

First ecological evaluation of the ancient Balkan Lake Megali Prespa based on plankton

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The ecological water quality of one of the ancient world lakes, Lake Megali Prespa, is examined for the first time on the basis of its plankton communities. During the study period (October–November 2008) the lake's plankton showed signs of a moderate ecological water quality. This quality was reflected in: i) phyto- and zoo-plankton species composition which was characteristic of eutrophic conditions, ii) phytoplankton functional groups typical for eutrophic waters, iii) phytoplankton biovolume values, iv) the occurrence of blooms of several nanoplanktic species (cyanobacteria, diatoms, prymnesiophytes and cryptophytes) and v) the presence of known potentially toxin-producing cyanobacterial species. These plankton characteristics suggest a link to eutrophication and water quality degradation in the ancient Lake Megali Prespa. Water managers are advised to consider the data on plankton community and the species exchange between the lakes Megali and Mikri Prespa (high overlap of phyto- and zoo-plankton species) in their strategies for the conservation of this precious ancient lake of the world.

Key words: large ancient lake, phytoplankton, zooplankton, eutrophic, ecological water quality.

INTRODUCTION

Eutrophication of freshwater systems is a worldwide phenomenon and one of the most serious processes degrading their water quality over past decades. With the expected increase in this phenomenon in the future, due to economic development and global warming (Jeppesen *et al.*, 2007) there is a growing need for the conservation and restoration of freshwater systems. This becomes even more important in the case of systems of great importance such as ancient world lakes (estimated age between 2 and 35 million years) which are about a dozen and have long been recognized as unique ecosystems in terms of their exceptional high biodiversity and levels of endemism (Martens, 1997). In Europe, ancient lakes are restricted to the Balkan region (Albrecht & Wilke, 2008). With an estimated age of 2-5 million years, the Balkan lakes Megali Prespa, Ohrid, Mikri Prespa and Maliq (it has

been drained) are considered as the oldest lakes in Europe and of the few long-lived lakes worldwide. These four lakes used to constitute the European lake group of Dessarettes formed during the Pliocene (Stanković, 1960).

Lake Megali Prespa (40°45' N, 21°01' E) is a transboundary lake shared by Greece, the Former Yugoslavian Republic of Macedonia and Albania. It is a monomictic lake located at ~850 m above sea level with a surface area of ~266 km² (Kagalou & Leonardos, 2009), maximum depth ~50 m, and mean depth ~20 m (Löffler *et al.*, 1998). In the light of its great importance for nature conservation, since it harbors an important number of endemic species (Crivelli *et al.*, 1997; Albrecht & Wilke, 2008), Lake Megali Prespa has been designated as part of the largest national park in Greece and is covered by the Ramsar Convention (<http://www.ramsar.org>). However, over the last few decades there has been increasing concern over its ecological status due to ongoing eutrophication and water level decline (Löffler *et al.*, 1998;

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Matzinger *et al.*, 2006).

The implementation of the Water Framework Directive (WFD) (2000/60/EC) (European Parliament, Council, 2000) in 2000 was a decisive step towards the protection of water quality in Europe. In the WFD, biological parameters, such as phytoplankton are considered as key elements for the assessment of the ecological status of lakes and reservoirs. Phytoplankton is a good indicator of change in ecological water quality because of its sensitivity and dynamic responses to changes in the surrounding environment (Padisák *et al.*, 2006). Though zooplankton is not included as an element to be assessed in the WFD, it is an important component of lake food webs (Lampert & Sommer, 1997).

In Greece, several lakes and reservoirs have been reported with severe symptoms of eutrophication (e.g. Vardaka *et al.*, 2005; Moustaka-Gouni *et al.*, 2007; Michaloudi *et al.*, 2009). Although comprehensive data on plankton communities are available for some freshwater ecosystems in the Balkan Peninsula, for other lakes the corresponding knowledge is lacking. Present knowledge concerning the phytoplankton of Lake Megali Prespa is based only on qualitative data (Schröder, 1921; Löffler *et al.*, 1998), and data on phytoplankton biomass for 2001–2003 have been derived indirectly from chlorophyll measurements (Petrova *et al.*, 2008). Data on zooplankton are also sparse (Serafimova-Hadzisce, 1958).

