# BACTERIOPLANKTON FROM TWO HUNGARIAN DANUBE RIVER WETLANDS (BEDA-KARAPANCSA, DANUBE-DRAVA NATIONAL PARK) AND ITS RELATIONS TO ENVIRONMENTAL VARIABLES

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**KEYWORDS**: bacterioplankton, wetlands, environmental variables, Hungary.

**ABSTRACT**

Seasonal and spatial distribution of bacterioplankton from two Hungarian oxbow lake type wetlands, Mocskos-Danube and Riha, was studied. They were both covered by macrophytes and they had different hydrological connectivity to the Danube. The six sampling campaigns from April to October 2014 included parallel samples from the Danube River at Mohács, Hungary. Bacterial abundance was the highest in spring and in Mocskos-Danube, followed by Mohács and Riha. Positive relationships existed between bacterioplankton and temperature on one hand and suspended solids, pH, $PO_4$-P and chl-a on the other. Negative correlations were with DOC, dissolved oxygen and $NH_4$-N.

**ZUSAMMENFASSUNG**: Das Bakterienplankton von zwei ungarischen Donaufeuchtgebieten (Beda-Karapancsa, Donau-Drau Nationalpark) und ihre Beziehungen zu Umweltfaktoren.

Die jahreszeitiliche und räumliche Verteilung des Bakterienplanktons von zwei ungarischen Altwasser-Feuchtgebieten, Mocskos-Donau und Riha, wurde untersucht. Sie waren mit Makrophyten bedeckt und hatten eine unterschiedliche hydrologische Konnektivität zum Hauptfluss. Die Feuchtgebiete wurden zwischen April und Oktober 2014, mit parallelen Probenahmen vom Donauhauptkanal bei Mohács, Ungarn sechs Mal untersucht Die Bakterienzahl war am höchsten im Frühling und in der Reihenfolge Mocskos-Donau, gefolgt von Mohács und Riha. Positive, statistisch signifikante Beziehungen wurden zwischen Bakterienabundanz und Temperatur, Schwebstoffen, pH, $PO_4$-P und Chlorophyll-a nachgewiesen. Negativ waren die Korrelationen mit der DOC, gelöstem Sauerstoff und $NH_4$-N.

**REZUMAT**: Bacterioplanctonul din două zone ale părţii ungare a fluviului Dunărea (Beda-Karapancsa, Dunăre-Parcul Naţional Drava), și relațiile sale cu variabilele de mediu.

A fost studiată răspândirea sezonieră și spațială a bacterioplanctonului din două zone umede de tip Oxbow din Ungaria, Mocskos-Dunarea și Riha. Ambele zone umede studiate au fost acoperite de macrofite și au diferite legături hidrologice cu Dunărea. Cele șase campanii de prelevare de probe, din aprilie până în octombrie 2014, au inclus eșantioane paralele din fluviul Dunărea la Mohács, Ungaria. Cea mai mare abundenţă a bacteriilor a fost primăvara, apoi la Mocskos-Dunăre, urmată de Mohacs şi Riha. Relaţii pozitive există între bacterioplancton şi temperatură, pe de o parte, substanţe solide în suspensie, pH, $PO_4$-P şi clorofilă, pe de altă parte. Corelații negative au fost cu CCO, oxigenul dizolvat şi $NH_4$-N.

## INTRODUCTION

Bacterioplankton, as a major component of plankton communities, plays an important role in the microbial food web as a food source, in the utilisation of dissolved organic carbon (DOC), in the decomposition of the dead organic matter and in the remineralisation of nutrients in the aquatic ecosystems (Havens, 1998; Cole, 1999; Jürgens and Matz, 2002; Vadstein et al., 2003). Factors controlling its abundance, size and morphology are important for the prognosis of organic matter removal from the aquatic systems or for the self-purification potential (Freese et al., 2007; Kalcheva et al., 2014).

Wetlands play an important role in the conservation of biodiversity, because of their high species richness and habitat diversity (Kalchev et al., 2010; Momeu et al., 2012; Tarjányi and Berczik, 2014). In large rivers, in particular the Middle and Lower Danube, the wetlands play an important role in nutrient cycling (Kalchev et al., 2014).

