Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 47, Issue 2, June 2018
pages (118-127)

ISSN 1730-413X
eISSN 1897-3191

Size-fractionated chlorophyll *a* and phycocyanin temporal variations in a highly eutrophic lake and its isolated karstic springs

by

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DOI: **10.1515/ohs-2018-0013**
Category: **Original research paper**
Received: **September 26, 2017**
Accepted: **December 8, 2017**

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Abstract

Monthly variations of size-fractionated chlorophyll *a* and phycocyanin were studied in Lake Pamvotis between August 2016 and January 2017. Sampling was conducted at two sampling sites: in the main lake (Site 1: Lake) and in an adjacent man-made water ski lake with karstic springs (Site 2: Springs). Samples were fractionated into three size classes: 0.2–2 µm (pico), 2–20 µm (nano) and 20–180 µm (micro). According to chlorophyll *a* values, eutrophic to hypereutrophic conditions prevail at Site 1 and oligotrophic to mesotrophic conditions – at Site 2. Similarly, Site 1 was distinguished by higher concentration of phycocyanin compared to Site 2. Fractionated chlorophyll *a* showed monthly variations at Site 1 with alternations in the dominance between the two larger fractions. The maximum of the 0.2–2 µm fraction was observed in October but it contributed less to the total chlorophyll *a* content than nano- or microphytoplankton. Its contribution was higher at Site 2, reaching occasionally ~ 40% of the bulk chlorophyll *a*. However, nanophytoplankton was the fraction found to respond faster when disturbances occurred. At Site 1, phycocyanin correlated well with total chlorophyll *a* as well as with the micro- and nanophytoplankton fractions, indicating that cyanobacteria represent an important component of the large-sized phytoplankton in Lake Pamvotis.

Key words: photosynthetic pigments, size fraction, primary producers, eutrophy, hypereutrophy, Lake Pamvotis, man-made lake

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Introduction

Phytoplankton constitutes a major group of primary producers in lakes and is often dominant in production, especially in eutrophic lakes (Smith et al. 1999). Three major groups of phytoplankton can be distinguished based on its size: pico- (0.2–2 µm), nano- (2–20 µm) and microphytoplankton (20–200 µm). Given that phytoplankton is the basis of the food web, its distribution to the above size fractions is of great ecological importance, because it determines the flow of matter and energy in the ecosystem (Fenchel 1988).

Chlorophyll *a*, present in all different representatives of phytoplankton, is commonly used as a proxy for its biomass and the trophic state of ecosystems (Hadjisolomou et al. 2016). Previous studies conducted in ocean or freshwater ecosystems have shown that the size distribution of phytoplankton varies with nutrient enrichment. In general, it has been shown that the microphytoplankton biomass increases and dominates over the smaller fractions (e.g. Watson & McCauley 1988; Kormas et al. 2002). With regard to the nano- and picophytoplankton, the findings are contrasting, because both the decrease (e.g. Bell & Kalff 2001; Søndergaard 1997; Stockner & Shortreed 1991; Szelag-Wasielewska 1997; Takamura & Nojiri 1994) and the increase (e.g. Agawin et al. 2000; Bell & Kalff 2001; Chisholm 1992; Kormas et al. 2002; Sprules & Munawar 1986; Watson & McCauley 1988) in their biomass have been reported after the ecosystem transition to the eutrophic state. In addition to nutrient enrichment, the distribution of chlorophyll *a* in different size classes reflects the response of phytoplankton to top-down control, because large-sized phytoplankton is well protected against grazers (Moustaka-Gouni et al. 2006).

The objective of the present study was to measure monthly variations of chlorophyll *a* and its size fractions as well as total phycocyanin in Lake Pamvotis and its springs in Amfithea (NW Greece). Similarly to chlorophyll *a*, it has been suggested that phycocyanin is a useful proxy for the actual concentration or biovolume of cyanobacteria cells (Loisa et al. 2015). Blooms of cyanobacteria are common in eutrophic lakes. In Pamvotis, cyanobacterial blooms occur throughout the year, though on a smaller scale in winter (Vardaka et al. 2005). Moreover, significant amounts of microcystins have been found in the lake, posing health hazards to fish and humans (Papadimitrou et al. 2012).

