

Primary Research Paper

Alternation of factors affecting bacterioplankton abundance in the Danube River floodplain (Kopački Rit, Croatia)

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Abstract

The purpose of this investigation was to determine the influence of different phases of the hydrological cycle on the abundance of colony-forming units (CFU) of bacteria in the Kopački Rit floodplain and to assess temporal and spatial variations in the proportion of eutrophic and oligotrophic bacteria (r- and K-strategists) as a biological indicator of dissolved organic carbon (DOC) availability. Determination of bacterial abundance was performed with abiotic variables (water temperature, depth, Secchi disc transparency, dissolved oxygen, pH, electrical conductivity, ammonia, nitrates + nitrites, total nitrogen and total phosphorus) and one biotic (chl-*a*) variable, and dimensionality reduced with principal component analysis (PCA). The component scores were used as independent variables in a multiple regression and the relationship between scores (indirect variables) and bacterial abundance was examined. An elevated bacterial abundance was observed during the limnophase (floodplain isolated from the river) and potamophase (flood). During the limnophase also the highest chl-*a* concentrations were found and in Sakadaš Lake during September 2003 significantly higher numbers of eutrophic than oligotrophic bacteria emerged ($p = 0.026$). During potamophase the opposite state was established. Elevated bacterial abundance occurred in parallel with minimal chl-*a* concentrations and during July 2004 in the Čonakut Channel a significantly higher number of oligotrophic than eutrophic bacteria emerged ($p < 0.001$). Differentiation in the number of eutrophic and oligotrophic bacteria suggests the existence of a higher concentration of labile DOC during September 2003 than during July 2004. Multiple regression analysis explains 13.6% of the variation in abundance of eutrophic bacteria. The regression model for oligotrophic bacteria is not significant. The results suggest two different mechanisms control bacterioplankton numbers to some extent in the Kopački Rit floodplain. In addition, two sources of DOC differentiating the quality of organic matter predominate under totally different hydrological regimes. Under these conditions, eutrophic bacteria may be partially bottom-up controlled, whereas it is not clear how the abundance of oligotrophs is controlled.

Introduction

Floodplains are exposed to constantly changing water levels. The oscillation is the major environmental factor affecting biota directly or through alteration of abiotic variables (Anesio et al., 1997;

Castillo, 2000; Høberg et al., 2002; Zalocar de Domitrovic, 2003). An alternation of two different water regimes can be distinguished in the floodplain: the floodplain isolation phase, or limnophase, and the flood phase, or potamophase (Neiff, 1996). One of the effects of the flooding

pulse is an acceleration of the decomposition of detritus (Lockaby et al., 1996; Glazebrook and Robertson, 1999; Anderson and Smith, 2002), which is also known for other types of intermittently flooded systems (Maamri et al., 1997). Considering that decomposition processes give rise to remineralisation, flooding has a stimulating effect on nutrient release, especially of dissolved organic carbon (DOC), a driving substrate of heterotrophic bacterioplankton. However, not all DOC is biologically available and ready for bacterial utilization (Chrost, 1986; Jugnia et al., 2000).

A major carbon pathway involves the labile fraction of DOC (Wetzel, 2001), which forms a limiting factor of bacterioplankton growth (Kirchman, 1994). In various studies, bacterial abundance was found to be positively related to the abundance of phytoplankton (Chrost, 1986; Karner et al., 1992; Anesio et al., 1997), whereas in some other investigations such relationship was not noticed (Vörös et al., 1996; Carvalho et al., 2003; Gocke et al., 2004). The latter lack of relationship suggests sources of organic carbon of biological importance for heterotrophic bacterioplankton other than phytoplankton.

The population of heterotrophic bacteria can be separated in two major groups with relation to their ability to uptake organic matter. Species with low nutrient affinity and rapid growth (r-strategists), depending on high carbon concentrations, are called polycarbophylls, copiotrophs or eutrophs, while those with high nutrient affinity and slow growth (K-strategists), that are able to cope with low concentrations of carbon, are called oligocarbophylls or oligotrophs (Morita, 1997). The differences in carbon use by heterotrophic bacteria can be used as a marker of bioavailability of DOC (Margolina, 1989; Hu et al., 1999; Kotsou et al., 2004). Different sources of DOC provide substrates of different susceptibility to heterotrophs, hence a change in the proportion of labile fraction of DOC results in a change in the proportions of eutrophic and oligotrophic bacteria.

