# 5 Macroinvertebrates

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## 5.1 Introduction

Benthic macroinvertebrates are one biological quality element used within the Framework of the European Water Framework Directive (EC, 2000/60; WFD) to assess the ecological water quality and were therefore monitored in all previously conducted Joint Danube Surveys (JDS). The methods applied were differing due to availability of devices, financial issues and the scientific focus. While in JDS1 grabs were used to investigate hard rocky substrates (Literathy et al., 2002), in JDS2 air-lift samples were taken to study the faunal composition of deep water habitats (Liška et al., 2008). During JDS3 a modified Multi-Habitat-Sampling (MHS) approach has been 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 JDS3 can be seen as an important documentation of the current distribution of specific taxa and a completion regarding faunistics of earlier studies, (Russev, 1998; Slobodnik et al., 2005; Csányi & Paunovic, 2006) and of all previous JDS expeditions. The results will significantly contribute to the currently ongoing discussions regarding the WFD compliant assessment methods of large rivers either for field work as well as the analysing aspects.

## 5.2 Methods

### 5.2.1 Sampling

Sampling of benthic macroinvertebrates for JDS3 had three approaches carried out by three separate sampling groups:

Main approach:

– **Multi-Habitat-Sampling**, MHS: A standardised, WFD compliant method for the ecological (status) assessment (AQEM Consortium, 2002). Sampling of different habitats in the actual littoral zone was done with a Multi-Habitat-Sampling net (BOKU).

Additionally approaches:

– **Deep Water Sampling**, DWS: Cross-sectional survey by dredging in the deep water area (Laboratory of MTA (Hung. Acad. Sci.), Centre for Ecological Research, Danube Research Institute). This approach was decided for comparability reasons with the Airlift-data, a deep water sampling method which was applied during JDS2 in 2007.

– **Kick and Sweep Sampling**, K&S: Sampling with a hand net at the shore region (Siniša Stanković, University of Belgrade (IBISS)) in order to provide comparisons with the K&S data from JDS2.

The aim of the additional K&S sampling was to extend the investigated zone adding further mussel data to the results of the near-littoral MHS sampling program.

Sampling procedure and taxonomic resolution greatly influences the results of bioassessment (e.g. Birk et al., 2012; Hering et al., 2004). Therefore the standardised MHS approach was used for the ecological status assessment together with the DWS as well as to investigate habitat preferences of specific taxa. Samplings from the riparian zones are influenced by hydrological conditions. Therefore dredging (DWS) was used additionally to include deep water habitats of the Danube River. Until now only the Air Lift method provided systematic data on macroinvertebrates from the extended depths but the whole cross section of the river was not involved during former surveys (JDS1, AquaTerra, JDS2).

All three approaches are complementing each other, especially in terms of biodiversity and longitudinal distribution issues. Experiences of the JDS3 can therefore substantially contribute to the development of a comprehensive sampling methodology in large rivers.

#### 5.2.1.1 Multi Habitat Sampling (MHS)

The habitat specific macroinvertebrate sampling at the littoral zone was done with a Multi-Habitat-Sampling (MHS) net with a frame of 25 x 25 cm. This semi-quantitative instrument provides a sampling area of 0.0625 m² per sampling unit and is positioned upstream in the riverbed whereas the sediment in front of the frame is stirred up so that the animals are drifting into the collecting net with a mesh size of 500 µm and minimum lengths of 1 m. This method can be applied in wadeable zones up to a maximum water depth of 1.5 m.

The original method focuses on a multi-habitat scheme designed for sampling major habitats in proportion to their presence within a sampling reach. A MHS-sample consists of 20 "sampling units" taken from all habitat types at the sampling site, each with a share of at least 5% coverage (AQEM-consortium, 2002).

During JDS3 at each sampling site all available habitats, regarding substrate type, such as lithal banks (of different grain sizes), rip-rap zones, macrophytes, woody debris (xylal), etc. were sampled and stored separately. The habitat types were selected by surveying shore-lines by motor boat. For each defined habitat five sampling units were taken for statistical reasons. Additionally water-depth and flow velocity were taken for each sampling unit. The sampling units of a habitat were pooled and stored separately. In case of homogeneous substrate diversity, the same substrate type was sampled under different hydraulic conditions. In total a minimum of 20 sampling units, representing at least four different habitats per sampling site were taken. All samples were fixed with formaldehyde (final concentration: 4%).

On the basis of this methodology, two approaches can be conducted:

- habitat preferences of different macroinvertebrate taxa can be ascertained and
- one WFD-compliant MHS, consisting of 20 sampling units, can be combined for standard analyses (e.g. Saprobity).

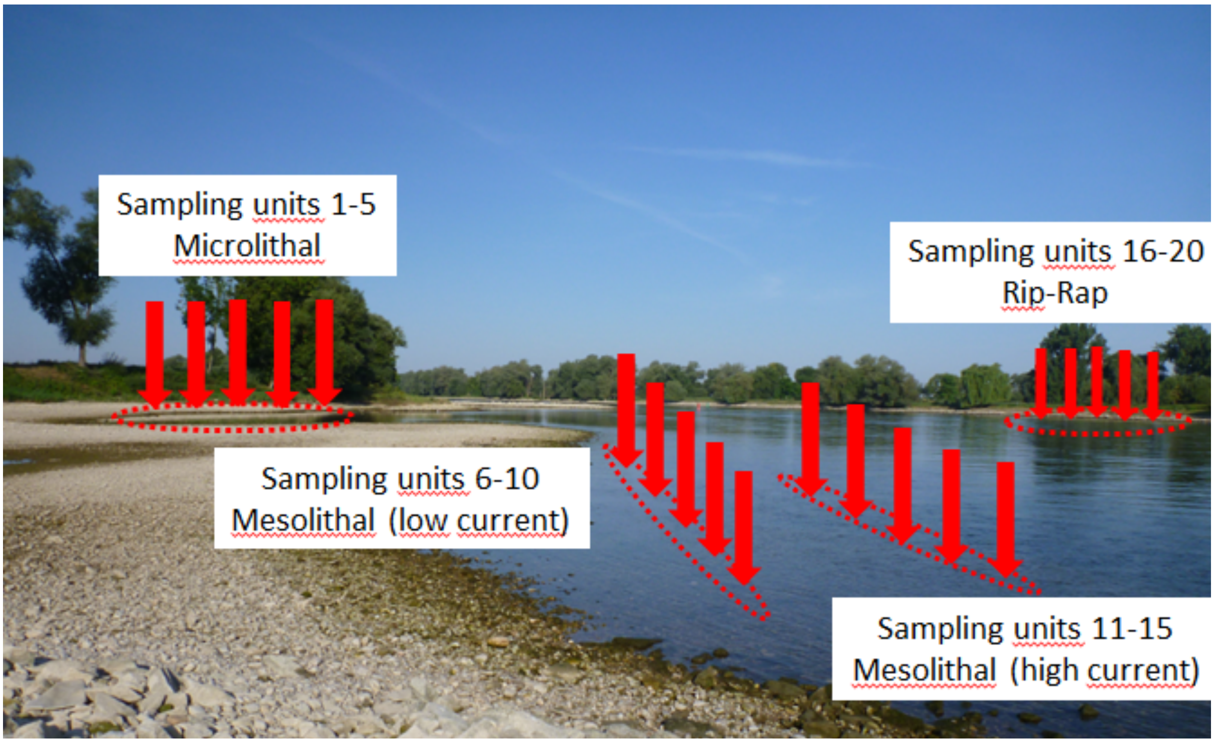


Figure 50: Habitat-specific sampling; example from JDS-site 5

The MHS methodology is based on the Rapid Bioassessment Protocols (Barbour et al., 1999), the procedures of the Environment Agency of England and Wales (Murray-Bligh, 1999), the Austrian Guidelines for the Assessment of the Saprobiological Water Quality of Rivers and Streams (Moog et al., 1999), ISO 7828, the AQEM sampling manual (2002), the AQEM & STAR site protocol (2002), the German methodology as described in www.fliessgewaesserbewertung.de, and the Austrian Standards M 6232 and M 6119-2.

