

Kostadin Dochin¹
Angelina Ivanova¹
Ivan Iliev²

The phytoplankton of Koprinka Reservoir (Central Bulgaria): species composition and dynamics

Authors' addresses:

Institute of Fisheries and Aquaculture,
Department of Aquaculture and Water
Ecosystems, 248 Vasil Levski Str.,
Plovdiv 4003, Bulgaria.

University of Plovdiv „Paisii
Hilendarski“, Faculty of Biology,
Department of Biochemistry and
Microbiology, 24 Tzar Asen Str.,
Plovdiv 4000, Bulgaria.

Correspondence:

Kostadin Dochin

Institute of Fisheries and Aquaculture,
Department of Aquaculture and Water
Ecosystems, 248 Vasil Levski Str.,
Plovdiv 4003, Bulgaria.
Tel.: +359 32 956 031
Fax: +359 32 953 924
e-mail: doksi11@abv.bg

Article info:

Received: 25 August 2017

In revised form: 27 September 2017

Accepted: 5 October 2017

ABSTRACT

The aim of the study was to determine the species composition and dynamics of the phytoplankton community of Koprinka reservoir. We have identified a total of 109 taxa assigned to 6 divisions including Chlorophyta (37), Ochrophyta (26), Cyanoprokaryota (22), Euglenophyta (11), Streptophyta (11) and Pyrrophyta (2). The highest phytoplankton biomass (PhB) and numbers (PhN) and the lowest species richness were detected in September. In October with the decrease of the PhB the species diversity has increased. The highest species richness was observed at station 3, situated in the riverine area, with the phytoplankton abundance being significantly higher compared to the other two stations. The species *Hariotina polychorda* (Korshikov) E.Hegewald dominated in the water samples in the summer at all stations. In the early autumn, a bloom of the potentially toxic species *Microcystis wesenbergii* (Komárek) Komárek ex Komárek was detected, which is an indicator for eutrophication process in the reservoir. Cluster analysis (CA) based on the phytoplankton composition isolated the samples from each sampling periods in a separate water cluster, characterized by significant spatial heterogeneity. The phytoplankton species composition, and the values for biomass, and chlorophyll *a* are evident for the eutrophic state of the reservoir.

Key words: phytoplankton, species composition, reservoir, blooms, potentially toxic species, eutrophication process

Introduction

Reservoirs in Bulgaria are essential for the economy and are used for power production, water supply, irrigation and fish farming. Unlike natural lakes, reservoirs are characterized by ecosystem instability, caused by the frequent water level fluctuations (Kenderov *et al.*, 2014). Attention to reservoirs and their phytoplankton communities started at the end of the 50s of the 20th century, generally with the impoundment of new water reservoirs (Dochin & Stoyneva, 2014). In previous studies of 23 Bulgarian reservoirs has been published data for 250 phytoplankton species (Stoyneva & Temniskova-Topalova, 2007; Stoyneva, 2014). Over the past years a number of investigations focused on the development of phytoplankton in large reservoirs in Bulgaria were published (Belkinova *et al.*, 2007; Teneva *et al.*, 2010; Stoyanov *et al.*, 2013; Belkinova *et al.*, 2014; Beshkova *et al.*, 2014; Dochin & Stoyneva, 2014, 2016). Several studies were conducted in Koprinka reservoir on the influence of environmental factors on the diversity of biological communities. Ognjanova-Roumenova *et al.* (2013) recorded 136 taxa of benthic diatom and explored the relationships

between environmental variables and patterns in the epilithic diatom assemblages. Water transparency and the amount of chlorophyll *a* were used for the assessment of the trophic state in the reservoirs Zhrebchevo and Koprinka (Kenderov *et al.*, 2014). Koprinka reservoir was built in 1950. It is situated on River Tundzha and is one of the oldest in Bulgaria. It has been under anthropogenic pressure for over 50 years. The eutrophication of the reservoir may have a negative effect on the biological communities in the Tundzha river ecosystem. The use of phytoplankton as the main indicator that immediately responds to changes in the environment would allow a clearer assessment of the status of this significant economic water basin. However, data concerning the phytoplankton, which is an important component of the reservoir Koprinka during the last years, is scarce. Therefore, the objective of this paper was to determine species composition, trophic status, and dynamics of algal communities in the water column of the Koprinka reservoir, situated in Central Bulgaria.

Materials and Methods

RESEARCH ARTICLE

Koprinka reservoir (IBW2062) is located in Central Bulgaria, situated on the Tundzha River. Its main morphometric characteristics are presented in Table S1. Samples were collected from three stations for the period July-November 2015 (Figure 1). Water samples with an analytical volume of one liter for each test were collected with Niskin-Type water sampler 5L model (Hydro-Bios Apparatebau GmbH, Germany) from the epi-, meta- and hypolimnion at each station. The samples for phytoplankton analysis were processed by the standard method of fixation with formalin to final concentration 4% and further sedimentation (ISO 5667-1:2006/AC:2007; ISO 5667-3:2003/AC:2007). Water temperature (TMP) and dissolved oxygen (DO) were measured *in situ* with an oxygen meter (WTW OXY 1970i). The depth of the euphotic layer was determined by measuring the water transparency (Z_S) with a 20 cm diameter Secchi disk. Electrical conductivity (Cond) and pH were measured with WTW Conductivity meter (Cond3310/SET) and WTW pH-meter (315/SET) respectively. Ammonium (N-NH₄), nitrate (N-NO₃) and total nitrogen (TN), and manganese III COD (COD_{Mn}) were

measured in the laboratory using standard analytical methods (ISO 8467:1993; ISO 5664:1984; ISO 7890-1:1986; ISO 6878:2004). Total phosphorus (TP) concentration was measured by the Phosphate Cell Test (114543, Merck Millipore). Chlorophyll *a* (Chl *a*) concentration was determined by the spectrophotometric method in an ethanol extract after filtration (ISO 10260:2002).



