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# PHYTOPLANKTON OF THE RESERVOIR "DOSPAT" (RODOPI MTS, BULGARIA) AS INDICATOR OF NEGATIVE TREND IN RESERVOIR DEVELOPMENT DUE TO LONG-TERM CAGE FISH FARMING

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> The paper is dedicated to Prof. D. Temniskova on the occasion of her 80th jubilee

**Abstract:** The paper presents the results of the recent (2011) investigation of phytoplankton composition and abundance, with its seasonal and spatial dynamics in the reservoir "Dospat" (the first in Bulgaria used for cage fish farming) and shows the changes in its waters after 30 years of exploitation. Totally 55 taxa from 7 divisions have been identified: Cyanoprokaryota (8), Pyrrhophyta (3), Euglenophyta (4), Cryptophyta (1). Ochrophyta (Bacillariophyceae – 26; Synurophyceae – 2), Chlorophyta (9) and Streptophyta (2), Cyanoprokaryotes have been recorded for first time in the reservoir and their harmful species *Planktothrix rubescens* and *Aphanziomenon flos-aquae* were found among the dominants and subdominants. The highest number of species was detected in June (42), the phytoplankton communities near to the dam were more species-rich and with higher abundance in comparison with those in the tailwaters, most probably due to the effect of the fish cages situated in the uppermost part of the reservoir.

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According to the average phytoplankton biomass  $(1,39 \text{ mg } l^{-1})$  in 2011 the reservoir "Dospat" could be classified as mesotrophic. However, it has to be noted that this value is twofold higher in comparison with 1972–1975 period, when the biomass was 0.7 mg  $l^{-1}$ . Most probably, the changes in the species composition, the increase in the phytoplankton biomass and of the reservoir trophic status are related with the effects from the cage fish farming.

Key words: cage fish farming, cyanoprokaryotes, harmful algae, mesotrophic status, anthropogenic impact

# INTRODUCTION

"Worldwide use in the increase of water bodies and anthropogenic pressures on them alter their health, which is of significant importance for maintaining of the water quality, biodiversity and fisheries" (ANNEVILLE ET AL. 2008, p. 1122). This is especially valid for the reservoir ecosystems, which have key role in human life. Therefore accumulation of data on their planktonic and benthic communities and natural or anthropogenically speeded-up successional changes with their possible causative factors and driving forces, is a permanent task for limnologists, and reservoir protection, based on good ecological evaluation, is among the cornerstone conservation activities. Phytoplankton is commonly used for water quality and ecological state assessments (*e.g.* HANPONGKITTIKUL 2005, DOMINGUES ET AL. 2008, JAKHAR 2013). Long-term data sets or comparison of phytoplankton structure during different periods are considered to be reliable indicators of environmental changes and trends (*e.g.* NASELLI-FLORES 2013 among the many others).

The reservoir "Dospat" was created by impoundment of a former large peatbog. Its exploitation started in 1968 and since then it is used for aquaculture (cage farm for rainbow trout *Oncorhynchus mykiss*), as electric power source and for irrigation (STOYNEVA & MICHEV 2007). The first data on the phytoplankton, its structure and dynamics (seasonal and annual), as well as on some abiotic parameters of the newly formed reservoir, were published by NAIDENOW & SAIZ (1977) for the period 1972–1975. These authors explained the relatively low number of taxa found (45) with the short insolation period for this long and narrow reservoir, situated in a deep mountain valley.

The aim of the present paper is to provide recent data (2011) on the phytoplankton of this important Bulgarian reservoir with an attempt to outline the changes in the aquatic system, caused by long-term cage fish farming and 30-years long reservoir succession.