Lake Pamvotis is located in northwestern Greece, 470 m above sea level, with a mean depth of 4 m, a maximum depth of 11 m and a surface area of 22.8 km^2 (Kagalou et al. 2008a). In the past, its waters were entirely renewed through karstic springs, but this has changed in the last few decades due to the construction of an embankment (see Papastergiadou et al. 2010 and references contained therein on the history of the lake and human impact). At present, there is a water ski lake with an area of about 1 km^2 situated behind the embankment, northeast of the main lake. The drainage of water from Pamvotis takes place through a system of underground reservoirs that transfer water from the lake into the Arachthos, Louros and Kalamas rivers (Kagalou et al. 2008a). It is generally described as a shallow polymictic lake with unstable stratification in the summer season and hypoxia events (Kagalou et al. 2001). It is classified as an ecosystem of global importance (Krystufek & Reed 2004), which supports local agriculture, tourism and various fishing activities (Kagalou et al. 2008a). For the past 40 years, however, it has been a typical example of a eutrophic lake as a direct result of anthropogenic interference, especially irrigation, domestic sewage and the use of fertilizers and agrochemicals (Albanis et al. 1986; Kagalou et al. 2001; Kagalou et al. 2008a; Kotti et al. 2000; Stalikas et al. 1994).

As mentioned above, shifts in the dominance of different size fractions of chlorophyll *a* could be related to nutrient availability and may indicate vulnerability/resistance to grazing but also flushing and settlement processes in the lake (Moustaka-Gouni et al. 2006). For example, the increase in the internal P load in Pamvotis in summer and the decrease of N:P ratios (Kagalou et al. 2003) may lead to a change in the dominance of the largest size fraction (Masson et al. 2000). Similarly, we would expect a decrease in small size fractions in summer/autumn due to the dominance of microphagous herbivores i.e. Rotifera and Cladocera (Romero et al. 2002), which are unable to use colonial cyanobacteria. Thus, photosynthetic pigments and their size fractions could be considered as alternative (Gaedke 1995; Mouillot et al. 2006) but also complementary to phytoplankton taxonomic descriptors used previously to study Lake Pamvotis (e.g. Kagalou et al. 2008b; Vardaka et al. 2005). Considering the above, fluctuations in photosynthetic pigments could give interesting insights into ecosystem functioning. Within this framework, the objective of our study was to investigate monthly variations in (a) the distribution of chlorophyll *a* in different size classes and (b) phycocyanin in eutrophic Lake Pamvotis and the adjacent newly constructed water ski lake as well as to investigate possible interactions with other biotic or abiotic parameters of the ecosystem.

Materials and methods

Sampling strategy

Sampling was conducted monthly at two sites in Lake Pamvotis, Ioannina, Greece (Fig. 1) between August 2016 and January 2017, covering both the warm and cold period of the year, as suggested by the EU Water Framework Directive 2000/60. The first sampling site is located in the city of Ioannina, (Site 1 – Lake). The second site is behind the embankment which isolates Pamvotis karstic springs from the main water body of the lake and in particular the adjacent, newly constructed (2013) man-made water ski lake (Amfithea, Site 2 – Springs). Physicochemical parameters were measured in situ at both sites and water was collected from the surface (0–40 cm) into 5 l polyethylene containers after filtration through a 180 µm plankton net. Samples were transferred to the laboratory at 4°C in the dark for further processing.

Physicochemical parameters

Water transparency was estimated using a Secchi disk. Temperature (°C), salinity (mg l^{-1}), total dissolved solids (TDS, mg l^{-1}) and conductivity (EC, µS cm^{-1}) were measured by a conductivity meter (Hach sension+ EC5), while pH was determined using pH paper. The dissolved oxygen (mg l^{-1}) in the water column was measured by the Winkler method (Carpenter 1965) as modified by Labasque et al. (2004). For this purpose, amber narrow mouth ground glass stopper bottles were filled with water (130 ml) and adequate volumes of manganese chloride (3M) and alkaline iodide (NaOH 8M, NaI 3M) solutions were added immediately. Samples were acidified using sulfuric acid (10N) within 3 h after collection and absorbance was measured at 466 nm within 5 min using a U2800 Hitachi spectrophotometer.

