ABSTRACT: Floodplains are lateral river extensions in which lotic, semi-lotic and lentic habitats are formed resulting in high habitat heterogeneity. Consequently, biota development is highly influenced by its location within the floodplain and by the hydrological cycle. In the present paper, the development of planktonic and biofilm bacteria associated with artificial substrates were investigated in the floodplain lake of the Danube River (Lake Sakadaš, Croatia) during different hydrological situations. The aim of the study was to investigate if there was any difference in the bacterial development between two compartments – plankton and biofilm, and how the floods influence these communities. The samples were taken monthly (July–November 2007) from surface and bottom water layer (plankton) and exposed glass slides (biofilm) at two sampling stations. For these purposes bacterial abundance was estimated by the determination of number of colony forming units (CFUs). The development of bacterioplankton was equal between the sites and had its maximum at the time of falling water after the flood pulse. Bacterioplankton abundance correlated significantly with water properties, and it had predictable dynamics comparable with the previous results established in the same floodplain area (Kopački Rit). The development of biofilm bacteria differed between the sites, and had its maximum prior to the flood pulse, or during the flood. The abundance of attached bacteria correlated with biofilm biomass while it was not significantly correlated with the water properties. Such results describe different development of planktonic and biofilm bacteria. Biofilm bacteria are more independent, compared to bacterioplankton, from the floodplain hydrology.

KEY WORDS: bacterial dynamics, bacterioplankton, attached, flood pulse, Lake Sakadaš, Kopački Rit

1. INTRODUCTION

Floodplains are lateral river extensions in which lotic, semi-lotic and lentic habitats are formed (Ward 1998) resulting in high habitat heterogeneity (Junk et al. 1989). Consequently, these areas support high diversity and production. Beside others bacterial dynamics are often the subject of investigations regarding floodplain functioning (e.g. Hein et al. 1999, Castillo 2000, Carvalho et al. 2003, Besemer et al. 2005, Palijan and Fuks 2006, Luef et al. 2007, Winter et al. 2007, Lemke et al. 2009). Only the planktonic bacteria have been investigated frequently and major factors that determine plankton development in floodplains have been described (Hein et al. 1999). At the same time the investigations of attached bacterial communities (i.e. biofilms) are scarce.
in river-floodplain systems and they became more frequent only recently (Besemer et al. 2005, Luef et al. 2007, Lemke et al. 2009). The attached communities investigated in these papers have been occurring in the water column attached to the seston particles. By definition such communities would be highly influenced by the flood pulses as well as the freely occurring planktonic cells. On the other hand, biofilms developed on submerged objects are to my knowledge uninvestigated in the context of river-floodplain systems and the floodplain functioning at this level of organization is completely unknown.

Biofilms are three-dimensional structures that protrude into the surrounding water enhancing on that way exchange of matter. The formation of the three-dimensional structures is supported by the excretion of the extracellular polysaccharide substance which is a result of the change in the gene expression in comparison to the same bacterial species which live unattached. Costerton (1999) has established the difference in 30 to 40% of bacterial membrane proteins between the biofilm and planktonic bacteria of the same species. Hence, biofilms are functional entities where cells display characteristic adaptations to biofilm way of life (Nadell et al. 2008 and references therein). Hence there exists supportive information that the functioning of the floodplains on the biofilm level of organization would considerably differentiate in relation to the bacterioplankton level of organization, not only because biofilms are immobilized in space but also because genetic expression differs between different counterparts of the same bacterial species. Beside that particles attached communities exhibit higher number of taxonomic units and this community is more heterogenous in time and space compared to free-living bacteria (Besemer et al. 2005). Biofilms grow over any submerged biotic or abiotic substrate, while the attachment stimulates the bacterial activity (van Loosdrecht et al. 1990). New submerged substrates would thus enable further development of biofilms, hence increasing bacterial metabolic activity of the system. This is especially important for shallow ecosystems. Namely, in such ecosystems the development of submerged vegetation is enhanced, while at the same time the contribution of the water column to the development of bacterioplankton is reduced. The activity (secondary production) of biofilm bacteria in such ecosystems has been reported to be three orders of magnitude higher that in the surrounding water (Theil-Nielsen and Sondergaard 1999). Furthermore, it has been estimated that a huge majority of bacteria in the environment prefers formation of biofilms. Costerton et al. (1995) estimated that in aquatic ecosystems less than 0.1% of bacteria live as bacterioplankton. Consequently, the attached bacteria could exert a considerably higher impact on the environment than the planktonic ones.

