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## **Environmental factors associated with phytoplankton succession in a Mediterranean reservoir with a highly fluctuating water level**

**Ali Fadel<sup>1,2\*</sup>, Ali Atoui<sup>3</sup>, Bruno J. Lemaire<sup>1,4</sup>, Brigitte Vinçon-Leite<sup>1</sup>, Kamal Slim<sup>3</sup>**

<sup>1</sup> Université Paris-Est, LEESU (UMR MA-102), Université Paris-Est-Créteil, Ecole des Ponts ParisTech, AgroParisTech, F-77455 Marne-la-Vallée, France; E-Mails: [bruno.lemaire@leesu.enpc.fr](mailto:bruno.lemaire@leesu.enpc.fr); [bvl@leesu.enpc.fr](mailto:bvl@leesu.enpc.fr)

<sup>2</sup>National Center for Remote Sensing, National Council for Scientific Research (CNRS), P.O. Box 11-8281, Riad El Solh, 1107 2260 Beirut, Lebanon; E-Mail: [afadel@cnrs.edu.lb](mailto:afadel@cnrs.edu.lb)

<sup>3</sup>Laboratory of Microorganisms and Food Irradiation, Lebanese Atomic Energy Commission-CNRS, P.O. Box 11-8281, Riad El Solh, 1107 2260 Beirut, Lebanon; E-Mail: [a.atoui@cnrs.edu.lb](mailto:a.atoui@cnrs.edu.lb), [kslim@cnrs.edu.lb](mailto:kslim@cnrs.edu.lb)

<sup>4</sup>AgroParisTech, F-75005 Paris, France

\*Author to whom correspondence should be addressed; E-Mail: [afadel@cnrs.edu.lb](mailto:afadel@cnrs.edu.lb); Tel.: + 961 (0)1 45 08 11; Fax: + 961 (0)1 45 08 10

### **Abstract**

Eutrophication and harmful algal blooms have become a worldwide environmental problem. Understanding the mechanisms and processes that control algal blooms is of great concern. The phytoplankton community of Karaoun Reservoir, the largest water body in Lebanon, is poorly studied, as in many freshwater bodies around the Mediterranean Sea. Sampling campaigns were conducted semi-monthly between May 2012 and August 2013 to assess the dynamics of its phytoplankton community in response to changes in physical-chemical and hydrological conditions. Karaoun Reservoir is a monomictic waterbody and strongly stratifies between May and August. Changes in its phytoplankton community were found to be a result of the interplay between water temperature, stratification, irradiance, nutrient availability and water level. Thermal stratification established in spring reduced the growth of diatoms and resulted in their replacement by green algae species when nutrient availability was high and water temperatures lower than 22 °C. At water temperature higher than 25 °C and low nutrient concentrations in summer, blooms of the cyanobacterium *Microcystis aeruginosa* occurred. Despite different growth conditions in other lakes and reservoir, cyanobacterium *Aphanizomenon ovalisporum* dominated at temperatures lower than 23 °C in weakly stratified conditions in early autumn and dinoflagellate *Ceratium hirundinella* dominated in mixed conditions, at low light intensity and a water temperature of 19 °C in late autumn. We believe that the information presented in this paper will increase the knowledge about phytoplankton dynamics in the Mediterranean region and contribute to a safer usage of reservoir waters.

**Keywords:** Cyanobacteria, Irradiance, Middle East, Nutrients, Thermal stratification.

# 1 Introduction

Eutrophication threatens freshwater bodies as it promotes the development and persistence of harmful algal blooms during warm conditions (Reynolds, 2006a). Many lakes and reservoirs throughout the world are contaminated by harmful algal species (Li et al., 2013), mostly toxic cyanobacteria that can produce cyanotoxins. These toxins cause numerous illnesses and deaths to livestock, pets, wildlife and humans that ingest water contaminated with toxic cyanobacterial cells, or toxins released from decaying cyanobacterial cells (Lance et al., 2010). In addition, these blooms decrease water transparency and reduce the dissolved oxygen concentration in the hypolimnion; they result in fish kills, due to hypoxia and cyanotoxins, and hinder the recreational use of the water bodies (Smith, 2003). The development of phytoplankton populations is mainly controlled by nutrient concentrations, light intensity, the water residence time, water temperature, mixing and grazing (Reynolds, 2006b). Understanding the processes that control algal blooms remains of great concern.

The response of fresh water bodies to water level fluctuations (WLF) is an under-studied field (Zohary and Ostrovsky, 2011). Most of the research studies performed on the environmental factors affecting algal succession and composition were done on lakes and reservoirs with a stable water level. Water level fluctuations can affect phytoplankton biomass and species composition through their effect on mixing processes, biogeochemical processes, oxygen dynamics, and underwater light climate (Naselli-Flores and Barone, 1997; Valdespino-Castillo et al., 2014).

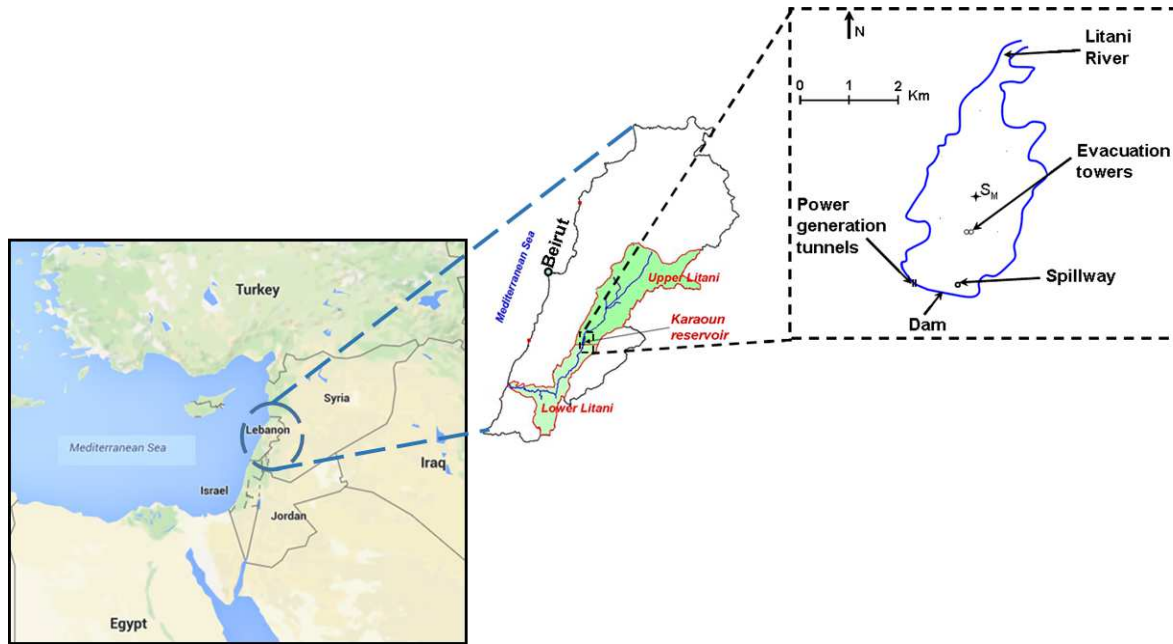
Karaoun Reservoir, the largest water body in Lebanon, was built up in 1965, primarily for agricultural irrigation and hydroelectricity production. This artificial ecosystem now serves varied purposes such as commercial fishing, recreation, tourism, and irrigation. These different uses result in high fluctuation in its water level (Fadel et al., 2014a).