#### MATERIAL AND METHODS

The reservoir "Dospat" (N 41°41`54`` E 24°05`10``) is situated in the Western Rodopi Mts, at 1 200 m a.s.l. and is oriented in NW-SE direction. It is the first water

body of the largest Bulgarian hydro-energetic water cascade "Dospat-Vucha" and is one of the largest reservoirs in Bulgaria – 18 km long, ca. 2 km broad with a total area > 2005,6 ha, maximum volume of 446,4 mln.  $m^3$  and minimum – 20 mln.  $m^3$ , with an average depth of 32 m, which varies from ca. 40 m at the dam to 20–30 m in the other parts of the water body (STOYNEVA & MICHEV 2007).

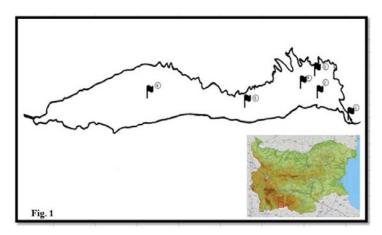


Fig. 1. Map of Bulgaria with the position of the reservoir "Dospat" and its scheme with the location of the sampling sites.

Table 1. Chemical and physical characteristics of the reservoir "Dospat" in the studied sites (1–6) from April (IV) till October (X) 2011.

Station №	Month	Coordinates	Temperature, °C	Transperency (Secchi), m	O², mg/l	Saturation O <sup>2</sup> , %	pН
	IV		9,8	2,2	11	112,4	6,96
1	VI	N 41º38`69``; E 24º09`13``	18,9	4,8	8,74	109	7,05
	VIII		20,5	4,7	7,96	101,7	7,23
	X		11,7	3,3	9,24	97,5	6,38
	IV		9,8	2,15	10,95	112,3	7,28
2	VI	N 41º39`45``; E 24º09`06``	19,1	5	8,7	108,9	8,15
	VIII		21,9	4	8,78	115,3	7,17
	X		11,5	3,3	8,44	88,9	6,79
	IV		9,7	2,2	10,35	105,8	7,41
3	VI	N 41º39`80``; E 24º09`18``	18,3	4,2	7,63	84,2	7
	VIII		22,2	4,8	7,44	98,4	7,05

	X		11,6	3,3	8,44	88,6	6,62
	IV		8,9	2,1	11,37	113,7	7,17
4	VI	N41º39`72``; E 24º8`89``	18,7	5,3	8,8	109,1	7,7
	VIII		21,7	4,8	7,8	101,9	7,29
	X		11,4	3,4	8,28	86,9	6,83
	IV		8,7	2,3	11,45	114	7,44
5	VI	N 41º40`41``; E 24º07`46``	19	5,2	8,9	110,7	7,59
	VIII		21,3	5,6	7,58	98,3	7,47
	X		11,3	3,5	8,85	92,8	5,71
	IV		8	2,15	11,58	113,5	7,67
6	VI	N 41º42`45``; E 24º04`55``	18,6	5,3	8,85	109,8	7,13
	VIII		21,2	5,6	7,44	96	7,28
	X		11,1	3,1	8,85	92	6,76

In total, 24 phytoplankton samples were collected at 6 sites of the reservoir "Dospat" (Fig. 1) in the period from April till November 2011. It has to be noted especially that sites 1–4 were in the main reservoir bed, where site 1 was situated near to the dam and site 2 - near to the cage fish farm, while sites 5 and 6 were more near to the "tail" of the reservoir. The samples were taken from depth 0-0.5 m by batometer of "Danish" type and were 1 200 ml in volume. They were fixed in formaldehyde (2–4% final concentration) and stored in glasses with volume of 1 1. Additional living samples were collected from the same sites. The quantitative analysis was done on Bürker blood-counting chamber (LAUGASTE 1974). The species composition was determined in parallel way on fixed and living samples by light microscope "Carl Zeiss Axioscope 2" with magnification 200x and 400x, and diatoms were identified after Cox (1996). The main counting unit was the cell and biomass was estimated by the method of stereometrical approximations (ROTT 1981; DEISINGER 1984). The following standard parameters were measured: water temperature (T°C), dissolved oxygen ( $O_2$  mg l<sup>-1</sup>), saturation ( $O_2$ %), pH and transparency (as Secchi depth) – Table 1. Their values in 2011 were similar to those registered in 2010 (HADJINIKOLOVA & ILIEV 2011).

