Joint Danube Survey 3

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International Commission for the Protection of the Danube River

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Full report report on: Macroinvertebrates

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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 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 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 JDS 3 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.

2 Methods

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 JDS 2 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 JDS 2.

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.

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 (Figure 2). 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 JDS 3 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:

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

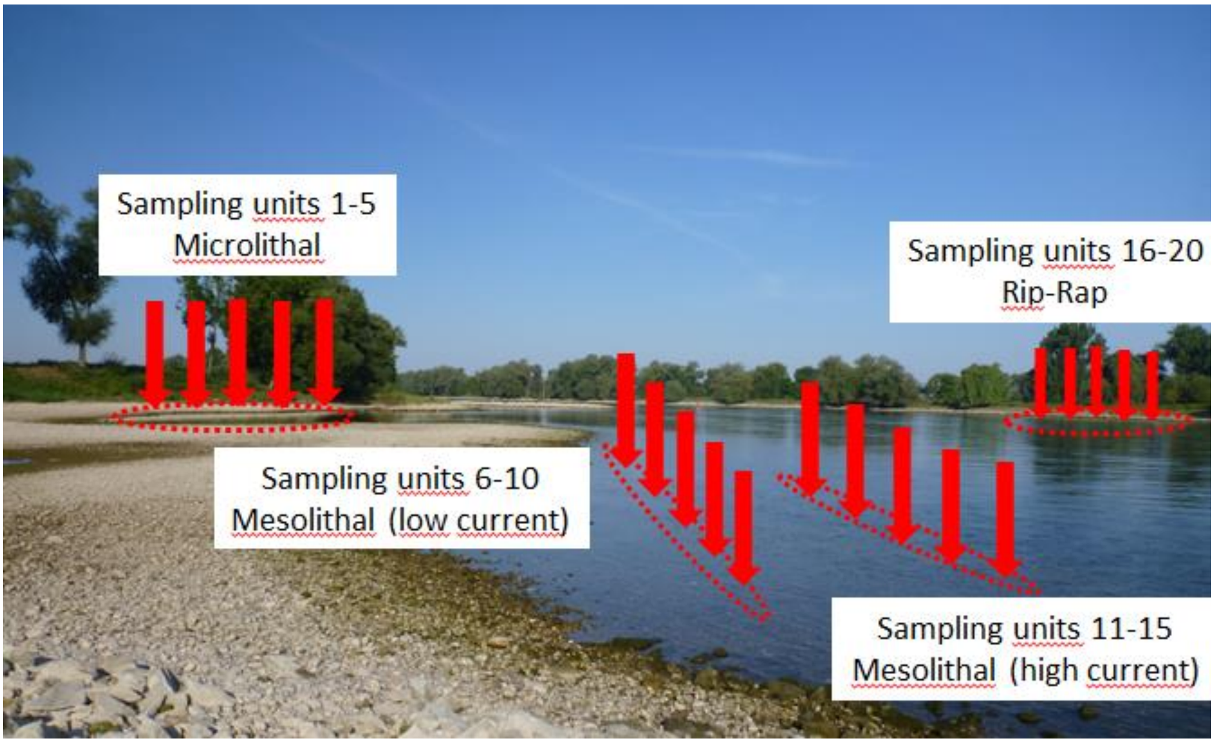


Figure 1: Habitat-specific sampling; example from JDS-site 5

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.

Figure 2: MHS netsampler (Photo: UWITEC)

2.1.2 Deep Water Sampling (DWS)

This dredging program provided rough information how these 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 3). 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 3: 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).

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.

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).

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 JDS 3 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.

2.3.1 Saprobic index and calculation of metrics

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.

$$SI = \frac{\sum_{i=1}^{n} s_i \times A_i \times G_i}{\sum_{i=1}^{n} A_i \times G_i}$$

s_i = saprobic value of the i^{th} taxon

A_i = abundance value of the i^{th} taxon

G_i = indicative weight of the i^{th} taxon

n = number of taxa

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 defined or agreed (officially) (BUIJS, 2006), the intercalibration procedure is still in progress (BIRK et al., 2013, SCHÖLL et al., 2012).

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 1 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 1: Threshold values for the indication of water quality classes based on organic pollution.

Ecological status class	Saprobic reference condition (range of Saprobic Index)			
	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

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 JDS 3 was to find valuable biotic scores that can be integrated into future assessment systems.

Hence, the recently developed Slovak method for large rivers (NARIADENIE VLÁDY 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 (URBANIČ, 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.

The relevant metrics for the Slovak method for each river type and benchmarks are listed in

Table 2:

Table 2: Metrics for 'large rivers at altitudes below 200 m' and 'large rivers at altitudes between 200 and 500 m' including benchmarks

Metric	large rivers at altitudes below 200 m	large rivers at altitudes between 200 and 500 m
Saprobic index - SI (Zelinka & Marvan)	$EQR = \frac{TV - 3{,}5}{2{,}00 - 3{,}5}$	$EQR = \frac{TV - 3{,}5}{1{,}75 - 3{,}5}$
[%] Oligosaprobic classified taxa (scored taxa = 100%)	$EQR = \frac{TV - 0}{22{,}1 - 0}$	$EQR = \frac{TV - 0}{32{,}6 - 0}$
BMWP	$EQR = \frac{TV - 1}{71{,}5 - 1}$	$EQR = \frac{TV - 1}{119{,}3 - 1}$
[%] Metarhithral classified taxa (scored taxa = 100%)		$EQR = \frac{TV - 0}{39{,}3 - 0}$
Rhithron Type Index (Biss et al., 2002)	$EQR = \frac{TV - 0}{7{,}25 - 0}$	$EQR = \frac{TV - 0}{11{,}4 - 0}$
Index of biocoenotic regions (IBCR)	$EQR = \frac{TV - 8{,}4}{4{,}65 - 8{,}4}$	$EQR = \frac{TV - 8{,}4}{3{,}5 - 8{,}4}$
[%] preferences for akal+lithal+psammal (scored taxa = 100%)	$EQR = \frac{TV - 0}{67{,}5 - 0}$	$EQR = \frac{TV - 0}{77{,}9 - 0}$
# EPT-Taxa		$EQR = \frac{TV - 0}{20 - 0}$

2.3.2 Multivariate analyses

2.3.2.1 Cluster analysis – MHS-data

The purpose of cluster analyses is to define groups of items based on their similarities (McCUNE et al., 2006). For determining the distance measure, the Sorensen (Bray & Curtis) coefficient (SØRENSEN, 1948) was used. The chosen group linkage method was Flexible Beta (Beta = -0.25). The taxa-abundances are log+1 transformed; the results are presented as a dendrogram.

2.3.2.2 Non-metric Multidimensional Scaling (NMS; KRUSKAL 1964) – MHS-data

For exploring similarities or dissimilarities in data, based on taxa composition in our case, the statistical technique non-metric multidimensional scaling (NMS) was used. It is a special case of ordination to verify the existing Danube sections. An NMS algorithm starts with a matrix of item-item similarities, then assigns a location of each item in a low-dimensional space, suitable for graphing or 3D visualisation. Similar objects are near each other and dissimilar sites are further from each other. The Sorensen (Bray-Curtis) -coefficient (SØRENSEN, 1948) was used to determine the distance measure, the number of runs was 50 and the number of axes *k* was, depending on the number of evaluated sites, 2 or 3. The taxa-abundances are log+1 transformed; the results presented as scatterplots. According to KRUSKAL (1964, in HARTUNG & ELPELT, 1999; LEYER & WESCHE, 2008) stress values < 5 are described as good results, values between 10 und 15 as satisfying and values between 15 und 20 as sufficient.

2.3.2.3 Correspondence analyses (CA) – K&S-data:

Data for sampling sites obtained by the K&S techniques were analysed using Correspondence analyses by employing Flora Software package (KARADŽIĆ, 2013). Basic variant ordination with

Singular Value Decomposition (SVD) algorithm was used (KARADŽIĆ, 2013), as more precise method in compare to Weighted Averaging.

2.3.2.4 Indicator Species Analysis – MHS-data:

A very common goal in community analysis is to detect and describe the value of different species for indicating environmental conditions. The method combines information on the concentration of species abundance in a particular group and the faithfulness of occurrence of a species in a particular group, producing indicator values for each species in each group which are tested for statistical significance by a randomization technique (McCUNE & MEFFORD, 2006).

Groups are commonly defined by categorical variables, such as Danube reaches/sections or habitat types. This method can be also used to choose a stopping point in cluster analysis (DUFRENE & LEGENDRE, 1997). Good indicators only occurring in one habitat type for example provide a high value; heterogeneous spread taxa and single findings show low values. The values range from 0 to 100 (McCUNE & MEFFORD, 2006). Only significant species ($p<0.05$) were categorized as indicators.

3 Results and Discussion

During JDS 3 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.

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 applied during JDS 3 regarding distribution of the main taxonomic groups is given in Figure 4.

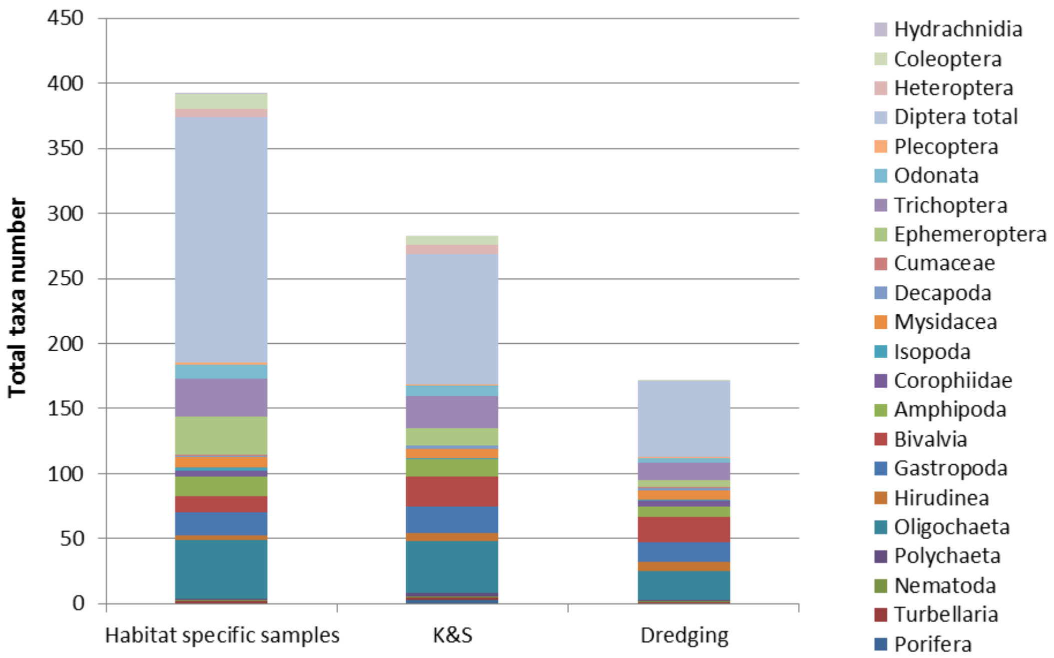


Figure 4: Number of taxa per taxonomic group recorded by habitat specific sampling method (MHS), K&S and Dredging (DWS)

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).

A detailed taxalist is given in Figure 5.

It needs to be emphasized that the higher taxa number (517 compared to 460) in this list is based on duplications resulting from inconsistent identification levels (species combinations) like e.g. *Tvetenia discoloripes/verralli*. Both species (*T. discoloripes* and *T. verralli*) and also the mentioned species combination (*T. discoloripes/verralli*) are listed but the latter must not be counted as a separate valid taxon.

Figure 5: List of taxa per sampling method and Danube reach

TAXON	MHS			DWS			K&S		
	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH
PORIFERA									
Ephydatia fluviatilis									x
Spongilla lacustris									x
NEMATODA									
Nematoda Gen.sp.	x	x	x		x				
TURBELLARIA									
Turbellaria Gen.sp.	x	x	x						
Dendrocoelum romanodanubiale		x		x	x		x	x	
Dugesia lugubris								x	x
GASTROPODA									
Bithynia tentaculata	x	x	x	x	x	x	x	x	x
Lithoglyphus naticoides				x	x	x	x	x	x
Potamopyrgus antipodarum				x	x	x	x	x	x
Lithoglyphus naticoides	x	x	x						
Radix auricularia									x
Radix balthica	x	x	x			x		x	x
Fagotia daudebartii acicularis		x	x		x	x		x	x
Fagotia esperi		x	x		x	x		x	x
Holandriana holandrii		x	x			x			x
Theodoxus danubialis		x	x		x	x	x	x	x
Theodoxus fluviatilis	x	x	x	x	x	x	x	x	x
Theodoxus transversalis			x			x		x	
Physella acuta				x	x			x	x
Physella sp.	x	x	x						
Ancylus fluviatilis	x	x	x					x	
Ferrissia sp.		x	x						
Gyraulus laevis							x		
Gyraulus sp.		x	x						
Potamopyrgus antipodarum	x	x	x						
Borysthenia naticina				x	x			x	
Valvata piscinalis								x	
Valvata sp.		x							
Viviparus acerosus		x			x			x	x
Viviparus sp.				x					
Viviparus viviparus		x	x		x	x		x	x
BIVALVIA									
Corbicula fluminalis								x	
Corbicula fluminea				x	x	x	x	x	x
Corbicula sp.	x	x	x						
Dreissena bugensis		x	x	x	x	x	x	x	x
Dreissena polymorpha	x	x	x	x	x	x	x	x	x
Pisidium amnicum					x	x	x	x	x
Pisidium casertanum				x	x		x	x	
Pisidium henslowanum				x			x	x	
Pisidium moitessierianum				x	x		x	x	
Pisidium nitidum				x		x	x	x	
Pisidium sp.	x	x	x		x				
Pisidium subtruncatum				x				x	
Pisidium supinum				x			x	x	
Pisidium tenuilineatum							x	x	
Musculium lacustre								x	
Sphaeridae Gen.sp.	x	x							
Sphaerium corneum				x	x			x	
Sphaerium rivicola				x	x	x		x	
Sphaerium solidum				x	x		x	x	
Sphaerium sp.	x	x	x						
Anodonta anatina			x	x	x	x	x	x	x
Pseudanodonta complanata		x	x					x	x

