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Bloom of the potentially toxic cyanobacterium *P. rubescens*: seasonal distribution and possible drivers of its proliferation in the Vrutci reservoir (Serbia)

by

Ana Blagojević Ponjavić^{1,*}, Dušan Kostić², Prvoslav Marjanović², Ivana Trbojević¹, Slađana Popović³, Dragana Predojević¹, Gordana Subakov Simić¹

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¹Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

²The Jaroslav Černi Institute for Development of Water Resources, Jaroslava Černog 80, 11226 Belgrade, Serbia

³Institute of Chemistry, Technology and Metallurgy, National Institute, Center of Ecology and Technoeconomics, Universtity of Belgrade, Karnegijeva 4/II, 11000 Belgrade, Serbia

Abstract

Planktothrix rubescens is a filamentous cyanobacterium with a worldwide distribution in lakes and reservoirs of varying size and morphology. The objective of this study was to identify the population dynamics and the major drivers of P. rubescens blooms. Samples for qualitative and quantitative analysis of phytoplankton were collected every month in 2014 and 2015 from stationary depths, while the main physical parameters were measured in situ along the water column. Samples for chemical analysis were collected below the surface, at mid-depth (metalimnion) and near the bottom. In the periods of thermal stratification, P. rubescens formed the maximum biomass in the metalimnion, however, its volume-weighted biomass reached the maximum value in the winter mixing period (4.40 mm³ l⁻¹). Changes in the P. rubescens population in the reservoir were related to changes in the measured environmental parameters. Significant physical, hydrological, meteorological and chemical parameters were distinguished based on the multivariate analysis. The variable associated with water-level fluctuations showed the highest positive correlation with P. rubescens biomass. We conclude that it is necessary to establish water quality monitoring and prevent excessive water-level fluctuations to ensure a high quality of water supplies from the reservoir.

Key words: *Planktothrix rubescens*, bloomforming cyanobacteria, water-level fluctuations, drinking water reservoir, water supply, water retention time



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^{*} Corresponding author: blagojevicana88@gmail.com

Introduction

Eutrophication and climate change are currently the major concerns for surface water management (Xia et al. 2016), as nutrients and climate factors lead to more intensive and more frequent occurrence of algal blooms (Mariani et al. 2015). Extreme events, including heat waves, storms and flooding effects, are expected to increase across Europe (Beniston et al. 2007; IPCC 2007). According to Elliott (2010) and Carvalho et al. (2011), the most important factor affecting the mass growth of cyanobacteria, especially in deep stratified reservoirs, is water retention time. However, only little information is available about the effect of water-level fluctuations (WLF) and the flushing rate on the spread of cyanobacteria and their taxonomic variability (Padisák et al. 1999; Romo et al. 2013; Pawlik-Skowrońska & Toporowska 2016). In deep stratified reservoirs, where interannual changes have affected the strength of stratification, irregular hydrological events override the seasonal cycle of phytoplankton and cause irregular proliferation of fastgrowing species (Padisák 1991; Naselli-Flores 1999). Proliferation of cyanobacteria in lakes and water supply reservoirs is a growing issue due to the presence of many potentially toxic species (Carmichael 1992; Codd 2000), which may affect organoleptic properties of water (Watson 2003; Izaguirre & Taylor 2004), cause water treatment problems and, in the worst case scenario, pose a threat to public health (Falconer 1999).

In deep lakes, which stratify in summer, some planktonic cyanobacteria proliferate in the metalimnion and form deep chlorophyll maxima (DCM). This has been observed for *Planktothrix rubescens* (de Candolle ex Gomont) Anagnostidis et Komárek (1988), a filamentous cyanobacterium that has a global distribution in lakes of varied shapes and sizes (Padisák et al. 2010). *P. rubescens* develops when total phosphorus (TP) concentrations are relatively low



Vrutci reservoir on the Đetinja River (Western Serbia). The yellow marker indicates the sampling location referred to as Vodozahvat

(Chorus et al. 2011). The species is adapted to low light levels (Reynolds 1997; Zotina et al. 2003) and is often associated with low water temperatures (Nürnberg et al. 2003). It is well documented that *P. rubescens* is a taxon capable of inhibiting the growth of other phytoplankton species through the production of microcystins (among other produced substances and secondary metabolites; Feuillade 1994). Compared to other cyanobacteria, the genus *Planktothrix* has the highest concentration of microcystins (MC) per unit of dry weight (Ernst et al. 2009).