Both major sources of organic carbon in aquatic ecosystems—autochthonous and allochthonous—are important for bacteria (Kritzberg et al., 2004). Thus, during the hydrological cycle in which the change of lotic and lentic influence occurs, different sources of organic carbon can be

established. During the limnophase, predominated by lentic influences, one would expect that phytoplanktonic primary production is the major source of organic carbon (Anesio et al., 1997). In contrast, during the potamophase, with predominantly lotic influences, washout from the floodplain may be the major source of carbon (Anesio et al., 1997; Carvalho et al., 2003).

The purpose of the present study was to determine the influence of different phases in the hydrological cycle of the Kopački Rit floodplain on bacterial abundance as well as on temporal and spatial dynamics of the proportions of oligotrophic and eutrophic bacteria as a biological indicator of carbon availability.

Materials and methods

Study area

Kopački Rit is a floodplain of the Danube River located in eastern Croatia (45° 34' latitude, 16° 24' longitude—near the town of Osijek) with a surface area of ca. 100 km² (Fig. 1). Floods enter Kopački Rit through the Vemeljski Dunavac, an old Danube branch (north part), and the Hulovo Channel (south part). The Hulovo Channel is connected with the Čonakut Channel which flows into Sakadaš Lake.

Sampling stations were at some distance from the main Danube flow and located in the Čonakut Channel (7.5 km away) and Sakadaš Lake (9.5 km away). Sakadaš Lake has a surface area of ca. 0.12 km² and a mean depth of 7 m; the sampling station was located at ca. 15 m from the shore opposite to the Čonakut Channel mouth. The Čonakut Channel has a length of ca. 3 km, a width of up to 30 m and a depth between 2 and 5 m; the sampling station was located in the central section at 1.5 km from Sakadaš Lake. Primary production in this part of the floodplain is limited by nitrogen (Horvatić et al., 2003a) and the area is characterised as eutrophic (Vidaković et al., 2002).

The inundated area is tenfold larger during high water periods than during low water periods. The start of flooding has been considered to coincide with +300 cm at the Danube water level gauge at Apatin (Mihaljević et al., 1999). The

