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ICPDR's efforts towards ensuring a healthy and clean Danube: Joint Danube Survey 3

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The Joint Danube Survey 3 (JDS3), was the world's biggest river research expedition of its kind in 2013, the UN International Year of Water Cooperation. JDS3 catalyzed international cooperation of the ICPDR Contracting Parties being a unique opportunity to assess the water quality in the whole Danube and providing the largest ever amount of knowledge about the Danube water pollution collected within a single scientific exercise.

The general objective of the JDS 3 was to undertake an international longitudinal survey that would produce comparable and reliable information on water quality for the whole of the length of the Danube River including the major tributaries on a short-term basis. The outcomes of the JDS 3 covered the information gaps necessary for the implementation of the EU Water Framework Directive (WFD).

The specific objectives and added values of the JDS 3 included:

- Support to the revision of Danube River Basin District Management Plan by 2015;
- Assessment of methods for large rivers;
- Monitoring of new candidate priority substances;
- Identification and prioritization of Danube River Basin District specific substances;
- Trend analysis for Danube River Basin District relevant substances;
- Highlighting the link between surface water and groundwater pollution;
- Investigation of quality of sediments;
- Harmonization of sampling methods for WFD biological quality elements;
- Investigation of invasive alien species;
- Improvement of hydromorphological assessment with the view of developing a harmonized approach for the Danube;
- Interlinking hydromorphology and biology (habitat quality);
- Interlinking chemistry – biology – microbiology;
- Support to future Intercalibration exercise in the Danube River Basin District;
- Specific investigations (zooplankton, microbiology, bioassays);
- Testing new methods;
- Training/learning by doing;
- Public awareness raising.

During JDS 3 altogether 68 sites were sampled by the Core Team of experts along a 2581 km stretch of the Danube, 15 of which were located in the mouths of tributaries or side arms. The results obtained cover a wide area of expertise on aquatic chemistry, biology, microbiology and hydromorphology and their findings create a comprehensive knowledge base for further assessment of water quality in the Danube River Basin and beyond. The findings of JDS 3 are supportive to the implementation of EU WFD as they provide an extensive homogeneous dataset production of which was mainly based on WFD compliant methods commonly used by the Danube experts. Even though these data have no ambition of replacing the national data used for the assessment of the ecological and chemical status they are an excellent reference database serving for future efforts of method harmonization in the Danube River Basin, especially concerning the development of a concerted type-specific approach to the status assessment of large rivers, and of the prioritization of the Danube river basin specific pollutants.

The final report of JDS3 has been completed and after its adoption by the ICPDR will be published in early 2015.

Figure 1. The JDS3 fleet anchoring in Belgrade.
Vessels 'Wien', 'Sounding Vessel I', 'Argus', 'Istros' (left to right).

Macro-invertebrates
A short report on the Biological Quality Element Benthic Invertebrates

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Introduction

Benthic macroinvertebrates are one biological quality element defined by the European Water Framework Directive (EC, 2000/60; WFD) to assess the ecological water quality and were therefore monitored in all previous Joint Danube Surveys (JDS). The methods applied differed due to the availability of devices, financial issues and the scientific focus. While in JDS 1 grabs were used to investigate hard rocky substrates (Literáthy et al., 2002), in JDS 2 air-lift samples were taken to study the faunal composition of deep water habitats (Liška et al., 2008). During JDS 3 a modified Multi-Habitat-Sampling (MHS) approach was performed to highlight the importance of specific micro-habitats in terms of biodiversity and, additionally, as a sound basis for river restoration efforts and water management issues in general. The data gained from JDS 3 can be seen as an important documentation of the current distribution of specific taxa and species, of specific habitat preferences and as a completion regarding faunistic results of earlier studies (e.g. Russev, 1998; Slobodnik et al., 2005; Csányi & Paunovic, 2006), and of all previous JDS expeditions. The results will significantly contribute to current discussions regarding the WFD compliant assessment methods for large rivers, both regarding the field work as well as the analysing aspects.

Methods

Sampling of benthic macroinvertebrates for JDS3 had three approaches carried out by three separate sampling groups: 1) **Multi-Habitat-Sampling,** MHS: A standardised, WFD compliant method for the ecological (status) assessment (AQEM Consortium, 2002; CEN, 2012a). Sampling of different habitats in the actual littoral zone was done with a Multi-Habitat-Sampling net (executed by the Institute of Hydrobiology and Aquatic Ecosystem Management, University of Natural Resources and Life Sciences, Vienna); 2) **Deep Water Sampling,** DWS: Cross-sectional survey by dredging in the deep water area (executed by the Laboratory of MTA, Hungarian Academy of Sciences, Centre for Ecological Research, Danube Research Institute). This approach was selected for reasons of comparability with the Airlift-results, the deep water sampling method applied during JDS 2 in 2007; 3) **Kick and Sweep Sampling,** K&S: Sampling with a hand net at the shore region (executed by the Institute or Biological Research "Siniša Stanković", University of Belgrade (IBISS)). The aim of the additional K&S sampling (CEN, 2012b) was to extend the investigated zone and to add additional data on molluscs to the results of the near-littoral MHS sampling program.

Results and discussion

According to the selected main sampling method the following chapters are based mainly on the evaluation of the MHS data set.

Overall taxa richness

During JDS 3 a total of 460 macroinvertebrate taxa were identified by the three sampling techniques. Insects, with 319 taxa, were the dominant component of the communities. Diptera were the richest insect order with 222 taxa, with 200 species belonging to the family Chironomidae. Other heterogeneous groups were: Oligochaeta (55 taxa), Mollusca (43 taxa - Bivalvia 23 and Gastropoda 20), Trichoptera (40 taxa), Ephemeroptera (32 taxa), Coleoptera (15 taxa), Amphipoda (15 taxa) and Odonata (13 taxa). Other groups of taxa were less diverse.

Diversity and abundances

In total the MHS-samples comprised 345 invertebrate taxa. When including samples taken from habitats which were added, but proportionately under-represented at a certain site (such as deadwood) an overall number of 393 taxa was documented.

The most heterogeneous groups were Diptera (162 taxa) and Oligochaeta (42 taxa) followed by Trichoptera (28 taxa), Ephemeroptera (24 taxa) and Molluscs (Gastropoda 17 taxa, Bivalvia 13 taxa, respectively). Coleoptera (11 taxa), Amphipoda (15 taxa) and Odonata (9 taxa) are as well noteworthy; other groups were important but less diverse. Along the three reaches of the Danube, Trichoptera and Ephemeroptera were decreasing in diversity, all other groups were quite constant or showed a peak at the Middle Reach *(Figure 1)*.

Regarding Amphipoda a high number of ponto-caspian, invasive species was documented, e.g. *Chelicorophium*

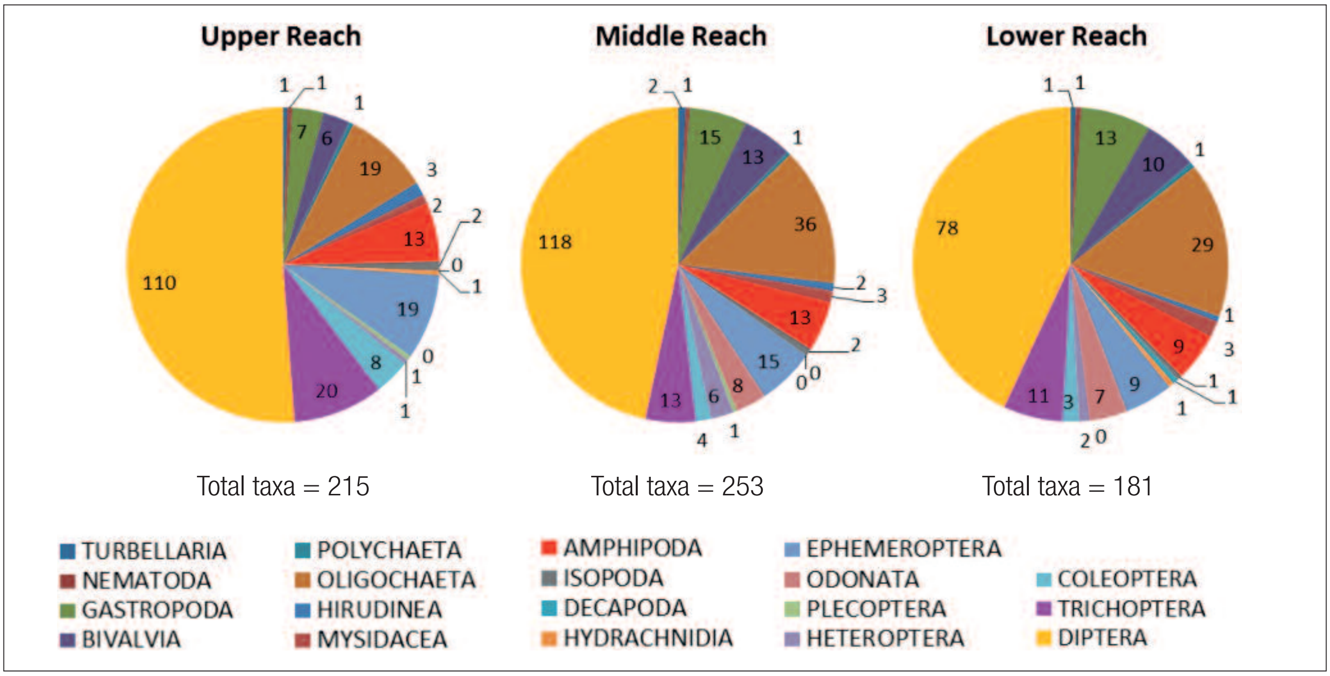

Figure 1. Number of taxa per taxagroup along the different reaches of the Danube (MHS-Data)

