

Planktothrix rubescens: a perennial presence and toxicity in Lake Sapanca

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Abstract: Cyanobacteria are expected to adjust to new conditions better than other primary producers since they have a long history of adaptation as one of the first organisms on earth. Their ecophysiological adaptations differ widely among cyanobacterial species. As a stratified species, *P. rubescens* forms a population in the metalimnion during summer months in lakes with clear water and disperses in the whole water column during the period. However, after a dry season in 2006 and 2007, a prolonged surface bloom in late winter was observed in Lake Sapanca. The maximum biomass of *P. rubescens* reached 13.9 mg L⁻¹ in surface water in February 2007 and microcystin concentrations varied between 0.12 and 8 µg L⁻¹ MC-LR equiv. in the open water throughout the year. The maximum microcystin value was measured at 79 µg L⁻¹ when accumulations of filaments were observed on the shoreline. As *P. rubescens* is a shade-adapted species, lower insolation in February together with stable water conditions and lower water temperature promoted the surface bloom. In conclusion, the presence of toxic *P. rubescens* throughout the year with changing depths poses severe health hazards to people and also an economic burden to the water treatment facility, since the lake is used as a drinking-water reservoir.

Key words: Lake Sapanca, *Planktothrix rubescens*, surface blooms, microcystin, Cyanobacteria

1. Introduction

Climate variability is expected to have a significant impact on inland waters, including lakes. It is widely accepted that water temperature is a key parameter in most biological systems, directly influencing water chemistry, biochemical reactions, and aquatic biota. Increasing water temperatures can change the hydrodynamics and expand the thermal stratification period of lakes (Komatsua et al., 2007). On the other hand, climatic effects on phytoplankton are also of considerable importance, since the composition and quantity of phytoplankton are important parameters of water quality (Shams et al., 2012) and a good indicator of the ecological status of water bodies (Demir et al., 2014).

Regional impacts of climate warming on water ecosystems represent a new and growing threat (Liboriussen et al., 2005). Climate change has been considered a potential tool for the further expansion of harmful cyanobacterial blooms, particularly in eutrophic waters with rising temperatures, enhanced stratification, increased residence time, and high nutrient loading all favoring cyanobacterial dominance (Jöhnk et al., 2008; Paerl and Huisman, 2009; Nöges et al., 2010).

Recent studies have shown that the increase of metalimnetic *Planktothrix rubescens* in a number of lakes is most probably caused by a synergistic effect of increased transparency due to the reduction in the phosphorus

loads, the deepening of the P-depleted zone, and increased water column stability (Anneville et al., 2005; Jacquet et al., 2005; Teubner et al., 2006).

On the other hand, there is always competition for resources between organisms and, as a metalimnetic species, *P. rubescens* has advantages over other phytoplankton species to reach nutrients from the hypolimnion in the stratified season, which allows the species to reach high numbers in summer months. Normally, during the mixing period, conditions are not good enough for *P. rubescens*; its population get smaller and other phytoplankton species take this opportunity to grow. On the other hand, if the conditions become favorable for *P. rubescens*, a surface bloom could occur in winter months (Walsby et al., 2005).

The present study was carried out in Lake Sapanca, which is located in the northeast of the Marmara Region of Turkey. The lake is used as a source of drinking water by the cities of Adapazarı and Kocaeli (Albay et al., 2003; Akcaalan et al., 2007). In 1997, the lake was considered polluted because of its red color due to the surface scum of dead *P. rubescens* filaments. After this phenomenon, metalimnetic populations of *P. rubescens* were recorded in the lake and microcystin was detected in the samples (Albay et al., 2003). Being a source of drinking water, it is important to monitor the lake according to presence of cyanobacteria and cyanotoxins. To understand the

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occurrence and distribution of *P. rubescens* in the lake, this paper aims to characterize the environmental factors that control spatial and seasonal abundance of *P. rubescens* and the variability of microcystin content.