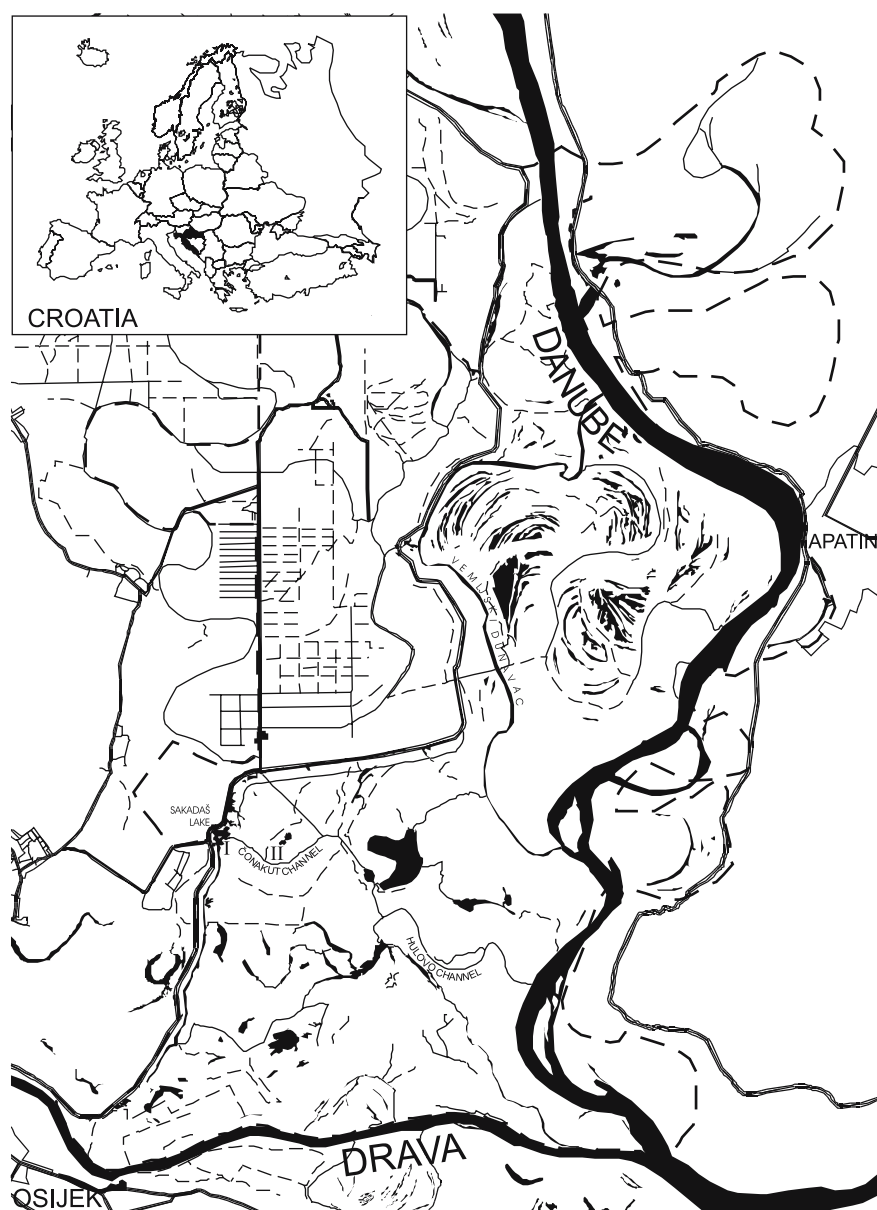


Figure 1. Study area of the Danube River floodplaine Kopački Rit. Whole region (dashed line) is protected as Natural park.

proposed start of flooding is not in agreement with our investigation. We recommend a lower threshold for start of floods about +250 cm at the Danube water level gauge at Apatin, supported by findings of Duroković and Brnić-Levada (1999). The Danube water enters channels which fill the lakes of the floodplain. When the river level reaches +400 cm at Apatin (Fig. 2), water in the

channels and lakes starts to overflow the surrounding lowland area of Kopački Rit, which is covered with forests that mainly consist of white willow (*Salix alba* L.), black poplar (*Populus nigra* L.) and pedunculate oak (*Quercus robur* L.) and with vast stands of emergent macrophytes, mainly common reed (*Phragmites communis* Trin.) and bulrush (*Carex* sp.).

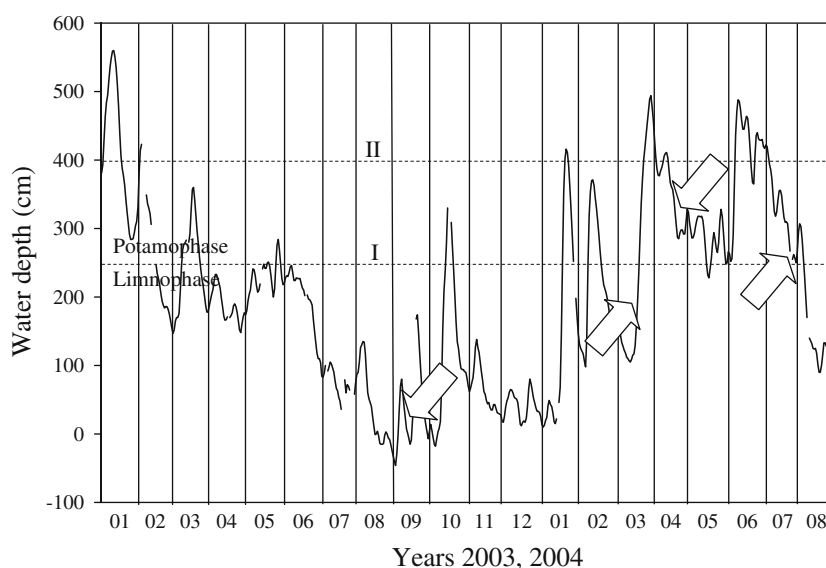


Figure 2. Water level of the Danube at Apatin gauge. First dashed line (I) marks the boundary water level between potamophase and limnophase. The second dashed line (II) represents the water level above which water in channels and lakes of floodplain start to overflow and flood surrounding lowland areas in Kopački rit. Arrows marks samplings in months when the number of bacteria was elevated.

Sampling

Water samples were collected monthly from March 2003 to July 2004 in the Čonakut Channel and Sakadaš Lake. On each sampling station two samples were collected, one from the surface and one from the bottom. Sampling was disrupted from December 2003 to February 2004 when the area was ice-covered. Chemical variables (pH, electrical conductivity, dissolved oxygen, ammonia, nitrates + nitrites, total N and total P) were determined in the laboratory according to APHA (1985) standard methods. Total nitrogen was obtained by addition of concentrations of oxidised forms of inorganic nitrogen (nitrates + nitrites) to the Kjeldahl nitrogen concentration, while total phosphorus was determined after sulfuric-nitric acid digestion.