TAXON	MHS			DWS			K&S		
	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH
Sinanodonta woodiana		x	x		x	x		x	x
Unio crassus					x	x		x	x
Unio pictorum		x	x	x	x	x	x	x	x
Unio sp.juv.	x	x	x						
Unio tumidus		x	x		x	x	x	x	x
HIRUDINEA									
Dina punctata	x	x		x	x				
Erpobdella octoculata					x			x	
Erpobdella vilnensis					x				
Erpobdellidae Gen.sp.			x		x				
Glossiphonia complanata					x			x	
Helobdella stagnalis	x			x				x	
Haemopis sanguisuga								x	
Italobdella sp.					x				
Piscicola geometra			x						
Piscicolidae Gen.sp.	x	x							
POLYCHAETA									
Hypania invalida	x	x	x	x	x	x		x	
Manayunkia caspica								x	x
OLIGOCHAETA									
Enchytraeidae Gen.sp.	x	x						x	x
Enchytraeus sp.							x		
Henlea ventriculosa							x		
Criodrilus lacuum	x	x	x						
Haplotaxis gordioides		x							
Eiseniella tetraedra	x	x	x	x	x	x		x	
Lumbricidae Gen.sp.		x		x	x	x			
Lumbriculus variegatus					x				
Bythonomus lemani		x	x						
Rhynchelmis limosella		x							
Stylodrilus brachystylus		x							
Stylodrilus heringianus	x	x		x	x		x	x	x
Stylodrilus sp.			x			x			
Dero digitata		x	x		x			x	x
Dero obtusa								x	x
Nais alpina	x								
Nais barbata	x	x						x	
Nais bretscheri	x	x	x				x	x	x
Nais christinae	x	x	x						
Nais communis								x	x
Nais elinguis								x	
Nais pardalis	x	x	x				x	x	
Nais sp.			x			x	x	x	x
Ophidonais serpentina	x	x	x					x	x
Paranais frici							x		
Piguetiella blanci							x		
Pristina aequiseta			x						x
Pristina rosea									x
Specaria josinae		x	x					x	
Stylaria lacustris	x	x	x					x	x
Uncinais uncinata		x					x	x	
Oligochaeta Gen.sp.				x	x	x			
Propappus volki	x	x	x		x			x	x
Aulodrilus japonicus		x	x						
Aulodrilus pluriseta	x								
Bothrioneurum vejdovskyanum				x				x	x
Branchiura sowerbyi		x	x	x	x		x	x	x

TAXON	MHS UPPER REACH	MHS MIDDLE REACH	MHS LOWER REACH	DWS UPPER REACH	DWS MIDDLE REACH	DWS LOWER REACH	K&S UPPER REACH	K&S MIDDLE REACH	K&S LOWER REACH
Embolocephalus velutinus		x	x			x			x
Haber speciosus		x	x						
Isochaetides michaelseni	x	x	x	x	x	x	x	x	x
Limnodrilus claparedeanus	x	x	x	x	x	x	x	x	x
Limnodrilus hoffmeisteri	x	x	x	x	x	x	x	x	x
Limnodrilus profundicola		x							
Limnodrilus sp.	x	x	x						
Limnodrilus udekemianus		x	x	x	x		x	x	x
Potamothrix bavaricus									x
Potamothrix danubialis		x	x				x		
Potamothrix hammoniensis	x	x	x	x	x	x	x	x	x
Potamothrix sp.		x	x				x	x	
Potamothrix vejdovskyi	x	x	x	x			x	x	x
Psammoryctides albicola					x		x	x	x
Psammoryctides barbatus	x	x	x	x	x	x	x	x	x
Psammoryctides moravicus		x	x						
Spirosperma ferox	x								
Tubifex ignotus	x	x							
Tubifex sp.						x			
Tubifex tubifex		x	x		x		x	x	x
Tubificidae Gen.sp.	x	x	x						
AMPHIPODA									
Chelicorophium curvispinum	x	x	x	x	x	x	x	x	x
Chelicorophium robustum	x	x	x	x	x	x	x	x	x
Chelicorophium sowinskyi	x	x	x	x	x	x	x	x	x
Chelicorophium sp.	x	x	x	x	x	x	x	x	x
Gammaridae Gen.sp.				x	x	x	x	x	x
Gammarus fossarum	x								
Gammarus roeselii	x						x		
Niphargoides spinicaudatus						x			
Niphargus hrabei		x		x			x		
Dikerogammarus bispinosus	x	x		x	x		x	x	
Dikerogammarus haemobaphes	x	x	x	x	x	x	x	x	x
Dikerogammarus sp.	x	x	x				x	x	x
Dikerogammarus villosus	x	x	x	x	x	x	x	x	x
Echinogammarus ischnus	x	x	x	x	x	x	x	x	x
Echinogammarus trichiatus	x	x	x					x	
Echinogammarus warpachowskyi			x						
Obesogammarus crassus			x						
Obesogammarus obesus	x	x	x	x	x	x	x	x	x
Pontogammaridae Gen.sp.	x	x	x						
Pontogammarus robustoides			x						x
Pontogammarus sarsi			x			x	x		x
DEKAPODA									
Astacus leptodactylus			x		x	x		x	x
Pacifastacus leniusculus							x		
Orconectes limosus					x			x	x
ISOPODA									
Asellus aquaticus	x	x							
Proasellus sp.	x								
Jaera istri	x	x	x	x	x	x	x	x	x
MYSIDA									
Hemimysis anomala			x						
Katamysis warpachowskyi	x	x	x		x		x		x
Limnomysis benedeni	x	x	x		x	x	x	x	x
Mysidae Gen.sp.					x	x			
Paramysis bakuensis			x			x			x
Paramysis intermedia			x			x			x

TAXON	MHS UPPER REACH	MHS MIDDLE REACH	MHS LOWER REACH	DWS UPPER REACH	DWS MIDDLE REACH	DWS LOWER REACH	K&S UPPER REACH	K&S MIDDLE REACH	K&S LOWER REACH
Paramysis lacustris		x	x		x	x	x	x	x
Paramysis sp.		x	x						x
Paramysis ullskyi			x			x			x
CUMACEA									
Schizorhamphus scabriusculus			x			x			
HYDRACHNIDIA									
Hydrachnidia Gen.sp.	x		x						
EPHEMEROPTERA									
Ametropus fragilis		x							
Baetis alpinus	x								
Baetis calceratus/tricolor			x						
Baetis fuscatus	x	x							x
Baetis lutheri								x	
Baetis rhodani	x								
Baetis sp.	x								
Baetis vernus	x	x							
Centroptilum luteolum	x	x	x					x	x
Cloeon dipterum	x	x	x					x	x
Caenis luctuosa/macrura	x	x	x	x	x		x	x	
Caenis macruraAd.		x							
Caenis pseudorivulorum	x		x						
Caenis robusta	x	x	x				x		
Caenis sp.	x	x	x	x		x			
Ephemerella ignita	x								
Ephemera danica	x								
Ephemera lineata		x					x	x	
Ecdyonurus helveticus								x	
Ecdyonurus insignis		x							
Ecdyonurus sp.	x	x	x		x			x	
Ecdyonurus venosus							x		
Electrogena affinis		x							
Electrogena sp.juv.	x								
Heptagenia flava	x	x	x					x	x
Heptagenia longicauda	x								
Heptagenia sp.			x						
Heptagenia sulphurea	x	x		x	x	x	x	x	
Rhithrogena sp.	x								
Paraleptophlebia submarginata	x								
Ephoron virgo	x	x		x	x				
Potamanthus luteus	x	x					x		
ODONATA									
Aeshna cf.mixta		x							
Calopteryx splendens		x	x					x	
Coenagrion pulchellum								x	x
Coenagrionidae Gen.sp.juv.		x	x						
Ischnura elegans					x				
Pyrrhosoma nymphula								x	
Gomphus flavipes		x	x		x	x		x	x
Gomphus vulgatissimus		x	x	x	x		x	x	x
Onychogomphus forcipatus		x					x	x	
Ophiogomphus cecilia		x							x
Libellulidae Gen.sp.		x	x						
Orthetrum brunneum					x				
Orthetrum cancellatum		x	x						
Orthetrum sp.			x						
Platycnemis pennipes	x						x		
PLECOPTERA									
Leuctra sp.	x	x			x		x		

TAXON	MHS			DWS			K&S		
	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH
HETEROPTERA									
Aphelocheirus aestivalisssp.		x							
Corixidae Gen.sp.		x	x						
Micronecta sp.	x	x	x					x	
Sigara dorsalis							x		x
Aquarius najas							x		
Mesovelia sp.							x		
Ilyocoris cimicoides		x							
Plea minutissima		x					x	x	
Ranatra linearis		x							
Microvelia sp.							x	x	
NEUROPTERA									
Sisyra sp.									x
COLEOPTERA									
Coleoptera Gen.Sp.							x		
Dytiscidae Gen.sp.Lv.	x								
Oreodytes sp.Ad.			x						
Platambus maculatus	x								
Elmis aenea								x	
Elmis sp.	x	x							
Esolus sp.	x	x	x						
Limnius sp.	x	x							
Limnius volckmari								x	
Oulimnius sp.	x						x		
Riolus sp.	x								
Orectochilus villosus	x								
Haliplus sp.	x		x						
Hydraena sp.		x							
Hydrophilidae Gen.sp.	x	x							
Elodes marginata								x	
TRICHOPTERA									
Brachycentrus subnubilus	x	x		x	x		x	x	
Micrasema sp.	x								
Ecnomus tenellus	x	x	x					x	
Cheumatopsyche lepida	x							x	
Hydropsyche angustipennis					x				
Hydropsyche bulgaromanorum	x	x	x	x	x	x	x	x	x
Hydropsyche contubernalis	x	x	x	x	x	x	x	x	
Hydropsyche exocellata	x	x			x				
Hydropsyche incognita	x	x		x	x				
Hydropsyche modesta		x			x				
Hydropsyche pellucidula								x	
Hydropsyche sp.	x	x	x	x	x	x	x	x	x
Agraylea sexmaculata			x						
Hydroptila occulta						x			
Hydroptila sp.	x	x	x						x
Hydroptila tineoides							x		
Lepidostoma hirtum							x		
Athripsodes sp.juv.	x								
Ceraclea cf.annulicornis	x								
Leptoceridae Gen.sp.juv.			x					x	
Mystacides azurea	x								
Mystacides longicornis	x								
Oecetis notata						x			
Oecetis ochracea			x						
Oecetis sp.juv.			x						
Orthotrichia sp.Pu.		x							
Setodes punctatus	x	x				x		x	

TAXON	MHS			DWS			K&S		
	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH
Allogamus auricollis	x								
Halesus digitatus							x		
Potamophylax cingulatus							x		
Stenophylax permistus								x	
Cyrnus sp.							x		
Cyrnus trimaculatus	x						x		
Holocentopus sp.								x	x
Holocentropus picicornis								x	
Holocentropus stagnalis							x		
Neureclipsis bimaculata		x	x		x	x		x	x
Polycentropus flavomaculatus	x								
Lype phaeopa	x								
Psychomyia pusilla	x	x		x	x			x	
Tinodes waeneri	x								
Rhyacophila dorsalis	x								
Sericostoma sp.							x		
Trichoptera Gen.sp.								x	x
DIPTERA									
Diptera Gen.sp.					x				
Tetanocera sp.								x	
Brachycera Gen.sp.	x	x							
Ceratopogonidae Gen.sp.	x	x	x		x		x	x	x
Forcipomyia sp.								x	
Ablabesmyia longistyla	x	x	x				x	x	x
Ablabesmyia phatta			x			x			x
Beckidia zabolotzkyi		x						x	
Brillia flavifrons		x						x	
Cardiocladius capucinus	x								
Cardiocladius fuscus	x	x							
Cardiocladius sp.				x	x		x		
Chernovskiia cf.orbicus		x	x						
Chironomidae Gen.sp.				x	x	x			
Chironominae Gen.sp.	x	x	x						
Chironomini Gen.sp.	x	x	x						
Chironomus(Ca.) pallidivittatus			x						
Chironomus(C.) acutiventris	x	x	x						
Chironomus(C.) acutiventris/obtusidens				x	x	x	x	x	x
Chironomus(C.) annularis									x
Chironomus(C.) annularius-Gr.					x				
Chironomus(C.) cf.bernensis	x		x				x	x	x
Chironomus(C.) dorsalis			x						
Chironomus(C.) nudiventris	x	x	x	x	x	x	x	x	
Chironomus(C.) plumosus		x	x					x	x
Chironomus(C.) plumosus-Gr.			x	x	x	x			
Chironomus(C.) riparius		x							
Chironomus(C.) sp.	x	x	x		x				x
Cladopelma sp.		x	x					x	x
Cladotanytarsus atridorsum		x							
Cladotanytarsus conversus		x	x						
Cladotanytarsus mancus	x	x	x						
Cladotanytarsus mancus-Gr.	x	x	x						
Cladotanytarsus sp.		x	x		x	x	x	x	x
Cladotanytarsus sp.3		x							
Cladotanytarsus vanderwulpi	x	x	x						
Cladotanytarsus vanderwulpi-Gr.	x	x	x						
Conchapelopia agg.								x	
Conchapelopia pallidula	x								
Conchapelopia sp.	x		x						