curvispinum, C. robustum, C. sowinskyi, D. bispinosus, D. haemobaphes, D. villosus, Echinogammarus ischnus, E. trichiatus, and *Obesogammarus obesus.*

Regarding abundance (individuals/m²) Amphipoda were the dominant group in all Danube reaches and increased downstream (varying from 27 to 45 %), while Diptera played an essential part in the Upper Reach (32 %) and decreased downstream (17 %). Oligochaeta and Mollusca were found in increasing numbers in the Middle and Lower Reach. Higher abundances of EPT-Taxa (Ephemeroptera, Plecoptera and Trichoptera) were only documented for the upper stretch, whereas Trichoptera dominated this group with highest abundances. Regarding aquatic insects, only Chironomidae (Diptera) played a major role along the whole Danube *(Figure 2).*

Neozoa

Neozoa originating from the Ponto-Caspian area, Asia, Australia and North America are a crucial fact influencing the

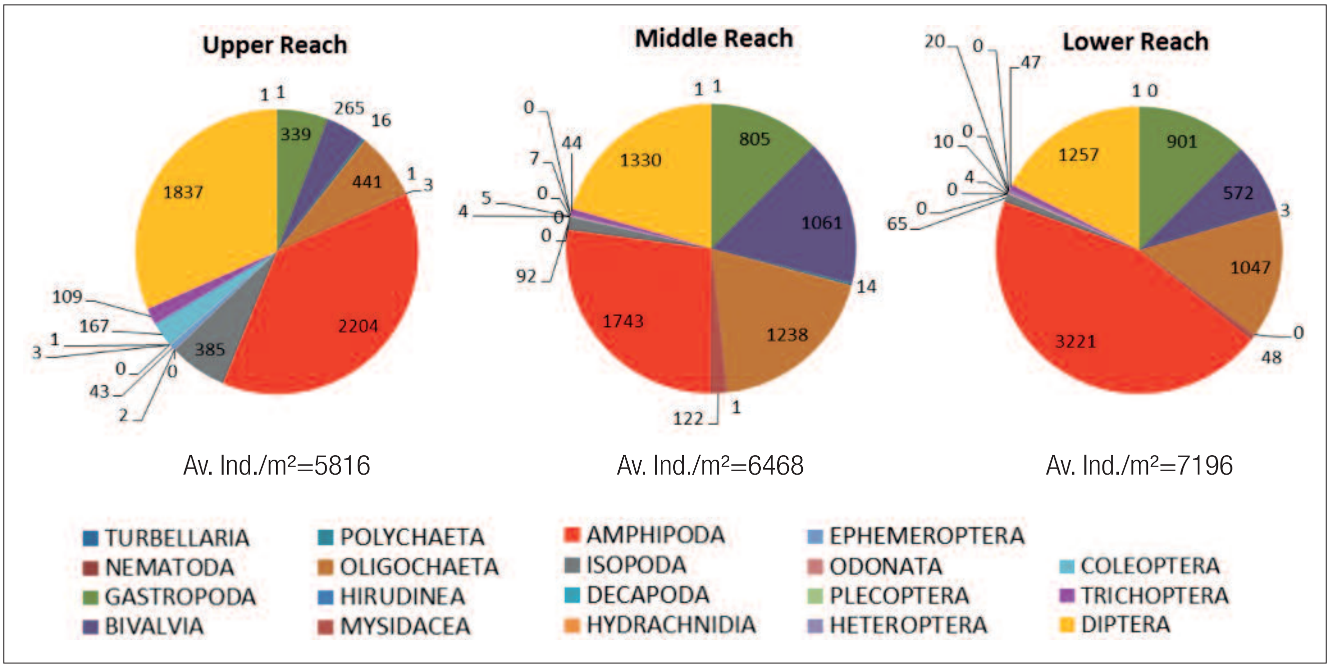

Figure 2. Average density (individuals/m²) per taxagroup along the different reaches of the Danube (MHS-Data)

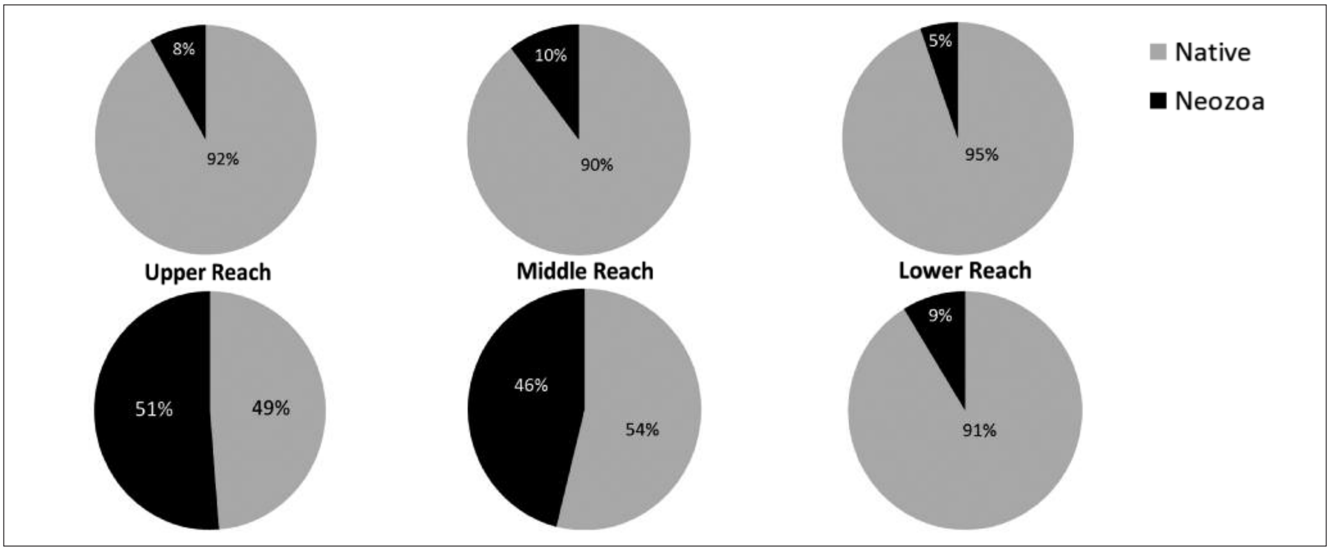

Figure 3. Proportion of taxa percentage (upper row) and average density (lower row) of native and neozoa taxa along the different reaches of the Danube (MHS-Data)

macrozoobenthic community of the Danube. The Danube is a part of the Southern Invasive Corridor (Black Sea – Danube – Main/Danube Canal – Main – Rhine – North Sea waterway), one of the four European most important routes for invasive species (Galil et al., 2007). The river is exposed to intensive colonisation by Aquatic Invasive Species which are currently spreading throughout the Danube Basin. Most neozoa of the Danube belong to Crustacea and Mollusca.

Although the taxa number of neozoa in the Danube ranges only between 8 to 10 % in the Upper and Middle Danube Reach, their total abundance is up to 50 % *(Figure 3).* Many taxa which are classified as neozoa in the Upper and Middle Reach (mostly Amphipoda) belong to the native fauna in the Lower reach. Hence, their proportion in taxa number and especially abundance decreases significantly. Regarding the most dominant taxa of the Danube, 8 out of 10 most frequent taxa are neozoa, while 6 of them belong to Crustacea.