Physical parameters determined in the field were depth, transparency (Secchi disc depth) and temperature.

Concentrations of chlorophyll *a* were determined spectrophotometrically after filtration through Whatman GF/C filters (APHA, 1985).

Bacterial abundance

Water samples for bacteriological examinations were taken in 200 ml sterilized bottles and

transported to the laboratory in a cooler at sampling temperature. To determine the number of colony-forming units (CFU), heterotrophic plate counts were conducted on two media that differed in their content of organic matter. Eutrophs were cultivated on MPA (nutrient agar) plates poured in triplicate and oligotrophs on 0.1 × MPA (Margolina, 1989; Kotsou et al., 2004), both at 25 °C. Conforming to Kuznetsov and Dubinina (1989) the numbers of CFU were counted after a prolonged period of incubation (MPA: three days; diluted MPA: 3 weeks). The abundance of colonies on plates was expressed as log CFU/ml.

Colonies on diluted nutrient agar were not tested for growth on richer media so it is not certain that they are strict oligotrophs (Morita, 1997), although they are considered as oligotrophs in this research.

Statistical analysis

Prior to statistical processing the data were log-transformed to fulfill the terms of parametric statistical analyses. Dimensionality of physical and chemical data and of chl-*a* concentrations was reduced by principal component analysis (PCA) after which VARIMAX rotation of significant components was performed. As a stopping rule in

the PCA, Parallel Analysis (PA) was used (Franklin et al., 1995; O'Connor, 2000; Peres-Neto et al., 2005). Significant loadings were determined by the Broken-Stick method (Peres-Neto et al., 2003).

Multiple regression was performed to investigate the influence of different phases of the hydrological cycle on bacterial abundance. For the purpose bacterial counts were regressed with rotated scores of the significant principal components for eutrophs and oligotrophs separately. Single variable examinations of abundance of oligotrophic and eutrophic bacteria were carried out by a *t*-test (Sokal and Rohlf, 1995). All results were considered significant if $p < 0.05$.

Results

Physical and chemical data

During the investigation the floodplain was inundated several times and periods of high versus periods with low water levels could be clearly delineated (Fig. 2). The difference between mean high and mean low water level was 1.55 m (Table 1). Mean values of Secchi disc transparency and nitrates + nitrites concentrations at high water levels were significantly higher than at low water levels. Mean values of chl-*a* concentration, ammonia and total nitrogen were significantly higher during low water levels.

PCA was performed to reduce dimensionality of the physico-chemical and chl-*a* concentration data. According to PA the first two principal components are significant, accounting for 52.3% of total variance (Table 2). Significant loadings on the first axis were found for chl-*a* concentration, ammonia and electrical conductivity (all positively correlated), and water depth, transparency and concentration of nitrates + nitrites (all negatively correlated). On the second axis significant loadings were found for water temperature, total nitrogen, total phosphorus, ammonia and chl-*a* (all positively correlated), and dissolved oxygen concentration (negatively correlated). Loadings for pH were not significant (Table 3).

Communality values (Table 3) represent the proportion of each variables' variance accounted for by principal components. Chl-*a* and total

Table 2. Eigenvalues and percents of explanation of variability among measured variables accounted by first five principal components

PC	Eigen values	% of variation	Cumulative % of variation	Eigenvalues from the parallel analysis
1	3.752	34.1	34.1	2.003
2	1.997	18.2	52.3	1.693
3	1.195	10.9	63.1	1.488
4	0.948	8.6	71.8	1.308
5	0.914	8.3	80.1	1.667

Eigenvalues greater than that from the parallel analysis are considered for interpretation (bold values).

