Indeks funkcionalnih skupina fitoplanktona kao pokazatelj promjena ekološkog stanja jezera u poplavnom području Dunava

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Graduate university study programme in Biology

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PERFORMANCE OF THE PHYTOPLANKTON ASSEMBLAGE INDEX IN EVALUATION OF ENVIRONMENTAL CHANGES OF A DANUBIAN FLOODPLAIN LAKE

Master's Thesis

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INDEKS FUNKCIONALNIH SKUPINA FITOPLANKTONA KAO POKAZATELJ PROMJENA EKOLOŠKOG STANJA JEZERA U POPLAVNOM PODRUČJU DUNAVA

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Rad je izrađen: Zavod za ekologiju voda, Odjel za biologiju, Sveučilište Josipa Jurja Strossmayera u Osijeku

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Indeks funkcionalnih skupina fitoplanktona (Q indeks) koristi se kao pokazatelj promjena ekološkog stanja slatkovodnih sustava te se istražuju mogućnosti njegove primjene u ocjeni stanja različitih tipova jezera. Cilj ovog rada je na temelju dinamike fitoplanktonskih funkcionalnih skupina i primjenom Q indeksa ocijeniti ekološko stanje Sakadaškog jezera, plitkog jezera u sustavu poplavnog područja Dunava. Istraživanje je provedeno u razdoblju srpanj 2011-listopad 2012, u hidrološkim uvjetima uobičajene dinamike poplava. Rezultati su uspoređeni s povijesnim podatcima dinamike fitoplanktona u razdoblju srpanj 1972-rujan 1973, kada je jezero bilo pod jakim antropogenim utjecajem. Dinamika i zastupljenost pojedinih funkcionalnih skupina fitoplanktona značajno su se razlikovali između dva uspoređena razdoblja. Česta pojava masovnog razvoja jedne vrste ili funkcionalne skupine karakteristične za zagađene vode, bile su značajke dinamike fitoplanktona u uvjetima velikog zagađenja okoliša na što ukazuju niske vrijednosi Q indeksa i ocjena *loše* do *umjereno loše* stanje. U uvjetima bez antropogenih pritisaka i očuvanog hidrološkog stanja, u fitoplanktonskoj su zajednici bile zastupljene funkcionalne skupine karakteristične za prirodna eutrofna jezera, a vrijednosti Q indeksa pokazuju da je jezero u statusu između *umjerenog* i *dobrog* stanja. Pomoću Q indeksa mogu se vrlo precizno odrediti promjene ekološkog statusa jezera.

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PERFORMANCE OF THE PHYTOPLANKTON ASSEMBLAGE INDEX IN EVALUATION OF ENVIRONMENTAL CHANGES OF A DANUBIAN FLOODPLAIN LAKE Katarina Kajan

Thesis performed at Subdepartment of Water Ecology, Department of Biology, Josip Juraj Strossmayer University of Osijek

Supervisor: Melita Mihaljević, PhD, Associate Professor

Co-supervisor: Judit Padisák, PhD, Full Professor, Department of Limnology, University of Pannonia, Veszprém, Hungary

As a monitoring tool for ecological status assessment of freshwater bodies, phytoplankton assemblage index (Q index) was so far successfully applied in the evaluation of different types of lakes. Currently, its wider application possibilities are being investigated. The aim of this study was to evaluate ecological status of the floodplain lake, Lake Sakadaš, part of the complex river-floodplain system in the Middle Danube (Croatia), by assessment of phytoplankton functional groups and applying the Q index. Phytoplankton research was conducted in the period July 2011-October 2012, in conditions of more or less usual flooding pattern. Results were compared with the historical data of phytoplankton dynamics within the period July 1972-September 1973, when the lake was under the strong anthropogenic pressure, which resulted in rapid eutrophication and deterioration of its ecological status. The data set of phytoplankton functional groups and the main group arrangements in the multivariate nMDS analysis showed variation related to the past and recent conditions. The frequent appearance of heavy bloom of only one species or assemblages characteristic for polluted waters was the most distinctive feature of phytoplankton dynamics in the past. In such an impacted environment, values of the Q index were low and indicated *bad* to *tolerable* ecological status. Recent data suggest that water quality improvement and near-natural hydrological condition support algal assemblages characteristic for naturally eutrophic lakes and the values of the Q index varied between *medium* to *good* ecological status. Altogether, results of this study showed that Q index can very precisely reflect anthropogenic pressure what was recognized as its major advantage in comparison with other indices used for ecological status assessment.

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1. INTRODUCTION

The ecological status estimation of surface waters according to the Water Framework Directive (Directive 2000/60/EC; WFD, 2000) is based on the assessment of biological elements supported by hydromorfological, chemical and physicochemical elements. The phytoplankton as an extremely diverse, photoautotrophic group of organisms responsible for the majority of primary oxygen and organic carbon production in aquatic ecosystems (Reynolds, 2006) is one of the five biological quality elements proposed for ecological status assessment of surface waters. Phytoplankton species are good indicators of water quality because they reflect the changes very rapidly both in their quantitative and qualitative composition. Dynamics of phytoplankton are mainly determined by environmental changes occurring at different temporal scales (short-term and annual variations) and are regulated by both internal and external factors (Reynolds, 1993; Reynolds, 2006).

Traditional phytoplankton monitoring based on the taxonomic level of community structure, including phytoplankton biomass and chlorophyll *a* concentration, appeared to be only partially useful for determining water quality. The main disadvantage of this approach is that numerous species of phytoplankton are included in the broader taxonomic groups despite their very different ecological properties (Reynolds et al., 2002).

The monitoring of phytoplankton was supplemented with phytoplankton trait-based approaches which grouped species with similar morphological and functional properties. Several phytoplankton classification concepts were developed: the functional group (FG) classification (Reynolds et al., 2002; Padisák et al., 2009), the morpho-functional group (MFG) classification (Salmaso and Padisák, 2007) and morphology-based group (MBFG) classification (Kruk et al., 2010; Kruk and Segura, 2012).

