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## PHYTOPLANKTON SPECIES COMPOSITION IN SEVEN FISH PONDS WITH A GRASS CARP POLYCULTURE (2018-2019)

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**Abstract.** The present article is an attempt to analyze the seasonal changes in the structure and the biomass of phytoplankton in fish ponds with a polyculture with two-year-old grass carp as a mean of biological control of unwanted aquatic vegetation. During a two-year study (2018-2019), 259 planktonic algae were identified with considerably higher number of species during the first year (216) in comparison with the second year (150), when the grass carp stocking densities were twice less. This decrease in the biodiversity was accompanied by a significant change in the dominant structure: in 2018, the most intense blooms were caused by potentially toxic cyanoprokaryotes *Dolichospermum planctonicum* (Brunnthal) Wacklin, L. Hoffmann & Komarek and *D. spiroides* Klebhan) Wacklin, L. Hoffmann & Komarek L. Hoffmann & K. Sivonen, while in 2019 the most abundant species were from Pyrrophyta (*Ceratium furcoides* (Levander) Langhans), Euglenophyta (*Euglena gracilis* G.A. Klebs) and Ochrophyta, Raphidophyceae (*Gonyostomum* cf. *ovatum* Fott and *Gonyostomum depressum* (Lauterborn) Lemmermann).

**Key words:** algae, cyanoprokaryotes, dominants, species alteration, toxic algae

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## INTRODUCTION

Biodiversity in small ponds is higher than the biodiversity in the larger water bodies, but despite this fact they are rarely studied due to their variable water balance, small volume and spatial heterogeneity. Water basins supplied with organic matter, as a result of agricultural activity, have rich algal flora (Borics et al. 2003). The loads of agricultural chemicals and the mismanagement of artificial fish ponds, can lead to eutrophication. Fertilizers are often used in fisheries to stimulate the development of the primary production and to increase the yields, which is why fish ponds are eutrophic with frequent algal blooms (Radojicic & Kopp 2016). Due to their different hydrology and small water depth they lack seasonal temperature stratification (Kopp et al. 2016). The use of high fish stocking densities increases the trophic status of the water bodies, which commonly causes cyanobacterial blooms, fluctuations in oxygen and high levels of nitrogen that destabilize these aquatic ecosystems.

Cyanoprokaryotes often are the main contributors to the total phytoplankton biomass in the summer, causing intense blooms and death among fish due to oxygen depletion. The specific features of cyanobacteria make them more adaptable to specific conditions, such as reduced light and depletion of nitrogen (Sevrin-Reyssac & Pletiković 1990; Komarkova 1998). At the same time, many cyanoprokaryotes are widely known as toxin producers which cause severe harm to human and ecosystem health. Therefore, the study of the summer ecosystems of small fish-breeding ponds achieves greater importance.

According to Michev & Stoyneva (2007) the species composition of algae found in fish farms in Bulgaria consisted of approximately 600 species. Detailed data on algal diversity in fish ponds in the country were published by Vodenicharov et al. (1974), Ludskanova & Paskaleva (1975), Paskaleva (1975), Kiryakov et al. (1982), Paskaleva & Vodenicharov (1984) and Douchin et al. (2020; in press). The aim of the present study is to report the general changes in the phytoplankton composition in fish ponds with polyculture with two-year-old grass carp as a means of biological control of unwanted aquatic vegetation.

## MATERIAL AND METHODS

The study was carried out during a two-year period (2018-2019) in the experimental ponds of the Institute of Fisheries and Aquaculture, Plovdiv, Bulgaria. During the study, in these ponds common carp (*Cyprinus carpio* L.), hybrid bighead carp (*Hypophthalmichthys nobilis* Rich, x *Hypophthalmichthys molitrix* Val.) and grass carp (*Ctenopharyngodon idella* Val.) were grown. During the second year (2019) the stocking density of grass carp in the experimental ponds was twice lower than the stocking density in 2018.