### RESULTS

### Taxonomic structure of the phytoplankton

Totally 55 taxa from 7 divisions were established in the reservoir phytoplankton: Ochrophyta (28: Bacillariophyceae – 26; Synurophyceae – 2), Chlorophyta (9), Cyanoprokaryota (8), Euglenophyta (4), Streptophyta (2), Pyrrhophyta (3) and Cryptophyta (1). Their percentage representation was as follows: Ochrophyta (50,9%: Bacillariophyta – 47,3%; Synurophyceae – 3,6%), Chlorophyta (16,4%), Cyanoprokaryota (14,5%), Euglenophyta (7,3%), Streptophyta (3,6%), Pyrrhophyta (5,5%) and Cryptophyta (1,8%) – Fig. 2.

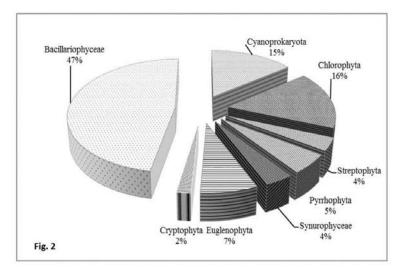


Fig. 2. Taxonomic structure of the phytoplankton of the reservoir "Dospat" (2011).

In April, 21 taxa from 6 divisions were identified (Table 2). Most of the species belong to Bacillariophyceae (6, or 52,3%) and Cyanoprokaryota (5, or 19%). From each of the other taxonomic groups (Synurophyceae, Euglenophyta, Cryptophyta, Chlorophyta and Streptophyta,) only 1 taxon was found (4,7%) – Fig. 3A. *Asterionella formosa* Hassall (66,8%), *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek (23%) and *Tabellaria fenestrata* (Lyngbye) Kützing (10,2%) dominated the phytoplankton all over the reservoir aquatory, while the diatoms *Hannea arcus* (Ehrbg.) Patrick, *Meridion circulare* (Grev.) Ag. and *Diatoma vulgare* Bory were found only more near to the "tail" of the reservoir. The number of taxa per site was low and almost similar: in each of the sites 1–4, situated in the reservoir's main bed, 8 taxa were found, while in the sites 5 and 6, situated more near to the tail, 6 and 7 taxa, respectively, were found (Table 2).

+9 ++++ S + 4 +× c +2 + + ++ + + + 0 + n +4 + VIII c ++ +2 ++++Site No -++9 ++ S +++4 + +-++Ν c + ++ + + + 2 +-++++ + + 9 + S 4 + +  $\mathbf{N}$ m + +2 + + + -+ + Gomont) Planktothrix agardhii (Gomont) Anagnostidis et Komárek Pandorina morum (O. F. Müller) Bory de Saint-Vincent Aphanizomenon flos-aquae Ralfs ex Born. et Flahault Tetrastrum glabrum (Y. V. Roll) Ahlstrom et Tiffany eх Candolle Scenedesmus communis (Breb.) Hegewald Microcystis aeroginosa (Kützing) Kützing Cyanoprokaryota Anabaena sphaerica Bornet et Flahault Chroococcus limneticus Lemmermann Chlorophyta Planktothrix rubescens (De Taxa Oocystidium ovale O.Korshikov Scenedesmus bijugatus Kutzing Anabaena flos-aquae G.S. West Coelastrum sphaericum Nägeli Elakatothrix gelatinosa Wille Pediastrum duplex Meyen Anagnostidis et Komárek Oocystis borgei J.Snow Aphanocapsa sp.

Table 2. Species composition of the phytoplankton in the reservoir "Dospat" by sites and months during the studied period (2011).