According to physiological, morphological and ecological attributes of phytoplankton species, Reynolds et al. (2002) proposed an approach assigning phytoplankton species into functional groups (coda). Groups of species are defined with specific habitat, tolerances and sensitivities based on several different combinations of physical, chemical and biological properties of the water body environment (such as depth of mixing layer, light, temperature, P, N, Si, CO₂, grazing pressure). The approach was further developed by Padisák et al. (2009) stipulating a detailed description of the typical misplacements and by modifying some of the original habitat templates and species allocations. Functional classification, altogether with more than 40 groups (coda) described, simplifies the comparison of seasonal changes in various water body types and evaluates the responses to environmental changes (Kruk et al., 2002; Naselli-Flores et al., 2003). The classification has been applied worldwide from temperate to tropical regions in different ecosystems, particularly in shallow lakes (Huszar et al., 2003; Mieleitner et al., 2008; Rangel et al., 2009; Barone et al., 2010; Borics et al., 2012; Izaguirre et al., 2012; Crossetti et al., 2013; Hu et al., 2013). This classification was also shown as successful approach in monitoring the changes of phytoplankton in reservoirs (Fonseca and Bicudo, 2008; Borges et al., 2008; Becker et al., 2009a; Xiao et al., 2011), estuaries (Costa et al., 2009) and rivers (Borics et al., 2007; Abonyi et al., 2012; Stanković et al., 2012).

Following the functional group classification, Padisák et al. (2006) developed the phytoplankton assemblage index (Q index) initially proposed to assess the ecological status of different lake types in line with the WFD (2000). The assemblage index provides 5 degrees of water quality based on the relative share of FG multiplied by factor number (*F*) defined for each FG. The most crucial step of the assessment is a determination of factor *F* values because they reflect values of FG in the reference condition of the studied water body (pristine status). The Q index was initially developed to assess the ecological status of different Hungarian lake types (Padisák et al., 2006). According to that time existing typology, there are 8 lake types in Hungary to which belong large lakes, alkaline lakes and oxbows outside flood control dams. Values of factor *F* were established for each functional group within all lake types.

Hajnal and Padisák (2008) used the phytoplankton biomass and assemblage structure expressed by the Q index to reconstruct the history of water quality in Lake Balaton and to quantify the changes in water quality during the eutrophication and restoration phases. This research represents the first attempt at historical reconstruction of the ecological status and compares it with changes in trophic state and current water quality. The results showed that this method could be successfully used to reconstruct water quality from historical archives and it is helpful in defining reference condition of the water body.

Up to now, the Q index was successfully applied to evaluate ecological status of various types of lakes (Demir et al., 2014; Teneva et al., 2014; Ochocka and Pasztaleniec, 2016; Ongun Sevindik et al., 2017) and other types of water bodies such as reservoirs (Crossetti and Bicudo, 2008; Becker et al., 2009b; Becker et al., 2010; Wang et al., 2011; Çelekli and Öztürk, 2014; Molina-Navarro et al., 2014; Vieira et al., 2015; Silva and Costa, 2015; Santana et al., 2017) regardless of geographic regions. It should be emphasized that the Q index was successfully applied for assessment of the water bodies from existed lake types in Bulgaria according to the accepted typology (Belikinova et al., 2014).

Floodplain lakes, also known as oxbow lakes, fluvial lakes, river lakes, limnocren, crescent lakes (Dawidek and Ferencz, 2012 and cites therein) remain to be the least known group of water bodies due to their complexity and diversity. A floodplain lake includes every inland water body, whose basin originates from fluvial processes, and its limnological functioning derives from irregular, but periodical limnophases and potamophases (Dawidek and Ferencz, 2012). Characterization of a floodplain lake is based on hydrogeological, hydrological and hydrochemical features. Due to the high anthropogenic impact, which includes the alteration of natural flow pulses, channelization, dredging, artificial levee construction, groundwater abstraction and enrichment with nutrients (wastewaters, nutrient inflow from agricultural areas), floodplains are nowadays one of the most threatened and sensitive ecosystems (Amoros and Bornette, 2002). The majority of large active floodplains have disappeared over the last two centuries, including up to 80% of the historical floodplain area along the Danube and its larger tributaries (Schwarz, 2010).

Floodplain along the Middle Danube section, known as Kopački Rit Nature Park, is one of the largest preserved natural floodplains of the Danube River. A diversity of aquatic and wet biotopes composed of ephemeral and perennial water bodies, mostly channels, oxbows, marshes and shallow lakes, continuously changing the area covered by water depending on the inflow of riverine waters. One of the significant water bodies in this floodplain is Lake Sakadaš, the deepest water depression located in the western part of the floodplain. During the 1970s, when the first limological investigations were carried out in the Lake Sakadaš (primarily focused on the water quality and phytoplankton), high variability in physical and chemical parameters was detected by water quality assessment. High variabilities in parameters indicated the high anthropogenic impact on the lake due to the direct income of wastewaters from the surrounding agricultural areas, which resulted in rapid eutrophication and deterioration of the ecosystem (Gucunski, 1975). Efforts for protection of the whole floodplain habitats resulted in strong protection of the area with the status of Nature Park. Moreover, measures for the revitalization of Lake Sakadaš applied in 1984 included complete isolations from the incoming of wastewaters and sediment removal.

During the past few decades, the lake was in eutrophic/hypertrophic state according to the OECD system (OECD, 1982) with yearly average of TP concentration higher than 100 μ g L⁻¹, water transparency less than 1.5 m, and maximum chlorophyll *a* concentration higher than 50 µg L-1 (Mihaljević et al., 1999; Vidaković and Bogut, 2007; Horvatić et al., 2003; Stević et al., 2005). The results of permanent monitoring of phytoplankton in the lake were carried out from 2003 till nowadays, showed that depending on the time scale occurrence, flood pulses can be a stimulating or a disturbance factor for phytoplankton development in Lake Sakadaš (Mihaljević et al., 2009). However, the intensity and duration of flooding can be qualified as the primary cause for the changes of phytoplankton assemblages in this floodplain lake (Stević et al., 2013).

1.1.Study aim

The aim of this study was to evaluate ecological status of the floodplain lake, Lake Sakadaš applying phytoplankton assemblage index. Due to the fact that there are no historical data for evaluation of pristine condition in this floodplain lake, current research of phytoplankton assemblages dynamic will be compared with the historical data of phytoplankton dynamics when the lake was under strong anthropogenic pressure. The hypothesis is that Q index will indicate changes between impacted and semi-natural conditions of the floodplain lake.