2. Materials and methods

2.1. Study location

Lake Sapanca (40°41'N to 40°44'N and 30°09'E to 30°20'E) is situated in a tectonic depression at an altitude of 30 m. It is elongated in an east-west orientation (17 km long and 5 km wide) with an area of 46.8 km² (Figure 1). Maximum and average depths are 55 and 26 m, respectively. It is a warm monomictic lake, with stratification generally starting in late April and ending in mid-November. Complete circulation, including the lake bottom, is achieved. The main water sources for the lake are small rivers and groundwater. The catchment area is 250 km² and agriculture is not very intense. On the other hand, the southern shores have a high number of summer cottages. In the southern part of the lake there is mountain terrain with forests, and more than 10 companies produce bottled natural spring water from the groundwater that is a source for the lake.

2.2. Sampling

Sampling was carried out at monthly intervals in 2007 in the eastern part of the lake, where water abstraction for drinking purposes is done. Samples were taken from the surface and 5, 10, 15, 20, and 30 m for chemical measurements and *P. rubescens* biomass and microcystin analysis with a closed water sampler.

2.3. Limnological and meteorological parameters

Water transparency was measured using a Secchi disk. Euphotic depths (Zeu) were calculated from respective Secchi depths as $Z_{eu} = 2.5 \times Z_s$. Vertical profiles of pH, temperature and dissolved oxygen were measured with a portable multiparameter (6600, YSI, USA) on each sampling date.

Nutrients [nitrate, nitrite, soluble reactive phosphorus (SRP), and total phosphorus] were measured according to

the American Public Health Association (APHA, AWWA, WEF, 1989). Total nitrogen (TN) was measured with an Elementar analyzer (Elementar, Germany). Chlorophyll *a* (chl-*a*) was determined using the method of Nusch (1980).

Climatological data were provided from the Turkish State Meteorological Service.

2.4. *P. rubescens* biomass

Water samples were fixed immediately with Lugol's iodine solution and stored in the dark. *P. rubescens* enumeration was done with an inverted microscope according to Utermöhl (1958). The biovolume of *P. rubescens* was calculated following the formulae of Hillebrand et al. (1999) and converted to biomass.

2.5. Microcystin analysis

The analysis of microcystins was performed using high-performance liquid chromatography with diode array detection (PerkinElmer, USA) according to Lawton et al. (1994). Briefly, lyophilized bloom samples were extracted in 70% (v/v) aqueous methanol with ultrasonication and centrifuged at $14,000 \times g$ for 5 min. Clear supernatants were injected into the HPLC column (Waters Symmetry C18, 3.9×150 mm, 5 μ m particle size). Microcystins were identified by their characteristic absorption maximum at 238 nm and quantified using MC-LR as an external standard.

2.6. Statistical analysis

The Spearman rank correlation test was used to evaluate the relationships among *P. rubescens* biomass, microcystin concentration, and physicochemical parameters.

3. Results

3.1. Meteorological parameters

Both annual mean air temperatures in Adapazarı Province and surface water temperatures measured in the lake showed a slight increase between 2000 and 2007 (Figure 1). The amount of rainfall had decreased in the last 10 years and the lowest rainfall was recorded in 2006 as 651 mm (Figure 1).

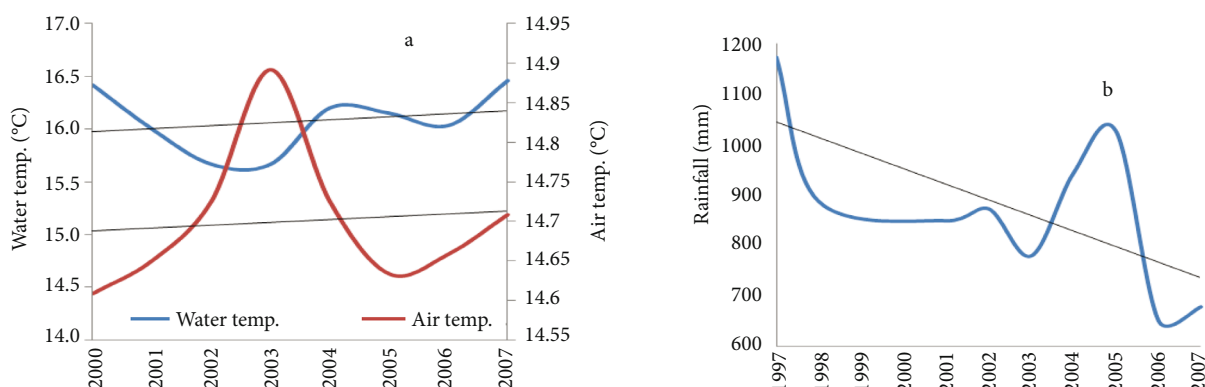


Figure 1. a) Yearly changes of air temperature in Adapazarı Province and surface water temperature in Lake Sapanca; and b) yearly changes of rainfall in Adapazarı Province.