Whereas studies have been carried out in Karaoun Reservoir on toxins (Fadel et al., 2014b) and metal and nutrient concentrations (Korfali and Jurdi, 2010), the few articles which report the occurrence of toxic cyanobacterial blooms do not describe the physical-chemical factors that control phytoplankton blooms (Atoui et al., 2013; Slim et al., 2014). In this study, we try to 1) present the temporal variation of physico-chemical parameters in Karaoun Reservoir, 2) identify its phytoplankton composition, and 3) understand the processes controlling phytoplankton succession in this reservoir with a high water level fluctuation.

## 2 Materials and methods

### 2.1 Study site

Karaoun Reservoir, located in the southern part of the Bekaa valley, between the two Lebanese mountain chains, is the largest freshwater body in Lebanon (Figure 1, Table 1). The reservoir was constructed between 1958 and 1965 on the Litani River (170 km length) for power production and irrigation. The river inflow occurs mainly in the wet season, from October to April, while the withdrawals are much more regular in the year, which causes a large water level variation in the year, and can reach up to 30 m (Fadel et al., 2014b).



**Figure 1** Karaoun Reservoir and sampling site:  $S_M$  ( $33^{\circ} 34' 05''N$ ,  $35^{\circ} 41' 44''E$ ).

**Table 1** Karaoun Reservoir morphometric and hydrologic characteristics.

Surface area at full capacity	12 km <sup>2</sup>
Maximum storage capacity	$224 \times 10^6$ m <sup>3</sup>
Maximum depth	60 m
Mean depth at full capacity	19 m
Altitude at maximum level	858.28 m above sea level
Catchment area	1,600 km <sup>2</sup>
Mean residence time of water	9 months (Fadel et al., 2014a)

## ***2.2 Sampling procedure***

Measurements and samples were taken at the most representative point ( $S_M$ ), located in the middle of the lake (33° 34' 05"N, 35° 41' 44"E). To choose this point, the sampling of several physical-chemical parameters was performed at 6 points at different locations throughout the reservoir (north, east, south and west). The results of profiles measured at these 6 points were relatively close with a variation of about 35%. The sampling point  $S_M$  was selected as the most representative because its values were comparable to the average of the measurements taken at the 6 different locations. Campaigns were performed bi-weekly between 11:00 and 13:00. Water samples were collected at 0.5 m depth from May to November 2012 and at 0.5, 5 and 10 m depths from March to August 2013 with a vertical Niskin bottle of 2.2 L capacity (Wildco 1120-D42, Florida, United States). Samples were stored at 4°C until further processing in the laboratory. Different volumes and bottles were used for phytoplankton identification and counting, nutrient analysis and chlorophyll-a quantification.

## ***2.3 Physical measurements and nutrient analysis***

Transparency measurements were performed at  $S_M$  with a Secchi disk. Measurements of dissolved oxygen concentration (in mg/L) were conducted at subsurface using an oxymeter (Hanna HI 9146, Woonsocket, USA).

Water temperature was measured continuously at the spillway with temperature sensors (Starmon mini, Star-Oddi, Gardabaer, Iceland) fixed on a buoy at 1, 7, 10, 13 and 16 m depths to monitor thermal stratification. The sensor measuring temperature range is -2 to 40 °C with an accuracy of  $\pm 0.05$  °C.

Subsamples used for the analysis of nutrients (total phosphorus, orthophosphate, nitrate, and ammonium) were preserved at 4 °C after addition of 2 mL of 18 M  $H_2SO_4$ . Soluble phosphorus (orthophosphate), nitrate, and ammonium subsamples were then filtered through a 0.45  $\mu m$  cellulose acetate filter (MF-Millipore, HAWP04700, Utah, United States).

Nitrate and ammonium concentrations were then estimated by colorimetry with a photometer (Palintest Photometer 7000se, Gateshead, England). Total phosphorus and orthophosphate concentrations were determined at 880 nm by UV/visible spectrophotometry (Thermospectronic, LaboTech, Beirut, Lebanon) using the colorimetric ascorbic acid method (EPA Standard Method 365.3, Washington, United States). The quantification range for nitrate nitrogen was 0.1 - 30 mg N L<sup>-1</sup>, for ammonium nitrogen 0.1 - 12 mg N L<sup>-1</sup>, and for phosphorus 0.001 to 1.2 mg P L<sup>-1</sup>.

## ***2.4 Phytoplankton analysis***

The phytoplankton species were determined on the sampling day according to taxonomic keys based on cell structure and dimensions, colony morphology, and mucilage characteristics (Komárek and Anagnostidis, 1999, 2005). Microscopic identifications and enumeration were carried out under a phase contrast microscope (Nikon TE200, Nikon, Melville, New York, USA). The subsamples used for counting were fixed by formaldehyde (4% formaldehyde of sample volume) and preserved at 4 °C. Phytoplankton counting was carried out under a  $\times 40$

objective using Nageotte chamber that accepts 100  $\mu\text{L}$  on 40 bands. The number of bands counted depended on sample concentration. Each subsample was counted on triplicate.

Total biovolumes of each phytoplankton species was calculated by multiplying the counted number of cells per millilitre by the average biovolume of a cell of that species. Cell biovolumes of each species were calculated according to the most suitable geometric models (Sun and Liu, 2003).

Chlorophyll-a quantification, used to estimate total phytoplankton biomass, was carried out according to Lorenzen method (Lorenzen, 1967). A duplicate of each sample was filtered using Whatman GF/C filters that were then kept frozen at  $-20\text{ }^{\circ}\text{C}$  for 16 h. Chlorophyll a was extracted from these filters in 90 % acetone by ultrasonication and agitation. The extracts were centrifuged at 3500 rpm for 10 min to reduce the turbidity. About 2 mL were used for chlorophyll-a quantification by spectrophotometry, then a correction was performed by adding 60  $\mu\text{L}$  of 0.1 M HCl to these 2 mL to measure the amount of chlorophyll-a degradation product, pheophytin-a.

## ***2.5 Zooplankton analysis***

Zooplankton samples were collected using a net with a mesh size of 100  $\mu\text{m}$  by lowering the nets to approximately two meter below the water surface. Samples were stored in 5% formalin. Major taxonomic groups that were identified and enumerated according to Stemberger (1979), Smith and Fernando, (1978), and Lynne (2004).