This work represents a first attempt to examine the current ecological water quality of Lake Megali Prespa on the basis of its plankton community (species composition, abundance and biovolume).

MATERIALS AND METHODS

Two samplings were conducted at the deepest point (~30 m) of the Greek part of the lake in October and in November 2008. October represents the last stage of seasonal phytoplankton development as it occurs in neighboring Lake Volvi, Greece (Moustaka-Gouni, 1993). This study period is considered as appropriate for the assessment of lake ecological status since early-autumn phytoplankton community of a lake integrates the preceding successional events (see Padisák *et al.*, 2006).

A Niskin sampler (2 L) was used for collecting water samples for phyto- and zoo-plankton analyses. Integrated water samples for phytoplankton analyses were collected from the surface to the end of the euphotic zone (maximum depth 7 m). Sub-samples were

preserved in Lugol solution. For zooplankton, at least 30 L of water were collected from the whole water column and filtered through a net with a mesh size of 50 µm. The collected samples were preserved in 4% formalin. The euphotic zone was calculated as 2.5 times the Secchi depth, which was 2.5 m in October exhibiting a slight increase at 2.8 m in November.

For phytoplankton analysis fresh and preserved samples were examined under a light inverted microscope (Nikon Eclipse SE 2000) and species were identified using taxonomic keys. Counts were performed using Utermöhl's (1958) sedimentation method. At least 400 phytoplankton individuals were counted in each sample. For the colonial cyanobacterium *Microcystis aeruginosa*, the number of cells per colony (y) was determined using the equation: $y = 1.475 \times (\text{colony diameter})^{1.55}$ according to Moustaka (1988). Since colonies were not always spherical, the colony diameter used in the final computation was the diameter of a sphere with an equal colony volume according to Reynolds & Jaworski (1978). Mean cell or filament volume estimates were calculated using appropriate geometric formulae (Hillebrand *et al.*, 1999). For this, the dimensions of 30 individuals (cells, filaments or colonies) of each species were measured using tools of a digital microscope camera (Nikon DS – L1). Species and taxonomical groups comprising more than 10% (v/v) of the total phytoplankton biovolume were considered to be dominant. Functional phytoplankton classification (Reynolds *et al.*, 2002 supplemented by the review of Padisák *et al.*, 2009) was applied. For assessing the ecological status of the lake on the basis of phytoplankton, the taxonomic and functional composition, abundance (blooms) and biomass (total biovolume) were examined as outlined by the WFD (JRC European Commission, 2009). Zooplankton species were identified in fresh and preserved samples using taxonomic keys under a light microscope (Leitz Laborlux S). For each sample (total volume of 100 ml), five counts of 1 ml subsamples were made on a Sedgwick-Rafter cell to estimate abundance. Biomass was calculated using individual dry weight data and length-weight relationships (McCauley, 1984; Michaloudi, 2005).

In order to evaluate differences in species composition between the two months the non parametric chi-square test was used.

RESULTS AND DISCUSSION

A total of 46 phytoplankton taxa were identified from Lake Megali Prespa in the study period (see online

supplementary material, Table S1), 38 out of which are reported for the first time. Chlorophytes (13), cyanobacteria (13) and diatoms (7) were the most diverse taxonomical groups. Many of the lake's phytoplankton species have a cosmopolitan distribution (e.g. *Microcystis aeruginosa*, *Aulacoseira granulata*, *Synedra acus*, *Rhodomonas minuta*, *Chroococcus limneticus*, *Pediastrum duplex*) and are reported as dominants in eutrophic freshwaters (Padisák et al., 2009). Sixty-seven per cent of the lake's phytoplankton species have also been recorded in neighboring Lake Mikri Prespa (Tryfon et al., 1994). Among the cyanobacteria, the known toxin-producing species *Microcystis aeruginosa*, *Aphanizomenon flos-aquae* and *Planktothrix* sp. were observed in low numbers (< 1 individual ml^{-1}). These

species have been reported as dominant in toxic water blooms in many eutrophic freshwaters of the Mediterranean region (e.g. Cook et al., 2004) and Europe (Sivonen & Jones, 1999). The high number of cyanobacteria species found in this study is also a feature of eutrophic lakes (e.g. Lake Mikri Prespa; Tryfon & Moustaka-Gouni, 1997).

