Implementation of the EU Water Framework Directive for the development of
the Danube River Basin Management Plan
(WATERDRB-2009)

Supportive analysis of the second Joint Danube Survey data (typology, intercalibration) and Technical support of the Eastern Continental Geographical Intercalibration Group

Final Report

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April 2009

Table of contents

List of tables

List of figures

List of abbreviations

BQE	Biological Quality Element
CB GIG	Central Baltic Geographical Intercalibration Group
DCA	Detrended Correspondence Analysis
DIS	Danube Intercalibration Stretch
EC GIG	Eastern Continental Geographical Intercalibration Group
EQR	Ecological Quality Ratio
ICPDR	International Commission for the Protection of the Danube River
IndVal	Indicator Value analysis (Dufrene & Legendre 1997)
JDS	Joint Danube Survey
JDS 2	Second Joint Danube Survey (Liska et al. 2008)
LDC	Least Disturbed Condition (Stoddard et al. 2006)
MRPP	Multi Response Permutation Procedure (McCune & Grace 2002)
PCA	Principal Component Analysis
TNMN	TransNational Monitoring Network (e.g. ICPDR 2007)
VAST	Valley segment affinity Search Technique (Brenden et al. 2008)
WATERDRB-2009	Short code for the project “Implementation of the Water Framework Directive for the development of the Danube River Basin Management Plan”
WFD	Water Framework Directive 2000/60/EC

1 Preface

One of the key elements of the European Water Framework Directive (WFD) is to ensure that all waters meet “good status” by 2015. The first step towards this aim is to create a River Basin Management Plan by 2009 based on the outputs of a basin-wide characterisation process. Furthermore, pursuant to Article 13 (5) of the WFD, the Member States may supplement the River Basin Management Plan by the production of more detailed programmes and management plans for sub-basins.

The main objective of the project “Implementation of the EU Water Framework Directive for the development of the Danube River Basin Management Plan (WATERDRB-2009)” is the completion of the Danube River Basin Management Plan in line with the WFD requirements, also considering the preparation of sub-basin plans.

Main actions of the project comprise

- the development of the Joint Programme of Measures for the Danube River Basin,
- the preparation for the Danube River Basin Management Plan and
- the analysis of the second Joint Danube Survey (JDS2) data and the respective intercalibration exercise of the Danube River.

The report at hand delivers first outcomes gained in the latter action on JDS2 data analysis and intercalibration. In particular, the tasks covered

- the preparation of an intercalibration approach for the Danube River using JDS2 data and
- the technical support of the Eastern Continental Geographical Intercalibration Group (EC GIG).

JDS2

Basis for all analyses presented in this report are data sampled during the second Joint Danube Survey cruise. JDS2 was an international longitudinal ship survey that produced homogeneous data on water quality for the complete length of the Danube River including the major tributaries. It was organized from August to September 2007. During the survey 96 sites were sampled along the 2600 km stretch of the Danube, from which 18 sites were in the mouths of the tributaries or side arms.

Sampling at the JDS2 stations included five different sample types - water, sediment, biology, suspended particulate matter and biota (mussels and fish), each with a different determinant list, which were taken at different sampling points (left, middle, right) at the station's cross sections of the main river, and in the middle of the cross-section of the tributaries.

A continuous observation of hydromorphological parameters in approximately 50 km stretches had been carried out during sailing, whereas more detailed screening was carried out at each of the 96 sampling sites.

2 Setting biological benchmarks for the Danube River intercalibration exercise: Global definition of least disturbed conditions and its relevance for the type-specific river coenosis

2.1 Summary

In the first part of this scientific report we establish criteria for Least Disturbed Conditions (LDC; Stoddard et al. 2006) at the Danube River, and identify monitoring stations complying with these criteria. We derive four Danube Intercalibration Stretches (DIS) based on the ten Danube Section Types (Moog et al. 2008). These stretches build the foundation to intercalibrate the ecological status classifications of Danube riparian states. Using data sampled during the second Joint Danube Survey (Liska et al. 2008) we generate a complex abiotic gradient of general anthropogenic disturbance. This gradient covers the whole length of the Danube River and reflects impairments, for instance, from major abutting cities or impoundments, as well as National Parks and other sub-natural reaches (e.g. Wachau, Danube-Auen National Park, Duna Drava National Park, Lower Danube Wetland System, Danube Delta) (Figure 2.1). We set a threshold value on the pressure gradient based on the classification of chemical and hydromorphological quality. This threshold allows for the assignment of sites in least disturbed conditions (LDC sites).