## ***2.6 Meteorological and hydrological data***

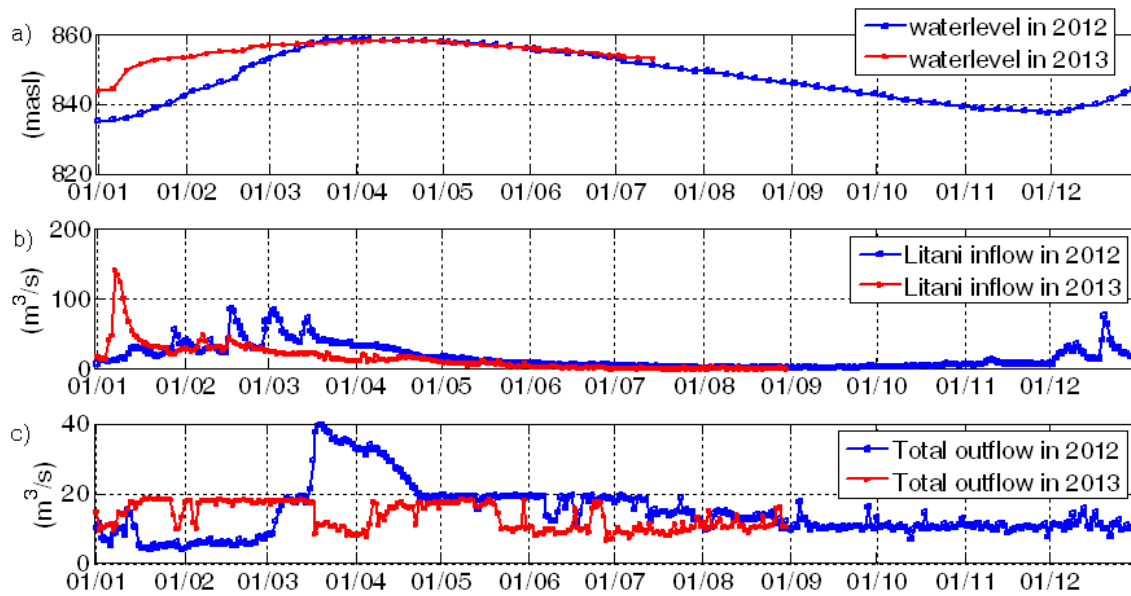
Solar radiation were obtained from Tal-Amara meteorological station of the Lebanese Agriculture Research Institute located in the Bekaa valley ( $33^{\circ} 51' 50''\text{ N}$ ,  $35^{\circ} 59' 06''\text{ E}$ ), 40 km North of Karaoun Reservoir. Weekly water levels measured on a graduated scale at the spillway were provided by the Litani River Authority, responsible for the management of the reservoir. The main withdrawal is through hydropower canals and irrigation canals; the outflow volume is measured directly on a canal transect. The main reservoir inflow is from the Litani River. Since it was not measured in 2012 and 2013, it was estimated by difference from the water level and the measured withdrawals. This method underestimates the inflow rate when the reservoir is full, since overflows through the spillway are not counted. More details about the reservoir functioning and hydrology are presented in (Fadel et al., 2014a).

# **3 Results**

## ***3.1 Hydrological conditions***

The evolution of the water level and the inflow and outflow rates in 2012 and 2013 in Karaoun Reservoir is presented in Figure 2. The reservoir reached its maximum capacity of  $224 \times 10^6\text{ m}^3$  (858.28 m above sea level) in both 2012 and 2013. The total outflow rate was rather regular except during high waters and ranged from 0 to  $40\text{ m}^3.\text{s}^{-1}$  (Figure 2c). Withdrawals from Karaoun Reservoir occur through three outlets: 1) the Markabi hydropower tunnels, that collect about 98% of the total outflow except for the periods in which the evacuation tunnels are used, 2) the main pumping station (MPS) that is used for irrigation

through Canal 900, that collects less than 2% of the total outflow throughout the dry season from April to October or November, 3) the evacuation tunnels that are seldom used; they were not used in 2013 but they were operated between the mid of March and April 2012 because of high inflow volumes from the Litani River (Figure 2c) while the reservoir was full.

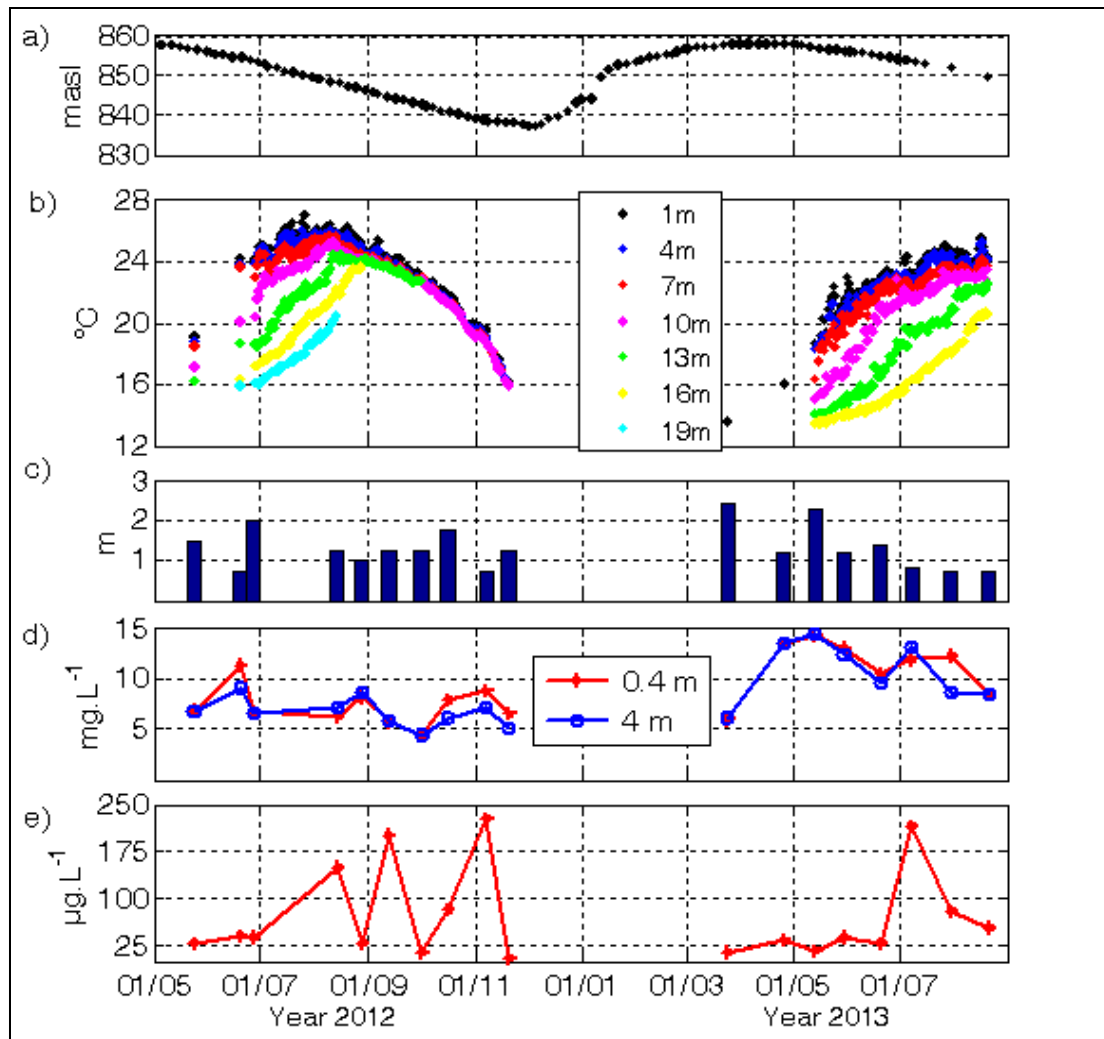


**Figure 2 a) measured water level, b) inflow computed from outflow rate and water level and c) outflow rate at Karaoun Reservoir in 2012 and 2013**

The total inflow volume of Litani River to Karaoun reservoir between January and August was  $407.10^6 \text{ m}^3$  for the year 2012 and  $320.10^6 \text{ m}^3$  for the year 2013. Higher inflow rates in January 2013 (Figure 2b) led to higher water levels between January and March 2013 (Figure 2a). Between mid-March and July the water level was comparable in both years but the withdrawal volume was smaller in 2013.