The objective of this study was to identify and describe the major drivers of *P. rubescens* proliferation and distribution in the Vrutci water supply reservoir. We hypothesized that the inflow of nutrients, water-level fluctuations and weather conditions are the factors determining the *P. rubescens* bloom.

Materials and methods

Study site

The Vrutci reservoir (43°50'34"N, 19°41'36"E) was created in 1984 by damming the Detinja River in Western Serbia (Fig. 1). It is a temperate, mid-altitude reservoir. The normal operational level of the reservoir was set at 621.3 m a.s.l. The reservoir is elongated in shape and is 7 km long, with a maximum depth (located near the dam) of 60 m (Table 1). The geological substrate consists mainly of limestone and serpentines. The average retention time is estimated at 250 days (JCI 1971). Apart from the Detinja River, there are 28 other permanent and intermittent tributaries. The Vrutci reservoir is the main source of water supply for the city of Užice. It is also used for flood protection, sediment retention, and low-flow management during periods of drought. The total area of the catchment is 160 km². It is sparsely populated and has no industry (JCI 1971). Mixing patterns in the Vrutci reservoir are highly complex and dependent on meteorological conditions and water withdrawals. Monitoring of the reservoir has been insufficient over the last 20 years and carried out by the officials only once a year.

Sampling procedure and laboratory analysis

At the end of December 2013, the upstream part of the reservoir turned into a reddish pulp that spread all over the reservoir in just a few days, which was followed by the formation of red surface patches. The study period lasted from January 2014 to December 2015. Water temperature (WT), pH, conductivity (Cond) and chlorophyll a concentration (Chl-a) were



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Table 1

Main morphological features of the studied Vrutci reservoir and characteristics of the catchment

Characteristics of the reservoir	Value	Unit
Area	1.92	km²
Volume	40.2×10^{6}	m ³
NPL (Normal Pool Level)	621.3	m a.s.l.
Max depth	60	m
Mean depth	20.9	m
Water retention time	250	days
Characteristics of the catchment		
Area	160	km²
Max altitude	1544	m a.s.l.
Mean altitude	915	m a.s.l.

measured in situ monthly (along the entire water column), using a multiparameter probe (YSI 6600V2-2), while water transparency was determined using a Secchi disk. Samples for the analysis of nutrients – ammonium (N-NH₄), nitrites (N-NO₂), nitrates (N-NO₃), total nitrogen (TN), orthophosphate (ortho-P) and total phosphorous (TP) – were collected in January, June, September, October and December 2014, as well as from March to December 2015. Samples were collected below the water surface, in the metalimnion (or at the mid-depth during circulation) and near the bottom, and analyzed at the Jaroslav Černi Institute Water Quality Laboratory, in accordance with the standard procedures and methods (Franson et al. 1975; Eaton et al. 2005; SRPS European Norms 12260:2008).

Phytoplankton samples were collected monthly throughout the study period with the exception of November 2014, January and February 2015, due to the lack of funding during these months. The sampling site was located near the dam and water intake and was referred to as Vodozahvat (Fig. 1). Representative samples were collected from stationary depths: 0, 3, 10, 14, 18, 27, 31, 35 and 43 m. Water samples for qualitative analysis of phytoplankton were collected using a plankton net (Ø 22–23 µm). A vertical (beta) Van Dorn bottle of 2.2 I was used to collect samples for quantitative analysis. Samples were put into plastic bottles and preserved with Lugol's solution according to European Standards 15204 (2006).

Phytoplankton analyses

The identification of taxa was performed using a light microscope (Karl Zeiss Imager M1), camera (Carl Zeiss AxioCam MRC5) and fluorescence microscope (Olympus BX 5) according to the standard literature (Huber-Pestalozzi et al. 1983; Starmach 1983; Krammer & Lange-Bertalot 1986; 1988; 1991; John et al. 2002;

Komárek & Anagnostidis 1998; 2005; Komárek 2013). The Shannon diversity index (H') was calculated according to the standard formula, based on the number and biomass of species (Shannon & Weaver 1949).

The quantitative analysis of phytoplankton was based on the Utermöhl method (EN 15204 2006), using a Leica inverted microscope. The number of cells was converted into the phytoplankton biomass by geometric approximations, using the standard formulas (Hillebrand et al. 1999; Sun & Liu 2003), while the biomass data were expressed as $mm^3 l^{-1}$.