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Cryptophyta							
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Ochrophyta							
Synurophyceae							
Mallomonas elongata Reverdin +			+	+ + +	+++++++++++++++++++++++++++++++++++++++	+ +	+ +
Mallomonas caudata Iwanoff			+				
Bacillariophyceae							
Asterionella formosa Hassall	+++++++++++++++++++++++++++++++++++++++	+++++++++++++++++++++++++++++++++++++++	++	+	+++++++++++++++++++++++++++++++++++++++	++	+ +
Aulacoseira granulata (Ehrenberg) Simonsen + +	+ +	+ + +	+				+
Aulacoseira islandica O. Mull.	+						
Cocconeis placentula Ehrenberg +	·	+					
Cocconeis pediculus Ehrenberg				+			

Caloneis amphisbaena (Bory de Saint Vincent) Cleve			+			+							
Cymbella cymbiformis C.Agardh	+												+
Cyclotella meneghiniana Kützing		+	+	+	+	+	+	+ +	+	+		+	+
Diatoma vulgare Bory		+	+	+									
Diatoma elongatum (Lyngbye) C. Agardh			+										
Diploneis ovalis (Hilse) Cleve	+			+									
Fragilaria crotonensis Kitton	+	+	+ +	+ +	+	+	т	+ +	+	+			
Meridion circulare (Greville) C. Agardh		+											
Hannea arcus (Ehrenberg) Patrick		+							+				
Gomphonema constricum Ehrenberg													+
Gomphonema acuminatum Ehrenberg				+									
Gomphonema truncatum Ehrenberg			+ +										
Melosira varians C.Agardh													+
Navicula rynchocephala Kützing													+
Navicula radiosa Kützing													+
Navicula sp.		+		+							+		+
Synedra ulna (Nitzsch) Ehrenberg	+		+	+ +	+			+					+
Synedra acus Kützing				+	+								
Tabellaria fenestrata (Lyngbye) Kützing	+ + + +	+++++++++++++++++++++++++++++++++++++++	+ +	+ + +	+++++++++++++++++++++++++++++++++++++++	+	т	+ +	+++++++++++++++++++++++++++++++++++++++	+ +	+	++	+++
Tabellaria floccuosa (Roth) Kützing			+										
Stephanodiscus hantzschii Grunow	+		+ + +	+	+		+	+			+		
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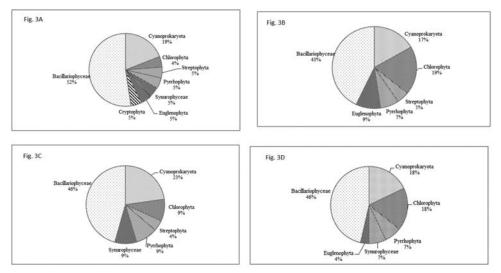


Fig. 3. Taxonomic structure of the phytoplankton of the reservoir "Dospat" during the studied period: A – in April 2011; B – in June 2011; C – in August 2011; D – in October 2011.

In June, 42 taxa from 6 divisions were found: Bacillariophyceae (26, or 42,8%), Chlorophyta (11, or 19%), Cyanoprokaryota (10, or 16,7%), Euglenophyta (9, or 9,5%), Pyrrhophyta (3, or 7,14%), Synurophyceae (2, or 4,76%) and Streptophyta (1, or 2,38%) – Fig. 3 B. In the same time the number of species of Bacillariophyceae decreased, but those of Chlorophyta and Euglenophyta increased. Dominants by numbers and biomass were *Fragilaria crotonensis* Kitton (31,5%), *Gymnodinium uberrimum* (G. J. Allman) Kofoid et Swezy (39%) and *Planktothrix rubescens* (22%), while *Aulacoseira granulata* (Ehrenberg) Simonsen was subdominant in almost all sites. Frequently occurring, but in small numbers, were the green algae *Scenedesmus communis* (Breb.) Hegewald, *Pediastrum duplex* Meyen and *Oocystidium ovale* Korshikov. The number of taxa per site was low, but showed more pronounced differences in comparison with the previous period: the richest in taxa was site 4 (25 taxa) in the reservoir bed, and the smallest number of taxa (7) was found in site 6, situated more near to the tail of the reservoir (Table 2).