Different groups of aquatic communities, macrophytes and macroinvertebrates (Tarjányi and Berczik, 2014), zooplankton (Kiss et al., 2015), etc., have been studied in some Hungarian wetlands of Béda-Karapancsa floodplain, the Middle Danube, Danube-Drava National Park, but bacterioplankton development and its relation to environmental factors have not been studied yet, like in some wetlands along the Lower Danube River, in Bulgaria (Naumova and Kalcheva, 2012; Kalcheva et al., 2014).

The aim of the study was to determine the seasonal and spatial dynamics of bacterioplankton total number, biomass and the morphological and size structure in two wetlands of Béda-Karapancsa floodplain, oxbow lakes Mocskos-Danube and Riha, and in the Danube River near Mohács, situated in Danube-Drava National Park, Hungary. Furthermore, using statistical analyses, we tested their relations to the environmental factors.

## MATERIAL AND METHODS

Different sites of two oxbow lake type wetlands, densely covered by macrophytes and with different hydrological connectivity, namely Mocskos-Danube (MDU) and Riha (RIH), first dominated by *Trapa natans* and the second with a *Ceratophyllum demersum* dominance were studied (Fig. 1). The wetlands are located in the protected side of Béda-Karapancsa, a highly valuable and protected (Natura 2000) area, part of the largest active floodplain of the Middle Danube River between rkm 1,447 and 1,440 (Fig. 1). Mocskos-Danube side arm (rkm 1,442-1,440), is approximately 3.4 km long, 60 m wide, with shallow water (average depth: 1.5 m) and very dense macrovegetation. It has a temporary connection with the Danube, the water flowing at 700 cm (gauge of Mohács rkm 1,447) at the upper end (MDU7, 45º58’18.3” N, 18º45’57.1” E) and at 550 cm at the lower end of the oxbow (MDU1, 45º57’24.8” N, 18º46’24.7” E, in details, Kiss et al., 2015). The water of MDU rarely flows (one to five times per year). Riha oxbow (46°00’ N, 18°44’ E) is located on the protected side of the floodplain and it has no connection with the main channel. It is 4.5 km long and 80 m wide. The average water depth is approximately 1-1.5 m. It is a strictly protected nature reserve area covered by dense macrovegetation.

The research was carried out in 2014 during six sampling visits and parallel samples were also taken from the main channel of the river Danube at gauge of Mohács (MOH, rkm 1,447), beyond the harbour of the ferry (45º56’ N, 18º46’ E) (Fig. 1). The sampling dates were established according to the Danube water regime, given the fact that the downstream connectivity of Mocskos-Danube is around 550 cm. A total of 61 water samples were taken in April-May, July-August and September-October.