Figure 1. Map of the Koprinka Reservoir and location of the sampling stations.

Table 1. List of phytoplankton species observed in Koprinka Reservoir for the studied period.

Taxa	Month								
	VII			IX			X		
Station №	1	2	3	1	2	3	1	2	3
Cyanoprokaryota									
<i>Anabaena</i> sp.	*	*							
<i>Anabaena sphaerica</i> Bornet & Flahault	*		*			*			
<i>Anabaenopsis circularis</i> (G.S.West) Woloszynska & V.Miller							*		
<i>Anathece clathrata</i> (W.West & G.S.West) Komárek, Kastovsky & Jezberová	*		*						
<i>Aphanizomenon flosaquae</i> Ralfs ex Bornet & Flahault	*	*	**	**		*	**	**	**
<i>Aphanocapsa delicatissima</i> West & G.S.West		**	*		*				
<i>Aphanocapsa incerta</i> (Lemmermann) G.Cronberg & Komárek	*	*	*				*	*	
<i>Aphanocapsa</i> sp.	*	*	*		*				*
<i>Aphanothece</i> sp.									*
<i>Chroococcus minutus</i> (Kützing) Nägeli								*	
<i>Chroococcus turgidus</i> (Kützing) Nägeli	*	*	**		*		*	*	
<i>Dolichospermum scheremetieviae</i> (Elenkin) Wacklin, L.Hoffmann & Komárek	**								*
<i>Gomphosphaeria</i> sp.	*	*	*						
<i>Limnococcus limneticus</i> (Lemmermann) Komárková, Jezberová, O.Komárek & Zapomelová		*	*		*				*
<i>Microcystis aeruginosa</i> (Kützing) Kützing		*	**	*		**			
<i>Microcystis</i> sp.									*
<i>Microcystis wesenbergii</i> (Komárek) Komárek ex Komárek	*		*	**	**	**	**	**	**
<i>Planktolyngbya limnetica</i> (Lemmermann) Komárková-Legnerová & Cronberg	*			**	**	*			*
<i>Pseudanabaena catenata</i> Lauterborn			*						
<i>Snowella lacustris</i> (Chodat) Komárek & Hindák			*						
<i>Synechococcus linearis</i> (Schmidle & Lauterborn) Komárek	*								
<i>Woronichinia naegelianiana</i> (Unger) Elenkin	*	*							*
Chlorophyta									
<i>Actinastrum hantzschii</i> Lagerheim			*	*					*
<i>Ankistrodesmus fusiformis</i> Corda									*
<i>Ankyra ocellata</i> (Korshikov) Fott			*						

RESEARCH ARTICLE

<i>Characium</i> sp.								*	
<i>Chlamydomonas</i> sp.		*							
<i>Coelastrum microporum</i> Nägeli		*		*	*		*		
<i>Coelastrum</i> sp.									*
<i>Coenochloris</i> sp.							*		
<i>Crucigenia tetrapedia</i> (Kirchner) Kuntze							*	**	
<i>Crucigeniella irregularis</i> (Wille) P.M.Tsarenko & D.M.John					*				
<i>Desmodesmus bicaudatus</i> (Dedusenko) P.M.Tsarenko					*				
<i>Desmodesmus communis</i> (E.Hegewald) E.Hegewald	*	**	*	*	*		*	**	*
<i>Desmodesmus protuberans</i> (F.E.Fritsch & M.F.Rich) E.Hegewald				*					
<i>Golenkinia radiata</i> Chodat							*		*
<i>Hariotina polychorda</i> (Korshikov) E.Hegewald	**	**	**	**	*	*			
<i>Hyaloraphidium contortum</i> Pascher & Korshikov			*						
<i>Korshikoviella limnetica</i> (Lemmermann) P.C.Silva							*		
<i>Korshikoviella</i> sp.				*			*		
<i>Messastrum gracile</i> (Reinsch) T.S.Garcia			*						
<i>Monactinus simplex</i> (Meyen) Corda				*	*	*			
<i>Monoraphidium contortum</i> (Thuret) Komárková-Legnerová				*			*		
<i>Monoraphidium</i> sp.					*				*
<i>Mucidosphaerium pulchellum</i> (H.C.Wood) C.Bock, Proschold & Krienitz	*	*							
<i>Oocystis lacustris</i> Chodat	*								
<i>Oocystis</i> sp.			*	*					
<i>Pandorina morum</i> (O.F.Müller) Bory			**				*	**	**
<i>Pediastrum duplex</i> Meyen	*	*		*	*	*		*	
<i>Pseudoschroederia robusta</i> (Korshikov) E.Hegewald & E.Schnepf									*
<i>Pseudotetrastrum punctatum</i> (Schmidle) Hindák								*	*
<i>Radiococcus polycoccus</i> (Korshikov) I.Kostikov, T.Darienko, A.Lukesová & L.Hoffmann							*		*
<i>Scenedesmus acuminatus</i> var. <i>elongatus</i> G.M.Smith									*
<i>Tetradismus obliquus</i> (Turpin) M.J.Wynne			*	*		*			
<i>Tetraedriella</i> sp.									*
<i>Tetraedron minimum</i> (A.Braun) Hansgirg	*	*	*	*					
<i>Tetraedron</i> sp.						*	*		
<i>Tetrastrum</i> sp.					*	*			
<i>Willea apiculata</i> (Lemmermann) D.M.John, M.J.Wynne & P.M.Tsarenko				*	*				
Streptophyta									
<i>Closterium aciculare</i> T.West	*	*		*	*			*	
<i>Closterium acutum</i> Brébisson in Ralfs	*	*							*
<i>Closterium pronum</i> Brébisson					*				
<i>Cosmarium margaritifera</i> Meneghini ex Ralfs	*		*						
<i>Cosmarium</i> sp.	*	*	*		*				
<i>Elakatothrix gelatinosa</i> Wille	*		*						
<i>Elakatothrix genevensis</i> (Reverdin) Hindák	*								
<i>Elakatothrix lacustris</i> Korshikov		*							
<i>Staurastrum gracile</i> Ralfs ex Ralfs	*	*	**						
<i>Staurastrum pingue</i> var. <i>planctonicum</i> (Teiling) Coesel & Meersters	**	**	**	*	*	*			*
<i>Staurastrum</i> sp.	*								
Euglenophyta									
<i>Euglena granulata</i> (G.A.Klebs) F.Schmitz								*	*
<i>Euglena</i> sp.				*	*	*			*
<i>Lepocinclis acus</i> (O.F.Müller) B.Marin & Melkonian		*							
<i>Phacus longicauda</i> (Ehrenberg) Dujardin		*							*
<i>Strombomonas</i> sp.									*
<i>Trachelomonas nigra</i> Svirenko							*		
<i>Trachelomonas</i> sp.		*	*		*			**	**

