000; Larsen *et al.*, 2001; Culley and Welschmeyer, 2002; Payet and Suttle, 2008) indicate that most of what we called the VLP1 group is likely to consist of bacteriophages. Using statistical analysis, we found a significant correlation between heterotrophic bacteria and virioplankton. However, we did not find any significant correlation between viral abundance and chlorophyll *a* concentrations. In contrast, VLP2, which we had thought was more specific to phytoplanktonic or cyanobacterial viruses, was observed to be significantly linked to chlorophyll *a* ($r = 0.41$, $P < 0.05$). These results, suggesting firstly that phytoplankton viruses do not contribute significantly to the total virus pool, and secondly that the positive effect of an increase in chlorophyll *a* on heterotrophic bacteria is not directly beneficial to virus production(Personnic *et al.*, 2009).

This is the first report of analytical flow cytometry (AFC) which was used as the tool choice to determine concentration of different groups within the whole microbial community, from viruses and bacteria, through to phytoplankton essentially the picocyanobacteria of the surface water in Tunisia. The selected microorganisms played a very important role in maintaining particular ecosystem structures and functions in the freshwater lake; however, very few studies have been conducted on the characteristics of the components in the microbial, viral and planktonic communities in the retentions. In the current study, we simultaneously observed the distribution patterns and biomass levels of heterotrophic bacteria, virus and picocyanobacteria. Their biomass was important in the upper water column. No significant correlations were found between nutrients, bacterial and viral groups. However, picocyanobacteria have a significant correlation with temperature, oxygen and chlorophyll *a*.

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