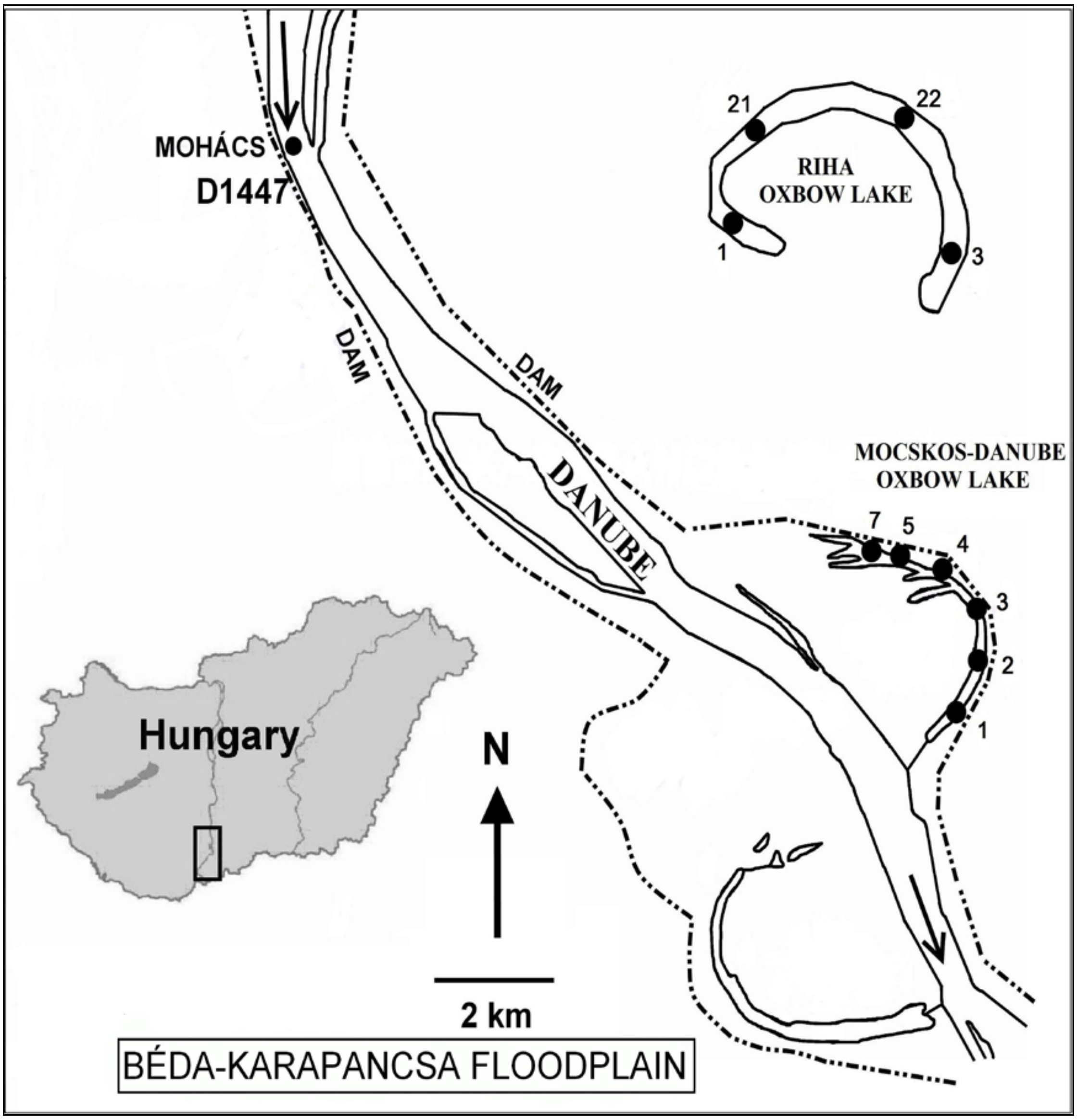


Figure 1: Overview of the study area in Hungary,
Béda-Karapancsa floodplain, Danube-Drava National Park,
and localisation of the sites in Mocskos-Danube (MDU; 1, 2, 3, 4, 5 and 7),
Riha (RIH; 1, 21, 22 and 3) and Mohács (MOH; 1, 2 and 3).

The number of bacteria was determined by the method of a direct count with a phase-contrast microscope (Carl Zeiss, Jena, Germany) by the ocular grid at a magnification of 1,600x after preliminary fixation with 2% formalin and staining with erythrosine (Razumov's method, updated by Naumova in Grudeva et al., 2006), described in detail in Kalcheva et al. (2008). Biomass was calculated in carbon content using Norland's formula (Straškrabová et al., 1999) after determination of the mean cell volume (MCV). Bacterioplankton was counted separately for cells (cocci and rods) freely dispersed on the filter (with 0.2 µm pore size) and for cells that were associated with detritus particles, since morphological groups were provisionally divided in four groups (free cocci and rods and attached cocci and rods). Biovolumes of cells of different morphological groups were determined by the geometric method (Antipchuk, 1983) and Naumova (Grudeva et al., 2006) provided simplified versions of formulas that use the median of the size class (for example the median of the sizes between 0.2 and 0.5 µm was 0.35 µm). The sizes of bacteria, from 0.2 to 4.2 µm, were divided into size classes (Pernthaler et al., 1996; Kalcheva et al., 2008; Chróst et al., 2009). Morphological index (M index = %rods/%cocci) was calculated, which allowed to determine the extent of pollution and the stage of self-purification, because the increase in the quantity and morphological diversity of rod cells, especially with the relatively large sizes was an indication of increased organic content in the water (Pernthaler, 2005). The number of detritus particles with attached bacteria was also counted.

Water samplings for environmental variables included in situ measurements of temperature, pH, dissolved oxygen, oxygen saturation and conductivity by a WTW Multi 403i meter. In the laboratory TOC (total organic carbon), DOC (dissolved organic carbon) and TN (total nitrogen) were determined by a TOC analyzer (Elemetar-liqui-TOC). Standard analytical methods (Golterman et al., 1978) were used to determine suspended solids, $NO_3$-N, $NH_4$-N, $PO_4$-P, TP (total phosphorus), and Chl-a (chlorophyll-a). The macrophyte cover in the wetlands was examined in % (Tarjányi and Berczik, 2014).