Table 1. Mean values, standard deviations and results of two-tailed *t*-test for measured variables during high and low water levels

Variables:	Low water levels		High water levels		<i>t</i>	<i>p</i>
	Mean	SD	Mean	SD		
Dissolved oxygen (mg/l)	8.00	4.14	7.09	3.16	-0.436	0.665
Chlorophyll- <i>a</i> (μ g/l)	42.97	19.25	27.67	17.58	-3.483	0.001
Water temperature ($^{\circ}$ C)	17.66	6.65	15.86	5.14	-0.791	0.432
Secchi disc transparency (m)	0.57	0.20	1.35	0.87	6.279	<0.001
Water depth (m)	2.63	0.89	4.18	1.33	5.184	<0.001
pH	8.04	0.57	7.82	0.34	-1.643	0.106
Electrical conductivity (μ S/cm)	589.69	146.99	564.14	171.12	-0.981	0.331
Ammonia (mg/l)	0.81	0.56	0.32	0.27	-3.759	<0.001
Nitrates + Nitrites (mg/l)	0.82	0.20	1.15	0.39	3.792	<0.001
Total nitrogen (mg/l)	2.92	1.58	1.94	0.97	-2.396	0.020
Total phosphorus (mg/l)	0.47	0.67	0.22	0.23	-1.712	0.093

Table 3. Loadings and communalities of measured variables on first two principal components

Variables:	PC1	PC2	Comm.
Dissolved oxygen	0.307	-0.689	0.570
Chlorophyll- <i>a</i>	0.724	0.426	0.705
Water temperature	0.108	0.709	0.514
Secchi disc transparency	-0.696	-0.249	0.547
Water depth	-0.697	-0.169	0.515
pH	0.407	0.127	0.182
Electrical conductivity	0.674	-0.163	0.482
Ammonia	0.479	0.558	0.541
Nitrates + Nitrites	-0.712	0.105	0.518
Total nitrogen	0.110	0.837	0.713
Total phosphorus	0.272	0.624	0.463

Significant loadings according to the broken-stick method are in bold.

nitrogen variables showed the best representation by significant principal components.

Low water levels are associated with positive loadings on the axis determined by chlorophyll and nitrogen while high water levels are associated with negative loadings (Fig. 3).

Bacterial abundance

The abundance of colony-forming units ranged from 4.1×10^2 to 6.5×10^7 CFU/ml for eutrophs

and 7.6×10^2 to 3.5×10^8 CFU/ml for oligotrophs (Fig. 4). The same figure shows that increased numbers of bacteria were present during September 2003 and March, April and July 2004. In Sakadaš Lake the number of eutrophs was significantly higher than the number of oligotrophs in September 2003 ($p=0.026$) while at Čonakut sampling station the number of CFU of oligotrophs was significantly higher in July 2004 ($p < 0.001$).

Multiple regression analysis, performed separately for eutrophs and oligotrophs (dependent variables) with the significant principal component scores (independent variables), showed that the coefficient of determination (R^2) was 0.136 for eutrophs ($p=0.021$), whereas the regression model for oligotrophs was not significant ($p=0.147$). The results suggest that 13.6% of the variability in eutroph abundance would be associated with variation in the first two axes. Standardized partial regression coefficients (β) were significant (PC1: $\beta = -0.259$, SE = 0.184, $p=0.047$; PC2: $\beta = 0.262$, SE = 0.184, $p=0.045$).

The abundance of eutrophic bacteria was correlated with each of the variables having significant loadings on the first and second principal components, especially those with the highest communalities (Table 3). The correlations were not significant except for dissolved oxygen which was negative.

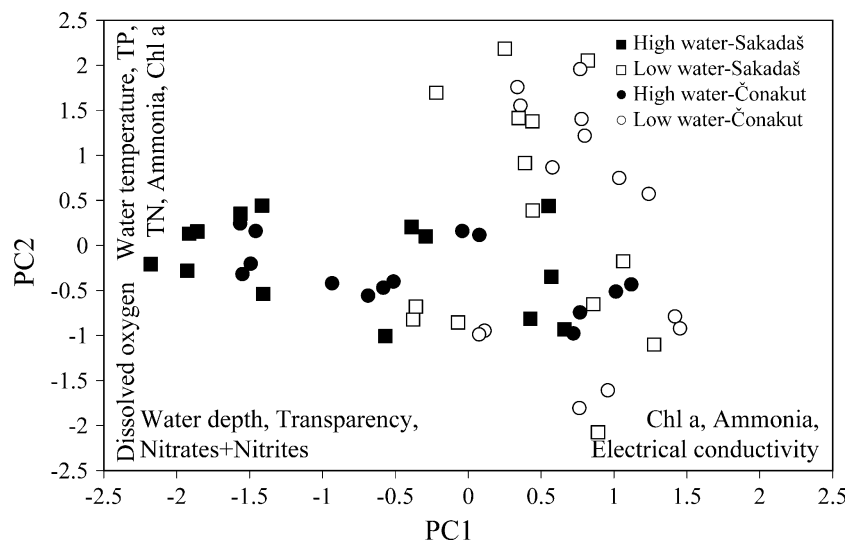


Figure 3. Principal component plot of first two axes after VARIMAX rotation with distribution of sampling stations: circles = Čonakut Channel, squares = Sakadaš Lake; closed symbols = high water level, open symbols = low water level. Variables with significant loadings on principal components are indicated.