2. MATERIALS AND METHODS

2.1. Study area

Kopački Rit Nature Park is one of the largest conserved natural riverine floodplains located in North-East Croatia between the Drava and the Danube River (Figure 1.). It is internationally recognized as the Ramsar Area (No: 3HR002) protected by the Ramsar Convention and included in the Important Bird Area (IBA) list, covering approximately 180 km^2 . Since the Pleistocene and Holocene epochs, when the Kopački Rit was formed, its ecological equilibrium and existence depends on the flooding regime of Danube and Drava River (Tadić et al., 2014). The floodplain complex provides a diversity of biotopes composed of periodic and permanent water bodies, such as marshes/pools, ponds, swamps on organic soils, shallow lakes, riverside arms and natural channels. The hydrological dynamics of the floodplain is under the impact of horizontal (inflows and outflows) and vertical (precipitation, evaporation and transpiration) components of water balance (Tadić et al., 2014).

The Drava River has a minor influence due to the high embankments constructed in the middle of the 20th century between the river and the floodplain (Bonacci et al., 2002). The intensity of the flooding primarily depends on the hydrological regime of the Danube, and it may occur in any season of the year. In the middle section, the Danube (1,410- 1,383 r. km) is a typical lowland river with the mean annual water level of 2.63 m and mean annual discharge of 2,085 m^3s^{-1} (from a database of daily recordings in the period 1987-2008 at the gauge station at river 1,401.4 km). Generally, during the first half of the year (mid-spring), the flow of the Danube is the highest, while in the second half of the year (June-October) the flow is characterized by a decrease (Buijse et al., 2002). During low river water discharge, the permanent water biotopes in the floodplain are isolated from one another.

The floodplain area can be divided into two subsystems due to the hydrological connectivity with the main river channel. Subsystem A is impounded by the river through the backwater system (side arm), while subsystem B through a network of perennial channels. According to Schwarz (2005), minor floods (3-3.5 m) inundate only 18% of the area in subsystem B, while extremely high floods (more than 5 m) inundate almost the whole floodplain area (more than 90% of subsystem B).

Figure 1. Location of the sampling site Lake Sakadaš in the study area Nature Park Kopački Rit. The dotted line marks the border of Nature Park Kopački Rit and dashed line marks the embankment of rivers Danube and Drava (arrows indicate the flow direction). Modified from Mihaljević et al. (2013).

The study was carried out in the western part of the floodplain in the Lake Sakadaš (Figure 1., Figure 2.). The average depth of the lake is about 5 m and a maximum depth of ca. 12 m, with a surface water area of about 0.15 km^2 . It is in direct hydrological connection with the main river channel through two channels (Čonakut and Hulovo channels) with total length ca. 10 km, and it is close to the embankment which delineates the inundation area. Flooding of the lake begins when the Danube water level at the gauge station river 1,401.4 km rises above 3 m (Mihaljević et al., 1999). The mixing regime is closely connected to flood events, and thermal stratification is usually expressed during the summer (June-September), while during the winter months (December-February) the entire lake is ice-covered.

In shallow parts of the lake, submerged macrophytes are well developed (*Ceratophylum demersum* L., *Myriophyllum spicatum* L. and *Potamogeton gramineus* L.). The common reed (*Phragmites communis* Trin.) occurs around the lakeshore adjoined by floodplain forests of white willow (*Salix alba* L.) and black poplar (*Populus nigra* L.).

Figure 2. Sampling site Lake Sakadaš. (Pictures from Subdepartment of Water Ecology archives)

2.2. Sampling and analysis of physical and chemical parameters

Sampling was conducted at monthly intervals during the period from July 2011 to October 2012 (except in February 2012) in the central part of Lake Sakadaš. The main studied physical parameters were water temperature, water depth, transparency, conductivity, pH and dissolved oxygen. Water temperature, pH, conductivity and dissolved oxygen were measured *in situ* at subsurface (*ca.* 0.2 m) using portable instrument Multi 340i WTW (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany). Water depth of the lake was measured using a weighted rope, and the transparency was estimated with a Secchi disc. Samples for the chemical analyses of ammonium (NH₄⁺), nitrates (NO₃⁻), nitrites (NO₂⁻), organic nitrogen (orgN), total nitrogen (TN) and total phosphorus (TP) were taken at a depth of *ca*. 0.2 m below the water surface. Chemical variables were analyzed in the laboratory according to standard methods (APHA, 1992). In order to determine the concentration (μl^{-1}) of chlorophyll *a* (Chl *a*), -*b* (Chl *b*) and -*c* (Chl *c*), sample of 1-L was carried on ice to the laboratory and filtered through Whatman GF/C glass fibre filters (Whatman International Ltd., Maidstone, England). Filtered samples were subsequently extracted with acetone. Absorbance was measured with a Hach DR 2010 spectrophotometer (Hach Company, USA) at four different wavelengths (630, 645, 663 and 750 nm). Chl *a,* -*b* and -*c* concentrations were calculated according to UNESCO (1966) and Strickland and Parsons (1972).

Similar methods were applied during the period from July 1972 to September 1973 (except in August 1973), with data available in the doctoral thesis of Professor Dragica Gucunski (Gucunski, 1975). From the doctoral thesis, the following values of physical variables were available: water temperature, water depth, transparency, pH and dissolved oxygen. Values of chemical variables could not be used, wherein only their presence or absence is indicated. Daily records of Danube water level were obtained for the gauge station at river 1,401.4 km.

2.3. Phytoplankton analyses

Depth-integrated samples intended to assess the quantitative composition of phytoplankton were collected from the entire water column and fixed *in situ* with Lugol's acidified solution. Additional samples for qualitative taxonomic analysis were obtained with a 22.5 μ m pore net and fixed with 4% formalin. Phytoplankton species were identified with light microscopic observations (Carl Zeiss Jena, Jena, Germany) using the standard literature for species determination (Hustedt, 1976; Hindák et al., 1978; Meffert et al., 1981; Anagnostidis and Komárek, 1985, 1988; Komárek and Anagnostidis, 1989). The taxonomic classification was updated with recent findings, and the nomenclature was updated according to Algaebase website (Guiry and Guiry, 2014). Quantitative assessment of phytoplankton was done according to Utermöhl (1958) using the inverted microscope (Axiovert 25, Carl Zeiss, Inc., Göttingen, Germany) at multiple magnifications (100×, 400×) counting 400 individuals. The counting unit was the individual (unicell, coenobium, filament or colony). Each species abundance was presented as the number of individuals per liter (ind. L^{-1}). Biovolumes were calculated following Rott (1981) where individuals were measured and their volumes calculated by relating cell shape with the corresponding geometric body. Volume calculations of colonial organisms with mucilage included entire colonies together with mucilage. Biomass was estimated multiplying each phytoplankton species abundance with mean biovolume of species (Javornický and Komárková, 1973; Sournia, 1978) and expressed as milligrams per liter (mg l-¹) fresh mass.