Based on different biological data (macrozoobenthos, macrophytes, benthic diatoms and phytoplankton) we test if the abiotic pressure gradient is having an effect on the shape of the biological communities in the Danube River. We apply indirect gradient analysis to investigate relationships between the pressure gradient and the biological assemblages. The outcomes reveal that the pressure gradient is significantly influencing the macrozoobenthos and macrophyte communities. Except for the Lower Danube Intercalibration Stretch the multivariate community descriptors (second DCA-axis explaining approximately 25 percent of total variance) are significantly different between LDC and non-LCD sites for each stretch. Several biological metrics are reflecting these differences on the stretch-specific level for the macrozoobenthos community, e.g. the Lotic-Invertebrate Flow Evaluation (Extence et al. 1999), the percentage of Crustacean taxa or the Portuguese GOLD index (Buffagni et al. 2005). For the macrophytes only the Austrian Index for Macrophytes AIM is responsive to the pressure gradient in the Upper DIS. We propose a sensitivity metric for the Southern

Pannonian DIS composed of the scores of 13 macrophyte indicators that we deduced from a correlation analysis of taxa abundance with the pressure gradient. This metric clearly distinguishes LDC sites from impaired sites.

This report sets the groundwork for the intercalibration of the Danube River within the Eastern Continental Intercalibration Group. Moreover, with this study we want to deliver an essential contribution to the international discussion on the intercalibration of large river classification schemes. Although our work faced incomplete biological assessment methods for the Danube River and we had to tackle issues of data quality, this case study provides a general approach to define biological benchmarks (Birk & Hering 2009) to be used in the Europe-wide large river intercalibration exercise.

