

## Microbiology – Part II

### Variation pattern of ecological bacterial parameters in the Danube River: Are tributaries a determining factor?

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#### Introduction

River networks fundamentally differ from most other ecosystems because (i) they are open systems with tight functional linkages to their adjacent ecosystems and (ii) they are nested systems with their physical and ecological structure and function changing over several spatial and temporal scales. Their hierarchical organization and their tight link to adjacent terrestrial and subterranean ecosystems have stimulated the development of concepts such as the River Continuum Concept (RCC) and more recent concepts on river-floodplain functioning (TOCKNER ET AL. 2000, THORP ET AL. 2006). While traditional perceptions during the last 25 years focus on the question whether the longitudinal continuum (VANNOTE ET AL., 1980) or the lateral connectivity driven by the flood pulse (JUNK 1989, BAYLEY 1991) control the organic matter supply, recent concepts discuss the importance of physical discontinuities (POOLE 2002, BENDA ET AL. 2004) and local processes in rivers (THORP & DELONG 2002) as substantial contribution to understand habitat structure and carbon cycling in lotic ecosystems. Since the integration of the microbial loop concept in aquatic ecosystems (POMEROY, 1974) it is recognized that a major part of the organic carbon from primary production is channelled through the bacterial compartment, usually quantified as rates of bacterial production and related estimations of required bacterial carbon demand. Only a small percentage of this energy is known to leave the microbial loop to fuel the macro-food chain and while this trend was often confirmed for lentic systems, we notice a lack of studies confirming or rejecting this observation for lotic systems in general and especially for large rivers. In this context it should be emphasized that the effect of the bacterial load of small rivers merging into larger ones was only sporadically investigated although such information is of primordial importance for the understanding of the functioning of large rivers (Winter et al, 2007).

Large rivers, as the river Danube, are known to experience the impact of an important number of tributaries, but while there is little doubt that these tributaries may influence the physico-chemical development of the main river, the magnitude of this influence is still a matter of debate. The Danube river basin is characterized by the tribute of some 40 rivers merging into the main stream on its way to the Black Sea and reveals a great number of side arms and oxbow lakes, thus making of the Danube river basin a unique aquatic system in Europe.

Within the frame of the JDS 2007 we had the opportunity to subsample a representative number of stations between Kelheim and the Danube delta and to determine basic microbial parameters (bacterial numbers, biomass, morphotypes and bacterial secondary production) which were so far largely missing in biological records concerning the Danube River. We attempted to address a major issue with this investigation: To provide new and additional data to test and/or reinforce the validity of the river continuum concept by investigating whether continuous, linear changes are found for the recorded bacterial parameters or whether

discontinuities and local processes control the bacterial performance. For this purpose special attention was given to bacterial input data from tributaries.

### Aims and Goals

Bacteriological data were collected during the Joint Danube Survey 2 (2007) along the longitudinal stretch of the River Danube from the upper section (km 2600) to the Delta (km 0) for the following goals:

- Monitor the above mentioned microbial-ecological parameters to obtain an overview of the microbial-ecological status for the Danube River and its tributaries.
- Analyse the obtained data in order to detect variation patterns in the Danube River, necessary for a better understanding of the carbon cycling in the river.
- Compare bacterial parameters from the tributaries with those from the Danube River to assess the potential impact of the tributaries on the bacterial community in the main stream.
- Investigate whether detected patterns of the bacterial parameter values confirm the idea of the river continuum concept.
- Evaluate the obtained parameter values for their applicability within the established EU Water Framework Directive (2000) as an additional tool to assess the ecological status of large rivers

### Methods

**Collection of water samples:** Water samples were collected in the middle of the river from the ship in sterile 1 L Schott-flasks with a sampling rod at a water depth of approx. 20 to 30 cm. Samples were immediately processed on-board. The water sample was divided into two fractions by filtration through 3 µm pore-size aluminium oxide filters (Whatman Anodisc).

**Bacterial numbers:** Bacterial numbers were estimated according to the acridine-orange direct count method (HOBBIE ET AL. 1977), modified after KIRSCHNER & VELIMIROV (1997). 10 ml of water sample (total and 3 µm filtrate) were fixed with 0.5 ml 37% formaldehyde and stored at 4°C in the dark. Bacterial cells were counted within 25 days and grouped in four morphotype classes (rods, cocci, vibrios and filaments) using an epifluorescence microscope.

**Biometry:** Bacteria were sized by an eyepiece micrometer. Cell volume estimations were derived from the assumption that bacteria have spherical or cylindrical shape with two hemispherical caps. At least 100 bacteria per sample were measured. Cellular carbon content expressed in fg C cell<sup>-1</sup> was calculated from estimated cell volumes (V; µm<sup>3</sup>) assuming the allometric relation  $C = 120V^{0.72}$  after NORLAND [1993].

**Leucine incorporation - bacterial secondary production:** Bacterial production was determined for both untreated and 3µm filtered samples (4 samples, 2 blanks). <sup>3</sup>H-Leucine was used as tracer for incorporation into the bacterial protein pool, following the protocol of EILER ET AL. (2003) and using the conversion factor of SIMON & AZAM (1989). Additionally, 8 saturation experiments along the Danube stretch were conducted in order to verify that the administered radioisotope concentration was adequate to saturate the uptake by the respective bacterial population.

**DOC analysis:** Dissolved organic carbon (DOC) analysis was made with Phoenix 8000 TOC Analyser (JVC, Wiener Neustadt, Austria). Samples were frozen on board of the ship at -20°C after filtration through 0.2µm filters and kept frozen until processing.

**Statistical evaluations:** All derived correlations were made by Pearson's *r*-test, where compared variables were quantitatively measurable. Parameters not fitting normal distribution were log10 transformed before correlation (\*\*: highly significant)

**Additional parameters:** All additional parameters, namely chlorophyll a, O<sub>2</sub>, NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub>, PO<sub>4</sub>, total phosphorus, temperature, total suspended solids, inorganic and organic suspended solids were obtained from the ICPDR data sheets.

## Results and interpretation

### Bacterial parameter variations for JDS2 sampling points

The majority of the bacteria within the samples from both the longitudinal Danube sections and the tributaries were found in the fraction below 3  $\mu\text{m}$ , which implies that the majority of the cells occur as free living cells. Table 1 shows that in tributaries some 67% of the bacteria are free cells, while in the Danube River this percentage is with 71% slightly higher. A similar trend is noticed for the bacterial biomass. Bacterial production of the attached fraction, however, is only slightly below the values of the planktonic fraction.

**Table 1:** Mean percentage of free versus attached bacteria in the Danube River and its tributaries plus side arms (SA).  $n = 21$  for tributaries and side arms,  $n = 75$  for the Danube river.