Habitat specific assessment

The focus of the habitat-specific sampling was to investigate the habitat preferences of taxa as a basis for river restoration and management in general. The Non-metric Multidimensional Scaling (NMS) – scatterplot in *Figure 4 (left)* shows a distinct faunal gra-dient from fine (pelal to akal) to coarse substrates (micro-/meso-/macro-lithal; gravel to boulders), rip-rap and woody debris (xylal). Other organic habitats as macrophytes and roots are widely spread over the scatterplot. This indicates a clear correlation between taxa composition and habitat type along the whole Danube course having a higher explanatory value regarding biological composition than the longitudinal distribution along the 3 reaches of the Danube *(Figure 4, right)* as especially the samples of Middle and Lower Danube Reach show no distinct separation. This implies a relatively homogenized fauna (except in the Upper Danube Reach) and that the occurrence of specific taxa is predominantly habitat-determined.

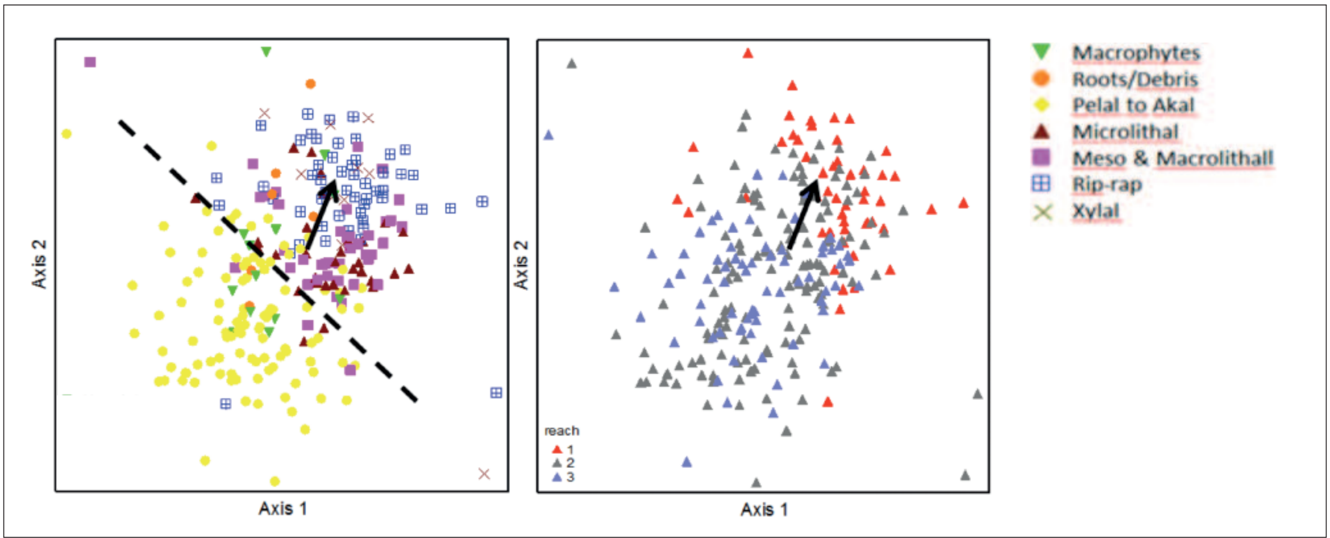

Figure 4. NMS scatterplot, based on taxa assemblages per sample (each point represents a pooled habitat sample of 5 single units); overlay: substrate types, partly combined (left), Danube reaches (1 = Upper, 2 = Middle, 3 = Lower Danube Reach), (right); final stress for 3-d solution: 16.7, final instability: 0.00338, iterations: 250; black vector: correlation between substrate type, Danube Reach and the number of invasive Crustacea (cutoff value $r^2 = 0.30$).

The number of significant indicator taxa per taxonomic group is presented in Figure 5 for the defined substrate types. Organic habitats provided the highest numbers of indicator taxa, with Diptera, detected as the most frequent and dominating taxa group along the Danube. The highest diversity of indicators was found in samples of roots/woody debris representing 19 taxa. Coarse lithal substrates like meso- and macrolithal as well as rip-rap comprise 4 indicators in total only, whereas rip-rap is preferred only by two taxa groups. Indicators of the sensitive group of EPT-Taxa were allocated to roots/ woody debris and meso-/macrolithal.

In a nutshell, organic habitats share a highly diverse indicator fauna compared to lithal habitats, especially artificial substrates as rip-rap which presence is correlated with the number of invasive Crustacea *(see Figure 4, arrow).*

Neozoa taxa reached highest average densities on hard substrates (mostly due to the mud shrimp *Chelicorophium* sp.) like meso- and macrolithal, rip-rap and xylal; highest species numbers were found in organic habitats like macrophytes and roots/woody debris.

Conclusions

During JDS3 samples were taken at wadeable and riparian areas (MHS and K&S), as well as in deeper parts (DWS) of the river at 55 sites along the Danube course. According to the different sampling methods the following main conclusions are stated:

General characteristics of the Danubian Fauna

- Altogether 460 macroinvertebrate taxa were identified by means of all sampling techniques.
- Insects, with 319 taxa, were the dominant component of the communities. Diptera were the richest insects order with 222 taxa, with 200 species belonging to the family Chironomidae. In terms of abundance, Diptera play an essential part in the Upper Reach and decrease in importance downstream.
- Amphipoda (mostly invasive Corophiidae) are the dominant group in all Danube reaches and increase downstream, while
- Oligochaeta and Mollusca were found in increasing numbers in the Middle and Lower Reach, whereas the Asian clam *Corbicula fluminea* occurred in high densities in all the three river reaches.
- Higher abundances of EPT- Taxa (Ephemeroptera, Plecoptera and Trichoptera) are restricted to the Upper Reach, whereas Trichoptera showed the highest abundances within these sensitive groups. Regarding aquatic insects Chironomidae played a major role along the entire Danube course.
- Highest taxa-richness was recorded with the MHS-approach. Some species were detected only in the middle region of the river bed at the deepest part of the Danube by dredging, as there are *Paramysis ullskyi, Schizoramphus scabriusculus, Niphargoides spinicaudatus.*