3.2. Limnological variables

Lake Sapanca has a slightly alkaline character (mean pH: 8.3). Mean annual conductivity was 248 $\mu\text{S cm}^{-1}$. Temperature profiles showed that thermocline formed between May and October. Minimum temperature was measured at 8.4 °C in December and the maximum was 27.5 °C in July at the surface. Water temperatures gradually decreased when cooling started in late fall and complete overturn was observed in December with a temperature of 10 °C (Figure 2). The lowest dissolved oxygen concentration was measured as 2.4 mg L^{-1} in

the hypolimnion and the highest was 11.2 mg L^{-1} in the epilimnion. Anoxic conditions were never detected in the hypolimnion. Euphotic depth was changed between 4.8 and 13.8 m.

Nutrient concentrations were highly variable throughout the investigation period. SRP ranged from below the detection limit ($2 \mu\text{g L}^{-1}$) to 48 $\mu\text{g L}^{-1}$ in the whole water column seasonally with a mean value of 7.5 $\mu\text{g L}^{-1}$. It was under detectable limits ($2 \mu\text{g L}^{-1}$) in February and mean water column values reached the highest concentrations in September (Figure 3). Mean TP value was 33.6 $\mu\text{g L}^{-1}$, TN

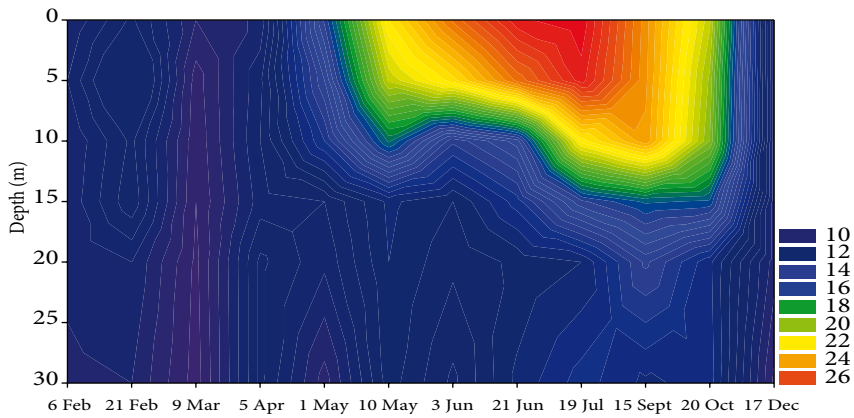


Figure 2. Spatiotemporal changes of water temperature in Lake Sapanca.