Historical data of phytoplankton quantitative analysis from July 1972-September 1973 was used from the doctoral thesis of Professor Dragica Gucunski (Gucunski, 1975) with her family's permission. Data of phytoplankton abundance (cell L^{-1}) was converted to biomass (mg L^{-1}), calculated according to cell biovolume of phytoplankton species database published by Gucunski and Popović (1984). Historical phytoplankton data was updated with currently used taxonomic classification and nomenclature.

2.4. Data analysis

Each taxon, determined in the study period from July 2011 to October 2012 and in historical data from July 1972 to September 1973, was classified into the functional group proposed in the classification of Reynolds et al. (2002) revised by Padisák et al. (2009). The assemblage index (Q index) followed by Padisák et al. (2006) was applied to assess the ecological status of floodplain Lake Sakaš during the periods.

The assemblage index was calculated according to the following formula:

$$
Q = \sum_{i=1}^{n} p_i F
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p_i = n_i/N
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p_i
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F – factor number established for the *i*-th functional group

The relative share of FGs in total biomass was calculated, and factor numbers (*F*) were established for each FG registered in chosen periods. Factor number was determined in the range from 0 to 5. Determination of factor numbers was based on the previous knowledge and experience of experts, together with the literature data of phytoplankton composition in Lake Sakadaš from 1972 up to the present day. Given factor numbers were compared with *F* defined for similar water body types (Padisák et al., 2006; Crossetti et Bicudo, 2008; Becker et al., 2010; Belkinova et al., 2014). According to the WFD requirements, values of Q index between 0 and 1 were classified as bad, between 1 and 2 as tolerable, between 2 and 3 as medium, between 3 and 4 as good, and between 4 and 5 as excellent (Padisák et al., 2006).

2.1. Statistical analyses

Pearson's correlation coefficient was used to analyze the correlation between the physical and chemical parameters of the periods using Statistica 8.0 software (StatSoft, Inc., USA). Non-metric multidimensional scaling (nMDS) was used to display similarity between the biomass of phytoplankton FGs of chosen periods using the statistical program PRIMER version 5.0 (Clarke and Warwick, 2001). Biomass of phytoplankton FGs was the square root transformed and applied on Bray-Curtis similarity coefficient. Increasing distance between samples in nMDS plot reflects higher dissimilarities between the biomass of FGs in the periods.

3. RESULTS

3.1. Physical and chemical characterization of the environment

Flooding dynamics of the river-floodplain system is determined by the fluctuation of the Danube water level. Daily courses of the Danube water level at river 1,401.4 km are shown in Figure 3. During the periods of July 1972-October 1973 and July 2011-October 2012, the floodplain was characterized by the periodical occurrence of limnophase (dry periods) and potamophase (flood periods). Flooding patterns were defined by the timing (season), duration of flooding and the total surface of the flooded area (Table 1.).

Danube water level daily courses of chosen periods showed a statistically significant positive correlation ($r = 0.27$, $p < 0.05$), despite the higher maximal water level (5.84 m) and extremely high flooding (27 days in total) with more than 75% of the flooded area recorded in 1972-1973. Most extended flood with 81 days in continuum was present during the spring-summer period of 1973. The absence of flooding with 155 days in total characterized the longest dry phase of studied period 2011-2012. After the dry phase, short-time flood pulses were frequent during the late winter and spring with two major flood pulses (maximum 4.91 m). Extremely low Danube water level influenced the outflow of the water from the floodplain to the main channel, with a minimal water level of -0.30 m recorded in December 2011. In summer during 1973 and 2012 floodplain went through a second dry phase during chosen periods, lasting till the end of the studies.

Table 1. Danube water level (maximum, minimum and mean) and flooding dynamics of the river-floodplain system (categorization and duration) during the period of July 1972-October 1973 and July 2011-October 2012. (*) Approximation after Mikuska (1979) and Schwarz (2005)

Figure 3. Daily courses of the Danube water level at 1,401.4 r. km during the period of July 1972-October 1973 (*dashed line*) and July 2011- October 2012 (*solid line*). Flooding categorization is shown in Table 1.

Parameter (abbreviation, SI)	1972-1973			2011-2012		
	Mean	Min.	Max.	Mean	Min.	Max.
Water depth (WD, m)	7.8	6.7	9.9	6.5	5.4	7.7
Transparency (T, m)	0.9	0.2	1.8	1.0	0.7	1.9
Water temperature (WT, $^{\circ}$ C)	16.0	3.0	30.0	18.4	4.1	30.6
Dissolved oxygen (DO, mg L^{-1})	9.9	2.2	21.8	11.6	7.1	15.3
pH	8.2	7.7	9.7	8.3	7.7	9.0
Ammonium (NH_4^+ , μ g L^{-1})				123.2	5.0	454.0
Nitrates $(NO3$, μ g $L-1)$				861.6	20.0	3,950.0
Nitrites $(NO2^{\circ}, \mu g L-1)$				23.9	5.3	83.6
Organic nitrogen (orgN, μ g L ⁻¹)				2,047.1	322.6	5,600.0
Total nitrogen $(TN, \mu g L^{-1})$				3,008.7	587.9	8,799.8
Total phosphorus $(TP, \mu g L^{-1})$				211.8	60.7	422.0
Conductivity (Cond, μ S cm ⁻¹)				405.1	354.0	568.0
Chlorophyll a (Chl-a, μ g L ⁻¹)				45.5	14.3	90.8
Chlorophyll b (Chl-b, μ g L ⁻¹)				7.9	1.3	24.1
Chlorophyll c (Chl-c, μ g L ⁻¹)				16.5	4.1	40.5

Table 2. Mean, minimum and maximum values of physical and chemical parameters in Lake Sakadaš during the period of July 1972-September 1973 and July 2011-October 2012.