### ***3.2 Physical-chemical parameters***

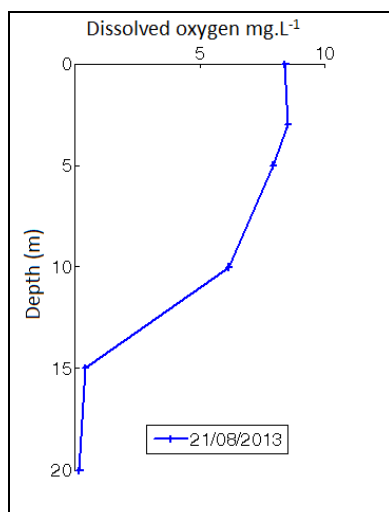
Water transparency was low; it ranged between 0.5 and 2.5 m (Figure 3c) and showed little variation throughout the study period. Dissolved oxygen ranged from 4 to 14 mg/l (oversaturation by 55%), at both measured depths 0.4 and 4 m (Figure 3d). This oversaturation was not correlated to phytoplankton concentrations. Oversaturation was observed for both low and high phytoplankton biomasses (Figure 3e). The vertical dissolved oxygen profile performed in 18 August (Figure 4) showed anoxia at 20 m and a drop in oxygen concentration between 10 and 15 m representing the oxycline depth during the summer season. Specific conductivity in Karaoun Reservoir was only measured twice during the study period. It ranged between 405 and 490  $\mu\text{S}/\text{cm}$ . Subsurface chlorophyll-a concentration ranged between  $6 \mu\text{g}\cdot\text{L}^{-1}$  (20 November) and  $226 \mu\text{g}\cdot\text{L}^{-1}$  (07 November) in 2012 (Figure 3e). It also reached a comparable maximum concentration of  $214 \mu\text{g}\cdot\text{L}^{-1}$  (08 July) in 2013 (Figure 3e).



**Figure 3** Variation of a) water level, b) water temperature, c) transparency, d) dissolved oxygen concentration at 0.4 and 4-m depths and e) subsurface chlorophyll-a concentration at  $S_m$  in Karaoun Reservoir in 2012 and 2013

Karaoun Reservoir was at its full capacity in mid May 2012 (Figure 3a). Due to continuous withdrawals, the water level decreased by 22 m until mid-November 2012. Water level then increased in winter to reach its maximum capacity in May 2013 where an overflow occurred for one week. Surface water temperature showed high seasonal variations at Karaoun Reservoir during the study period (Figure 3b). The reservoir was warm monomictic in both 2012 and 2013. Thermal stratification was already established in May, strong persistent stratification continued between June and July, then stratification started to debilitate at the end of July. The water temperature difference between the 1 and 16-m depths ranged from 0 °C in November and December 2012 to more than 10 °C in July 2012 and 2013 (Figure 3b).





**Figure 4 Vertical profile of dissolved oxygen at Karaoun Reservoir on 21 August 2013.**

Ammonium concentration was always below detection level while nitrate concentration reached a maximum of  $0.47 \text{ mg N.L}^{-1}$  (on 16 October 2012) and a minimum of  $0.02 \text{ mg N.L}^{-1}$  (on 01 October 2012) (Table 2).

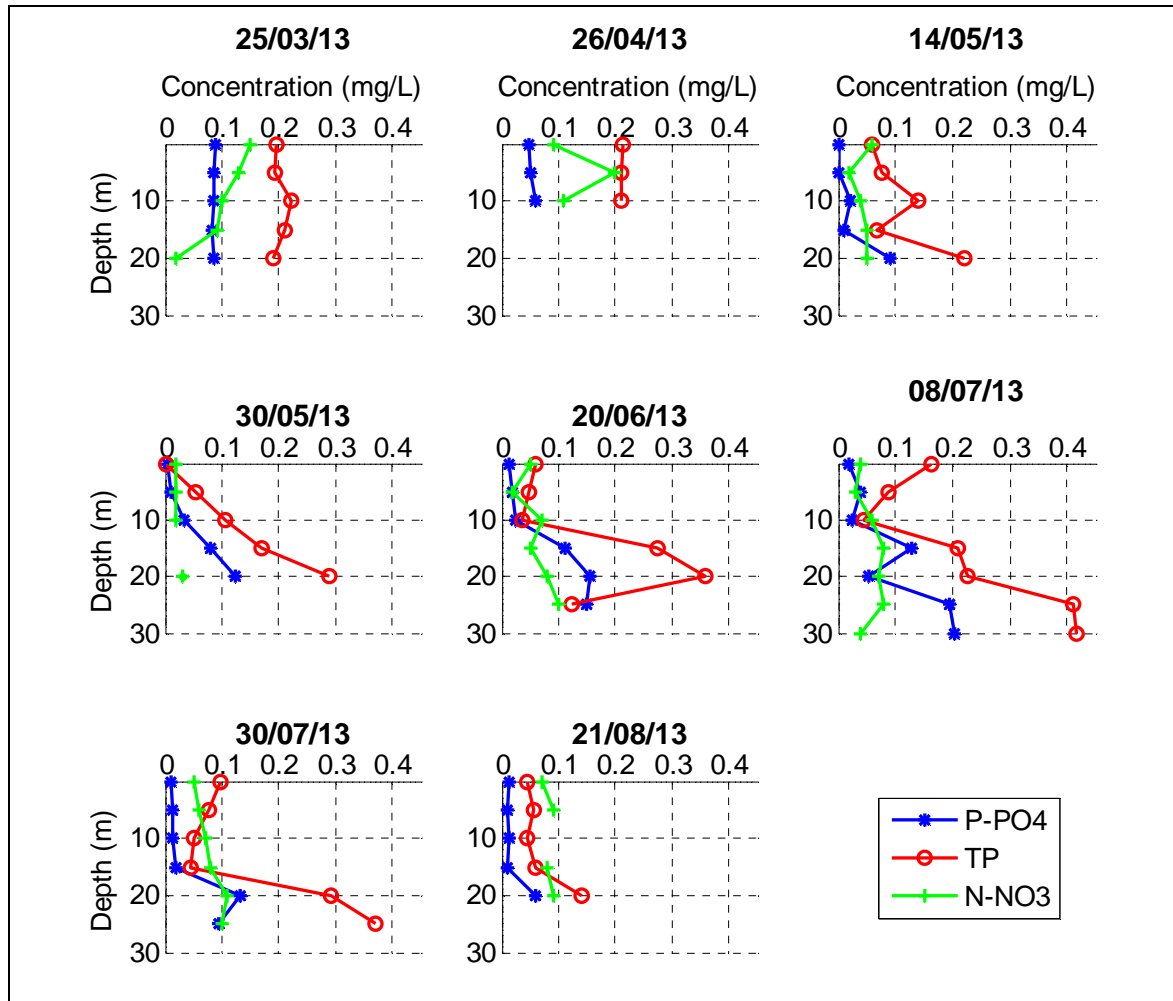
**Table 2 Subsurface nutrient measurements at Karaoun Reservoir in 2012, (-: not measured).**

Date	N-NO <sub>3</sub> (mg N/L)	P-PO <sub>4</sub> (mg P/L)	Total P (mg P/L)
07/06/2012	-	$4.7 \times 10^{-3}$	0.042
19/06/2012	-	$1.19 \times 10^{-2}$	0.034
15/07/2012	0.16	-	-
14/08/2012	0.05	-	0.039
28/08/2012	-	$2.34 \times 10^{-3}$	0.035
12/09/2012	0.05	$4.76 \times 10^{-3}$	0.149
01/10/2012	0.04	$2.71 \times 10^{-3}$	0.020
16/10/2012	0.47	$1.19 \times 10^{-3}$	0.025
07/11/2012	0.02	$1.19 \times 10^{-3}$	0.132
20/11/2012	0.03	$2.67 \times 10^{-3}$	0.028

In 2013, ammonium concentration always remained below detection level. At all measurement depths and during the whole monitored period, nitrate concentration did not exceed  $0.2 \text{ mg N.L}^{-1}$  (Figure 5). At the beginning of the 2013 campaigns on 25 March, the

nitrate profile showed higher concentrations in the epilimnion than in the hypolimnion. Nitrate concentrations then decreased gradually at the surface and became higher in the hypolimnion by the end of the study period in 2013.