Photosynthetic pigments

To determine the concentration of photosynthetic pigments (chlorophyll *a* and phycocyanin), the lake water was filtered through nylon and membrane filters with different pore sizes. The total amount of each pigment (0.2–180 µm) was measured after filtration of water (0.1–0.8 l) on a 0.2 µm GTTP filter (Isopore membrane filters 47 mm, Millipore). The fractions of 0.2–2 µm (picophytoplankton) and 2–20 µm (nanophytoplankton) were obtained after sequential filtration through a 20 µm nylon filter; 2 µm TTTP filter and 0.2 µm GTTP filter (Isopore membrane filters 47 mm, Millipore). To minimize any potential measurement biases due to filter clogging, filtering was performed initially with gravity and then at low vacuum (≤ 100 mm Hg) (Wetzel & Likens 2000). A modification of the widely accepted size range for microphytoplankton (20–200 µm) was used in this study and the upper threshold was set as 180 µm (Kormas et al. 2002; Lima-Mendez et al. 2015). The 20–180 µm fraction was calculated from the difference

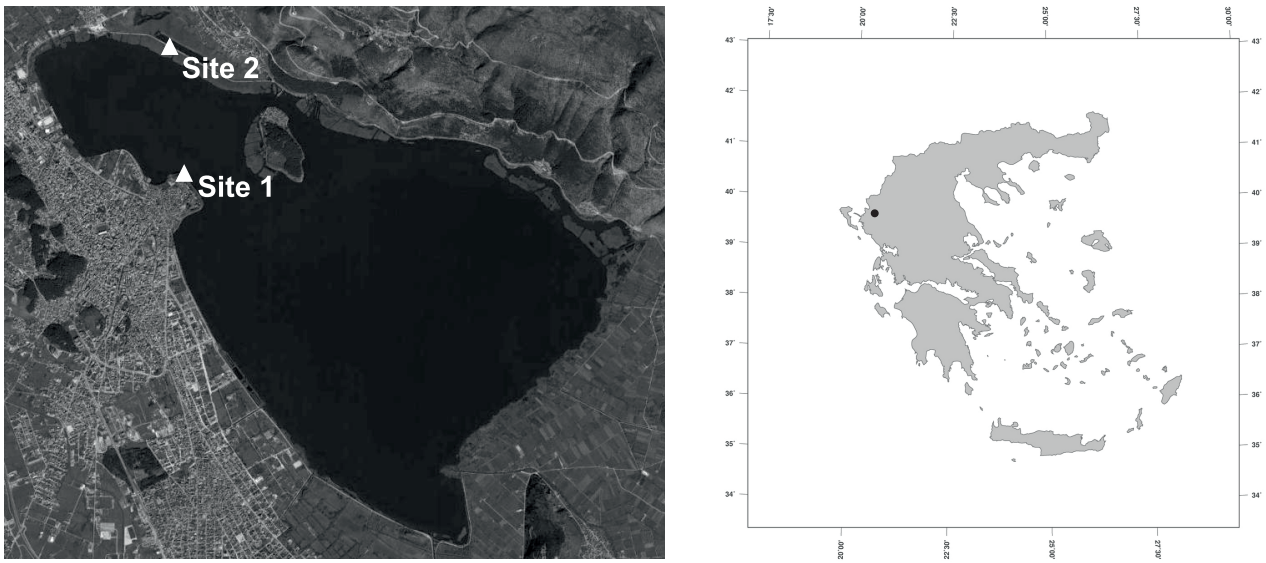

Figure 1

Sampling sites in Pamvotis (Ioannina) Lake (Greece). Site 1: Lake, Site 2: Springs. The location of Ioannina is indicated on the right map (from GinkgoMaps – project http://www.ginkgomaps.com)

between the total chlorophyll and the sum of pico- and nano- fractions ($Chl_{20-180} = Chl_{0.2-180} - [Chl_{0.2-2} + Chl_{2-20}]$). The above procedures were followed for both chlorophyll *a* and phycocyanin.

For the extraction of chlorophyll *a*, the filters were incubated for 24 h at 4°C in 90% acetone solution. The samples were then centrifuged at 2300 g (Kubota 4200 with head 053–5840) for 30 min. The supernatant absorbance was measured at 750, 664, 647 and 630 nm with a 1 cm quartz cuvette against 90% acetone. Photosynthetic pigment concentration (µg l^{-1}) was calculated in different fractions as described in Parsons et al. (1984).