	BN		BBM		BSP	
	% planktonic	% attached	% planktonic	% attached	% planktonic	% attached
<b>All samples</b>	70.1	29.9	62.5	37.5	55.8	44.2
<b>Tributaries+SA.</b>	67.2	32.8	58.8	41.2	50.9	49.1
<b>Danube river</b>	71.0	29.0	63.5	36.5	57.2	42.8

The comparison of the mean biomass/production ratios over all section types of free (1.19) and attached (0.88) bacteria indicated that attached bacteria have relatively higher secondary production per unit biomass compared to the free living fraction. BN and BBM values for attached bacteria correlated significantly with total suspended solids (TSS) and particulate inorganic matter (PIM). All three bacterial parameters of the attached fraction correlated significantly with particulate organic matter (POM) (BN  $r=0.32^{**}$ , BBM  $r=0.49^{**}$  and BSP  $r=0.33^{**}$ ), which was not observed for free living bacteria.

In Fig. 1 it can be seen that most of the peaks for bacterial numbers, biomass and secondary production within the longitudinal Danube transect are derived from tributaries or side arms of the river Danube. Bacterial numbers below  $2.0 \times 10^9$  cells  $\text{L}^{-1}$  and biomasses below  $110 \mu\text{g C L}^{-1}$  are characteristic for Danube River stretches, while tributaries display abundances and biomasses ranging generally between  $2.0 \times 10^9$  cells  $\text{L}^{-1}$  and  $7.30 \times 10^{10}$  cells  $\text{L}^{-1}$  and  $150 \mu\text{g C L}^{-1}$  to  $6140 \mu\text{g C L}^{-1}$ , respectively. However, there are exceptions like the river Inn, which import amounts to  $1.27 \times 10^9$  cells  $\text{L}^{-1}$ , corresponding to some  $83 \mu\text{g C L}^{-1}$ . Low bacterial imports are also recorded for the Rackeve-Soroksar arm 2, the rivers Hron, Drava, Tisza, and Sava, where abundances ranged from  $1.26 \times 10^9$  cells  $\text{L}^{-1}$  to  $1.80 \times 10^9$  cells  $\text{L}^{-1}$ , and biomasses from 33 to  $102 \mu\text{g C L}^{-1}$ , respectively.

Correspondingly, high values are noticed for bacterial secondary production of tributaries with high biomasses and vice versa. The maximum bacterial production value of  $14.80 \mu\text{g C L}^{-1} \text{h}^{-1}$  was recorded for Arges River which had also the highest bacterial abundance and biomass of all tributaries. For all other tributaries with high abundances and biomasses we recorded production values between 1.5 and  $5.44 \mu\text{g C L}^{-1} \text{h}^{-1}$ . It is noteworthy that the tributary Hron which had both a low bacterial abundance and biomass manifested a relative production peak of  $2.40 \mu\text{g C L}^{-1} \text{h}^{-1}$  while all other tributaries with low abundance and biomass values gave production values between 0.37 and  $0.80 \mu\text{g C L}^{-1} \text{h}^{-1}$ . Among the tributaries with production values below  $1.0 \mu\text{g C L}^{-1} \text{h}^{-1}$  are also the rivers Timok, Olt, Jantra and Siret despite abundance and biomass values above  $2.80 \times 10^6$  cells  $\text{L}^{-1}$  and  $110 \mu\text{g C L}^{-1}$ .

BN, BBM and BSP were highly correlated, despite the heterogeneity along the river (Pearson correlation BN/BBM:  $r=0.96^{**}$ , BN/BSP:  $r=0.59^{**}$ , BSP/BBM:  $r=0.63^{**}$ ). Although the overall trend indicates high bacterial abundances, biomasses and production values for the

majority of the tributaries but low values for the samples from the Danube River, it was surprising to notice that the water body of the main river remained largely unaffected by the tributaries input. This becomes even more apparent when calculating the specific bacterial biomass input for each tributary by considering the respective discharge rates of the tributaries and when comparing the calculated load of the tributary with the biomass load of the following down stream Danube sample station. This was true for tributaries with both large and small volume discharges (see below).

### **Bacterial parameter variations in the main stream**

A different pattern of parameter variations becomes obvious when studying the values from Danube sampling points alone. Observing the values of Danube samples from the Inn downstream, we can see that bacterial numbers (Fig. 2) are constantly increasing. In contrast, the mean cell volume becomes smaller as the river approaches its mouth (Fig. 3). As a consequence bacterial biomass reveals only a slight increase over the longitudinal transect (Fig. 4). No such continuous trend was observed for bacterial production rates, and it was attempted to group them according to similar magnitude of rates, shown below in the chapter “Magnitude regions of BSP”. Allocation of BSP values to the different section types gave no recognizable patterns or trends.

### **Assessment of bacteriological parameters for the sections of the Danube and tributaries**

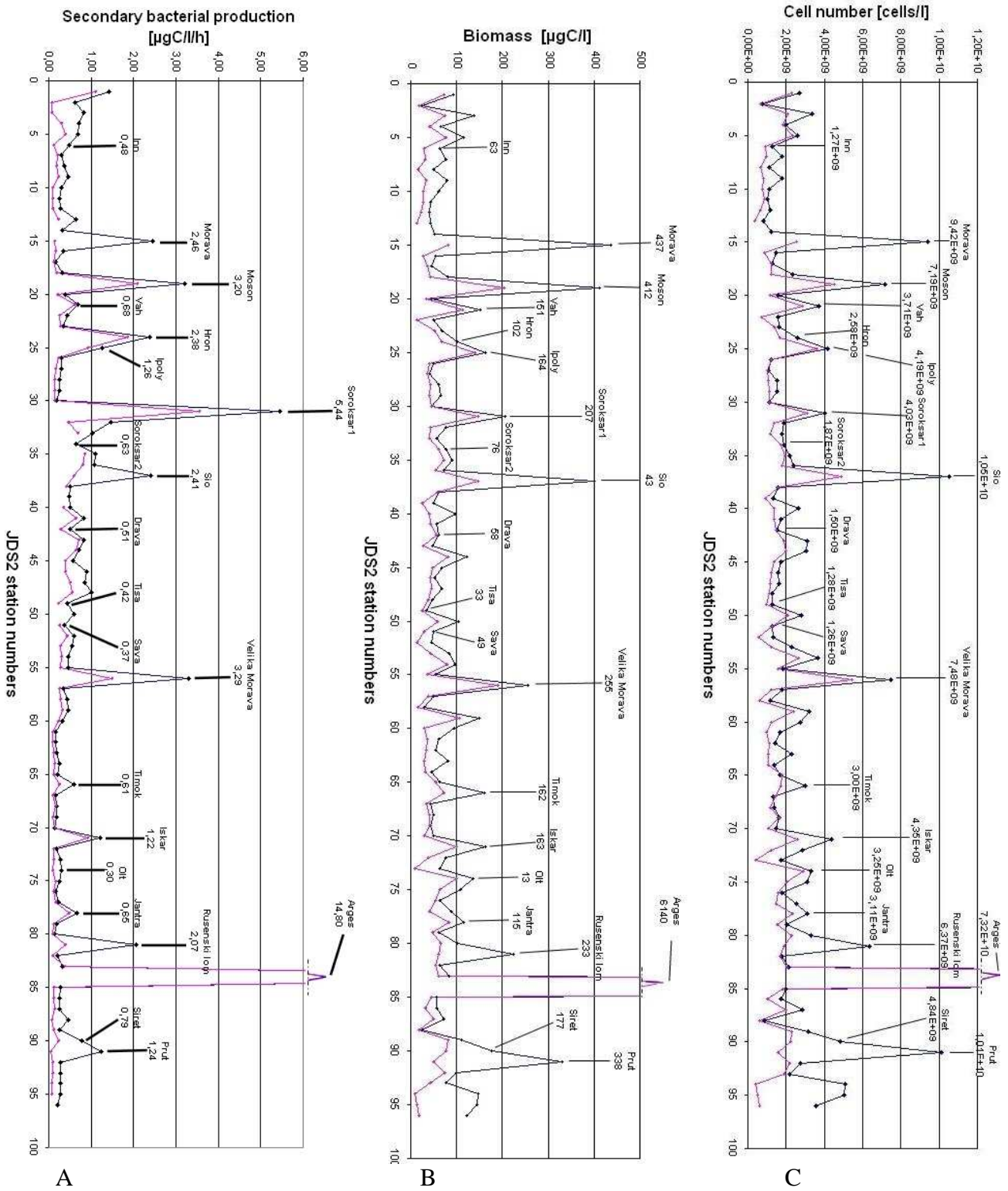
There are 10 defined geomorphological section types along the Danube River. Over those 10 sections, abundances vary from  $1.40$  to  $3.90 \times 10^9$  cells  $L^{-1}$ , biomasses from  $52$  to  $124 \mu g C L^{-1}$  and BSP from  $0.20$  to  $1.40 \mu g C L^{-1} h^{-1}$ . Yet it should be noted that minimum biomasses do not correspond to minimum secondary productions or abundances, indicating a distinct heterogeneity of bacterial parameter between sections.