TAXON	MHS			DWS			K&S		
	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH
Conchapelopia/Arctopelopia-Gr. sp.				x	x				
Corynoneura coronata								x	
Cricotopus festivellus							x	x	
Cricotopus sp.	x	x	x				x	x	x
Cricotopus intersectus				x			x		
Cricotopus(C.) cf.annulator	x	x							
Cricotopus(C.) sp.	x	x	x						
Cricotopus(C.) tremulus-Gr.	x								
Cricotopus(C.) triannulatus	x	x	x						
Cricotopus(C.) trifascia	x								
Cricotopus(I.) bicinctus	x	x	x	x	x	x	x	x	x
Cricotopus(I.) cf.dobrogicus	x	x	x						
Cricotopus(I.) dobrogicus/sylvestris-Gr.	x	x	x		x	x			
Cricotopus(I.) triannulatus							x	x	
Cricotopus(I.) triannulatus-Agg.				x	x				
Cryptochironomus defectus			x						
Cryptochironomus obreptans		x	x						
Cryptochironomus obreptans/supplicans	x	x	x				x	x	x
Cryptochironomus rostratus	x	x	x	x	x	x	x	x	x
Cryptochironomus sp.	x	x	x	x	x	x			
Cryptotendipes sp.	x	x		x			x	x	
Demicryptochironomus sp.	x		x					x	
Demicryptochironomus vulneratus				x		x			
Dicrotendipes cf.nervosus	x	x	x		x	x	x	x	x
Einfeldia sp.	x	x	x						
Endochironomus albipennis	x	x	x						
Endochironomus tendens		x							
Eukiefferiella cf.claripennis		x							
Eukiefferiella clypeata	x				x		x	x	
Eukiefferiella devonica/ilkleyensis	x								
Eukiefferiella dittmari	x								
Eukiefferiella lobifera	x	x							
Eukiefferiella sp.		x							
Glyptotendipes cf.pallens	x	x							
Glyptotendipes imbecillis		x							
Glyptotendipes sp.								x	
Harnischia angularis		x							
Harnischia sp.	x	x	x	x	x	x	x	x	x
Kiefferulus tendipediformis		x						x	
Kloosia pusilla		x							
Limnophyes sp.	x	x							
Lipiniella araenicola					x	x		x	x
Lipiniella moderata		x	x						
Macropelopia adaucta				x			x		
Macropelopia notata									x
Microchironomus tener		x	x	x	x	x	x	x	x
Micropsectra atrofasciata-Agg.	x								
Micropsectra bidentata								x	
Microtendipes cf.britteni	x	x							
Microtendipes chloris-Gr.	x	x							
Microtendipes pedellus	x	x					x	x	
Microtendipes pedellus-Gr.	x			x					
Microtendipes sp.	x								
Monodiamesa sp.	x	x		x			x		
Monopelopia tenuicalcar		x							
Nanocladius bicolor							x	x	
Nanocladius dichromus	x	x							
Nanocladius dichromus/distinctus	x	x							
Nanocladius rectinervis	x								
Nanocladius sp.		x							
Neozavrelia cf.fuldensis	x								
Neozavrelia luteola	x								
Neozavrelia luteola/fuldensis	x								
Neozavrelia sp.	x	x							
Nilotanypus dubius	x								
Nilothauma brayi	x								
Orthocladiinae Gen.sp.	x	x	x						
Orthocladiini CO	x	x	x						
Orthocladiini COP	x	x							
Orthocladiini CP	x	x	x						
Orthocladius holsatus						x			
Orthocladius(E.) sp.	x								
Orthocladius(O.) rubicundus	x	x							
Orthocladius(O.) sp.	x	x					x		
Parachironomus arcuatus							x	x	x
Parachironomus arcuatus-Gr.	x	x	x			x			
Parachironomus biannulatus								x	
Parachironomus frequens	x	x						x	x
Parachironomus sp.	x	x	x						
Paracladius conversus			x				x		
Paracladopelma laminatum							x		
Paracladopelma nigritulum			x				x		
Paracricotopus niger	x								
Paralauterborniella nigrohalteralis		x	x	x	x		x	x	
Parametriocnemus stylatus	x								
Paratanytarsus dissimilis	x		x				x	x	
Paratanytarsus dissimilis/inopertus	x	x	x						
Paratanytarsus dissimilis-Agg.						x			
Paratanytarsus lauterborni								x	x
Paratanytarsus sp.	x	x							
Paratendipes albimanus	x	x					x	x	
Paratendipes connectens			x						
Paratendipes intermedius		x	x						
Paratendipes nubilus					x			x	x
Paratrichocladius rufiventris	x	x	x		x		x	x	
Pentaneurini Gen.sp.		x							
Phaenopsectra sp.		x					x		
Polypedilum sp.	x	x	x		x	x	x	x	
Polypedilum sp."Pucking"		x							
Polypedilum(Pe.) uncinatum							x	x	
Polypedilum(P.) albicorne	x						x	x	
Polypedilum(P.) albicorne/cultellatum	x								
Polypedilum(P.) cf.apfelbecki		x							
Polypedilum(P.) cf.nubifer		x	x						
Polypedilum(P.) laetum								x	
Polypedilum(P.) nubeculosum	x	x	x	x	x	x	x	x	x
Polypedilum(P.) nubifer									x
Polypedilum(P.) pedestre	x	x			x				
Polypedilum(T.) acifer	x	x	x			x		x	
Polypedilum(T.) aegyptium	x	x	x						
Polypedilum(T.) bicrenatum		x	x		x			x	
Polypedilum(T.) bicrenatum-Gr.		x	x						
Polypedilum(T.) scalaenum		x		x	x	x	x	x	x
Polypedilum(T.) scalaenum-Gr.	x	x	x						
Polypedilum(T.) sp.		x							
Polypedilum(U.) convictum	x	x							

TAXON	MHS			DWS			K&S		
	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH	UPPER REACH	MIDDLE REACH	LOWER REACH
Polypedilum(U.) cultellatum	x	x			x				
Potthastia gaedii	x				x		x	x	
Potthastia gaedii-Gr.	x	x							
Procladius sp.							x	x	x
Procladius (H.) choreus	x	x	x						
Procladius (H.) sp.	x	x	x	x	x	x			
Procladius/Tanypus sp.		x							
Prodiamesa olivacea	x	x		x			x	x	
Prodiamesa rufovittata	x								
Psectrotanypus(P.) varius		x							
Rheocricotopus(P.) chalybeatus	x	x	x	x	x	x	x	x	x
Rheopelopia ornata	x	x	x						
Rheopelopia sp.	x	x	x	x	x	x	x		x
Rheotanytarsus pentapoda	x								
Rheotanytarsus pentapoda/reissi	x								
Rheotanytarsus pentapoda/rhenanus	x								
Rheotanytarsus rhenanus	x	x	x						
Rheotanytarsus sp.	x	x	x	x	x	x	x	x	x
Robackia cf.demeijerei		x	x						
Robackia sp.				x	x				
Saetheria sp.								x	
Stempellina bausei		x	x						
Stempellina sp.							x	x	
Stempellinella edwardsi								x	
Stempellinella minor		x							
Stenochironomus (S.) gibbus	x	x							
Stictochironomus maculipennis	x	x							
Stictochironomus pictulus	x	x			x		x	x	
Stictochironomus pictulus/maculipennis		x							
Stictochironomus sp.	x	x					x	x	
Stictochironomus sticticus								x	
Synorthocladius semivirens	x			x			x	x	
Tanypus cf.kraatzi			x					x	
Tanypus punctipennis		x	x		x			x	x
Tanypus sp.			x						
Tanypus vilipennis					x	x			
Tanytarsini Gen.sp.	x	x	x						
Tanytarsus brundini	x	x	x						
Tanytarsus brundini/curticornis	x	x	x						
Tanytarsus ejuncidus	x	x							
Tanytarsus eminulus	x	x	x						
Tanytarsus sp.	x	x	x		x	x	x	x	x
Tanytarsus sp."Traun"	x	x							
Telopelopia fascigera		x	x						
Thienemanniella sp.	x								
Thienemannimyia Gr.,Gen.indet.	x	x	x						
Thienemannimyia sp.	x	x							
Tvetenia calvescens	x								
Tvetenia discoloripes	x							x	
Tvetenia discoloripes/verralli	x								
Tvetenia discoloripes-Agg.				x	x				
Tvetenia sp.		x							
Tvetenia verralli	x	x							
Tvetenia vitracies	x								
Virgatanytarsus cf.arduennensis	x	x	x						
Xenochironomus xenolabis	x	x	x				x		x
Empididae Gen.sp.	x	x	x						
Hemerodromia sp.									x
Ephydridae Gen.sp.							x		
Antocha sp.	x	x						x	
Cheilotrichia sp.								x	
Hexatoma sp.	x	x							
Limoniidae Gen.sp.	x	x	x	x				x	
Ulomyia fuliginosa							x	x	
Simuliidae Gen.sp.					x			x	
Simulium sp.juv.	x	x							
Simulium(E.) cf.angustipes	x								
Simulium(S.) reptans	x								
Simulium(W.) balcanicum		x							
Simulium(W.) sp.		x							
Stratiomys longicornis									x
Tabanus sp.							x	x	x

The results of the different applied methods during JDS 3 are discussed in separated chapters.

3.1 Analyses of samples from Multi Habitat Sampling (MHS)

3.1.1 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 6).

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.

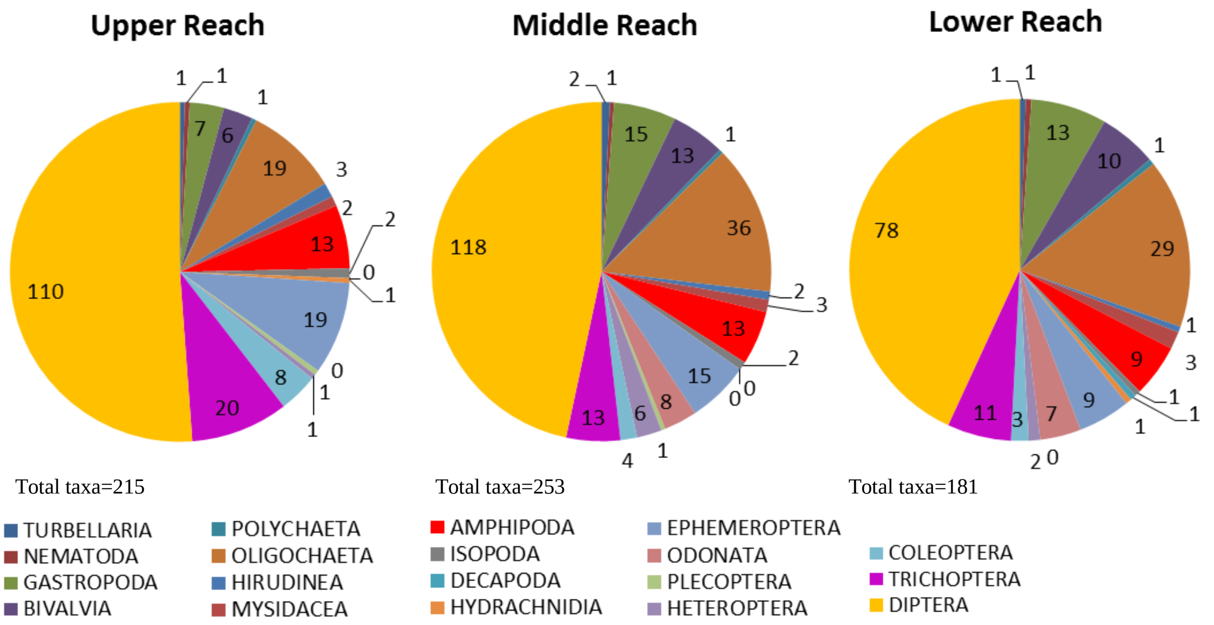


Figure 6: Number of taxa per taxagroup along the different reaches of the Danube (MHS-Data)

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 7).

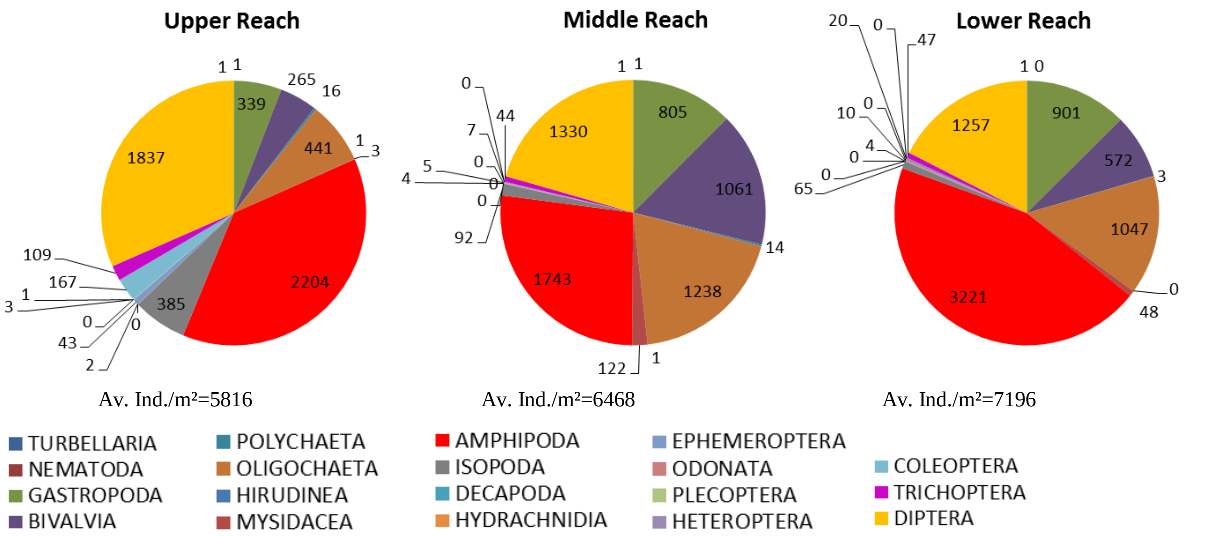


Figure 7: Average density (individuals/m²) per taxagroup along the different reaches of the Danube (MHS-Data)

3.1.2 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 8 (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 8, 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.

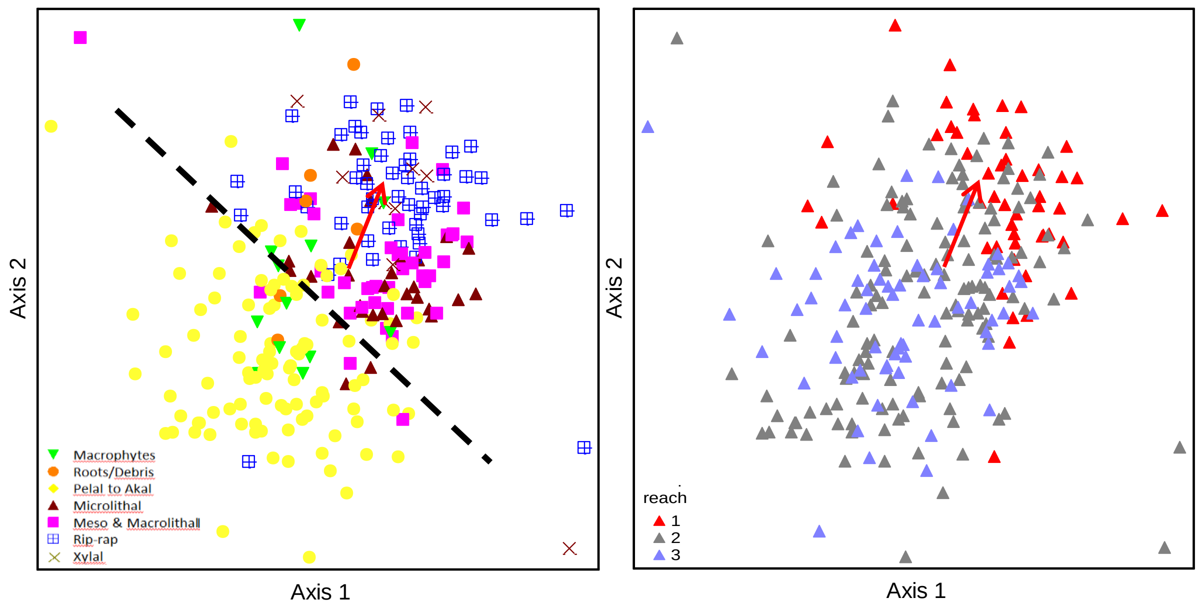


Figure 8: NMS scatterplot, based on taxa assemblages per sample (each point represents a pooled habitat sample of 5 single units); overlay: substrate types, partly combined (left), Danube reaches (1=Upper, 2=Middle, 3= Lower Danube reach), (right); final stress for 3-d

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 ($p \leq 0.05$) indicator taxa per taxonomic group for the defined substrate types are presented in Figure 10.