Multivariate statistical analysis Redundancy Analysis (RDA) with the program CANOCO for Windows 4.5 (ter Braak and Smilauer, 2002), single factor analysis of variance (one-way ANOVA), nonparametric correlations of Spearman ($R_{Sp}$) and regressions (linear correlations) with the computer program STATISTICA 7.0 (Fowler et al., 1998) were used. Bacterioplankton variables were included in RDA as dependent variables, while environmental factors were included as independent variables. Statistical evaluations were performed using a level of significance p (probability) with 5% risk of error ($\alpha$ or $p \leq 0.05$).

## RESULTS AND DISCUSSION

### Bacterioplankton dynamics

The total number of bacterioplankton ranged from $8.12 \times 10^4$ to $6.93 \times 10^5$ cells.ml$^{-1}$. It was higher in Mocskos-Danube, increasing in sites moving away from the lower inflow of the river, followed by samples from Mohács distinguished by many detritus particles with attached bacteria in the deeper areas (Figs. 2 and 3). The lower values were observed in Riha oxbow where the macrophyte cover was significant. Most probably the reason in RIH also is the lack of connection with the river, which would import additionally nutrients and organic matter necessary for the bacteria. The maximum in abundance was in MDU3, measured only in spring (April and May, MDU1 and MDU2 were without sampling), suggesting that the water from the Danube River inflow, due to spring flooding in 2014, was rich in organics. Kovalova et al. (2010) observed the decrease in bacterioplankton number in wetlands further from the Danube River and in their opinion this was caused by a decrease in the distance from the Danube River and from the Danube discharge influence carrying allochthonous organic matter and nutrients. The decrease of bacteria from MDU7 (upper inflow) to MDU1 (lower inflow) might be explain considering that opinion. Palijan et al. (2007) in Kopački Rit, Croatia, and Kalcheva et al. (2014) in Danube wetlands on Belene Island, Bulgaria, found spring maximum of bacterial abundance during high water level of the Danube River, as observed in this study.