Meteorological and hydrological data

Meteorological data (air temperature – AT, wind speed – Wind, precipitation – Prec, insolation and cloudiness) were obtained from annual reports of the Republic Hydrometeorological Institute of Serbia (RHMZ), from the nearest weather station (Zlatibor). Data on the water outflow and water level were obtained from the Užice Public Utility Company.

Data analysis

Complex external drivers produced complex-shaped vertical temperature profiles that deviated from those commonly observed. In most cases, the distinction between seasonal and ephemeral thermocline was not obvious. A modified van Genuchten fitting curve (Rimmer et al. 2005; Jennings et al. 2012) was employed to model the observed temperature profiles. The thermocline was then positioned to match the depth at which the second derivative of the temperature plot equals zero.

To answer the question as to which environmental factors shaped the dynamics of the P. rubescens population, redundancy analysis (RDA) was performed using CANOCO for Windows, Version 5.0 (Ter Braak & Šmilauer 2012). The RDA showed the relationship between the biomass of P. rubescens and other phytoplankton taxa as well as preselected environmental (hydrological, meteorological, physical and chemical) parameters - 11 variables included in total. The linear method was used since the gradient on the first axis was 0.9 SD units long. Before the RDA was performed, meteorological and chemical factors were processed separately using the "interactive forward selection" option, where statistical significance of each variable was tested by the Monte Carlo permutation test (p < 0.05). The significance of all other variables was also tested, so that all variables included in the ordination diagram were significant. The explanatory variables included physical (WT, Cond and Secchi



depth – Secchi), hydrological (water flushing – Wflush), meteorological (AT, Prec and Wind) and chemical (NH_4 , NO_3 , ortho-P and TP) parameters. The terms indicating lake stratification (epilimnion – E, metalimnion – M, hypolimnion – H) and water mixing (C) were used as supplementary variables. In addition, response curves that represent the relationship between *P. rubescens* biomass and two parameters separately (WT and Wflush) were also included for more detailed analysis.

Variation partitioning with three groups of variables was performed. The first group included physical and hydrological parameters (WT, Cond, Secchi, and Wflush), the second group included meteorological parameters (AT, Prec and Wind) and the third group was represented by nutrients or chemical parameters (NH_4 , NO_3 , ortho-P and TP). The objective of these analyses was to assess how much variability in our data is described by every group of variables, taking in account their simple and conditional effect. The significance of each fraction was also presented.

Results

Seasonal and vertical distribution of the *P. rubescens* population

Species diversity of the phytoplankton in the Vrutci reservoir, expressed as the Shannon diversity index, was generally low (1.18 in 2014 and 1.20 in 2015) and higher in the epilimnion than in the metalimnion. The phytoplankton biomass was characterized by the dominance of the cyanobacterium P. rubescens throughout the study period, except autumn 2014 and spring 2015 when the highest growth of Bacillariophyta was observed (Fig. 2). P. rubescens volume-weighted biomass was higher in 2014 (max 4.40 mm³ l⁻¹; Fig. 2a) than in 2015 (Fig. 2b). During the winter mixing, the P. rubescens population showed a higher biomass in the upper water layers and its gradual decline with increasing water depth (Fig. 3a). After that, at the beginning of the stratification period (April 2014), a sharp decline of P. rubescens biomass was observed. It is important to note that in the period of the highest abundance of P. rubescens (winter 2014), the water level was 5 to 6 m lower than usual (Fig. 4) and red scum appeared on the surface in areas sheltered from the wind. Immediately after the high floods in May 2014, a large amount of inactive P. rubescens filaments formed the purple-red floating patches on the water surface (May and June). After this extreme event and a large outflow of water, P. rubescens reappeared in the metalimnion during the period of stable summer stratification, at a depth of 3 to 7 m (Fig. 3a). The





Volume-weighted averages of *P. rubescens* and total

phytoplankton biomass in the Vrutci reservoir: in 2014 (a) and 2015 (b)

volume-weighted biomass of *P. rubescens* during the metalimnetic bloom was twice as low as the biomass during the surface bloom in winter (2.19 mm³ l⁻¹). The maximum volume-weighted biomass of *P. rubescens* in 2015 was recorded in July (3.95 mm³ l⁻¹; Fig. 2b). After that, the biomass of *P. rubescens* was characterized by a high value in September at a depth of 6 m (8.54 mm³ l⁻¹) and October (0–3 m; Fig. 3a).