RESEARCH ARTICLE

<i>Trachelomonas hispida</i> (Perty) F.Stein			*		*	*		*	*
<i>Trachelomonas oblonga</i> Lemmermann		*						*	*
<i>Trachelomonas planctonica</i> Svirenko			*	*	**		**	*	**
<i>Trachelomonas volvocina</i> (Ehrenberg) Ehrenberg							**		
Pyrrhophyta									
<i>Ceratium hirundinella</i> (O.F. Muller) Dujardin	*	**	**	*	*			**	**
<i>Peridinium</i> sp.				*	*	*			*
Ochrophyta									
Chrysophyceae									
<i>Chrysococcus</i> sp.									*
Synurophyceae									
<i>Mallomonas acaroides</i> Perty								*	**
<i>Mallomonas</i> sp.								*	**
<i>Mallomonas tonsurata</i> Teiling									*
<i>Rhodomonas</i> sp.	*								
Bacillariophyceae									
<i>Amphora</i> sp.								*	*
<i>Asterionella formosa</i> Hassall	*			*	*	**	**	**	*
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen				**	**	*		*	*
<i>Cocconeis pediculus</i> Ehrenberg								*	
<i>Cocconeis placentula</i> Ehrenberg				*					
<i>Cocconeis placentula f. euglypta</i> (Ehrenberg) Hustedt									*
<i>Cocconeis</i> sp.				*		*			
<i>Cyclotella</i> sp.				*	*	*		*	
<i>Cymbella</i> sp.									*
<i>Diploneis</i> sp.	*								
<i>Fragilaria acus</i> (Kützing) Lange-Bertalot								*	
<i>Fragilaria capucina</i> Desmazières	*								
<i>Fragilaria crotonensis</i> Kitton	*	**	*	**	**	**	*	**	*
<i>Fragilaria</i> sp.				*					
<i>Gomphonema gracile</i> Ehrenberg									
<i>Gomphonema</i> sp.					*			*	
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst									*
<i>Navicula</i> sp.	*		*	*		*		*	*
<i>Nitzschia</i> sp.									*
<i>Stephanodiscus hantzschii</i> Grunow	**	**	**	**	**	**	**	*	**
<i>Ulnaria ulna</i> (Nitzsch) Compère					*	*			

Legend: ** dominant species

The quantitative analysis of phytoplankton was done on Bürker blood-counting chamber (Laugaste, 1974). The species composition was determined by light microscopy at magnification x200 and x400 on “Carl Zeiss, Axioscope 2” microscope using standard taxonomic literature with the critical use of AlgaeBase (Guiry & Guiry, 2017). Diatoms were identified after Cox (1996). The main counting unit was the cell and biomass was estimated by the method of stereometric approximations (Rott, 1981; Deisinger, 1984). The numbers (PhN) were expressed as ($\times 10^{-6}$ cells L⁻¹). The biomass (PhB) was expressed as (mg L⁻¹).

Statistical analysis was performed with SPSS, v. 23.0 (IBM Analytics). Pearson correlation coefficients were calculated for estimations of the relationships between environmental parameters and the phytoplankton community. Hierarchical cluster analysis (CA) was performed on the normalized data set by means of Ward’s method, using

squared Euclidean distances as a measure of similarity between stations.

Results

Water temperature ranged from 11.7 °C to 29 °C. The water was neutral to slightly alkaline (pH 7.85-9.27). The values of DO varied from 0.05 to 9.9 mg L⁻¹. More detailed information on the physicochemical parameters of the water in Koprinka reservoir is given in Table S2.

A correlation matrix was used as a basis for the application of the cluster analysis. We used Pearson’s linear correlation coefficients for the determination of the environmental variables with the highest impact on the phytoplankton. During the research period, significant correlation dependency was determined for most of the analyzed indicators (Table S3). According to the correlation coefficients, indices with the strongest correlation with the

RESEARCH ARTICLE

quantitative development of phytoplankton in Koprinka reservoir include Chl *a*, Z_s, pH, N-NO₃, and TN.

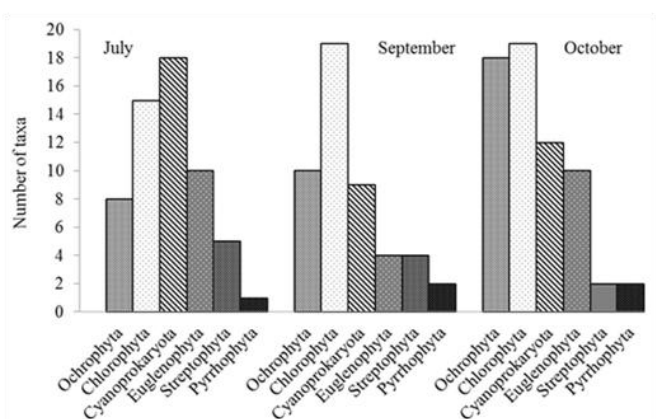


Figure 2. Seasonal distribution of main phytoplankton divisions in Koprinka Reservoir.