The total phosphorus concentration reached a maximum of 0.15 mg P.L<sup>-1</sup> (on 12 September 2012) and a minimum of 0.02 mg P.L<sup>-1</sup> (on 01 October 2012) while orthophosphate concentration reached a maximum of 0.012 mg P.L<sup>-1</sup> (on 19 June) and a minimum of 0.001 mg P.L<sup>-1</sup> (on 16 October and 07 November 2012, Table 2).



**Figure 5** Vertical profiles of orthophosphate (P-PO<sub>4</sub>), total phosphorus (TP) and nitrate (N-NO<sub>3</sub>) concentrations at S<sub>M</sub> in Karaoun Reservoir in 2013.

In 2013, the maximum values of both total phosphorus and orthophosphate concentrations were recorded near the bottom of the reservoir (Figure 5). Through different depths in the water column and during the whole monitored period, total phosphorus reached maxima of 0.42 mg P.L<sup>-1</sup> (08 July 2013, 30 m at S<sub>M</sub>) and minima of 0 mg P.L<sup>-1</sup> (30 May 2013, subsurface at S<sub>M</sub>) while orthophosphate reached maxima of 0.204 mg P.L<sup>-1</sup> (08 July 2013, 30 m at S<sub>M</sub>) and minima under detection limit (30 May 2013, subsurface at S<sub>M</sub>). The relative vertical distribution of orthophosphate paralleled the distribution of total phosphorus within the water column (Figure 5). At the beginning of the survey in March 2013, both total phosphorus (TP)

and orthophosphate (PO<sub>4</sub>) vertical profiles were homogeneous through the water column. Phosphorus concentrations decreased gradually in the top 10 m to below 0.1 mg P.L<sup>-1</sup> in May 2013, and stayed lower than 0.1 mg P.L<sup>-1</sup> until the end of the survey except for the end of May 2013 and beginning of July 2013 where an increase in total phosphorus concentration at the subsurface occurred. At the end of the survey in August 2013, phosphorus concentration in the top 20 m reached its lowest values.

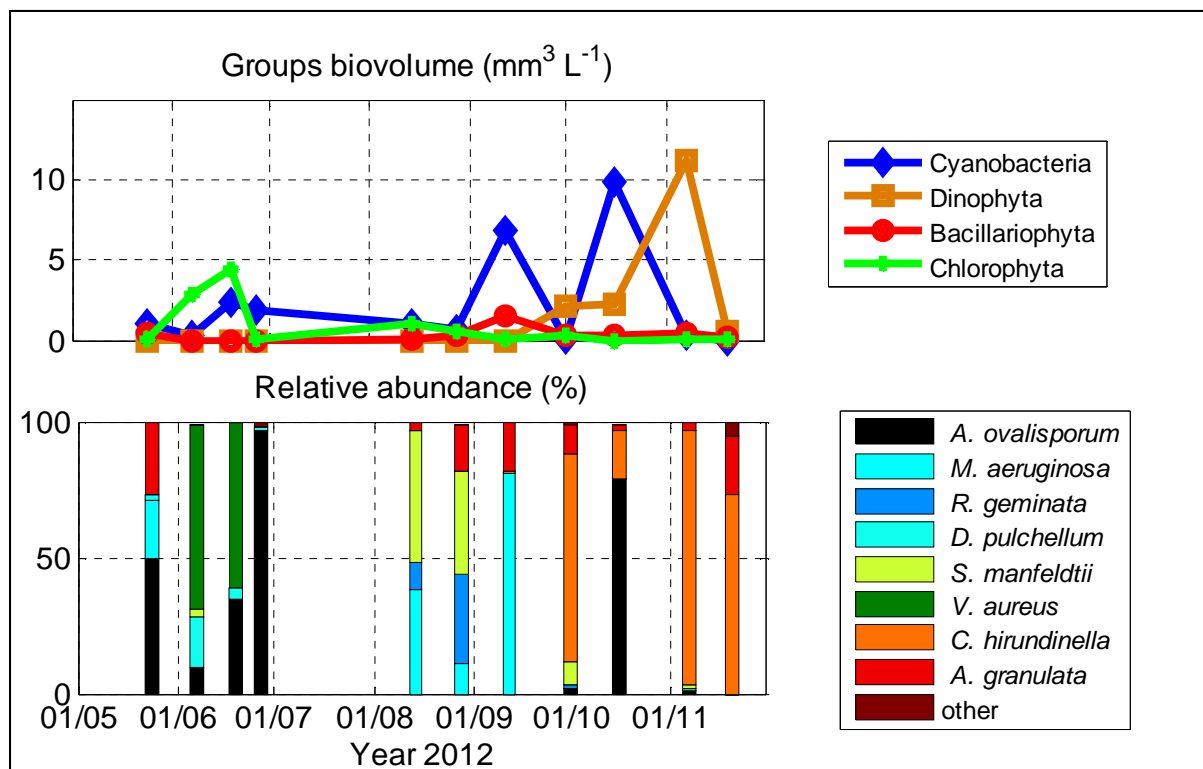
### ***3.3 Phytoplankton composition, biovolumes and seasonal succession***

The phytoplankton community structure varied greatly from May to November 2012 and March to August 2013, the period when phytoplankton cell viability was examined. A total of 30 phytoplankton species were identified (Table 3) in the water samples examined throughout both years. Chlorophytes contributed with the highest number of species (11) followed by Cyanobacteria (10), Bacillariophyta (8) and Dinophyta (1).

Throughout the study period, the total biovolume ranged from 0.3 to 12.4 mm<sup>3</sup> L<sup>-1</sup> (Figure 6e and Figure 7). The lowest total biovolume was recorded on March 2013 while the highest total biovolumes in summer and autumn, in October 2012 during cyanobacterium *Aphanizomenon ovalisporum* bloom at 12.4 mm<sup>3</sup> L<sup>-1</sup>, November 2012 during dinophytum *Ceratium hirundinella* bloom at 11.9 mm<sup>3</sup> L<sup>-1</sup> (Figure 6) and July 2013 during cyanobacterium *Microcystis aeruginosa* bloom at 12.3 mm<sup>3</sup> L<sup>-1</sup>.

The four phytoplankton groups were detected in both monitored years, but the pattern was not the same. Some groups were detected earlier but as an overall: Chlorophyta dominated in spring, cyanobacteria dominated in summer and early autumn, Dinophyta dominated in late autumn and diatoms occurred in low biovolumes in spring, summer and autumn.

Diatoms were present in low biovolumes throughout the study period; this group was dominated by *Melosira varians* and *Aulacoseira granulata* with a very different pattern in 2012 and 2013. *Aulacoseira granulata* occurred in both mixed and stratified water columns. *Melosira varians* was detected only at the end of April 2013 in low biovolume in a stratified water column, with high irradiance, high water level and water temperature of 16 °C. However, it declined after 2 weeks at the beginning of May 2013. *Aulacoseira granulata* was also detected at the end of April 2013 and it was detected frequently in low biovolumes between May and November 2012 in a wide range of water temperature, irradiance, nutrient availability, thermal stratification and water level conditions (Figure 6).



**Figure 6 Biovolumes of phytoplankton groups and relative abundance of phytoplankton species at the subsurface in Karaoun Reservoir in 2012.**

In 2012 and 2013, Chlorophyta were dominated by *Botryococcus braunii*, *Dictyosphaerium pulchellum*, *Volvox aureus*, *Staurastrum manfeldtii* and *Coelastrum microporum*. Only 3 species were detected in subsurface samples of 2012 (*Dictyosphaerium pulchellum*, *Volvox aureus*, *Staurastrum manfeldtii*). *Volvox aureus* dominated in June 2012 with the presence of *Dictyosphaerium pulchellum* in high irradiance, a stratified water column, a high water level and a water temperature of 24 °C. *Staurastrum manfeldtii* dominated in August with the cyanobacterium *Microcystis aeruginosa* in lower irradiance and water level, in a thermal stratification period with a water temperature of 26 °C (Figure 6).