Environmental factors related to the bloom of *P*. *rubescens*

Due to the exceptionally mild winter of 2013/2014, the ice cover was not formed, which induced the monomictic mixing behavior of the Vrutci reservoir. High air temperatures in summer induced warming of surface layers and formation of a stable vertical thermal structure. Maximum epilimnetic water temperatures were recorded in August 2014 and July 2015 (Tables 2 and 3). More pronounced differences in mean temperatures between the water layers were observed in 2015.

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Figure 3

Biomass of P. rubescens (a); water temperature and location of the thermocline (black lines) in the Vrutci reservoir (b)



Figure 4

Annual water-level fluctuations in the Vrutci reservoir in 2014 and 2015



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Mean, minimum and maximum values of hydrological, meteorological, physical and chemical (Chl-*a*) parameters in 2014

Hydro- and meteorological parameters	Min.			Mean			Max		
Water flushing (m³ s⁻¹)	-3.71			0.86			6.79		
Air temp. (°C)		-0.09		9.07			17.33		
Wind speed (m s ⁻¹)	1.19			1.85			4.66		
Insolation (h)	3.34			5.35			8.91		
Precipitation (mm)	1.38			7.37			12.36		
Physicochemical parameters									
Secchi depth (m)	1.2			1.9			2.8		
	Epilimnion		Metalimnion			Hypolimnion			
	Min.	Mean	Max	Min.	Mean	Max	Min.	Mean	Max
Water temp (°C)	4.9	14.5	22.7	5.2	10.7	15.9	4.9	8.5	12.5
Conductivity (µS cm ⁻¹)	222.0	259.2	288.5	219.0	247.1	290.5	204.5	239.8	276.0
рН	8.2	8.7	9.2	7.8	8.2	8.5	7.7	7.8	8.2
Chl- <i>a</i> conc. (μ g l ⁻¹)	3.1	7.2	13.9	3.7	7.4	9.9	4.5	6.1	9.8

Table 3

Mean, minimum and maximum values of hydrological, meteorological, physical and chemical parameters in 2015									
Hydro- and meteorological parameters	Min.			Mean			Max		
Water flushing (m ³ s ⁻¹)	-1.10			0.15			3.38		
Air temp. (°C)		-0.76		9.29			20.71		
Wind speed (m s ⁻¹)	1.00			1.41			2.20		
Insolation (h)	3.94			6.61			10.89		
Precipitation (mm)	1.25			5.76			9.82		
Physicochemical parameters									
Secchi depth (m)	1.1			2.8			4.3		
	Epilimnion		1	Metalimnio	n	Hypolimnion			
	Min.	Mean	Max	Min.	Mean	Max	Min.	Mean	Max
Water temp. (°C)	6.4	16.5	25.3	5.4	9.7	13.2	4.6	6.7	9.7
Conductivity (μS cm⁻¹)	145.0	267	320.5	223.2	244.4	256.5	222.6	251.5	301.0
рН	8.3	8.6	9.0	7.3	8.2	9.0	7.2	7.6	8.6
Chl- <i>a</i> conc. (μ g l ⁻¹)	3.6	4.8	6.5	3.3	11.2	32.8	2.9	3.8	5.0
N-NH ₄ (μg l ⁻¹)	20	120	400	10	113	276	10	99	198
N-NO ₂ (μg l ⁻¹)	5	6	9	14	35	90	5	14	90
N-NO ₃ (μg l ⁻¹)	50	280	810	200	373	820	340	624	838
TN (μg l ⁻¹)	450	920	1430	710	1101	1601	784	1228	1846
ortho-P (ug l ⁻¹)	10	20	50	9	17	31	9	9	10
	10								
TP (μg ⁻¹)	30	120	470	34	135	417	34	156	324

In both years, the location of the seasonal thermocline was observed from a depth of about 3 m in spring to a depth of about 12 m in autumn (Fig. 3b). As a consequence of the extreme inflow in May 2014, the temperature of hypolimnetic water increased to about 4°C and the thermocline shifted 3 m upward. The pH value was relatively constant, with slightly lower values in deeper strata of metalimnion and hypolimnion compared to the upper strata (Tables 2 and 3).