For the extraction of phycocyanin, the filters were placed in centrifuge tubes with 8 ml of KH_2PO_4–KOH buffer (pH = 6.8). The samples had to undergo three freeze/thaw cycles, one 24-hour and two 3-hour cycles, during which time they were initially placed at –20°C and then at 9°C. Samples were then sonicated (sonicator probe, 10 kHz, cycle 50%) (Omnisonic raptor ultrasonic homogenizer 400) for 4 min and centrifuged at 2300 g (Kubota 4200 with head 053–5840) for 30 min. The supernatant was used for the photometric determination of phycocyanin at 652 and 615 nm with a 1 cm quartz cuvette against the buffer (Siegelman & Kycia 1978; Horváth et al. 2013). The concentration of phycocyanin (µg l^{-1}) in various samples was calculated according to Siegelman & Kycia (1978).

Bacterial abundance

To determine the bacterial abundance, samples (5–11 ml) were stained with DAPI (4,6-diamidino-2-phenylindole, 1 mg l^{-1}, UV-blue) after adding 37% formalin and filtered on 0.2 µm GTTP filters (Isopore membrane filters 25 mm, Millipore) (Porter & Feig 1980). Bacteria were counted under an epifluorescence microscope (LEICA DM LS2) at ×1000 magnification. At least 10 fields and 300 cells were measured in each sample (Kirchman 1993). Bacterial abundance was measured in lake water < 2 µm to avoid large particles that interfere with the counting under an epifluorescence microscope.

Statistical analysis

To check for statistically significant correlations between different parameters studied, the Pearson correlation coefficient (r) was calculated. When the *p*-value was ≤ 0.05, the correlation was considered statistically significant. The Kruskal-Wallis test was used to check for statistically significant differences in total chlorophyll *a* and its different size fractions as well as in phycocyanin between the two sampling sites. All statistical analyses were performed using the Past3 software.

Results

Physicochemical parameters

Temperature decreased gradually during the sampling period. The lowest values were recorded in January for Site 1 (Site 1, Lake, 4°C) and December for Site 2 (Site 2, Springs, 8°C) and the highest values in August (28°C and 25°C respectively, Fig. 2). Salinity in the lake ranged from 114 to 141 mg l^{-1}, whereas at the second site (Springs) – from 292 to 311 mg l^{-1}. Electric Conductivity (EC) and Total Dissolved Solids (TDS) were 240–294 µS cm^{-1} and 155–190 mg l^{-1} respectively for Site 1 (Lake) and 602–640 µS cm^{-1} and 387–412

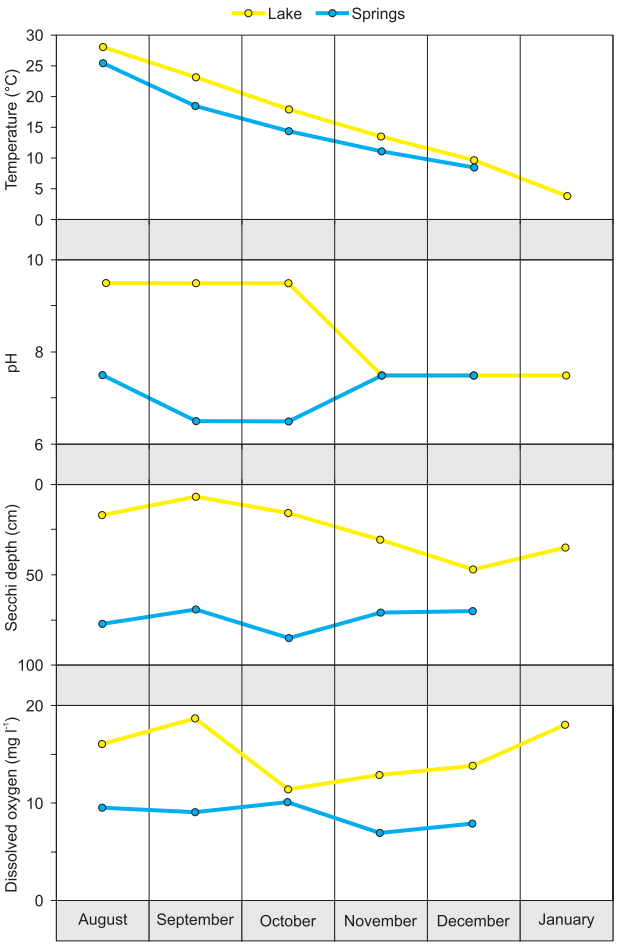

Figure 2

Temperature, pH, Secchi depth, Dissolved Oxygen at Sites 1 (Lake) and 2 (Springs) between August 2016 and January 2017

