ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., Suppl. 7, 2014: 153-158

Application of Morpho-Functional Classifications in the Evaluation of Phytoplankton Changes in the Danube River

Melita Mihaljević*, Filip Stević, Dubravka Špoljarić, Tanja Žuna Pfeiffer

Department of Biology, Josip Juraj Strossmayer University of Osijek, Cara Hadrijana 8/A, HR-31000 Osijek, Croatia; E-mails: mmihaljevic@biologija.unios.hr, fstevic@biologija.unios.hr, dspoljaric@biologija.unios.hr, tzuna@biologija.unios.hr

Abstract: The traditional taxonomic approach is today being supplemented by ecological classifications of phytoplankton following the concepts of functional groups (FG), morpho-functional groups (MFG), and morphology-based functional groups (MBFG). In this study, we compared the potential of all three concepts in the evaluations of phytoplankton changes in the Danube River (rkm 1388). Redundancy analysis revealed that a higher percentage of variance is explained by using ecological classifications than by using the taxonomic approach. Diatoms were the most abundant representatives. Applying the MBFG classification, whereas all diatoms were sorted into a single group (G6), only the changes of total diatom abundance can be followed. According to the FG classification, diatoms were distributed into seven groups, among which the dominant codons were C, D, and T_B, while the MFG classification separated diatoms into five groups. Different temperature requirements and sensitivity to flushing were found to be the key driving factors for the successions of dominant species within small centrics, sorted into only one group. The need for phytoplankton species integration according to their capability to cope with the specific river environments still persists and further investigations, focused on the changes of phytoplankton along the Danube River, would aid the improvement of the phytoplankton classification schemes.

Keywords: Phytoplankton, Danube River, ecological classification, diatoms

Introduction

Traditional phytoplankton monitoring, based on phytoplankton biomass and/or chlorophyll-a, cannot reflect species or functional trait level properties. The interest in finding a substitute for the taxonomic approach to understand the phytoplankton dynamics in freshwater ecosystems contributed to the creation of the three most relevant ecological classification concepts: the functional group (FG) classification introduced by Reynolds *et al.* (2002) and updated by Padisák *et al.* (2009), the morpho-functional group (MFG) classification by Salmaso, Padisák (2007), and the morphology-based functional group (MBFG) classification (Kruk *et al.* 2010, Kruk, Segura 2012). In a functional group, ecologically, morphologically or morpho-functionally similar species are assembled together and are expected to represent a more or less well-defined functional trait. All three systems in their original descriptions were developed for lakes, whereas the FG approach has been applied in different lakes worldwide (see Salmaso *et al.* 2012 for review). In our previous studies, we tested the applicability of all three approaches in evaluating changes of phytoplankton in the floodplain waters of the Kopački Rit (Mihaljević *et al.* 2009, 2010, 2013, 2014, Mihaljević, Stević 2011, Stević *et al.* 2013). Beshkova *et al.* (2010) used phytoplankton

---

[*] Corresponding author: mmihaljevic@biologija.unios.hr

taxonomic and functional groups for evaluation of phytoplankton in Bulgarian floodplain habitats (lake and wetlands). However, the proposed concepts are still far from their application in phytoplankton monitoring in river ecosystems. As was summarised by Abonyi *et al.* (2012), the longitudinal succession of phytoplankton in large rivers is redrawn by inflowing tributaries, by natural dead zones or by human modifications on the river bed (dikes, reservoirs, flow modifications, stone disposal). Based on residence time, nutrient availability, and light conditions, maximum phytoplankton production occurs at middle sections of rivers where phytoplankton is mainly dominated by centric diatoms (Abonyi *et al.* 2012, Borics *et al.* 2007, Schmidt 1994).

According to the FG classification, diatoms are distributed into seven groups defined by their morphological, physiological, and ecological adaptations to different types of habitats. The MFG classification separates diatoms into five groups (large or small centrics or pennates and colony-forming large pennates based on easily recognisable traits including shape, size, and cell aggregation. The least sensitive MBFG scheme assigns taxa to groups based on purely morphological features, sorting diatoms as non-flagellated organisms with siliceous exoskeletons to only one group (G6).