### 5.2.1.2 Deep Water Sampling (DWS)

This dredging program provided rough information how the animal populations are distributed in the cross section the deep water space along the river bed.

Dredging was carried out with the help of the motor boat of the ARGUS. The iron-forked mouth of the triangle shaped dredge had a collecting net with 500 µm mesh size (Figure 51). Pulling the dredge was carried out with a rope downstream direction. The upstream-heading boat was driven backwards; so that the dredging was done from the frontal part of the boat. The dredging speed of the sampler on the bottom had to exceed the actual current velocity in order to avoid the washing out of the material from the net. The first 2 m of the pulling device was a heavy iron chain in order to keep the dredge horizontal on the bottom during dredging. We tried to keep the angle of the rope less than 25° during the procedure because this orientation made the dredge capable to dig in the bottom material efficiently.

Dredging locality was recorded with a GPS device, water depth was measured by hydro-acoustic equipment. The dredged material was filled into buckets marked with serial numbers I-V (Number I is near to right bank, II is far from right, III is in the middle, IV is far from left, V is near to left). Photos were taken to illustrate grain size distributions of the sample.

Usually 10 L of bed material was collected. Abundance data of dredging can theoretically be regarded as semi-quantitative: dredging 5 cm thick layer and 25 cm wide bed layer will provide this 10 l of volume if we pull the dredge roughly along a 80 cm long distance. This surface area (25x80 $cm^2$) represents 0.2 $m^2$. Thus the individual number of the sample multiplied by five roughly provides the individual number per square meter.

Figure 51: Bottom dredge with chain and rope for macroinvertebrate sampling

Deep water sampling was carried out in depths that are bigger than the wadeable, usually littoral (1.5 m) deep zone. The deepest part where the dredging was successfully applied was more than 20 m (Chilia arm).

#### 5.2.1.3 Kick and Sweep Sampling (K&S)

Kick & Sweep (K&S) sampling (EN 27828:1994) carried out in a wet diving suit was used in the near-shore region. This way the sampling depth was bigger than 1.5 m in the littoral zone (up to 2.0 m) A hand net with 500µm mesh size was used. Free diving was also done in order to increase the sampling depth principally for collecting more data on freshwater mussels (up to 4 m water depth).

However, the results of the three sampling methods are complementing each other: MHS data are used for status assessment, DWS and K&S data provide more information characterizing biodiversity and analysing the spatial-temporal distribution of native and invasive taxa.

### 5.2.2 Sorting and Identification

In case of the habitat specific macroinvertebrate sampling at the littoral zone, the samples collected from a defined habitat were stored separately for further determination in the laboratory at the BOKU in Vienna. After a curing time of at least 2 weeks the material of each sample was sorted completely. The animals were counted, separated into their specific orders and determined by taxonomic experts to the best level possible. Additionally the crustacean order Amphipoda and the Bivalvia genus *Corbicula* were divided into size-classes for further investigation.

The following taxonomic experts were involved:

MHS – Ferdindand Sporka (Oligochaeta); Peter Borza (Crustacea); Wolfram Graf (Plecoptera, Trichoptera), Thomas Huber (Ephemeroptera); Patrick Leitner (Simuliidae); Berthold Janecek (Chironomidae/Odonata)

The samples collected by dredging (DWS) and K&S were partially processed in the field. Reduction of sample volume was done by rinsing (mesh size 500 µm) to separate organic from mineral fractions. The material was preserved with 4% formaldehyde.

Further sorting of material collected by dredging was performed in the Laboratory of MTA (Hung. Acad. Sci.), Centre for Ecological Research, Danube Research Institute, while the sorting of material collected by K&S was done in the Laboratory of the Institute for Biological Research “Siniša Stanković”, University of Belgrade (IBISS).

The following taxonomic experts were involved:

DWS – Péter Borza (Crustacea); Béla Csányi (Mollusca, Hirudinea, Insecta); József Szekeres (Mollusca, Crustacea, Insecta); Ana Atanacković (Oligochaeta); Đurađ Milošević and Dubravka Čerba (Chironomidae)

K&S – Péter Borza (Crustacea); Ana Atanacković (Oligochaeta); Đurađ Milošević, Dubravka Čerba (Chironomidae); Jelena Tomović, Vanja Marković, Momir Paunović (Mollusca), Bojana Tubić, Momir Paunović (Insecta other than Chironomidae) and Stefan Anđus (Porifera).

### 5.2.3 Analyses

To ensure harmonised data storage the species-list per sampling unit including all measured parameters was filled into the Access-based software ECOPROF 4.0 (Moog et al., 2013), which is compatible with the ICPDR database. For the calculation of metrics and saprobic indices only WFD compliant (semi-)quantitative and area related approaches, represented by 20 combined sampling units (MHS-method) were used. Species list, diversity as well as cluster/NMS analyses for typological conclusions were based on all data collected during JDS3 including all habitat specific sampling units per site.

In the case of dredging and K&S method, data harmonization in respect to systematics was ensured using ASTERICS/PERLODES entering coding system. Coding system is principally harmonised with the ICPDR database and ECOPROF 4.0, which ensured comparability of the data.

#### 5.2.3.1 Saprobic index and calculation of metrics

##### 5.2.3.1.1 Saprobic Index

Saprobic indices based on the Fauna Aquatica Austriaca ed. by Moog (1995) were calculated based on available national methods using the software packages ECOPROF 4.0. and ASTERICS/PERLODES (www.fliessgewaesserbewertung.de). For calculations based on the Makovinska-catalogue (Sommerhäuser et al., 2003), a database has been created and linked with ECOPROF. For the calculation of saprobic indices based on German and Czech Standards, data have been exported to Excel and imported into the AQEM assessment software.

##### 5.2.3.1.2 WFD-compliant criteria for assigning the ecological status

Much information has already been compiled with respect to hydrobiological (reference) conditions in the Danube basin (e.g. 'WFD Roof Report' ANNEX 3: Typology of the Danube River and its reference conditions [ICPDR, 2005]). Nevertheless, currently no WFD-compliant metrics for large rivers have been officially defined or agreed (Buijs, 2006), the intercalibration procedure is still in progress (Birk et al., 2013, Schöll et al., 2012).

##### 5.2.3.1.3 Organic pollution

For monitoring the organic pollution the saprobic system has a long tradition – the WFD compliant implementation of this system is based on the deviation of the Saprobic Index from saprobic reference conditions (Stubauer & Moog, 2003; Ofenböck et al., 2010; Rolauffs et al., 2003). BMWP and ASPT are alternative indices that are widely used for assessment.

For the indication of water quality classes the threshold values of the Saprobic Index given in Table 6 were applied (Buijs, 2006). For the Upper Danube reach (from site 1 to site 8) the existing national classifications are used. In Germany the reference values are 1.80 for national type 9.2 and 1.85 for type 10 respectively (Rolauffs et al., 2003). In Austria the reference conditions are defined as 1.75 for ecoregion 9 (Stubauer & Moog, 2003) and 2.0 for ecoregion 11 which are changing between JDS site 8 and 9. Stubauer & Moog suggested in Sommerhäuser et al. (2003) a Saprobic Index of 2.0 as the highest threshold reference value for the Danube sections downstream. This value is consequently used as the saprobic basic condition for the Middle and Lower Danube reach. The same classification scheme was employed in the case of results obtained by the K&S sampling technique.

**Table 6: Threshold values for the indication of water quality classes based on organic pollution.**

| | Saprobic reference condition (range of Saprobic Index) | | | |
|---|---|---|---|---|
| Ecological status class | Germany national type 9.2 | Germany national type 10 | Austria Saprobic basic condition 1.75 | Austria Saprobic basic condition 2.0 |
| I – High | 1.65 – 1.80 | 1.75 – 1.85 | ≤ 1.75 | ≤ 2.00 |
| II – Good | 1.81 – 2.25 | 1.86 – 2.30 | 1.76 – 2.21 | 2.01 – 2.40 |
| III – Moderate | 2.26 – 2.85 | 2.31 – 2.90 | 2.22 – 2.68 | 2.41 – 2.80 |
| IV – Poor | 2.86 – 3.40 | 2.91 – 3.45 | 2.69 – 3.14 | 2.81 – 3.20 |
| V – Bad | >3.40 | >3.45 | >3.14 | >3.20 |

#### 5.2.3.1.4 General Degradation

Due to the absence of commonly agreed metrics for the assessment of large rivers, up to now the river quality of large rivers was mainly assessed by organic pollution. To achieve the demands for an integrated biological assessment for macroinvertebrates and to assess the ecological status of a water body the taxonomic composition, abundance, ratio of disturbance sensitive taxa to insensitive taxa, and the diversity of biological indicators, have to be considered and compared to respective target values under reference conditions. The aim of JDS3 was to find valuable biotic scores that can be integrated into future assessment systems.