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 8, arrow)

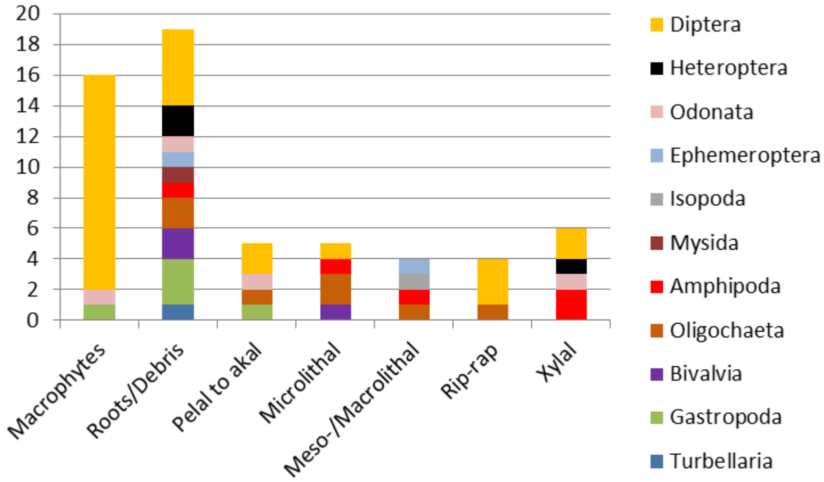
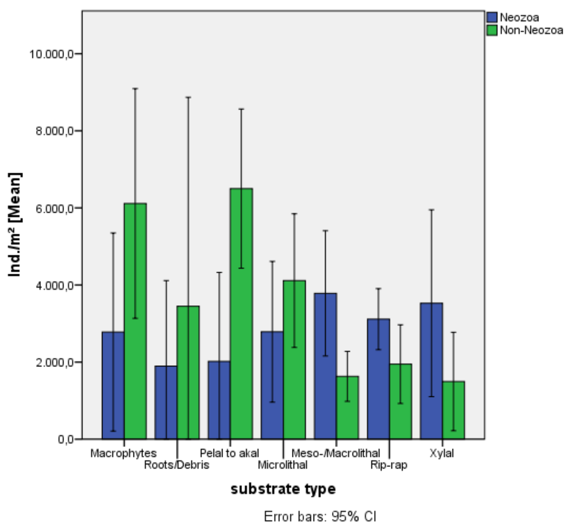


Figure 9: Significant indicator species per substrate type

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 10).



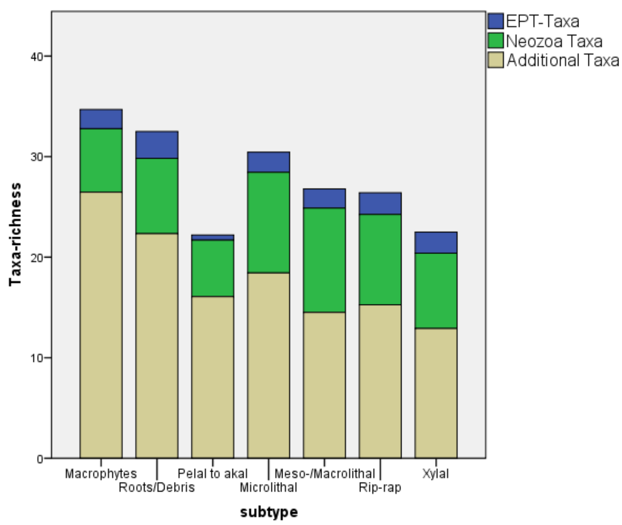


Figure 10: Average density of neozoa and indigenous taxa on different substrate types (left); Taxa richness and substrate type (right);

3.1.2.1 Habitat specific assessment per Danube reach

Separated into the 3 defined Danube reaches a quite clear differentiation of macro-invertebrate's habitat preferences between Upper reach and the other reaches is given.

After the exclusion of impounded sections (due to a quite discrete faunal composition) and sites with doubtful data (due to increasing water level at sampling date) the NMS scatterplots indicate a distinct habitat classification regarding taxa composition (Figure 11). As the dominating substrate type in the Upper Danube reach is rip-rap a clear separation from fine sediments is indicated, while macrophytes and xylal show a higher similarity to this artificial substrate. In the Middle reach organic macrophytes and roots/debris are forming a rather separated cluster indicating a unique fauna in comparison with coarse lithal substrates. Artificial substrates (rip-rap) play a minor in the Lower reach; coarse lithal substrates together with organic substrates show a high dissimilarity towards fine substrates.

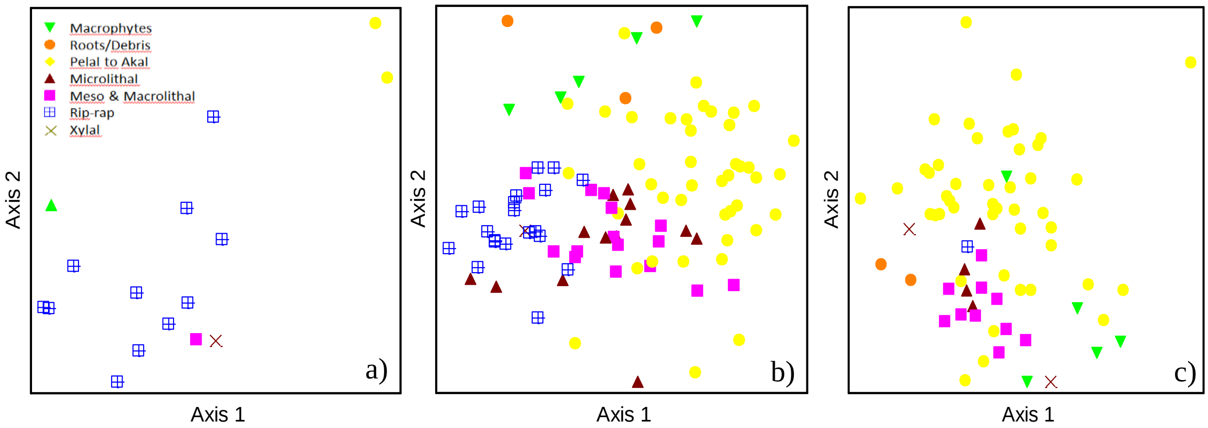


Figure 11: NMS scatterplot, based on taxa assemblages per sample (each point represents a pooled habitat sample of 5 single units); overlay: substrate types, Danube reaches (a=Upper, b=Middle, c= Lower Danube reach); final stress for 2-d solution: 6.7, final instability: <0.00000, iterations: 93 (Upper reach); final stress for 3-d solution: 13.5, final instability: <0.00000, iterations: 125 (Middle reach); final stress for 3-d solution: 13.7, final instability: <0.00000, iterations: 170 (Lower reach)

These findings are underlined by the following indicator species analyses. Habitats with a high number of indicator species generally postulate a preference by a specific fauna and rather high dissimilarity to other habitats, those with low numbers of indicators a colonisation by generalists.

In the Upper reach fine sediments (pelal to akal) and rip-rap show the highest number of indicator taxa representing a high dissimilarity of habitat quality followed by xylal and microlithal (Figure 12, left). Macrophytes, roots and debris do not play a major role regarding habitat preference due to their rare occurrence.

In contrast the Middle and Lower Danube reaches show the highest numbers of indicator taxa for organic substrates whereas especially for roots/debris in the Middle reach an extremely high number of taxa with habitat preferences are indicated (Figure 13 and Figure 14, left).

Another important topic is the composition of indicator taxa at each substrate type. A clear preference of neozoa taxa for artificial lithal substrates (rip-rap) in the Upper reach is given, while lithal substrates of high grain-size and deadwood (xylal) respectively are preferred in the Middle and Lower reach. Organic substrates as macrophytes and roots/debris show generally a low ratio of neozoa (Figure 12 to Figure 14, right). It must be noted that strictly speaking most of the taxa which are handled as neozoa in the Upper and Middle Danube reach should be counted as native in the Lower reach (downstream Iron Gate). For better comparison they are counted as neozoa for the whole Danube stretch in this study. The detailed taxa-lists of significant indicators per Danube reach are given from Table 3 to Table 5)

Summarized the importance of organic habitats for macro-invertebrates in the Middle and Lower Danube reach is obvious while in the Upper Danube these habitats are indicated as less important because of their rare occurrence due to morphological degradation along the Upper stretch. Regarding neozoa the highest habitat preference is given for artificial rip-rap in the Upper reach and coarse lithal substrates in the Middle and Lower reach where rip-rap habitats are decreasing.

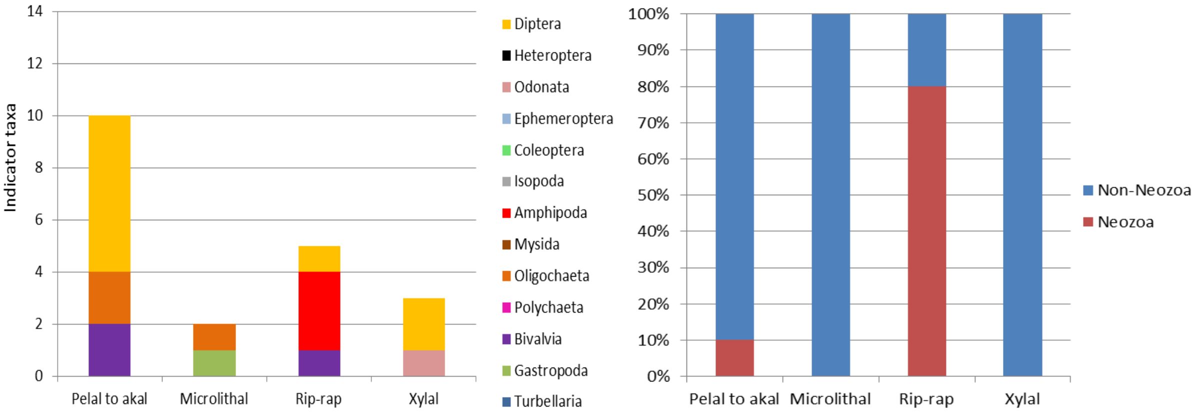


Figure 12: Significant indicator species per substrate type (left) and percentage of neozoa taxa per substrate type (right); Upper Danube reach

Table 3: Significant (p≤0.05) indicator taxa per substrate type; impounded sites (3) excluded; Upper Danube reach

Taxagroup	*Family*	*Genus*	*Species*	*Neozoon*	*Substrate type*	*Value (IV)*	*Mean*	*S.Dev*	*p*
Odonata	Platycnemididae	Platycnemis	pennipes		Xylal	66.7	18.1	9.61	0.0126
Diptera	Chironomidae	Cricotopus (I.)	dobrogicus/ sylvestris-Gr.		Xylal	56.2	30.6	14.07	0.0368
Diptera	Chironomidae	Orthocladiini	CP		Xylal	55.3	20.1	11.05	0.0182
Bivalvia	Sphaeridae	Pisidium	sp.		Pelal to akal	98.8	22.4	12.08	0.0002
Bivalvia	Corbiculidae	Corbicula	sp.	yes	Pelal to akal	89.9	38	14.18	0.001
Diptera	Chironomidae	Chironomini	Gen. sp.		Pelal to akal	74.9	22.7	12.07	0.0034
Oligochaeta	Tubificidae	Potamothrix	moldaviensis		Pelal to akal	72.3	29.9	13.72	0.016
Diptera	Chironomidae	Procladius (H.)	choreus		Pelal to akal	66.7	18.5	9.54	0.014
Diptera	Chironomidae	Chironomus (C.)	nudiventris		Pelal to akal	60.2	26.2	13.85	0.0184
Diptera	Chironomidae	Tanytarsus	ejuncidus		Pelal to akal	58	21.8	12.22	0.0236
Diptera	Chironomidae	Cryptochironomus	sp.		Pelal to akal	54.5	19.4	10.78	0.0238
Oligochaeta	Tubificidae	Psammoryctides	barbatus		Pelal to akal	50.8	24.3	12.4	0.0378
Diptera	Chironomidae	Harnischia	sp.		Pelal to akal	46.2	19.3	10.73	0.0412
Oligochaeta	Tubificidae	Tubificidae	Gen. sp.		Microlithal	77.8	37.5	15.77	0.027
Gastropoda	Planorbidae	Ancylus	fluviatilis		Microlithal	33.3	19.4	10.3	0.0462
Amphipoda	Corophidae	Chelicorophium	curvispinum	yes	Rip-rap	71.6	37.4	10.17	0.001
Amphipoda	Corophidae	Corophium	sp.	yes	Rip-rap	67	34.8	8.87	0.001
Bivalvia	Dreissenidae	Dreissena	polymorpha	yes	Rip-rap	49.9	25.1	12.48	0.05
Diptera	Chironomidae	Cricotopus (C.)	sp.		Rip-rap	49.2	30	8.16	0.0236
Amphipoda	Pontogammaridae	Dikerogammarus	villosus	yes	Rip-rap	45.4	31	5.2	0.0118