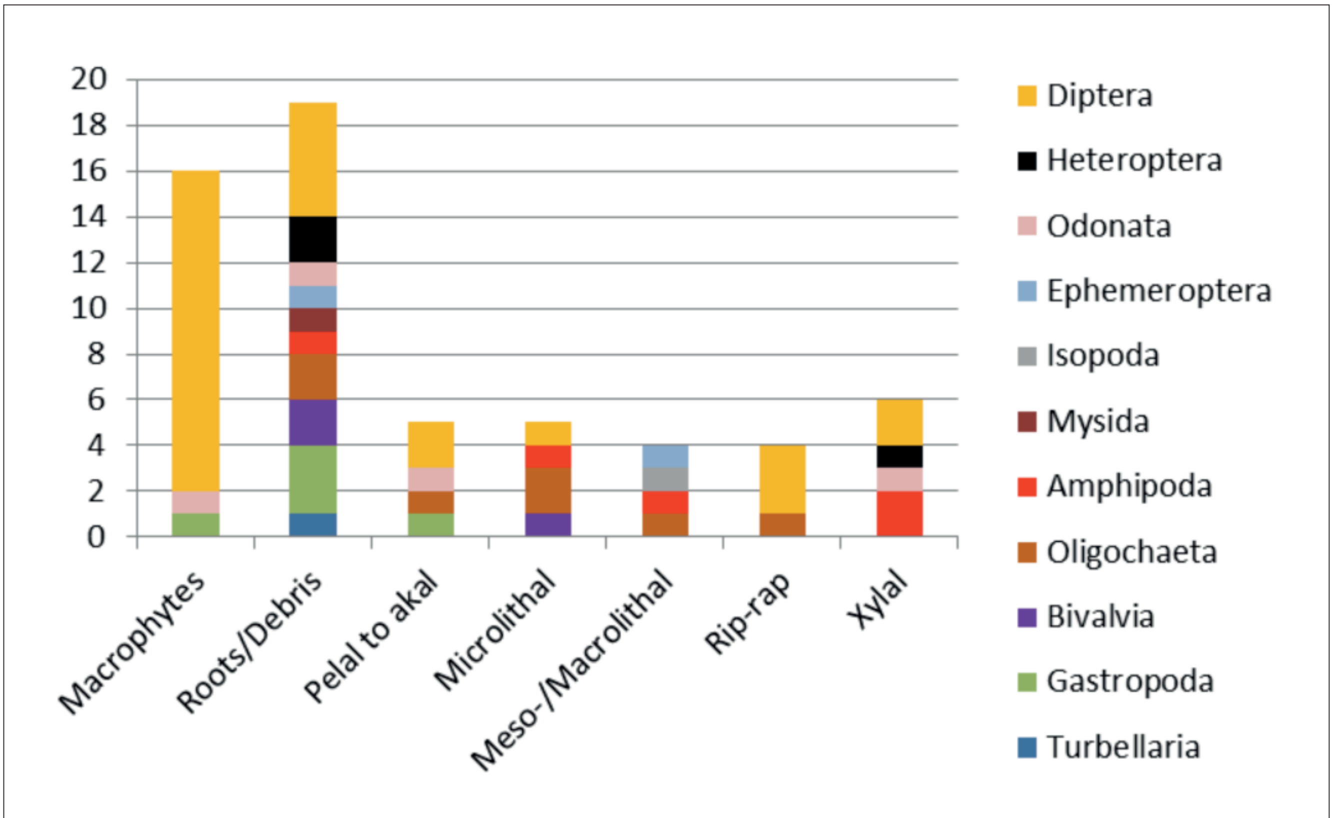

Figure 5. Significant indicator species per substrate type

Habitat preferences of indicators with implications on management actions

- As habitat degradation is one main stressor in large rivers the preferences of taxa were a main focus during JDS 3. Organic habitats provided the highest numbers of indicator taxa. The highest diversity of indicators was found in samples taken from roots or woody debris.
- Coarse lithal substrates like meso- and macrolithal as well as rip-rap were inhabited by only four indicator taxa in total.
- Invasive crustaceans show high affinities to stable substrates, especially rip-rap.
- Indicators of the sensitive group of EPT-Taxa (Ephemeroptera, Plecoptera, Trichoptera) were allocated to roots/ woody debris and meso-/macrolithal.

More detailed analyses and conclusions on this subject are given in the JDS 3 final report (Liska et al. in prep).

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Phytoplankton

Phytoplankton of the River Danube: August/September 2013 (JDS3)

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Introduction

An essential quality element in all lakes and larger rivers is the autotrophic phytoplankton. Photosynthetic processes by primary producers are important in the cycling of carbon and in the oxygen budget. The accumulated biomass can serve as food for other trophic levels. The composition of the phytoplankton assemblage and the biomass produced primarily indicates the trophic status of the water body. Within the framework of the EC-WFD, metrics have been developed to evaluate the trophic situation (Mischke and Oppitz 2005). Chlorophyll-a is used as an additional measure of biomass.

Species composition of phytoplankton may also be used to evaluate impacts from certain chemicals or to evaluate changes in hydromorphology which affect phytoplankton assemblages. Regulated stretches decrease retention time resulting in reduced biomass development. Impounded or artificially deepened river sections are more similar to lakes indicated by an increase in species more common in standing waters and a reduction in the contribution from benthic taxa usually common in free flowing rivers.

Within the Danube River Basin phytoplankton assessment is particularly relevant because the River Danube as well as several of the larger tributaries have a great potential of producing large amounts of phytoplankton biomass. Some stretches may even carry self-sustaining plankton populations (potamoplankton). Monitoring of phytoplankton diversity will help to assess changes in nutrient input and pollution control. The development of the nutrient levels and the associated phytoplankton biomass in the Danube River Basin finally has a large impact on the Black Sea.

Methods

Samples were taken from the surface of the river on the left (L), middle (M) and right (R) side with a black bucket (8 L) and used for all further analysis. A qualitative sample was taken with a plankton net (10 µm mesh size), Secchi-depth was measured at each point. On-board analysis included the immediate measurement of 'active' chlorophyll-a by delayed fluorescence (DF, Gerhardt and Bodemer 2000). Sub-samples were filtered onto GF/C filters for total chlorophyll-a analysis, stored at -35°C until analysis in the laboratory. Filters were extracted and analysed in the spectrophotometer according to DIN 38412 later in the laboratory replacing 90 % Methanol by 90 % Acetone to allow HPLC analysis for

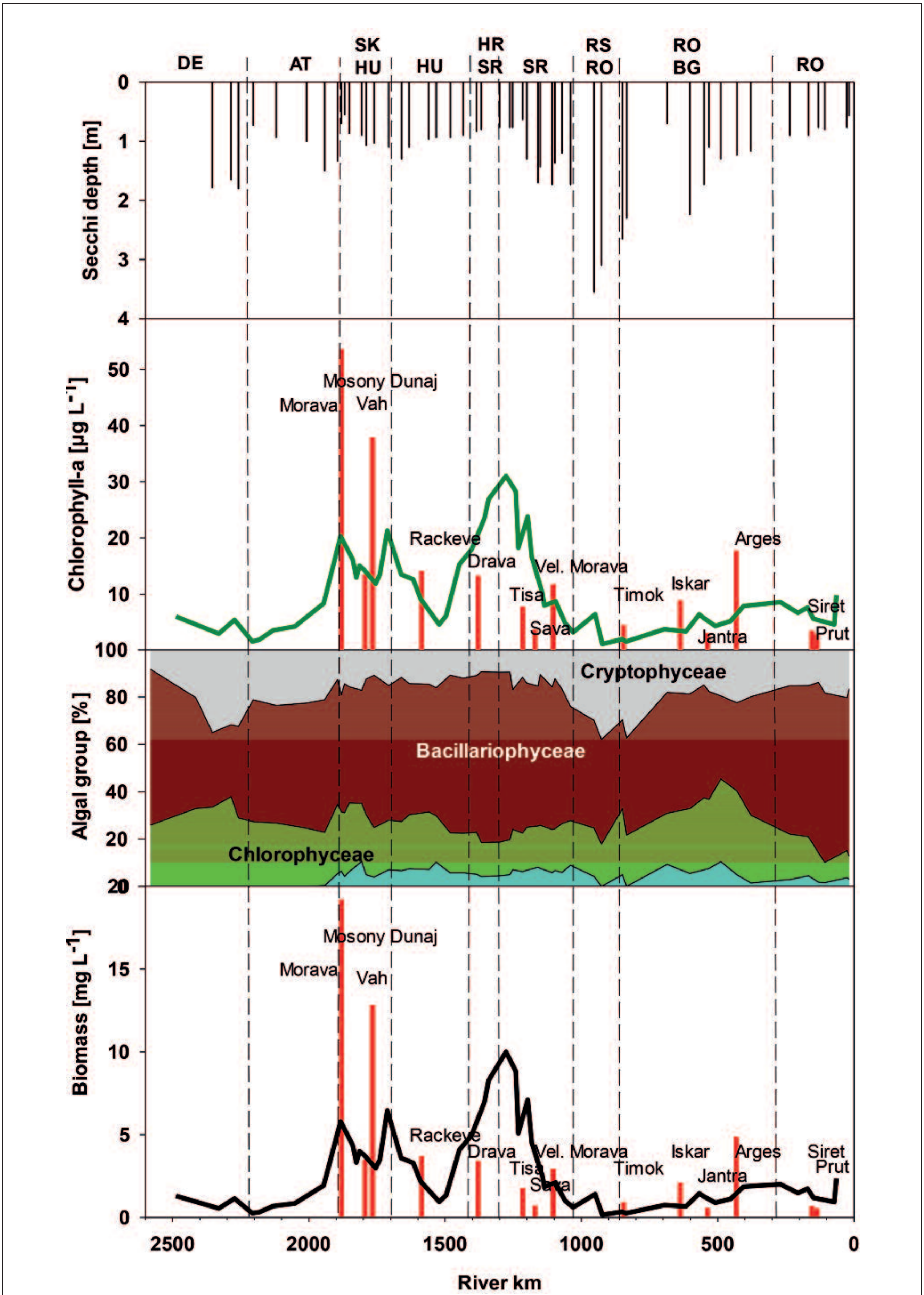

Figure 1. Longitudinal transect of the River Danube from river km 2600 to the Black Sea obtained during JDS3, August/September 2013. Variables from top to bottom: Secchi depth (SD), Chlorophyll-a in the river (green solid line) and in the tributaries (red bars); Contribution of the main algal groups (%); phytoplankton biomass in the river (black solid line) and in the mouth of the tributaries (red bars). Units are indicated on the axes. Delineations and abbreviations of countries inserted.