Hence, the recently developed Slovak method for large rivers (Nariadenie Vlady Slovenskej republiky, 2012; Sporka et al., 2009) of catchment sizes >1000 km² (separated into altitude classes between 200 and 500 m and <200 m respectively) was tested with the MHS-data, calculating the ecological status by means of this national method that combines Saprobity and selected (degradation-) metrics for each river type. This assessment method was chosen because it was already tested with prior Austrian Danube data (Leitner, 2013) providing reasonably results. The Slovenian multimetric index (Urbanović, 2012) is based on an analogue functional metric and was not tested therefore separately. Additionally Marković et al. (2012) developed a multi-metric index for the Middle Danube region which was not analysed further because of its type-specificity.

All relevant metrics for the Slovak method for each river type and benchmarks are listed in the Full report on the attached CD.

### 5.2.3.2 Multivariate analyses

For the following analyses the JDS-sites 11, 13, 28 & 32 were excluded from the calculation because of questionable results due to increasing water level or bad status and accordingly under-represented taxa numbers.

For the MHS-data the following statistical methods were applied by using PC-Ord Software Version 5.33 (McCune & Mefford, 2006).

- Cluster analysis – Distance measure: Sørensen (Bray & Curtis) coefficient (Sørensen, 1948); group linkage method: Flexible Beta (Beta = -0.25)
- Non-metric Multidimensional Scaling (NMS; Kruskal 1964) – Distance measure: Sørensen (Bray-Curtis)
- Indicator Species Analysis (ISA) – Dufrene and Legendre's (1997) method.

Data for sampling sites obtained by the K&S techniques were analysed using Correspondence analyses (CA) by employing Flora Software package (KARADŽIĆ, 2013). Basic variant ordination with Singular Value Decomposition (SVD) algorithm was used (KARADŽIĆ, 2013), as more precise method when compared to the Weighted Averaging.

## 5.3 Results and discussion

According to the selected main sampling method the following chapters are based mainly on the evaluation of the MHS data set. Due to spatial limitations the detailed discussion of DWS and K&S data is given in the CD supplement of this Report.

### 5.3.1 Overall taxa richness

During JDS3 a total of 460 macroinvertebrate taxa were identified by three applied 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. 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 taxagroups were less diversified.

### 5.3.2 Diversity and abundances

The following statistics provide the data of the **MHS-samples** (20 subsampling units per site) representing only the taxa of the proportional estimation of habitats for each single site. Additional samples of under-representative habitats (<5%) are not included to avoid deviations of means due to varying numbers of samples.

In total the combined MHS-samples comprised 345 invertebrate taxa; including the additional habitat-samples (of habitats which were additionally sampled but proportionately under-represented at a certain site, such as deadwood) an overall number of 393 taxa were 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 are important but less diverse. Along the three reaches of the Danube, Trichoptera and Ephemeroptera are decreasing in diversity, all other groups are quite constant or showing a peak at the middle reach (Figure 52).

Regarding Amphipoda a high number of invasive species (*Chelicorophium curvispinum, C. robustum, C. sowinskyi, D. bispinosus, D. haemobaphes, D. villosus, Echinogammarus ischnus, E. trichiatus* and *Obesogammarus obesus*) was documented.

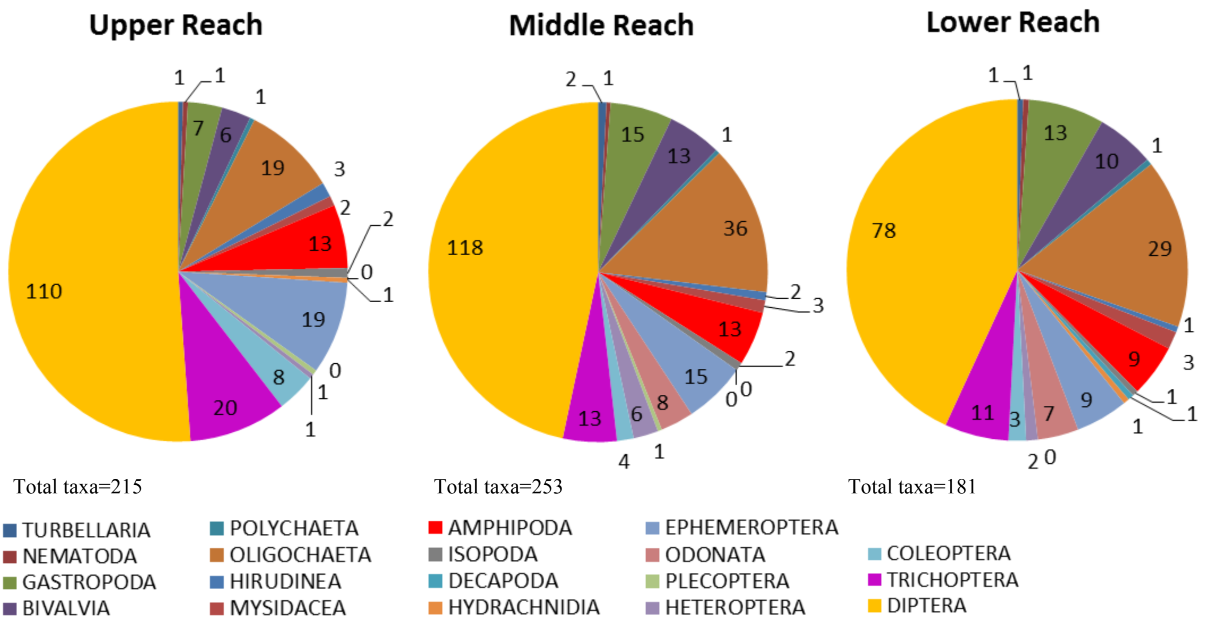


Figure 52: Number of taxa per taxa group along the different reaches of the Danube (MHS-Data)

Regarding abundance (ind./m²) Amphipoda are the dominant group in all Danube reaches and increase downstream (varying from 27 to 45%), while Diptera play an essential part in the Upper Reach (32%) and decrease 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 showed highest abundances within this group. Regarding aquatic insects, only Chironomidae play a major role along the whole Danube stretch (Figure 53).

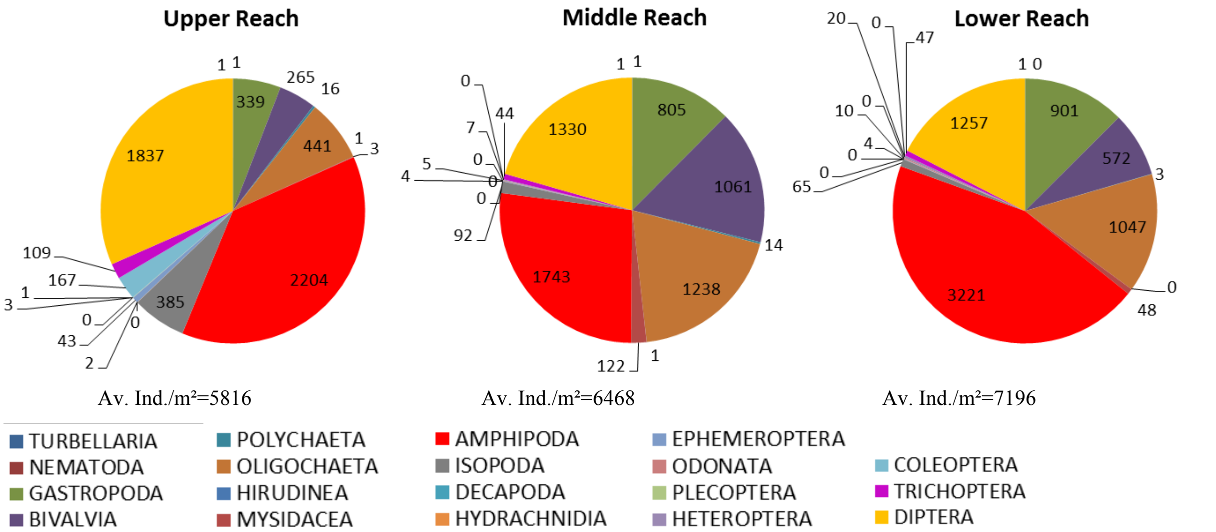


Figure 53: Average density (individuals/m²) per taxa group along the different reaches of the Danube (MHS-Data)