Phytoplankton biovolume was $5.8 \text{ mm}^3 \text{ l}^{-1}$ in October and decreased to $1.9 \text{ mm}^3 \text{ l}^{-1}$ later in November (Fig. 1A). These values are indicative of eutrophic conditions (Smith, 2003) throughout the whole euphotic zone, that was limited to 7 m, and are similar or lower than the ones reported in the shallow sister Lake Mikri Prespa in autumn 1990-1991 (Tryfon & Moustaka-Gouni, 1997). Mean phytoplankton bio-

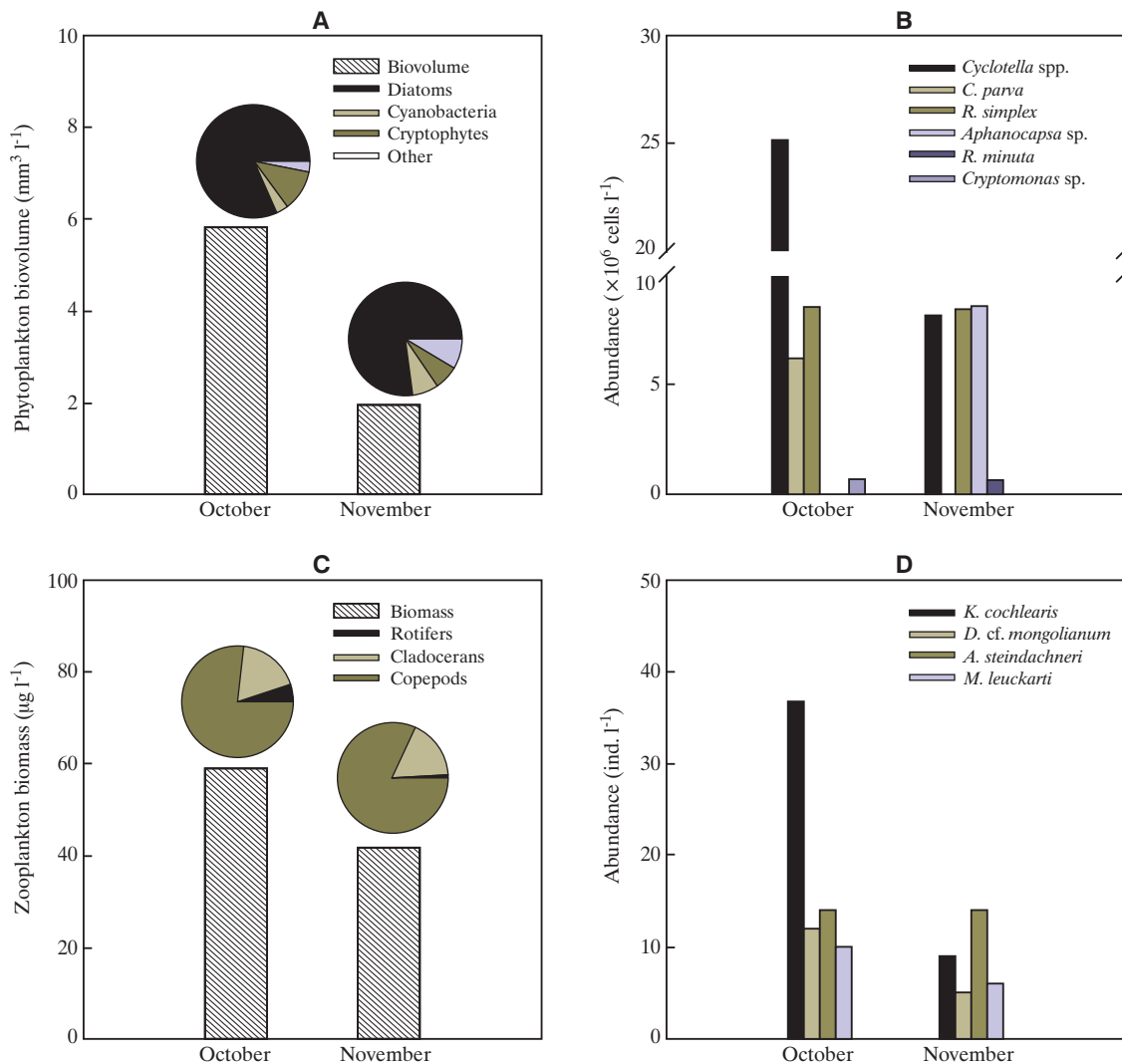


FIG. 1 Phyto- and zoo-plankton data in October and November 2008. (A) Total phytoplankton biovolume (bars) and the contribution of taxonomic groups to biovolume (inserted pies), (B) most abundant phytoplankton species, (C) total zooplankton biomass and the contribution of zooplankton groups to biomass (inserted pies) and (D) most abundant zooplankton species.

lume ($3.85 \text{ mm}^3 \text{ l}^{-1}$) was higher than the boundary between good and moderate (G/M) ecological status for total phytoplankton biovolume ($2.1 \text{ mm}^3 \text{ l}^{-1}$) proposed by the Mediterranean Geographical Intercalibration Group (GIG) for Mediterranean reservoirs (JRC European Commission, 2009) during the warm period. This comparison was made, though Lake Megali Prespa is not a reservoir, since reference conditions and ecological classification of natural lakes of the Mediterranean region have yet to be established. Compared to the phytoplankton biovolume, boundary of G/M proposed by the Alpine GIG (Wolfram *et al.*, 2009) for lake-type L-AL4 (G/M: $1.9\text{--}2.7 \text{ mm}^3 \text{ l}^{-1}$) and even to boundaries used for the classification of large shallow lakes (e.g. Lake Balaton; Padišák *et al.*, 2006) the phytoplankton biovolume of Lake Megali Prespa does not indicate a high or good ecological water quality. This contradicts the findings of Kagalou & Leonardos (2009) who suggested a good ecological status for this lake based on chlorophyll *a* measurements, Secchi depth and total phosphorus concentration. Their data though refer to a period before 1997 (Kagalou, personal communication), so they do not reflect the present status of the lake.