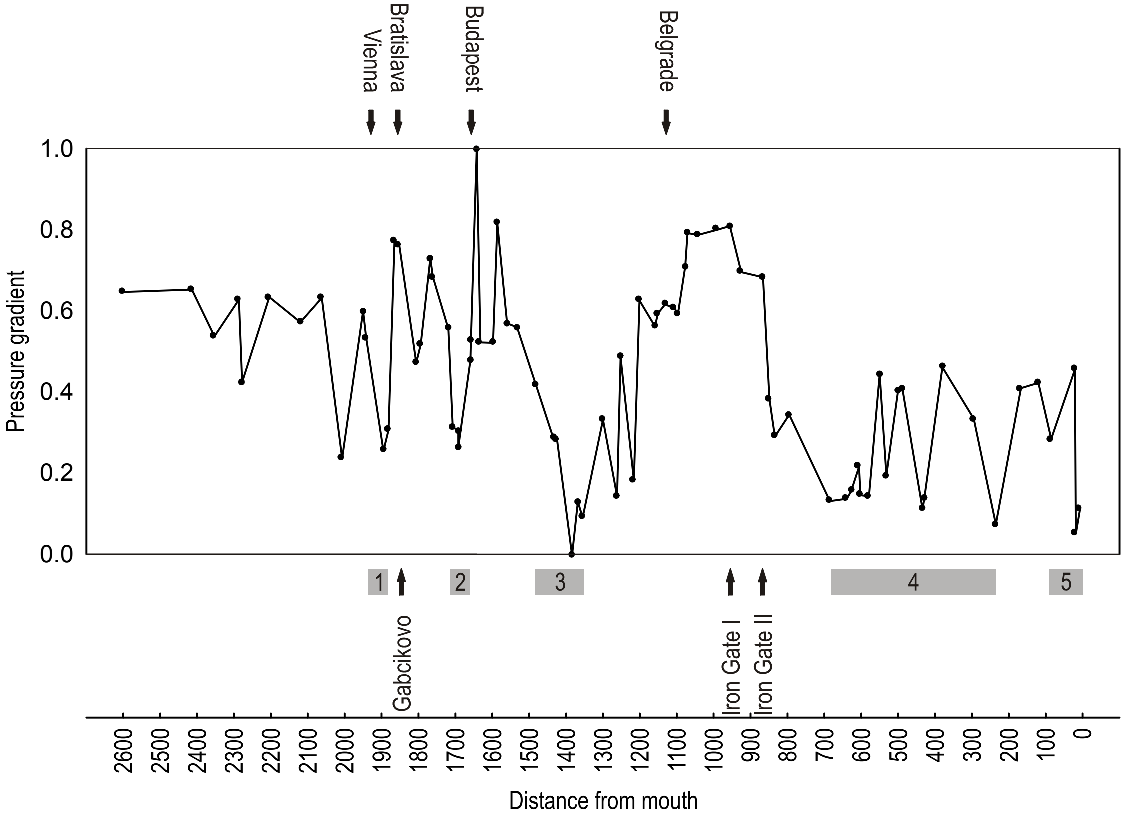

Figure 2.1: Pressure profile for the Danube River, specifying the locations of major cities, impoundments and important wetlands. The complex pressure gradient is measured in values between 0 (near-natural) to 1 (severely impaired). See Chapter 2.2 for details. 1=Danube-Auen National Park; 2=Danube bend; 3=Duna Drava National Park; 4=Lower Danube Wetland System; 5=Danube Delta.

2.2 Defining least disturbed conditions for the Danube River

2.2.1 Introduction

The near-natural reference state of a water body type represents the benchmark for ecological quality assessment of individual water bodies. The status classification of a water body depends on how much the biological community (or related aspects) is deviating from undisturbed conditions. This deviation is thus not measured in absolute terms, but relative to the reference state. This relation is expressed in the so-called "Ecological Quality Ratio" (EQR). Member States have defined the good quality status by setting class boundaries on the relative EQR scale ranging from 1 (reference state) to 0 (non-natural state). These boundaries reflect the national interpretations of what is regarded as 'good'. The intercalibration exercise aims at comparing and harmonizing these national interpretations.

In this regard, it is crucial that the intercalibration exercise is carried out against a common baseline. Even if measured on a comparable scale, the same class boundary values may reflect different levels of human impairment, if the reference state is defined differently (Figure 2.2). In Central Europe, Member States recently intercalibrated river diatom and invertebrate classifications by common metrics (CB GIG Rivers 2008). These metrics were correlated with the national assessment methods, and regression analyses inferred the values of the common metrics that corresponded to the national quality class boundaries. To compare common metric values between countries they had to be standardized. For this purpose the participating countries provided data on undisturbed reference sites, which were selected with harmonized criteria (CB GIG Rivers 2008). The biological community of these undisturbed sites yielded the reference value of the common metrics and provided EQR scales that were comparable between countries.

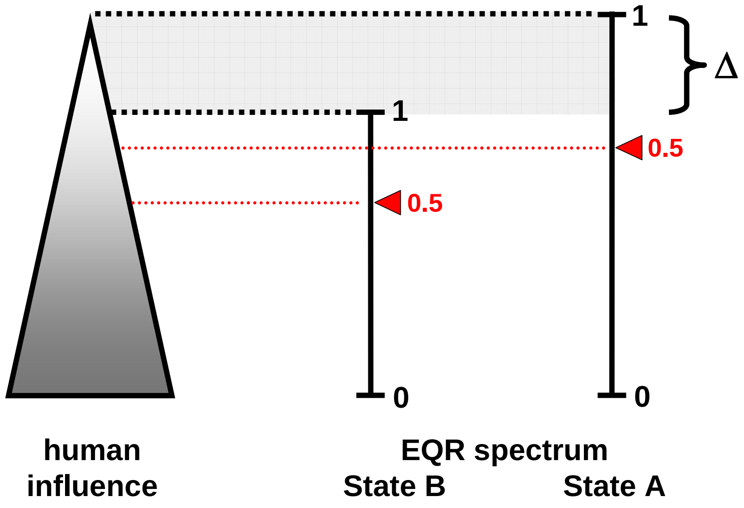

Figure 2.2: On the importance of a common definition of near-natural reference conditions in intercalibration. If the national assessment methods of two countries refer to different levels of human influence (Δ), the same Ecological Quality Ratios (EQR) represent different levels of impairment (Birk & Böhmer 2007, modified).