A hundred and nine taxa distributed in 6 divisions were identified. In our study, the phytoplankton community composition and abundance was characterized by clear seasonal variations. Detailed species composition for the studied period is given in Table 1. In the samples from July, were registered a total of 57 taxa. Division Cyanoprokaryota was the most representative of 18 species followed by Chlorophyta (15), Streptophyta (10), Ochrophyta (8), Euglenophyta (5), and Pyrriophyta (1) (Figure 2). The analysis revealed spatial differences in the composition of the dominant groups. At St1, 35 taxa from 5 divisions were established. The dominant species include *Hariotina polychorda*, *Pediastrum duplex* Meyen, *Staurastrum pingue* var. *planctonicum* (Teiling) Coesel & Meersters and *Stephanodiscus hantzschii* Grunow. (Table 1) At St2, 29 taxa from 6 divisions were identified. The community was dominated by *Hariotina polychorda*, *Aphanocapsa delicatissima* West & G. S. West, *Desmodesmus communis* (E. Hegewald) E.Hegewald, *Ceratium hirundinella* (O.F. Muller) Dujardin and *Fragilaria crotonensis* Kitton. At St3, 35 taxa from 6 divisions were identified. The most abundant were *Hariotina polychorda*, *Aphanizomenon flosaquae* Ralfs ex Bornet & Flahault, *Chroococcus turgidus* (Kützing) Nägeli, *Pandorina morum* (O. F. Müller) Bory, *Staurastrum gracile* Ralfs ex Ralfs, and *Ceratium hirundinella*. In July at all stations were recorded blooms ($14.3-20.5 \times 10^{-6}$ cells L⁻¹) of the green algae *Hariotina polychorda* (Table 1).

In September, 48 taxa from 6 divisions were identified (Figure 3). Near the reservoir walls (St1), 34 species were identified. Dominant species include *Microcystis wesenbergii*, *Aphanizomenon flosaquae*, *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg, *Aulacoseira granulata* (Ehrenberg) Simonsen, *Fragilaria crotonensis* and *Stephanodiscus hantzschii*.

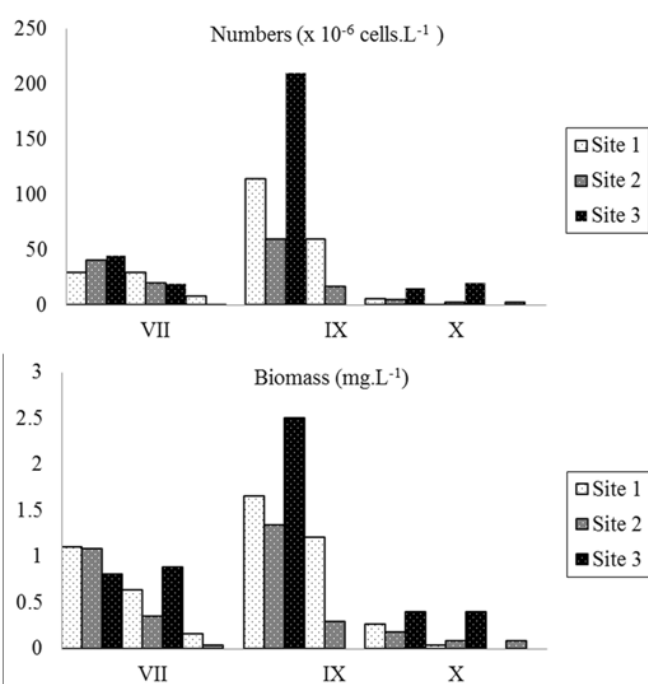


Figure 3. Numbers ($\times 10^{-6}$ cells L⁻¹) and biomass (mg L⁻¹) of phytoplankton in Koprinka Reservoir.

At the St2, 31 taxa were registered: *Microcystis wesenbergii*, *Trachelomonas planctonica* Svirenko and *Fragilaria crotonensis* dominated the phytoplankton at the cage farm. Phytoplankton composition at St3 was characterized by 23 taxa with domination of *Microcystis aeruginosa*, *Hariotina polychorda*, *Asterionella formosa* Hassall, and *Fragilaria crotonensis*. A massive bloom of blue-green algae *Microcystis wesenbergii* was registered at all stations in September (Table 1). In our study, the taxonomic diversity was highest in October, with a total of 63 identified taxa (Figure 2). At St1, 22 species were registered. Dominants were *Aphanizomenon flosaquae*, *Microcystis wesenbergii*, *Trachelomonas planctonica*, *Trachelomonas volvocina* (Ehrenberg) Ehrenberg, *Asterionella formosa* and *Stephanodiscus hantzschii*. At St2, 28 taxa were identified. The most abundant were *Aphanizomenon flosaquae*, *Microcystis wesenbergii*, *Trachelomonas planctonica*, *Trachelomonas volvocina*, *Desmodesmus communis*, *Pandorina morum*, and *Fragilaria crotonensis*. For the whole study period Station 3 is outlined as the most heterogeneous regarding the phytoplankton composition with a twice higher number of identified taxa (46) than the other two stations. Dominant species include *Aphanizomenon flosaquae*, *Microcystis wesenbergii*, *Pandorina morum*, *Trachelomonas planctonica*, *Ceratium hirundinella*, *Mallomonas* sp., *Mallomonas acaroides* Perty and *Stephanodiscus hantzschii* (Table 1).