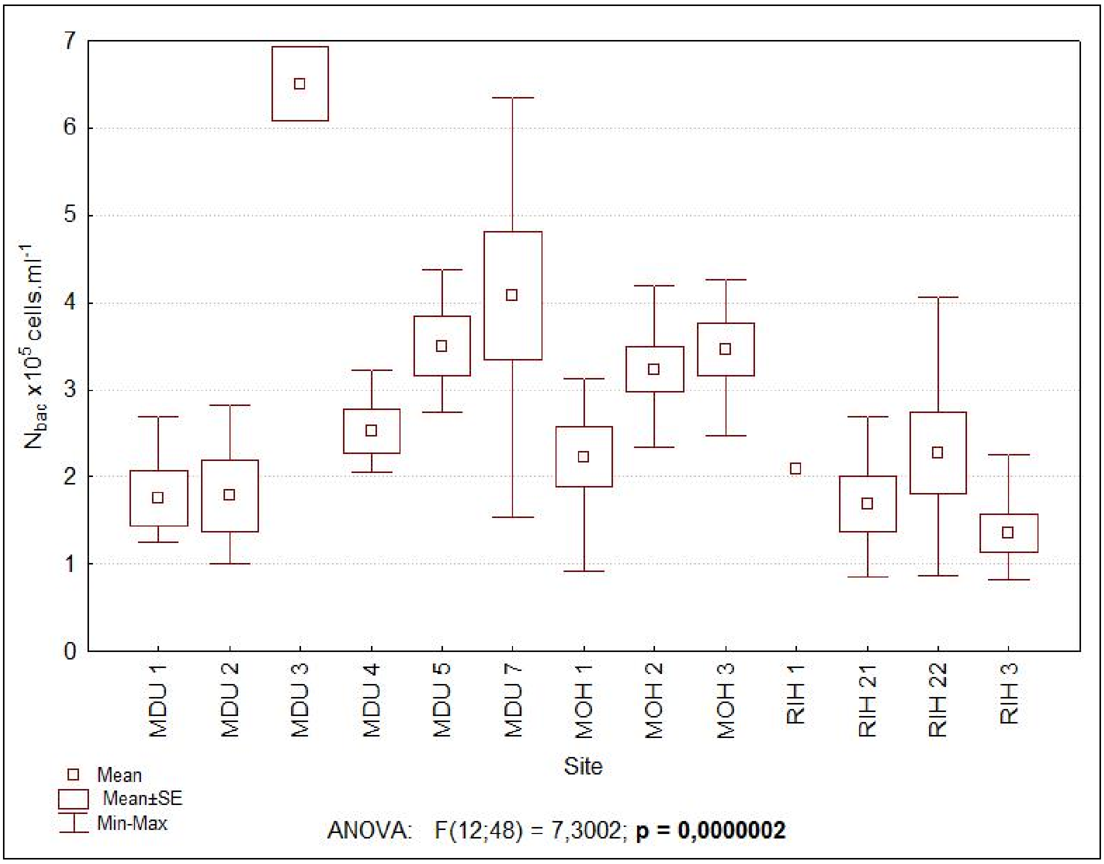


Figure 2: Spatial distribution of bacterioplankton total number (N bac)
in different points of three sites, Mocskos-Danube (MDU, 1, 2, 3, 4, 5 and 7),
Riha (RIH, 1, 21, 22 and 3) and Mohács (1-3),
situated in Béda-Karapancsa floodplain in 2014.

The biomass varied from 0.91 to 10.80 μgC.L$^{-1}$ and its dynamics followed the abundance's dynamics, except the maximum in summer (August), while the mean cell volume (MCV) ranged from 0.033 to 0.08 μm$^3$ (Figs. 3 and 4), which was a relatively small biovolume because of the prevalence of bacteria from the smallest size class, which was the group 0.2-0.5 μm. Domination of bacteria from the smallest size class has been found in freshwater ecosystems with different trophic state (Pernthaler et al., 1996; Šimek et al., 1997; Cole, 1999; Jürgens and Matz, 2002; Pernthaler, 2005; Chróst et al., 2009) and is a normal phenomenon owing to abiotic factors outside of the optimum (temperature, pH, etc.), nutrient limitation (mainly of organic C or inorganic P), increased number of bacterivores or inactive state of the bacterial cells (Kalcheva et al., 2014).

The total number of bacteria (Figs. 2 and 3) and the biomass and MCV (Fig. 3) showed significant differences between the sampling sites ($p < 0.01$, ANOVA), while the biomass and MCV showed significant differences between the seasons ($p < 0.05$, ANOVA, Fig. 4). Morphotypes and cell sizes differed significantly by sites ($p < 0.01$) with prevalence of free-living cocci (36-88%). Only the rod-shaped bacteria differed significantly by seasons ($p = 0.03$, ANOVA) having a minimum in autumn. The average ratio free/attached bacteria in % was 78:22, indicating that a significant part of the bacterioplankton community in the water column is included in detritus particles with attached bacteria from the bottom (sediment) during continuous processes of resuspension and sedimentation of organics which is typical for shallow water bodies. Detritus particles had the highest values in MOH with a level of significance by sites $p = 0.0002$. Morphological index (Mindex) was with significant spatial ($p = 0.00004$) and seasonal differences ($p = 0.047$), higher in MOH3 and MDU7, but less than one, which was an indication of easily degradable organic matter in the water column.

