# Phytoplankton composition and abundance in Srebarna Lake and adjacent temporary wetlands (Bulgarian floodplain of the Lower Danube River)

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## 1 Introduction

Environmental changes taking place in lakes and small ponds located in the floodplains are generally fast and closely related to the hydrological regime of the river. In response to these changes the structure of phytoplankton is also changing rapidly, making phytoplankton a good indicator of the aquatic ecosystem status. The pattern of phytoplankton response of small water bodies to the environmental factors, however, is not as well understood as in large lakes (Padisák et al., 2003; Ortega-Mayagoitia et al., 2003). In these shallow environments the relative importance of abiotic and biotic factors may change dramatically among seasons. Furthermore, such factors like sediment should be taken into consideration when trying to understand the sequence of alterations of phytoplankton structure in wetlands (Ortega-Mayagoitia et al., 2003). Yet another open question is how phytoplankton structure can be used best to indicate the relationship of organisms with their environment. It could be expressed either by the distribution of organisms within major taxonomic groups or on the basis of morpho-functional (m/f) groups (Padisák & Reynolds, 1998, Reynolds et al., 2002). The phytoplankton functional groups have been found to be more precise to indicate environmental conditions (Huszar et al., 1998, Kruk et al., 2002).

In this investigation we are focusing on phytoplankton structure, which will be presented through functional and taxonomical groups. Further, we want to analyse its relationship to a variety of environmental factors, such as the type and density of the sediment (mud), the presence of macrophytes, the water depth and water temperature, the distance from the center and shores of the lake and from the nearby village. Our aim is to compare phytoplankton of the natural permanent shallow Srebarna Lake and different kinds of small ponds (wetlands) located in the area between the lake and the Danube River and to try to explain their differences and similarities.

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# 2 Materials and Methods

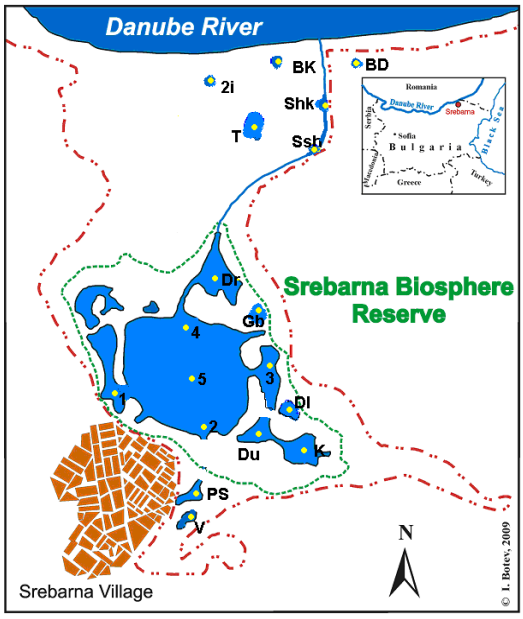


***Figure 1.*** *(modified after Beshkova et al., 2009). Map of Srebarna Lake (M: 1:18000) with location of sampling sites indicated as follows: 1,2,3,4,5 – lake sampling stations, pool 'Kulneza'(K); pudle 'Durvoto' (Du); pool 'Dulboka' (Dl); pools 'Varban Bozun' (V); 'Pod seloto' (PS); pool 'Tanyova' (T); 'Gabritsa' (Gb); 'S shluz' (Ssh); 'S shluz kana' (Shlk), pool 'Dragayka' (Dr); 'Bauz Devnya' (BD); 'Bauz Komluka' (BK); 'Il yama' (2i)*

The location of sampling sites is presented in Fig.1.

Srebarna Lake (SL, 44°07 N, 27° 04 E) has an area of 120 ha, a depth of 1.7 to 4.7 m dependent on Danube water level, and a volume of 2.81-14.35 $km^3$; lake status is eu-hypertrophic. Characteristics of pools (area/depth): (K): 9.2 ha/2.1 m; (Du): 3.4 ha/2.50 m; (Dl): 2.2 ha/2.60 m; (V) and (PS): 1.2 ha/2.4 m; (T): artificial basin, drying out at low Danube water level; (Gb): 2 ha/3.5 m; (Ssh) and (Shlk) are located in the canal connecting the Danube with Srebarna Lake during high flow; (Dr): 6.8 ha/3 m, located in the south end of the canal, separated by reed beds; (BD), (BK) and (2i) are temporary water bodies, regularly flooded at high water level, around 1 ha/1.5 m.