The principal problem with this approach was the scarcity of reference sites, since unimpacted conditions no longer exist (e.g. Birk et al. 2007; Gabriels 2007) or data were not available as monitoring focused on impacted sites. Several countries could therefore not intercalibrate their methods, especially those applied for large rivers. Thus, the question arose: Does the intercalibration of class boundaries necessarily require data on near-natural reference sites or are there alternative approaches? In a review of the reference concept in freshwater bioassessment Stoddard et al. (2006) introduce the term “Least Disturbed Conditions” (LDC) that refer to the best available physical, chemical, and biological habitat conditions given today’s state of the landscape. The consistent definition of LDC can theoretically provide a solution for the issue of lacking near-natural reference sites in intercalibration.

Against this background, Wasson et al. (2005) proposed a practical concept for the intercalibration of very large rivers. They suggested defining physico-chemical and hydromorphological criteria for the status boundary between either high and good, or good and moderate ecological quality (Figure 2.3). Based on the biological communities found at the sites complying with the abiotic criteria, a harmonised biological quality class boundary should be derived. In this regard, Birk & Hering (2009) developed an applied approach establishing “biological benchmarks” based on data from sites of at least good environmental status. The biological benchmark was defined as the condition of the biological community that represents the transnational reference point for harmonization. This benchmark was given for selected aspects of the biological community measured by common metrics. The approach allowed for the intercalibration of national assessment methods in the Danube River Basin without reference to data from undisturbed sites.

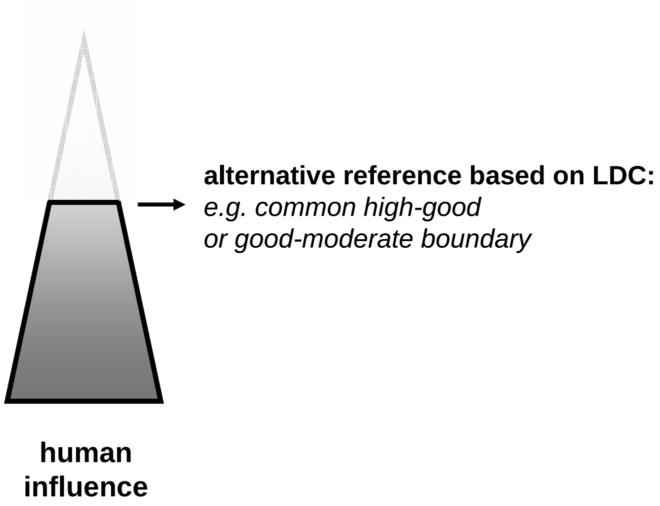

Figure 2.3: Definition of an alternative reference in intercalibration by the use of sites in Least Disturbed Conditions (LDC) impacted by a similar level of impairment instead of near-natural reference sites.

This chapter describes a case study to consistently define Least Disturbed Conditions (LDC) at very large rivers, and to identify sampling sites meeting the LDC criteria (i.e. “LDC sites”). Based on Principal Component Analysis (PCA) we extract the main environmental gradients from the abiotic data sampled during JDS2. Among these gradients we identify the component that best reflects anthropogenic pressure, i.e. the “pressure gradient”. To validate the selected gradient, we apply an abiotic classification scheme to the JDS sites covering parameters of general water quality and hydromorphological evaluation. We use the pressure gradient range among sites in good abiotic status to define a threshold value for the allocation of LDC sites.

2.2.2 Methods

Description of data basis

The basis for our analysis were environmental and biological data samples at 78 out of the 96 sites (those situated in tributaries excluded) of the Danube during JDS2 from August 13th to September 8th, 2007. Sampling included different sample types: water, sediment and biology, which were taken at different locations in the river profile (left, middle, right) at the station's cross-sections of the main river.

Five groups of environmental data were recorded:

- The basic physico-chemical data (temperature, oxygen, conductivity and pH) was sampled and analysed in situ.
- Chemical quality elements like nitrate, alkalinity, ammonium, orthophosphate, and dissolved oxygen concentration were sampled in the sediment and the water. They were analysed on-board.
- Organic nitrogen and total phosphorus were sampled in the sediment and water, processed, preserved and then analysed in the laboratory.
- Basic hydromorphological data was recorded for each station (e.g. river width and depth, shoreline index, amount of large woody debris). However, some of this data was not sampled per site, but per river stretch of approximately 50 km length.
- Abiotic data collected from the macrophyte survey was sampled at 3 km stretches near the site on both sides of the river.

Summary statistics of the selected variables are shown in Table 2.1.