The species composition found in the two months studied was significantly different ($df = 14$, chi-square: 94.2, $p < 0.05$). Diatoms were the most important group in terms of biovolume ($> 80\%$ contribution to the total) (Fig. 1A) and were dominated by species of the genus *Cyclotella* (*C. ocellata*, *C. meneghiniana*), corresponding to Reynolds' Codon B and C, respectively. *Cyclotella* species were also the most abundant phytoplankton organisms during the study period reaching $25 \times 10^6 \text{ cells l}^{-1}$ in October (Fig. 1B). Cryptophytes were found to co-dominate the lake's phytoplankton in October, but their share of total biovolume decreased the following month below 10% (Fig. 1A). *Rhodomonas minuta* and *Cryptomonas* sp. (Reynolds' Codons X2 and Y, respectively), their main representatives, were among the abundant phytoplankton species with numbers $\sim 5 \times 10^5 \text{ cells l}^{-1}$ (Fig. 1B). The small prymnesiophyte *Chrysochromulina parva* (Reynolds' Codon X2) was also very abundant in October, showing a density of $\sim 6 \times 10^6 \text{ cells l}^{-1}$. Cyanobacteria exhibited high numbers ($> 2 \times 10^6 \text{ cells l}^{-1}$) with the colonial species *Romeria simplex* (second most abundant species in the lake's phytoplankton after *C. ocellata*) and *Aphanocapsa delicatissima*, both belonging to Reynolds' Codon K, being most abundant (Fig. 1B). Nevertheless, they were not dominant in the lake's phytoplankton biovolume. The non-dominance

of cyanobacteria and their $< 10\%$ contribution to total biovolume (Fig. 1A) could suggest a good ecological water quality. However, low cyanobacterial contribution was due to the small cell size of the abundant cyanobacteria. The presence of several cyanobacterial species in the lake's phytoplankton including known toxin-producing species and the high abundance of pico- ($> 1 \times 10^7 \text{ cells l}^{-1}$) and nanophytoplankters ($> 3 \times 10^7 \text{ cells l}^{-1}$) belonging to functional groups (B, C, X2, K, Y) typically found in eutrophic waters (Padišák *et al.*, 2009) could not be considered indicative of high or good ecological water quality according to the WFD.