During the two-year period, 108 phytoplankton samples were taken from seven

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ponds (P6, P7, P12, P16, P18, P19 and P23), each with area between 0.18 to 0.40 ha. The macrophytic vegetation in the ponds was represented by *Ceratophyllum demersum* L., *Nuphar lutea* (L.) Sm. and *Typha angustifolia* L.).

The sampling was conducted at the depth of 0.5 m bimonthly in the late spring-summer period (from April/May to September) of both years with the results discussed as average values per month. The phytoplankton samples were collected and processed by standard methods of fixation with formalin to final concentration 4% and further sedimentation (ISO 5667-1:2006/AC:2007; ISO 5667-3:2003/AC:2007) with some additional living samples for identification of raphidophytes. Microscope work was done on Burker chamber. The species composition was determined by light microscope (Carl Zeiss, Axioscope 2 plus) with magnification 400x using standard taxonomic literature with critical use of AlgaeBase (Guiry & Guiry 2020). Diatoms were identified after Cox (1996). The main counting unit was the cell and the biomass was estimated by the method of stereometrical approximations (Rott 1981; Deisinger 1984). Counting units were cells, filaments and colonies. The total biomass of each sample was assessed and it was defined as the amount of biomass of all species summarized in separate taxonomic groups. Dominant species were determined according to the percentage of individual species to the total biomass.

## RESULTS AND DISCUSSION

During a two-year study (2018-2019), totally 259 taxa of planktonic algae from 6 divisions were identified (**Table 1**).

During the first year of investigation, the total number of identified taxa was 216 and during the second year it was 150 (**Table 1, Fig. 1-3**). The number of species ranged around 100 per month during the first year, and was about twice less during the second year (**Figs. 1, 3**). These pronounced differences in total number of taxa during each studied month (**Figs. 1, 2**) were accompanied with changes in the dominant structure of the phytoplankton (**Table 1**).

In April 2018, only 10 phytoplankton taxa, mostly from Ochrophyta, were identified. In May 2018, among the 102 taxa identified the most abundant were *Aulacoseira granulata* (Ehrenberg) Simonsen, *Anabaena sphaerica* Burnett & Flahault and *Trachelomonas hispida* (Perty) F. Stein. In June 2018, number of species was 100 and *Dolichospermum spiroides*, *Dolichospermum planctonicum* and *Aphanizomenon flosaquae* Ralfs ex Bomet & Flahault reached the highest biomass. In July 2018, 105 taxa were identified, with the highest biomass of *D. spiroides*, *A. sphaerica* and *A.*



*Limnothrix redekei* (Goor) Meffert \*

*Merismopedia elegans* A. Braun ex Kützing \* *Merismopedia glauca* (Ehrenberg) Kützing \*\*

*Merismopedia punctata* Meyen \*\*\*

*Merismopedia* sp. \*\* 61

**T axa/Y ear 2018 2019 M onth** IV V VI VII VIII IX V VI VII VIII IX *Merismopedia tenuissima* Lemmermann

\*\*\* *Microcystis aeruginosa* (Kützing) Kützing \* \* \* \* \* *Microcystis* sp. \* \* \* \*

*Microcystis wesenbergii* (Komárek) Komárek ex Komárek \* \* \* \* \*

*Noctoc* sp. \*

*Oscillatoria* sp. \* \* \* \* \* *Oscillatoria limosa* C. Agardh ex Gomont \* \* \* \* \*  
\*\*

*Phormidium* sp.

*Planktolyngbya limnetica* (Lemmermann) \* \* \* \* \*  
Komáreková- Legnerová & Cronberg

*Planktolyngbya* sp. \*

*Planktothrix agardhii* (Gomont) Anagnos  
tidis & Komárek

*Pseudanabaena catenata* Lauterborn \*\* *Pseudanabaena galeata* Böcher \* \*  
\*

*Pseudanabaena limnetica* (Lemmermann)  
Komárek

*Pseudanabaena* sp. \*

*Synechococcus linearis* (Schmidle & Lauterborn) \*  
Komárek

*Snowella lacustris* (Chodat) Komárek & Hindák \* \* \*

## Chlorophyta

*Actinastrum hantschii* Lagerheim \* \* \* \* \*

*Ankistrodesmus bibraianus* (Reinsch) Korshikov \* \* \* \* \*

*Ankistrodesmus falcatus* (Corda) Ralfs \* \* *Ankistrodesmus fusiformis* Corda \* \* \* \* \*