Table 2.1: Summary statistics of the JDS2 samples

Variable	Unit	Mean	Range	Standard deviation
Catchment size	km^2	382,393	4,100 – 801,500	253,742
Altitude	m	90.8	0 – 480	91.0
Distance from mouth	km	1,233.9	7 – 2,600	656.2
Conductivity	$\mu S\ cm^{-1}$	386.9	346 – 577	35.1
pH	-	7.76	7.3 – 8.1	0.16
Alkalinity	$mmol\ l^{-1}$	2.71	2.3 – 4.4	0.32
Ammonium ($N\text{-}NH_4$)	$mg\ l^{-1}\ N$	0.029	0 – 0.379	0.059
Nitrate ($N\text{-}NO_3$)	$mg\ l^{-1}\ N$	1.66	0.78 – 3.12	0.36
Orthophosphate ($P\text{-}PO_4$)	$mg\ l^{-1}\ P$	0.061	0.044 – 0.095	0.010
DO concentration	$mg\ l^{-1}$	8.18	3.49 – 10.10	0.99

Several techniques have been used to fill gaps or replace values in the environmental data set (Lepš & Šmilauer 2003). Missing values were replaced by linear regression when possible. For example, at site JDS005 a missing value for *catchment size* was filled using the *distance from mouth*, as linear regression between the two yielded an R^2-value of 0.97. When this was not possible and the variable varied along the longitudinal gradient, the mean of adjacent sites was inserted, as has been done with the *discharge at high and low water* at site JDS004. When there was no clear gradient the mean of the variable was used, like for a number of missing values for *organic nitrogen in the sediment*. For binary variables like *naturalness of bank slope*, the summarized variable was assigned the value "natural", if both location in profile variables were "natural" and "neither", if these were "natural" and "non natural". Data from the 50 km stretches was inserted into the sites that were situated along it.

As an additional data source we used nutrient data continuously sampled within the TransNational Monitoring Network (TNMN; e.g. ICPDR 2007) between January and September 2007 at monitoring stations across the Danube (see Table 2.2). Missing values in this dataset were replaced by linear regression. Values of the nearest upstream JDS site were replaced by TNMN values, since they were deemed more relevant for the BQEs and better for site characterization. N-NH_4, N-NO_3 and P-PO_4 concentrations in the water showed a wider distribution for the TNMN data (Figure 2.4).

Selected methods were then used to transform all data into normal distributions (Table 2.3).

Table 2.2: TNMN values per site, including country, distance from mouth (RKM), the number of samples taken between January and September 2007 (N) and mean and 90th percentile values (90th) in mg l^{-1}. Values printed in bold have been modelled.