mg l^{-1} for Site 2 (Springs). At Site 1 (Lake), alkaline pH was recorded for all months studied and ranged from 7.5 in winter to 9.5 in summer. At Site 2 (Springs), pH ranged from 6.5 to 7.5 (Fig. 2). Secchi depth at the lake sampling site was lower compared to the springs (Fig. 2). Furthermore, Site 1 (Lake) showed low values in summer/early autumn (13 ± 5.5 cm) and increased values in November till the end of the sampling period (38 ± 8.3 cm). At Site 2, Secchi depth was ≥ 70 cm. Dissolved Oxygen (DO) varied in the Lake (Site 1) between 11.4 (October) and 18.7 mg l^{-1} (September, Fig. 2). The Springs site (Site 2) had DO values ranging from 6.9 mg l^{-1} (November) to 10.1 mg l^{-1} (October).

Chlorophyll a and phycocyanin

Total chlorophyll *a* (Fig. 3) showed a decreasing gradient at Site 1 (Lake) with the highest values occurring in August (314 µg l^{-1}) and the lowest values in December (37 µg l^{-1}). An increase in total chlorophyll *a* occurred in January (57 µg l^{-1}). Size fractionation indicated that microphytoplankton (20–180 µm) dominated in August (214 µg l^{-1}, 68% of the total chlorophyll *a* content), October (65 µg l^{-1}, 51%) and November (41 µg l^{-1}, 60%) and nanophytoplankton (2–20 µm) in September (132 µg l^{-1}, 51%), December (20 µg l^{-1}, 54%) and January (47 µg l^{-1}, 80%) (Fig. 3). Picoplankton occurs in all the months studied, without ever exceeding the concentrations measured for the two other size fractions. Picoplankton maxima occurred in October (4.3 µg l^{-1}). Its relative biomass ranged from 0.3 (August and September) to 3.5% (October).

In the Springs (Site 2), chlorophyll *a* reached lower concentration compared to the Lake (Site 1). The maximum occurred in September (17.7 µg l^{-1}) and the minimum in November (1.2 µg l^{-1}, Fig. 3). Furthermore, it appears that the nanophytoplankton dominated over the micro- and pico- fractions in August (3.2 µg l^{-1}, 65%), September (13.3 µg l^{-1}, 75%) and December (0.7 µg l^{-1}, 51%). The larger fraction (20–180 µm) dominated only in November (55% of the total chlorophyll *a* content). The picophytoplankton fraction occurred throughout the study period with the highest concentration measured in October (1.7 µg l^{-1}) and the lowest in November (0.02 µg l^{-1}) (Fig. 3). The picoplankton fraction contributes considerably more at Site 2 than at Site 1, with values ranging from about 1% in November to nearly 45% in December. The Kruskal-Wallis test indicated statistically significant differences ($p < 0.001$) for total chlorophyll *a* and its different size fractions between the sampling sites.

Figure 4 shows the phycocyanin concentrations measured at both sites. Site 1 (Lake) had higher values than Site 2 (Springs), with the highest value recorded in August (1.3 µg l^{-1}) and the lowest in January (0.12 µg l^{-1}). Phycocyanin biomass at Site 2 was very low throughout the study period (< 0.05 µg l^{-1}). Statistically significant differences were found between Site 1 and 2 in the phycocyanin concentration (Kruskal-Wallis test, $p < 0.05$).

Bacterial abundance

At Site 1 (Lake), bacterial abundance ranged from 18.2 × 10^7 cells ml^{-1} in December to 56.4 × 10^7 cells ml^{-1}