*Ankistrodesmus longissimus* (Lemmermann) Wille  
*Ankyra ancora* f. *issajevii* (Kisselev) Fott  
 \*  
 \* \* \* \* \*

*Ankistrodesmus spiralis* (W. B. Turner) Lemmermann

*Ankyra judayi* (G. M. Smith) Fott \* \* \*  
*Ankyra ocellata* (Korshikov) Fott \*  
*Ankyra* sp. \*  
*Carteria klebsii* (P. A. Dangeard) Francé \*

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*Characium angustum* A. Braun \* \* \* \*

*Characium* sp. \* \*  
*Chlamydomonas* cf. *incerta* Pascher \* \*  
*Chlamydomonas simplex* Pascher \*\*  
*Chlamydomonas* sp. \*  
*Chlorella vulgaris* Beyerinck \*  
*Chlorolobion braunii* (Nägeli) Komárek \*  
*Coelastrum astroideum* De Notaris \* \* \* \* \*  
*Coelastrum microporum* Nägeli in A. Braun \* \* \* \* \*  
 \* \* \*  
*Coelastrum* sp. \* \*  
*Coelastrum sphaericum* Nägeli \*  
*Coenochloris* sp. \*  
*Crucigenia quadrata* Morren \* \* \* \* \*  
*Crucigenia* sp. \*  
*Crucigenia tetrapedia* (Kirchner) Kuntze \* \* \* \* \*

*Crucigeniella pulchra* (West & G. S. West) Komárek  
*Desmodesmus protuberans* (F. E. Fritsch & M. F. Rich) Hegewald \* \* \* \* \*

*Desmodesmus bicaudatus* (Dedusenko) P. M. Tsarenko

*Desmodesmus brasiliensis* (Bohlin) Hegewald \* \* \* \* \*

*Desmodesmus communis* (Hegewald) Hegewald \*

*Desmodesmus denticulatus* (Lagerheim) S. S. An, T. Friedl & Hegewald \* \* \* \* \*  
 \* \* \* \* \*

*Desmodesmus intermedius* (Chodat) Hegewald \*

*Desmodesmus opoliensis* (P. G. Richter) Hegewald \*

*Desmodesmus perforatus* (Lemmermann) Hegewald \* \* \* \* \*  
 \* \* \* \* \*

*Desmodesmus spinosus* (Chodat) Hegewald \* \* \* \* \*  
*Dictyosphaerium ehrenbergianum* Nägeli \*

*Eudorina elegans* Ehrenberg \*  
*Golenkinia radiata* Chodat \* \* \* \* \*  
*Gonium pectorale* O. F.

Müller \*

*Hariotina polychorda* (Korshikov) Hegewald  
\*

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Taxa/Year 2018 2019 Month IV V VI VII VIII IX V VI VII VIII IX

*Hyaloraphidium contortum* Pascher & Korshikov \* \* \* \*

*Hyaloraphidium rectum* Korshikov \*

*Kirchneriella lunaris* (Kirchner) Möbius \* \*

*Kirchneriella obesa* (West) West & G. S. West \* \*

*Koliella longiseta* (Vischer) Hindák \*

*Korshikoviella limnetica* (Lemmermann) P. C. Silva \* \* \* \*

*Lagerheimia ciliata* (Lagerheim) Chodat \* \* \* \*  
*Lagerheimia genevensis* (Chodat) Chodat \* \* \* \*  
*Lagerheimia* sp. \* \* \* \*  
*Lambertia* sp. \*

*Lemmermannia triangularis* (Chodat) C. Bock & Krienitz \* \* \* \* \*

*Messastrum gracile* (Reinsch) T. S. Garcia \* \* \* \* \*  
*Micractinium pusillum* Fresenius \* \*  
\* \* \* \* \*