the assessment of pigment composition. Quantitative samples (100–200 ml) for phytoplankton counting and sizing were fixed with Utermöhl's acetic acid Lugol solution, preserved with a few drops of formalin in brown screw cap glass bottles and stored in a cool dry place (Utermöhl 1958, Hillebrand et al. 1999,). Algae were largely determined on board using the unpreserved concentrated 10 µm-net samples. Fresh-weight biomass was calculated from chlorophyll-a concentrations using three independent conversion equations (not intercalibrated):

(1) Chl-a = 0.5 % fresh-weight biomass (Reynolds 2006)
(2) Chl-a = 0570 + 4.131*B (derived from JDS1 and 2 data, Dokulil unpubl.)
(3) Chl-a = 4.063*B0.66 (Felip and Catalan 2000)

Results

During the observation period, samples were taken at 68 locations (53 in the Danube and 15 from tributaries) resulting in 159 river samples (L/M/R) plus 15 from the inflows. Results from the variables measured are shown in *Figure 1.*

Secchi-disk (SD) readings were 1.6 to 1.8 m in the German river section and dropped to values between 0.7 and 0.9 m after the confluence with the River Inn. Visibility remained moderate in the Austrian section reaching 1.3 m in Wildungsmauer (rkm 1895). Secchi depth varied between 0.6 and 1.3 m throughout Slovakia, Hungary, Croatia and Serbia. Higher values of 1.3 to 1.7 m were reached following the confluence with the River Sava (SD 1.8 m) and remained high until both Iron Gate reservoirs had been passed. Maximum Sechi depth of 3.6 and 3.1 m occurred below the Iron Gate at Vrbica/Simijan, rkm 926 and upstream of the Timok (km 849). The rather turbid Timok (SD 0.9m) reduced Secchi depth which was further diminished by the inputs from the tributaries downstream of km 235 leading to readings of 0.8-0.9 m.

Both chlorophyll-a and phytoplankton biomass concentration remained below 10 µg L-1 chl-a or 2 mg L-1 algal biomass in the upstream section until km 1942 (Klosterneuburg, AT), below Budapest, HU (rkm 1632 – 1533) and downstream of rkm 1151 (Pancevo) as indicated in *Figure 1, panel 2 & 4.* Values higher than those occurred from Klosterneuburg (AT, km 1942) till upstream Budapest (HU) and between Baja (HU, km 1481) and downstream of the Sava (SR, km 1159). Highest concentrations of up to 31 µg L-1 chl-a or 9.9 mg L-1 biomass were reached in the Drava/Tisa region between km 1384 and km 1262.

Chlorophyll-a input from tributaries to the river Danube ranged from 3 µg L-1 chl-a in the Jantra to the exceptionally high 53 µg L-1 from the Morava *(Figure 1, panels 2 & 4).* Similarly, biomass ranged from 0.5 to 20 mg L-1.

The phytoplankton of the River Danube was dominated by diatoms (Bacillariophyceae) and green algae (Chlorophyceae) with a significant contribution from Cryptophyceae *(Figure 3, 3rd panel from top).* Their average contribution was 55.8, 22.3 and 16.5 %, respectively. Cyanobacteria were of minor importance in the river (4.6 % contribution). In the region of greatest phytoplankton development, diatoms and green algae together contribute about 90 % to total biomass. Centric diatoms are most abundant and quantitatively most important among the Bacillariophyeae, such as *Aulacoseira granulata, Skeletonema potamus* and *Melosira varians.* Although numerous benthic diatom species were identified their contribution to total biomass was negligible. A wide variety of green algal species from the order Chlorococcales (particularly the genera *Kirchneriella, Monoraphidium, Ankistrodesmus* and *Scenedesmus)* quantitatively contributed to phytoplankton biomass. Cyanobacteria were of greater importance in several of the tributaries such as Drava (8.5 %) and Timok (7.2 %). In the river Arges 41.6 % of the biomass originated from the Cyanobacterial species *Microcystis aeruginosa* and *Microcystis flos-aquae.*

Conclusions

The distribution of phytoplankton chlorophyll-a and biomass along the river corridor was significantly different from previous investigations. From the findings during JDS1 and JDS 2 three river sections had been defined: An upstream section with low values, a middle section where values increased to a maximum and a downstream section with generally low values. During the 2013 survey, these distinct sections were somewhat replaced by alternating sections of low and high concentrations. As previously, the highest chlorophyll and biomass concentrations occurred in the middle section of the river between km 1481 (Baja) and 1159 (downstream Sava). Different from earlier observations however, chlorophyll-a and biomass concentrations exceeded threshold values between Klosterneuburg (km 1942) and upstream of Budapest (km 1660). These high values most likely were a reflection of the heat wave preceding the investigation period and low discharge associated with. Both the concentrations of chlorophyll-a and the phytoplankton biomass are higher compared to 2007 during JDS2 particularly in the section between Vienna and Budapest. It must be emphasized however that direct comparison of chemical and biological concentrations of the two investigation periods might be inconclusive because of different hydrological discharge situations. The smaller concentrations during JDS2 can partly be a reflection of dilution due to higher run-off reported during this earlier survey.

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Fish Fauna

The JDS3 Survey on Danube Fish

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Introduction

In total 102 species of freshwater fish inhabited the Danube along its entire course, covering various ecological and functional guilds (Schiemer & Waidbacher 1992, Schiemer 2003, Eros et al. 2005). The introduction of new species is an ongoing process with about ten new species that have been recorded since 1992 upstream the Iron Gates as migrants from the lower Danube (Schiemer 2003). The appearance of new species is known to cause negative impacts on autochthonous species due to new parasites and diseases, but also leads to drastic changes in fish communities and food chains as a consequence of increased predation, competition for food and ecological requirements (Wiesner et al. 2010, Essl & Rabitsch 2003; Brandner et al. 2013). To investigate the abundance of allochthonous (alien) species along the Danube and to identify potential impacts of these alien species on the Danube fish fauna was one of the main goals of the fish survey during JDS3.

Methods

After the first fish ecological investigations along the entire Danube during JDS2 in 2007, the sampling effort and applied methods were increased during JDS3, where a core fish team, consisting of seven experts from Austria, Bulgaria and Hungary, sampled a total of 32 sites along the Danube. The investigation of the Danube fish fauna followed the joint approach of JDS2, combining fish sampling efforts of the core team with field investigations of the various national teams. The core team sampled the littoral area in the main Danube channel by electric fishing during day and night, and for the first time, the river bottom using an electrified benthic frame trawl net. The national teams mainly focused on additional electric fishing in the littoral zones and of the main channel and its tributaries and also used additional sampling methods (e.g. trammel nets) at some sites. In many cases experts from the core team joined the national teams and vice versa during sampling to facilitate the exchange of expertise and to learn about different sampling methods.

The sampling effort during JDS3 appeared to be feasible for a fish ecological investigation of a large river, despite the tight time schedule, some technical problems and occasio-nally unsuitable weather conditions. Due to the high sampling effort (day & night sampling), the fish core team had to be independent from the two lab ships and was based on a separate ship, the “Wien”, towing two additional smaller electro-fishing boats for the littoral and benthic fishing.

Electrified benthic frame trawl

As the electrified benthic frame trawl proofed to be a very effective additional sampling method for detecting species not caught by littoral sampling, a schematic picture of this fishing method is shown in *Figure 1.* For more details see Szalóky et al. (2014).

Results

Total catch

In total more than 139.000 individuals representing 67 fish taxa and one jawless species were caught during the JDS3 sampling by the core team (littoral and benthic) and national teams. Two species, namely bleak, *Alburnus alburnus*

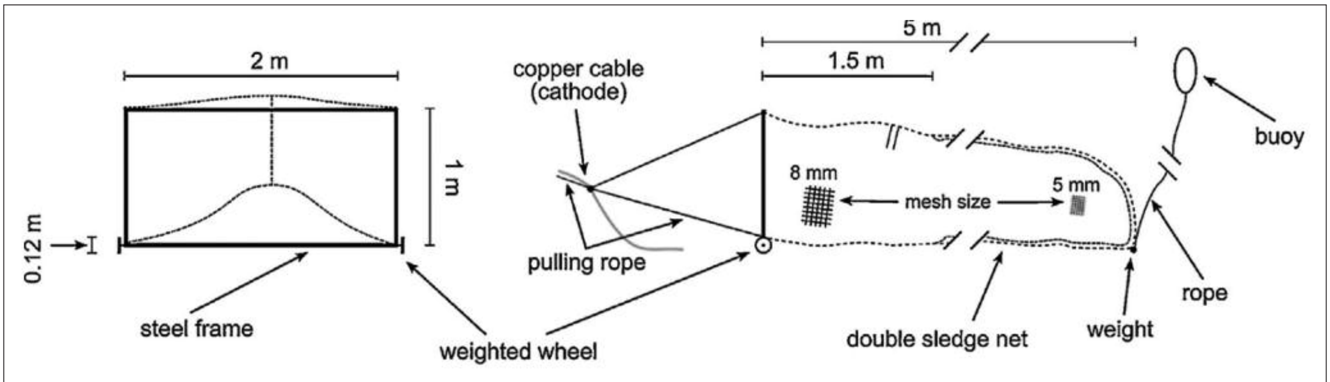

Figure 1. Schematic picture and parameters of the electrified benthic framed trawl. After Szalóky et al. (2014)