Previous investigations in the Kopački Rit floodplain described different mechanisms of control of bacterioplankton (Palijan and Fuks 2006, Palijan et al. 2007, 2008). In these investigations copiotrophic bacteria (also called r-strategists which depend on higher concentrations of labile fraction of dissolved organic carbon) have been developed during increased phytoplankton biomass (Palijan and Fuks 2006), or on more isolated locations within the floodplain, such as isolated lake and inner channel (Palijan et al. 2008). On the contrary, oligotrophic bacteria (also called K-strategists which grow at low concentrations of labile carbon) primarily depend on the flood occurrence and have the highest abundance during or immediately after the flood-pulse (Palijan et al. 2007, 2008).

In the present study the development of free-living (planktonic) and attached (biofilm) bacteria was investigated at two sampling stations in a floodplain lake. Because of known differences between planktonic and biofilm bacteria functioning I supposed that the development of culturable biofilm bacteria would have different dynamics compared to planktonic bacteria and that they would exhibit dependence on different environmental variables.

2. STUDY AREA

The investigation was conducted at Lake Sakadaš (Fig. 1), a floodplain lake connected with the Danube River by a system of channels for most of the year. It is quite isolated as it is situated ca. 10 km from the Danube within the Kopački Rit floodplain. It is best
characterized as paleopotamal site (Ward et al. 2002), although it is not reconnected with the river only during flood pulse (as oxbow lakes) but also during flow pulse through the system of floodplain channels. When the lake is disconnected (Lake Sakadaš water level $\leq +80$ cm), the flow pulse occurs when the Danube water level at Apatin gauge reaches $+167$ cm ($\pm 5$ cm). Further rise of the water level results in the development of the flood pulse at approximately $+350$ cm ($\pm 10$ cm) at the same gauge (Lake Sakadaš water level $= +260$ cm) (personal observation). The surface area of the lake is ca. 0.12 km$^2$ with mean depth of 7 m. During summer periods of isolation from the river the lake is thermally stratified. The sampling stations were located ca. 10 m from the steep lake shore, one in the main water body of the lake ca. 100 m from the connecting channel (S2) and the other in the small bay at ca. 320 m from the connecting channel (S1) (Fig. 1). Lake Sakadaš is nitrogen limited throughout the hydrological cycle with higher limitation during flooding period (Peršić et al. 2009).

3. MATERIALS AND METHODS

3.1. Sample collection

The sampling was conducted monthly from July to November 2007 at two stations (S1 and S2) (Fig. 1). The stations were located ca. 10 m from the shore. One water sample was collected from each station ca. 10 cm beneath the surface and one from the contact water layer ca. 40 cm above the lake bottom. Water samples were collected with weighted bottle with plastic plug. To investigate dynamics of CFUs in biofilms microscopic glass slides (37.5 cm$^2$) were used as model artificial substrate. Prior to submergence, slides were washed with detergent and acid. The first sampling of slides was conducted after one month of submergence. Seven slides (subsamples) were collected at each of the sampling stations/dates and transported to the laboratory in separate containers. Three slides were used for the determination of chlorophyll concentrations and another three for determination of biofilm biomass (dry weight, ash weight...
and ash free weight). One slide was used for the bacteriological analyses.