Site	Country	RKM	N	Ammonium ($N\text{-}NH_4$)		Nitrate ($N\text{-}NO_3$)		Nitrite ($N\text{-}NO_2$)		Orthophosphate ($P\text{-}PO_4$)		Total phosphorus	
				Mean	90th	Mean	90th	Mean	90th	Mean	90th	Mean	90th
JDS007	Germany/Austria	2204	29	0.037	0.070	1.859	2.790	0.011	0.015	0.030	0.049	0.074	0.107
JDS014	Austria	1881	18	0.029	0.056	1.855	2.760	0.016	0.023	0.012	0.035	0.059	0.074
JDS016	Slovakia	1865	57	0.034	0.060	1.943	2.950	0.011	0.020	0.042	0.060	**0.101**	**0.151**
JDS018	Slovakia/Hungary	1806	9	0.010	0.037	1.732	2.600	0.012	0.021	0.014	0.024	0.086	0.130
JDS020	Slovakia/Hungary	1768	27	0.012	0.047	1.854	2.850	0.013	0.023	0.017	0.028	0.090	0.130
JDS026	Hungary	1707	2	0.140	0.160	2.430	2.600	0.016	0.024	0.038	0.046	0.138	0.180
JDS039	Herzegovina	1434	18	0.056	0.140	1.740	2.712	0.014	0.023	0.016	0.026	0.126	0.150
JDS040	Herzegovina/Serbia	1424	16	0.068	0.200	1.741	2.700	0.018	0.025	0.033	0.067	0.116	0.151
JDS043	Herzegovina/Serbia	1368	14	0.081	0.220	1.748	2.630	0.018	0.026	0.037	0.069	0.116	0.154
JDS045	Herzegovina/Serbia	1300	20	0.053	0.100	1.910	3.164	0.016	**0.020**	**0.054**	0.089	0.119	0.170
JDS047	Serbia	1252	18	0.110	0.200	1.484	2.640	0.022	0.029	0.081	0.087	0.119	0.158
JDS053	Serbia	1151	14	0.096	0.160	1.231	1.760	0.019	0.028	0.038	0.054	0.148	0.213
JDS058	Serbia	1077	17	0.146	0.230	1.362	1.940	0.022	0.029	0.058	0.073	0.109	0.134
JDS059	Serbia/Romania	1071	46	0.331	0.551	0.993	1.491	0.030	0.038	0.038	0.110	0.079	0.160
JDS062	Serbia/Romania	956	18	0.043	0.160	1.418	2.240	0.017	0.041	0.060	0.104	0.095	0.146
JDS065	Serbia/Romania	849	33	0.203	0.349	0.931	1.240	0.026	0.042	0.044	0.080	0.077	0.140
JDS067	Romania/Bulgaria	834	51	0.219	0.411	1.050	1.356	0.027	0.045	0.034	0.068	0.065	0.120
JDS070	Romania/Bulgaria	639	9	0.389	0.700	1.189	2.800	0.027	0.056	0.084	0.100	0.120	0.200
JDS077	Romania/Bulgaria	550	8	0.071	0.296	1.214	2.090	0.021	0.025	0.246	0.400	0.635	1.640
JDS080	Romania/Bulgaria	500	9	0.102	0.209	1.183	1.990	0.018	0.032	0.213	0.297	0.174	0.352
JDS083	Romania/Bulgaria	434	22	0.225	0.408	1.562	2.090	0.024	0.029	0.057	0.070	0.107	0.144
JDS086	Romania/Bulgaria	380	51	0.193	0.339	1.454	1.980	0.023	0.032	0.081	0.135	0.146	0.286
JDS092	Romania/Ukraine	120	56	0.212	0.412	1.363	2.240	0.043	0.114	0.043	0.095	0.080	0.135
JDS093	Romania/Ukraine	18	29	0.203	0.434	1.300	2.130	0.033	0.076	0.055	0.108	0.088	0.143
JDS095	Romania	21	30	0.172	0.416	1.316	1.960	0.040	0.097	0.061	0.112	0.099	0.150
JDS096	Romania	85	30	0.182	0.347	1.270	1.940	0.035	0.089	0.064	0.119	0.113	0.197