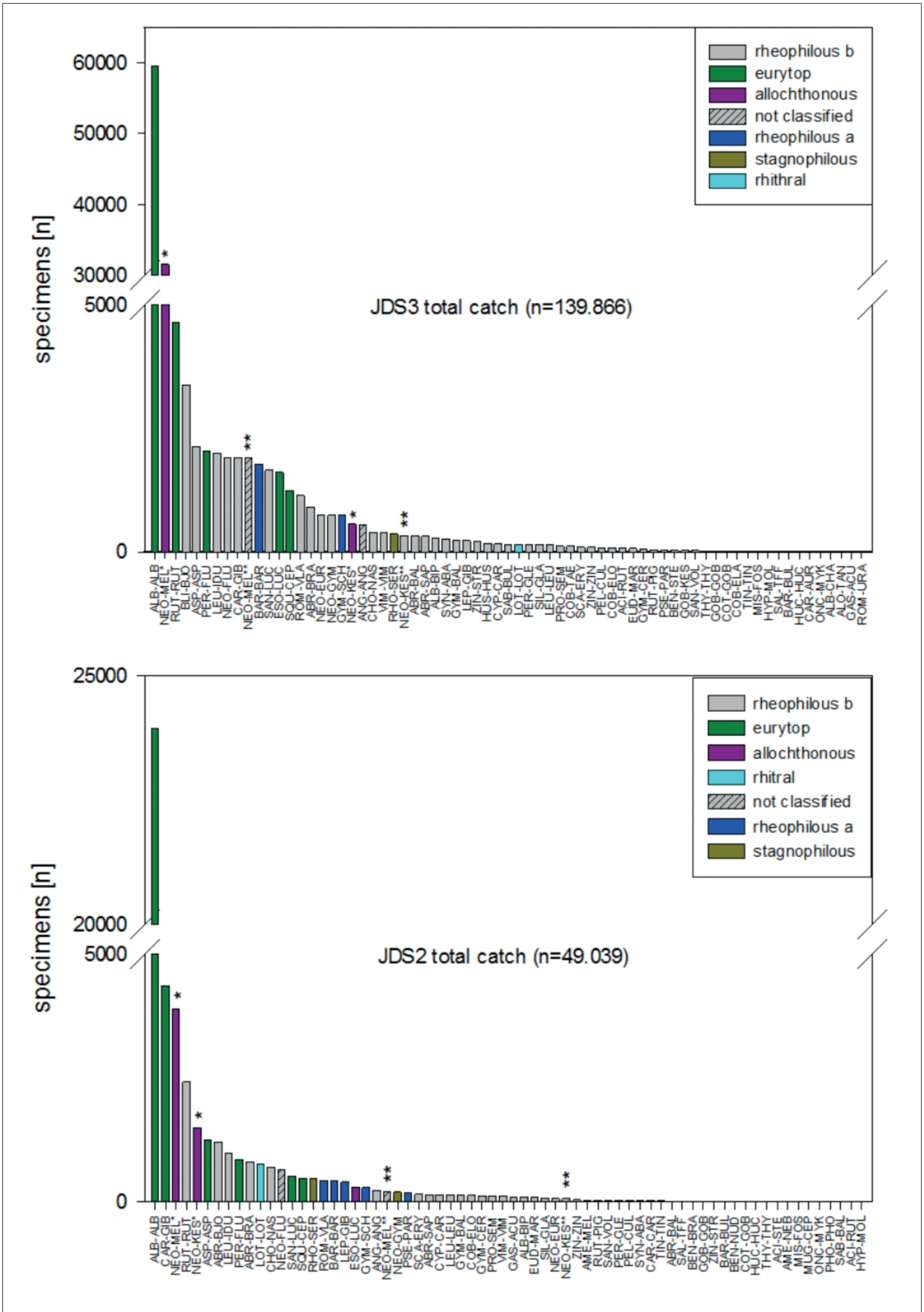

Figure 2. Total catch (individuals per species) of JDS3 (above) and JDS2 (below). Please note that the occurrence of Neogobius species is shown separately for the Danube downstream the Iron Gate dam, where it is autochthonous (indicated by a single star) and upstream, where it is allochthonous (indicated by a double star)

and round goby, *Neogobius melanostomus* dominated the catches by far *(see Figure 2)* with a relative proportion of 46 % and 26 % of the total catch, respectively. Most abundant (>55 %) were eurytop (ubiquitous) individuals, followed by allochthonous (alien) species (>30 %). A comparison with the total catch results of JDS2 shows that there has been a drastic shift of the total species frequency: allochthonous species, most notably the round goby, were caught more often during JDS3 than JDS2 outside of their range of natural occurrence (31.491 vs. 3.389 specimens). This underlines research results showing a dramatic, active distribution of the round goby in the Danube basin.

Figure 3. Acipenser ruthenus

Figure 4. Alburnus mento

Figure 5. Neogobius fluviatilis

Electrified benthic frame trawl

During JDS3 a total of 4445 specimens from 38 species could be collected by electrified benthic frame trawl samp-ling. The results show, that *Neogobius melanostomus* (36.5 % relative abundance) is the dominant species even in benthic habitats, followed by *Romanogobio vladykovi* (14.7 %) and *Blicca bjoerkna* (10.1 %). The relative abundance of the other species was below 10 %. Electrified benthic frame trawls caught species which could not be detected by other methods, like sterlet, *Acipenser ruthenus (Figure 3)* and Danube bleak *Alburnus mento (Figure 4).* Moreover, it could detect the monkey goby *(Neogobius flu-viatilis)* for the first time in the Austrian section of the River Danube *(Figure 5).*

Alien species

The proportion of allochthonous species to the total catch differed significantly between sampling sites upstream and downstream of the Iron Gate Dam. Between 2007 and 2013 the share of alien species has more than doubled from 17.8 to 37 % at sampling sites upstream the migration barrier, whereas downstream the dam a decrease from 2.6 to 0.3 % could be detected. *Neogobius melanostomus* was highly dominant outside its natural range of occurrence (above the Iron Gate) representing 56.7 % of all alien species in the entire Danube River during JDS2 and even 92.8 % during JDS3.

The share of the second most abundant allochthonous species, *Neogobius kessleri,* declined from 20.9 % in 2007 to 1.8 % in 2013. The abundance of other alien species can be seen as negligible.

Conclusions

The standardised representative data set collected by the core team during JDS3, based on the combination of two quantitative sampling methods, provides a sound basis for the comparison of different sampling methods and different assessment approaches. The additional sampling effort conducted by the national teams was essential for a concise description of the Danubian fish fauna.

Both the abundance and the quantitative proportion of alien species change along the course of the Danube reflected the differences in habitat types and shoreline structure (e.g. rip rap). Hence, allochthonous *Neogobius* species, especially the round goby, *N. melanostomus,* were found in high or even dominating abundance along the rip-rap protecting the river banks in the upper and middle reach of the Danube. Downstream the Iron Gate, where this habitat is not so common, the abundance of these species was much lower.

The electrified benthic frame trawl indicated the commonness of specific benthic species along the Danube and added valuable information which would have remained hidden using only shoreline surveys. It revealed the common occurrence and relatively high abundance of Zingel species, especially of *Z. streber* which occurred at 16 sampling sites with 127 individuals (cf. with all the other methods only 84 individuals were caught at 8 sites). To emphasize the importance of the application of the electrified benthic frame trawl, note that the JDS2 survey, without this method, could not prove the occurrence of *Z. streber* in the whole Hungarian river section of the Danube (Wiesner et al. 2007). This large scale spatial survey revealed that benthic offshore areas are intensively used by a variety of species which are distributed relatively homogenously along the entire river course. Their abundance and species composition, however, can vary largely among the sample stretches standardised by the JDS 3 sampling grid.