Slides were positioned vertically in the modified plastic slide-boxes which were placed at the fixed depth of 25 cm. As the water level of the floodplain oscillates the slide-holder was constructed to compensate that constant change (Fig. 2). The device consists of two parts: one being fixed with a weight to the lake bottom and tightened up by the lift of the sunken buoy (Fig. 2A). The tighten rope is acting as a solid support and in that way it ensures horizontal positioning of the sampling station. The second part was assembled to the tightened rope with the metallic ring (Fig. 2B). The ring enables the upper portion to freely slide up and down. The upper portion is connected with the surface buoy which is caring the slides in modified slide-boxes. In that way vertical positioning of the sampling station was ensured. The only usage limitation of the slide-holder is that the maximal change in the water level must be lower than the minimal depth of the lake on the place of the sampling station, i.e. it is not suitable for shallow ecosystems. Otherwise the buoy with slides could be pooled below the water surface.

3.2. Environmental variables

Water samples were collected for the laboratory determination of ammonia, nitrites, nitrates, total nitrogen, total phosphorus, total suspended solids (APHA, 1985) and chlorophyll a concentrations (Strickland and Parsons 1968). Concentration of dissolved oxygen, pH, electrical conductivity and water temperature were determined with portable multimeter (Multi 340i/SET) in the field as well as water depth and transparency (Secchi disc depth). Determination of dry weight, ash weight and ash free weight were conducted according to APHA (1999), while periphyton chlorophyll concentration was conducted according to Strickland and Parsons (1968) after the material was scraped off the slides. Autotrophic index (AI) was calculated as a ratio of ash free weight and chlorophyll a content of biofilms to describe their autotrophic/heterotrophic nature.

3.3. Bacterial heterotrophic plate counts

For bacterioplankton investigations water samples were collected in 100 ml sterilized bottles, while slides where collected in the glass bottles with sterile physiological solution. Samples were transported to the laboratory in a cooler and processed within 6 hours after collection. Bacterial abundance in the plankton and biofilm samples was estimated by the determination of colony forming units (CFUs). Abundance of CFUs was determined by cultivation of 1 ml of sample on two agar media with
different carbon content to develop colonies of copiotrophic and oligotrophic bacteria. Copiotrophs were cultivated on meat-pepton agar (MPA) plates poured in triplicate and oligotrophs on MPA:10 (Gorbenko 1961, Margolina 1989). The developed colonies were counted after three days (copiotrophs) and three weeks (oligotrophs) of incubation at 25°C. Biofilm samples were scraped off the slides by the sterile razor blade, resuspended in 100 ml of sterile physiological solution, thoroughly mixed and further processed as the water samples.

3.4. Statistical analyses

Environmental variables except pH were log transformed prior to analysis to fulfill the terms of parametric statistical tests. This was done a priori as reliable testing of normality could not be conducted because of small sample size. Outliers were removed from the data. The removed values were replaced either by the mean value of the rest of the data from the station or by the value calculated from the equation of linear regression line when the correlation between the data and the trend-line was high ($r^2 > 0.96$) (Lepš and Šmilauer 1999). Statistical tests were considered significant if $P < 0.05$ if not otherwise stated, and were conducted with Statistica software v. 7.1.

To test for significant differences between stations in environmental data and abundance of CFUs a t-test was used with month samples as replicates. To investigate relationships between the abundance of CFUs and physical, chemical and biological water properties and biofilm biomass Pearson correlation coefficient was calculated (Zar 1999) with month samples as replicates.

4. RESULTS

4.1. Environmental variables

The established values of environmental variables are represented by Figs 3 and 4.

Fig. 3. Physical, chemical and biological water properties at two sampling stations (S1, S2) from surface and bottom water layer of Lake Sakadás, and water level of the Danube River at Apatin gauge during the period of investigation. Horizontal lines represent flow and flood pulse threshold water levels.
The dynamics of measured water properties were similar between the surface water layer samples and between the bottom water layer samples between sampling stations. Substantial differences existed for bottom water layer samples of total nitrogen and total phosphorus and surface water layer samples of total suspended solids. For these variables considerable differences were established during the start of the investigation (Fig. 3).