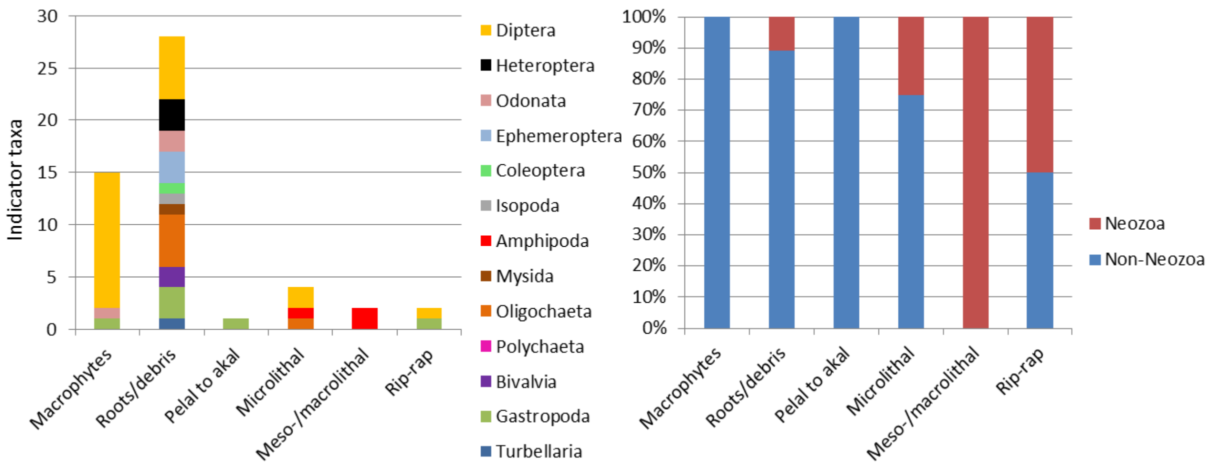


Figure 13: Significant indicator species per substrate type (left) and percentage of neozoa taxa per substrate type (right); Middle Danube reach

Table 4: Significant (p≤0.05) indicator taxa per substrate type; impounded sites (4) excluded (MP=Macrophytes; R/D=Roots/Debris); Middle Danube reach

Taxagroup	*Family*	*Genus*	*Species*	*Neozoon*	*Substrate type*	*Value (IV)*	*Mean*	*S.Dev.*	*p **
Diptera	Chironomidae	Cricotopus (I.)	dobrogicus/ sylvestris-Gr.		MP	88.5	14.5	8.84	0.0004
Diptera	Chironomidae	Cricotopus (I.)	cf. dobrogicus		MP	78,9	11,1	8,13	0,0002
Diptera	Chironomidae	Cricotopus	sp.		MP	75,4	13,6	8,84	0,0012
Diptera	Chironomidae	Parachironomus	arcuatus-Gr.		MP	66,2	9,6	7,48	0,0004
Odonata	Coenagrionidae	Coenagrionidae	Gen. sp. juv.		MP	61,9	11,4	7,96	0,0006
Diptera	Chironomidae	Nanocladius	dichromus/ distinctus		MP	56,7	10,8	8	0,0028
Diptera	Chironomidae	Chironomini	Gen. sp.		MP	53,2	21,5	10,18	0,0224
Diptera	Chironomidae	Microchironomus	tener		MP	49,8	20,8	10,47	0,0288
Diptera	Chironomidae	Dicrotendipes	cf. nervosus		MP	49,1	18,9	9,4	0,015
Gastropoda	Lymnaeidae	Radix	ovata/peregra		MP	38,8	10,8	8,06	0,0112
Diptera	Chironomidae	Procladius (H.)	sp.		MP	38,7	16,2	9,31	0,0406
Diptera	Chironomidae	Cryptochironomus	sp.		MP	35,5	10,9	7,6	0,0194
Diptera	Chironomidae	Cladopelma	sp.		MP	35,1	9,5	7,71	0,0088
Diptera	Chironomidae	Cryptochironomus	obreptans/ supplicans		MP	32,8	11	8,03	0,0202
Diptera	Chironomidae	Tanypus	punctipennis		MP	21	8,1	6,89	0,0394
Mysida	Mysidae	Limnomysis	benedeni	yes	R/D	80,4	20,4	10,25	0,0018
Gastropoda	Planorbidae	Ferrissia	sp.		R/D	66,7	7,4	6,81	0,0008
Ephemeroptera	Caenidae	Caenis	sp.		R/D	66,7	7,4	6,81	0,0008
Gastropoda	Physidae	Physella	sp.	yes	R/D	65,2	12,8	8,8	0,0008
Oligochaeta	Naididae	Dero	digitata		R/D	64,6	9	7,33	0,0004
Oligochaeta	Naididae	Specaria	josinae		R/D	58,8	8,3	6,98	0,0014
Bivalvia	Unionidae	Pseudanodonta	complanata complanata		R/D	51,5	10,2	7,24	0,0026
Diptera	Chironomidae	Polypedilum (P.)	nubeculosum		R/D	46,2	18,1	9,94	0,0304
Diptera	Ceratopogenidae	Ceratopogonidae	Gen. sp.		R/D	45	12,2	8,08	0,0096
Oligochaeta	Naididae	Ophidonais	serpentina		R/D	35,1	8,9	7,25	0,0066
Oligochaeta	Naididae	Stylaria	lacustris		R/D	33,6	12,8	8,7	0,0264
Bivalvia	Unionidae	Sinanodonta	woodiana	yes	R/D	33,3	6,1	6,22	0,031
Oligochaeta	Propappidae	Propappus	volki		R/D	33,3	6	6,07	0,027
Isopoda	Asellidae	Asellus	aquaticus		R/D	33,3	6	6,16	0,0304

Taxagroup	*Family*	*Genus*	*Species*	*Neozoon*	*Substrate type*	*Value (IV)*	*Mean*	*S.Dev.*	*p **
Odonata	Libellulidae	Libellulidae	Gen. sp.		R/D	33,3	6	6,16	0,0304
Odonata	Libellulidae	Orthetrum	cancellatum		R/D	33,3	6,1	6,22	0,031
Heteroptera	Corixidae	Corixidae	Gen. sp.		R/D	33,3	6	6,16	0,0304
Heteroptera	Naucoridae	Ilyocoris	cimicoides		R/D	33,3	6	6,16	0,0304
Coleoptera	Hydrophilidae	Hydrophilidae	Gen. sp.		R/D	33,3	6	6,16	0,0304
Diptera	Chironomidae	Endochironomus	tendens		R/D	33,3	6	6,16	0,0304
Diptera	Chironomidae	Polypedilum (U.)	convictum		R/D	33,3	6	6,07	0,027
Gastropoda	Planorbidae	Gyraulus	sp.		R/D	32	8,8	7,19	0,0306
Heteroptera	Pleidae	Ranatra	linearis		R/D	31,6	7,9	6,85	0,035
Ephemeroptera	Baetidae	Cloeon	dipterum		R/D	31,5	8,8	7,08	0,0386
Turbellaria	[Kl:Turbellaria]	Turbellaria	Gen. sp.		R/D	29	9,6	7,7	0,0404
Diptera	Chironomidae	Glyptotendipes	cf. pallens		R/D	28,1	7,6	6,89	0,0486
Diptera	Chironomidae	Chironomus (C.)	cf. plumosus		R/D	27,7	11	7,58	0,031
Ephemeroptera	Caenidae	Caenis	robusta		R/D	26,7	9	7,22	0,0346
Gastropoda	Lithoglyphinae	Lithoglyphus	naticoides		Pelal to akal	63,8	26,5	9,74	0,0046
Amphipoda	Pontogammaridae	Dikerogammarus	bispinosus	yes	Microlithal	69,7	21,3	9,88	0,004
Diptera	Chironomidae	Cryptochironomus	rostratus		Microlithal	47,6	22,1	10,08	0,0252
Oligochaeta	Lumbriculidae	Stylodrilus	heringianus		Microlithal	36,2	18,5	8,82	0,0498
Diptera	Chironomidae	Polypedilum	sp. "Pucking"		Microlithal	23,1	8,2	6,63	0,035
Amphipoda	Corophidae	Chelicorophium	sowinskyi	yes	Meso-/ macrolithal	53,5	22,2	10,56	0,0224
Amphipoda	Pontogammaridae	Dikerogammarus	haemobaphes	yes	Meso-/ macrolithal	47,9	22	10,1	0,0314
Gastropoda	Neritidae	Theodoxus	fluviatilis	yes	Rip-rap	45,2	24	8,96	0,0354
Diptera	Chironomidae	Potthastia	gaedii-Gr.		Rip-rap	20	8,9	7,07	0,046

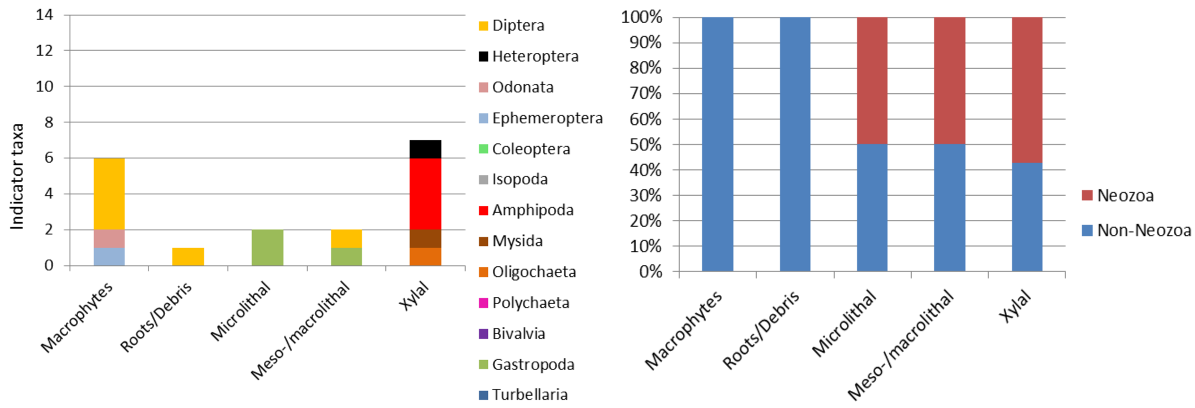


Figure 14: Significant indicator species per substrate type (left) and percentage of neozoa taxa per substrate type (right); Lower Danube reach

Table 5: Significant (p≤0.05) indicator taxa per substrate type (*strictly speaking no neozoon in the Lower reach) ; Lower Danube reach

Taxagroup	Family	Genus	Species	Neozoon	Substrate type	Value (IV)	Mean	S.Dev	p *
Diptera	Chironomidae	Cricotopus (I.)	cf. dobrogicus		Macrophytes	73,5	22,3	14,47	0,0156
Ephemeroptera	Baetidae	Cloeon	dipterum		Macrophytes	63,3	21,3	13,42	0,0208
Diptera	Chironomidae	Cricotopus	sp.		Macrophytes	56,5	21,8	14,34	0,0252
Diptera	Chironomidae	Tanypus	punctipennis		Macrophytes	55,1	20,2	13,71	0,016

Taxagroup	Family	Genus	Species	Neozoon	Substrate type	Value (IV)	Mean	S.Dev	p *
Odonata	Coenagrionidae	Coenagrionidae	Gen. sp. juv.		Macrophytes	51,5	21,8	14,47	0,0278
Diptera	Chironomidae	Procladius (H.)	sp.		Macrophytes	50,7	22,9	14,42	0,0364
Diptera	Chironomidae	Rheotanytarsus	sp.		Roots/Debris	46,6	17,6	13,25	0,0386
Gastropoda	Melanopsidae	Esperiana	daudebartii acicularis	*	Microlithal	64,8	27,3	12,79	0,0182
Gastropoda	Viviparidae	Viviparus	viviparus	*	Microlithal	62,6	22,7	13,84	0,0222
Gastropoda	Neritidae	Theodoxus	fluviatilis	*	Meso-/macrolithal	83,8	34	14,65	0,0054
Diptera	Chironomidae	Xenochironomus	xenolabis		Meso-/macrolithal	50	18,6	13,56	0,0114
Heteroptera	Corixidae	Micronecta	sp.		Xylal	89,5	20,7	13,61	0,0042
Amphipoda	Corophidae	Chelicorophium	curvispinum	*	Xylal	84,1	43,5	15,06	0,0032
Amphipoda	Pontogammaridae	Pontogammarus	sarsi	*	Xylal	79,4	21,4	13,55	0,0088
Oligochaeta	Naididae	Specaria	josinae		Xylal	50,8	20,9	13,34	0,0246
Amphipoda	Pontogammaridae	Pontogammarus	robustoides	*	Xylal	49,5	15,8	12,87	0,024
Mysida	Mysidae	Paramysis	sp.		Xylal	48,9	13,7	13,38	0,045
Amphipoda	Pontogammaridae	Pontogammaridae	Gen.sp.	*	Xylal	48	19,2	13,93	0,0256

3.1.3 Sectioning of the Danube River based on MHS results

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-representative taxa numbers.

On the basis of the cluster analysis in Figure 15 the macrozoobenthic community indicates 6 well-defined typological sections (clusters). These sections are:

1. JDS site 1 – 13A: Böfinger Halde to downstream Bratislava
2. JDS site 14 – 25: Gabcikovo reservoir to Paks
3. JDS site 26 – 36: Baja to downstream Tisa
4. JDS site 38 – 43: upstream Pancevo to Banatska Palanka/Bazias (including site 61 – Giurgeni)
5. JDS site 44 – 53: Irongate reservoir (Golubac) to downstream Zimnicea/Svishtov
6. JDS site 55 – 68: downstream Jantra to St. Gheorghe arm (excluding site 61)

According to this classification the first sampling site can actually be seen as own cluster at a more detailed view because of different hydromorphological (e.g. stream size, substrate, depth) and biological characteristics (taxa richness, abundance, taxa composition) compared to downstream sites. Section 6 which comprises the taxa assemblages of the lowest part of the Danube is surprisingly clustered close to section 3 which represents the region upstream Belgrade. All other sections follow a successional gradient. The only outlier is site 61 which is clustered to section 4.

In comparison with the three main reaches proposed by LITERÁTHY et al. (2002) and the ICPDR (2005, 'WFD Roof Report' ANNEX 3: Typology of the Danube River and its reference conditions) data from reach 2 and 3 are rather mixed up because of the unexpected clustering of section 6 (see overlay in Figure 15). The typology of these reaches was used as prerequisite for the development of a type specific assessment system.