In 2013, *Volvox aureus* was detected earlier at the end of April, not in similar conditions as in 2012, water temperature was below 18 °C. *Botryococcus braunii* and *Coelastrum microporum* were detected for the first time in Karaoun Reservoir and dominated at the beginning of May in high irradiance, a stratified water column, with a high water level and a water temperature of 19 °C. After the increase of water temperature to 23 °C and as well thermal stratification at the end of May, *Botryococcus braunii* and high biovolume *Coelastrum microporum* were replaced by *Staurastrum manfeldtii* that dominated and also occurred in low biovolumes in June. *Dictyosphaerium pulchellum*, *Kirchneriella obesa*, *Desmodesmus communis*, *Haematococcus pluvialis*, *Closterium acutum*, *Pediastrum duplex*, *Pediastrum boryanum* were also detected in Karaoun Reservoir in 2013 but in low biovolumes.

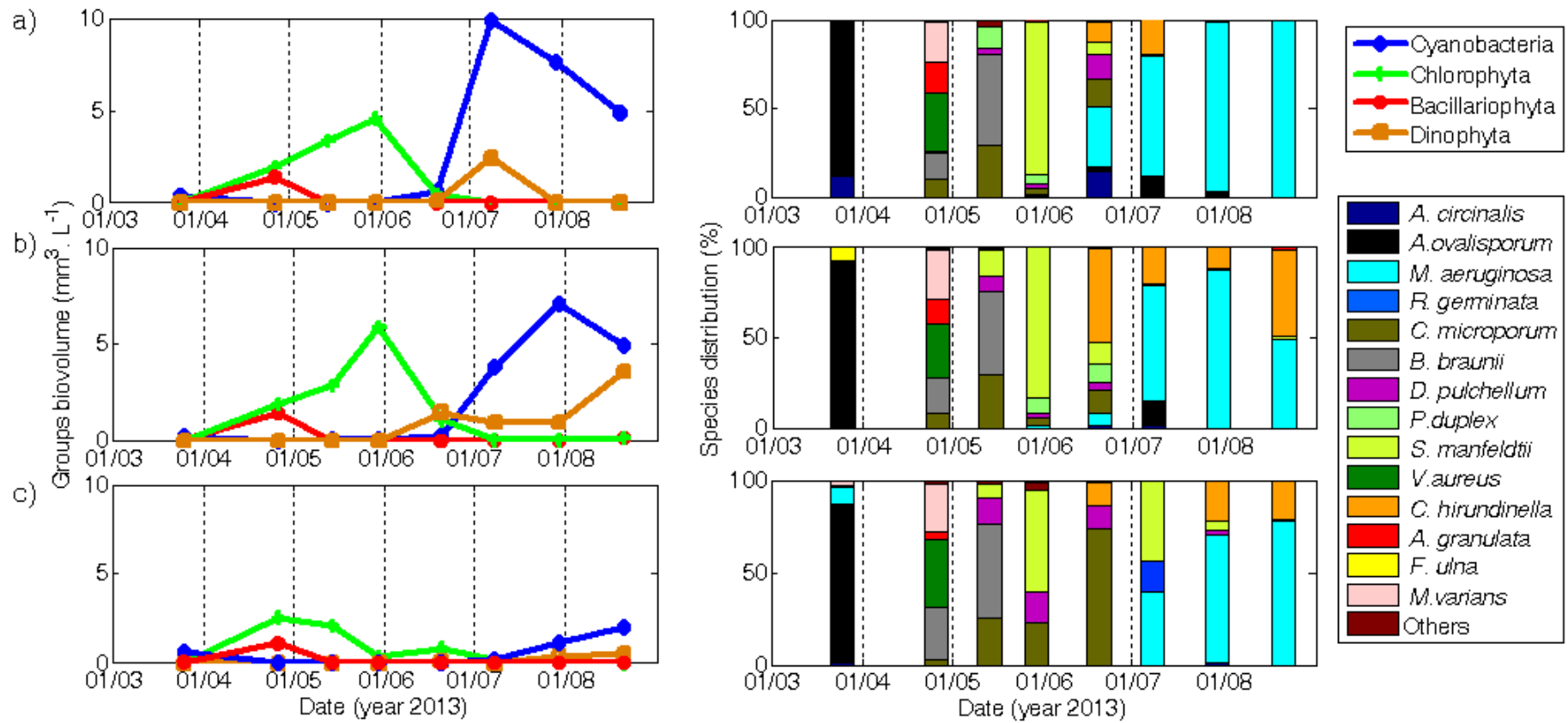


Figure 7 Variation of the biovolumes of phytoplankton groups and their species distribution at a) subsurface, b) 5m and c) 10 m.

1 Cyanobacteria group was dominated by *Aphanizomenon ovalisporum* and *Microcystis*  
2 *aeruginosa*. *Aphanizomenon ovalisporum* occurred in low biovolumes with Chlorophyta and  
3 diatoms between the end of May and the end of June during periods of high irradiance and  
4 water level, thermal stratification and surface water temperature ranging between 18 and 25  
5 °C. *Aphanizomenon ovalisporum* was then replaced by another subsurface bloom-forming  
6 cyanobacterium, *Microcystis aeruginosa*, that developed populations in August 2012  
7 (coinciding with *Staurastrum manfeldtii*) and had its biovolume peak in September 2012 in a  
8 period of lower irradiance and water level, weaker stratification and subsurface water  
9 temperature of 25 °C. In October 2012, after the decline of *Microcystis aeruginosa*,  
10 *Aphanizomenon ovalisporum* emerged as the dominant genus and had its biovolume peak.  
11 Then the decline in the *Aphanizomenon ovalisporum* population coincided with the  
12 development of *Ceratium hirundinella*.

13 In 2012, *Anabaena circinalis* were not detected; it was first detected in low biovolumes in  
14 March 2013 (coinciding with *Aphanizomenon ovalisporum* that contained akinetes, Figure 6)  
15 and then in June 2013 (coinciding with *Microcystis aeruginosa*, Chlorophyta and *Ceratium*  
16 *hirundinella*).

17 *Microcystis aeruginosa* was most prevalent at subsurface and was detected earlier in June and  
18 had its biovolume peak at the end of July in a stratified water column, with a high water  
19 level, high solar irradiance and temperature ranging between 22 and 24 °C.

20 *Ceratium hirundinella* was the only dinoflagellate observed in Karaoun Reservoir in 2012 and  
21 2013. Biovolume peaks of *Ceratium hirundinella* occurred at the beginning of November  
22 2012 during destratified water column, low water level, low irradiance and water temperature  
23 of 20 °C. The increases in biovolume of *Ceratium hirundinella* between October and  
24 November 2012 coincided with a decrease in temperature and irradiance. *Ceratium*  
25 *hirundinella* was then detected in June, July and August 2013 in a stratified water column,  
26 with the highest irradiance (240 W.m<sup>-2</sup>), a high water level and high water temperatures  
27 ranging between 20 and 25 °C. However, it was detected in small biovolumes during this  
28 period and was most prevalent at 5 m depth.