**Section type 1 (Upper course of the Danube, rkm 2786: – rkm 2581):** Average bacterial number ( $2.70 \times 10^9$  cells  $L^{-1}$ ) and biomass values ( $92.6 \mu g C L^{-1}$ ) are high and secondary production is the highest recorded of all sections ( $1.41 \mu g C L^{-1} h^{-1}$ ). Since only one sample has been made over a stretch of 200 km within this section, it cannot be decided whether the monitored values are really representative for the bacterial compartment.

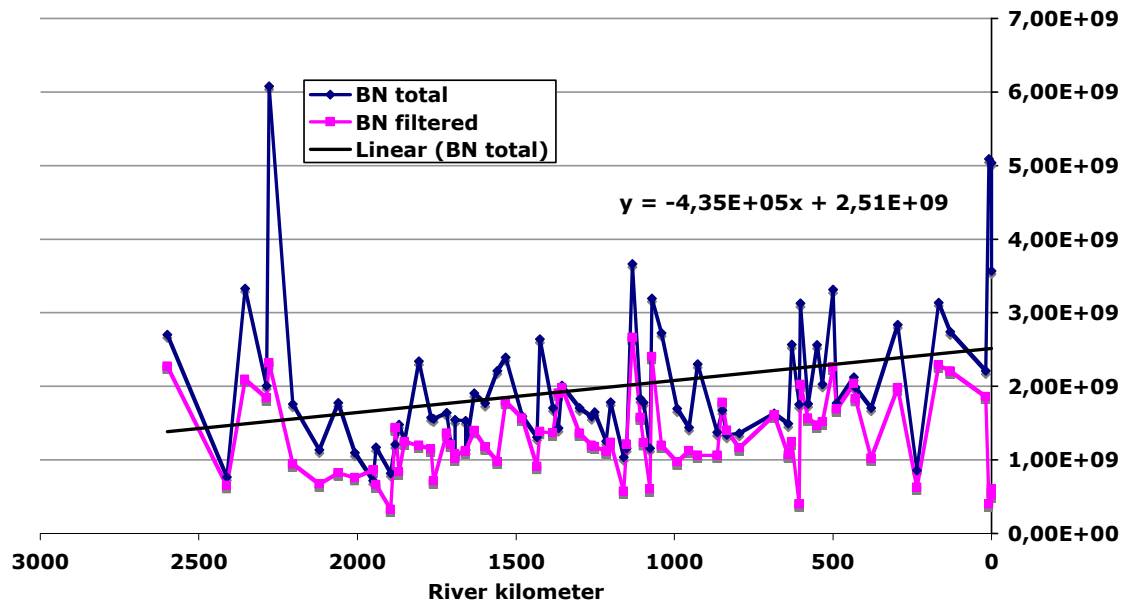
**Section Type 2 (Western Alpine Foothills Danube: rkm 2581- rkm 2225):** All parameters are extremely high with mean values of bacterial number ( $3.05 \times 10^9$  cells  $L^{-1}$ ), biomass ( $125 \mu g C L^{-1}$ ) and secondary production ( $0.71 \mu g C L^{-1} h^{-1}$ ). The end of the section is marked by the input of the River Inn, with low biomass and BSP values but with a large water volume, which doubles the water volume of the main stream.

**Section Type 3 (Eastern Alpine Foothills Danube: rkm 2225 - rkm 2001):** In this section a sudden decrease of all mean bacterial parameter values down to half of the previous section values is recorded (BN =  $1.44 \times 10^9$  cells  $L^{-1}$ , BBM =  $66.70 \mu g C L^{-1}$  and BSP =  $0.36 \mu g C L^{-1} h^{-1}$ ).

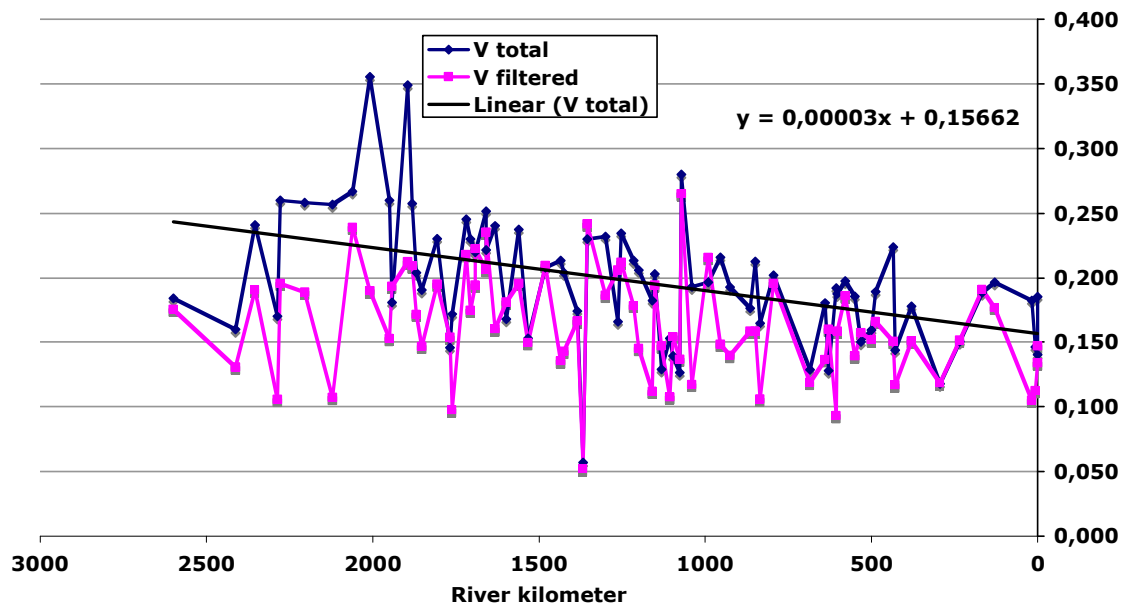
**Section Type 4 (Lower Alpine Foothills Danube: rkm 2001 - rkm 1789.5):** Bacterial parameters keep declining, showing their minimum at Bratislava i.e. Gabčíkovo dam (BN =  $1.29 \times 10^9$  cells  $L^{-1}$ , BBM =  $52.28 \mu g C L^{-1}$  and BSP =  $0.33 \mu g C L^{-1} h^{-1}$ ). However, the inputs of Morava River and Moson arm are characterised by high bacterial numbers, biomasses and production rates, which are higher by a factor 6, 8 and 9 respectively. Nonetheless it is remarkable that bacterial parameter values from Danube samples at the vicinity of Bratislava remain low.