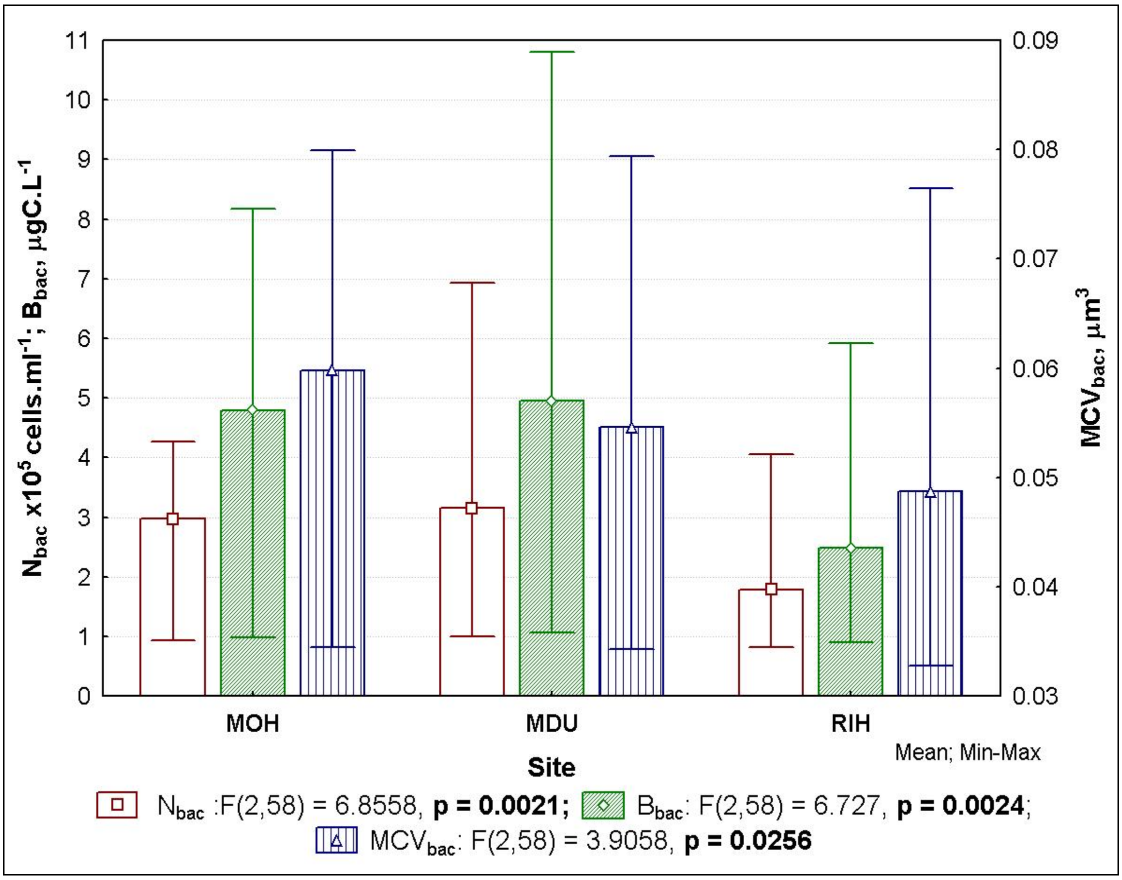


Figure 3: Spatial dynamics of bacterioplankton total number (N bac), biomass (B bac) and mean cell volume (MCV bac) in two wetlands, Mocskos-Danube (MDU) and Riha (RIH) and in the Danube River at the harbour of Mohács (MOH), situated in Béda-Karapancsa floodplain in 2014.