Variations of environmental parameters during the period of 1972-1973 (physical parameters) and 2011-2012 (physical and chemical parameters) are shown in Table 2. and Figures 4.-8. During the period 1972-1973, when Danube water level rose above 5 m and most of the floodplain area was flooded, the water depth of the lake reached 9.9 m. Lower water depth characterized the period of 2011-2012 with a maximum of 7.7 m. Water depth of the lake showed a significant positive correlation with Danube water level in 1972-1973 ($r = 0.78$, $p <$ 0.05) and in 2011-2012 ($r = 0.73$, $p < 0.05$). During the summer months, transparency values were minimal in both periods, while in the winter months transparency reached maximal values. Water temperature was in opposite trending than transparency, which resulted in a significant negative correlation of parameters in both periods (1972-1973 ($r = -0.60$, $p < 0.05$) and in 2011-2012 ($r = -0.78$, $p < 0.05$)). Values of pH were higher than 7 during both periods indicating alkaline conditions. In the period of 1972-1973, pH and water temperature were in a significant positive correlation ($r = 0.64$, $p < 0.05$), while in 2011-2012 they were in a significant negative correlation ($r = -0.54$, $p < 0.05$). Higher mean value of dissolved oxygen was in 2011-2012, while in 1972-1973 values of dissolved oxygen were highly variable with a range from 2.2 to 21.8 mg L^{-1} .

Chlorophyll *a* in 2011-2012 oscillated between 14.3 to 90.8 μ g L⁻¹ and was in a significant negative correlation (*r* = -0.56, *p* < 0.05) with transparency. Maximal values of Chlorophyll *a*, *b* and *c* were measured in October 2011, while minimum values were measured in January and May 2012. Conductivity (354-568 μ S cm⁻¹) was in a significant negative correlation ($r = -0.62$, $p < 0.05$) with the water temperature.

Chemical parameters of Lake Sakadaš in 2011-2012 were highly variable. Highest values of ammonium (454 μ g L⁻¹) and nitrates (3,950.0 μ g L⁻¹) were noted in December 2011 during the highest transparency. Values of ammonium and nitrates showed a significant positive correlation (NH₄ ($r = 0.55$, $p < 0.05$); NO₃ ($r = 0.58$, $p < 0.05$)) with transparency. Concentrations of total nitrogen were higher in 2012 with a maximum of 8,799.8 μ g L⁻¹. Values of total phosphorus had high oscillations (60.7-422 μ g L⁻¹) during the whole period of 2011-2012.

Figure 4. Temporal variations of water depth and transparency in Lake Sakadaš during the periods of July 1972-September 1973 (*dashed line*) and July 2011-October 2012 (*solid line*).

Figure 5. Temporal variation of water temperature in Lake Sakadaš during the periods of July 1972-September 1973 (*dashed line*) and July 2011-October 2012 (*solid line*).

Figure 6. Temporal variation of dissolved oxygen in Lake Sakadaš during the periods of July 1972-September 1973 (*dashed line*) and July 2011-October 2012 (*solid line*).

Figure 7. Temporal variation of pH in Lake Sakadaš during the periods of July 1972-September 1973 (*dashed line*) and July 2011-October 2012 (*solid line*).

Figure 8. Temporal variations of ammonium (A), nitrates (B), nitrites (C), total nitrogen (D), total phosphorus (E), conductivity (F) and Chlorophyll *a* (G) in Lake Sakadaš during the period of July 2011-October 2012.

3.2. Taxonomical and functional diversity of phytoplankton

A total number of 317 phytoplankton taxa were registered during the periods in Lake Sakadaš. Chlorophyta and Bacillariophyceae achieved high species richness (Figure 9.). During the period 2011-2012, Cyanobacteria were represented by 33 taxa and differed from 1972-1973 with 23 taxa. In the period 1972-1973, there were 202 taxa identified with a range of 21 to 88 taxa per sample (Figure 10. (A)) with high oscillations of phytoplankton biomass ranging from 24.3 to 9,075.9 mg l^{-1} in addition to the mean value of 995.0 mg l^{-1} . During the studied period 2011-2012, 190 taxa were identified with a range of 26 to 85 taxa (Figure 10. (B)) and lower values of biomass ranging from 3.1 to 146.2 mg l^{-1} with the mean value of 34.5 mg l^{-1} . Species number of 1972-1973 had a significant positive correlation with water temperature ($r = 0.69$, p) < 0.05) and pH ($r = 0.78$, $p < 0.05$), while biomass significantly correlated with transparency (*r* $= 0.59, p < 0.05$), dissolved oxygen ($r = 0.64, p < 0.05$) and pH ($r = 0.94, p < 0.05$). Number of species in 2011-2012 significantly correlated only with water temperature ($r = 0.60$, $p < 0.05$).

Figure 9. Percentage of phytoplankton taxonomical groups based on the total number of species in Lake Sakadaš during the periods of July 1972-September 1973 (A) and July 2011-October 2012 (B).

Figure 10. Temporal variations in phytoplankton total biomass and number of species in Lake Sakadaš during the periods of July 1972-September 1973 (A) and July 2011-October 2012 (B)

The identified phytoplankton taxa were sorted into 28 functional groups (FG): **A**, **B**, **C**, **D**, **E**, F, G, H1, H2, J, K, L₀, M, MP, N, P, S1, S2, S_N, T, T_B, W1, W2, W_S, X1, X2, X3 and Y. Functional groups and representative species with their maximal contribution to total biomass within chosen periods are shown in Table 3.

The period of 1972-1973 was characterized with 27 FGs of which 16 FGs had relative biomass higher than 5% (Figure 11. (A)). Groups **B**, **D**, **G** and **S2** had the highest contribution to total biomass. Among 25 FGs from 2011-2012, 17 FGs had relative biomass higher than 5% (Figure 11. (B)) with best-developed groups **C**, **D** and **S1**. Comparison of dominant species within FGs in both periods had shown a significant difference in five groups: **B** (*Aulacoseira italica* \rightarrow *Lindavia comta*), **C** (*Asterionela formosa Cyclotela meneghiniana*), **H1** (*Anabaena planctonica Cuspidothrix issatschenkoi*), **S1** (*Limnothrix redekei Planktothrix agardhii*) and \mathbf{S}_N (*Raphidiopsis mediterranea* \rightarrow *Cylindrospermopsis raciborskii*). In 1972-1973 group **D**, **G**, **B** and **S2** had relative biomass higher than 70% of total biomass (Figure 12. (A)). In June 1972 group **D** reached 83.75% of total biomass with the high dominance of centric diatoms *Stephanodisucus hantzschii* and *Cyclostephanos dubius*. Group **G** reached up to 82.98% of total biomass in July 1973 with the dominance of *Eudorina elegans*, *Pleodorina illinoisensis* and *Pandorina morum*. Dominant species *Aulacoseira italica* from group **B** was massively developed in June 1973 with 77.88% of total biomass. Group **S2** with monodominant species *Spirulina* sp. reached up to 70.42% in September 1972.