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Microbial life

The Microbiology Program of the Joint Danube Survey 2013

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Introduction

In rivers, microorganisms are a key component for the assessment of water quality. On the one hand, microbes – especially bacteria – represent the largest living surface and are responsible to a large extent for organic matter degradation (including self-purification processes), for steering carbon fluxes including CO_2 production and for ecosystem nutrient cycling. On the other hand, the extent of microbial faecal pollution from anthropogenic and natural sources has significant influence on the diverse ways of human water utilization (e.g. drinking water production, recreation, water used for industrial applications). In comparison to their importance, microorganisms are a neglected issue in the EU Water Framework Directive (EU-WFD, 2000).

During Joint Danube Survey in 2013 (JDS 3), implementing a comprehensive microbiological program covering both aspects of microbiological water quality emerged as a central objective in order to significantly advance the current state of knowledge in this underrepresented field.

Map of microbial faecal pollution

Within the frame of JDS3, a detailed microbiological water quality map of the Danube (including 16 tributaries/branches) was drawn and the “hot-spots” of microbial faecal pollution were identified. The faecal indicators *E. coli* and enterococci were measured on-board of the cruise ship using standardised methods. With the exception of the smaller tributaries/branches, samples were taken at each station from the left side of the river, from the middle and from the right side. For the microbiological program two sites were sampled in addition to the JDS 3 program (Inn River, and downstream of Vienna).

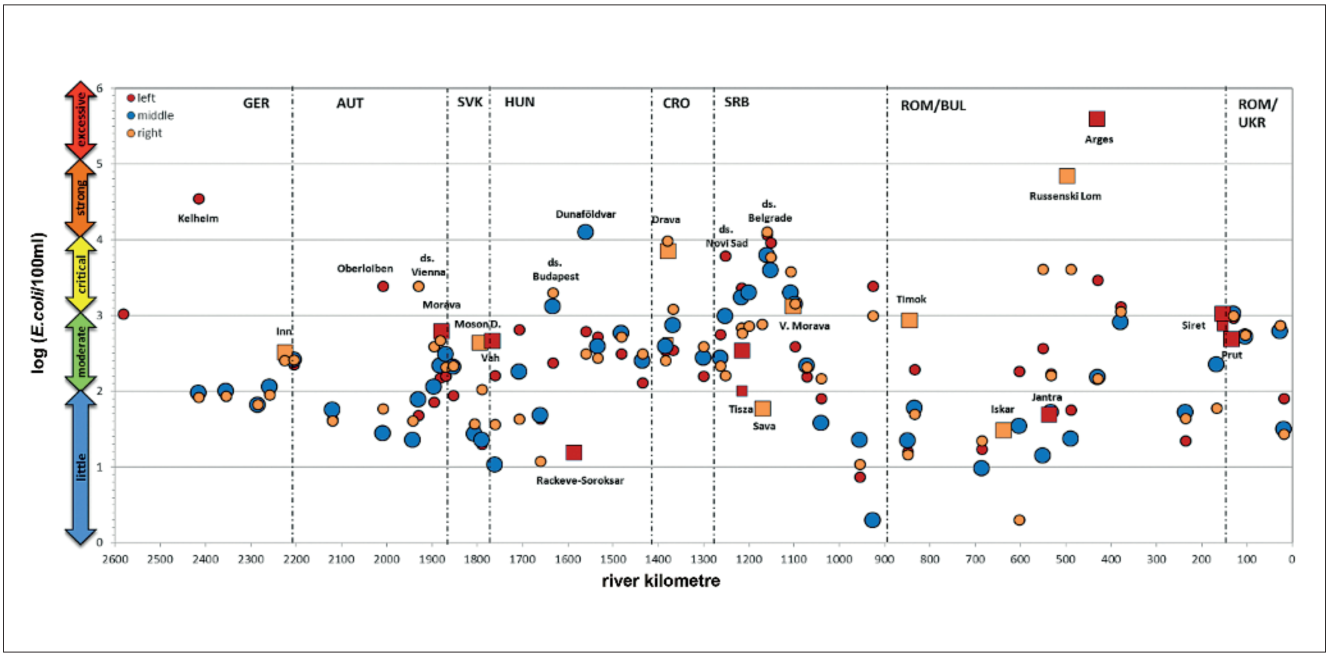

Figure 1. Concentrations of the faecal indicator bacterium E.coli along the Danube (circles) and in selected tributaries/branches (squares). Data are log-transformed: 1 = 10 E.coli / 100 ml, 2 = 100 E.coli / 100 ml, 3 = 1.000 E.coli /100 ml, 4 = 10.000 E.coli / 100 ml, 5 = 100.000 E.coli / 100 ml, 6 = 1.000.000 E.coli / 100 ml. The samples were taken left (red), middle (blue, large symbols) and right (orange) at all 54 Danube stations and at the tributaries Inn, Drava, Tisza, Save and Siret. Smaller tributaries/branches were sampled only in the middle. Tributaries merging at the left side of the Danube are marked red; tributaries at the right side are marked orange. Coloured arrows along the y-axis depict the level of microbial faecal pollution, from little (blue), moderate (green), critical (yellow), strong (orange) to excessive (red) pollution (figure taken from: Kirschner et al 2014a).

Figure 1 shows the *Escherichia coli* concentrations along the Danube, results for Enterococci gave a similar picture.

Fourty-two samples (of in total 186) were classified as critically (34), strongly (5) or excessively (3) polluted. The tributary Arges (Romania) and the branch Russenski Lom (Bulgaria) were identified as "hot spots" of excessive pollution. Surprisingly, the site with the highest contamination in the Danube occurred in the upper reaches in Kelheim (Germany, left river side, upstream of the confluence with the Rhine-Main-Danube Canal), a section with generally little to moderate pollution and state-of-the-art wastewater treatment. Additional hot-spots of faecal pollution in the Danube were the river sections downstream of Budapest (right side, Hungary) and Dunaföldvar (mid-river, Hungary), between Novi Sad and downstream of Belgrade (Serbia), as well as downstream of Zimnicea and the confluence with the Arges (both Romania).

At many sampling stations, the influence of wastewater was registered only at one of the two river sides, most prominently in Kelheim (left side), downstream of the town of Ruse (Bulgaria, right side) and downstream of the river Arges (left side). The same impairment of water quality, though less pronounced, was recorded in Oberloiben (Austria, left side), downstream of Vienna (Austria, right side), following the confluence with the river Vah (Hungary, left side) and downstream of the Iron Gate at Vrbica/Simijan (Serbia, Romania, both river sides).

However, it has to be stated that the results obtained during JDS3 have single measurement character and represent a "snap-shot" analysis of microbial faecal pollution. The data is able to depict the general trends along the Danube, but for definitive site-specific statements a more detailed analysis with temporal and spatial replicate sampling is necessary.

Tracking the origin of faecal pollution

Not only the amount of faecal pollution is crucial, but also – and most important – the specific determination of its origin, a research field called 'Microbial Source Tracking'. Not before having identified the main sources of pollution, one can counter-act with specific management measures.

In the case of the Danube with its high numbers of large municipalities and intensive agriculture in many parts of the catchment, it can be hypothesized that microbial faecal pollution is attributed to direct or indirect anthropogenic influence to a large extent. Other natural sources (wildlife, birds, fish) may play a role in specific regions, e.g. extensive nature conservation zones.

As part of JDS3 activities, a new set of genetic faecal markers was applied for microbial source tracking and the marker concentrations were determined for the first time along the whole Danube and its most important tributaries. Using this marker set it could be shown that microbial faecal pollution in the Danube is predominantly caused by human excreta *(Figure 2)*. Animal sources (pigs, cattle) obviously play only a minor role.

In detail, the linear regression analysis indicates that for all investigated samples 67% (HF183II) and 58% (BacHum) of the variation in E. coli concentrations could be explained with the concentrations of the human faecal markers. Again, this data set represents just an initial "snap-shot" analysis and must be corroborated by further in-depth investigations.

Self-purification

The microbial-ecological water quality component comprises – aside from bacteria – viruses and protozoa (heterotrophic flagellates, ciliates). Together, these microorganisms are building the basis for the effective degradation of organic material that is either produced autochthonously within the ecosystem by primary producers like planktonic and benthic algae or it is introduced into the ecosystem from allochthonous sources. These degradation processes are often denoted as "self-purification". The bacterial biomass that is built up is consumed to a large extent by protozoa which themselves are consumed by metazoa (mainly zooplankton and fish), representing an important nutritional source for the entire river ecosystem. The activities and the composition of the microbial populations and the factors controlling them are of prime importance for understanding the ecology of large rivers and the self-purification processes therein.