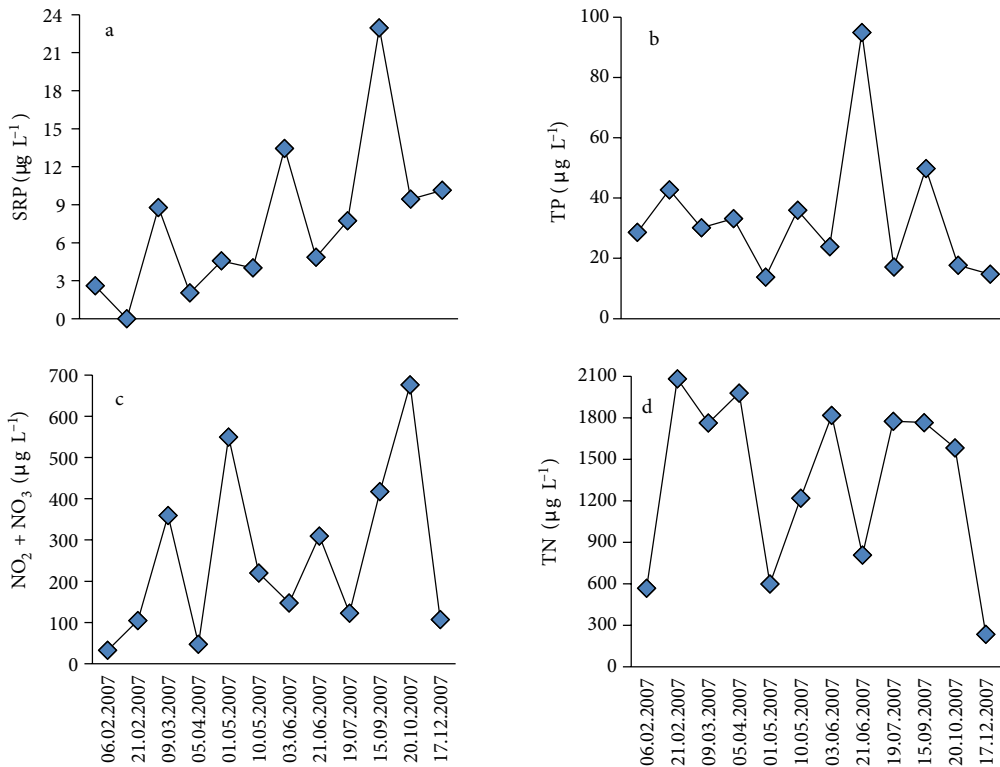


Figure 3. Seasonal changes of mean values of SRP ($\mu\text{g L}^{-1}$) (a), TP ($\mu\text{g L}^{-1}$) (b), $\text{NO}_2\text{-N}+\text{NO}_3\text{-N}$ ($\mu\text{g L}^{-1}$) (c), and TN ($\mu\text{g L}^{-1}$) (d) in the water column in Lake Sapanca.

was $1350 \mu\text{g L}^{-1}$, and nitrate was $258 \mu\text{g L}^{-1}$ in the sampling period (Figure 3). TN/TP rates were calculated along the water column and ranged between 9 and 104.

Mean chl-*a* of water column ranged between 1.8 and $26.1 \mu\text{g L}^{-1}$. Maximum chl-*a* was detected as $101 \mu\text{g L}^{-1}$ at the surface in February. Chl-*a* values were above $10 \mu\text{g L}^{-1}$ in winter and spring and dropped to below $5 \mu\text{g L}^{-1}$ in summer and fall (Figure 4).

3.3. The seasonal dynamics of *Planktothrix rubescens*

P. rubescens was present on all sampling days in 2007. At the beginning of February, a surface bloom of *P. rubescens*

was observed with a biomass of 9.4 mg L^{-1} on the surface. After 2 weeks, another surface bloom occurred in the lake when the weather was calm and sunny, at which point sampling was done on 3 consecutive days. The highest biomass of *P. rubescens* in open water was 13.9 mg L^{-1} on the first day of the second surface bloom. During the following days, the biomass decreased to 3 mg L^{-1} . The *P. rubescens* biomass was not restricted to the surface; it was present in the whole water column as the dominant species in spite of its lower biomass in depths compared to surface waters (Figure 5). Accumulations were also observed on the shoreline.

In March and April, *P. rubescens* was still in the whole water column, although its biomass was reduced dramatically. After the beginning of stratification in May, the population was nearly absent in the epilimnion and established itself in deeper waters until December, especially with higher biomass in June, September, and October (Figure 5). When the upper mixed layer reached greater depths, the *P. rubescens* population was evenly dispersed in the whole water column with a mean biomass of 0.63 mg L^{-1} in December.

The main variables affecting *P. rubescens* biomass were temperature and euphotic depth. Both parameters showed a considerable negative correlation with biomass. On the other hand, the relationship between nutrients and *P. rubescens* biomass was not strong. Both SRP and $\text{NO}_3\text{-N}$ were negatively correlated with biomass (Figure 6).