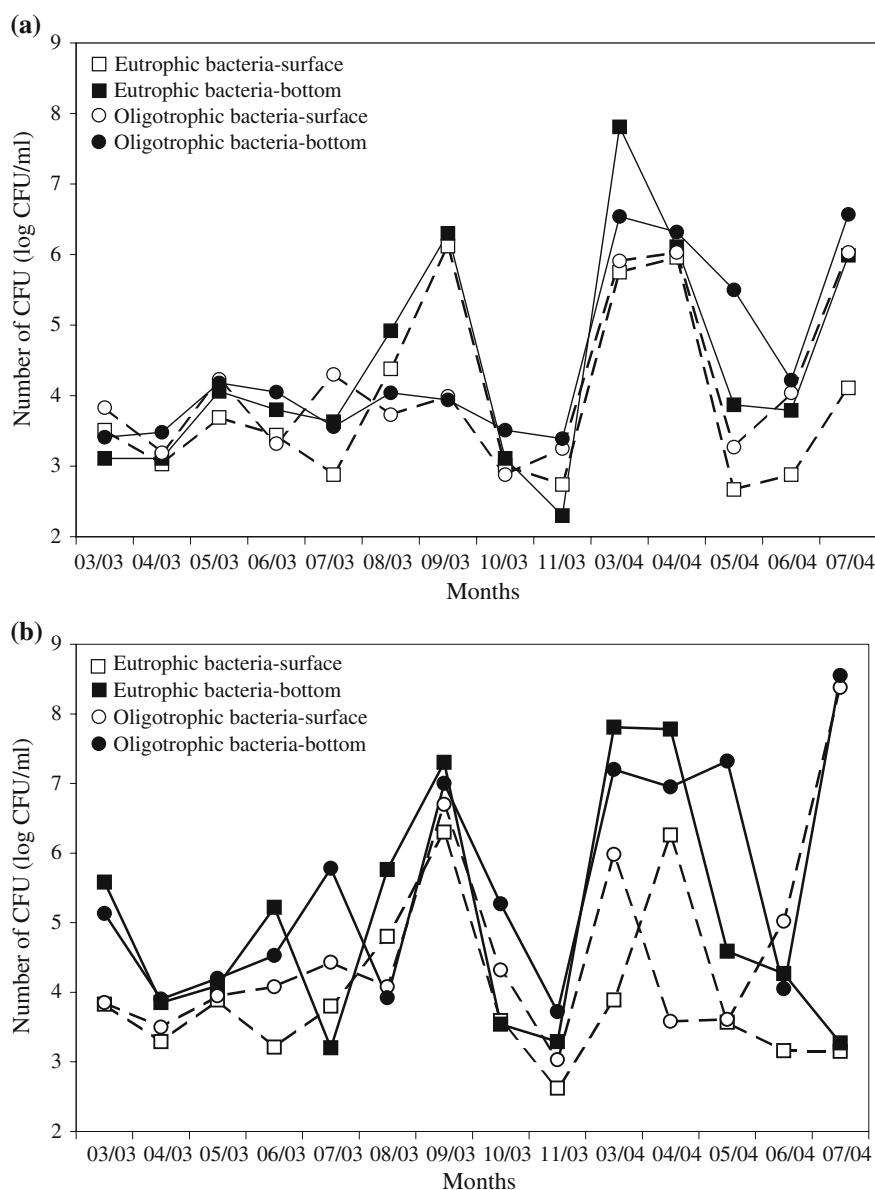


Figure 4. Values of CFU number in Sakadaš Lake (a) and in the Čonakut Channel (b). Eutrophs (□-surface, ■-bottom), oligotrophs (○-surface, ●-bottom). Significant difference between number of eutrophs and oligotrophs emerge during September 2003 in Sakadaš Lake and during July 2004 in the Čonakut Channel.