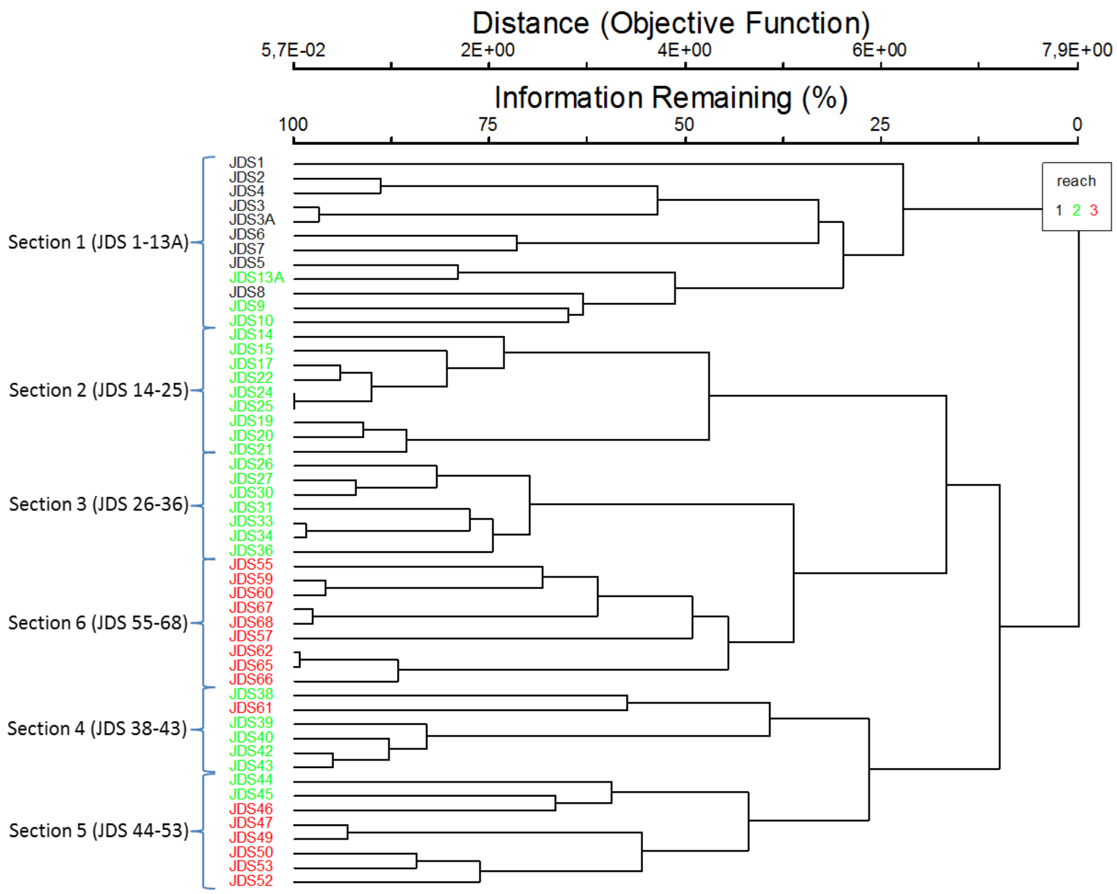


Figure 15: Cluster analysis; MHS samples; definition of 6 typological sections; overlay: Danube reaches (1=Upper Danube reach, 2=Middle Danube reach, 3=Upper Danube reach)

A more detailed view on the MHS-data from JDS 3 in comparison with the defined section types by the ICPDR (2005) shows significant deviations (Figure 16). Following the results from the cluster analysis in Figure 15 the new defined section 1 ranges from Böfinger Halde up to Bratislava comprising section types 1, 2, 3 and more than the half stretch of section type 4 of the classification from 2005. Section 2 of JDS 3 covers the rest of section type 4 and the entire section type 5. JDS 3 sections 3 and 4 cover the whole section type 6. The last JDS 3 sections 5 and 6 are corresponding section types 7, 8, 9 and 10.

In summary the borders of the new section types only partly go along with the definitions of the ICPDR (2005), whereas also the borders between the Danube reaches cannot be categorically confirmed based on macroinvertebrate distribution patterns.

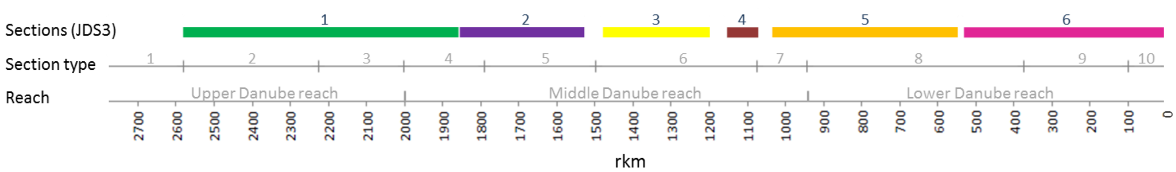


Figure 16: Comparison of new defined section types based on JDS 3 data (coloured bars) with section types and Danube reaches (grey) by the ICPDR (2005)

3.2 Comparison of methods

A comparison of the main approaches applied during JDS3 (MHS) and JDS2 (Airlift) based on taxa assemblages (abundances log(n+1) transformed) shows a distinct separation of the methods regarding NMS-analyses (Figure 17, 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 17, right).

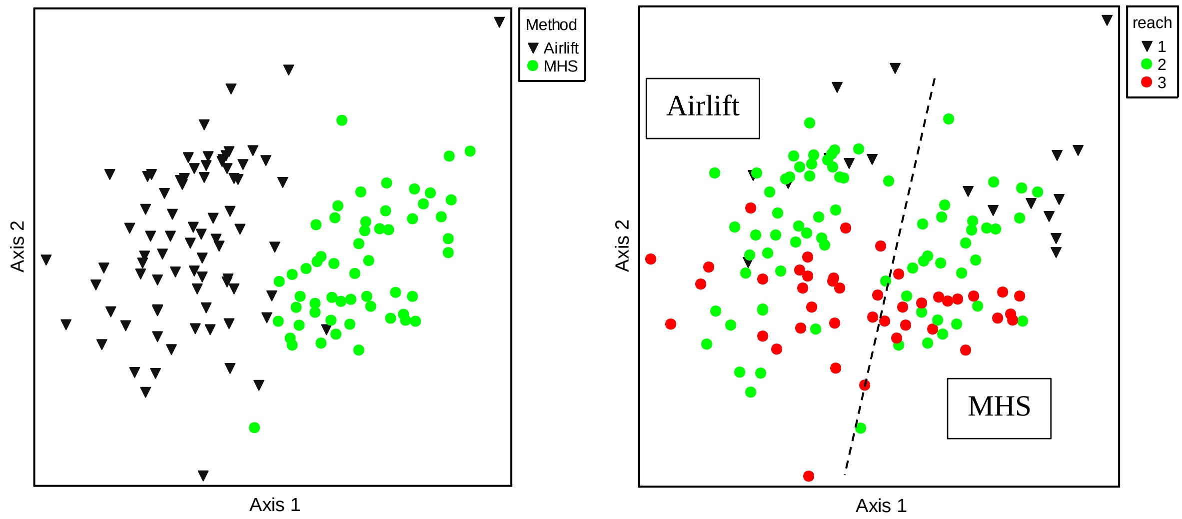


Figure 17: NMS scatterplot based on taxa assemblages of the Airlift method (JDS 2) compared to MHS data (JDS 3); overlay: sampling method (left), Danube reaches (right); final stress for 3-d solution: 14.56, final instability: 0.000, iterations: 194; data log(n+1) transformed

Comprising all applied methods during JDS 3 the NMS scatterplot based on presence/absence transformed faunal composition in Figure 18 (left) gives a quite equal distribution of the sites. None of the methods is forming a separate cluster, indicating a high accordance of shared taxa. In comparison with the Airlift-data from JDS 2 (Airlift method) a sequential arrangement of the methods is noticeable (Figure 18, right). Airlift samples show a higher similarity with DWS samples (due to higher contiguousness in the scatterplot) followed by MHS and K&S data. These results are absolutely comprehensible according to the fact that the deep water fauna differs from the littoral fauna to a certain degree. Following the scatterplot the DWS method captures animals of both river zones, the MHS and K&S method the littoral fauna mainly.

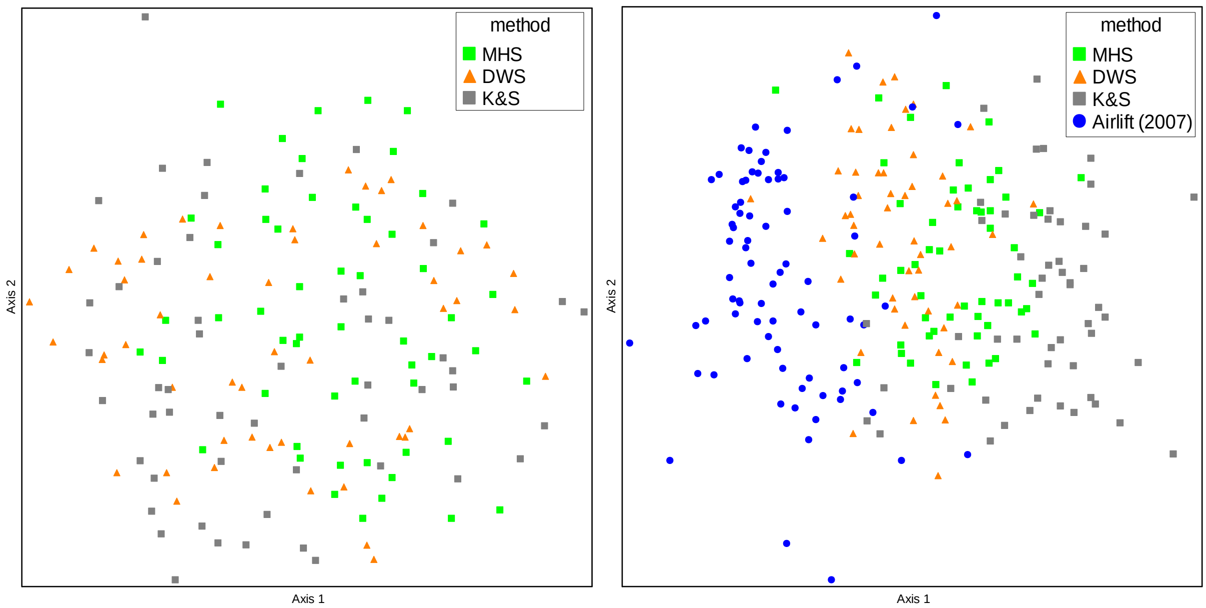


Figure 18: NMS scatterplots based on taxa assemblages captured by MHS, DWS and K&S method (left) and including Airlift method from JDS 2 (right); final stress for 3-d solution: 19.56, final instability: 0.011, iterations: 250 (left), final stress for 3-d solution: 19.26, final instability: 0.012, iterations: 250 (right); data presence/absence transformed

If abundances (log+1 transformed) are included for NMS analyses a deviant picture is given, whereas each method is forming a separated cluster. It indicates that the three methods cover different amounts of macroinvertebrates although a transformation to square meter was done. Sampling efficiency seems to be consistent within the same method but differs significantly between the methods.

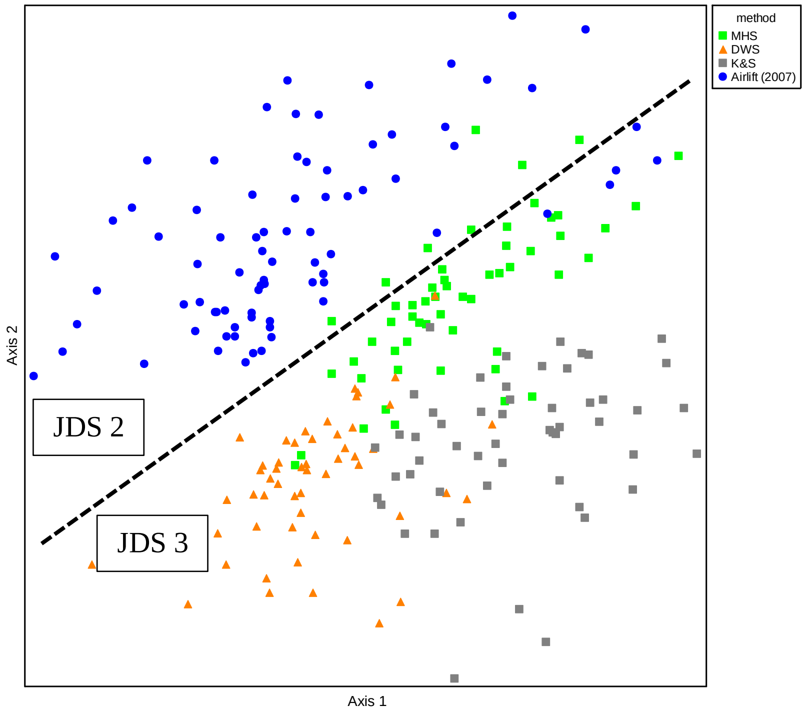


Figure 19: NMS scatterplots based on taxa assemblages captured by MHS, DWS and K&S method (left) and including Airlift method from JDS 2 (right); final stress for 3-d solution: 17.26, final instability: 0.011, iterations: 250; data log(n+1) transformed

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.

3.3 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.

3.3.1 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 (JDS 3) 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 JDS 2 (

Table 6). During JDS 3 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 (

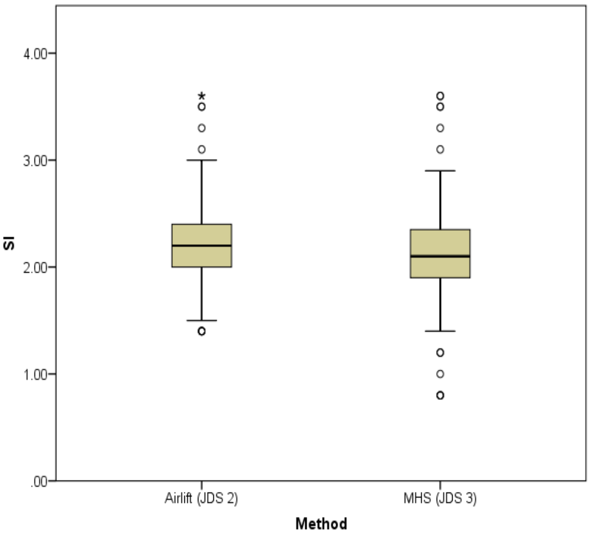
Table 6, indicated by italics).

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 JDS 2, 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 JDS 2 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 20) 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.