*Micractinium quadrisetum* (Lemmermann) G. M. Smith \* \* \* \*

*Monactinus simplex* (Meyen) Corda \* \* \* \* \* \* \* \* \*

*Monoraphidium contortum* (Thuret) Komárková-Legnerová \* \* \* \* \*

*Monoraphidium griffithii* (Berkeley)

*Monoraphidium* sp. \*

*Mucidosphaerium pulchellum* (H. C. Wood) C. Bock, Proschold & Krienitz \* \* \* \* \* \* \* \*

*Oocystidium ovale* Korshikov \* \* \*

*Oocystis borgei* J. W. Snow \* \*

\* \* \* \* \*

*Oocystis lacustris* Chodat \* \* \* \* \*  
*Oocystis* sp. \* \* \* \* \*  
\* \* \* \* \*  
*Pandorina morum* (O. F. Müller) \* \* \* \* \*

Bory *Pediastrum duplex* Meyen \* \* \*

*Pseudodidymocystis planctonica* (Korshikov)  
*Pseudoschroederia robusta* (Korshikov)  
Hegewald & Deason Hegewald & E. Schnepf  
*Pseudopediastrum boryanum* (Turpin)  
Hegewald 64

*Scenedesmus acuminatus var. biseriatus* Reinhard  
*Scenedesmus arcuatus* (Lemmermann)  
Lemmermann  
\* \* \* \* \*  
*Scenedesmus acuminatus var. elongatus* G. M. Smith  
*Scenedesmus apiculatus* (West & G. S. West) Chodat  
\* \* \* \* \*  
*Scenedesmus obtusus* Meyen \*  
*Scenedesmus producto-capitatus* Schmulz \* \* \*

*Scenedesmus* sp. \* \* \*

*Schroederia setigera* (Schröder) Lemmermann \*

*Schroederia* sp. \* \*  
*Schroederia spiralis* (Printz) Korshikov \* \*  
*Selenastrum bibraianum* Reinsch \*  
*Sphaerocystis* sp. \*  
*Stauridium tetras* (Ehrenberg) Hegewald \* \* \* \*

*Stichococcus* sp. \*

*Tetrachlorella alternans* (G. M. Smith) Korshikov \*

*Tetradesmus bernardii* (G. M. Smith) M.J.Wynne \*

*Tetradesmus lagerheimii* M. J. Wynne & Guiry \*\* \* \* \* \* \*  
*Tetradesmus obliquus* (Turpin) M. J. Wynne \*\*

*Tetraedron minimum* (A. Braun) Hansgirg \* \* \* \* \*  
*Tetraedron caudatum* (Corda) Hansgirg \* \*

*Tetraedron* sp. \*

*Tetrastrum* sp. \* \* \* \* \*

*Treubaria planctonica* (G. M. Smith) & Kovacik Korshikov \* \* \* \* \*

*Treubaria schmidlei* (Schröder) Fott

*Treubaria* sp. \* \* \*  
*Vitreochlamys velata* (Korshikov) Ettl \*



*Volvox aureus* Ehrenberg \*

*Willea apiculata* (Lemmermann) D. M.  
John, M. J. Wynne & P. M. Tsarenko  
\* \*

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## Streptophyta

*Closterium aciculare* T. West \*

*Closterium acutum* Brebisson in Ralfs \* *Closterium pronum* Brebisson \* \* \* \* \*

*Closterium* sp. \* \*

*Cosmarium margaritiferrum* \* \* \* \*

Meneghini ex Ralfs

*Cosmarium* sp. \* \* \* \* \* *Elakatothrix gelatinosa* Wille \* \* \* \* \* *Gonatozygon* sp. \* \*

*Sphaerosoma* sp. \*

*Spirogyra* sp. \*

*Staurastrum* cf. *cingulum* (West & G.S. West) G. M. Smith

*Staurastrum gracile* Ralfs ex Ralfs \* \* \* \* *Staurastrum hexacerum*

Wittrock \* \*

*Staurastrum pingue* var. *planctonicum* \* \* \* \*

(Teiling) Coesel & Meersters

*Staurastrum* sp. \* \* \* \* \* *Staurastrum tetracerum* Ralfs ex Ralfs \* \* \* \*