Discussion

Several studies have demonstrated the importance of hydrologic factors (primarily variations in water levels) on different characteristics of floodplains (e.g. Heiler et al., 1995; Anesio et al., 1997; Spink et al., 1998; Castillo, 2000; Johnston et al., 2001; Høberg et al., 2002; Zalocar de Domitrovic, 2003).

Our investigation focused on the development of bacterial communities in relation to the main hydrological characteristics in Kopački Rit.

During the limnophase a higher bacterial abundance occurred in parallel to increased chl-*a* concentration as commonly observed in many systems (Cole et al., 1988; Anesio et al., 1997). Considering positive loadings on the principal

components explaining differentiation of the water level (Table 3, Fig. 3), it can be concluded that water temperature, electrical conductivity, total phosphorus, ammonia, total nitrogen and chl-*a* concentration have a major influence on bacterial development during low water levels. Communal-ity values indicate that chl-*a* and total nitrogen have the best representation by the significant components. Also, negative value of standardised regression coefficient (β) for the first principal component indicates the negative influence of that axis on the bacterial abundance. Significant loading of water depth directs attention to the dilution effect described by Anesio et al. (1997) by which elevated abundance of eutrophs would be decreased during flood. After the initial burst of eutrophs and oligotrophs during the start of the potamophase ceases (Fig. 4), only oligotrophic bacteria increase their abundance again while eutrophic bacteria stay in low abundance permanently.

The literature suggests that bacterial abundance is primarily limited by the availability of organic substrate while requirements of inorganic nutrients (N and P) are sufficiently met under *in situ* conditions (Kirchman, 1994). In Kopački Rit, primary production has been shown to be N-limited (Horvatić et al., 2003a), a raised ammonia concentration could stimulate the development of phytoplankton. A possible consequence is the increased abundance of bacterioplankton driven by fresh organic matter released from phytoplankton. The positive loading of ammonia on the principal components as well as the negative loading of nitrates + nitrites (Table 3) accentuate the importance of ammonia as a primary nitrogen source for bacterioplankton (Kirchman, 1994) and phytoplankton (Presing et al., 1997) during low water levels. These results give some insight into the abundance of eutrophic bacteria. Hu et al. (1999) found an increase of eutrophic bacteria in amended soil samples immediately after the input of organic carbon, while the number of oligotrophs also increased but only after a decrease of the labile carbon concentration. Such response to carbon amendment is expected, arising from the competition relationship between r-(eutrophic bacteria) and K-(oligotrophic bacteria) strategists (Kuznetsov et al., 1979).

In the present study such differentiation was observed in Sakadaš Lake during September 2003 only, at a time of low water level (Fig. 4a). In preceding months bacterial abundance was low (Fig. 4a) so when nutrients probably became copious (September) eutrophs took advantage and outcompeted oligotrophs in conditions of high nutrient supply. The abundance of oligotrophs, however, was not increased subsequently, probably due to the increased water level during October 2003, and a dilution effect (Anesio et al., 1997). Still, infrequent sampling could also be considered as a reason. Possibly the number of oligotrophs vs. eutrophs would be much more differentiated on a smaller time scale.

Eutrophic and oligotrophic bacteria did not exhibit similar dynamics during potamophase. During July 2004, oligotrophs were significantly more abundant than the eutrophs in the Čonakut Channel (Fig. 4b). A considerably higher number of oligotrophs has been noticed in sediment samples of Lake Sakadaš in an earlier study (Vidaković et al., 2001). Considering that the multiple regression model for oligotrophs was not significant, their density could have been controlled by variables other than those measured. Vörös et al. (1996) explained the uncoupling of bacterial abundance from various biotic and abiotic factors as being the result of a change from bottom-up to top-down bacterial control at different times in the year. An earlier investigation of Lake Sakadaš also showed the absence of a positive relationship between the number of oligotrophic bacteria and water temperature (Vidaković et al., 2001), while the absence of a positive relationship between bacterial abundance and phytoplankton has been established by Vörös et al. (1996), Carvalho et al. (2003) and Gocke et al. (2004).

As Carvalho et al. (2003) hypothesized, changes in water level may increase bacterioplankton abundance indirectly through the input of nutrients and different sources of DOC from the floodplain. In contrast, Anesio et al. (1997) concluded that bacterial abundance may be reduced during high water probably as a result of dilution and the input of less labile DOC from floodplains. In any case, different authors have emphasized that during floods organic matter washed out from the floodplains is the major source of carbon. Our

method of bacterial investigation employed enables biological assessment of changes in the organic matter quality, as indicated by changes in the proportions of oligotrophic and eutrophic bacteria. Thus, an increased number of oligotrophs vs. eutrophs would coincide with a lower availability of carbon at that time.