In May 2014, heavy precipitation was observed when during four consecutive days (from 13 to 16 May)

110 mm of rainfall was recorded at the watershed gauge station. Almost 25% of the reservoir volume was replenished with surface runoff (Fig. 4). The water level varied in a wide range between 615 and 626.7 m a.s.l., and in over 30% of the study period the water level was higher than the usual water level (621.3 m a.s.l.; Fig. 4). Minimum water flushing values were recorded in October 2014 and July 2015, which indicated a higher outflow than inflow (Tables 2 and 3).

 $N-NH_4$ concentrations were higher in epi- than in meta- and hypolimnion, and the maximum value was







Nutrients in the Vrutci reservoir in 2014 and 2015: N-NO, concentration (a) and ortho-P concentration (b)

recorded in September 2015 (400 µg l⁻¹; Table 3). N-NO₂ mean concentrations were generally high, especially in meta- and hypolimnion (Table 3), but concentrations started to decrease rapidly in June, from the surface to a depth of 15 m (Fig. 5a). Ortho-P concentration was high in the epi- and metalimnion (Table 3) at a depth ranging from 0 to 10 m from July to September (Fig. 5b).

According to data from two years, P. rubescens was characterized by higher biomass during the mixing period and during stratification (metalimnion; Fig. 6). No correlation between the biomass of P. rubescens and other phytoplankton was observed (Fig. 6). P. rubescens biomass was positively correlated with the hydrological parameter Wflush and NH⁺. Slightly negative correlation was observed in the case of Wind, NO₂, WT and AT, and negative correlation in the case of physical parameters Cond and Secchi.

The response curves that include the relationship between the P. rubescens biomass and two parameters separately (WT and Wflush) are presented in Figure 7. It appears from Figure 7a that the maximum value of P. rubescens biomass is observed at a temperature close to 16°C. The biomass of P. rubescens was almost stable at Wflush values ranging from 2 to 8, and the highest biomass was observed at the value of about 2 (Fig. 7b).

According to the variation partitioning, physical and hydrological variables (WT, Cond, Secchi and



Figure 6

RDA ordination diagram showing the relationships between the biomass of P. rubescens and other phytoplankton hydrological, taxa as well as meteorological, physical and chemical parameters (nutrients). Water mixing layers are used as supplementary variables. The first RDA axis explained 21.90% and the first and second axes together explained 40.80% of the variability in our data, which accounted for the total explained variation in the analysis (F = 12.6, p = 0.0002).





Figure 7

Response curves that include the relationship between *P. rubescens* biomass and water temperature – WT (a) and water flushing – Wflush (b)

Wflush – fractions a + d + f + g) explained 21.8% of the variability in our data. Irrespective of the others, this group of variables explained 11.8% of the total variation (fraction "a"). Meteorological parameters (Prec, Wind and AT - fractions b + d + g + e) explained a total of 16.2%, and independently - 6.2% of the variability (fraction "b"). Nutrients (chemical parameters) used in the RDA analysis (NH_{4}^{+} , TP, ortho-P and NO_{2} – fraction c + e + f + g) explained 18.8% of the total variability. However, this group of variables with no shared effect with other groups explained 9.1% of the variation in our data (fraction "c"). The total explained variation was 40.8%. Comparing these three groups, physical and hydrological variables explained the highest portion of variability in our data and were followed by chemical (nutrients) and then meteorological parameters. The explained variability and the significance of the above-mentioned fractions are presented in Table 4.

Discussion

Our results confirmed the hypothesis that physical (WT) and hydrological parameters (Wflush) presented in Table 4 were crucial for the proliferation of *P. rubescens*, and contrary to our hypothesis, the biomass of *P. rubescens* did not depend on the input of nutrients. According to the RDA results, the biomass of *P. rubescens* showed a slightly negative correlation with water temperature. The optimum water temperature for the growth of *P. rubescens* in the Vrutci reservoir (7–16°C) coincides with the optimum temperature range for its growth in Lake Steinsfjorden and Lake Mondsee (Blikstad Halstvedt et al. 2007; Dokulil and Teubner 2012). According to Suda et al. (2002), the optimum of *P. rubescens* is between 10°C and 20°C, but our research has shown that the biomass

Table 4

Explained variation and significance tests of the variation partitioning that included three groups of variables – conditional (a, b, c) and simple effects (a + d + f + g, b + d + g + e, c + e + f + g) were tested