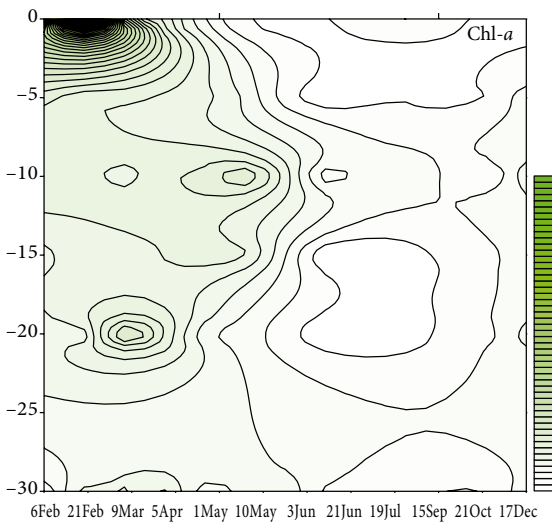


Figure 4. Spatiotemporal changes of chlorophyll-*a* ($\mu\text{g L}^{-1}$) in Lake Sapanca.

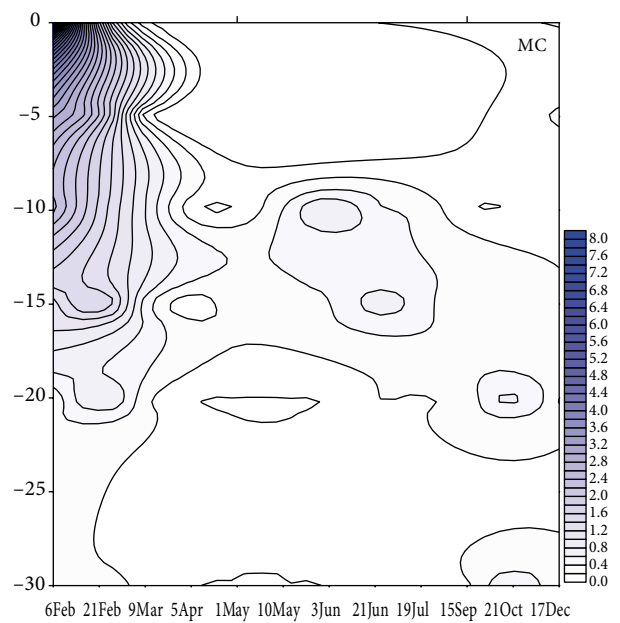
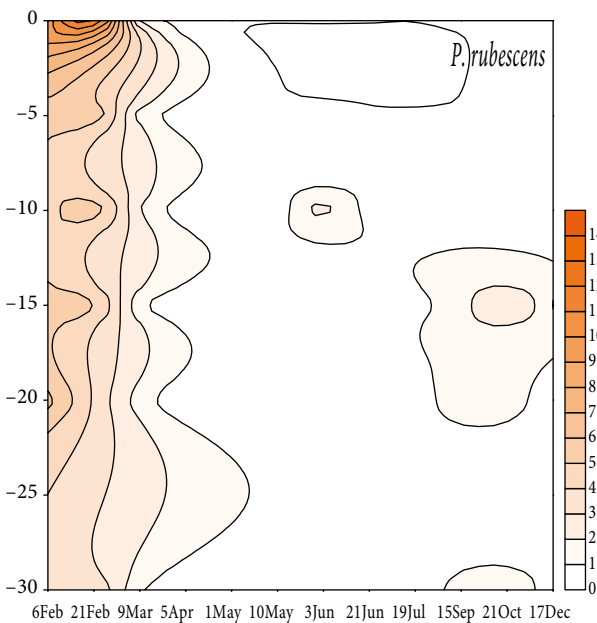


Figure 5. Spatiotemporal changes of *Planktothrix rubescens* biomass (mg L^{-1}) and microcystin concentration (MC; $\mu\text{g L}^{-1}$) in Lake Sapanca.

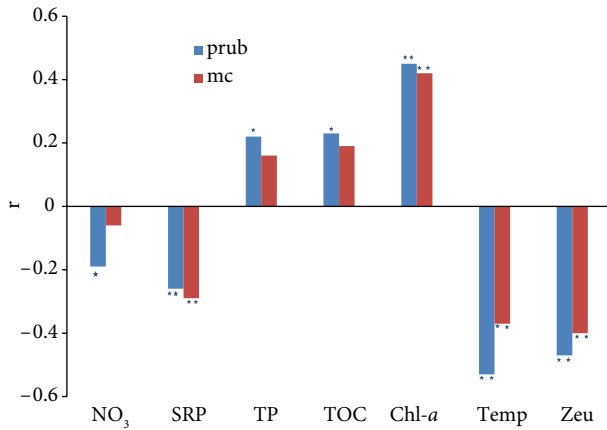


Figure 6. Spearman correlation coefficient between the variables and *P. rubescens* biomass (prub) and microcystin concentration (mc). *: $P < 0.05$, **: $P < 0.01$.