Also during the start of the investigation organic matter content of biofilms was higher at S1 while S2 biofilms had higher content of inorganic matter (Fig. 4). Biofilm chlorophyll content was similar between the stations throughout the investigation. Autotrophic index was considerably higher at S1 in the first month of the investigation and throughout the study it was higher at S1 compared to S2. Nevertheless, there was no statistically significant difference in the physical, chemical and biological properties of water between the stations nor between the biomasses of the biofilms (data not shown).

4.2. Hydrological conditions

During the investigation there were six flood events. During July flow and flood pulses occurred the second of which had its maximum 14 days before sampling. The same situation occurred in November when samples were collected 10 days after maximum of the second flood pulse. During August one flow pulse occurred with maximum water level 12 days before sampling. Flood pulse occurred in September and had its maximum 9 days before sampling. Prior to flood pulse in September the floodplain was isolated for 17 days, while prior to the flow pulse in August and November it was isolated 25 and 18 days respectively (Fig. 3). Prior to the investigation relatively low water levels were established in the floodplain with alteration of short isolation periods and flow pulses.

4.3. Bacterial heterotrophic plate counts

Dynamics of CFUs from bacterioplankton samples was similar between the stations, although the dynamics of copiotrophs and oligotrophs within the sampling station was different (Fig. 5). During the time of maximum of oligotrophs, the abundance of copiotrophs was almost the lowest during the investigation.

Dynamics of CFUs from biofilm samples from the two stations was different, while copiotrophs and oligotrophs within the station oscillated equally (Fig. 5). At S1 the highest abundance of CFUs was established in August (during low water level) while at S2 in September (during falling water) (Fig. 5). Also in August abundance of CFUs at S1 was considerably higher than at S2, while during the rest of the investigation the abundance of CFUs in biofilm samples was similar between the stations.
There was no significant difference in the abundance of CFUs of the plankton samples between sampling stations as not between biofilm samples from two sampling stations (data not shown).

Bacteria abundance in CFUs correlated significantly with different water properties and biofilm biomass (Table 1).

### Table 1. Correlation coefficients ($r$) between the abundance (measured in CFU-s) of copiotrophic (CO) and oligotrophic (OL) bacteria from plankton and biofilm samples and environmental variables for sampling stations S1 and S2 in Lake Sakadaš. Bold values are significant at $P = 0.05$ (marked with asterix *) or at $P = 0.052$ (marked with plus (+)). DW – dry weight, AW = ash weight

| Variable | Surface CO | OL | Bottom CO | OL | Biofilm CO | OL | Surface CO | OL | Bottom CO | OL | Biofilm CO | OL | Surface CO | OL | Bottom CO | OL | Biofilm CO | OL | Surface CO | OL | Bottom CO | OL | Biofilm CO | OL |
|----------|------------|----|-----------|----|------------|----|------------|----|-----------|----|------------|----|------------|----|-----------|----|------------|----|-----------|----|------------|----|-----------|----|------------|----|-----------|----|------------|----|-----------|----|
| Depth    | 0.784      | 0.454 | 0.838     | 0.511 | 0.424      | 0.4 | 0.956*     | 0.395 | 0.894*     | 0.386 | 0.776      | 0.725 |           |    |           |    |           |    |           |    |           |    |           |    |
| chl $a$  | -0.676     | -0.572 | -0.831    | -0.697 | -0.407     | -0.426 | -0.975*    | -0.388 | -0.813     | -0.405 | -0.508     | -0.522 |           |    |           |    |           |    |           |    |           |    |           |    |
| NO$_2$+NO$_3$ | **0.933** | 0.459 | **0.921** | 0.284 | -0.228    | -0.28 | 0.845      | 0.283 | **0.875**  | 0.153 | 0.669      | 0.583 |           |    |           |    |           |    |           |    |           |    |           |    |
| NH       | -0.708     | 0.491 | -0.728    | -0.79 | -0.42     | -0.214 | -0.737     | 0.124 | -0.879*    | -0.681 | -0.616     | -0.652 |           |    |           |    |           |    |           |    |           |    |           |    |
| TP       | -0.649     | -0.295 | -0.505    | -0.925* | 0.648     | 0.748  | 0.017      | 0.301 | -0.901*    | -0.645 | -0.611     | -0.654 |           |    |           |    |           |    |           |    |           |    |           |    |
| DW       | –          | –     | –         | –     | 0.851     | **0.944** | –          | –     | –          | –     | 0.754      | 0.773 |           |    |           |    |           |    |           |    |           |    |           |    |
| AW       | –          | –     | –         | –     | 0.787     | 0.882  | –          | –     | –          | –     | –          | –     |           |    |           |    |           |    |           |    |           |    |           |    |