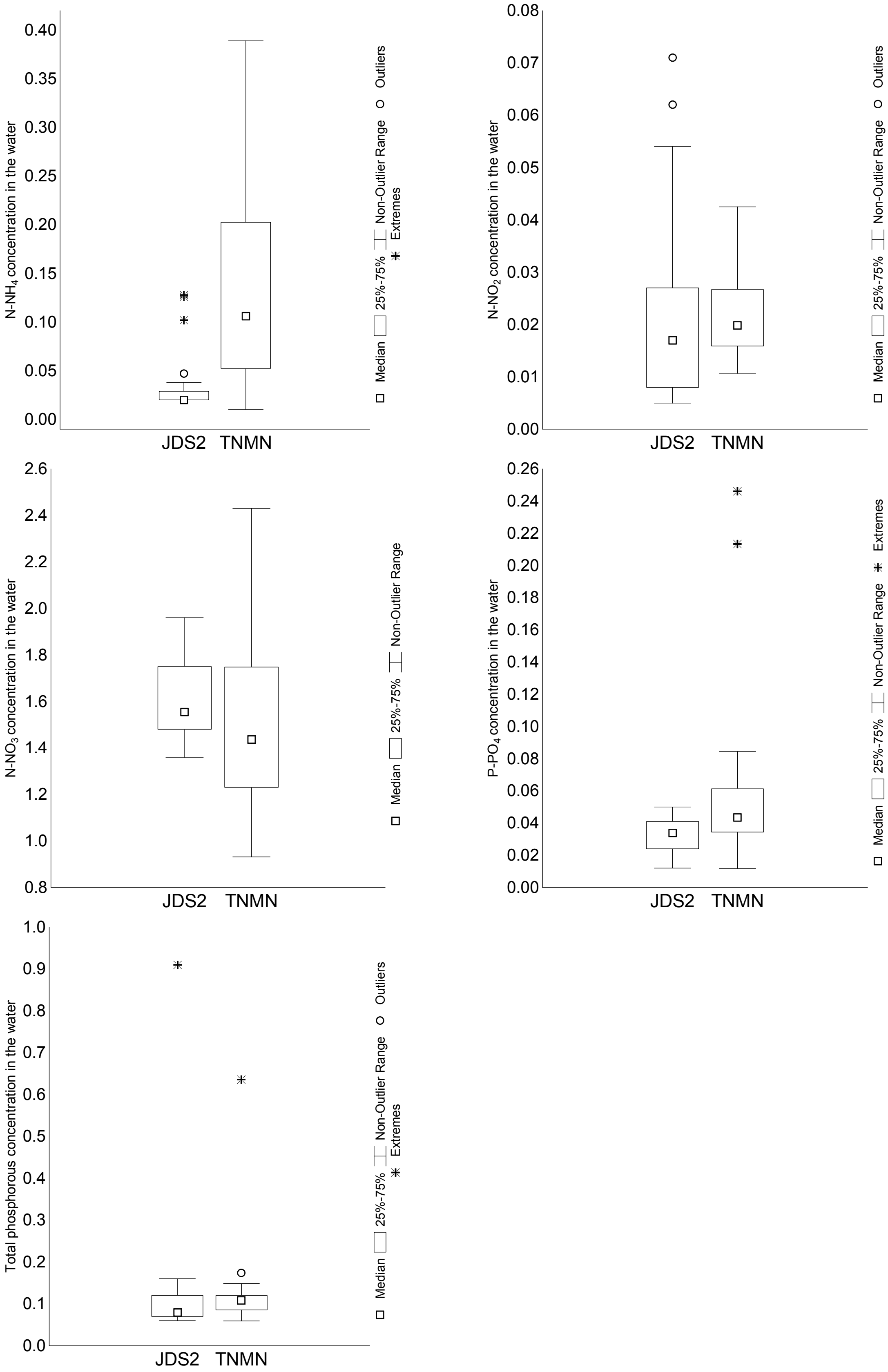

Figure 2.4: Box-plots of selected chemical parameters for JDS2 versus TNMN data.

Table 2.3: Parameters used in analysis, denominating their abbreviation in the PCA plot, their unit, mean/median, range and transformation methods.

Parameter	Abbreviation	Unit (Type of variable)	Mean / *Median	Range	Transform.
Distance from mouth	RIVER_KM	km	1,233.9	(7 – 2,600)	log10
Average width	WIDT_AVG	m	671.0	(50 – 3,500)	log10
Average depth	DEPTH_AVG	m	6.4	(0.5 - 20)	log10
Width of riparian corridor	W_RIPCOR	km	1.5	(0.1 - 8.5)	^0.2
Average surface velocity	VSURFAV	- (ranked)	2*	(0 - 4)	-
Naturalness of bank vegetation	B_VEG	- (ranked)	0.5*	0 – 1	-
Naturalness of bank slope	BSLNAT	- (ranked)	0.5*	0 – 1	-
Station in impounded section	STAT_BW	- (binary)	0*	0 – 1	-
Type of substratum	SUBSTR	- (ranked)	3.5*	1 – 7	-
Width of navigation channel	NAV	- (ranked)	4*	1 – 4	-
Large woody debris	LWD	- (ranked)	1.5*	1 - 3	-
Type of land use	LANDUSE	- (ranked)	0.5*	0 - 1	-
Current planform: sinuous	CP_SINUO	- (binary)	1*	0 - 1	-
Current planform: meandering	CP_MEAND	- (binary)	0*	0 - 1	-
Shoreline index	SHOREID	- (ratio)	1.18	(1.0 - 1.5)	-
Morphological evaluation: channel	EVCHAN	- (ranked)	3*	1 - 5	-
Morphological evaluation: banks	EVBANK	- (ranked)	3*	1 - 5	-
Morphological evaluation: floodplain	EVFLPL	- (ranked)	3*	1 - 5	-
N-NO$_2$ concentration in the water	NNO2	µg l^{-1} N	0.020	(0.005 – 0.072)	^0.2
N-NH$_4$ concentration in the water	NNH4	µg l^{-1} N	0.041	(0.016 – 0.379)	^0.2
P-PO$_4$ concentration in the water	PPO4	µg l^{-1} P	0.036	(0.010 – 0.093)	^0.2
Total phosphorous in the water	PWATER_M	µg l^{-1} P	0.12	(0.06 – 0.95)	^0.2
N-NO$_3$ concentration in the water	NNO3	µg l^{-1} N	1.66	(0.78 – 3.12)	-
Dissolved oxygen concentration in the water	DO_CONC	mg l^{-1}	8.18	(3.49 - 10.10)	log10
Organic nitrogen in the sediment	ORGNSED	µg kg^{-1} N	725	(110 – 1703)	log10
Phosphorous in the sediment	P_SED	µg kg^{-1} P	787	(447 – 1502)	log10
Total Organic Carbon in the sediment	TOCSED	µg kg^{-1} organic C	16,265	(1432 - 34022)	log10
Macrophyte Secchi depth	MPHSEC	cm	88.1	(20 – 165)	log10
Toxic Unit derived from macrozoobenthos data	TU_MZB	- (ratio)	-0.753	(-2.84 - 0.03)	log10+4