3.4. Microcystin analysis

Only particulate microcystin was presented in this study. Dissolved microcystin was measured in the bloom period and it was under the detection limits. The main microcystin variant was MC-RR throughout the study period. Total cell-bound microcystin concentrations ranged between 0 and $79 \mu\text{g L}^{-1}$. The highest amount of microcystin (as $79 \mu\text{g L}^{-1}$ MC-RR) was detected on the shoreline and $8 \mu\text{g L}^{-1}$ was measured in the middle of the lake at the beginning of February. Microcystin was detected in the whole water column between February and March, then descended to 10 m in May with the onset of stratification and was detected between 10 and 30 m until October. Microcystin concentrations varied between 0.12 and $0.94 \mu\text{g L}^{-1}$ and never exceed $1 \mu\text{g L}^{-1}$ in the metalimnion. It was found at low concentrations ($0.2 \mu\text{g L}^{-1}$) even in December. Microcystin concentration was negatively affected by temperature and euphotic depth and positively correlated with chl-a (Figure 6).

Microcystin concentration and *P. rubescens* biomass showed a very strong relationship in general (Figure 7). However, microcystin content per biomass ranged

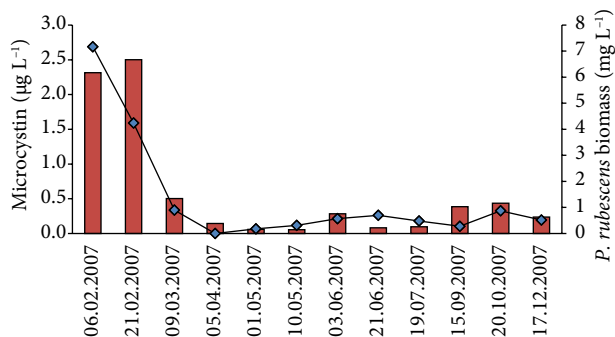


Figure 7. Seasonal changes of mean values of microcystin concentration (line) and *P. rubescens* biomass (column) in the water column.

between 0.02 and $1.92 \mu\text{g mg}^{-1}$ throughout the sampling period. Highest microcystin content was recorded at the end of June (Figure 8a.). When the second surface bloom occurred, *P. rubescens* biomass increased compared to the first bloom, although the MC concentration decreased markedly. At that time, samples were taken on 3 consecutive days. Microcystin content was detected as $0.12 \mu\text{g mg}^{-1}$ at the surface with a mean of $0.25 \mu\text{g mg}^{-1}$ for the whole water column on the first day. On the second day, values of mean toxin content were similar, with the exception of the surface water. On the third day, *P. rubescens* biomass was decreased in the whole water column. However, microcystin concentrations did not show a distinct decrease. Consequently, microcystin content increased 2-fold (Figure 8b).

4. Discussion

P. rubescens dominates the phytoplankton biomass in stratified, oligo- to mesotrophic lakes frequently in the summer period. It has been found in the metalimnion in Lake Sapanca since 1997 and deep chl-a maxima have been recorded in the lake for 30 years (Akcaalan et al., 2007). With decreasing temperatures, the mixing depth reaches the metalimnion and filaments disperse in the whole water column with much lower density. The described situation for Lake Sapanca is also quite a common phenomenon for many deep lakes around Europe, especially after the improved trophic state of lakes (Jacquet et al., 2005; Legnani et al., 2005; Ernst et al., 2009). With increasing transparency in summer months after the reduction of epilimnetic phosphorus concentrations, the metalimnion receives enough irradiance to allow the *P. rubescens* population to increase its biomass.