In August, 22 taxa from 5 divisions were registered: Bacillariophyceae (10, or 45,5%), Cyanoprokaryota (5, or 22,7%), Chlorophyta (2, or 9%), Pyrrhophyta (2, or 9%), Synurophyceae (2, or 9%) and Streptophyta (1, or 0,5%) – Fig. 3C. Dominants by both numbers and biomass were *Cyclotella meneghiniana* Kützing (35,7%), *Chroococcus limneticus* Lemmermann (37,7%), *Stephanodiscus hantzschii* Grunow (14,1%) and *Tabellaria fenestrata* (9,2%), while *Asterionella formosa* and *Aphanizomenon flos-aquae* Ralfs ex Bornet et Flahault were subdominants. The number of taxa per site was the highest detected, and was relatively similar in all studied sites. It was the biggest in sites 2 (11) and 1 (10) and was more or less the

same in sites 5 (9) and 6 (10), while the smallest number of taxa (7) was found in site 3 (Table 2).

In the end of October, 28 taxa from 5 divisions were identified: Bacillariophyceae (13, or 46,4%), Cyanoprokaryota and Chlorophyta (each of them with 5 taxa, or 17,85%), Synurophyceae (2, or 7,14%), Pyrrhophyta (2, or 17,85%) and Euglenophyta (1, or 3,57%) – Fig. 3D. The most abundant was the group of diatoms, from which *Tabellaria fenestrata* (85,2%) and *Asterionella formosa* (11,1%) dominated. Subdominants were the pyrrhophytes *Gymnodinium uberrimum* and *Ceratium hirundinella* (O. F. Müller) Dujardin. The number of taxa per site increased significantly and its maximum for the studied period was detected. The maximum number of taxa was found in site 6 (19), thus being almost twice higher in comparison with the previous periods, while the minimum was in site 5 (7) – Table 2.

#### Phytoplankton abundance (numbers and biomass)

In 2011, total phytoplankton numbers varied from 2,35 x10<sup>6</sup> cells/l<sup>-1</sup> (August) to 98,3 x10<sup>6</sup> cells/l (April), being 42,11 x10<sup>6</sup> cells l<sup>-1</sup> in average, and total biomass varied from 0,036 mg l<sup>-1</sup> to 3,100 mg l<sup>-1</sup>, being 1,39 mg l<sup>-1</sup> in average (Fig. 4). These values indicated the general mesotrophic status of reservoir waters. In the text below seasonal and spatial changes of phytoplankton abundance are briefly described.

In April, during the mass bloom of diatoms, phytoplankton numbers varied from  $13,7x10^6$  cells  $1^{-1}$  (site 3) to  $340x10^6$  cells  $1^{-1}$  (site 2) – Fig. 5A. Phytoplankton abundance was the biggest at sites 2 and 1 ( $340x10^6$  cells  $1^{-1}$  and  $71,7x10^6$  cells  $1^{-1}$ , respectively), situated near the cage farm and near to the dam. The lowest abundance was found at site 3 ( $13,7x10^6$  cells  $1^{-1}$ ). More near to the tail of the reservoir, at sites 5 and 6, the numbers were  $36,4x10^6$  cells  $1^{-1}$  and  $65,25x10^6$  cells  $1^{-1}$ , respectively. The biomass values varied from 0,457 mg  $1^{-1}$  (site 3) to 10,652 mg  $1^{-1}$  (site 2) – Fig. 5A. The month average value of the biomass was 3,103 mg  $1^{-1}$  and indicated the eutrophic state of the reservoir waters for this period.