**Fig 1:** Bacterial parameters from water samples of 75 stations along the Danube River and 21 tributaries and side arms. a) Bacterial numbers. b) Bacterial biomasses, c) Bacterial secondary production. Untreated samples (black lines) and 3µm filtered samples (purple line). Peaks of tributaries and side arms are labelled with specific names and obtained values.

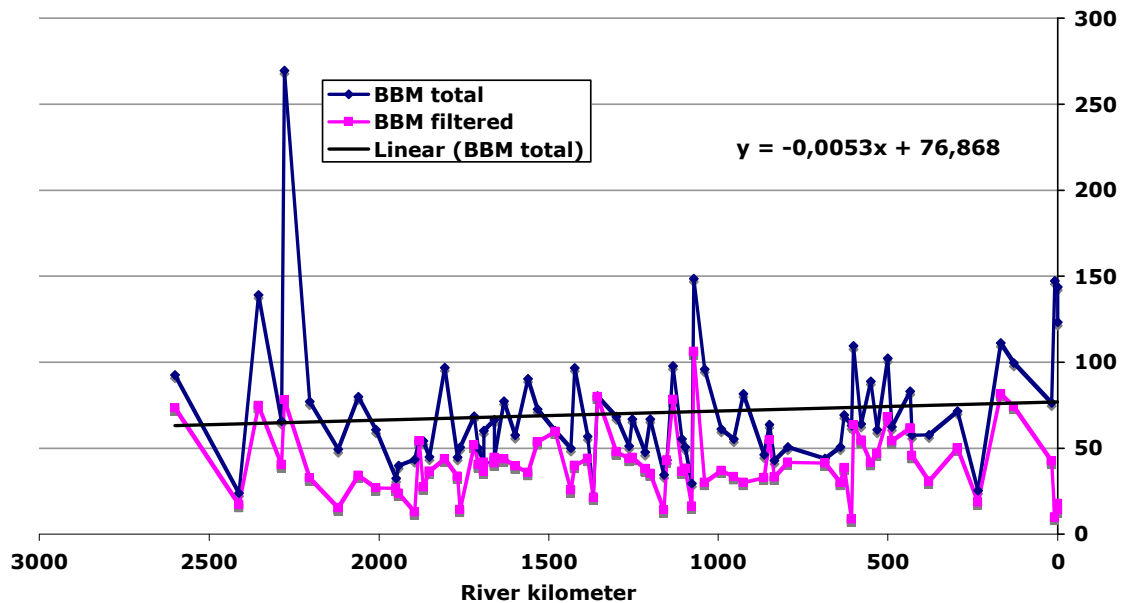


**Fig. 2:** Bacterial numbers in water samples of the Danube River without tributaries.



**Fig. 3:** Mean bacterial volume in water samples of the Danube River without tributaries.





**Fig. 4:** Bacterial biomass in water samples of the Danube River without tributaries

**Section Type 5 (Hungarian Danube Bend: rkm 1789.5 - rkm 1497):** The beginning of section 5 is characterised by the presence of tributaries Vah, Hron and Ipoly. These tributaries are characterised by low discharge volumes (less than 10% of the Danube) but relatively high bacterial parameter values as compared to the main stream. Differences by a factor of 2,6 and 2,5 for BBM, BN and BSP respectively, are observed. The bacterial parameter values of the first part of this Danube section are lower than mean values from section 4. A contrasting situation is recorded for the samples in the close vicinity of Budapest and thereafter, where all bacterial and most nutrient values show a significant increase as compared to samples taken upstream Budapest (BN=  $1.63 \times 10^9$  cells  $L^{-1}$ , BBM =  $60.30 \mu g C L^{-1}$  and BSP=  $0.58 \mu g C L^{-1} h^{-1}$ ). The influence of the river Sio is negligible.

**Section Type 6 (Pannonian Plain Danube: rkm 1497 - rkm 1075):** Bacterial parameters in this section are slightly higher than in the previous section (BN=  $1.82 \times 10^9$  cells  $L^{-1}$ , BBM =  $62.68 \mu g C L^{-1}$  and BSP =  $0.62 \mu g C L^{-1} h^{-1}$ ) with chlorophyll a showing its maximum after Novi Sad. Due to three big tributaries (Drava, Tisa and Sava) the water volume of the Danube is doubled.

**Section Type 7 (Iron Gate Danube: rkm 1075 - rkm 943):** Bacterial number and biomass keep increasing (BN =  $1.96 \times 10^9$  cells  $L^{-1}$ , BBM =  $70.72 \mu g C L^{-1}$ ), whereas bacterial secondary production reveals a sudden decrease comparable to section type 3 (BSP =  $0.22 \mu g C L^{-1} h^{-1}$ ), again a stretch of impounded water (Iron Gate reservoir).

**Section Type 8 (Western Pontic Danube: rkm 943 - rkm 375.5):** After a stretch of slow water current in the previous section, bacterial numbers remain almost at the same level as in section 7 ( $1.99 \times 10^9$  cells  $L^{-1}$ ) but biomass decreases ( $66.54 \mu g C L^{-1}$ ) together with the average cell volume (8.5% smaller than in section type 7). Bacterial secondary production reaches its minimum in the Danube River (BSP =  $0.21 \mu g C L^{-1} h^{-1}$ ). This comparably long section (570km) is characterised by 6 important tributaries: Timok, Iskar, Olt, Jantra, Russenski Lom and Arges. The first four rivers show low bacterial parameter values, nutrient concentrations and flow rates. Russenski Lom and the Arges River had low discharge volumes, yet bacterial parameter values and nutrient concentrations were conspicuously high, due to city waste waters of Russe and Bucharest, respectively.