In this paper, we compared the application of all three concepts in the phytoplankton monitoring of the Danube River, focusing on diatoms as the most relevant component of potamoplankton worldwide.

Material and Methods

The Danube River in its middle section (rkm 1410-1383) shows lowland river characteristics with a mean annual discharge of 2085 m^3 s^{-1} and mean annual water level of 2.63 m (data source: daily recordings at the gauge station at river 1401.4 km). The average monthly flow of the Danube River is the highest in the first half of the year (in mid-spring) followed by a decrease from June through October and a subsequent increase thereafter. The research was conducted in 2006, 2008, and 2009. The sampling point was located on the main river course (rkm 1388). *In situ* measurements of water temperature (WT), pH, conductivity and dissolved oxygen (DO) were done using the portable instrument WTW Multi 340i. Concentration of nutrients was analysed according to APHA (1992). To assess the qualitative and quantitative composition of phytoplankton, depth-integrated samples (10 L volume) were collected from the entire water column. Phytoplankton species were identified by light microscopic observations, using standard literature for species determination. For the diatom analysis, samples were subsequently treated with H_2O_2 and HCl. Quantitative assessment of species was conducted according to Utermöhl (1958). The counting unit was the individual (single cell, coenobium, filament or colony). For colonial organisms with mucilage, volume calculations were made for entire colonies including mucilage. The abundance of each species is expressed through the number of individuals per litre. For biovolume estimation, individuals were measured and their volumes calculated according to geometrical solids, and converted to biomass. Biomasses were sorted into FGs (Reynolds *et al.* 2002, Padisák *et al.* 2009), MFGs (Salmaso, Padisák 2007, Tolotti *et al.* 2012) and MBFGs (Kruk *et al.* 2010, Kruk, Segura 2012). Detrended correspondence analysis suggested the use of the linear method and redundancy analysis (RDA) was performed with the CANOCO for Windows version 4.5 (Ter Braak, Šmilauer 2002). The RDA analysis was based on field data, on the biomass of the phytoplankton taxa, biomass of functional groups and on the environmental variables. Non-metric multidimensional scaling (nMDS) analysis was performed on the same biomass data using the statistical program PRIMER version 5.0 (Clarke, Warwick 2001).

Results and Discussion

The hydrological regime of the Danube River varied significantly on a yearly scale with the water level ranging from 0.2–8.1 m in 2006, 0.4–4.7 m in 2008 and 0.4-7.3 m in 2009 (Fig. 1). Notable patterns in water properties during the whole research period included: temperature range from 4.2 to 24.7°C; DO permanently above 7 $mg.l^{-1}$; variations in total phosphorus (TP) in the range of 87-402 $\mu g.l^{-1}$ (mean value 193.6 $\mu g.l^{-1}$), and variations in total nitrogen (TN) in the range of 219.2-3581.3 $\mu g.l^{-1}$ (mean value 2013.6 $\mu g.l^{-1}$). Temporal changes of phytoplankton biomass (4.96-9.19 $mg.l^{-1}$ in 2006, 0.31-23.49 $mg.l^{-1}$ in 2008 and 0.30-10.02 $mg.l^{-1}$ in 2009) indicated that higher biomass coincides with low water level in the river (Fig. 1). At these conditions the suspended matter content substantially decreases, the water column