**Sampling frequency:** Samples of 0.5 l were taken from 5 permanent lake points and from the pools during the vegetation periods (March-October) during 2004-2006, covering spring, summer and autumn. The number of visits (v) were as follows: SL -12 v.; K and Du -11 v.; Dl -7v.; V -6 v.; Dr -4 v; BD, BK , Ssh and 2i - 2 v.; unmentioned sites got a single visit only. Since lake site no. 3 is separated from the main lake by thick reed belt it was analyzed in the group of the pools.

**Methods**: The samples were preserved with formalin (4%) to count and calculate phytoplankton numerical abundance as individuals per liter (ind $l^{-1}$) and biomass (mg $l^{-1}$). The counting was done on a light microscope (200x) in the hemocytometer type of Bürker's chambers. Biomass was obtained on the base of individual algal volume measured and calculated by the method of geometrical approximations (Rott, 1981). The variables mud density (MudD), degree of macrophyte coverage (DMC) and degree of macrophyte remains (DMR) were evaluated through relative scale: 0 - absent, 1 – low, 2- middle, 3-high. The type of the mud (MudT) was characterized as: brown - 1, black - 2, black-gray – 3. Other variables measured were temperature (T,°C), water depth (m), distance from the center of lake mirror ($D_1$, m); distance from the closest lake shore ($D_2$, m), distance from the village ($D_3$, m).

**Statistics**: Partial canonical analysis (CCA) (Ter Braak & Šmilauer, 2002) was applied to reveal spatial and temporal variations of phytoplankton taxonomic and functional groups in relation to the environmental factors. Explanatory variables were considered: depth, T, MudD, DMR, DMC, $D_1$, $D_2$, $D_3$. Response variables were biomasses of different taxonomical and functional groups. The taxonomical groups (divisions) Cyanophyta, Chlorophyta, Bacillariophyta, Chrysophyta, Cryptophyta, Pyrrhophyta, Euglenophyta, and Xanthophyta are presented in figures by abbreviation composed from the first three letters. Functional groups are indicated with letters according Reynolds et al. (2002).

# 3 Results and discussion

During the study period phytoplankton numerical abundance in the ponds showed a far wider range (from 540 to 187668 .$10^3$ ind./l) than in Srebarna Lake (from 4418 to 201594.$10^3$ ind./l). Biomass changed from 0.52 mg/l to 51.65 mg/l and from1.99 mg/l to 62.66 mg/l respectively. The main m/f groups prevailing in biomass of phytoplankton were $S_1$, D, $H_1$,Y, $X_1$ and J, available in biomass shares of 35:16:7:7:7:6 respectively. The biomass shares of the main taxonomic groups were as follows: Cyanophyta - 46, Bacillariophyta -22, Chlorophyta -12 and Cryptophyta -7. Other m/f or taxonomical groups include shares smaller than 5 %.