**Section Type 9 (Eastern Wallachian Danube: rkm 375.5 - rkm 100):** Bacterial secondary production values from this section ( $BSP = 0.31 \mu\text{g C L}^{-1} \text{h}^{-1}$ ) can be compared with those from section 4, but abundance and biomass values ( $BN = 2.40 \times 10^9 \text{ cells L}^{-1}$ ,  $BBM = 76.89 \mu\text{g C L}^{-1}$ ), show a continuous increase along the river, indicating uncoupling between BSP and BBM. Rivers Siret and Prut are the last tributaries before the delta. Their bacterial parameters values are moderate but still higher than the Danube values.

**Section Type 10 (Danube Delta: rkm 100 - rkm 7):** The Danube Delta is characterized by extreme concentrations of particulate organic matter as well as by high bacterial abundance and biomass ( $BN = 3.98 \times 10^9 \text{ cells L}^{-1}$ ,  $BBM = 122.66 \mu\text{g C L}^{-1}$ ), especially in the attached bacterial fraction. Bacterial secondary production remains at the same level or even slightly lower than in the previous section ( $BSP = 0.25 \mu\text{g C L}^{-1} \text{h}^{-1}$ ).

### Magnitude regions of BSP

On the basis of regions (i.e. a consecutive number of JDS sampling stations) with comparable bacterial production we distinguished four magnitude regions of mean bacterial secondary production (Table 2). JDS sampling point 1 was considered to be unrepresentative for the Danube River (see above) and was therefore not included in the estimations of means to characterize the regions. The first magnitude region corresponds to the upper Danube with a mean BSP of  $0.71 \mu\text{g C L}^{-1} \text{h}^{-1}$ , followed by the region between the river Inn and the sampling point upstream Budapest, the Alpine Foothills with an average BSP of  $0.33 \mu\text{g C L}^{-1} \text{h}^{-1}$ . The third region corresponds to the Pannonian Lowland, between the city of Budapest and Iron Gate (again a mean BSP of  $0.71 \mu\text{g C L}^{-1} \text{h}^{-1}$ ), and the fourth magnitude region extends from Iron Gate downstream to the Danube delta with a mean BSP of  $0.23 \mu\text{g C L}^{-1} \text{h}^{-1}$ .

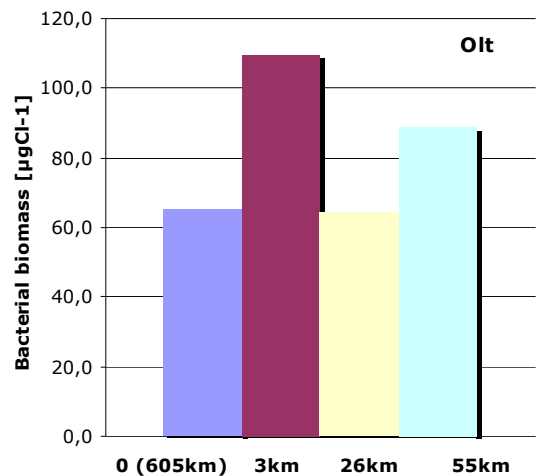
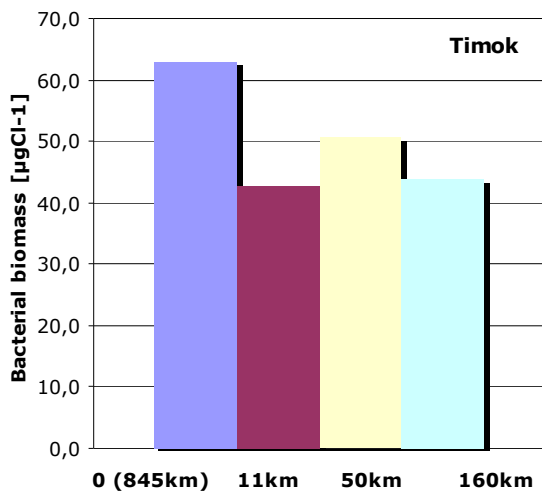
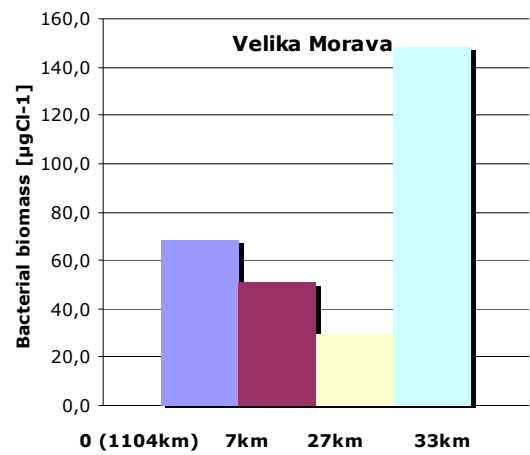
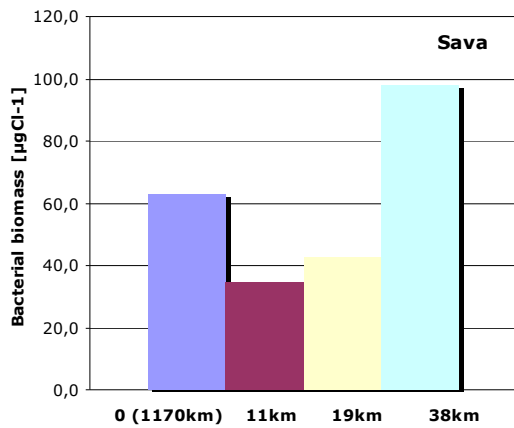
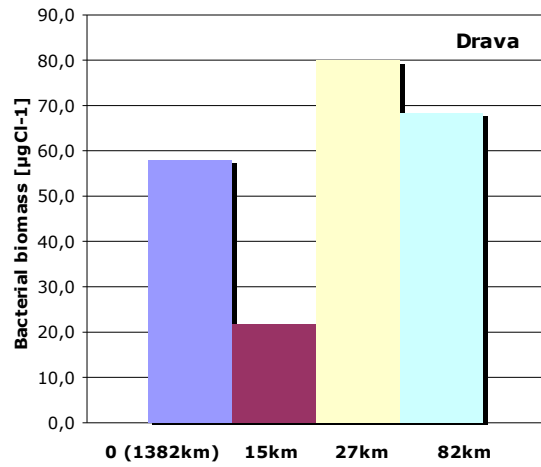
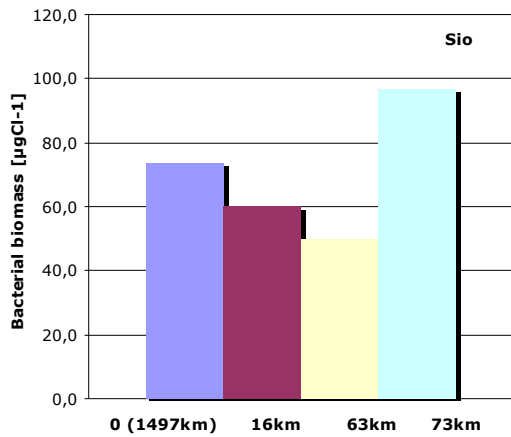
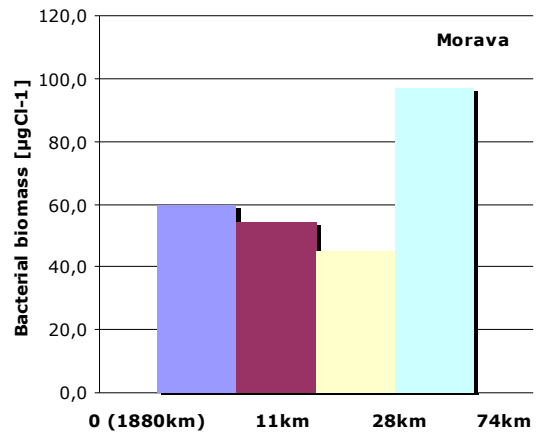
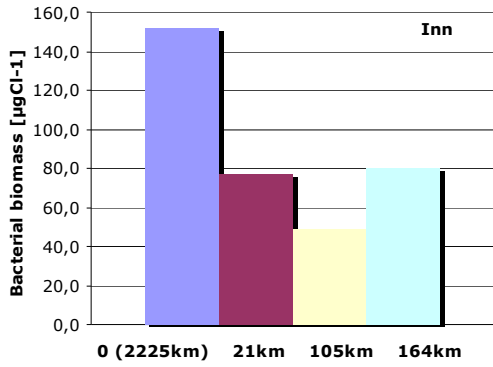
**Table 2:** Magnitude regions of BSP along the Danube without tributaries. \*\* indicate highly significant differences in mean BSP values between consecutive magnitude regions.