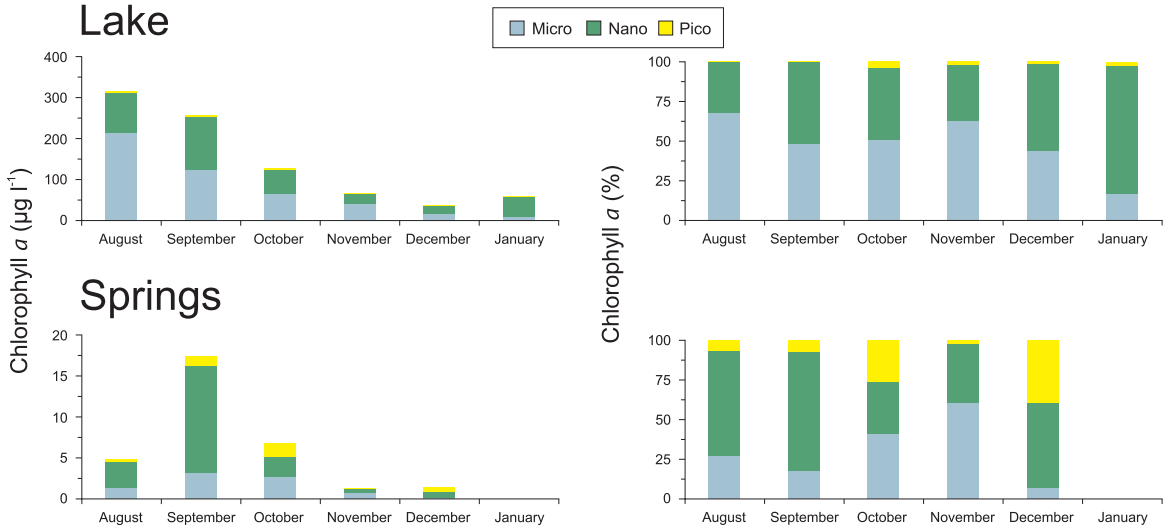

Figure 3

Size-fractionated chlorophyll *a* concentration (in µg l^{-1}) and as a percentage (%) of total chlorophyll (< 180 µm) at Sites 1 (Lake) and 2 (Springs) between August 2016 and January 2017

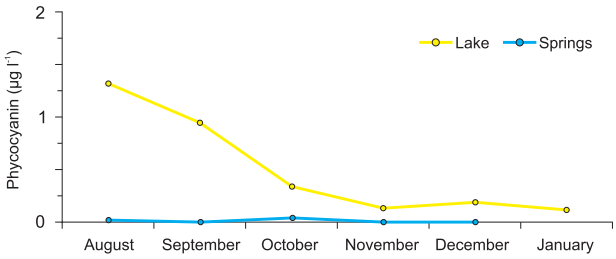

Figure 4

Phycocyanin concentration at Sites 1 (Lake) and 2 (Springs) between August 2016 and January 2017

in January. At Site 2, bacterial abundance varied in the same order of magnitude, except in August when it reached 95.1 x 10^7 cells ml^{-1} (Fig. 5).

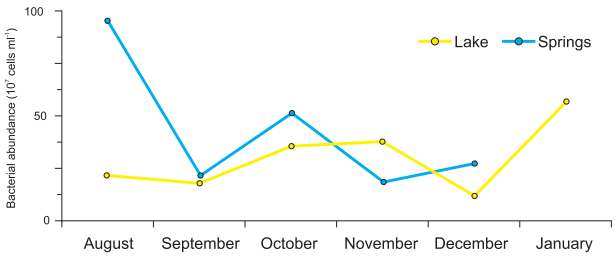

Figure 5

Bacterial abundance at Sites 1 (Lake) and 2 (Springs) between August 2016 and January 2017

Discussion

Chlorophyll *a* is a useful indicator for assessing the trophic status and water quality of surface waters, as it can be used as a proxy for phytoplankton biomass. During the study, chlorophyll *a* concentration in Lake Pamvotis (Site 1) was always higher than 36 µg l^{-1}, reaching values > 120 µg l^{-1} in summer/early autumn. Our results indicate eutrophic to hypereutrophic ecological status in Lake Pamvotis with values similar to those recorded in the late 1980s (Koussouris et al. 1991) before opening and starting the wastewater treatment plant for the city of Ioannina (Papastergiadou et al. 2010). These values are five to six orders of magnitude higher than those recorded for the years 1998–1999, after changing the municipal sewage system in the city (Kagalou et al. 2001). A shift to eutrophic conditions was also recorded in 2005 by Kagalou et al. (2008b). The eutrophic state of Lake Pamvotis can also be perceived macroscopically by observing phytoplankton blooms throughout the year, especially in summer when they color the water and increase its turbidity. Although sampling was not performed in a network of sites which would enable us to investigate possible spatial variability, we believe that our results are quite representative of the whole ecosystem. In fact, Papadimitriou (2010) has previously indicated the lack of statistically significant differences between coastal (2 sites) and pelagic (2 sites) sampling sites for different photosynthetic pigments in Lake Pamvotis.