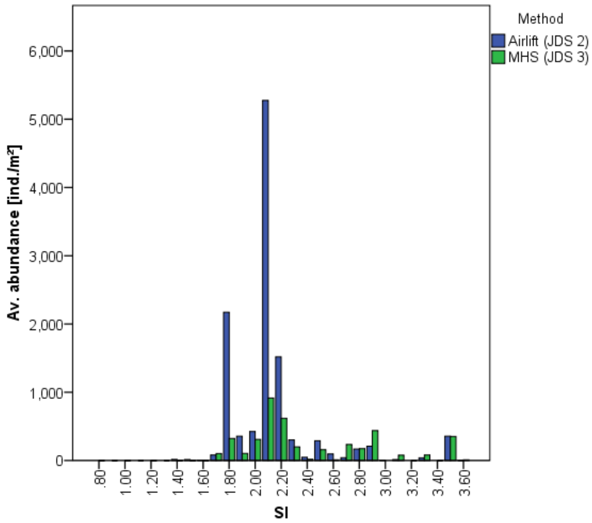


Figure 20: Boxplots of Saprobic Indices of all classified taxa found during JDS 2 by Airlift method and JDS 3 by MHS method (left); average abundances [ind./m²] of taxa per Saprobic Index class of all samples per method (right)

3.3.2 General Degradation

The results of the Slovak method for large rivers applied for the JDS 3 MHS-data (

Table 6) 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 JDS 3 results which are ranging between good and poor status (MHS) in this certain stretch.

Table 6: Saprobic indices (SI) and indication of water quality classes for all Danube sites; results from JDS 2 (Airlift) in grey, results from JDS 3 (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 JDS 2 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*					

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	SK Class
/ 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					
/ 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

3.3.3 Proposal for assigning the ecological quality class

As the calculation of the overall ecological quality on the basis of the Slovak method combines metrics indicating saprobity and general degradation to one index, highly polluted sites under less morphological stress show generally a better status class than the saprobic index as single parameter (e.g. site 32, upstream Novi-Sad in **Error! Reference source not found.**). In compliance with e.g. the Austrian assessment method for rivers the evaluation of the overall ecological quality based on the results of 2 separated modules, organic pollution and general degradation, preferably using the worst case (as shown in Figure 5), is recommended.

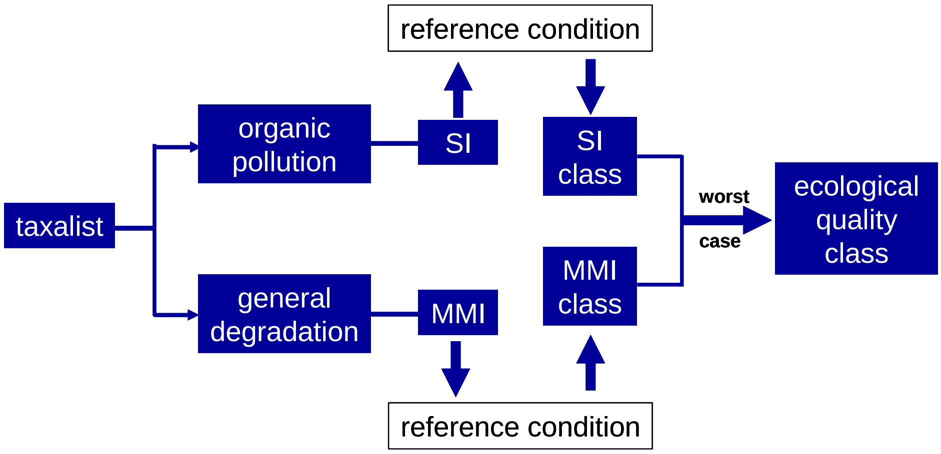


Figure 21: Evaluation of the river quality using two modules, for instance saprobic index and a multimetric index (MMI)

3.4 Neozoa

Neozoa originating from the Ponto-Caspian area, Asia, Australia and North America are a crucial fact influencing the macrozoobenthic community of the Danube. The Danube is a part of the Southern Invasive Corridor (Black Sea-Danube-Main/Danube Channel-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 of Aquatic Invasive Species and further spreading throughout the Danube Basin. Most neozoa of the Danube belong to Crustacea and Mollusca (more detailed information is given in chapter 10 “invasive species” of the JDS 3 report).

Neozoa dominate the Danube not only locally but they are distributed along the entire stretch.

Regarding the most dominant taxa, 8 out of 10 most frequent taxa are neozoa, while 6 of them are belonging to Crustacea. Indigenous taxa occurring in more than 50 % of all sampling sites belong, with the exception of the gastropod *Lithoglyphus naticoides*, mostly to Chironomidae and Oligochaeta.

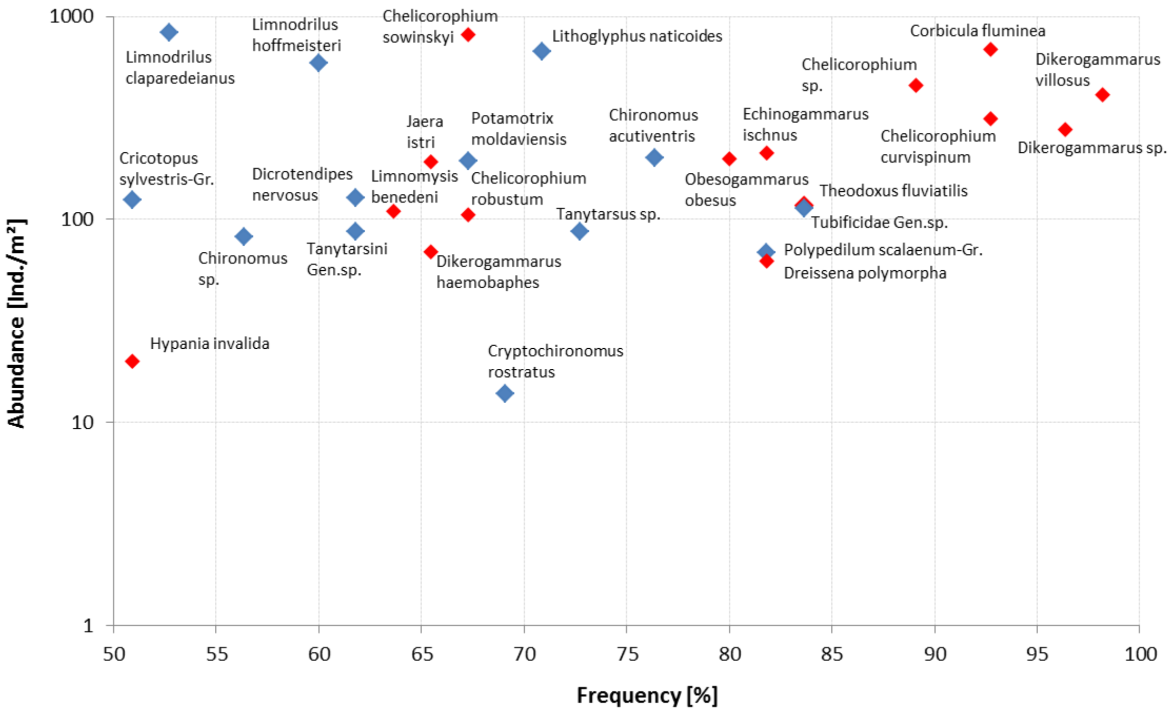


Figure 22: Most dominant taxa (frequency > 50 %) and their average abundances (when present) in the Danube during JDS 3 (MHS method); Neozoa marked red

3.4.1 Distribution of Crustacea

3.4.1.1 Peracarida

3.4.1.1.1 Longitudinal distributions

Based on almost 70000 identified specimens altogether 28 Peracarida species representing four orders (17 Amphipoda, 1 Cumacea, 3 Isopoda, 7 Mysida) were recorded during the survey. Only in the case of 9 can we assume that their longitudinal distribution is not considerably constrained within the investigated section of the river (*Chelicorophium curvispinum* (G. O. Sars, 1895), *Dikerogammarus haemobaphes* (Eichwald, 1841), *Dikerogammarus villosus* (Sowinsky, 1894), *Echinogammarus ischnus* (Stebbing, 1899), *Hemimysis anomala* G. O. Sars, 1907, *Jaera sarsi* Valkanov, 1936, *Katamysis warpachowskyi* G. O. Sars, 1893, *Limnomysis benedeni* Czerniavsky, 1882, *Obesogammarus obesus* (G. O. Sars, 1894); Annex). All nine are successful invaders occurring in several river basins throughout Europe and some of them even beyond the continental coast (Bij de Vaate et al., 2002; Audzijonyte et al., 2008; MacNeil et al., 2010). The relatively scattered occurrence of *H. anomala* and *K. warpachowskyi* can be attributed to their rather special habitat use necessitating specific methods for their effective collection (Borza et al., 2011). Literature data prove their continuous presence along the river, so their presentation elsewhere would have been misleading (Wittmann, 2007, 2008; Borza et al., 2011).

All the remaining species are limited by abiotic or biotic factors in their distribution. The most curious pattern is when a considerable gap divides the range into two parts, which could be observed in three species. *Echinogammarus trichiatus* (Martynov, 1932) and *Chelicorophium robustum* (G. O. Sars, 1895) are recent invaders in the upper and middle reaches of the river (Weinzierl et al., 1997; Bernerth & Stein, 2003). They have arrived by jump dispersal (probably by ships), so in their case the gap indicated that their subsequent downstream spread has not been complete. The records of the survey indicate that *C. robustum* is still rapidly expanding; in 2009 the invasion front was detected at Nagymaros (rkm 1694) (Borza, 2011), whereas by 2013 it has colonized upstream part of Serbian Section. On the contrary, the spread of *E. trichiatus* has slowed down; no expansion could be observed since the last published records in the region (Borza, 2009). The third species, *Chelicorophium sowinskyi* (Martynov, 1924) is an early invader in the Upper and Middle Danube (recorded already in the 1910s) (Borza, 2011), so the pattern in this case must be in connection with the environmental tolerance of the species. The records of the survey do not show considerable change in the size of the gap since the last observations (Borza, 2011).

Three other species showed affinity to the upstream parts of the river. The presence *Gammarus fossarum* Koch, 1836 and *Gammarus roeselii* Gervais, 1835 at the upstream sampling site in the German section, where most Ponto-Caspian species cannot penetrate (with the exception of *D. villosus*) is not unexpected. On the contrary, the distribution of the invasive *Dikerogammarus bispinosus* Martynov, 1925 (i.e. its total absence in the Lower Danube, its native range) is the most puzzling case of all. *D. bispinosus* was an early invader of the Middle Danube; it was first reported in the Hungarian section in the 1920s (Dudich, 1927). We can only assume that at that time it was more abundant in the Lower Danube, but due changes in environmental conditions the population collapsed. It might still not be extinct, perhaps it could be found with higher sampling effort especially in the Delta.

The 10 species occurring only downstream (*Echinogammarus warpachowskyi* (G. O. Sars, 1894), *Obesogammarus crassus* (G. O. Sars, 1894), *Paramysis bakuensis* G. O. Sars, 1895, *Paramysis intermedia* (Czerniavsky, 1882), *Paramysis lacustris* (Czerniavsky, 1882), *Paramysis ullskyi* (Czerniavsky, 1882), *Schizorhamphus scabriusculus* (G. O. Sars, 1894), *Pontogammarus robustoides* (G. O. Sars, 1894), *Pontogammarus sarsi* (Sowinsky, 1898), *Uroniphargoides spinicaudatus* (Carausu, 1943)) are non-expansive Ponto-Caspian peracarids with the exception of P. lacustris, which has recently been found in the River Tisza as upstream as Tokaj (Borza & Boda, 2013). In the Danube itself its most upstream presence corresponded to the mouth of this tributary. It is also noteworthy that *O. crassus, P. robustoides*, and *E. warpachowskyi* were found rarely, whereas in other river basins they are actively expanding (Bij de Vaate et al., 2002).

Asellus aquaticus (Linnaeus, 1758) and Niphargus hrabei S. Karaman, 1932 cannot be regarded as a resident of the Danube main arm; their sporadic occurrence is a result of drift from adjacent waters. The same can be supposed in *Proasellus coxalis* (Dollfus, 1892), which prefers smaller waters (Kaiser, 2005).

3.4.1.1.2 Abundance patterns (based on MHS data)

In general, the frequency of occurrence of the species corresponded well with their abundance; common species were abundant and those occurring rarely were present in low numbers (Figure 23). Only a couple of species deviated from this pattern to a considerable extent; *C. sowinskyi* and *E. trichiatus* were more abundant than expected (due to a few mass occurrences), while *K. warpachowskyi* was present rather frequently but in very low numbers. The Peracarida assemblage of the river was dominated by ten invasive species, whereas the frequency of non-invasive species barely exceeded 5% each.

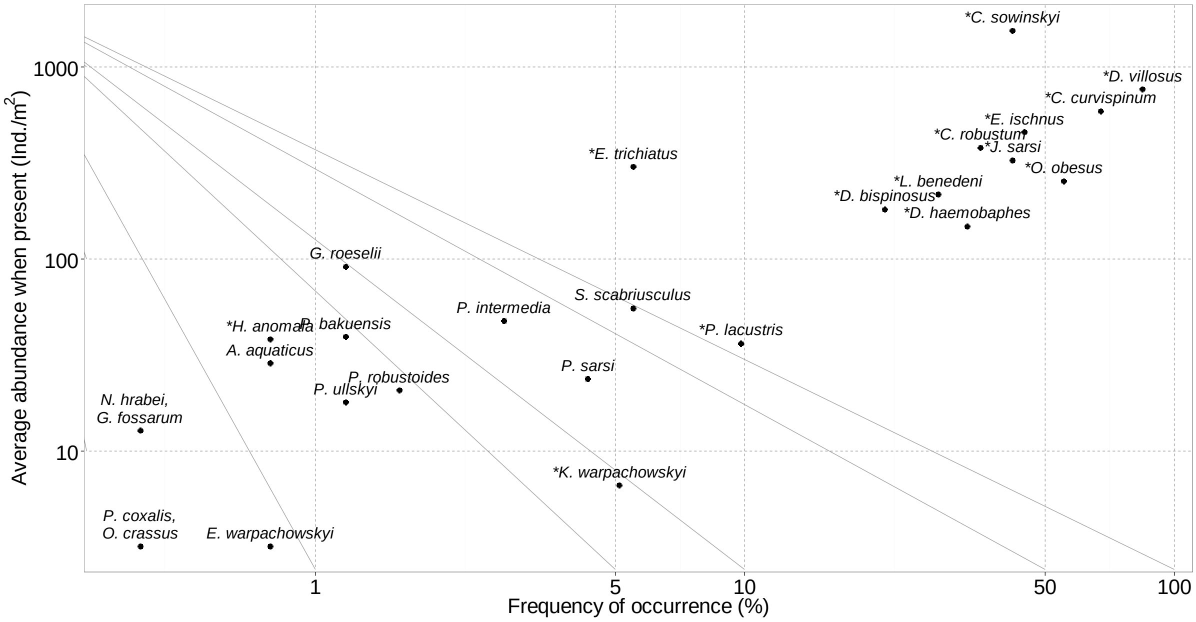


Figure 23: Frequency of occurrence versus average abundance (zeros not included) of the Peracarida species collected during the survey. *: invasive species (within the Danube basin).