Fig. 5. Dynamics of abundance (measured in CFU-s) of copiotrophic and oligotrophic bacteria from surface water layer, bottom water layer and from biofilm samples from two sampling stations (S1, S2) in Lake Sakadaš.

There was no significant difference in the abundance of CFUs of the plankton samples between sampling stations as not between biofilm samples from two sampling stations (data not shown).

Bacteria abundance in CFUs correlated significantly with different water properties and biofilm biomass (Table 1).

### 5. DISCUSSION

The attached bacteria are considerably different compared to the same species in the free-living form in many aspects (Nadell et al. 2008 and references therein) so it can be expected that they will respond differently to changes in the environment. Also, the development of biofilms dominates the microbial metabolism in ecosystems with high surface-area to water-column ratios (Theil-Nielsen and Søndergaard, 1999), as well as in particle rich environments (Luef et al. 2007). Both, high surface-area to water-column ratio and abundant suspended particles are characteristics of floodplains during the time of flood. During that time aquatic/terrestrial transition zone becomes flooded (Junk et al. 1989, Wantzen et al. 2008), while all submerged objects represent potential colonization surface. As the slope of the terrain in the Kopački Rit floodplain is small, the flooded area i.e. the amount of potential colonization surface suddenly becomes extensively enlarged. Submerged surfaces are rapidly colonized by bacteria, hence the flooding
also increases the metabolically active area in the sense of bacterial metabolism. Taking into account the fact that bacteria attached to submerged surfaces would not be physically translocated as it would be planktonic and particle-attached ones, it can be supposed that biofilm bacteria will function more independently from the flood than would planktonic and/or particle-attached bacteria. Abundance of biofilm bacteria would possibly be influenced by the processes established within the biofilm community perhaps by the organic matter production. The results of this investigation support such an idea as biofilm bacteria reached their maximum prior to flood pulse, while planktonic bacteria had its maximum during falling water after the flood. Moreover, biofilm dynamics were unequal between the stations suggesting more heterogeneous system compared to bacterioplankton, although water physico-chemistry was similar at the two stations. The cause of the difference in the dynamics of CFUs from biofilm samples could be the result of different dynamics of biofilm biomass. Organic content of the biofilm at S1 in July was higher than at S2. Also at S2 a considerably higher content of inorganic matter was established in July, which could negatively affect development of invertebrate fauna (Graham 1988, Quinn et al. 1992) and in that way result in lower amount of organic matter at S2. Consequently, elevated bacterial abundance at S2 was established later than at S1. In other words, maximal abundance of CFUs occurred after the maximum in biofilm organic matter content and following different timing of organic matter maximums at different stations. Also, the abundance of biofilm CFUs correlated with biofilm biomass suggesting a relation with organic matter content of biofilms. Other possible source of organic matter for biofilm bacteria was that of biofilm algae as it is indicated with increased chlorophyll content in September but not with significant correlation relationship between biofilm CFUs and chl a, b or c concentrations. Apart from the perception of biofilm biomass as a source of organic carbon for bacteria, it should be noted that biofilm is a three-dimensional structure so its biomass also represents bacterial habitat. Increased biofilm biomass would have the potential to harbor a higher number of bacterial cells resulting in dynamics of CFUs established in this investigation. Such perception of biofilms could also explain the earlier maximum of CFUs at S1 as organic matter content at that station was increased earlier and already in July this station had more heterotrophic nature as it is indicated by autotrophic index.