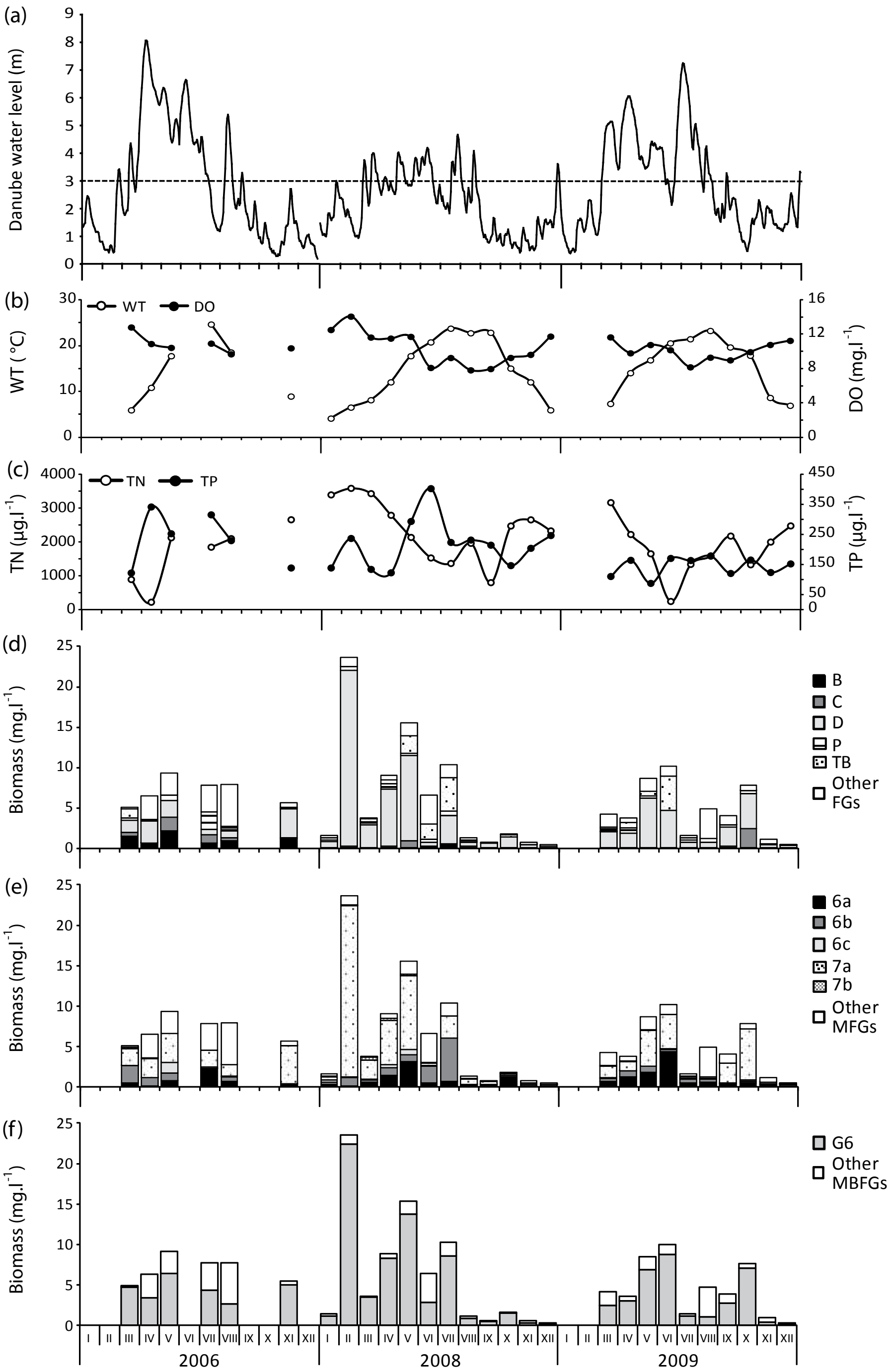

Fig. 1. The annual changes in: (a) the Danube River water level; (b) water temperature (WT) and dissolved oxygen (DO); (c) total nitrogen (TN) and total phosphorus (TP); (d) biomass of phytoplankton functional groups (FGs); (e) biomass of phytoplankton morpho-functional groups (MFGs); and (f) biomass of phytoplankton morphology-based functional groups (MBFGs) in the Danube River. See Legend of Fig. 2. for group representatives

can become transparent almost to the bottom and the phytoplankton density can double in a matter of days (Kiss *et al.* 1996).