On the basis of the **DWS method** altogether 172 taxa were detected in 53 different cross sections (5dredges/site). The most abundant groups are Insecta (82 taxa, Chironomidae with 54 taxa) and Mollusca (15 Gastropoda- and 20 Bivalvia-taxa). The Annelida group contains 22 Oligochaeta, 7 Hirudinea taxa and one Polychaeta taxon. The 23 Crustacea-taxa are characterised by 8 Amphipoda, 7 Mysididae, 4 Coropiidae, 2 Decapoda and 1-1 Isopoda and Cumacea.

14 of these taxa are considered as invasive. Most of these species are of Ponto-Caspian origin. Their presence on the Lower Danube should be regarded as natural (native species for that reach). Only two taxa are relatively new in the Danubian Fauna: *Theodoxus fluviatilis* was firstly reported from the Budapest section of the Danube not long ago (Frank et al. 1990). Similarly, *Corbicula fluminea* was found at first in the lower Hungarian Danube in 1998 (Csányi 1998-1999) as a new species for Hungaria.

Based on the **K&S sampling** procedure, all together 282 macroinvertebrate species were identified. Aquatic insects were found to be the dominant component of the communities, with 160 taxa recorded.

The number of taxa per sampling site ranged from 13 (JDS32, Upstream Novi Sad) to 63 (JDS14, Gabčikovo Reservoir).

The number of taxa of the main taxonomic groups per sampling method is given comparatively in Figure 57.

### 5.3.2.1 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. For the following analysis all samples (also from proportionally under-represented habitats) taken by the MHS method were integrated.

The NMS scatterplot in Figure 54 (left) shows a distinct faunal gradient from fine (pelal to akal) to coarse substrates (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 stretch having a higher explanatory value regarding biological composition than the longitudinal distribution along the 3 reaches of the Danube (Figure 54, 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 the occurrence of specific taxa is predominantly habitat-determined.

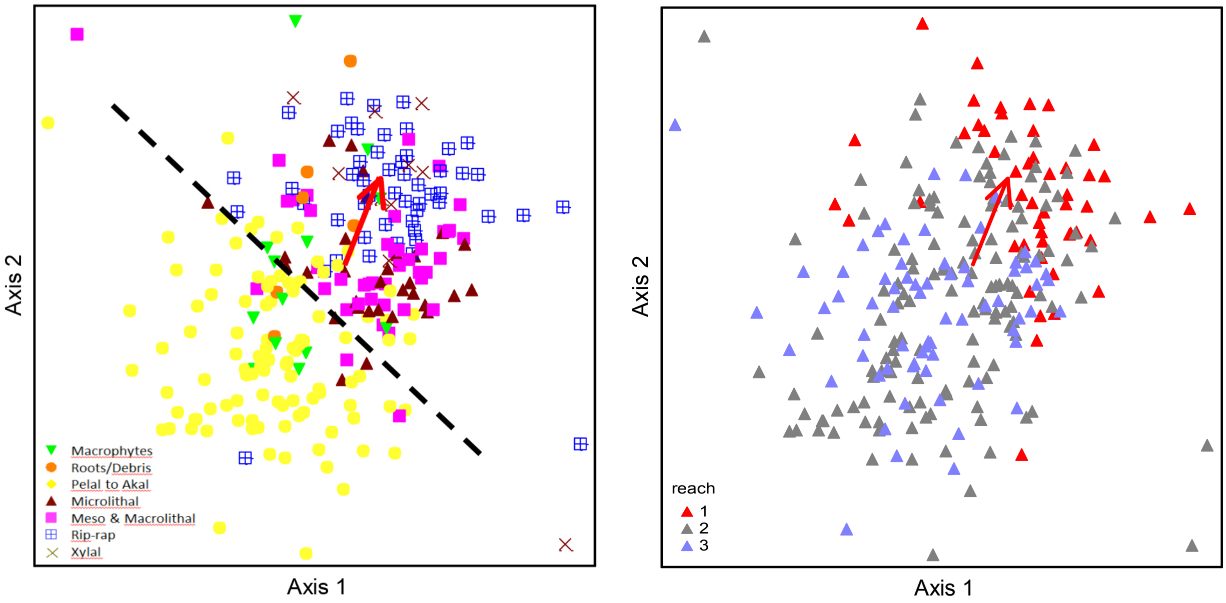


**Figure 54: NMS scatterplot, based on taxa assemblages per sample (each point represents a pooled habitat sample of 5 single units); overlay: substrate types, partly combined (left), Danube reaches (1=Upper, 2=Middle, 3= Lower Danube reach), (right); final stress for 3-d solution: 16.7, final instability: 0.00338, iterations: 250; red 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 for the defined substrate types are presented in Figure 56.

Organic habitats provide the highest numbers of indicator taxa, whereas Diptera, as the most frequent taxa group along the Danube, are dominating. 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 55).

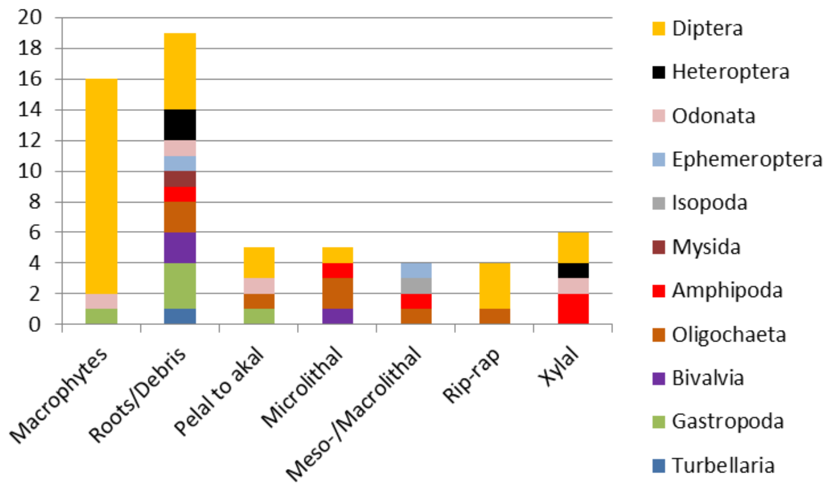


Figure 55: Significant indicator species per substrate type

Neozoa taxa reach 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 are found in organic habitats like macrophytes and roots/woody debris (Figure 56).