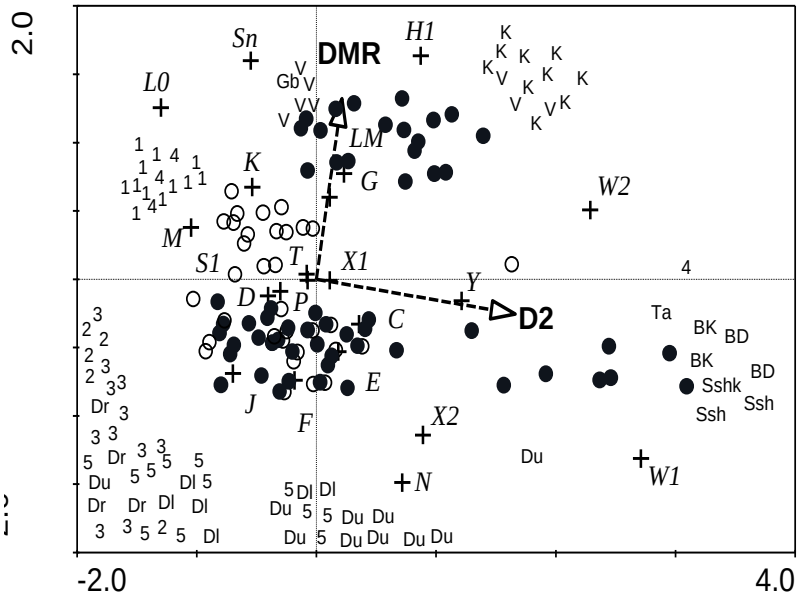


***Figure 2.*** *Partial CCA triplot of sample spatial distribution with phytoplankton functional groups as response variables and $D_2$ and DMR as explanatory variables after elimination of seasonal and annual influences. + functional groups; ● - pools, ○ - lake sampling stations; DMR - degree of macrophyte remains, $D_2$ – distance from the neighboring lake shore; Total inertia = 4.840, Sum of all eigenvalues 3.714, Explained EV = 0.393, P = 0.0020*

The distribution of sampling points on the base of m/s groups (partial CCA) shows clear differentiation of pond samples by the first two main axes formed by $D_2$ and by DMR explanatory variables (Fig.2). Accordingly the pools seem to form three distinct groups. The first group unifies the ponds located far from the lake shore towards the Danube River (BK, BD, T, Ssh, Sshk). They are characterized by higher presence of $W_1$, $W_2$ and Y functional groups. All these groups consist of motile organisms, capable to myxothrophic nutrition and often coexisting in small, mixed ponds, rich of organic matter (for example dead vegetation after flooding).

The second group brings together ponds with high DMR - V, K and Gb. They are characterized by predominance of $H_1$ and $S_N$ m/f groups and to some extent, of $L_M$ and G groups.

The third pool group consists of sites Du, Dl and Dr containing mainly C, E, F, J, P, D functional groups of algae. This group overlaps with half of lake sampling stations (No 2,3 and 5) which contain the same list of functional groups. This overlap between pools and lake stations is explained by their proximity (southeastern part of the lake area). The other part of lake stations (No 1 and 4) does not overlap with pools i.e. their phytoplankton has different composition including functional groups L0, K and M differentiating them from third pool group and lake stations No 2, 3 and 5. The representatives of these functional groups are colonial blue-greens. The phytoplankton in the third pool group has a larger amount of organisms belonging to groups F, J and E.

Figure.3 shows that three factors - the water depth together with $D_2$ and DMR explain a significant share of temporal (inter annual) and spatial variations of biomass of phytoplankton functional groups.

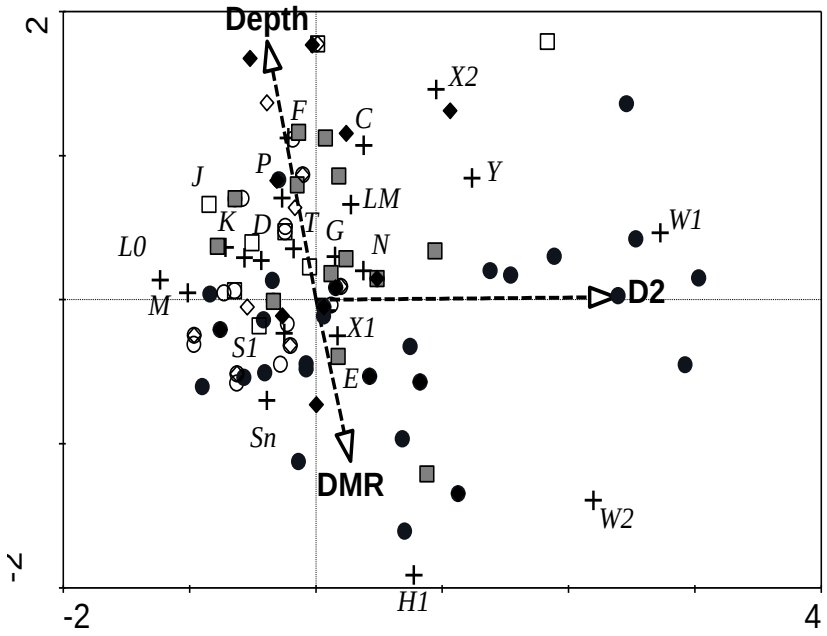


***Figure 3.*** *Partial CCA triplot of inter annual and space distribution of samples, phytoplankton functional groups as response variables and depth, DMR and D2 as explanatory variables after elimination of seasonal influences.* **+** *functional groups;* ● *2004 pools,* ○ *2004 lake,* ■ *2005 pools,* □ *2005 lake,* ♦ *2006 pools* ◊ *2006 lakes; Total inertia = 4.840, Sum of all eigenvalues 4.126, Explained EV = 0.561, P = 0.0020.*

