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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 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" (Annevilleetal. 2008, p. 1122). This is especially valid for the reservoir ecosystems, which have key role in hum an 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. Hanpongkittikul2005, Dominguesetal. 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 im poundment of a form er large peat bog. 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 Naiden ow & Saiz(1977) for the period 1972-1975. These authors explained the relatively low num ber 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 M ETHODS

The reservoir "Dospat" (N  $41^{\circ}4T$  54'' E  $24^{\circ}05'10"$ ) 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

the largest reservoirs in Bulgaria - 18 km long, ca. 2 km broad with a total area > 2005,6 ha, m aximum volume o f 446,4 mln. m 3 and minimum - 20 mln. m 3, with an average depth o f 32 m, which varies from ca. 40 m at the dam to 20-30 m in the other parts o f the water body (Stovneva & 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 Temperature, oC O2,

Transperency mg/l O2, % pH

Coordinates (Secchi), m Saturation

IV 9,8 2,2 11 112,4 6,96 1  $_{
m VI}$  N 41038'69";

E 24009'13" 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  $_{
m VI}$ N 41039'45";

E 24009'06" 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 VIN 41039'80";

E 24009'18" 18,3 4,2 7,63 84,2 7 VIII 22,2 4,8 7,44 98,4 7,05

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X 11,6 3,3 8,44 88,6 6,62 IV 8,9 2,1 11,37 113,7 7,17 4 VIN4 1039'72";

E 2408'89" 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 VIN 41040'41";

8,85 92,8 5,71 IV 8 2,15 11,58 113,5 7,67 6 VI N 41042'45";

E 24004'55" 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 N ovem ber 2011. It has to be noted especially that sites 1-4 were in the m ain 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 batom eter 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 l. Additional living samples were collected from the same sites. The quantitative analysis was done on Bürker blood-counting cham ber (La u g a s t e 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 (ToC), dissolved oxygen (O2 mg 1-1), saturation (O2%), pH and transparency (as Secchi depth) - Table 1. Their values in 2011 were similar to those registered in 2010 (Hadjinikolova & Iliev 2011).

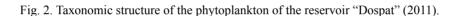
#### RESULTS

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),

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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.



In April, 21 taxa from 6 divisions were identified (Table 2). M ost of the species belong to Bacillariophyceae (6, or 52,3%) and Cyanoproxaryota (5, or 19%). From each of the other taxonom ic groups (Synurophyceae, Euglenophyta, Cryptophyta, Chlorophyta and Streptophyta,) only 1 taxon was found (4,7%) -

Fig. 3A. Asterionella form osa Hassall (66,8%), Planktothrix rubescens (De Candolle ex Gom ont) A nagnostidis & Kom ârek (23%) and Tabellaria fenestrata (Lyngbye) Kützing (10,2%) dom inated the phytoplankton all over the reservoir aquatory, while the diatom s H annea arcus (Ehrbg.) Patrick, M eridion circulare (Grev.) Ag. and D iatom a vulgare Bory were found only m ore near to the "tail" of the reservoir. The num ber of taxa per site was low and alm ost similar: in each of the sites 1-4, situated in the reservoir's m ain bed, 8 taxa were found, w hile in the sites 5 and 6, situated more near to the tail, 6 and 7 taxa, respectively, were found (Table 2).

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

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Taxa Site №

IV VI VIII X

1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6 1 2 3 4 5 6

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Cyanoprokaryota
Anabaena sphaerica Bomet et Flahault
Anabaenaflos-aquae G.S. West
Aphanocapsa sp.
Aphanizomenon flos-aquae Ralfs ex Bom. et Flahault Chroococcus limneticus
Lemmermann
Microcystis aeroginosa (Kützing) Kützing
Planktothrix rubescens (De Candolle ex Gomont) Anagnostidis et Komârek
Planktothrix agardhii (Gomont) Anagnostidis et Komârek Chlorophyta
Coelastrum sphaericum Nägeli
Elakatothrix gelatinosa Wille
Oocystidium ovale O.Korshikov
Oocystis borgei J.Snow
Pandorina morum (0. F. Müller) Bory de Saint-Vincent Pediastrum duplex Meyen
Scenedesmus bijugatus Kützing
Scenedesmus communis (Breb.) Hegewald
Tetrastrum glabrum (Y. V. Roll) Ahlstrom et Tiffany
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+

++++++

++ + + ++ + ++++ + ++ ++++++++ + + + + + + + + +++++++ +++

+++++

+++

+

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+
+
+
Streptophyta
Cosmarium sp.
Staurastrum planctonicum Teiling
Pyrrhophyta
Ceratium hirundinella (0. F. Müller) Dujardin Peridinium sp.
+++++
++++
+++++
++
+ + + + +
+++++++ Euglenophyta
Euglena acus (0. F. Müller) Ehrenberg
Phacus orbicularis K. Hübner
Strombomonas sp.
Trachelomonas volvocina (Ehrenberg) Ehrenberg + Cryptophyta
Cryptomonas sp. ++ Ochrophyta
Synurophyceae
+
++
+
++
Mallomonas elongata Reverdin Mallomonas caudata Iwanoff Bacillariophyceae
+++++
+++++
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Asterionella formosa Hassall

Aulacoseira granulata (Ehrenberg) Simonsen Aulacoseira islandica 0. 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 + + 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 + + + + + Tabellariafloccuosa (Roth) Kützing + Stephanodiscus hantzschii Grunow + + + + + + + + +

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 num ber 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* M eyen and *Oocystidium ovale* Korshikov. The num ber 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 num ber 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%), Cyanoproxaryota (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 form osa* and *Aphanizomenon flos-aquae* Ralfs ex Bornet et Flahault were subdominants. The num ber 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 num ber o f taxa (7) was found in site 3 (Table 2).