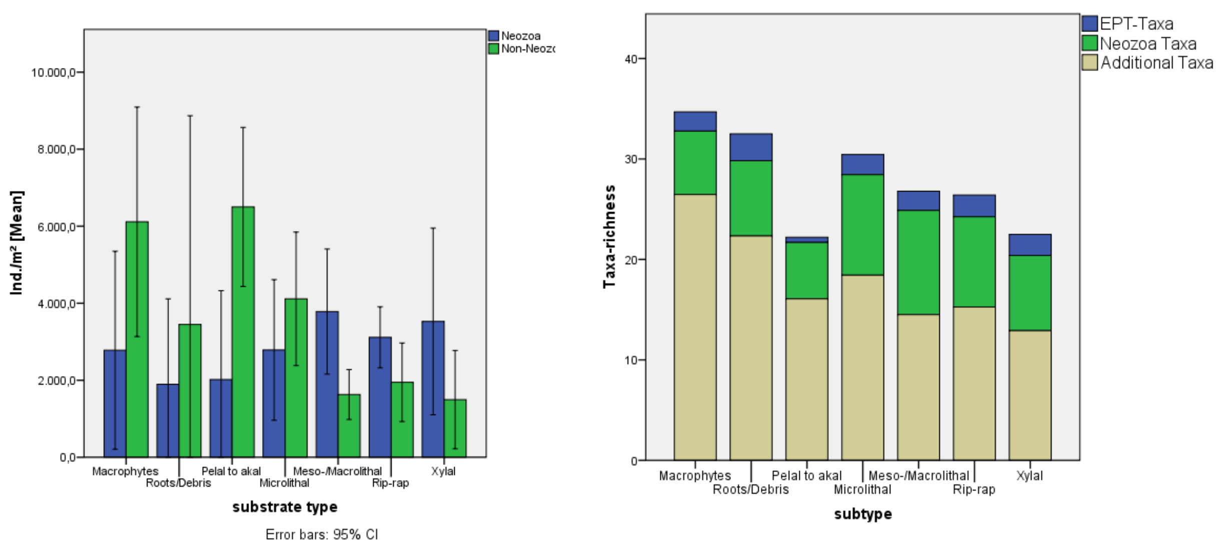


Figure 56: Average density of neozoa and indigenous taxa on different substrate types (left); Taxa richness and substrate type (right)

A more detailed analysis per section type and reach with a comprehensive splitting into all substrate types with detailed information about the indicator taxa is given in Full Report on macrozoobenthos on the attached CD.

### 5.3.3 Comparative analysis of the different applied methods

Large rivers consist of two distinct habitats: a lentic riparian zone and a much wider, non wadeable deep water area with higher water current. While margin habitats reveal more local conditions, the lotic environment tends to be shaped by the whole catchment. MHS and K&S were performed in the wadeable zones, DWS focused on the deeper, lotic habitats.

A comparison of the three sampling methods of JDS3 is given in Figure 57.

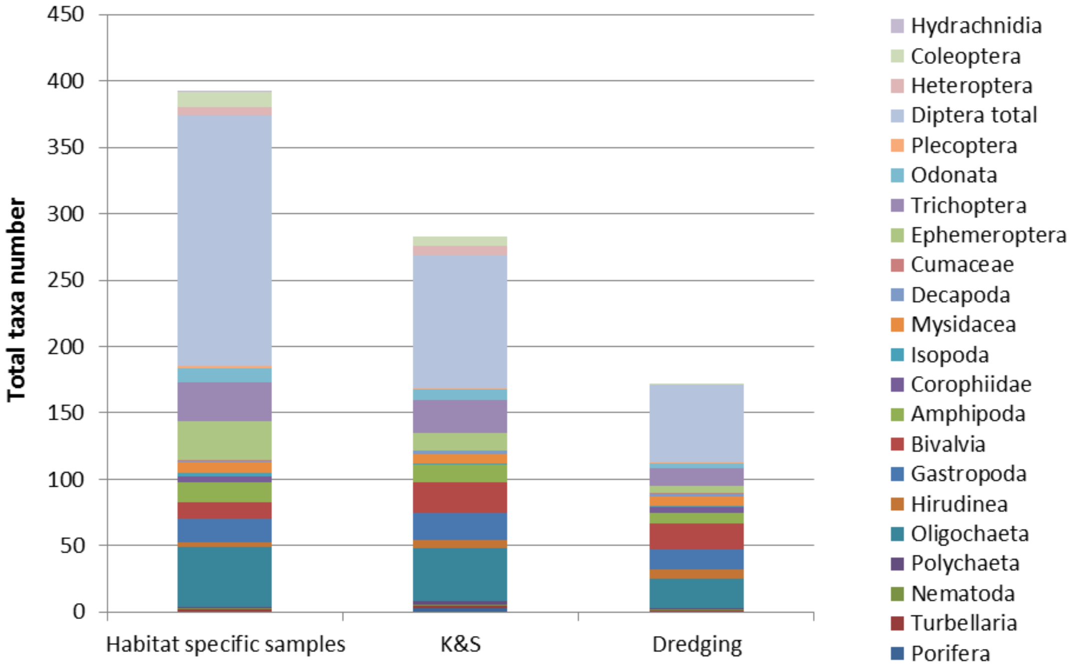


**Figure 57: Number of taxa per taxonomic group recorded by habitat specific sampling method, K&S and Dredging**

Less taxa were detected in the lotic deep water region (DWS) than either by MHS or K&S sampling in the littoral wadeable zone. This can be explained by the fact that deep water sections of large rivers are generally less densely and diversely colonized mostly caused by instable sediment conditions (Moog et al., 2000; CSÁNYI et al. 2012).

These results are confirmed by comparing MHS data from JDS3 with the Airlift data from JDS2 Figure 58 (left). The number of taxa shared by both methods is 220 only, which is quite low compared to the total taxa number. It indicates that each method provides a unique fauna – a deep-water fauna and a riparian related fauna. The allocation of the samples into the 3 main Danube reaches shows comparable accuracy; faunas from both methods indicate a similar gradient regarding longitudinal zonation (Figure 58, right).

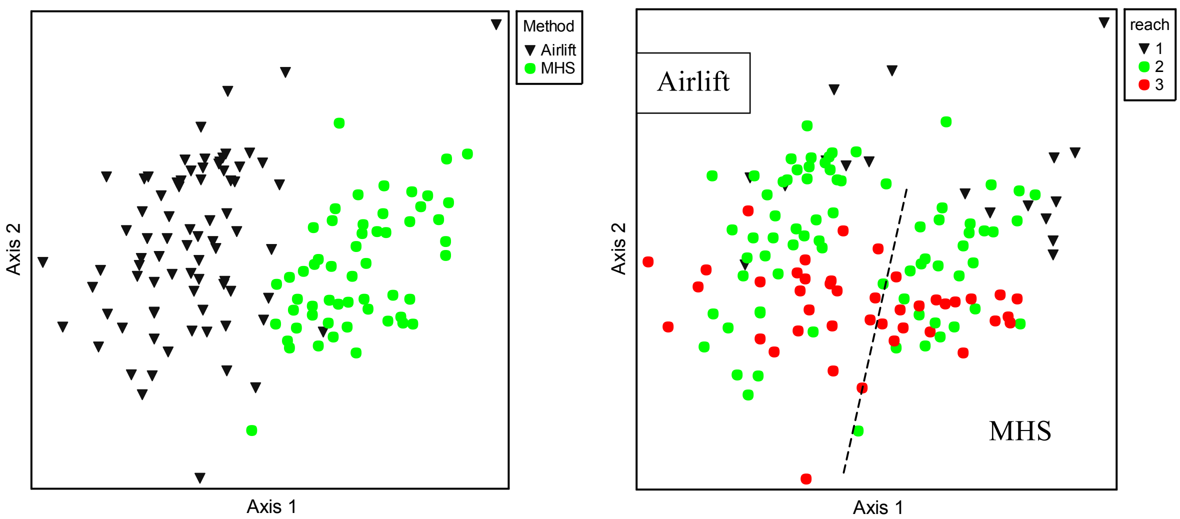


**Figure 58: NMS scatterplot based on taxa assemblages of the Airlift method (JDS2) compared to MHS data (JDS3); overlay: sampling method (left), Danube reaches (right); final stress for 3-d solution: 14.56, final instability: 0.000, iterations: 194**

Neale et al. (2006) compare the effectiveness and suitability (regarding the assessment system of Great Britain) of available techniques for sampling invertebrates in deep rivers (airlift, dredge, margin samples and long-handled pond net). They recommend the air-lift as the most suitable method but explicitly state: "to permit the effective assessment of river quality at deep water sites, sampling activity should target deep water habitats and margin habitats".

This is underlined by findings of JDS3. The combination of all habitat-specific approaches provides a more comprehensive insight in the faunal composition of a specific site for large lowland rivers. As JDS3 focuses equally on issues like ecological status, biodiversity and documentation of invasive species the precise study objectives are prerequisite for methodological recommendations.

Further discussion on all three spatial aspects of the macroinvertebrate community collected by the different sampling methods is provided in the Full Report on macrozoobenthos on the attached CD.