The samples are divided along the second main axis into such with small depth and high DMR (from 2004) and other with high depth and small DMR (from 2005 and 2006). The figure also shows that $H_1$ (nitrogen fixing cyanobacteria) and $S_N$, to a certain extend, prevail mainly in the shallower ponds with high DMR. However, these groups are considered as tolerant to low nitrogen and sensitive to mixing (Reynolds et al., 2002), a fact which does not correspond well to their occurrence in shallow ponds. In order to explain this discrepancy we have to refer to some other authors (Padisak & Reynolds, 1998; Huzar et al., 2000), cited after Reynolds et al. (2002), who stated that the solitary forms of H group are recognized to be more closer to S type, and in particular to $S_N$. Furthermore, groups $S_1$, $H_1$ and $S_N$ are often in competition because of their similar requirements to environmental conditions.

The increase of water depth favors the groups F, C, P and $L_M$. A common feature of these groups is that they are associated with epilimnia in eutrophic lakes, and are considered to be tolerant to carbon deficiencies or low nutrients (Reynolds et al., 2002). The increase of their share probably indicates that depth increase leads to a change in the conditions from polymictic to dimictic (with more stable stratification in the summer). This change immediately affects the structure of phytoplankton not only in the main lake but in the smaller ponds too. The groups $X_2$ (regarded as tolerant to stratification) and Y (tolerant to low light) are also favored by the increase of the depth, but they are also related to $D_2$, i.e. they predominate in the small distant ponds rather than in the lake. These groups of C-strategists have been often found in water bodies with low phytoplankton biomass and fish absence, and high zooplankton pressure respectively (Padisák et al. 2003).

As a whole, the functional groups that contribute mostly to the separation of lake and pool phytoplankton samples were $S_N$, $H_1$, $W_1$, $W_2$, $X_2$ and Y.

The partial CCA (spatial) of taxonomic group distribution (Fig. 4), shows that the main factors, explaining variations of samples are $D_2$ and MudD, pointing in similar direction. Cryptophyta, Chrysophyta and Euglenophyta are better represented in the ponds than in the main lake, their relative biomass raises with increase of D2 and MudD.

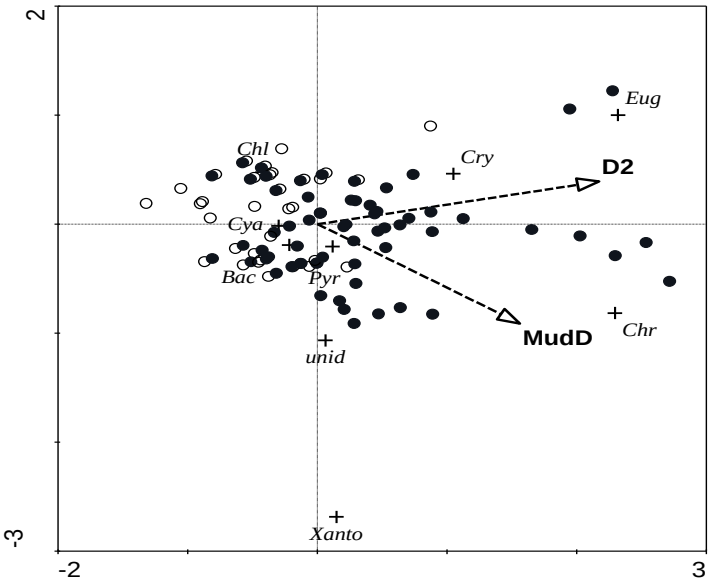


***Figure 4.*** *Partial CCA triplot (spatial variations) with phytoplankton taxonomical groups as response variables and $D_2$ and MudD as explanatory variables; abbreviation + taxonomical groups; ● - pools, ○ - lake stations; MudD – mud density, $D_2$ – distance from the nearest lake shore; Total inertia = 2.285, Sum of all eigenvalues 1.814, Explained EV = 0.301, P = 0.0020*

$D_2$ again like in Fig.1 explains a significant share of spatial variations of sample division composition. However, the variation of phytoplankton samples (total inertia) defined by algal divisions is much smaller than that defined by functional groups. Accordingly the separation between different sample groups defined by algal divisions is worse than that by functional groups. The increase of density of the sediment mud in the ponds, remote from the lake (BK, BD) is probably due to their temporary nature. They probably often dry up and have no time to accumulate a thick layer of a loose slime. As regard the ponds Ssh and Sshk, situated in the canal between the Danube River and Srebarna Lake, the loose slime is probably periodically washed by in- and outcoming waters between the river and the lake.