Phytoplankton species diversity during the three years of investigation accounted for a total of 183 taxa, among which 43 taxa achieved biomass higher than 5% of total biomass and can be considered as dominants. The taxa were sorted into morpho-functional groups, and a total of 16 MFGs (1b, 1c, 2a, 2d, 3a, 3b, 5a, 5d, 6a, 6b, 6c, 7a, 7b, 9c, 10a, 11a), 15 FGs (B, C, D, G, H1, J, L0, P, S1, T, TB, W1, X1, X3, Y) and 7 MBFGs (G1-G7) were found to be dominant. Redundancy analysis revealed that a higher percentage of variance is explained by us-

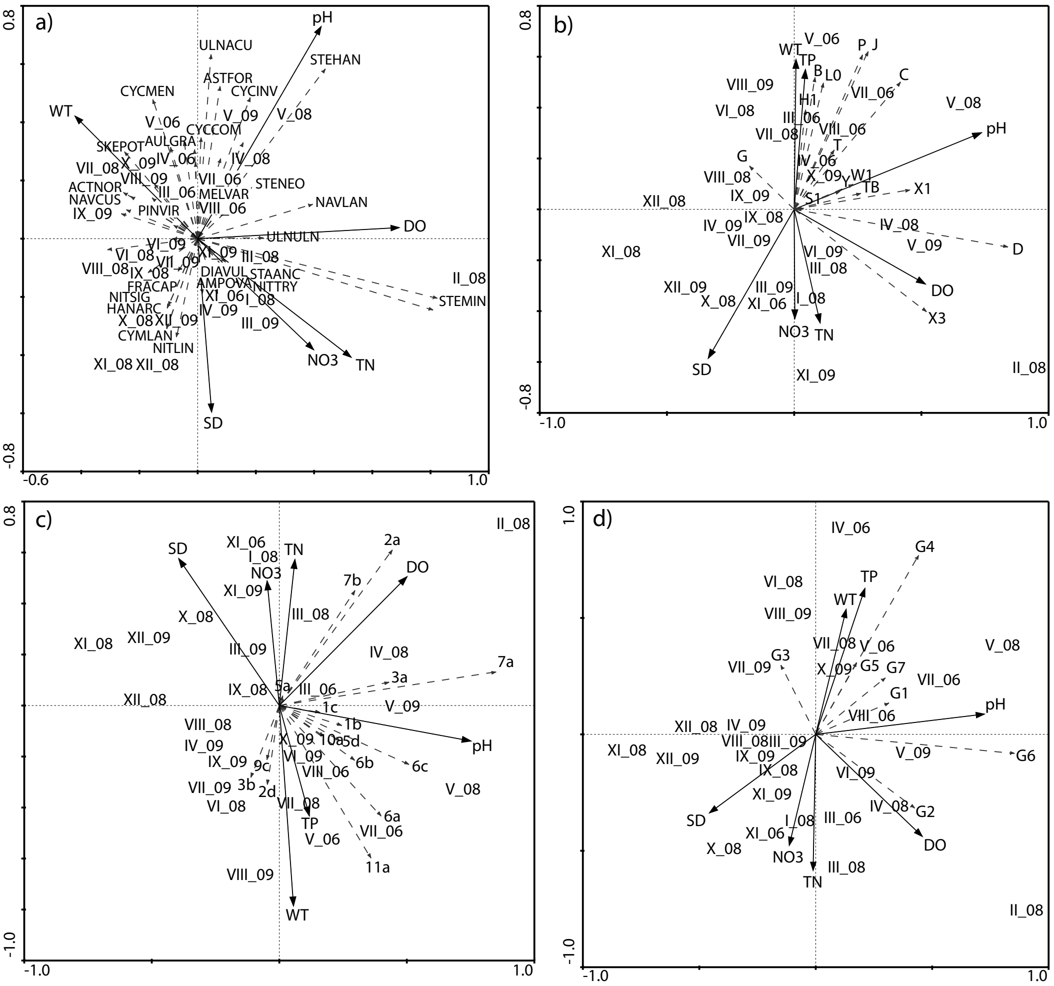

Fig. 2. RDA triplot based on the Danube River phytoplankton: (a) taxa (only diatom codes are shown); (b) FGs; (c) MFGs; and (d) MBFGs biomass. Thick solid arrows indicate environmental variables (water temperature - WT, transparency - SD, dissolved oxygen - DO, nitrates - NO$_3$, total nitrogen - TN, total phosphorus - TP); thick dashed arrows indicate phytoplankton taxa and group vectors; open circles and the corresponding month and year indicate samples. Taxa names (with taxa code and group representatives - FG, MFG, MBFG): *Actinocyclus normanii* (Greg.) Hust. (ACTNOR, D, 7a, G6); *Amphora ovalis* (Kütz.) Kütz. (AMPOVA, $T_{B,}$ 6b, G6); *Asterionella formosa* Hass. (ASTFOR, C, 6c, G6); *Aulacoseira granulata* (Ehrenb.) Simons. (AULGRA, P, 6a, G6); *Cyclotella meneghiniana* Kütz. (CYCMEN, C, 7a, G6); *Cyclotella comta* (Ehrenb.) Kütz. (CYCCOM, B, 7a, G6); *Brebissonia lanceolata* (Ag.) Mah. & Reim. (BRELAN, $T_{B,}$ 6b, G6); *Diatoma vulgaris* Bory (DIAVUL, $T_{B,}$ 6c, G6); *Fragilaria capucina* Desm. (FRACAP, P, 6c, G6); *Ulnaria ulna* (Nitzsch) P. Comp. (ULNULN, D, 6b, G6); *Ulnaria ulna* var. *acus* (Kütz.) Lange-Bert. (ULNACU, D, 6b, G6); *Hannaea arcus* (Ehrenb.) Patr. (HANACU, $T_{B,}$ 6b, G6); *Melosira varians* Ag. (MELVAR, $T_{B,}$ 6a, G6); *Craticula cuspidata* (Küt.) Mann (CRACUS, $T_{B,}$ 6b, G6); *Navicula lanceolata* Ehrenb. (NAVLAN, $T_{B,}$ 7b, G6); *Nitzschia linearis* (Ag.) Smith (NITLIN, D, 6b, G6); *Nitzschia sigma* (Kütz.) Smith (NITSIG, D, 6b, G6); *Nitzschia tryblionella* Hantzsch (NITTRY, D, 6b, G6); *Pinnularia viridis* (Nitzsch) Ehrenb. (PINVIR, $T_{B,}$ 6b, G6); *Skeletonema potamos* (Weber) Hasle (SKEPOT, D, 7a, G6); *Stauroneis anceps* Ehrenb. (STAANC, D, 6b, G6); *Stephanodiscus hantzschii* Grun. (STEHAN, D, 7a, G6); *Cyclostephanos invisitatus* (Hohn & Hell.) Ther., Stoer. & Håk. (CYCINV, D, 7a, G6); *Stephanodiscus minutulus* (Kütz.) Cleve & Möll. (STEMIN, D, 7a, G6); *Stephanodiscus neoastraea* Håk. & Hickel. (STENEO, D, 6a, G6)

ing ecological classifications than by the taxonomic approach. When using species, the first two axes of RDA accounted the lowest value of the variance in the species-environment relationships (57.8%, axis 1: 34.3% and axis 2: 23.5%) (Fig. 2).

The first axis was mainly correlated with water temperature, DO, nitrates and TN, and the second axis was mainly defined by transparency and pH (Fig. 2). On the contrary, when species were grouped into MBFGs, the MBFG-environment relations of RDA axis 1 (75.5%) and RDA axis 2 (12.0%) explained the highest percentage of variance (87.5%). The first axis was mainly correlated with transparency, DO and pH, and the second axis was mainly defined by water temperature, nitrates, TN and TP. The two main axes of RDA explained the similar cumulative percentages of variance when species were grouped into FGs (72.6%, axis 1: 52.4% and axis 2: 20.2%) and MFGs (76.3%, axis 1: 61.3% and axis 2: 15.0%). The first axis was mainly correlated with DO and pH, and the second axis was mainly defined by transparency, water temperature, nitrates, TN and TP. Also, according to the nMDS analysis (data not shown) there was the lower stress using the ecological classifications (from 0.12 to 0.17) while the stress values in nMDS analysis based on species was at the highest permissible limit (0.20).

Among the dominant phytoplankton species, more than half are diatoms (25 taxa) and they accounted for 23.4-96.8% of the total phytoplankton biomass. According to MBFGs concept all diatom taxa are sorted to G6 group (Fig. 1) and applying this concept only the changes of total diatom abundance can be followed.