### 5.3.4 WFD-compliant criteria for assigning the ecological status

The lack of appropriate methods to assess the ecological status in large rivers like the Danube is a fundamental obstacle in implementing the WFD compliant monitoring (Birk, 2003). In the past the river quality was mainly evaluated by assessing organic pollution. To achieve the demands of the WFD for an integrated biological assessment of macroinvertebrates and to assess the ecological status of a water body, further attributes of the species assemblage have to be considered and evaluated.

As already applied and proved in several EU member states a modular assessment system is recommended (Ofenböck et al., 2010; Hering et al., 2004; Birk et al., 2012) for the biological quality indicator 'benthic invertebrates' based on:

1. the assessment of **organic pollution** (saprobic condition) and
2. the assessment of the **general degradation** (hydromorphological and hydrological impact like damming, impoundment etc.) e.g. using multimetric indices (MMI) or predictive models.

#### 5.3.4.1 Organic pollution

For monitoring the organic pollution the saprobic system has a long tradition – the WFD compliant implementation of this system is based on the deviation of the Saprobic Index from saprobic reference conditions (Stubauer & Moog, 2003; Ofenböck et al., 2010; Rolauffs et al., 2003). It has to be clearly pointed out that a WFD compliant assessment of the ecological status based exclusively on saprobic indices can provide only a rough indication of the status as several other pressures are not revealed by assessment tools based on saprobic systems.

The data gathered by MHS method (JDS3) were analysed using all available national systems of saprobic indices and transferred to water quality classes and are given for each single site investigated during both surveys in comparison with Airlift from JDS2 (Table 7). During JDS3 all saprobic classes from high to bad status were assessed. Serious organic pollution was detected upstream Novi-Sad (indicating bad status). Saprobically "poor status" was indicated upstream Drava, downstream Velika Morava and at Vrbica/Simjan in the Irongate reservoir.

In some cases questionable results – underlined by a statistically under-represented number of total taxa – were obtained due to rising water level (Table 7, indicated by italics).

A proportion of 73% (=40 sites) of all 55 sampling sites can be classified as "indication of good ecological status", nine sites (16%) as "indication of moderate ecological status" and two sites (4%) actually as "high ecological status" according to the WFD.

During JDS2, the highest values of Saprobic Indices indicating serious organic pollution (poor status) were detected downstream Pancevo and at Giurgeni. Regarding organic pollution 74% (=58 sites) of all 78 sampled Danube sites were classified as "indication of good ecological status" according to the WFD. For eight sites the SI showed an "indication of moderate ecological status", for three sites "poor ecological status" and for nine a "high ecological status" was indicated.

Compared to the JDS2 data, the proportions of sites per status class are generally comparable, although a change of the quality class is detected at certain sites. About 60% of the shared sampling sites at both surveys indicate the same status; at 12% of the sites a better ecological status is indicated and at 28% of the sites a worse status. This must not be interpreted as an aggravation of organic pollution; it is a result of the applied methodologies: Airlift samples are usually taken at higher depths in lotic parts of the river which are colonised by a different fauna than riparian zones. Saprobic Indices of both faunas (riparian and lotic) show a similar range but abundances of saprobic indicators are different regarding the two methods (Figure 59) leading to deviations of the overall ecological status. In a case study at the Austrian Danube Moog et al. (2000) found similar results comparing Saprobic Indices from cross-sectional samples.

As mentioned earlier riparian habitats provide information on more local conditions, deep water areas reveal the overall characteristics. Both habitats are essential for ecological processes and the functioning of the ecosystem. We therefore propose a worst-case approach to overcome this dilemma and to include indications in a holistic way.

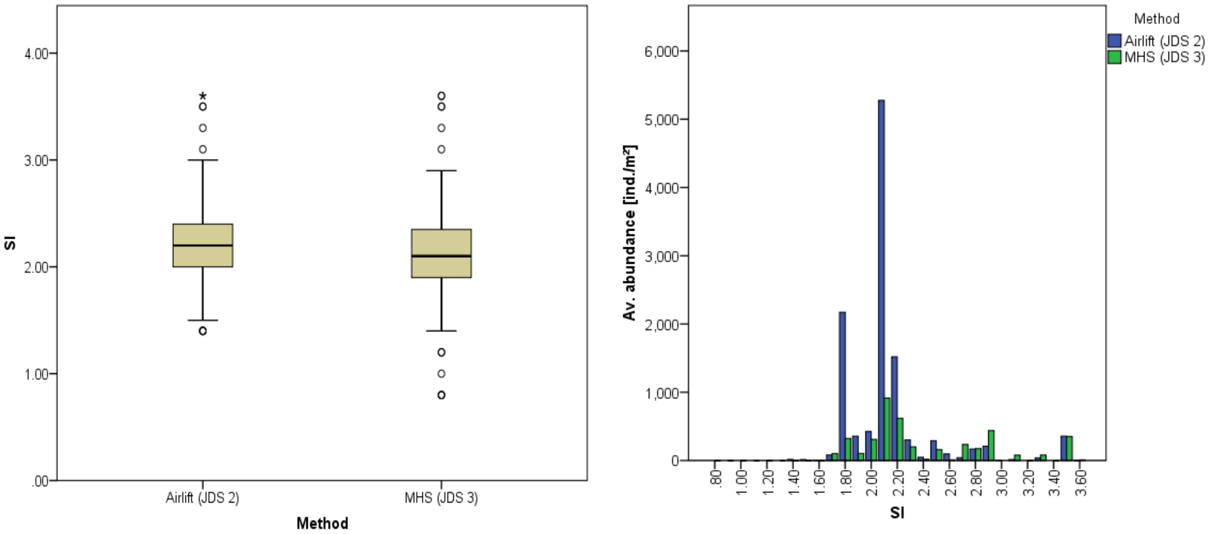


**Figure 59: Boxplots of Saprobic Indices of all classified taxa found during JDS2 by Airlift method and JDS3 by MHS method (left); average abundances [ind./m²] of taxa per Saprobic Index class of all samples per method (right)**

### 5.3.4.2 General Degradation

The results of the Slovak method for large rivers applied for the JDS3 MHS-data (Table 7) indicate quite balanced ecological classes of good (26 sites) and moderate (27 sites) status. Only Klosterneuburg indicates class 1 (high status) and site 32 upstream Novi-Sad class 4 (poor status). The results are thoroughly comprehensible as the sampling site Klosterneuburg provided a high variation of different substrate types and current velocity classes and therefore a diverse fauna sharing a comparatively high number of (EPT-) taxa. At Novi-Sad the Saprobic Index already indicated an alteration compared to other sites.

On the basis of this method the morphological high degraded sites (channelized or impounded, with rip-rap dominating at the shore zones) in the Upper Danube reach indicate moderate status, while sites with less morphological impact, providing adequate gravel banks, indicate generally good status. The parameter saprobity only indicates quite constantly a good status in the Upper reach not capturing hydromorphological degradation. The results implicate that the general degradation of large rivers can be largely covered by this assessment method. A compatibility of the Slovak method in the Lower Danube reach has to be further tested and possible adaptations of boundary values have to be critically revised due to the fact that the environmental conditions show a distinct change along the Danube stretch and deviate considerably from reference conditions used by the Slovak method.

Marković et al. (2012) report on moderate ecological status at 7 sampling sites along the Iron Gate reservoir (rkm 849-1,077) by using 7 selected metrics. This partly deviates from the JDS3 results which are ranging between good and poor status (MHS) in this certain stretch.

More details are given in the Full Report, whereas this information could be used to implement a multimetric index in a national assessment method or within the Danube intercalibration process.