During the studied period 2011-2012 only three functional groups developed with a contribution to total biomass higher than 40% (Figure 12. (B)). Highest contribution to total biomass was recorded in September 2011 during the massive development of filamentous Cyanobacteria from groups **S1**, **H1** and **SN**, when only the **S1** reached up to 50.39% of total biomass with the dominance of *Planktothrix agardhii* (48.12%). Group **H1** reached maximal contribution to total biomass of this group during the studied period with 23.00% of *Dolichospermum sigmoideum* in August 2012. Filamentous Cyanobacteria functional groups were present during the whole studied period with higher contribution of **S1** group to the total biomass, except in June and July during the summer flood when phytoplankton composition was characterized with group **D**. *Ulnaria ulna* (31.48%) and *Stephanodisucus hantzschii* (4.43%) took dominant position during the whole period in group **D**. Group **C** with *Cyclotella meneghiniana* reached 48.69% of the total biomass in November 2011.

Group **Y** was continuously present during the periods with the highest contribution to total biomass in March 1973 (65.25%) and March 2012 (37.22%).

Besides mentioned FGs, significant difference in species composition within FGs comparing two periods was also found in groups **E**, **F**, **J**, **MP**, **T**, **W2** and **X2**. In May 2012 group **J** had a significant development with 24.08% of total biomass, while in the period 1973-1972, had a contribution to total biomass lower than 10%. Group **W2** was highly contributed with 45.68% of the total biomass in December 1972 with dominant species *Strombomonas annulata*.

Table 3. The maximal contribution of functional groups and species to total biomass in Lake Sakadaš during the period of July 1972-September 1973 and July 2011-October 2012.

Figure 11. Dynamics of dominant functional groups on relative biomass in Lake Sakadaš during the period of July 1972-September 1973 (A) and July 2011-October 2012 (B). FGs with relative biomass higher than 5%.

Figure 12. The maximal contribution to total biomass of best developed functional groups in Lake Sakadaš during the period of July 1972-September 1973 (A) and July 2011-October 2012 (B).

Figure 13. Non-metric multidimensional scaling (nMDS) plot based on biomass of phytoplankton functional groups in Lake Sakadaš during the periods July 1972-September 1973 and July 2011-October 2012. Circles indicate samples from 1972-1973, while triangles indicate samples from 2011-2012. *Solid lines* separate groups, *dashed lines* separate subgroups.

The multivariate nMDS analysis displayed a clear separation of samples into three groups based on the biomass of phytoplankton FGs in the lake during 1972-1973 and 2011-2012 (Figure 13.). Group 2011-2012 encompassed samples from the period of 2011-2012 with the division in three subgroups characterized with Bacillariophyceae, Cryptophyceae and Cyanophyceae species. Lower biomass characterized subgroup with the dominance of **Y**, **D**, **S1** and **X2** groups including samples from December 2011-April 2012 with the highest contribution of group **Y**. The subgroup with the dominance of **Y**, **J**, **D** and **S1** included samples from May-July and September-October 2012, while subgroup with the dominance of **S1**, **H1**, **Y** and **D** groups included samples from July-October 2011 and August 2012.

Group 1972-1973 encompassed subgroup with samples from December 1972-February 1973 and May 1973 with high contribution to total biomass of **D** and **W2** group, and the subgroup with the dominance of **Y** and **D** (September-November 1972 and March-April 1973).

Group 1972-1973 with monodominant FGs represented samples with the dominance of one FG whose contribution was higher than 70% of the total biomass.

Samples from November 2011 and August 1972 had high dominance of **D** group and were not included in any of current subgroups, but they were listed in current groups while they had more similarity with samples from the group than with one another.

3.3. Phytoplankton assemblage index

Factor numbers were determined for each functional group identified in Lake Sakadaš from the period of July 1972-September 1973 and July 2011-October 2012. Determined *F* values were presented in Table 4. with *F* values of similar water body types.

Q index values of the lake in period 1972-1973 varied from 0.17 to 3.10 (Figure 14. (A)). The ecological status of the lake was under the *medium* classification during the most of the period, except in July and September 1973 when the lake was under the *bad* classification, December 1972 and January in *tolerable*, and in May 1973 when it was in *good* condition.

Q index values in the period 2011-2012 varied with season, changing the ecological status of Lake Sakaš from *tolerable* to *good* (Figure 14. (B)). The highest qualification with 3.68 occurred in November 2011, and the lowest evaluation with 1.04 in September 2011 when the cyanobacterial bloom occurred.

Mean values of Q index in chosen periods showed that Lake Sakadaš has *medium* ecological status.

Table 4. Factor number (*F*) of phytoplankton functional group of Lake Sakadaš with comparison to *F* for similar water body types: oxbow outside flood control dams (Hungary)- Padisák et al. (2006); shallow eutrophic reservoir (Brazil)-Crossetti and Bicudo (2008), watersupply reservoir (Brazil)-Becker et al. (2010); small and middle-sized lowland lakes (Bulgaria)- Belkinova et al. (2014).

Figure 14. Dynamics of Q index and ecological status evaluation of Lake Sakadaš during the period of July 1972-September 1973 (A) and July 2011-October 2012 (B).

4. DISCUSSION

A comparison of historical records (1972-1973) with recent data (2011-2012) showed important differences in the abundance and dynamics of the dominant phytoplankton assemblages in the investigated floodplain lake.