Magnitude regions of BSP	JDS points	Average $\mu\text{g C L}^{-1} \text{h}^{-1}$	Standard deviation	Minimum	Maximum	t-test
Upper Danube	2 - 5	0.71	0.09	0.61	0.83	-
Alpine foothills	7 - 31	0.33	0.10	0.15	0.63	**
Pannonian Lowland	32 - 60	0.71	0.28	0.35	1.46	**
Lower Danube	61 - 96	0.23	0.07	0.14	0.47	**

### Bacterial parameter variations in tributaries and their impact on the River Danube

Except for the large tributaries like Inn, Drava, Tisa and Sava, confluent rivers have usually higher BN, BBM and BSP values than the specific Danube sections where the tributaries merge. Since it is a major challenge in microbial ecology to develop models which predict evolutionary trends in ecosystems under specific environmental conditions it was attempted to use available data sets to predict the magnitude and direction of carbon flow of the defined Danube section which are impacted by tributaries. For this purpose, we calculated the expected BBM values for the first sampling point downstream of the tributary mouth and compared it with the monitored BBM values downstream. Care was taken to obtain comparisons with data from at least three consecutive sampling points downstream (Fig.5).





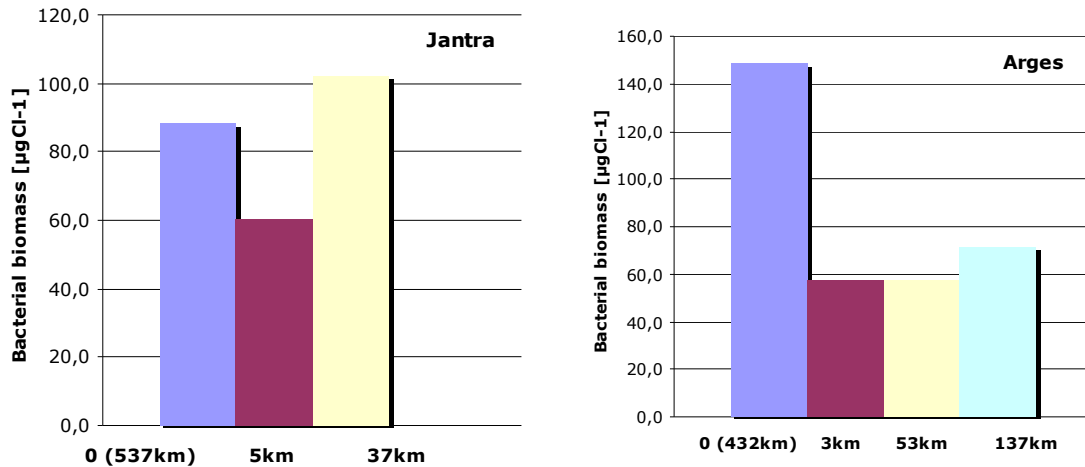


Fig. 5: Expected bacterial biomass load in the Danube after the tributary input and observed biomass loads in following JDS- stations. Expected value is on point zero (with river kilometre as a distance of the tributary mouth from Danube delta), and observed values are shown with the distances of sampling points from the mouth of tributary. Expected load calculation = (Biomass load from Danube River + biomass load from tributary) / (Discharge from Danube River + discharge from tributary). The premise for the calculation of the expected biomass load is based on the assumption that homogenous mixing of the merging water bodies (tributary and main river) occurs.

We choose 10 representative tributaries and an exception is noted for the situation of the river Jantra with only 2 consecutive sampling points. The third sampling point downstream of the river Jantra was again the mouth of an important side arm (Russenski Lom). It could be shown that in the majority of all cases tributaries had a rather negligible impact on the main stream of the Danube River. In the majority of all cases the expected BBM values were always between 5 to 50 % higher than the monitored BBM value at the following JDS station downstream (Fig. 5). In 60% of all cases, even the second JDS station downstream of the tributaries mouth was still below the expected value. Also, it is noteworthy that for all large tributaries with discharge volumes amounting to 20% or more of the water volume of the main stream, the monitored BBM values were usually by 40 to 60% below the expected values. At the actual state of knowledge with respect to the available data we have no explanation for this observation. Nonetheless it should be pointed out that tributaries with large discharge volumes such as the rivers Inn, Drava or Sava have mostly low bacterial biomass loads but are also assumed to have different water mixing and dilution dynamics than tributaries with small discharge volumes. It may therefore be speculated that the filter effect of the river bed sediments and especially of the sediments close to the river banks functions differently (i.e. more efficiently) when large rivers merge.

### Variations in bacterial morphotypes

In figures 6 and 7 it could be shown that cocci and to a lesser degree vibrio shaped cells become more abundant from section type 2 to section type 10. Similarly, the abundance of rod shaped cells, which are in most aquatic systems the dominant bacterial morphotypes, is

decreasing as cocci increase (Figures 6 and 7). Since we observed at the same time an increase in bacterial numbers but only a very moderate increase in bacterial biomass, it was obvious that the monitored overall decrease in bacterial volume would necessarily lead to a dominance of small bacterial cells. Consequently the dominance of small cells increases in the Danube river along the longitudinal transect as the stream approaches the Black Sea. The majority of these small cells are represented by cocci and vibrio shaped cells which reach their highest relative abundances in section type 10. Even though vibrio shaped bacteria are rather cells with volumes comparable to those of rods, the volume of vibrios experiences a decrease in size in the section types of the Lower Danube and close to the Black Sea (Figs 8 and 9. )

In section 2 these two morphotypes represent 15 % of all morphotypes in untreated samples and 28 % in filtered samples. In section 10, these two morphotypes make up some 53 % of all morphotypes in untreated samples and 59% in filtered samples, filamentous forms being negligible, yet decreasing.