According to MFGs concept, all of the five MFGs comprising diatoms were found (Fig. 1): large centrics (6a), large unicellular pennates (6b), colony-forming large pennates (6c), small centrics (7a) and small pennates (7b). Small centrics accounted for the largest portion of total phytoplankton biomass during the whole period of investigation, similarly as it was found during the intensive surveys of the Hungarian Danube stretch (Schmidt 1994 and cites therein). However, significant differences in the pattern of key species within this group were established. The massive blooms of *Stephanodiscus* species, as observed during the vernal period, represent the well-pronounced phase of the Danube River phytoplankton development (Verasztó *et al.* 2010). *Stephanodiscus hantzschii* together with *Cyclotella meneghiniana* and *C. comta* were the dominant species during conditions of high water discharge. Another increase in biomass of small centrics was established in the summer-autumn period with the dominance of *Skeletonema potamos*, accompanied by *S. hantzschii*, *C. meneghiniana* and *Actinocyclus normanii*. According to Kiss *et al.* (1994), higher water temperatures and lower water discharge favour the blooms of *S. potamos*. During the whole period of investigation, large centrics, mostly taxa of the genera *Aulacoseira* and *Melosira*, together with large unicellular pennatess, merely *Ulnaria* spp., comprised a large portion of the total biomass, being related to the strong mixing events during high-discharge periods.

Among seven codons in which diatoms were sorted according to the FGs approach, we found B, C, D, P and T_B as the dominant codons in the Danube River (Fig. 1). Most of these groups are characteristic for turbid mixed environments including rivers (Padisák *et al.* 2009). Functional group D (*Stephanodiscus* spp., *Ulnaria* spp. and *S. potamos*) was the most represented throughout the investigated period, oftentimes reaching more than 70% of the total phytoplankton biomass. Small celled and fast growing species which belong to this group were tolerant to the water mixing and low light levels which represent its advantages in highly turbulent conditions. Generally, centric diatoms from the codons C and D can be considered as typical and permanent potamoplankton species in the Danube River, while during high-discharge period tychoplanktonic diatoms (T_B) can become dominant (Stanković *et al.* 2012).

It is obvious that a fine partition of phytoplankton taxa within the morpho-functional classification enables a more satisfying description of the phytoplankton changes in a river ecosystem. However, the need to integrate the phytoplankton species according to their ability to cope with specific river environments still exists and further investigations, focused on the changes of phytoplankton along the Danube River, would undoubtedly improve the phytoplankton classification schemes.

References

Abonyi A., M. Leitão, A. M. Lançon and J. Padisák 2012. Phytoplankton functional groups as indicators of human impacts along the River Loire (France). - *Hydrobiologia*, **698** (1): 233-249.

APHA (American Public Health Association) 1992. Standard methods for the examination of water and wastewater, American Public Health Association, Washington, DC.

Beshkova M., R. Kalchev, L. Pehlivanov and V. Vassilev 2010. Phytoplankton composition and abundance in Srebarna Lake and adjacent temporary wetlands (Bulgarian floodplain of the Lower Danube River). – In: „Large River Basins – Danube meets Elbe; Challenges - Strategies - Solutions", 38th IAD Conference, 22–25 June, Dresden, Germany, 127 p.

Borics G., G. Várbíró, I. Grigorszky, E. Krasznai, S. Szabó and K. T. Kiss 2007. A new evaluation technique of potamoplankton for the assessment of the ecological status of rivers. - *Archiv für Hydrobiologie, Supplement*, **161** (3/4): 465-486.

Clarke K. R., R. M. Warwick 2001. Change in marine communities: An approach to statistical analysis and interpretation (2nd ed.), PRIMER-E, Plymouth.

Kiss K. T., É. Ács and A. Kovács 1994. Ecological observations on *Skeletonema potamos* (Weber) Hasle in the River Danube, near Budapest (1991-92, daily investigations). - *Hydrobiologia,* **289** (1-3): 163-170.

Kiss K. T., A. Schmidt and É. Ács 1996. Sampling strategies for phytoplankton investigations in a large river (River Danube, Hungary). - In: Whitton B. A., E. Rott (eds.): Use of algae for monitoring rivers II. Innsbruck, Institut für Botanik, Universität Innsbruck. 179-185 p.