Therefore, different modern technologies were applied to the JDS samples like epifluorescence microscopy, the uptake of radioactive amino-acids (Velimirov et al 2011) or next generation sequencing (Savio et al 2015). For the first time, basic hypotheses on the self-purification processes and on the development of the natural micro-flora along the whole river could be formulated. It was intriguing to see that the bacterial community collected in the middle of the Danube was progressively unaffected by external sources (wastewater, tributaries) with increasing width of the river. This was observed for the bacterial faecal pollution patterns but also for the general bacterial population dynamics. For example, despite the many purified and un-purified wastewater inputs and other sources of pollution, the bacterial community in the middle of the river was progressively dominated by small coccoid cells *(Figure 3).* This can be interpreted as an adaptation to increasing nutrient deprivation.

Additional activities

In addition to the program outlined above, samples were taken for the determination of the distribution of antibiotic resistance along the Danube and for the in depth characterization of the bacterial communities at four representative sites with different anthropogenic influence (agriculture, industry, urban, best available site) via microbial metagenomics. However, it is beyond the scope of this article to present these studies here. Detailed reports on the results analysed so far can be found in Zarfel et al (2014) and Lettieri et al (2014), respectively.

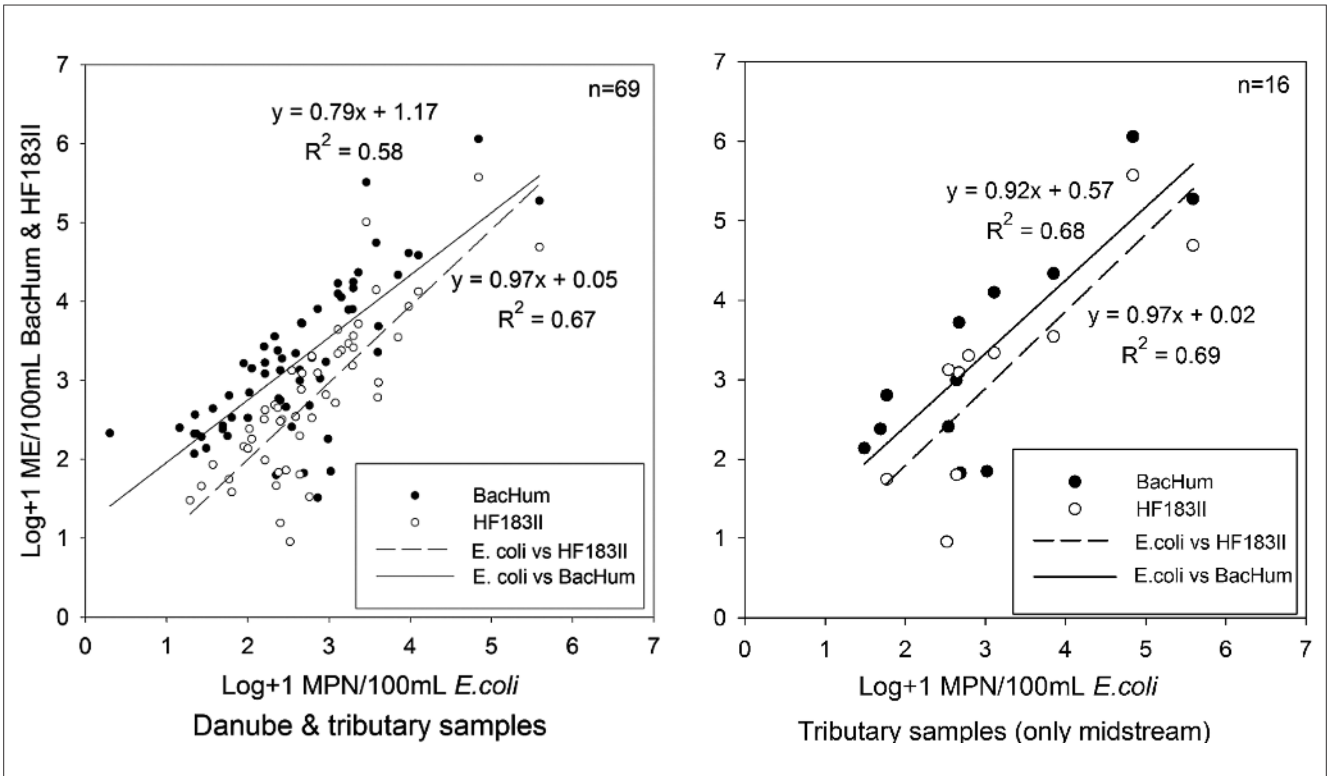

Figure 2. Regression analysis of human-associated genetic faecal markers (BacHum, HF183II) versus E. coli concentrations (as indicators of faecal pollution) in all investigated samples (left panel) and in the tributaries/branches (right panel). y-axis: logarithmic concentration of faecal markers in marker-equivalents (ME); X-axis: logarithmic concentration of E. coli bacteria in MPN (most probable number) units (figure taken from: Reischer et al 2014).

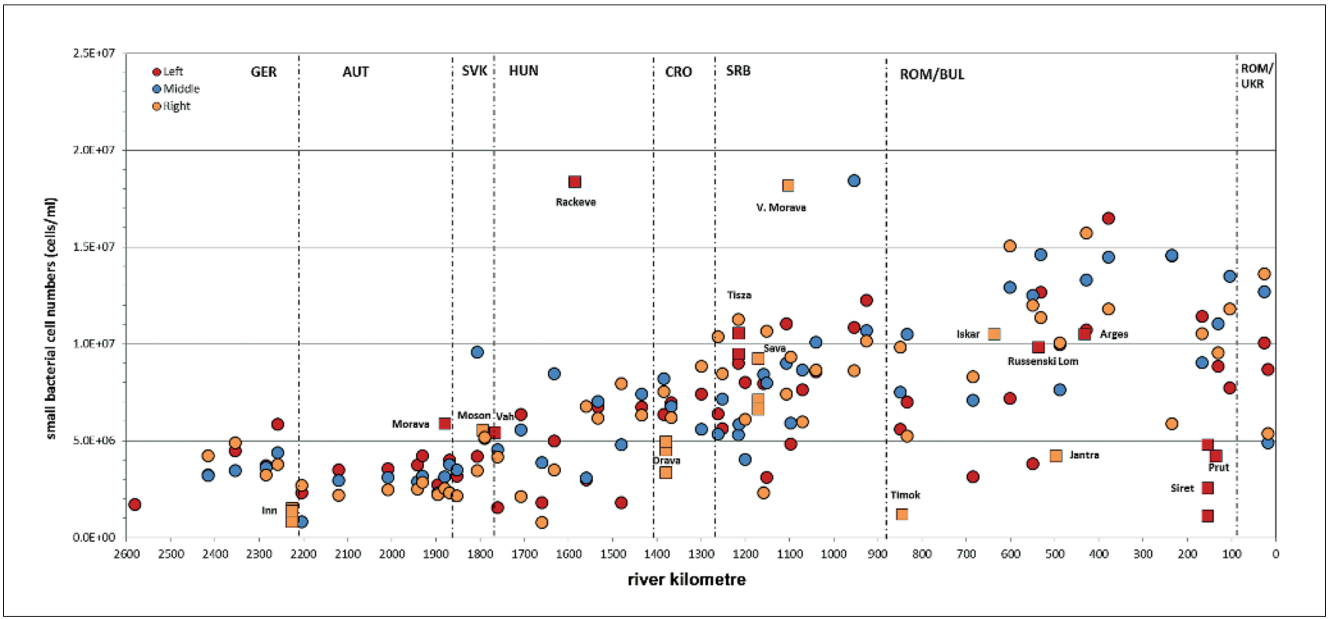

Figure 3. Increasing amount of coccoid bacterial cells (small cell numbers) along the Danube river (circles) and in selected tributaries/branches (squares). The samples were taken left (red), middle (blue, large symbols) and right (orange) at all 54 Danube stations and at the tributaries Inn, Drava, Tisza, Save and Siret. Smaller tributaries/branches were sampled only in the middle. Tributaries merging at the left side of the Danube are marked red; tributaries at the right side are marked orange (figure taken from: Kirschner et al 2014b).

Conclusion

The new insights will hopefully serve as a valuable basis for the sustainable management of the microbiological water quality of the Danube, and should stimulate more profound and detailed studies. The results from our investigations can thus be considered as highly relevant for the European water economy, especially for NGOs like the IAD (International Association of Danube Research), the IAWD (International Association of Water Supply Companies in the Danube River Catchment Area), the ICPDR (International Commission for the Protection of the Danube River) and/or the administration of the riparian countries.

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Hydrological catchment of the River Danube

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