*Zygnema* sp. \*

## Euglenophyta

*Euglena gracilis* Klebs \*\* \* \* \* \* *Euglena* sp. \* \* \* \* \* *Euglena viridis* (O. F. Müller) Ehrenberg \* \* \* \* \*

*Eugleniformisproxima* (Dangeard) M. S. Bennett & Triemer *Lepocinclis oxyuris* (Schmarda) Marin & Melkonian \* \* \* \*

*Lepocinclis acus* (O. F. Müller) Marin & Melkonian

*Lepocinclis ovum* (Ehrenberg) Lemmermann \* \* \* \* \* \* \* \* \*

\* \* \* \* \*

*Lepocinclis* sp.      *Phacus curvicauda* Svirenko      *Phacus longicauda*  
(Ehrenberg) Dujardin      *Phacus orbicularis* K. Hübner

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*Phacus* sp.      *Phacus tortus* (Lemmermann) Skvortzov      *Strombomonas* sp.  
*Trachelomonas hispida* (Perty) F. Stein  
*Trachelomonas planctonica* Svirenko      *Trachelomonas* sp.  
*Trachelomonas volvocina* (Ehrenberg) Ehrenberg

### Pyrrhophyta

*Ceratium furcoides* (Levander) Langhans  
*Ceratium hirundinella* (O. F. Müller) Dujardin  
\* \* \* \*

*Glenodinium* sp.      *Gymnodinium* sp.

*Parvodinium* cf. *inconspicuum* (Lemmermann)  
Carty

*Peridinium bipes* F. Stein      *Peridinium* cf. *aciculiferum* Lemmermann  
*Peridinium cinctum* (O. F. Müller) Ehrenberg

*Peridinium* sp.      **Ochrophyta**

### Chrysophyceae

*Dinobryon borgei* Lemmermann  
*Dinobryon divergens* O.E.Imhof      *Dinobryon sociale* (Ehrenberg) Ehrenberg      *Kephyrion* sp.  
*Uroglena* sp.

### Eustigmatophyceae

*Tetraedriella acuta* Pascher  
*Tetraedriella gigas* (Wittrock) Hansgird  
*Tetraedriella* sp.

*Tetraedriella spinigera* Skuja \* \* \*

**Synurophyceae**

*Mallomonas acaroides* Perty \*

*Mallomonas elongata* Reverdin \* \*

*Mallomonas* sp. \*

**Raphidophyceae**

*Gonyostomum* cf. *ovatum* Fott \* \* \* \* 67

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*Gonyostomum* cf. *semen* (Ehrenberg) Diesing \* \* \* \* \* \* \* \* \*

*Gonyostomum depressum* (Lauterbom)  
Lemmermann

*Gonyostomum* sp. \* \* \* \* \* *Vacuolaria* sp. \* **Xanthophyceae**

*Centrtractus belonophorus* (Schmidle)  
Lemmermann

**Bacillariophyceae**

*Amphiphora* sp. \*

*Amphora* sp. \* \* \* \* \* \* \* *Anomoeoneis* cf. *sphaerophora* Pfitzer \*

*Asterionella formosa* Hassall \* \*

*Aulacoseira granulata* (Ehrenberg) Simonsen

\* \* \* \* \* \* \* \* \* \* \* \* \* \*

*Aulacoseira islandica* (O. Müller) Simonsen \* \* \*

*Caloneis amphisbaena* (Bory) Cleve \* \* \* *Caloneis silicula* (Ehrenberg) Cleve \* \* \* *Cocconeis*

*pediculus* Ehrenberg \* \* *Cocconeis placentula* Ehrenberg \* \* \* \* \* \* \* \*

*Cocconeis placentula* var. *euglypta* \* \*  
(Ehrenberg) Grunow

*Cocconeis* sp. \*

*Ctenophora pulchella* (Ralfs ex Kützing) D.  
M. Williams & Round

*Cyclotella* cf. *glomerata* H. Bachmann \*

cf. *Discostella stelligera* (Cleve & Grunow) \* \*  
Houk & Klee

*Cyclotella meneghiniana* Kützing \* \* \* \* \*





cyanotoxin producers affecting ecosystem and human health (e.g. Meriluo et al. 2017), were found. The broad distribution of these genera and their relation with toxic blooms in Bulgaria was shown in the summary by Stoyneva-Gartner et al. (2017).