As a consequence of strong anthropogenic influence in the past, the lake environment was dramatically deteriorated by the inflow of wastewaters from surrounding agricultural area. Large amounts of suspended matter supported the intensive decomposition process and resulted in the periodical appearance of the hypoxic condition and dramatically increase of pH. After four decades of water protection in the whole floodplain area and applied revitalization measure (sediment removal) in the lake, it seems that the lake has recovered. Investigations done in the past few decades showed that flooding dynamic, still preserved in its near-natural state, is the key ecological factor that influenced phytoplankton dynamics (Mihaljević et al., 2009). Moreover, the results of the effectiveness of the functional approach designed to explain the phytoplankton changes associated with hydrological events showed that the functional classification allows representing of the hydrological phases (Stević et al., 2013). The instrument applied to assess the changes in the ecological status of this floodplain lake, Q index, was applied because unlike other indices it can reflect general anthropogenic pressure (Padisák et al., 2006). Due to the known fact that determination of factor number (*F*) for each FG is the most sensitive step in defining of Q index (Belkinova et al., 2014), a comparison between FGs in impacted and semi-natural conditions allows to allocated lower values to FGs found to be the most expressive in the undesirable conditions.

Given the dataset of phytoplankton functional groups and the main group arrangements in the multivariate nMDS analysis showed variation related to the past and recent condition. Heavy algal bloom (with extremely high biomass) of species assemblages from one FGs or bloom of only one species from particular FGs were the most distinctive feature of the environmental conditions in the past. The bloom of species from the group **G** (*Eudorina*, *Pandorina*, *Pleodorina*), when the relative biomass reached up to 80% of the total biomass, appeared in July 1973 in the highly alkaline conditions (pH 9.7). As was noted by Gucunski (1975), the lake water at that time had an intensively green color and "there was no fish in the lake". During the summer of 1973, Kiss (1977) also recorded intensively algal bloom of *Eudorina elegans* in the Tisza dead-arm in strongly polluted salt and alkaline waters, containing large amounts of decomposing organic matters and qualified those ecological state as "eutrophication with a

dangerous extent". Heavy polluted water has been widely recognized as a suitable environment for blooming of species from the group G. Almost monodominant *Eudorina* blooms were registered in the polluted shallow pond which receives large quantities of sewage from the nearby city (Munawar and Zafar, 1967). Immense bloom of *Pandorina morum* occurred in the Lake Erie (Laurentian Great Lakes), which has a long history of anthropogenic perturbation with excessive nutrient loading (Millie et al., 2009). According to Reynolds et al. (2002), habitat template for group **G** is characterized by nutrient-rich conditions in stagnating water columns, small eutrophic lakes and very stable phases in larger river-fed basins and storage reservoirs. Different weights of factor *F* were used in the qualification of the **G** in order to determine ecological status: value 4 for oxbow lakes outside flood control, value 0 for different types of alkaline lakes in Hungary (Padisák et al., 2006), value 3 for small and middle-size lowland lakes in Bulgaria (Belkinova et al., 2014) and value 1 for the reservoir in China (Wang et al., 2011). In this study, factor *F* with value 0 is used and calculated Q index for July 1973 is 0.17, reflecting the *bad* ecological status of the lake, which was affected by wastewaters and was in strongly alkaline condition.

Another heavy algal bloom occurred thereafter (September 1973), formed by filamentous bluegreen alga *Spirulina* sp. (group **S2**), which was contributed more than 70% to the total biomass, confirmed heavy polluted conditions in the lake. It is known that *Spirulina* bloom occurs widely in polluted lakes, particularly in tropical and sub-tropical lakes with high salinity (El-Bestawy et al., 1996). Warm, shallow and often highly alkaline waters are habitat template for the group **G** (Reynolds et al., 2002) and it is clear that used factor *F* value for the group **S2** is 0. Moreover, current research showed that the group **S2** can be associated with other shade tolerant cyanobacterial species from **S^N** assemblage, forming an **S2**-**S^N** association in shallow polymictic tropical lakes (Gebrehiwot et al., 2017). Accompanied species from **S^N** group during the bloom of *Spirulina* in the Lake Sakadaš was *Raphidiopsis mediterranea*. The occurrence of this subtropical cyanobacterial species was registered for the first time during summer 1973 in all floodplain habitats of Kopački Rit, and also in the main riverbed of the Danube (Gucunski, 1975). The first appearance of *Raphidiopsis mediterranea* in the Lake Balaton was also noted in summer of 1973 (Padisák, 1992). However, after these findings there are no records of *R. mediterranea* in waters of Kopački Rit, in spite that distribution pattern of this species showed its continually spreading throughout various European freshwater ecosystems from the beginning of this century (Kaštovský et al., 2010). It should be emphasized that the morphology of *Raphidiopsis* is highly similar to that of *Cylindrospermopsis*, especially in the nonheterocytous life-cycle stages of *Cylindrospermopsis* species where heterocystes and kinetes are not formed (Li et al., 2001; Moustaka-Gouni et al., 2009; Alster et al., 2010). According to Moustaka-Gouni et al. (2009), *R. mediterranea* can represent non-heterocytous stages of *Cylindrospermopsis* complex life cycle. Thus, overlapping features of *Cylindrospermopsis*/*Raphidiopsis* have led to the difficulty in distinguishing these two genera by the traditional taxonomic approach.

A great expansion of *C. raciborskii* in the temperate waters was noted during the past decades (Padisák, 1997). After its first records in the Danube Delta (Roll, 1961), its mass development was found in various floodplain habitats along the Danube River, from the Lower Danube (Stoyneva, 2003) till the Upper Danube (Dokulil and Mayer, 1996). The first finding of *C. raciborskii* bloom in the Lake Sakadaš was in the extremely dry summer of 2003 when its longlasting over-domination led to the establishment of equilibrium phase (Mihaljević et al., 2009). Afterwards, massive development of *C. raciborskii* has been noted in the summer of 2007 (Mihaljević and Stević, 2011). Given data showed that *C. raciborskii* was accompanied species (5.85 % of the total biomass) during the blooming of cyanobacteria, dominated by species from the **S1** group (46.87% of the total biomass, over-dominated by *Planktothrix agardhii*) and codominated by species from **H1** group (24.38 % of the total biomass). It is known that species belonging to the **S1**, **H1** and **S^N** group are frequently in competition (Padisák et al., 2003). A comprehensive research of cyanobacterial blooming in this floodplain lake showed that lownitrogen **H1** group was particularly sensitive to stress caused by flooding, while filamentous N2-fixing (**SN**) and non-N2-fixing species (**S1**) showed tolerance to short-term flooding (Stević et al., 2013). Thus, in the highly turbulent environmental conditions in the floodplain lake, species of the **H1** group are less successful in forming long-lasted bloom. Factor *F* value 1 is used for the group **H1**, while for the groups **S1**, **S2**, **SN** factor F is 0.