Table 3 List of phytoplankton species identified in Karaoun Reservoir in 2012 and 2013

Cyanobacteria	Chlorophyta	Diatoms	Dinophyta
<i>Oscillatoria tenuis</i>	<i>Kirchneriella obesa</i>		
<i>Microcystis aeruginosa</i>	<i>Dictyosphaerium pulchellum</i>	<i>Diatoma vulgare</i>	
<i>Microcystis ichthyoblabe</i>	<i>Desmodesmus communis</i>	<i>Fragilaria ulna</i>	
<i>Microcystis viridis</i>	<i>Botryococcus braunii</i>	<i>Navicula menisculus</i>	
<i>Microcystis botrys</i>	<i>Haematococcus pluviialis</i>	<i>Navicula cryptocephala</i>	
<i>Aphanizomenon ovalisporum</i>	<i>Closterium acutum</i>	<i>Cymatopleura elliptica</i>	<i>Ceratium hirundinella</i>
<i>Anabaena spiroides</i>	<i>Coelastrum microporum</i>	<i>Nitzschia gracilis</i>	
<i>Anabaena circinalis</i>	<i>Pediastrum duplex</i>	<i>Melosira varians</i>	
<i>Radiocystis geminata</i>	<i>Volvox aureus</i>	<i>Aulacoseira granulata</i>	
<i>Pilgeria brasiliensis</i>	<i>Staurastrum manfeldtii</i>		
	<i>Pediastrum boryanum</i>		



### 3.4 Zooplankton community

Zooplankton species dominated in Karaoun Reservoir when the cyanobacterial biomass was low. Nine zooplankton species were found in the lake in 2012 and 2013: two rotifers (*Asplanchna periodonta* and *Keratella cochlearis*), three cladocerans (*Chydorus sphaericus*, *Daphnia magna* and *Moina rectirostris*) and four copepods (*Eudiaptomus drichii*, *Mesocyclops ogunnus*, *Mr. leuckarti* and *cyclopoid nauplii*). No studies on the zooplankton microfauna have been done in Lebanon so far. This list is not exhaustive, more frequent monitoring must be done in the coming years to close the gap and understand the fluctuations of the zooplankton community.

## 4 Discussion

Diatoms in Karaoun Reservoir were rare, not only in terms of biomass, but also in terms of species number, contributing to low biovolumes during the study period. The reservoir was not monitored in winter, a season that can be favourable for the growth of this group. There was no limitation in phosphorus concentration in spring 2013. Silica limitation is possible but since it was not measured it cannot be confirmed. During persistent thermal stratification *Aulacoseira* species tend to sink out of the euphotic zone at a speed of 0.95 m.d<sup>-1</sup> (Sherman et al., 1998). In spring and summer, Karaoun Reservoir was stratified; this can partially explain why diatoms biomass which was mainly represented by *Aulacoseira granulata* was low at the top 10 m in 2012 and 2013.

Green algae blooms occurred in late spring at temperatures lower than 22 °C and high phosphorus concentrations in 2013. Thermal stratification in Karaoun Reservoir was already established in May 2013. This might explain the dominance of vertically migrating green algae like *Botryococcus braunii* that becomes buoyant by producing and accumulating oil (Niehaus et al., 2011; Weiss et al., 2012) and large colonies of *Volvox aureus* that can rise by 3.6 m in one hour (Sommer and Gliwicz, 1986). In 2013, green algae were able to dominate the reservoir in spring, for two months (April and May 2013) taking advantage of nutrient availability and favourable water temperature. At the end of May nutrient concentration decreased, most probably due to its consumption by green algae and a decrease in the Litani River inflow, the main nutrient influx to Karaoun Reservoir (Fadel et al., 2014a). Nutrient limitation together with the increase in water temperature could have resulted in the decline of green algae and their replacement by cyanobacteria.

In the catchment of Karaoun Reservoir, there were at the time of the study one million habitants, 570 km<sup>2</sup> of cultivated farmlands and several industries, mostly food, paper, tanning, plastic, cosmetic and detergent industries (Arif, 2013). This deteriorates the water quality of Karaoun Reservoir through agricultural effluents (pesticides and fertilizers), untreated industrial and municipal wastewaters, and solid and liquid wastes dumped into the Litani River (ELARD, 2011). In Summer, about 1.3 mg/L of NO<sub>3</sub>, 0.7 mg/L of NH<sub>3</sub>, and 1.2 mg/L of PO<sub>4</sub> enter Karaoun Reservoir through Litani River (USAID, 2012b). Wetland systems were designed to remove waste water loads coming from the watershed before they enter Karaoun reservoir (USAID, 2012a). The removal efficiency is 10 to 20% of total wastewater loads. Nutrient removal in winter is less effective due to the heavier loading of nutrients after storm events and the negative effect of low temperatures on microbial activity that reduces nitrogen levels (USAID, 2012b). These contamination sources along the Litani River and its

tributaries are the main causes of the high nutrient concentrations in Karaoun Reservoir leading to algal blooms.

Results of 2012 -2013 campaigns showed that buoyant cyanobacteria (*Aphanizomenon ovalisporum* and *Microcystis aeruginosa*) dominated Karaoun Reservoir between late spring and early autumn. Environmental conditions and their physiology allow them to dominate on other phytoplankton groups in Karaoun Reservoir. Unlike diatoms which tend to sink during stratification (Huisman et al., 2004), positively buoyant cyanobacteria like *Aphanizomenon ovalisporum* and *Microcystis aeruginosa* can regulate their location in the water column, floating upwards during weak or moderate mixing (Reynolds, 2006b).

The water level decreases continuously from the beginning of May (the reservoir was full) until the end of December where the reservoir volume usually reaches 25 % of the capacity. The decrease in water volume in the reservoir is mainly due to withdrawals for hydro-power generation. However, these withdrawals do not destroy the stable thermal stratification. Rainfall on the reservoir catchment has an important role in disrupting thermal stratification due to washout (Domis et al., 2013), but it is not the case of Karaoun. Precipitation events in Karaoun Reservoir become rare after the end of April. Segura et al. (2013) showed that the competitive ability of large cyanobacteria with gas vesicles was highest under low flushing rates. Romo et al. (2013) suggested that longer water residence time in the dry season increased total *Microcystis aeruginosa* populations and microcystin production in the lake water. The decrease in rainfall in Mediterranean lakes decreases the flushing rates and increases the dominance of cyanobacteria.

*Aphanizomenon ovalisporum* blooms develop at the beginning of spring and autumn while *Microcystis aeruginosa* blooms at higher temperature in summer. *Aphanizomenon ovalisporum* optimal temperature is lower than that of *Microcystis aeruginosa* (optimal temperature: 28 – 32 °C; minimal temperature: 20 °C); this may explain why *A. ovalisporum* is detected in spring and autumn (Imai et al., 2009; Yamamoto, 2010).

In 2012, *Aphanizomenon ovalisporum* bloomed in June and October while *Microcystis aeruginosa* bloomed in August. However, in 2013, *Aphanizomenon ovalisporum* occurred for a short period in March and it was then replaced by chlorophyta species, then *Microcystis aeruginosa* was detected earlier in June. In comparison with year 2012, surface water temperature was higher in May 2013 and lower in June and July. This variation in water temperature and thermocline location with other parameters like nutrient availability, solar irradiation and discharge management may have prevented a steady growth phase *Aphanizomenon ovalisporum* in May and June 2013 and supported an earlier bloom of *Microcystis aeruginosa*.