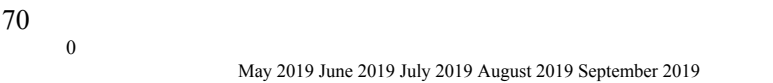


Fig. 2. Number of phytoplankton taxa in experimental fish ponds per month during the late spring-summer periods in 2019.

The results obtained during this study are in accordance also with the earlier results which have demonstrated that the high stocking density of grass carp can seriously affect the functioning of the aquatic ecosystems. For example, negative changes may be associated with alterations in the structure and abundance of plant communities, as well as in the environment they inhabit, such as changes in transparency, sediments, and increased levels of biogens after deposition of faeces (Pipalova 2006; Dibble & Kovalenko 2009). Earlier, Richard et al. (1984) reported that three years after the introduction of grass carp Chlorophyta and Bacillariophyta should significantly increase and the amount of Cyanoprokaryota should decrease. Holdren & Porter (1986) also demonstrated that after the introduction of grass carp, changes in the dominant phytoplankton species occurred.

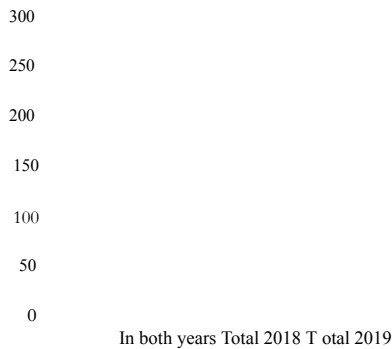


Fig. 3. Number of phytoplankton taxa in experimental fish ponds during the spring-summer periods of 2018, 2019 and in both years.

According to Boriceva et al. (2016) in small ponds, despite the expected development of small nannoplankton, summer conditions favor the development of large euglenoids, cyanoprokaryotes and chlorophytes but the size of the ponds clearly affects the detailed composition of the phytoplankton. In our study, algal biodiversity and abundance were significantly lower in 2018 compared to 2019 with registered changes in the dominant species. The most significant differences were observed in

the Chlorophyta, Streptophyta and Ochrophyta divisions from which more taxa were found in the first year than in the second, while there was almost no difference in the number of identified Cyanoprokaryota species. These results are in conformity with the data on the taxonomic structure of the phytoplankton obtained in our previous research (Dochin et al., in press).

In the early summer of 2018, after the removal of macrophytes from the grass carp, the development of phytoplankton rapidly increased and reached pronounced peaks in all experimental ponds (except P6), which will be described in details elsewhere. This rapid development with increase of the phytoplankton biomass was linked with high amount of nutrients released after the aquatic vegetation was removed by the grass carp and with the improved light regime in the water column (Dochin et al., 2020, in press). In turn, the intense development of phytoplankton can cause shading and suppression of aquatic plants (Bonare et al., 2002) and this is in accordance with the lack of macrophyte overgrowth observed during 2019 (this study; Dochin et al., 2020, in press).

## CONCLUSION

The changes in the phytoplankton composition in fish ponds stocked with grass carp polyculture observed by us showed relatively high algal biodiversity with considerably less identified species in 2019 than those in 2018. At the same time, a significant change in the structure of the phytoplankton dominants was detected: while the blooms of some potentially toxic species of Cyanoprokaryota were most intense in 2018, in 2019 the most abundant species were from Pyrrophyta, Euglenophyta and Raphidophyceae. The fact of detecting potentially toxic cyanoprokaryotes as dominants in fish ponds can serve as alarm for monitoring of the summer phytoplankton in these small water bodies.

## CONFLICT OF INTERESTS

The author declares that there is no conflict of interest regarding the publication of this article.

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