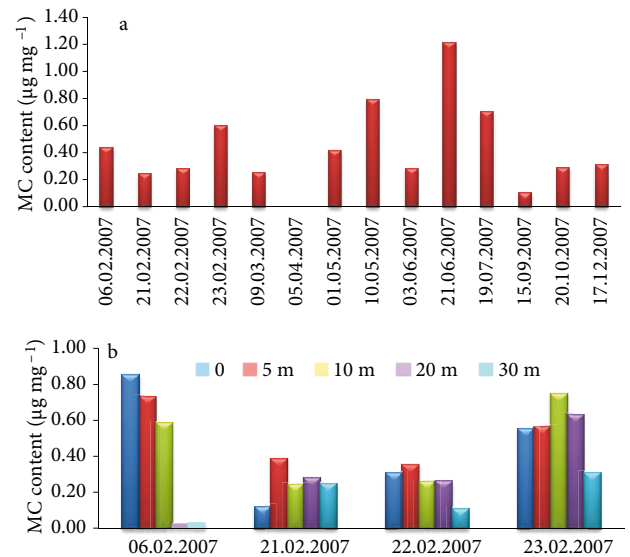


Figure 8. Mean microcystin content ($\mu\text{g mg}^{-1}$) of water column throughout the sampling period (a) and spatial distribution of microcystin content in the bloom period (b) in Lake Sapanca.

P. rubescens also forms surface blooms in winter with increasing frequency. Superficial blooms of *P. rubescens* were also observed in some lakes around Europe (Janse et al., 2005; Messiano et al., 2006; Naselli-Flores et al., 2007; Manganeli et al., 2010.). Three of these blooms occurred in central Italy and Sicily between November and February. Lake Zurich also experienced these blooms in November and December after deeper mixing, when the weather was calm and insolation was low, defined both by modeling and empirically (Walsby et al., 2005). Although *P. rubescens* has a long-term presence in Lake Sapanca, a surface bloom of *P. rubescens* was observed for the first time and it remained in the whole water column from February to March with a very high biomass.

It is unclear why *P. rubescens* did not show any surface blooms in the winter period in Lake Sapanca before. The winter of 2006/2007 was the mildest on record and was followed by an unusually warm spring (Dokulil et al., 2010). According to the National Oceanic and Atmospheric Administration, temperatures were warmer than average in Europe, Asia, and western Africa in January and February 2007. The most important winter effect of climate change is the year-to-year variation in rainfall (George, 2010). In this case, 2006 and 2007 had the lowest rainfall, at 651 and 678 mm, respectively, of all years between 1997 and 2010. Annual precipitation over northern Europe has increased by between 10% and 40% in the last century while the Mediterranean basin has experienced reductions of up to 20% (Dokulil et al., 2010). Whereas heavy rainfalls tend to transport more nutrients from catchment to lakes resulting in more or sometimes less primary production, depending on the lake retention time, lower precipitation will affect the retention time of the lakes and influence the composition of phytoplankton via reduction of flushing effects. Lower precipitation and calm water may have resulted in a superficial *P. rubescens* bloom in Lake Sapanca.

Air temperatures in the area of Lake Sapanca increased very slightly (0.06 °C) between 2000 and 2010; however, surface water temperatures showed a more pronounced increase (0.68 °C) in comparison to air temperature. The air temperature can be considered as a reliable indicator of biotic processes, such as cyanobacteria behavior within the epilimnion (Livingstone, 1998). Air temperature affects cyanobacteria directly through an increased growth rate and indirectly via the stabilization of the water column, favoring buoyant cyanobacteria.

Climate change impacts these ecosystems in various manners, including changes in temperature, ice cover, wind, and precipitation (Mooij et al., 2005). As a lowland lake (31 m a.s.l.), Lake Sapanca has no ice cover. *P. rubescens* was recorded at between 10 to 20 m depths where temperatures were 9–18 °C in the metalimnion; however,

it reached its highest biomass values at the surface waters in 2007 with water temperature of 11 °C. Naselli-Flores et al. (2007) found the same surface bloom in a Sicilian reservoir in December when lower water temperatures (9–10 °C) were recorded. In Lake Zurich, visible filaments on the surface were recorded in November and December with a temperature of 6–8 °C and calm weather (Walsby et al., 2005). *P. rubescens* prefers lower temperature to grow and Davis and Walsby (2002) stated that of 2 *Planktothrix* species, *P. rubescens* would outcompete *P. agardhii* within the temperature range of 10–21 °C.