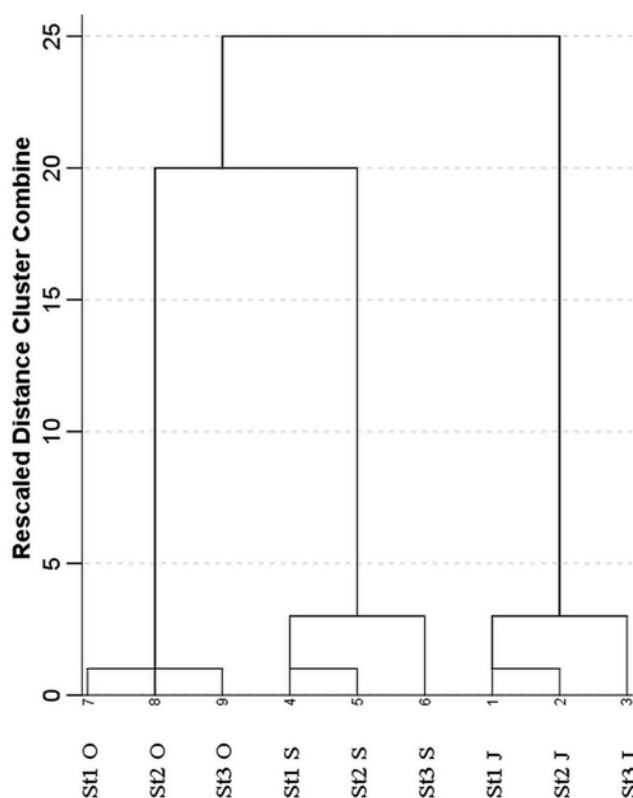


Figure 4. Dendrogram (using Ward Linkage) showing similarity between different sampling stations of Koprinka Reservoir.

In 2015, phytoplankton numbers (PhN) ranged from 0.8×10^{-6} at St 2 to 210.2×10^{-6} cells L^{-1} at St 3, and values for biomass (PhB) were in the range from 0.035 at St 2 to 2.508 $mg L^{-1}$ at St 3 (Figure 3). In July the highest abundance was registered in the superficial water layer at St3 and St2 - 44.3×10^{-6} and 40.7×10^{-6} cells L^{-1} , respectively (Figure 3). The lowest abundance was found at a depth of 14 m at St2 (0.8×10^{-6} cells L^{-1}). At St1, the numbers varied from 7.8×10^{-6} to 29.2×10^{-6} cells L^{-1} . The biomass values followed the trend of the PhN (Figure 4). In September, the numbers were three to five times higher than in July and varied from 16.33×10^{-6} (St2) to 210.2×10^{-6} cells L^{-1} (St3), (Figure 3). The biomass varied from 0.292 (St2) to 2.508 $mg L^{-1}$ (St1). The samples from October were characterized by the lowest phytoplankton numbers and varied from 1.7×10^{-6} (St1) to 19.91×10^{-6} cells L^{-1} (St3). The biomass ranged from 0.039 in a St1 to 0.402 $mg L^{-1}$ in St3. Maximum values of both parameters in this period were established at a St3 (Figure 3). Once again the abundance was higher at St3 in comparison with the other two stations. The highest average biomass (1.836 $mg L^{-1}$) which was an indication of eutrophication was registered in September when the water level was very low, and the lowest (0.284 $mg L^{-1}$) in October coinciding with a rise in the water level, leading to dilution of phytoplankton. In July the same indicator had value 0.999 $mg L^{-1}$. The established indicator species, average biomass (1.040 $mg L^{-1}$) and measured values

of chlorophyll a in the euphotic layer show eutrophic status of the reservoir.

Cluster analyses based on the phytoplankton diversity generated three water clusters, characterized by high cluster distance. The samples from the three different sampling periods are grouped into separate clusters. Each cluster was associated with different seasonal phytoplankton assemblages. The community structure within each cluster was characterized by a higher taxa similarity compared to the other two clusters (Figure 4).

Discussion

There are only a few previous studies focused on the taxonomic composition of the phytoplankton assemblages of the Koprinka reservoir. According to Stoyneva and Mitchev (2007) in the periods 1959-1961 and 1988-1989, each year mass fish mortality occurred in the summer as a result of massive blooms of *Peridinium bipes* f. *tabulatum* (Ehrenberg) Lefèvre and *Microcystis aeruginosa* (Kützing) Kützing. The authors described a total of 57 taxa of planktonic algae. In the present study, the number of identified species is twice as high compared with the previous data. In our study, significant seasonal and spatial variations in the horizontal distribution of the phytoplankton community were established. We have determined a negative relationship between taxonomic diversity and abundance with the largest number of taxa (63), identified in October. This period was characterized by a minimum in the development of phytoplankton. In September the diversity decreased with the increase of the abundance and biomass of the community and only 48 taxa were identified in the water samples. The results indicate that St3 is characterized by the highest diversity through the whole study period which is probably a result of the proximity to the river Tundzha. No significant differences in the development of the phytoplankton between the other two stations were identified. Moreover, the number and the biomass in St3 samples were two to three times higher than the other stations. A similar trend of increasing the abundance of the phytoplankton in the direction from the wall to the tail is in conformity with studies of previous researchers (Beshkova, 1996; Belkinova et al., 2007). Our results show two maxima in the abundance: summer peak in July, with the leading role of cyanoprokaryotes; and autumn peak in September, with the dominance of green, blue-green algae and diatoms. With the greatest species abundance were green algae (37 taxa) followed by cyanoprokaryotes (22) and diatoms (21). In July at all stations as well as at St1 in September the euphotic layer was dominated by the green algae *Hariotina polychorda*. According to Reynolds et al. (2002) and Padisak et al. (2009) *Hariotina* is typical for shallow, mixed systems. The same species has been reported for two Bulgarian reservoirs by Belkinova et al. (2014).