In the end o f october, 28 taxa from 5 divisions were identified: Bacillariophyceae (13, or 46,4%), CyanoproKaryota and Chlorophyta (each o f 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 m ost abundant was the group of diatoms, from which *Tabellaria fenestrata* (85,2%) and *Asterionella form osa* (11,1%) dominated. Subdominants were the pyrrhophytes *Gymnodinium uberrimum* and *Ceratium hirundinella* (O. F. M üller) Dujardin. The num ber of taxa per site increased significantly and its m aximum for the studied period was detected. The maximum num ber 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 x106 cells/l-1 (August) to 98,3 x106 cells/l (April), being 42,11 x106 cells l-1 in average, and total biomass varied from 0,036 mg l-1 to 3,100 mg l-1, being 1,39 mg l-1 in average (Fig. 4). These values indicated the general mesotrophic status o f reservoir waters. In the text below seasonal and spatial changes

o f phytoplankton abundance are briefly described.

In April, during the mass bloom o f diatoms, phytoplankton numbers varied from 13,7x106 cells l-1 (site 3) to 340x106 cells l-1 (site 2) - Fig. 5A. Phytoplankton abundance was the biggest at sites 2 and 1 (340x106 cells l-1 and 71,7x106 cells l-1, respectively), situated near the cage farm and near to the dam. The lowest abundance was found at site 3 (13,7x106 cells l-1). More near to the tail o f the reservoir, at sites 5 and 6, the numbers were 36,4x106 cells l-1 and 65,25x106 cells l-1, respectively. The biomass values varied from 0,457 mg l-1 (site 3) to 10,652 mg l-1 (site 2) - Fig. 5A. The month average value o f the biomass was 3,103 mg l-1 and indicated the eutrophic state o f 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 0,4x106 cells l-1 (site 5) to 36,4x106 cells l-1 (site 1) - Fig. 5B. Minimum numbers were registered at sites 5 and 6 - 0,4x106 cells l-1 and 0,76x106 cells l-1, respectively. Phytoplankton biomass varied from 0,013 mg l-1 (site 6) to 1,115 mg l-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 l-1, indicating the oligotrophic state of the reservoir waters.

In August, during the sum m er stratification, the phytoplankton numbers were the lowest and varied from 0,44x106 cells l-1 (site 6, in the tailwaters and in the vicinity of the inflow from the rivulet "Dospatska R eka") to 5,33x106 cells l-1 (site 1, near to the dam) - Fig. 5C.

Biomass values ranged from 0,017 mg l-1 (site 5)

56 to 0,091 mg l-1 (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-1 and indicated oligo- to ultraoligtrophic state o f the reservoir waters.

« »N um bers x!06 cells'!

April June August October

•Biomass mg 1

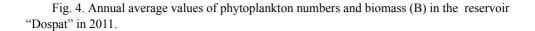


Fig. 5. Phytoplankton numbers and biomass in the reservoir "Dospat" during the studied period: A - in April 2011; B - in June 2011; C - in August 2011; D - in October 2011

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In October, during the autumn homothermic conditions, diatoms dominated again. Phytoplankton numbers varied from 12x106 cells l-1 (site 5) to 119,5x106 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 o f the biomass (1,702 mg l-1) indicated the m eso- to eutrophic status o f the reservoir waters.

#### DISCUSSION

The results obtained during this study show that tem poral and spatial distribution of the phytoplankton in the reservoir "D ospat" was heterogeneous in both quantitative and qualitative aspect. The phytoplankton abundance was with two pronounced maxim a (highest spring peak in April and lower autumn peak in October) and a low summer peak in June, with the relevant quantitative representation of taxonom ic 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 (Sommeretal. 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 mg l-1, while according to Naidenow & Saiz (1977) for the period 1972-1975 it was 0,7 mg l-1, 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 dam (1-2) in comparison with the more shallow sites situated more 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 D ospatska Reka, which could cause a dilution of the reservoir waters. However,

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the am ount 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.

o u r suggestion finds support in the data and conclusions published much earlier by N  $_{a}$  u  $_{m}$  o  $_{v}$  a &  $Z_{h}$  i  $_{v}$  k o  $_{v}$  (1988). They were the first authors, who noted the significant concentration o f biogenes (ammonium and nitrites in particular) in the aquatory o f several square kilometers near to the reservoir dam and explained it with the effects o f cage farming. According to them, enormous amount o f organic m atter (1 500-2 500 tons) had been spread annually in and near to the cages. A big part o f it remains unutilized and sinks on the bottom. Its degradation results in enrichment o f the waters with biogenes, followed by nuisance algal blooms combined with night oxygen depletions and even short-termed fish-kills. The effect is m ost 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  $N_{aumova}$  &  $Z_{hivkov}$  (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 taxonom ic 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 species rich development near to the dam, we can outline the negative trend in reservoir development due to long-term cage fish farming.

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