In June the abundance of cyanoprokaryotes and green algae increased. The phytoplankton numbers were 10 times lower than in April and varied from  $0,4x10^6$  cells  $1^{-1}$  (site 5) to  $36,4x10^6$  cells  $1^{-1}$  (site 1) – Fig. 5B. Minimum numbers were registered at sites 5 and 6 –  $0,4x10^6$  cells  $1^{-1}$  and  $0,76x10^6$  cells  $1^{-1}$ , respectively. Phytoplankton biomass varied from 0,013 mg  $1^{-1}$  (site 6) to 1,115 mg  $1^{-1}$  (site 1). Maximum values of both numbers and biomass were registered at sites 1 and 2, situated near to the dam and cage farm, respectively. The month average value of the phytoplankton biomass was 0,511 mg  $1^{-1}$ , indicating the oligotrophic state of the reservoir waters.

In August, during the summer stratification, the phytoplankton numbers were the lowest and varied from  $0,44 \times 10^6$  cells l<sup>-1</sup> (site 6, in the tailwaters and in the vicinity of the inflow from the rivulet "Dospatska Reka") to  $5,33 \times 10^6$  cells l<sup>-1</sup> (site 1, near to the dam) – Fig. 5C. Biomass values ranged from 0,017 mg l<sup>-1</sup> (site 5)

to 0,091 mg l<sup>-1</sup> (site 1), also decreasing from the dam and cage farm region to the tailwaters. The average phytoplankton biomass was the lowest detected - 0,090 mg l<sup>-1</sup> and indicated oligo- to ultraoligtrophic state of the reservoir waters.

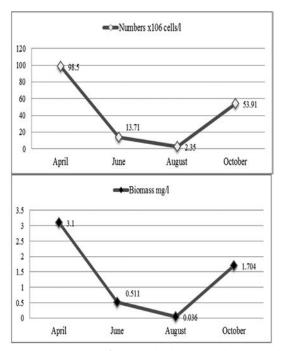


Fig. 4. Annual average values of phytoplankton numbers and biomass (B) in the reservoir "Dospat" in 2011.

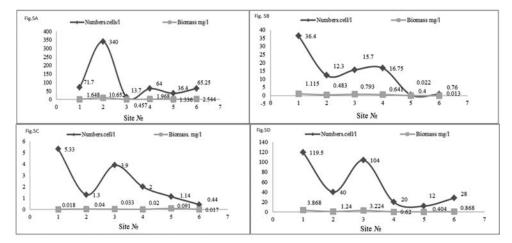


Fig. 5. Phytoplankton numbers and biomass in the reservoir "Dospat" during the studied period: A – in April 2011; B – in June 2011; C – in August 2011; D – in October 2011

In October, during the autumn homothermic conditions, diatoms dominated again. Phytoplankton numbers varied from  $12x10^6$  cells  $l^{-1}$  (site 5 ) to  $119,5x10^6$  cells  $l^{-1}$  (site 1), and the biomass ranged from 0,404 mg  $l^{-1}$  (site 5) to 3,868 mg  $l^{-1}$  (site 1) – Fig. 5D. The phytoplankton abundance decreased significantly from the dam region to the tailwaters. The month average value of the biomass (1,702 mg  $l^{-1}$ ) indicated the meso- to eutrophic status of the reservoir waters.