RESEARCH ARTICLE

In July and October among the dominant species were green algae *Pandorina morum* and *Ceratium hirundinella* from Pyrrophyta. Green algae *Pandorina* usually grow in nutrient-rich conditions in eutrophic lakes, and reservoirs (Padisak et al., 2009). According to the same researchers, *Ceratium hirundinella* is typically found in oligo- and eutrophic lakes. In October, with a relatively high number was presented *Trachelomonas planctonica* and *Trachelomonas* sp. as well as *Mallomonas acaroides* and *Mallomonas* sp. Reynolds et al. (2002) reported the presence of *Mallomonas* usually in small and shallow lakes or heterotrophic ponds. *Trachelomonas* occurs is generally found in nutrient-rich waters (Yamagishi, 1987). The *Trachelomonas volvocina* is inhabiting waters with a high organic matter content, and it's blooming results in deterioration of water quality (Philipose, 1988; Solorzano et al., 2011). The species *Trachelomonas planctonica*, *Trachelomonas volvocina*, and *Trachelomonas* sp. take part in dominant complexes in October at all stations. The diatoms *Aulacoseira granulata* and *Asterionella formosa* were also found in high number in autumn. In the recent years, both species were reported as dominant in several Bulgarian reservoirs (Belkinova et al., 2007; Belkinova et al., 2014; Dochin & Stoyneva, 2014). Other dominant species in this period include *Stephanodiscus hantzschii* and *Fragilaria crotonensis*. According to Padisak et al. (2009), *Stephanodiscus hantzschii* is common in turbid waters. *Fragilaria crotonensis* is a mass species in reservoirs Krichim, Kardzhali, and Dospat (Belkinova et al., 2014; Dochin & Stoyneva, 2014). We have registered 22 taxa of blue-green algae in the samples from Koprinka reservoir. Most of them take part in the dominant complexes and some of them are potentially toxic species. In July and October were recorded high numbers of *Aphanizomenon flosaquae*. In September at all research stations were established blooms of *Microcystis wesenbergii*. The abundance of the same species remains relatively high in October. Cyanobacteria species are important components of phytoplankton in summer and early autumn at meso- and eutrophic lakes (Trifonova, 1998). Our results suggest an increased eutrophication of the reservoir and are in accordance with the previous study of Stoyneva and Michev (2007). According to Cheshmedjiev et al. (2010), cyanoprokaryotes constituted 13.33% of the phytoplankton assemblage in Koprinka reservoir. In our results, the percentage of cyanoprokaryotes ranged from 18.8% to 31.6%.

Compared with the previous data (Stoyneva & Mitchev, 2007; Cheshmedjiev et al., 2010) the presented results also show the presence of potentially toxic species. Stoyanov et al. (2013) reported on the presence of *Aphanizomenon elenkinii* Kisselev (now known as *Cuspidothrix ussaczewii* (Proshkina-Lavrenko) Rajaniem, Komárek, Willame, Hrouzek,

Kastovská, Hoffmann & Sivonen) and *Aphanizomenon flosaquae* in reservoir Koprinka. Our results confirm the presence of *Aphanizomenon flosaquae* as part of the dominant complexes. Some of the dominant species identified in the present study were reported by previous researchers in Bulgarian reservoirs. *Aphanizomenon flosaquae* was reported in Koprinka reservoir (Stoyanov et al., 2013), in Vacha Dam (Teneva et al., 2010; Belkinova et al., 2014) and in Dospat reservoir (Dochin & Stoyneva, 2016). The habitat of *Aphanizomenon flosaquae* includes stratified lakes with low nitrogen, carbon and phosphorus levels (Reynolds et al., 2002; Padisak et al., 2009). *Microcystis wesenbergii* was found in Dourankoulak Lake and Bistritsa reservoir by Pavlova et al. (2014) and in Zhrebchevo reservoir (Beshkova et al., 2014). The genus *Microcystis* inhabits the eutrophic, small to medium-sized lakes (Padisak et al., 2009).

According to our research hierarchical cluster analysis revealed the formation of three clusters. The differentiation is based on the differences in the taxonomic structure of the dominant complexes and reflects the seasonal succession of phytoplankton. The applied CA demonstrate the presence of patterns of seasonal succession of phytoplankton communities in the reservoir. Cluster analysis is often a useful tool for classifying changes in different groups of communities (Kwon et al., 2009; Manoharan et al., 2014). According to Matos et al. (2011), the separation in clusters is influenced primarily by the spatial distribution of the sampling sites. In our study, the separation is based mainly on the temporal differences of the phytoplankton distribution and the spatial changes had a smaller impact. The segregation of St3 samples in separate sub-clusters in July and September is a clear evidence for the influence of the river waters on the community composition at the tail section of the reservoir. For instance, CA1 in July (summer) was characterized by the dominance of *Haricotina polychorda*, *Staurastrum pingue* var. *planctonicum* and *Stephanodiscus hantzschii*. CA2 includes the samples from September (early autumn) and is characterized generally by blooms of *Microcystis wesenbergii* and subdominants *Fragilaria crotonensis* and *Trachelomonas planctonica*. The samples in CA2 showed higher abundance of phytoplankton and chl *a* concentration. In CA3 dominated *Aphanizomenon flosaquae*, *Microcystis wesenbergii* and *Trachelomonas planctonica*. Similar findings have been reported for the seasonal development of the phytoplankton community in the eutrophic reservoir (Kwon et al., 2009). Results from the same survey show that samples in different seasons were grouped together in different clusters, and each cluster characterized by dominant indicator species.

Conclusions

RESEARCH ARTICLE

In conclusion, we have determined that the algal communities of the Koprinka reservoir were dominated by chlorophytes, cyanoprokaryotes and diatoms. The number of identified taxa was almost twice higher compared to previous studies. The presence of a large number of cyanoprokaryotes (22 taxa) in the dominant species, as well the detected blooms of potentially toxic species is also an indication of eutrophication. The highest species richness (46 taxa) was determined at the station in the riverine area with the abundance of phytoplankton being almost two to three times higher than the other stations. The dominant species in each cluster were determined and reflected the seasonal succession of phytoplankton. The green algae *Hariotina polychorda* dominated in the summer samples at all stations. The species composition of the phytoplankton, the average biomass, chlorophyll a concentration and the reported blooms of potentially toxic species *Microcystis wesenbergii* are evidence for the eutrophic condition of the reservoir for the studied period.