**Table 7: Saprobic indices (SI) and indication of water quality classes for all Danube sites;** results from JDS2 (Airlift) in grey, results from JDS3 (MHS, DWS and the multimetric Slovak method for large rivers (SK)) in black; Country specific Saprobic Indices were applied for the German, Austrian and Slovakian stretch; for all other countries the Romanian SI was calculated; values and indications of water quality based on under-represented (less than 10 taxa for DWS and JDS2 data; less than 27 taxa for Upper Danube reach and less than 20 for Middle and Lower Danube reach following standardised residuals for MHS data) indicator taxa are scientifically questionable and written in italic.

| JDS3JDSrkm [JDS2/JDS3] | | Site no. [JDS2/JDS3] | | Sampling Site | Saprobic basic condiciton | JDS2 Airlift SI | JDS2 Airlift Class | JDS3 MHS SI | JDS3 MHS Class | JDS3 DWS SI | JDS3 DWS Class | JDS3 SK Class |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2599.8 / | | 1 / | | Donaurieden | 1.65 | 1.94 | II | | | | | |
| / | 2581 | / | 1 | Böfinger Halde | 1.75 | | | 2.08 | II | | | 2 |
| 2412.4 / | 2415 | 2 / | 2 | Kelheim – gauging station | 1.75 | 2.23 | II | 2.14 | II | | | 2 |
| / | 2365 | / | 3 | Geisling power plant (upstream) | 1.75 | | | 1.94 | II | 2,19 | II | 3 |
| 2353.5 / | | 3 / | 3A | Geisling power plant (downstream) | 1.75 | 2.2 | *II* | 1.88 | II | 2,15 | II | 3 |
| 2287 / | 2285 | 4 / | 4 | Deggendorf | 1.75 | 2.18 | II | 1.93 | II | 2,14 | II | 3 |
| 2278 / | | 5 / | | Niederalteich | 1.75 | 2.16 | II | | | | | |
| / | 2258 | / | 5 | Mühlau | 1.75 | | | 1.90 | II | 2,10 | II | 2* |
| 2203,5 / | 2205 | 7 / | 6 | Jochenstein | 1.75 | 2.31 | III | 2,33 | III | 2,95 | *IV* | 4 |
| 2120,5 / | 2121 | 8 / | 7 | Upstream dam Abwinden-Asten | 1.75 | 2.12 | II | 2.18 | *II* | 2,11 | *II* | *3* |
| 2062 / | | 9 / | | up. KW Ybbs/Persenbeug | 1.75 | 2.2 | II | | | | | |
| 2007.5 / | 2007 | 10 / | 8 | Oberloiben | 1.75 | 1.87 | II | 2.00 | II | 2,02 | II | 3 |
| 1950.6 / | | 11 / | | Greifenstein | 2.00 | 2.54 | III | | | | | |
| 1942 / | 1942 | 12 / | 9 | Klostemeuburg | 2.00 | 1.84 | I | 2.06 | II | 2,19 | II | 1 |
| 1895 / | 1895 | 13 / | 10 | Wildungsmauer | 2.00 | 1.83 | I | 2.03 | II | 2,12 | II | 2 |
| 1881.9 / | 1882 | 14 / | 11 | Upstream Morava (Hainburg) | 2.00 | 1.95 | *I* | 2.02 | *II* | 2,16 | II | 2 |
| / | 1868 | / | 13 | Bratislava | 2.00 | | | 2.20 | *II* | 2,25 | *II* | 2 |
| 1865 / | 1865 | 16 / | 13A | Bratislava (downstream) | 2.00 | 2.27 | II | 2.30 | II | 2,23 | II | 2 |
| 1851.5 / | 1855 | 17 / | 14 | Gabcikovo resevoir | 2.00 | 2.3 | *II* | 2.27 | II | 2,25 | II | 2 |
| 1806 / | 1806 | 18 / | 15 | Medvedov/Medve | 2.00 | 2.09 | *II* | 2.03 | II | 2,20 | II | 2 |
| 1794 / | | 19 / | | Mosoni Danube | 2.00 | 2.84 | IV | | | | | |
| / | 1790 | / | 17 | Klizska Nema | 2.00 | | | 2.05 | II | 2,24 | II | 2 |
| 1768 / | | 20 / | | Komarno | 2.00 | 2.11 | II | | | | | |
| 1761 / | 1761 | 22 / | 19 | Iza/Szony | 2.00 | 2.09 | *II* | 2.13 | II | 2,08 | II | 2* |
| 1719 / | | 23 / | | Esztergom | 2.00 | 2.12 | II | | | | | |
| 1707 / | 1707 | 26 / | 20 | Szob | 2.00 | 2.11 | *II* | 2.12 | II | 2,02 | II | 2 |
| 1692 / | | 27 / | | Szetendre Island | 2.00 | 2.11 | II | | | | | |
| 1692 / | | 28 / | | Szetendre Island arm | 2.00 | 2.15 | *II* | | | | | |
| 1659 / | 1660 | 29 / | 21 | Budapest upstream – Megyeri Bridge | 2.00 | 2.07 | II | 2.16 | II | 2,05 | II | 3 |
| 1658 / | | 30 / | | Budapest up. Sidearm | 2.00 | 2.09 | *II* | | | | | |
| 1632 / | | 31 / | | Rockere-Sorokser Sidearm | 2.00 | 2.31 | II | | | | | |
| 1632 / | 1630 | 32 / | 22 | Budapest downstream – M0 bridge | 2.00 | 1.94 | I | 2.44 | III | 2,08 | II | 3 |
| 1598 / | | 33 / | | Adony/Lorev | 2.00 | 2.12 | II | | | | | |
| 1586 / | | 34 / | | Rockere-Sorokser Arm end | 2.00 | 2.28 | II | | | | | |
| 1560 / | 1560 | 35 / | 24 | Dunafoldvar | 2.00 | 2.06 | *II* | 2.13 | II | 2,38 | II | 2 |
| 1533 / | 1532 | 36 / | 25 | Paks | 2.00 | 2.26 | II | 2.24 | II | 2,11 | II | 2 |
| 1481 / | 1481 | 38 / | 26 | Baja | 2.00 | 2.35 | II | 2.06 | II | 2,01 | *II* | 2* |
| 1434 / | 1434 | 39 / | 27 | Hercegszanto | 2.00 | 2.23 | *II* | 2.17 | *II* | 2,05 | *II* | *3* |
| 1424 / | | 40 / | | Batina | 2.00 | 2.13 | *II* | | | | | |
| 1384 / | 1384 | 41 / | 28 | Upstream Drava | 2.00 | 2.2 | *II* | 3.05 | *IV* | 2,03 | *II* | *3* |
| 1367 / | 1367 | 43 / | 30 | Downstream Drava (Erdut/Bogojevo) | 2.00 | 2.17 | II | 2.51 | III | 2,16 | *II* | 3 |
| 1355.3 / | | 44 / | | Dalj | 2.00 | 2.2 | *II* | | | | | |
| 1300 / | 1300 | 45 / | 31 | Ilok/Backa Palanka | 2.00 | 2.13 | *II* | 2.27 | II | 2,14 | II | 3 |
| 1262 / | 1262 | 46 / | 32 | Upstream Novi-Sad | 2.00 | 2.25 | II | 3.32 | V | 2,00 | II | 4 |
| 1252 / | 1252 | 47 / | 33 | Downstream Novi-Sad | 2.00 | 2.15 | II | 2.33 | II | 2,01 | II | 3 |
| 1216 / | 1216 | 48 / | 34 | Upstream Tisa (Stari Slankamen) | 2.00 | 2.16 | *II* | 2.41 | *III* | 2,10 | II | *3* |
| 1200 / | 1199 | 50 / | 36 | Downstream Tisa/Upstream Sava | 2.00 | 2.11 | *II* | 2.03 | *II* | 2,01 | II | *2* |
| / | 1159 | 52 / | 38 | Upstream Pancevo/Downstream Sava | 2.00 | 2.22 | II | 2.12 | II | 2,13 | *II* | *3* |
| / | 1151 | 53 / | 39 | Downstream Pancevo | 2.00 | 3.09 | IV | 2.41 | III | 2,10 | II | 2 |
| / | | 54 / | | Grocka | 2.00 | 2.29 | *II* | | | | | |
| / | 1107 | 55 / | 40 | Upstream Velika Morava | 2.00 | 2.26 | *II* | 2.62 | III | 2,48 | III | 2 |
| / | 1095 | 57 / | 42 | Downstream Velika Morava | 2.00 | 2.27 | II | 2.86 | IV | 2,00 | II | 3 |
| / | | 58 / | | Starapalankaram | 2.00 | 2.43 | III | | | | | |