A site specific overview of the most abundant Crustacea-genera (excl. *Astacus, Niphargus, Proasellus, Asellus*) is given in Table 7.

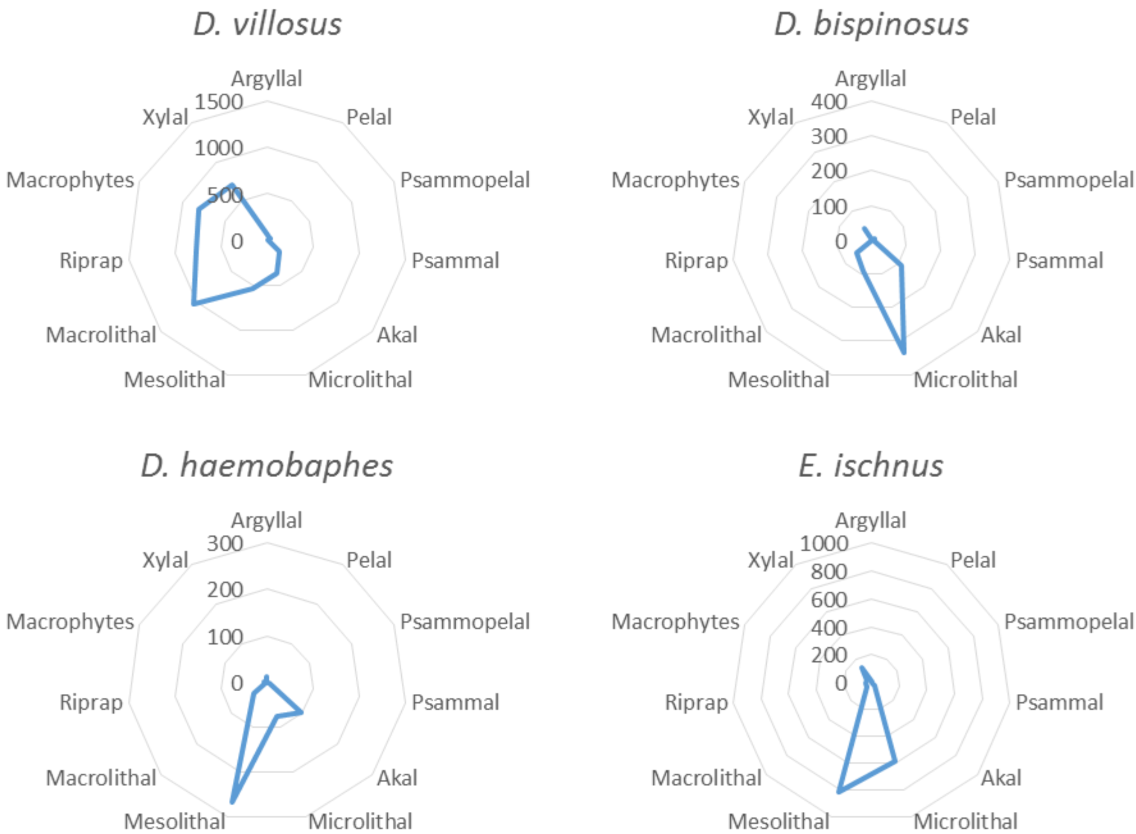
Table 7: Abundance classes of Crustacea-genera (excl. Astacus, Niphargus, Proasellus, Asellus) along the Danube ([a] = sampled at increasing water level; **= Isopoda; *= Cumacea)

		Amphipoda						I.**	Mysida				C.*
JDS site	Reach	Gammarus	Dikerogammarus	Chelicorophium	Echinogammarus	Obesogammarus	Pontogammarus	Jaera	Limnomysis	Katamysis	Paramysis	Hemimysis	Schizorhamphus
1	Upper Danube	◑	●	○	○	○	○	○	○	○	○	○	○
2		○	◕	◕	◕	○	○	◕	◔	○	○	○	○
3		○	●	◕	◔	○	○	◑	◔	○	○	○	○
3A		○	●	●	◑	◔	○	◑	◔	○	○	○	○
4		○	●	◕	◑	◑	○	◕	◔	◔	○	○	○
5		○	●	●	◕	◔	○	●	○	○	○	○	○
6		○	●	◑	◔	◔	○	○	◔	○	○	○	○
7		○	◕	◑	◑	◔	○	◑	○	○	○	○	○
8		○	◕	◕	◑	◑	○	◕	○	○	○	○	○
9	Middle Danube	○	●	◕	◑	◕	○	◕	○	○	○	○	○
10		○	◑	◑	◑	◔	○	◑	○	○	○	○	○
11		○	◕	◔	◑	○	○	◑	○	○	○	○	○
13		○	◕	◔	◑	◑	○	◑	◔	○	○	○	○
13A		○	◕	◕	◑	◔	○	◕	◔	○	○	○	○
14		○	◕	●	◑	◕	○	◑	◑	◔	○	○	○
15		○	◕	◕	◕	◑	○	◕	○	○	○	○	○
17		○	◕	●	◑	◑	○	◕	◔	○	○	○	○
19		○	◕	◕	◔	◕	○	◕	◑	○	○	○	○
20		○	●	●	◕	◕	○	◕	○	○	○	○	○
21		○	◕	◑	◑	◕	○	○	○	○	○	○	○
22		○	◕	◕	◔	◔	○	◕	○	○	○	○	○
24		○	◕	◕	◕	◕	○	◑	○	○	○	○	○
25		○	◕	◕	◑	◕	○	◑	◔	○	○	○	○
26		○	◕	●	○	◔	○	◑	◑	○	○	○	○
27		○	◕	◕	◔	○	○	◑	○	○	○	○	○
28[a]		○	○	○	○	○	○	○	○	◔	○	○	○
30		○	◕	◑	◔	◔	○	○	◔	◔	○	○	○
31		○	◕	◔	◔	◑	○	◔	◔	○	○	○	○
32		○	●	◑	◔	○	○	○	◔	◔	○	○	○
33		○	◕	◑	◔	◔	○	◔	◔	○	○	○	○
34		○	◕	◑	◔	◑	○	○	◔	○	○	○	○
36		○	◕	◕	○	◔	○	○	◔	○	◑	○	○
38		○	◑	◑	○	◔	○	○	◔	◔	○	○	○
39		○	●	●	◔	◔	○	◑	◑	○	○	○	○
40		○	◕	◕	◔	◕	○	◔	●	◔	◔	○	○
42		○	●	◑	◑	◑	○	○	◕	○	◑	○	○
43		○	●	◕	◑	◔	○	◑	◕	○	◑	○	○
44		○	●	◑	●	◔	○	◑	◕	○	○	○	○
45		○	◕	◑	◑	○	○	○	◕	○	○	○	○
46	Lower Danube	○	◕	○	◔	○	○	○	◕	○	○	○	○
47		○	◑	◑	○	◔	○	◔	◕	◔	◑	○	○
49		○	◕	●	◑	◕	○	◑	◕	○	◔	○	○
50		○	●	●	●	◔	◑	◔	◑	○	◑	○	○
52		○	◕	●	◕	◑	○	◕	◑	◔	○	◑	○
53		○	◕	◑	◕	◕	◔	◑	◔	◔	◔	○	○
55		○	◕	●	◑	●	◔	○	◔	○	○	○	○
57		○	◑	◑	◔	◕	◔	○	○	○	○	○	○
59		○	◕	●	●	◑	◑	◕	○	○	◔	○	○
60		○	◕	●	◑	◕	◔	◔	◔	◔	◑	○	○
61		○	◑	◔	○	◕	○	○	◔	○	◑	○	○
62		○	◕	◑	◔	◔	◔	○	○	○	◔	○	◑
65		○	◕	◑	◔	◔	◔	○	◔	○	◑	○	◑
66		○	◑	◕	◔	◔	○	◔	◔	◔	◔	○	○
67		○	◕	●	◔	◑	◔	○	◔	○	◔	○	◔
68		○	◕	●	○	◑	○	○	○	○	◑	○	◑

Ind./m²	
●	≥1000
◕	100-999
◑	10-99
◔	1- 9
○	0

3.4.1.1.3 Habitat preferences

The preliminary results on habitat preference (based on the MHS-data) revealed that most of the dominant peracarids preferred solid substrates (Figure 24). Only *O. obesus* showed clear preference towards pelal and argyllal; while psammal and psammopelal were largely avoided by all of the species. Among *Dikerogammarus* spp., *D. villosus* preferred organic substrates (macrophytes, xylal) and those offering large solid surfaces (riprap, macrolithal), whereas the other two were most abundant on gravel (*D. bispinosus* on microlithal, *D. haemobaphes* on mesolithal rather). *E. ischnus* and *C. robustum* showed a similar predilection for gravel but with less stress on grain size. The abundance of *C. curvispinum* seems to be positively related to grain size in the microlithal-macrolithal/riprap range, and xylal was also a preferred substrate. The occasionally extreme high abundances of *C. sowinskyi* resulted in a peculiar bipolar preference pattern between mesolithal and pelal, pointing at the importance of revealing the role of other factors. *J. sarsi* showed clear dependence on hard surfaces, while *L. benedeni* preferred organic substrates.



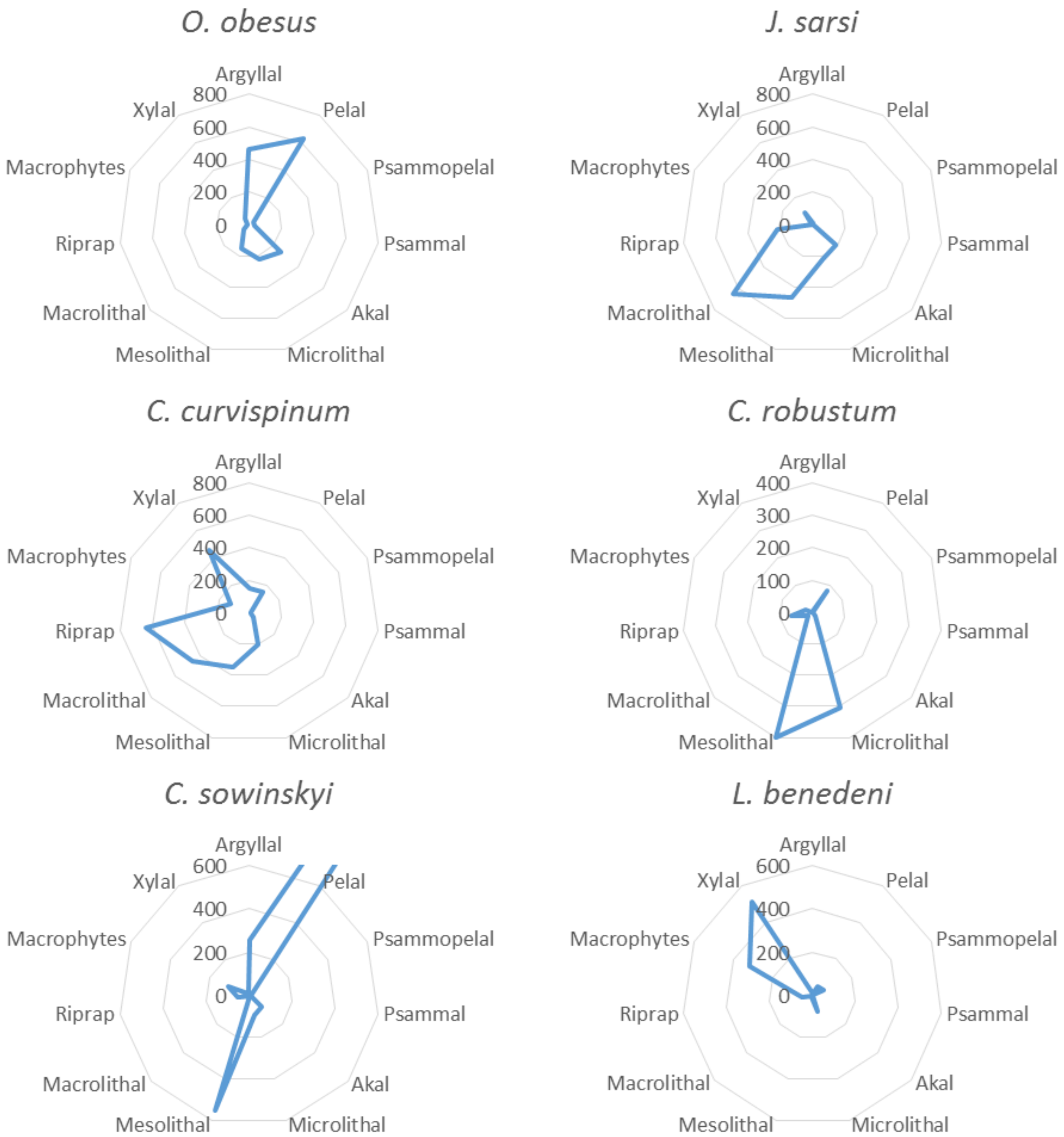


Figure 24: Average abundances (ind/m2, only considering the sections where the species was present) per habitat type (based on AQEM protocol) of the 10 most abundant peracarid species during the survey. C. sowinskyi rescaled due to the extreme high mean abundance (> 4500 ind/m2) on pelal.

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*.
- Regarding the most dominant taxa, 8 out of 10 most frequent taxa are neozoa, while 6 of them are belonging to Crustacea. Indigenous taxa occurring in more than 50 % of all sampling sites belong, with the exception of the gastropod *Lithoglyphus naticoides*, mostly to Chironomidae and Oligochaeta.

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 JDS 2 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 JDS 2 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 JDS 3. 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.

Comparison with the JDS 2 data

- A comparison of the main approaches applied during JDS3 (MHS) and JDS2 (Airlift) based on taxa assemblages shows a distinct separation of the methods, indicating that each method provides a unique fauna – a deep-water fauna and a riparian related fauna.
- Comprising all applied methods during JDS 3, Airlift samples show a higher similarity with DWS samples followed by MHS and K&S data according to the fact that the deep water fauna differs from the littoral fauna to a certain degree; consequently the DWS method captures animals of both river zones, the MHS and K&S method the littoral fauna mainly.

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