Among the changing physical and chemical properties, temperature, light transmission, and nutrients had greater effects on algal growth (Baykal Özer et al., 2012). Nutrients showed an increase in Lake Sapanca between 1997 and 2007, coincident with chl-*a* concentrations (Aykulu et al., 2006; Akcaalan et al., 2007). The increase in nutrient concentrations could be a result of the longer retention time of the lake, since 2006/2007 was the driest period of the last 14 years. This increase could be one of the reasons for the surface bloom of *P. rubescens* in Lake Sapanca. Similarly, the appearance of this cyanobacterium in the surface waters of Lake Mondsee was observed in the eutrophication period of the lake (Dokulil and Teubner, 2012).

On the other hand, *P. rubescens* dominates the phytoplankton biomass in stratified reoligotrophicated lakes frequently at total phosphorus concentrations of approximately 10 µg L⁻¹. (Dokulil and Teubner, 2000). Accordingly, Jacquet et al. (2005) stated that *P. rubescens* mass occurrence arose when phosphate had been severely depleted in Lake Bourget. Absence of SRP in the epilimnion will be negatively effective on phytoplankton biomass and in favor of *P. rubescens* forming metalimnetic blooms in summer months. This was the situation in the lake more so than in the last 2 decades (Akcaalan et al., 2006). However, the only period in which SRP was below detection limits was February in the investigation period in Lake Sapanca, since *P. rubescens* bloom may have utilized all SRP when it started to develop a bloom. Interestingly, with a decreasing biomass of *P. rubescens*, SRP concentrations increased and a negative relationship between *P. rubescens* biomass and SRP was detected.

The growth and vertical distribution of the *P. rubescens* population in Lake Sapanca is likely to be affected by many factors. As a buoyant species, it has a chance to change its position in the water column. In low light conditions with enough nutrients, *Planktothrix rubescens* could produce more gas vesicles to float up to surface, forming Burgundy-blood phenomenon. The other important physical factor was rainfall. There were double effects of lower precipitation on *P. rubescens* biomass. George et al. (2004) showed that heavy winter rains reduce the phytoplankton

biomass by flushing effects, and hence a dilution occurs in the inocula of the following summer's population of *P. rubescens*. Secondly, an effect of lower rainfall is the longer retention time of water in lakes, enabling an increase of nutrients reaching upper parts of the water column by mixing in winter. When weather is calm, this will result in a winter surface bloom of *P. rubescens* that is superior in competition with other phytoplankton species by regulating its buoyancy.

Microcystin production was positively correlated with *P. rubescens* biomass. On the other hand, the relationship between *P. rubescens* biomass and microcystin concentration was not consistent in successive days in February. Microcystin content per biomass of *P. rubescens* was highly variable; lower toxin content was detected when biomass was higher. Similarly, a higher percentage of genotypes with inactive *mcy* genes was observed at higher *Planktothrix rubescens* biomasses by Kurmayer et al. (2004). Similar results were found in Lake Klinckenberg and higher toxin peaks were detected before the biomass maximum (Janse et al., 2005).

In conclusion, the combination of light climate and temperature is responsible for the metalimnetic occurrence of *P. rubescens* when nutrient concentrations are moderate (Dokulil and Teubner, 2000). Low SRP levels and high euphotic depth give rise to a *P. rubescens* increase

in the metalimnion of Lake Sapanca during the summer. Mild and calm winters together with lower precipitation will result in increasing surface blooms of *P. rubescens* in winter months. Similar to reports on Sicilian reservoirs, this study demonstrates that health risks caused by MC-producing cyanobacteria in lakes are present during winter as well as the rest of the year. The most striking effect of climate change on the Mediterranean basin will be increasing drought together with temperature rise. The general perspective of climate change focuses on cyanobacteria blooms in shallow and eutrophic lakes, which will be affected severely in this situation. However, deep and oligo- to mesotrophic lakes with a stratifying type of *Planktothrix* will be subject to microcystin the whole year around. When considering water shortages in the Mediterranean area, the most adverse effect will be on intensive drinking-water production from lakes such as Sapanca.

Acknowledgments

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