| JDS3JDSrkm [JDS2/JDS3] | Site no. [JDS2/JDS3] | Sampling Site | Saprobic basic condiciton | JDS2 Airlift SI | JDS2 Airlift Class | JDS3 MHS SI | JDS3 MHS Class | JDS3 DWS SI | JDS3 DWS Class | JDS3 SK Class |
|---|---|---|---|---|---|---|---|---|---|---|
| / 1073 | 59 / 43 | Banatska Palanka/Bazias | 2.00 | 2.15 | II | 2.36 | II | 2,00 | II | 2 |
| / 1040 | 60 / 44 | Irongate reservoir (Golubac/Koronin) | 2.00 | 2.58 | III | 2.35 | II | 2,00 | II | 2 |
| / | 61 / | Donij Milanovac | 2.00 | 2.69 | III | | | | | |
| / 956 | 62 / 45 | Irongate reservoir (Tekija/Orsova) | 2.00 | 2.44 | III | 2.67 | III | 2,44 | III | 3 |
| / 926 | 63 / 46 | Vrbica/Simijan | 2.00 | 2.47 | III | 3.02 | IV | 2,16 | II | 3 |
| / | 64 / | Irongate II | 2.00 | 2.13 | II | | | | | |
| / 847 | 65 / 47 | Upstream Timok (Rudujevac/Gruia) | 2.00 | 2.21 | II | 2.39 | II | 2,26 | II | 3 |
| / 837 | 67 / 49 | Pristol/Novo Selo Harbour | 2.00 | 2.13 | II | 2.08 | II | 2,05 | II | 2 |
| / | 68 / | Calafat | 2.00 | 2.26 | II | | | | | |
| / 686 | 69 / 50 | Downstream Kozloduy | 2.00 | 2.29 | II | 2.02 | II | 2,01 | II | 2 |
| / | 70 / | up. Iskar | 2.00 | 2.06 | II | | | | | |
| / | 72 / | ds. Iskar | 2.00 | 1.78 | I | | | | | |
| / | 73 / | up. Olt | 2.00 | 2.14 | II | | | | | |
| / 604 | 75 / 52 | Downstream Olt | 2.00 | 1.9 | I | 2.36 | II | 2,09 | II | 2 |
| / | 76 / | ds. Turnu Magurele | 2.00 | 1.93 | I | | | | | |
| / 550 | 77 / 53 | Downstream Zimnicea/Svishtov | 2.00 | 2.38 | II | 2.27 | II | 2,01 | II | 3 |
| / 532 | 79 / 55 | Downstream Jantra | 2.00 | 2.32 | II | 2.00 | I | 2,01 | II | 2 |
| / | 80 / | up. Ruse | 2.00 | 2.18 | II | | | | | |
| / 488 | 82 / 57 | Downstream Ruse/Giurgiu | 2.00 | 1.48 | I | 2.00 | I | 2,03 | II | 3 |
| / | 83 / | up. Arges | 2.00 | 2.1 | II | | | | | |
| / 429 | 85 / 59 | Downstream Arges. Oltenita | 2.00 | 1.81 | I | 2.12 | II | 2,03 | II | 2 |
| / 375 | 86 / 60 | Chiciu/Silistra | 2.00 | 2.76 | III | 2.04 | II | 2,00 | II | 3 |
| / | 87 / | ds. Crnavoda | 2.00 | 2.16 | II | | | | | |
| / 232 | 88 / 61 | Giurgeni | 2.00 | 3.15 | IV | 2.49 | III | 2,02 | II | 3 |
| / 170 | 89 / 62 | Braila | 2.00 | 2.23 | II | 2.12 | II | 2,34 | II | 3 |
| / 132 | 92 / 65 | Reni | 2.00 | 2.16 | II | 2.19 | II | 2,00 | II | 3 |
| / 18 | 93 / 66 | Vilkova – Chilia arm/Kilia arm | 2.00 | 2.24 | II | 2.72 | III | 2,01 | II | 3 |
| / | 94 / | Bystroye Canal | 2.00 | 2.15 | II | | | | | |
| / 31 | 95 / 67 | Sulina – Sulina arm | 2.00 | 2.16 | II | 2.01 | II | 2,05 | II | 3 |
| / 104 | 96 / 68 | Sf.Gheorghe – Sf.Gheorghe arm | 2.00 | 2.11 | II | 2.08 | II | 2,00 | II | 2* |

* EQR values close to thresholds (≤ 0.01 points) are rounded up to the next best status class

## 5.4 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 stretch. 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 used 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 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* occurs in high densities.
- Higher abundances of EPT- Taxa (Ephemeroptera, Plecoptera and Trichoptera) are restricted to the upper stretch, whereas Trichoptera show the highest abundances within these sensitive groups. Regarding aquatic insects Chironomidae play a major role along the entire Danube stretch.

- Highest taxa-richness was recorded with the MHS-approach. Some species were detected only in the middle region of the river bed on the lowest part of the Danube by dredging: *Paramysis ullskyi*, *Schizoramphus scabriusculus*, *Niphargoides spinicaudatus.*

**Methodology**

- The MHS method is especially applicable for ecological status assessment of large rivers at low water period: it is standardized, stressor-specific and habitat-oriented.
- K&S and diving method can provide additional information particularly on mussel populations inhabiting deeper zones next to the bank.
- DWS is not affected by water level and discharge so much and is appropriate for data collection from all of deep parts and habitats of a large river. Carefully operation of the dredge can provide semi-quantitative data.
- Regarding detailed surveys of Mollusca a detailed habitat monitoring in the field is necessary.

**Saprobiological assessment**

- The different methodological approaches produce clearly different datasets leading to different assessment results. While Saprobic Indices from riparian habitats (obtained K&S and MHS) are largely comparable, DWS collates more lotic faunas associated with lower Saprobic Indices resulting in a better ecological status. To overcome this phenomenon a worst-case approach of deep water and riparian sampling is applied.
- Saprobic Indices and based on that, water quality status class per site, are comparable to the JDS2 data.
- Regarding Saprobity in total 73% of 55 sampled sites in 2013 can be classified as "indication of good ecological status", 15% of the sites as "indication of moderate ecological status" and 4% actually as "high ecological status" according to the WFD. This proportion is similar to the JDS2 results.
- Serious organic pollution was identified upstream Novi-Sad (bad status). Saprobically "poor status" was indicated in Jochenstein, upstream Drava, downstream Velika Morava and at Vrbica/Simjan in the Irongate reservoir.

**General degradation**

- On the basis of the Slovak assessment method for large rivers, the morphologically high degraded sites (channelized or impounded, with rip-rap dominating at the shore zones) in the Upper Danube reach indicate moderate status, while more natural sites at the Upper and Middle Danube reach indicate generally good status.
- These results implicate that the general degradation of the main channel of large mountainous rivers can be roughly covered by this assessment method.
- Compatibility of this method in the Lower Danube reach has to be further tested as substrate composition differs considerably from the Middle Danube.
- Additionally the inclusion of WFD- compliant assessment methods based on biological quality elements of associated floodplains of large rivers, is needed in respect of a holistic aquatic ecosystem approach.

**Habitat preferences of indicators with implications on management actions**

- As habitat degradation is one main stressor of large rivers the preferences of taxa were one main focus of JDS3. Organic habitats provide the highest numbers of indicator taxa. The highest diversity of indicators was found in samples of roots/woody debris.
- Coarse lithal substrates like meso- and macrolithal as well as rip-rap comprise only four indicator taxa in total, whereas rip-rap is preferred by only two taxa groups.
- Indicators of the sensitive group of EPT-Taxa (Ephemeroptera, Plecoptera, Trichoptera) were allocated to roots/woody debris and meso-/macrolithal.
- Invasive crustaceans show high affinities to stabile substrates, especially rip-rap.

The following topics are discussed in the Full report: on macrozoobenthos on the attached CD:

- Longitudinal, sectional and cross sectional change of the main taxonomic groups based on comparative analysis of results gained by different sampling methods
- Comments and conclusions about the Danube typology
- Analyses of the indicative power of selected taxa groups regarding organic pollution and habitat preferences
- Analyses of the distribution of Crustacea

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