Tested fraction	Explained variation (%)	F	р			
а	11.8	10.0	0.0002	First Group		Second Group
b	6.2	7.1	0.0002	а	d	Ь
с	9.1	7.8	0.0002		-	
a + d + f + g	21.8	14.5	0.0002	f	g	e
b + d + g + e	16.2	11.3	0.0002			
c + e + f + g	18.8	12.0	0.0002		С	Third
a + b + c + d + e + f + g	40.8	9.8	0.0002			c.oup



of *P. rubescens* rapidly decreased at temperatures above 16°C. *P. rubescens* was present in all seasons throughout the study period, with two annual, prolonged, consecutive blooms. The first extreme in *P. rubescens* biomass was recorded in water samples collected during the intense winter bloom at a very low water temperature (5.5°C) and during the summer bloom when the maximum temperature of metalimnion was 15.9°C. The adaptation of *P. rubescens* to low light conditions and low water temperatures allows this cyanobacterium to survive in winter, even with ice cover (Dokulil & Teubner 2012).

The prolonged autumn-winter mixing favored the spread of P. rubescens into the deeper water layers. The biomass of P. rubescens during winter mixing exceeded its biomass during the period of stratification, and similar patterns were observed in Lake Zürich (Van den Wyngaert et al. 2011). The maximum P. rubescens volume-weighted biomass in Vrutci (4.40 mm³ l⁻¹) was equal to the biomass occurring in Lake Mondsee before the effects of restoration measures were observed (Dokulil & Teubner 2012). The ecology and chemistry of the reservoir were strongly affected by heavy precipitation in May 2014. During that storm, part of the P. rubescens population that entered the mixed surface layer competed with other phytoplankton taxa, which are better adapted to the high irradiance (Davis et al. 2003). Intensive mixing leads to dilution of P. rubescens filaments. The high inflow caused strong horizontal adventive flow and pushed the phytoplankton populations closer to the dam. At the same time, torrential tributaries introduced additional amounts of nutrients from the catchment, which is prone to pluvial erosion (Kostić et al. 2016). After significant water withdrawals, the water level in the reservoir dropped in July 2014. At that time, the high P. rubescens biomass level was recorded again. According to the RDA results, the biomass of P. rubescens was positively correlated with the flushing rate. Previous studies (Naselli-Flores 2000) showed that reservoirs with large volume fluctuations are dominated by cyanobacteria. Weather conditions in 2015 were more stable, warm and dry, without extreme events, which is why the population of P. rubescens settled in the metalimnion during the period of stratification. Periodical appearance of red scum patches on the lake surface, after the flood in 2014 and September 2015, is a common phenomenon explained by the change of the buoyancy regulation mechanism (Walsby et al. 2006). There are different explanations regarding the annual cycle of P. rubescens (Reynolds 1984; Micheletti et al. 1998), however, the results of this study coincide with the previous research by Davis et al. (2003) showing that P. rubescens is a flexible organism that can become

dominant in different seasons and different conditions. It is not only a low-irradiance specialist in the summer metalimnion or autumn mixing, but can be competitive in both periods (Davis et al. 2003).

Although many studies emphasize the importance of phosphorus load management for cyanobacterial blooms, reducing its input is not always an effective way to prevent toxic blooms (Lewis & Wurtsbaugh 2008; Amano et al. 2010; Koreiviene et al. 2014) and our results are in accordance with this observation. As a result, P. rubescens has a competitive advantage over eukaryotic algae in the nitrogen-deficient Vrutci reservoir, considering its ability to store nitrogen in cells in the form of cyanophycin and phycocyanin (Van de Waal et al. 2010), and to absorb organic nitrogen from the external environment (Kurmayer et al. 2016). Smith (1983) reported that cyanobacterial blooms tend to occur mainly when the epilimnetic TN:TP ratio drops below 29. In addition to the limitation of nutrients, temperature and water column stability management is important for the disappearance of P. rubescens (Jacquet et al. 2014), while according to Romo et al. (2013) and Naselli-Flores and Barone (2005), restoration measures should include reservoir residence time management.

Conclusion

P. rubescens in the Vrutci reservoir prefers low temperatures and low irradiance and is characterized by prolonged winter and summer (metalimnetic) blooms similar to those observed in deep and cold pre-Alpine lakes. In addition to standard monitoring of water quality and eutrophication, it is important to maintain the constant water level in the Vrutci reservoir in order to reduce the volume fluctuations.

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P. rubescens distribution in the Vrutci reservoir

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