Considerably lower values (in general < 7 µg l^{-1}) were recorded at the second sampling site (Site 2) behind the Amfithea embankment that was constructed in the 1970s, causing changes in the hydraulic connection between the lake and its karstic springs (Papastergiadou et al. 2010). In 2013, the water ski lake was constructed at this site. With the exception of September when mesotrophic conditions prevail, the above values indicate an oligotrophic state (OECD1982) for the ecosystem adjacent to the main lake ecosystem. This is also confirmed by the large Secchi depth values. In general, a great Secchi depth corresponds to smaller values of chlorophyll or phycocyanin (Tilzer 1988). In the present study, the Secchi depth was found to increase at Site 1 over the months, which seems to be due to the decline in chlorophyll *a*. The statistically significant correlation between chlorophyll *a* and Secchi depth ($r = -0.81$, $p < 0.05$) seems to confirm this finding. A different pattern (great Secchi depth with basically no monthly fluctuations) was observed at Site 2 due to the inflow of fresh water from the surrounding mountains. It has to be noted, however, that during the sampling period, the water ski track was used only in September, and more precisely just before the sampling for this month was carried out. This probably explains the peak of chlorophyll *a* observed during that month (17.7 µg l^{-1}), which classifies the lake as mesotrophic. The discharge of the lake is interrupted during water ski races, resulting in a different flushing effect.

This study was the first attempt to investigate chlorophyll *a* size fractionation in Lake Pamvotis. Our findings indicate a decrease in the biomass of both nano- and microphytoplankton when total chlorophyll *a* decreased at eutrophic Site 1 (i.e. from August to January). This is in accordance with the previous studies that showed that both the large and small size fractions can be affected by shifts in nutrient concentrations (e.g. Agawin et al. 2000; Bell & Kalff 2001). Picophytoplankton showed small fluctuations, however, it is interesting to note that its concentration reached 4.3 µg l^{-1} in October. Microphytoplankton was found to be the dominant contributor to the total chlorophyll *a* content in late summer (August,

68.1%), while nanophytoplankton dominated in late winter (January, 81.2%). The two fractions were more or less equally represented from September to December. The positive correlation between microphytoplankton chlorophyll *a* and temperature ($r = 0.94$, $p < 0.01$) indicates that under eutrophic/hypereutrophic conditions, temperature may affect community structure. Furthermore, based on elemental ratios of N and P and divergences from the Redfield ratio, Kagalou et al. (2008b) have reported nitrogen limitation in the lake in summer/autumn. Also in another study conducted simultaneously with the current one, we found T-N > 80 μg l^{-1}, T-P > 700 μg l^{-1} and N:P ratios << 16:1 (data not shown). Nitrogen limitation shapes phytoplankton communities favoring the occurrence of N-fixers, which in turn may promote the growth of large *Microcystis* colonies (Beversdorf et al. 2013). Other, experimental studies have also shown that large phytoplankton dominates in nitrogen-limited systems, while in phosphorous--limited systems, picophytoplankton abundance and biomass increases (Stockner & Shortreed 1991; Masson et al. 2000). Finally, we should mention that large-size phytoplankton is considered well protected against grazing and thus dominant in eutrophic ecosystems (Moustaka-Gouni et al. 2006). At the second sampling site (Site 2, Springs), the contribution of microphytoplankton to the total chlorophyll *a* only once exceeded 50%. In all other cases, considering the criteria defined by Wang et al. (2013) for the dominant size class, nanoplankton contributed the most to chlorophyll *a*. However, we should mention that the contribution of picoplankton exceeded 25% twice. There is evidence from previous studies that pico- and nanoplankton, with a high surface to volume ratio, are better competitors for nutrients than larger plankton under oligotrophic conditions (Andersson et al. 1994). Interestingly, the disturbances that occurred in September at Site 2 favored nanophytoplankton. This indicates a close association between this size fraction and ecosystem nutrient enrichment that may have occurred because, as mentioned before, the track is cut off from the natural outflows during water ski races and the hydrology of the system is changing. Further studies are needed to investigate how these manipulations affect flushing rates of the system and consequently its physicochemical and biological characteristics.