Kruk C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lürling, C. S. Reynolds and M. Scheffer 2010. A morphological classification capturing functional variation in phytoplankton. - *Freshwater Biology,* **55** (3): 614-627.

Kruk C., A. M. Segura 2012. The habitat template of phytoplankton morphology-based functional groups. - *Hydrobiologia*, **698** (1): 191-202.

Mihaljević M., F. Stević, J. Horvatić and B. Hackenberger Kutuzović 2009. Dual impact of the flood pulses on the phytoplankton assemblages in a Danubian floodplain lake (Kopački Rit Nature Park, Croatia). - *Hydrobiologia*, **617** (1): 77-88.

Mihaljević M., D. Špoljarić, F. Stević, V. Cvijanović and B. Hackenberger Kutuzović 2010. The influence of extreme floods from the River Danube in 2006 on phytoplankton communities in a floodplain lake: Shift to a clear state. - *Limnologica*, **40** (3): 260-268.

Mihaljević M., D. Špoljarić, F. Stević and T. Žuna Pfeiffer 2013. Assessment of flood-induced changes of phytoplankton along a river–floodplain system using the morpho-functional approach. - *Environmental Monitoring and Assessment*, **185** (10): 8601-8619.

Mihaljević M., F. Stević, D. Špoljarić and T. Žuna Pfeiffer 2014. Spatial pattern of phytoplankton based on the morphology-based functional approach along a river-floodplain gradient. - *River Research and Applications*, DOI:10.1002/rra.2739.

Mihaljević M., F. Stević 2011. Cyanobacterial blooms in a temperate river-floodplain ecosystem: the importance of hydrological extremes. - *Aquatic ecology*, **45** (3): 335-349.

Padisák J., L. O. Crossetti and L. Naselli-Flores 2009. Use and misuse in the application of the phytoplankton functional classification: a critical review with updates. - *Hydrobiologia*, **621** (1): 1-19.

Reynolds C. S., V. Huszar, C. Kruk, L. Naselli-Flores and S. Melo 2002. Towards a functional classification of the freshwater phytoplankton. - *Journal of Plankton Research*, **24** (5): 417-428.

Salmaso N., J. Padisák 2007. Morpho-Functional Groups and phytoplankton development in two deep lakes (Lake Garda, Italy and Lake Stechlin, Germany). - *Hydrobiologia*, **578** (1): 97-112.

Salmaso N., L. Naselli-Flores and J. Padisák 2012. Impairing the largest and most productive forest on our planet: how do human activities impact phytoplankton? - *Hydrobiologia*, **698** (1): 375-384.

Schmidt A. 1994. Main characteristics of the phytoplankton of the Southern Hungarian section of the River Danube. - *Hydrobiologia*, **289** (1-3): 97-108.

Stanković I., T. Vlahović, M. Gligora Udovič, G. Várbíró and G. Borics 2012. Phytoplankton functional and morpho-functional approach in large floodplain rivers. - *Hydrobiologia*, **698** (1): 217-231.

Stević F., M. Mihaljević and D. Špoljarić 2013. Changes of phytoplankton functional groups in a floodplain lake associated with hydrological perturbations. - *Hydrobiologia*, **709** (1): 143-158.

Ter Braak C. J. F., P. Šmilauer 2002. CANOCO Reference manual and CanoDraw for Windows, User's Guide: Software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca 500 p.

Tolotti M., H. Thies, U. Nickus and R. Psenner 2012. Temperature modulated effects of nutrients on phytoplankton changes in a mountain lake. - *Hydrobiologia*, **698** (1): 61-75.

Utermöhl H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton - Methodik. - *Mitteilungen der internationale Vereinigung für theoretische und angewandte Limnologie*, **9** (9): 1-38.

Verasztó Cs., K. T. Kiss, Cs. Sipkay, L. Gimesi, Cs. Vadadi-Fülöp, D. Türei and L. Hufnagel 2010. Long-term dynamic patterns and diversity of phytoplankton communities in a large eutrophic river (the case of River Danube, Hungary). - *Applied Ecology and Environmental Research*, **8** (4): 329-349.