Concerning the appearance of cyanobacterial bloom in the investigated lake it must be emphasized that in the past decades, as a consequences of frequent occurrence of extreme flood events, the lake can be shifted between a state of turbid water, characterized by high phytoplankton biomass and regular appearance of cyanobacteria blooms, to a state of clear water with very low phytoplankton biomass and absence of cyanobacteria, and back to the turbid state (Mihaljević and Stević, 2011). It is evident that the appearance of cyanobacterial bloom has a strong influence on the ecological status of the lake and values of Q index were inversely proportional to the dominance of cyanobacterial groups. Thus, low values of Q index (1.04 and 1.54) during the cyanobacterial bloom in September-October 2011 substantially decreased the final assemblage index value on an annual scale (2.57).

A permanent component of phytoplankton in the lake are diatoms, sharing a certain percentage on a whole-year scale in both observed periods. A previous research (Stević et al., 2013) summarized that flooding of the lake and mixed waters are favorable for the development of diatoms belonging to the **B**, **C**, **D** and **P** functional groups. However, due to the dilution and washout effect, their biomass is low during the long-lasting flooding despite their input from the river. The similar pattern was established in the investigated period 2011-2012. However, comparison with the historical records showed that in particular conditions some diatoms species can be mass developed and form a heavy bloom. The most expressive diatom bloom had occurred in June of 1973, after extremely high flooding and in the hypoxic conditions, when species from the **D** group (*Stephanodiscus hantzschi* and *S. dubius*) contributed up to 83.75% of the total biomass. Similar physical and chemical characterization of the environment, hypoxic and alkaline condition after extremely high flooding in July of 1972, supported a heavy monotypic bloom of centric diatom *Aulacoseira italica* from the group **B**, which contributed up to 78.59% of the total biomass. The high abundance of *Stephanodiscus hantzschii* is considered as indicative of high nutrient levels and is a good indicator of eutrophic conditions in lakes (Tolotti et al., 2010, and cites therein). It is known that many species of *Stephanodiscus* and *Aulacoseira* form a vegetative resting cell in surficial lake sediments and are meroplanktonic, spending a part of their life in hypolimnion or the littoral zone and found in the plankton only during periods when the water column undergoes turbulent mixing (Lashaway and Carrick, 2010). Moreover, Yang et al. (2015) revised the physiological characteristics defined by Reynolds et al. (2002) for the groups **B** and **D** as follows. Habitat template for the group **B** (*Aulacoseira italica*): mixed, mesotrophic, small-medium lakes; adapted to low light; some species could form resting cells, meroplanktonic. Habitat template for the group **D** (*Stephanodiscus hantzschii*): shallow, nutrient-enriched, well-ventilated waters; sensitive to nutrient depletion; some species could form resting cells. Factor *F* value used till now for more or less similar lake types (Padisák et al., 2006; Belkinova et al., 2014; Wang et al., 2011) varied between 1 and 5 for the group **B** and between 2 and 3 for the group **D**. According to the given experience of heavy blooming of species from **B** and **D** in the condition of extremely high anthropogenic pressure and deteriorated waters factor *F* value 2 was used for both groups.

The appearance of diatom-dominated functional groups **C**, **P**, **T^B** and **A**, groups with maximal *F* factor value 5, is commonly associated with the conditions of flooding phase and clear water with very low phytoplankton biomass (Mihaljević and Stević, 2011; Stević et al., 2013), as confirmed by the obtained results. Species from the group **T^B** (*Navicula* spp*.*, *Cymbella*, *Melosira varians*) characteristic for highly lotic environments (Padisák et al., 2009; Abonyi et al., 2012) as well as from the group **A** (small centric diatoms) characteristic for clear, often mixed lakes with low P half-saturation (Reynolds et al., 2002) occasionally occurred with very small contribution (less than 5%) to the total biomass. Likewise, species from the group **P** (*Aulacoseira granulata*) which is characteristic for more eutrophic waters (Yang et al., 2016). It should be noted that the dependence of species from the group **P** upon physical mixing is strongly apparent, requiring a continuous or semi-continuous mixed layer of 2-3 m in thickness (Reynolds et al., 2002). Mixing of the water in the floodplain lake is related to the flood pulses, which can support re-suspension of meroplanktonic species, such as *A. granulata*, in the water column. However, flooding usually lasts a short time and is followed with dry phase with stagnant water unfavorable for further development of species from the group **P**. However, small centric diatoms from the group **C** were permanently present in phytoplankton during the period 2011-2012, while their occurrence in the past was insignificant. Their dominance (48.69% of the total biomass) in November 2012 coincided with the decrease of nutrients in the lake water and Q index reached a maximum value of 3.68, which indicated *good* ecological status of the lake in that time.

5. CONCLUSION

A comparison of previous and ongoing assessments of phytoplankton assemblage dynamics has greatly assisted in the evaluation of the ecological state of the floodplain lake using the assemblages Q index, especially because there is no data for the determination of pristine condition. The chosen historical assessment reflects a period of significant anthropogenic influences that resulted in rapid eutrophication, moreover deterioration of water environment. The frequent appearance of heavy bloom of only one species or assemblages characteristic for polluted lakes decreased the value of Q index to *bad* and *tolerable* state. Recent data suggest that water quality improvement and near-natural hydrological condition support algal assemblages characteristic for naturally eutrophic lakes and values of Q index varied between *medium* to *good* ecological status. Currently, the most important threat for this lake is a longlasting bloom of alien invasive cyanobacteria what might lead to shifting towards the *bad* condition. Altogether, by the evaluation of historical records and the application of assemblages Q index, natural changes can be distinguished from anthropogenic changes in the floodplain lake.

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7. APPENDIX

Appendix 1. Biomass of phytoplankton with pertaining functional group in the period July 2011-October 2012.

EUGLENOPHYTA

CRYPTOPHYTA

CHRYSOPHYTA

CHLOROPHYTA