Acknowledgements

The study was carried out in a framework of the project D-34-18/24.04.2015 „Conducting a exploratory monitoring program to assess the pressures and impacts of fish farming on surface water bodies and update the program of measures in the plan for river basin management in the East region”. FISHFARMING.

References

- Belkinova D, Mladenov R, Dimitrova-Dyulgerova I, Cheshmedjiev S, Angelova I. 2007. Phytoplankton research in Kurdzhali reservoir, *Phytologia Balcanica*, 13(1): 47-52.
- Belkinova D, Padišák J, Gecheva, GL, Cheshmedjiev S. 2014. Phytoplankton based assessment of ecological status of Bulgarian lakes and comparison of metrics within the Water Framework Directive. *Appl. Ecol. Env. Res.*, 12(1): 83-103.
- Beshkova M. 1996. Horizontal distribution of the phytoplankton and characteristics of the Struma River-Pchelina Reservoir ecotone, *Hidrobiologiya*, (40): 43-54.
- Beshkova M, Kalchev R, Kalcheva H. 2014. Phytoplankton in the Zhebchevo Reservoir (Central Bulgaria) before and after invasion of *Dreissena polymorpha* (Mollusca: Bivalvia). *Acta Zool. Bulgar.*, 66 (3): 399-409.
- Cheshmedjiev S, Belkinova D, Mladenov R, Dimitrova-Dyulgerova I, Gecheva G. 2010. Phytoplankton based assessment of the ecological status and ecological potential of the lakes type in Bulgaria, *Biotechnol. Biotec. Eq.*, 24(S1): 14-25.
- Cox JE. 1996. Identification of freshwater diatoms from live material. – Chapman and Hall, London, UK.
- Deisinger G V. 1984. Leit faden zur Bestimmung der planktischen Algen der Karntner Seen und ihrer Biomasse. – Karntner Institut für Seenforschung, Klagenfurt, Austria.
- Dochin KT, Stoyneva MP. 2014. Effect of long-term cage fish-farming on the phytoplankton biodiversity in two large Bulgarian reservoirs, *Ber. nat. -med. Verein. Innsbruck*, 99: 49-66.
- Dochin KT, Stoyneva MP. 2016. Phytoplankton of the Dospat Reservoir (Rhodopi Mts, Bulgaria) – indicator of negative trend in reservoir development due to long-term cage fish farming. *Annual of Sofia University, Book 2 – Botany*, 99: 47-60.
- Guiry MD, Guiry GM. 2017. *AlgaeBase*, World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>.
- Kenderov L, Pavlova M, Trichkova T, Tyufekchieva V, Vidinova Y, Kozuharov D, Botev I, Hubenov Z, Uzunov Y, Stoichev S, Beshkova M, Kalcheva H, Kalchev R. 2014. Trophic state and trophic structure in three Bulgarian reservoirs. *Acta Zool. Bulgar.*, 66(2): 247-254.
- Kwon YS, Hwang S J, Park KS, Kim HS, Kim BH, Shin KH, An KG, Song YH, Park YS. 2009. Temporal changes of phytoplankton community at different depths of a shallow hypertrophic reservoir in relation to environmental variables. *Ann. Limnol.-Int.J.Lim*, 45(2): 93-105.
- Laugaste R. 1974. Size and weight of the most widely distributed algae in the Tchudsko-Pskovsk and Vyrtsyarv lakes. *Gidrobiologicheskie issledovaniya*, 4: 7-23 (In Russian).
- Manoharan D, Isaiarasu L, Sureshkumar R. 2014. Cluster analysis of physical, chemical and nutrient parameters of three freshwater temple ponds at Thirupparankundram near Madurai, Tamil Nadu, India. *International Journal of Fauna and Biological Studies*, 1(6): 121-125.
- Matos JB, Sodr e DKL, da Costa KG, Pereira LCC, da Costa RM. 2011. Spatial and temporal variation in the composition and biomass of phytoplankton in an Amazonian estuary. *J. Coastal Res.*, 64: 1525-1529.
- Ognjanova-Rumenova N, Rashkova L, Botev I, Trichkova T. 2013. Diversity of benthic diatoms in relation to environmental factors in two Bulgarian reservoirs. *Phytologia Balcanica*, (19)3: 307-322.
- Padišak J, Crossetti LO, Naselly-Flores L. 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. *Hydrobiologia*, 621(1): 1-19.
- Pavlova V, Stoyneva M, Georgieva V, Donchev D, Spoo L, Meriluoto J, Bratanova Z, Karadjova I. 2014. New records of microcystins in some Bulgarian water bodies of health and conservation importance. *Journal of Water Resource and Protection*, 6(5): 446-453.
- Philipose MT. 1988. Contributions to our knowledge of Indian algae-3. Euglenineae Part 3. The genera *Trachelomonas* Ehrenberg and *Strombomonas* Deflandre. *Prog. Indian Acad. Sci.*, 98(5): 317-394.
- Reynolds CS, Huszar V, Kruk C, Naselli-Flores L, Melo S. 2002. Towards of functional classification of the freshwater phytoplankton. *J. Plankton Res.*, 24(5): 417-428.
- Rott E. 1981. Some result from phytoplankton counting intercalibration. *Schweis. Z. Hydrol*, 43(1): 34-62.
- Solorzano GG, Martinez MGO, Vazquez AL, Garfias MBM, Zuniga REQ, Conforti V. 2011. *Trachelomonas* (Euglenophyta) from a eutrophic reservoir in Central Mexico. *J. Environ. Biol.*, 32(4): 463-471.
- Stoyanov P, Teneva I, Mladenov R, Belkinova D. 2013. Diversity and ecology of the phytoplankton of filamentous blue-green algae (Cyanoprokaryota, Nostocales) in Bulgarian standing waters. *Ecologia Balcanica*, 5(2): 1-6.
- Stoyneva MP, Michev, T. 2007. Yazovir Koprinka. - In: Michev T M & Stoyneva M P. (eds), *Inventory of Bulgarian wetlands and their biodiversity. Database of Bulgarian Non-Lotic Wetland and their Biodiversity*, Ed., Elsi-M Publ., House, Sofia, IBW 3155.
- Stoyneva MP, Temniskova-Topalova DN. 2007. Cyanoprokaryotes and of Bulgarian Non-Lotic Wetlands and their Biodiversity. - In Michev T, & Stoyneva M P. (eds), *Inventory of Bulgarian*