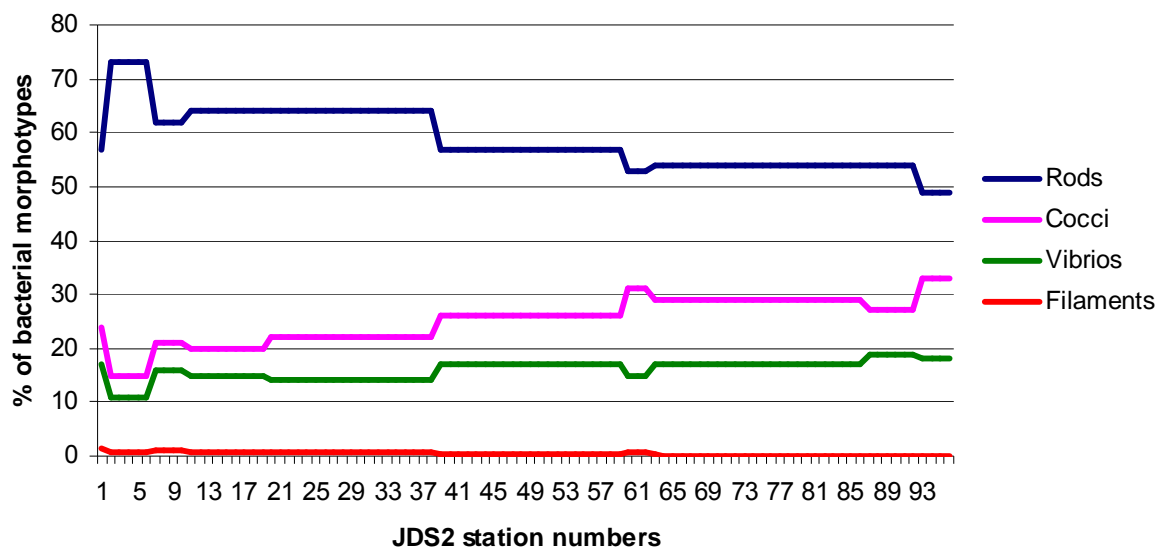


Figure 6: Mean percentage of bacterial morphotypes for 10 respective Danube sections from untreated water samples

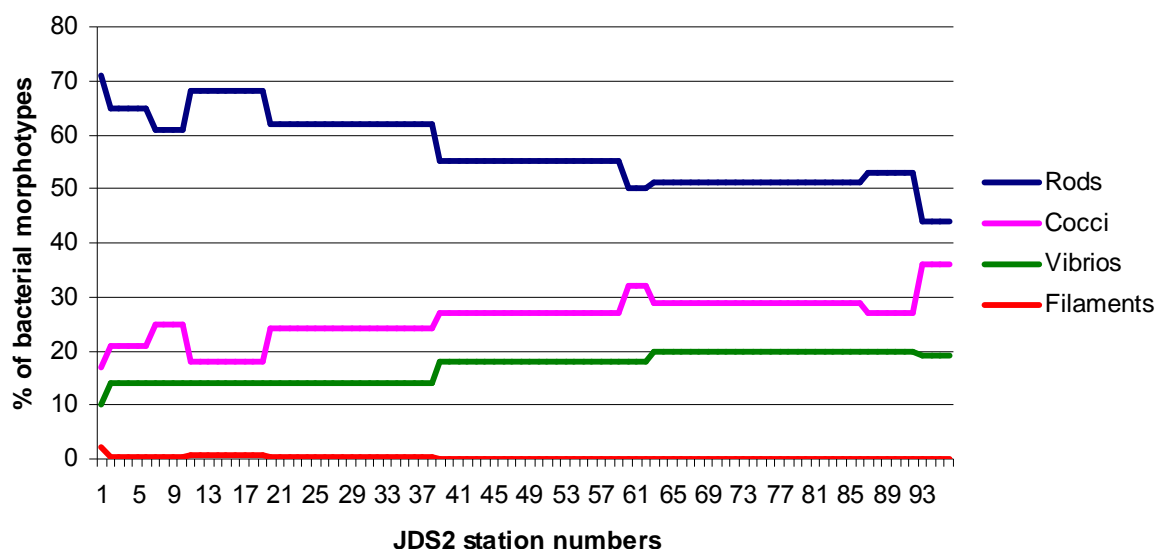


Figure 7: Mean percentage of bacterial morphotypes for 10 respective Danube sections from filtered water samples

This clear shift in morphotypes from large rods and filamentous forms to small cocci and smaller vibrios and the related overall decrease in cell volume may be seen as an indication for an insufficient amount of nutrients for growth and multiplication. Bacterial cells deprived of a key element for growth react generally with a global response resulting in a more adapted or resistant cell. In most cases the starvation adapted cell is smaller and may change its cellular morphology, whereby cells become smaller and often spherical (Lange and Hengge-Aronis, 1991). Alternatively, the imported water from tributaries along the investigated stretch may contain harming substances which accumulate in the main stream of the River Danube as it approaches the Black Sea, still permitting cell division but inhibiting normal bacterial cell growth. Our hypothesis on the starvation-survival phenomenon of the bacterial community is thus supported by the observed changes in the relative abundance of morphotypes (Figs 6 and 7), the monitored size decrease for both the cocci and vibrio shaped cells (Figs. 8 and 9) as the main stream approaches the Black Sea and the rather low bacterial production rates which were recorded for most section types (Table 2). Even though we had occasionally BSP values reaching  $1.46 \mu\text{g C L}^{-1} \text{h}^{-1}$  in the Pannonian Lowland (Table 2), the calculated BSP averages for the defined magnitude regions ranged from 0.23 to  $0.71 \mu\text{g C L}^{-1} \text{h}^{-1}$ , with lowest values in the Lower Danube region. Gradual changes in bacterial community composition along the longitudinal transect, were observed by Winter et al. (2007). This observation fits well to the observed changes of morphotype composition and cell volumes.. Such a change may be a reaction of the community to increasingly unfavourable nutrient situations, favouring bacterial species with low substrate uptake affinities.

Thus it seems to be obvious that the observed changes in bacterial community parameters along the Danube River are attesting events which are attributed to the specific functioning of the microbial loop in large rivers. The bacterial development is mainly controlled by the availability of utilizable DOM sources. According to the River Continuum Concept, the labile DOM fraction of mainly terrestrial origin is taken up rapidly, leading to an increase of the more recalcitrant fraction which is transported downstream. Therefore the key processes in the continuum of large rivers are those which induce the biological transformation of aged terrestrial carbon (Battin et al., 2008) and the related local events of primary production which

provide the energy to initiate this degradation. We assume that these processes are the determining events which shape the bacterial community composition and trigger off starvation-survival reactions among the prokaryotes. Thus, the proposed hypotheses are not excluding each other but should rather be seen as being complementary to each other.