Lake ecosystems, such as Lake Pamvotis, which owe their eutrophication state to human activities tend to experience a massive increase in cyanobacteria (Moustaka-Gouni 1993; Watson et al. 1997; Moustaka-Gouni et al. 2006). In this paper, the concentration of the blue pigment, phycocyanin, was used as a proxy for cyanobacterial abundance fluctuations during the sampling period. Several papers have been published on phycocyanin extraction methods (Horváth et al. 2013; Lawrenz et al. 2011; Zhu et al. 2007; Zimba 2012). In all cases, it was suggested that there is an underestimation of its concentration due to methodological issues. In the present study, the measurement of phycocyanin was based on the protocol of Horváth et al. (2013), with changes concerning the filter type, freeze/thaw cycles and the amount of buffer solution, to create the optimal conditions for pigment extraction from our samples. The results showed that Site 1 had higher concentration of phycocyanin compared to Site 2. This also suggests that Site 2, close to the springs, is less enriched with nutrients compared to Site 1 (Li et al. 2017). However, our data probably indicate an important underestimation of the total phycocyanin, given that cyanobacterial blooms occur in Lake Pamvotis throughout the year, though on a smaller scale in winter (Vardaka et al. 2005). Long-lasting blooms of these organisms color the water and create an unpleasant odor (Lee 2008). Both features were observed in Lake Pamvotis during the study period. Furthermore, previous studies of eutrophic freshwater ecosystems (Lyu et al. 2013; Randolph et al. 2008; Simis et al. 2005; Song et al. 2012; Pyo et al. 2017; Wang et al. 2014) have shown phycocyanin concentration one order of magnitude higher compared to this study. However, most of the above studies found a high standard deviation on a spatial or temporal scale, while some authors (Randolph et al. 2008; Song et al. 2012) concluded that the technique used for the calculation of phycocyanin gave results several classes lower than expected. In our study, phycocyanin was statistically significantly correlated with total chlorophyll ($r = 0.98$, $p < 0.01$) as well as with micro- ($r = 0.97$, $p < 0.01$) and nanophytoplankton ($r = 0.84$, $p < 0.05$) fractions, indicating that cyanobacteria represent an important component of large-sized phytoplankton in Lake Pamvotis. *Microcystis aeruginosa* (Kützing) Kützing is the most widespread and most often dominant species of cyanobacterial blooms in Lake Pamvotis and other Greek lakes (Katsiapi et al. 2011; Vardaka et al. 2005). Moreover, Cook et al. (2004) reported that the scum in Lake Pamvotis during the summers of 1997–2000 was caused by *M. aeruginosa* and *Dolichospermum flos-aque* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek (formerly *Anabaena flos-aque*). These two species were also identified in this work in November, when a sample check was carried out (data not shown).

An increase in the bacterial abundance was observed in late autumn (October–November) at Site 1.

This is probably due to the bacterial degradation of organic matter, produced during summer by the excessive growth of phytoplankton (Agustí & Duarte 2013; Buchan et al. 2014; Ye et al. 2014) or due to bacterial degradation of microcystins (Moustaka-Gouni et al. 2006). The reduction of dissolved oxygen in October at Site 1 supports this finding, which probably indicates an increase in the activity of the microbial loop (Azam et al. 1983). At Site 1, bacterial abundance peaks again in January, probably due to the inflow of allochthonous material in the form of sewage after heavy rain fall (Ye et al. 2014). Indeed, the rainfall in January was 19.8 mm compared to < 0.2 mm from August to December, except in October when it reached 3 mm (Pedini meteorological station, Ioannina; http://stratus.meteo.noa.gr/front). Similarly, the increase in bacterial abundance at Site 2 in October may be related to the high chlorophyll *a* content measured at this site in the previous months.

To sum up, the biomass of both nano- and microphytoplankton fractions increases when the total chlorophyll *a* increases under eutrophic/hypereutrophic conditions of Lake Pamvotis and the alternation in the dominance of these two size fractions was observed throughout the study period. Picophytoplankton contribution was low (≤ 0.03%) but its chlorophyll *a* content reached 4 μg l^{-1} in late autumn. At the same time, the enrichment with nutrients in the ski track adjacent to the main lake, which receives freshwater directly from the karstic springs of Mitsikeli Mountain, seems to be limited. Under these conditions, total chlorophyll *a* concentration is low and the nanophytoplankton fraction alone or together with picophytoplankton dominates. Interestingly, the nanophytoplankton seems to respond quickly and thrive when the ecosystem is disturbed. The above findings may have implications for the restoration of the eutrophic/hypereutrophic lake through water management and, in particular, the feeding of the lake through its natural springs.

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