During high water level an elevated total number of bacteria occurred in April and July 2004. Samples from these months were taken under similar conditions of declining water level after it had reached a maximum (Fig. 2). If the floodplain washout contains a small proportion of labile DOC (mainly recalcitrant organic matter) (Anesio et al., 1997; Carvalho et al., 2003), then it is not clear why during April 2004 in the Čonakut Channel both oligotrophs as eutrophs increased whereas during July only oligotrophs increased. We suggest that during April the allochthonous organic carbon did contain a labile fraction large enough to support the development of eutrophs just as oligotrophs, whereas in July it did not. The main distinction between the two flooding pulses was that the first one occurred after more than one year without flooding (Fig. 2), probably flushing out deposited organic matter of high lability from the lowland. In addition, flooding may cause an acceleration of decomposition rates of detritus (Lockaby et al., 1996; Maamri et al., 1997; Glazebrook & Robertson, 1999; Anderson & Smith, 2002), leaving behind only organic remnants of lower biodegradability. Since bacterial abundance was high in April (Fig. 4), it might have depleted the organic matter released by the washout, leaving a low concentration of the labile DOC fraction. In the same period (April–June) the concentration of chl-*a* was low (data not shown); the low values of bacterial abundance and chl-*a* concentration in June were coupled with the highest Secchi disc transparencies measured during the study. The “clearwater” phase observed in May and June is a common phenomenon reported to occur in Lake Sakadaš in May (Horvatić et al., 2003b). The flooding pulse occurring during the full swing of vegetation season in July may subsequently wash out only organic remnants that remain after the first pulse. The established DOC quality can now only support the development of adapted heterotrophs, K-strategists or oligotrophs. The change of bacterial community struc-

ture due to dependence on the DOC quality and concentration is a known consequence (van Hannen et al., 1999; Findlay et al., 2003).

Also, if we suppose control of oligotrophs by grazing then it must follow that grazing zooplankton were in low abundance during July which may be possible if we assume their removal during the “clearwater” phase. During that time oligotrophs relieved of grazing pressure took advantage and outcompeted eutrophs in conditions of low nutrient supply.

Castillo (2000) and Carvalho et al. (2003) hypothesised that high counts of bacteria in floodplains may be of allochthonous origin, being simply imported during floods and the rainy season. Accordingly, high counts of oligotrophs in July can be explained by bacterial washout from soil where oligotrophs are common microorganisms (Varnam, 2000) and where they increase in abundance after the availability of carbon decreases (Hu et al., 1999).

The two stages of the hydrological cycle, viz. a limnophase with a long period of isolation from the Danube River and a potamophase driving flooding pulses, affect the multiple variables that control bacterial abundance. It can be concluded from multiple regression models that the control is unequally manifested for oligotrophs and eutrophs. Although only 13.6% of the variation within the eutrophs’ abundance was explained by the model, oligotrophs were not explained at all. Hence the results suggest some level of divergence in the controlling mechanisms. Probably the use of more precise techniques of bacteriological examination (i.e. epifluorescence) in combination with heterotrophic plate counts would result in more accurate regression models, as would introduction of other abiotic variables (e.g. DOC concentration). Also, in our study grazers were not taken into account although they probably have an impact on bacterioplankton abundance.

Conclusion

The abundance of cultivable heterotrophic bacterioplankton shows a temporal variation in Lake Sakadaš and the Čonakut Channel with occasionally clear spatial differences. Two sources of organic carbon, autochthonous during limnophase

and allochthonous during potamophase, appear to drive the development of heterotrophic bacterio-plankton. During the limnophase, a significant relative increase of eutrophs was observed in Sakadaš Lake, whereas the opposite was noticed during the potamophase with a significant relative increase of oligotrophs in the Čonakut Channel. The results suggest the existence of separate ways of abundance control for the two bacterial groups. While eutrophs are to some extent bottom-up controlled, the mechanism that controls the abundance of oligotrophs is still unclear. However, neither the possible allochthonous origin of oligotrophs nor the probable grazing pressure on both groups of heterotrophs should be neglected.

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