RESEARCH ARTICLE

- Wetlands and their Biodiversity, part 1: Non-Lotic Wetlands. Ed., Publ. House Elsi-M, Sofia, p. 155-167.
- Stoyneva MP. 2014. Contribution to the studies on biodiversity of aero- and hydrobiontic pro- and eukaryotic algae in Bulgaria, DrSc Thesis, Sofia University St. Kliment Ohridski, Sofia, Bulgaria, p. 825.
- Teneva I, Belkinova D, Dimitrova-Dyulgerova I, Vlaknova M, Mladenov R. 2010. Composition and toxic potential of Cyanoprokaryota in Vacha Dam (Bulgaria). *Biotechnol. Biotec Eq.*, 24(1): 26-32.
- Trifonova IS. 1998. Phytoplankton composition and biomass structure in relation to trophic gradient in some temperate and subarctic lakes of north-western Russia and the Prebaltic. *Hydrobiologia*, 370: 99-108.
- Yamagishi T. 1987. The Euglenophyceae from Brophet in the Central Plain of Thailand. *Gen. Educ. Rev. Coll Apr. and Vet. Med. Nihon. Univ.*, 23: 39-51.

RESEARCH ARTICLE

Supplementary Tables

Table S1. Hydrological characteristics of Koprinka Reservoir.

Year	2015
Reservoir name	Koprinka (IBW2062)
Altitude (m)	382
Length (km)	7.4
Width (km)	1.2
Max depth (m)	15 (36)
Water volume (m)	140 x 10 ⁶
Tributary	Gyurlya River, Leshnitsa River
Source	Tundzha River
Mixion type	Dimictic
Built	1955
Location	Central Bulgaria
Area (ha ⁻¹)	853.8
Station	1 (Wall)
GPS coordinates	(42°36.960'N) (025°18.361'E)
Station	2 (Cages)
GPS coordinates	(42°37.238'N) (025°17.457'E)
Station	3 (Tail)
GPS coordinates	(42°37.310'N) (025°16.409'E)

Table S2. Average values of the physicochemical parameters in Koprinka Reservoir for the studied period.

Month	Stations	TMP	Cond.	Z _s	Chl _a	pH	DO	COD _{Mn}	NH ₄ -N	NO ₃ -N	TN	TP
Measure	№	T°C	µS cm ⁻¹	m	µg L ⁻¹		mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
July	St1	23.80	359.70	1.20	3.10	8.70	4.44	5.77	0.28	2.02	2.30	0.53
	St2	23.23	355.30	1.20	5.55	8.65	4.03	4.70	0.08	2.18	2.27	0.34
	St3	27.15	309.50	1.10	2.96	9.23	7.69	2.74	0.09	1.19	1.28	0.70
September	St1	21.83	392.00	0.40	28.87	8.50	5.08	3.98	0.18	0.85	1.03	0.11
	St2	22.10	432.00	0.35	20.67	8.28	4.57	4.23	0.18	0.86	1.04	0.13
	St3	22.10	408.00	0.30	38.74	8.45	4.66	4.04	0.18	0.86	1.05	0.15
October	St1	12.50	423.00	0.90	0.84	7.92	7.95	3.82	0.23	4.36	4.59	0.02
	St2	12.90	427.00	0.85	0.42	7.89	8.00	3.82	0.26	4.05	4.31	0.02
	St3	13.30	422.00	0.95	1.18	7.94	9.63	3.56	0.23	4.70	4.93	0.05

Table S3. Pearson correlation matrix of the physicochemical parameters and phytoplankton in Koprinka Reservoir.

	TMP	Cond	Z _s	Chl _a	pH	DO	COD _{Mn}	N-NH ₄	N-NO ₃	TN	TP	PhN	PhB
TMP	1												
Cond	-0.772*	1											
Z _s	0.217	-0.677*	1										
Chl _a	0.167	0.259	-0.866**	1									
pH	-0.909**	-0.936**	0.490	-0.067	1								
DO	-0.137	-0.170	0.916**	-0.915**	0.086	1							
COD _{Mn}	0.385	-0.327	0.395	-0.119	0.316	0.178	1						
N-NH ₄	-0.310	0.194	-0.484	0.095	-0.157	0.016	-0.122	1					
N-NO ₃	-0.881**	0.554**	0.194	-0.542	-0.785*	0.185	-0.279	0.155	1				
TN	-0.892**	0.560**	0.175	-0.527	-0.786**	0.183	-0.284	0.225	0.997**	1			
TP	0.578**	-0.489*	0.452	-0.194	0.450*	-0.322	0.087	-0.310	-0.231	-0.250	1		
PhN	0.332	-0.091	-0.709*	0.946**	0.221	-0.151	0.051	-0.086	-0.455*	-0.455*	-0.025	1	
PhB	0.517*	-0.286	-0.628*	0.924**	0.426*	-0.024	0.225	-0.150	-0.583**	-0.586**	0.080	0.930**	1

Legend: * Correlation in significant at the 0.05 level (2-tailed); ** Correlation in significant at the 0.01 level (2-tailed)