Contrary to biofilm samples, development of planktonic bacteria was more similar between stations, as were the water properties. Homogenization of water properties was especially pronounced after the flood pulse in September when thermal stratification disappeared together with other differences in water properties between surface and bottom water layer samples. Hence, horizontal homogenization between water habitats (Thomaz et al. 2007) also occurred vertically in the water column. Such effects were not accomplished by flood pulse in July due to its low intensity, so future work is needed to define the threshold water level of flood pulse which produces vertical homogenization in Lake Sakadaš.

Previous studies showed that the abundance of different groups of heterotrophic bacterioplankton was controlled by different factors in Kopački Rit floodplain (Palijan and Fuks 2006, Palijan et al. 2007, 2008). Copiotrophs are to some degree "bottom-up" controlled, while oligotrophs are not coupled with the established physical and chemical water properties. Their abundance coincides with the hydrological cycle with high bacterial abundance during flood or during falling water level (Palijan et al. 2008). Such pattern was also noted in this investigation. Abundance of CFUs of copiotrophs was significantly related to water properties, and this relationship to the nitrates+nitrites during the high water period was reported earlier (Palijan and Fuks 2006, Palijan et al. 2008). Abundance of CFUs of oligotrophs was related to falling water level after the flood pulse, while there was no significant correlation between oligotrophs and water properties (except with total phosphorus in bottom water layer samples at S1). Oligotrophs did not correlate significantly with the concentration of nitrates+nitrites. The highest abundance of oligotrophs coincided with the low concentration of nitrates+nitrites.
during the falling water in October. During that time nitrate was loaded in the water bodies of the floodplain from the land (data not shown). Possibly, such low concentration of nitrates+nitrites could be explained by high bacterioplankton demands for nitrogen as their abundance during that time was in maximum. Hence, they could deplete the nitrates from the water column. As during the flood nitrates+nitrites are the main source of nitrogen (Palijan and Fuks 2006), such bacterial relatedness to it could explain the development of nitrogen limitation during the flood (Persić et al. 2009), even though the Danube flooding water is considered to be the source of nitrates for the floodplain (Tockner et al. 1999). Similarly as nitrates, organic matter could also have been loaded during falling water level in the floodplain water bodies from the land. Benner et al. (1995) described higher bacterial growth efficiency during high water period in relation to less labile organic matter washed from the floodplain. The development of oligotrophs after the high water period could be explained by the establishment of less labile carbon in the water column washed from the floodplain.

6. CONCLUSIONS

The development of culturable bacteria was different between planktonic and biofilm samples. Moreover, bacterioplankton dynamics was highly similar between stations while development of biofilm bacteria was different between stations. Also, planktonic bacteria had predictable dynamics comparable with previous results established in the Kopacki Rit floodplain with maximum abundance after the flood pulse. Biofilm bacteria development was independent of the hydrology as its maxima occurred prior to the flood pulse or in parallel with it. The results suggest that the development of biofilm bacteria was connected with the biofilm biomass, i.e. it followed the dynamics of biofilm organic matter content. Such results show a higher degree of autonomy of biofilm regulating mechanisms compared to bacterioplankton from the floodplain hydrology. Nevertheless, it should be noted that the abundance of CFUs from biofilms ceased after the flood pulse possibly because of its negative impact on the biofilm organic matter content.

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7. REFERENCES


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