# DISCUSSION

The results obtained during this study show that temporal and spatial distribution of the phytoplankton in the reservoir "Dospat" was heterogeneous in both quantitative and qualitative aspect. The phytoplankton abundance was with two pronounced maxima (highest spring peak in April and lower autumn peak in October) and a low summer peak in June, with the relevant quantitative representation of taxonomic groups - leading role of diatoms in colder periods with increase of the participation of cyanoprokaryotes and green algae during summer stratification. These data are on general conformity with the classical PEG-model for seasonal phytoplankton succession (SOMMER ET AL. 1986). They completely coincide with the data on the general seasonal phytoplankton dynamics, published on the same reservoir by NAIDENOW & SAIZ (1977), who also indicated the lowest phytoplankton abundance for August and highest - for April. However, the results obtained by us on the species composition show pronounced difference with those published by NAIDENOW & SAIZ (1977). The highest total number of taxa detected by us was in June (42), being almost twice higher in comparison with number of species found in all other months (21-28), while in the 1972-1975 the highest number of taxa was in the summer periods (35), being almost twice higher in comparison with the spring periods (19).

The average biomass value in 2011 was  $1,39 \text{ mg} \text{ l}^{-1}$ , while according to NAIDENOW & SAIZ (1977) for the period 1972–1975 it was 0,7 mg l<sup>-1</sup>. In spite of the fact, that according to these values the reservoir yet has to be classified as mesotrophic (UZUNOV & KOVACHEV 2002; STOYNEVA & MICHEV 2007), this twofold increase in 30 years period has to be outlined, since, in our opinion, it is due mainly to the influence of cage fish farming on reservoir waters. NAIDENOW & SAIZ (1977) did not discuss the horizontal phytoplankton distribution and therefore no comparison is possible, but we would like to discuss the data obtained during this study. The strange, at first look, were the results on the spatial distribution of the species composition, according to which more taxa were generally found in the deeper sites near to the tail (5–6), and on the more abundant phytoplankton development near the dam, as well. It has to be taken into account that near to site 6 is the inflow of the rivulet Dospatska Reka, which could cause a dilution of the reservoir waters. However,

the amount entering from this rivulet is not high enough to explain alone such a significant decrease of phytoplankton quantity. It is possible to suppose that one of the most probable reasons for the higher phytoplankton amounts in the uppermost part of the reservoir is the influence from the cage farm, situated there.

Our suggestion finds support in the data and conclusions published much earlier by NAUMOVA & ZHIVKOV (1988). They were the first authors, who noted the significant concentration of biogenes (ammonium and nitrites in particular) in the aquatory of several square kilometers near to the reservoir dam and explained it with the effects of cage farming. According to them, enormous amount of organic matter (1 500–2 500 tons) had been spread annually in and near to the cages. A big part of it remains unutilized and sinks on the bottom. Its degradation results in enrichment of the waters with biogenes, followed by nuisance algal blooms combined with night oxygen depletions and even short-termed fish-kills. The effect is most strong in spring, when the homothermy is combined with the typical for the region south-eastern wind, and the accumulated biodegradation products rise to the surface water levels. The highest nutrient concentrations were registered by NAUMOVA & ZHIVKOV (1988) in the dam region, and were triple in amounts in comparison with the values of nitrites, and twofold higher for nitrates, iron and magnesium in comparison with the values, recorded for these ions earlier by NAIDENOV & SAIZ (1977).

Additional prove for the statement on the strong negative effect of cage farms on the reservoir could be found in the recent detection of cyanoprokaryotes with notable quantities and 8 taxa in the phytoplankton. This taxonomic group was not found during the studies by NAIDENOW & SAIZ (1977) and its occurrence is commonly accepted as related with higher trophic status of the inhabited waters. Among the cyanoporkaryotes, the abundant development of both harmful species *Planktothrix rubescens* and *Aphanizomenon flos-aquae* (GUIRY & GUIRY 2014) has to be outlined. These species are more typical for lowland eutrophic waters, and have been rare found in deep mountain waters (*e.g.* D'ALLELIO & SALMASO 2011). Therefore, taking into account the detected changes in phytoplankton quantitative and qualitative structure and its reverse (in comparison with standard horizontal distribution in reservoirs) spatial distribution with more abundant and speciesrich development near to the dam, we can outline the negative trend in reservoir development due to long-term cage fish farming.

#### ACKNOWLEDGEMENTS

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