*Microcystis aeruginosa* can outpace *Aphanizomenon ovalisporum* at high temperature due to its higher growth rate and competition for light. Although nitrogen limitation promotes *Aphanizomenon ovalisporum*, we think that it is a minor controlling factor in comparison to water temperature and the competitive eco-physiology of *Microcystis aeruginosa* in Karaoun Reservoir. Nitrogen limitation is not enough for *Aphanizomenon ovalisporum* to outgrow *Microcystis aeruginosa* that can also survive in low nitrogen availability conditions. Imai et al (2009) showed that *Microcystis aeruginosa* was able to grow in nitrogen-limited culture and its concentration was half the concentration reached after 12 days in culture without nutrient limitation. The availability of light has a major impact on the dynamics and structure of phytoplankton communities. *Microcystis* has a slightly lower critical light intensity than

*Aphanizomenon*. Huisman et al. (1999) demonstrated that when they were placed in mixed culture to compete for light, *Microcystis* strains outpaced *Aphanizomenon* ones (Huisman et al., 1999). Laboratory experiments on the growth rate of *Microcystis* and *Aphanizomenon* strains of Lake Mendota (Wisconsin) showed that *Microcystis* had an apparent doubling time of 2 days in culture while *Aphanizomenon* needed 5.5 days (Konopka and Brock, 1978). Other laboratory experiments showed that *Aphanizomenon ovalisporum* has a maximum growth rate below  $0.4 \text{ day}^{-1}$  (Hadas et al., 2002; Mehnert et al., 2010), lower than that of *Microcystis aeruginosa* that can reach  $0.8 \text{ day}^{-1}$  (Chu et al., 2007; Zheng et al., 2008).

In Karaoun Reservoir, *Aphanizomenon ovalisporum* blooms occurred in spring and autumn at surface water temperature below  $22 \text{ }^{\circ}\text{C}$ , while a *Microcystis aeruginosa* blooms occurred in summer at  $24 - 27 \text{ }^{\circ}\text{C}$ . This succession can be explained by the fact that the growth of *Microcystis* sp. stops below  $20 \text{ }^{\circ}\text{C}$  and is optimal at water temperatures ranging from  $27.5$  to  $32 \text{ }^{\circ}\text{C}$  (Imai et al., 2009; Robarts and Zohary, 1987). In contrast, *Aphanizomenon* sp. has an optimal growth temperature ranging from  $15$  to  $28 \text{ }^{\circ}\text{C}$  (Robarts and Zohary, 1987) and is able to outgrow *Microcystis* sp. at lower temperatures (Miller et al., 2013). The competitive eco-physiology of *Microcystis aeruginosa* at high temperature could explain why *Aphanizomenon ovalisporum* blooms never occurred at water temperatures higher than  $23 \text{ }^{\circ}\text{C}$  in Karaoun Reservoir.

*Ceratium hirundinella* was the only dinoflagellate detected in Karaoun Reservoir. It tended to develop in late summer 2013 during stratified conditions and had its biovolume peak in late autumn 2012 in a mixed water column after a decrease by 22 m in the water level. This does not agree with *C. hirundinella* populations in the close Lake Tiberias (Israel) that develop in winter and spring (Pollinger and Hickel, 1991). However, similarly to some Mediterranean reservoirs in Spain (Pérez-Martínez and Sánchez-Castillo, 2001), *C. hirundinella* dominated the phytoplankton assemblages in autumn and during both in stratified and mixed periods (Pérez-Martínez and Sánchez-Castillo, 2001). Matsumura-Tundisi et al. (2010) attributed *Ceratium* blooms in Billings Reservoir, Brazil, to the mixing and turbulence of the water column. In Karaoun Reservoir, *C. hirundinella* bloomed in November 2012 during mixing conditions after an increase in inflow volumes. An increase in nutrient concentrations due to inflows and mixing favoured by low water level could have removed *Ceratium* cysts from the sediment surface and promoted its growth as in Billings Reservoir.

Between June and July 2013, *C. hirundinella* in Karaoun Reservoir was more prevalent at 5-m depth rather than the surface due to the high irradiance that occurred in these months. Whittington et al. (2000) revealed that *Ceratium hirundinella* in Chaffey Reservoir, a subtropical reservoir in northern New South Wales, Australia, formed subsurface accumulations at depths only when the light intensity was favourable for photosynthesis and cell growth ( $212\text{--}552 \text{ } \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , equivalent to  $53\text{--}138 \text{ W.m}^{-2}$ ). At higher incident irradiance, *C. hirundinella* migrated downwards, avoiding high-light-induced.

The decrease in water level can indirectly affect the physical-chemical and biological characteristics of freshwater bodies, mainly through mixing enhancement (Valdespino-Castillo et al., 2014). Extreme decreases in reservoir water level can affect the thermal structure of the water column (Zohary and Ostrovsky, 2011). Our results show that in October 2012 (Figure 3), when the water level decreased by more than 15 m due to low inflow and continuous withdrawal, thermal stratification was weakened and the water column was mixed. Naselli-Flores (2003) made a similar observation in Lake Arancio that has a maximum depth of 29 m where strong wind resulted in destratification after a 15 m decrease in water level

(Naselli-Flores, 2003). The amount of energy required to mix the water column increases as the water level increases (Kling, 1988). The increase in maximum depth of Lake Elsinore from 5 to 10.5 m had a tremendous effect on the stability of its water column that increased as much as 4 times, from 18 J/m<sup>2</sup> to more than 60 J/m<sup>2</sup> (Lawson and Anderson, 2007).

Water level fluctuation affects nutrient profiles in reservoirs. External nutrient loading usually decrease with water level because of the decrease in inflows. However, the decrease in water level increases nutrient concentrations in the hypolimnion (Zohary and Ostrovsky, 2011) and can favour upward fluxes of sediment nutrients by internal waves (MacIntyre et al., 1999).

High water level and nutrient depletion at the epilimnion may have favoured the dominance of cyanobacteria on other phytoplankton groups in Karaoun Reservoir. During periods of high water level, thermal stratification is more stable and the hypolimnion is anoxic. Phosphorus is consumed by phytoplankton in the epilimnion and its concentration increases in the hypolimnion due to internal release from the sediments during anoxia (Nurnberg, 1984). This gives advantage to cyanobacteria that can overcome the epilimnetic phosphorus limitation during stratification due to their internal nutrient storage. They have a substantial storage capacity for phosphorus that allows them to increase their biomass by up to 32 folds (Chorus and Bartram, 1999). Even after depletion of this stock, cyanobacteria can migrate downwards to the metalimnion, where nutrient limitation is less severe (Camacho, 2006).

## 5 Conclusion

The information presented in this study increases the knowledge about phytoplankton dynamics in reservoirs with highly fluctuating water levels and in the Mediterranean region, and contribute to a safer water usage in Karaoun Reservoir. The phytoplankton community of Karaoun Reservoir has a seasonal pattern of spring chlorophyta and cyanobacteria blooms, summer-early autumn cyanobacterial blooms and late autumn dinoflagellate blooms. Variations in water temperature, stratification, irradiance and nutrient availability and water level result in different successions. Thermal stratification established in spring reduces the growth of diatoms that sink out of the euphotic zone. High nutrient availability during spring promotes green algae blooms at high water level and light intensity, stratified conditions and surface water temperatures lower than 22 °C. The increase in surface water temperature (over 25 °C) and high irradiance conditions promote blooms of cyanobacterium *Microcystis aeruginosa* blooms in summer. Despite different growth conditions in other lakes and reservoirs, cyanobacterium *Aphanizomenon ovalisporum* dominated at surface temperatures lower than 23 °C in weakly stratified conditions in early autumn and dinoflagellate *Ceratium hirundinella* dominated in mixed conditions, at low light intensity in late autumn at 19 °C. These different growth conditions might be a result of the high water fluctuation of the reservoir.

Reservoir managers in Karaoun Reservoir can take advantage of this repetitive succession pattern and reduce the nutrient influx from the upstream watershed to the reservoir to avoid the long steady state of these blooms.

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