The partial CCA of interannual and spatial variations of the phytoplankton samples presented by higher taxa selected water depth, D2 and MudD as main environmental factors explaining the significance of the structural variations of planktonic biota (Fig.6). In 2004 (distinguished by small depth) the lake and pond samples overlap, and only one group with predominance of Euglenophyta and Chrysophyta is separated. In 2005 and 2006 (with increase of water depth) the divisions of Cryptophyta, Pyrrhophyta and also unidentified species (mainly different small flagellates), become quantitatively more important in the pond samples.

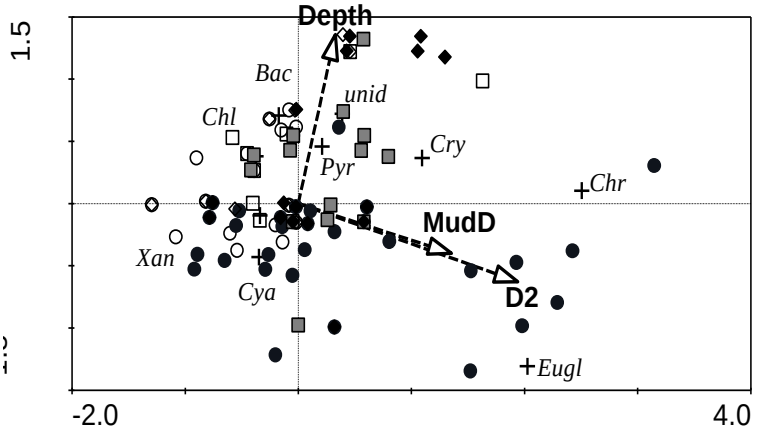


***Fig. 6.*** *Partial CCA triplot of inter annual and spatial variations of samples, with algal divisions as response variables and Depth, MudD and D2 as explanatory variables after elimination of seasonal influences. Meaning of applied signs: + taxonomical groups; black symbols – pools, white symbols - lake sites ● ○ 2004, ■ □ 2005, ♦◊ 2006; MudD - mud density, $D_2$ – distance from the closest lake shore; Total inertia = 2.285, Sum of all eigenvalues 2.089, Explained EV = 0.419, P = 0.0020.*

The taxonomic groups of Cryptophyta, Euglenophyta and Chrysophyta mostly contribute for the segregation of samples between lake and different small ponds. To a certain extent they coincide with the functional groups $W_1$, $W_2$, $X_2$ and Y. However, with regards to the other major taxonomic groups - Cyanophyta, Chlorophya and Bacillariophyta, they do not contribute to the separation between the investigated ponds.

# 4 Conclusions

The expression of phytoplankton structure through the functional groups provides a more detailed and informative picture on the similarities and differences between compared water bodies and on the relationship of phytoplankton variables with environmental factors in both spatial and temporal aspects. Nevertheless, the reliability of both classification schemes, by taxonomic and functional algal groups, is in accordance with other studies and they confirm each other with respect of their discriminatory power (K. Teubner, personal communication).

D2 and DMR are the factors significantly contributing for differentiation between lake and pond phytoplankton assemblages regarding their spatial distribution. The same factors, together with water depth explained the combination of interannual and spatial variations together, but MudT replaces DMR as explanatory factor of variations of phytoplankton samples presented by taxonomic groups. The water depth variations are closely connected with lake level changes. As the analyses showed the depth factor appears on interannual basis because these water level variations are stronger than the seasonal ones. These variations are related but are not a direct consequence of the river level changes. Functional groups that contribute most to the separation of samples both in spatial and temporal aspects are nitrogen fixing cyanobacteria ($S_N$, $H_1$) and motile unicellular species (Y, $W_1$, $W_2$ and $X_2$). These groups are positively related with DMR, water depth and D2 and are better represented in the pools.

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