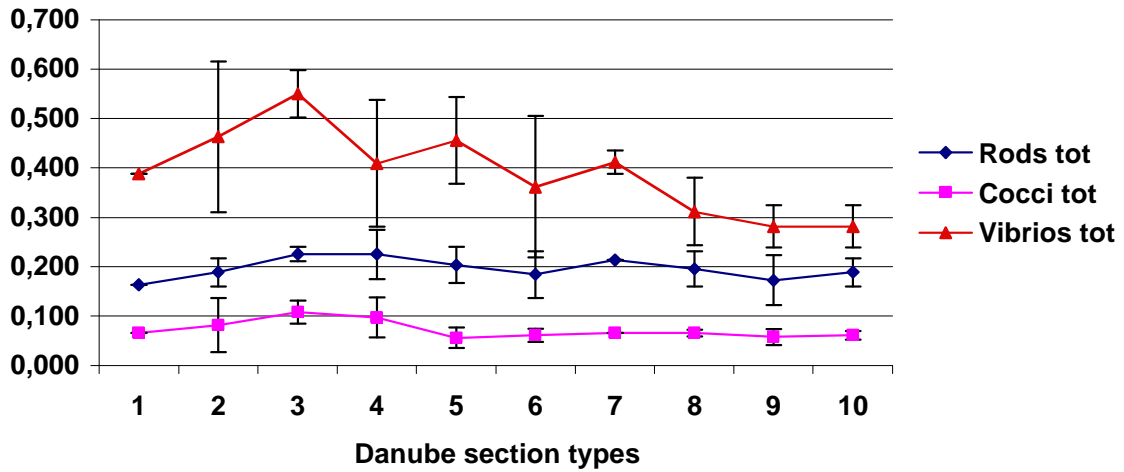


Figure 8: Mean volume of bacterial morphotypes in the various section types of the Danube River from untreated water samples. Vertical bars denote standard deviations

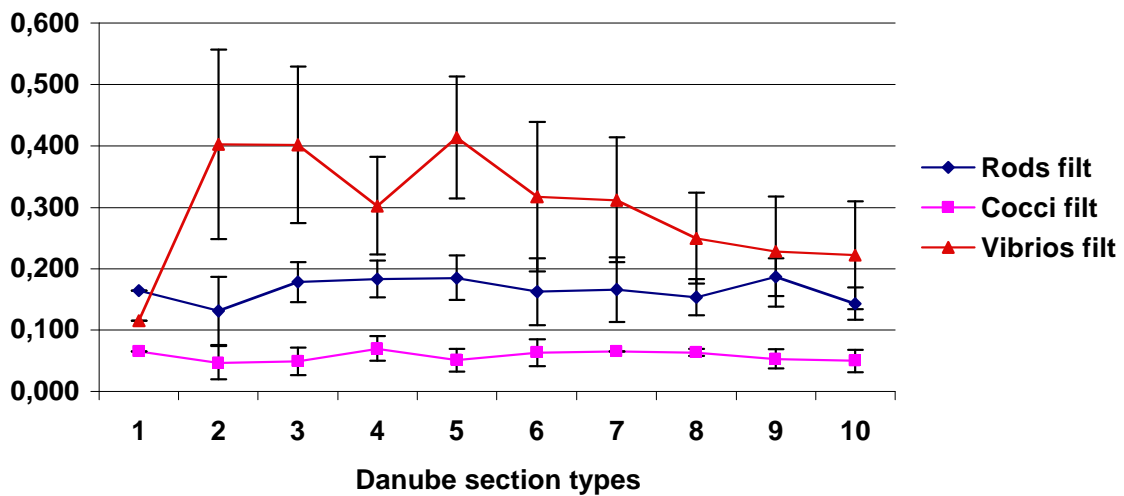


Figure 9: Mean volume of bacterial morphotypes in the various section types of the Danube River from filtered water samples. Vertical bars denote standard deviations

## Summary and conclusions

- The longitudinal study of the entire course of the Danube River and its tributaries revealed conspicuous differences between bacterial parameter values from the Danube River water samples and the merging tributaries
- Bacterial numbers, biomasses and secondary production values from tributaries were mostly higher than in the Danube River. Only in the case of tributaries with important discharge volumes, such as the rivers Inn and Drava, bacterial parameter values remained below those of the Danube River.

- Load calculations for tributaries allowed estimating expected parameter values for sampling stations downstream of the tributaries. However, in the majority of the cases the monitored bacterial parameter values were well below the expected values.
- Hence, the Danube River remains - as suggested by the obtained data – rather unaffected by the tributaries input, similar to what has been demonstrated earlier by Winter et al (2007). As measured parameter values were usually lower than the calculated expected values after the input of tributary waters, it may be assumed that beside the impact of bacterivory by microzooplankton, both the filter feeding macrozoobenthos and cell retention by river bank sediments are responsible for the observed decrease of bacterial biomasses. Thus, the Danube River may function as purification system for bacterial loads imported from tributaries. This is true for the sampling stations in the middle of the river but the trend can not be extended for the river banks since they were not included in the sampling strategy. Nonetheless, one should also consider the possibility that the water of the tributaries flows over longer stretch distances in the vicinity of the river banks and mixing with mid-river water is only achieved at distances of some 30 km or more after the tributaries mouths. Testing of this hypothesis is definitely required.
- The evolution of the bacterial parameters along the longitudinal transect reveals clear trends for an increase of bacterial numbers and biomasses and a marked decrease in bacterial cell volume. These observations are in agreement with the River Continuum concept.
- Bacterial secondary production does not follow a recognizable trend along the longitudinal transect and is therefore not in agreement with the River Continuum Concept. This parameter seems to be more prone to the influence of tributaries and the specific environmental conditions of regions encompassing several section types.
- A shift in the dominance of bacterial morphotypes along the longitudinal Danube transect was recorded. As large rod shaped and filamentous bacteria decrease from section type 2 to section type 10, the relative abundance of small cocci and vibrio shaped cells with decreasing cell volume increases. Since this phenomenon is related to an overall decrease of bacterial cell volume, it was hypothesized that most bacterial cells get adapted gradually to either nutrient exhaustion or to a missing key element for growth. The rather low BSP values, especially in the Lower Danube region, support the hypothesis of a starvation/survival reaction of the bacterial community.
- Free living bacteria are more abundant than attached bacteria in the Danube River and the tributaries but attached bacteria are more productive than free living bacteria, as indicated by mean BBM/BSP ratios.
- Although data on total phosphorus and primary production, required for the determination of the trophic situation of the various section types are now available it is yet not possible to decide whether the bacterial parameters can be used for the assessment of the ecological status in the various section types. Comparative analysis with macrozoobenthos data is definitely required in order to achieve final conclusions. However, the evolution of most parameters along the longitudinal transect is encouraging the planned construction of a new tool for the determination of ecological status classes via bacterial parameters. First attempts in constructing such a tool indicate the potential utility of microbial parameters possibly within the established EU-Water Framework Directive (2000) to assess the ecological status of large rivers.

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