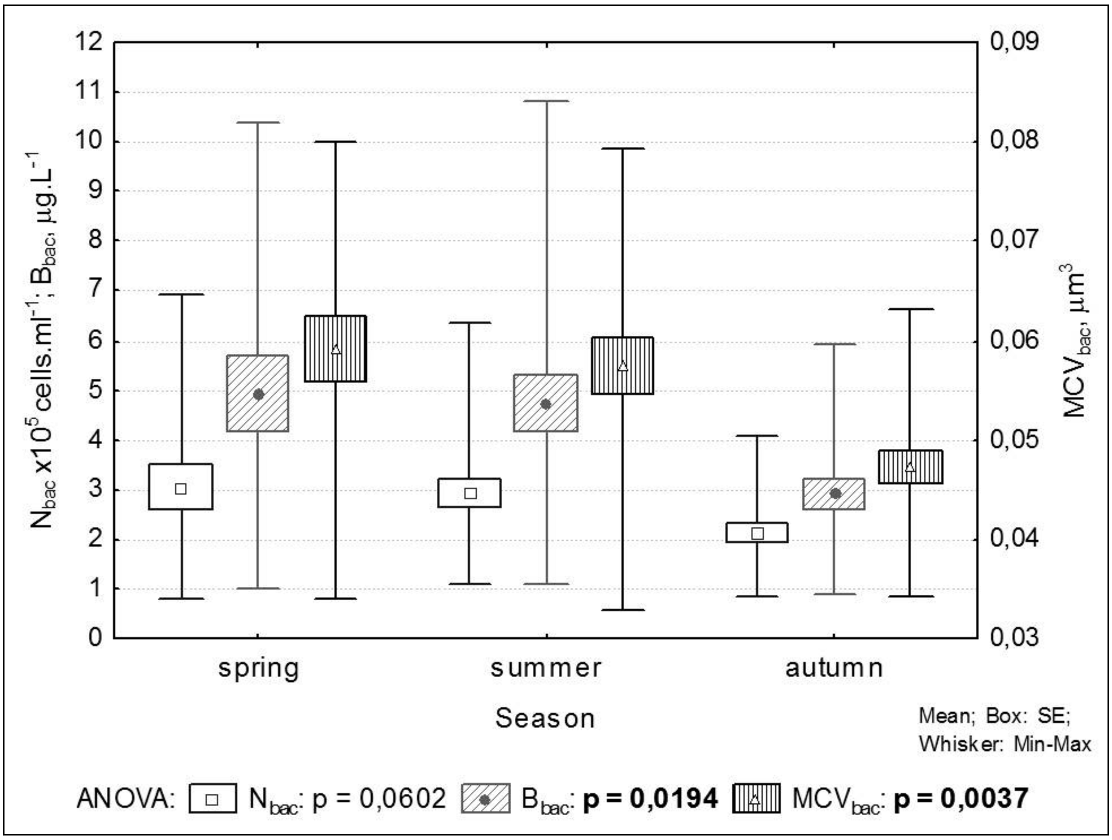


Figure 4: Seasonal dynamics (spring, summer and autumn) of bacterioplankton total number (N bac), biomass (B bac) and mean cell volume (MCV bac) in the wetlands Mocskos-Danube and Riha (Béda-Karapancsa floodplain) and in the Danube River near Mohács in 2014.

**Relations of bacterioplankton to environmental variables**

Direct relationships existed between bacterioplankton (total number and biomass) and temperature, suspended solids, pH, $PO_4$-P and Chl-a ($0.47 < r < 0.80$, $p < 0.5$). The multivariate redundancy analysis (Fig. 5) and Spearman's nonparametric tests also confirmed these correlations. As expected, the DOC decreased with the increase of planktonic bacteria, because of its utilization ($r = -0.49$, $p = 0.009$). Furthermore, the correlations of dissolved oxygen and $NH_4$-N with the attached bacteria and between conductivity and the number and biomass of bacteria were similar, negative. Macrophyte cover in %, the highest in summer in MDU, was related positively to the temperature ($R_{Sp} = 0.48$), negatively to TN ($R_{Sp} = -0.55$) and conductivity, but very weak negatively only to the quantity of the attached bacteria ($r = -0.18$, $p = 0.17$, not significant). *Ceratophyllum demersum* increases the dissolved oxygen and decreases COD and the pollution in the water and can be an effective biosorbent for phosphorus, ammonium and nitrate (Foroughi, 2011). Most probably such processes might have happened in Riha oxbow with dominance of this macrophyte species, reflecting with lower bacterioplankton development.