As regards the zooplankton community of the lake, 20 zooplankton taxa (13 Rotifera, four Cladocera, two Copepoda and one planktic mollusk larva) were identified in the water samples obtained from Lake Megali Prespa during the study period (see online supplementary material, Table S1). All the species recorded (e.g. *Mesocyclops leuckarti*, *Daphnia cucullata*, *Arctodiaptomus steindachneri*, *Bosmina longirostris*) have also been reported in the sister Lake Mikri Prespa (Michaloudi *et al.*, 1997) and are typical indicators of eutrophic waters (*Filinia longisetata*, *Keratella cochlearis* var. *tecta*, *Trichocerca cylindrica*, *B. longirostris* and *D. cucullata*) (Gannon & Stemberger, 1978; Michaloudi *et al.*, 1997).

The species overlap of both zooplankton and phytoplankton species between the sister lakes Megali and Mikri Prespa probably indicates a species exchange between them. This was expected since the two lakes are connected via a regulated man-made channel. Such management is considered as an agent that could facilitate plankton dispersal (Havel & Shurin, 2004).

Zooplankton abundance and biomass were higher (113 ind. l^{-1} and $57 \mu\text{g l}^{-1}$, respectively) in October and decreased by $\sim 50\%$ and 30% , respectively in November (Fig. 1C). These values were lower than the ones recorded in the eutrophic Lake Mikri Prespa (Michaloudi *et al.*, 1997) for October and November. Species composition in the two months studied were significantly different ($df = 16$, chi-square: 132.89, $p < 0.05$). The main representatives were *Keratella cochlearis*, *Synchaeta* sp. (rotifers group), *Diaphanosoma* cf. *mongolianum* (cladoceran group), and *Mesocyclops leuckarti* and *Arctodiaptomus steindachneri* (copepod group). Rotifers and copepods were the main contributors to the total abundance (Fig. 1D), whereas, due to rotifers' small size, copepods and cladocerans were the main contributors to the total bio-

mass (Fig. 1C). The abundance ratio calanoids/(cyclopoids + cladocerans) was 0.36 in October and 1 in November, suggesting eutrophic conditions during the study period (Gannon & Stemberger, 1978). Zooplankton/phytoplankton biomass ratio was low (0.01 and 0.02 in October and November, respectively) not indicative of an oligotrophic lake (Jeppesen *et al.*, 1997).

In conclusion, this is the first attempt to examine the ecological water quality of the ancient Lake Megali Prespa according to the WFD on the basis of phytoplankton data supplemented by information on zooplankton abundance and biomass. Results obtained in autumn of 2008 suggest signs of a moderate ecological water quality as indicated by: i) phyto- and zoo-plankton species composition (e.g. *Microcystis aeruginosa*, *Aulacoseira granulata*, *Filinia longiseta*, *Daphnia cucullata*) which was characteristic of eutrophic conditions, ii) dominance of phytoplankton functional groups (B, C, X2, Y, K) typical for eutrophic waters, iii) values of phytoplankton biovolume (3.85 mm³ l⁻¹) indicative of moderate eutrophic conditions, iv) high abundance (> 10⁷ cells l⁻¹) of several nanoplanktic species (cyanobacteria, diatoms, prymnesiophytes and cryptophytes) and v) the presence of known potentially toxin-producing cyanobacterial species (*Microcystis aeruginosa*, *Anabaena flos-aquae* and *Planktothrix* sp.). All these plankton characteristics do not suggest a high or good water quality in Lake Megali Prespa. However, additional data on species composition, abundance and seasonality of biological assemblages supported by physical and chemical data (i.e. nutrients) are needed for the final classification through regular monitoring. This preliminary examination underlines the general necessity to start monitoring activities aiming at acquiring limnological knowledge fundamental for i) any kind of preventative and conservation lake management and ii) the ecological classification of Mediterranean lakes.

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