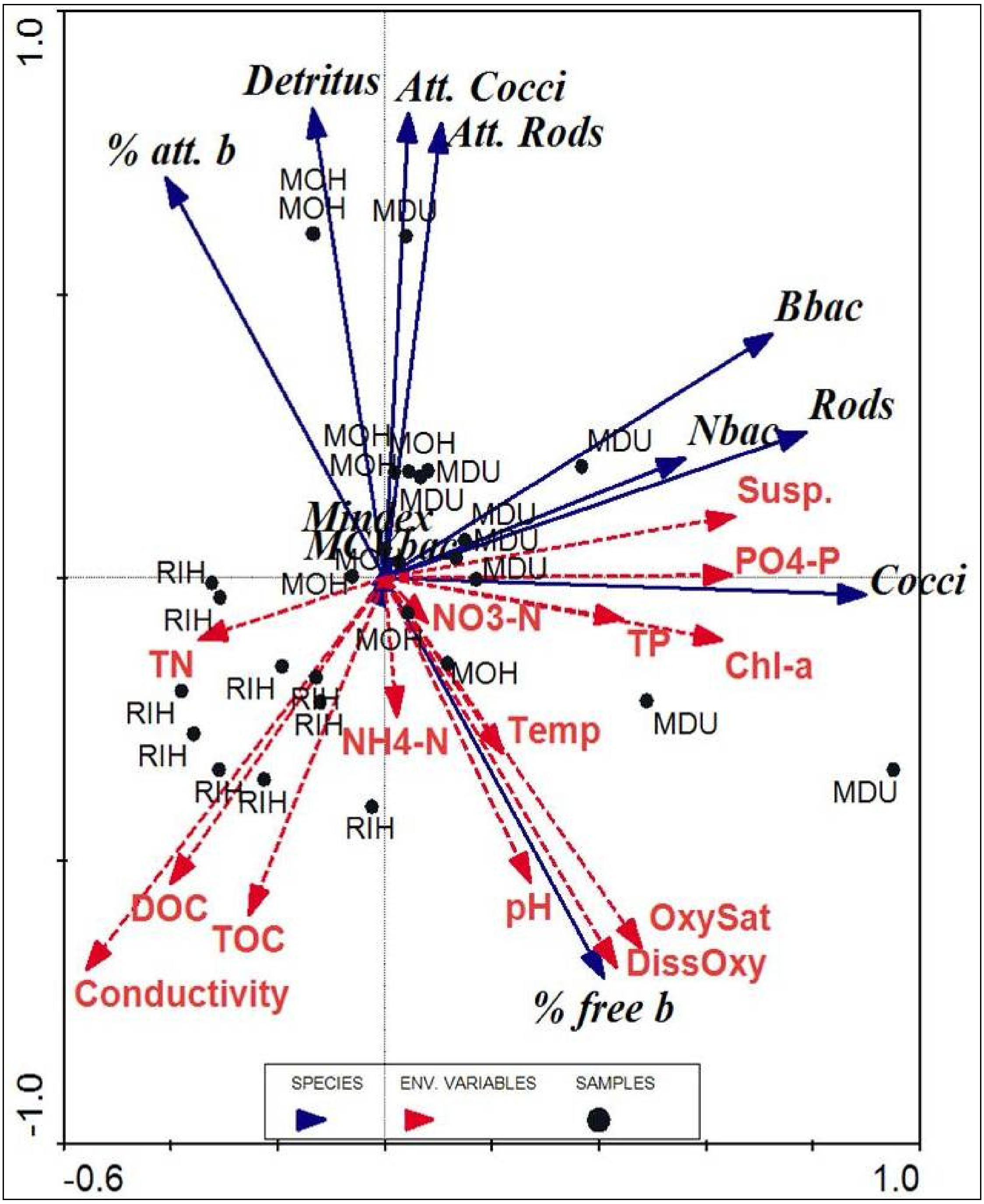


Figure 5: RDA triplot of correlations between bacterioplankton variables (species) and environmental variables (env. variables) by sites (samples) and the Monte Carlo test, given in the text, with p-value = 0.0240. Abbreviations: N − number, B − biomass, b = bac − bacteria, att. − attached, Mindex − morphological index, MCV − mean cell volume, RIH – Riha oxbow, MDU − Mocskos-Danube oxbow lake, MOH − Mohács, Temp − temperature, Oxy − oxygen, Sat − saturation.

The RDA analysis (Fig. 5) showed that 78.9% of all included environmental factors (canonical eigenvalues CanEV) explained the changes in all bacterioplankton variables by sites (MOH, MDU and RIH) and the correlations between them were significant (AllEV = 1.000, CanEV = 0.789, F-ratio = 3.478, p-value = 0.0240) of which 89% were correlations by the axis one. Suspended solids, $PO_4$-P, TP and chl-a, with their maximal values in MDU, correlated positively with the number and biomass of bacteria and with the numbers of free-living cocci and rods. Negative were the correlations with DOC, TOC, conductivity and TN, having the highest values in RIH. The % of free-living bacteria increased where the temperature and oxygen content were high. Attached bacteria and detritus particles, with the highest values in MOH, correlated negatively with the nutrients $NH_4$-N and $NO_3$-N, temperature, oxygen content and other factors. Mindex and MCV had weak variations by sites and the correlations were not clearly visible, but were positive with the nutrients, temperature and dissolved oxygen. P-limitation very often is the reason for weak utilization of DOC by bacterioplankton, because of C:N:P ratio out of the optimum, according to Vadstein et al. (2003).

## CONCLUSIONS

Seasonal dynamics of bacterioplankton in the wetlands Mocskos-Danube and Riha, Béda-Karapancsa floodplain, Danube-Drava National Park, Hungary, demonstrates spring maximum what is also typical for other wetlands along the Middle and Lower Danube River during spring floodings.

The seasonal and spatial development of bacterioplankton and its relation to DOC, $PO_4$-P, TP, $NO_3$-N, $NH_4$-N and chl-a suggest that bacterioplankton actively participates in the decomposition of the dead organic matter and self-purification of water and in remineralisation of nutrients to be used by primary producers (phytoplankton and macrophytes) in the wetlands. Macrophyte dominance of *Ceratophyllum demersum* probably also helps in the removal of nutrients and against the pollution of water.

The lower bacterioplankton number in summer and autumn is probably connected with the increase of zooplankton pressure and competition with macrophytes and phytoplankton for nutrients.

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