The pressure gradient

The environmental data were analysed by Principal Component Analysis (PCA) using PC-ORD version 5.0 (McCune & Mefford 1999). PCA is generally used for linear relationships in environmental data (Jongman et al. 1995). We extracted complex gradients that reflected the main variations of the dataset. First, a pre-selection of variables to be included in the analysis was done. All variables with a Spearman correlation coefficient of >0.70 or <-0.70 were removed, as well as those variables that were deemed not interesting for further analysis. For example, chemical parameters were excluded if their range did not cover a larger gradient, i.e. several quality classes as given by ICPDR (2007).

The PCA was finally executed for a total of 29 variables and 78 sites. A complete list of variables and their abbreviations is given in Table 2.3. The cross-products matrix was centered and variable scores were calculated using a distance based Bi-Plot. Rotation was applied for the selected gradients to coincide with the PCA axes. The PCA scores were then normalized.

In a next step of the analysis, key parameters were identified by Spearman correlation between the abiotic PCA gradients and the individual environmental parameters. A pressure profile covering the entire course of the Danube River was created in order to identify relevant pressure factors.

Definition of LDC sites

We classified the abiotic status of each JDS site using the TNMN water quality classification (e.g. ICPDR 2007) for selected chemical parameters (Table 2.4). This classification was supplemented by including the hydromorphological evaluation score of the Danube reach in which the JDS site was located (Schwarz & Kraier in Liska et al. 2008). Following a “one out-all out” concept the JDS sites were only classified, for instance, in good abiotic status if the respective criteria were met for all the classified parameters. We analysed the distribution of the pressure gradient scores among sites in different abiotic status. The worst gradient score obtained for sites in good status was defined as the threshold value to differentiate between LDC and non-LDC sites.

Table 2.4: Abiotic classification scheme to identify sampling sites in good and moderate status ($N-NH_4$=Ammonium, $N-NO_2$=Nitrite, $N-NO_3$=Nitrate, TP=Total Phosphorus, $P-PO_4$=Orthophosphate, DO=Dissolved Oxygen, HYMO= Overall hydromorphological quality class; all concentrations given in mg/l).

Abiotic status class	$N-NH_4$	$N-NO_2$	$N-NO_3$	TP	$P-PO_4$	DO	HYMO
good	≤ 0.3	≤ 0.06	≤ 3	≤ 0.2	≤ 0.1	≥ 6	≤ 2
moderate	≤ 0.6	≤ 0.12	≤ 6	≤ 0.4	≤ 0.2	≥ 5	≤ 3

2.2.3 Results

The pressure gradient

Rotation according to the variable *type of substratum* was applied to maximize co-incision of the selected gradient with the PCA axes. The PCA scores were normalized to values ranging from 0 to 1. The Bi-Plot in Figure 2.5 shows the results from the rotated PCA for the environmental parameters and the sites. Table 2.5 lists the correlation coefficients for the individual parameters. Parameters reflecting the longitudinal river gradient (*slope, average channel depth, average surface velocity* and *distance from mouth*) are mostly correlated to PCA-axis 1. Parameters that are related to abiotic pressure (*morphological evaluation of total, channel, floodplain and banks*, *dissolved oxygen concentration, station in impounded section* and *naturalness of bank slope*) determine PCA-axis 2.

Table 2.5: Spearman correlation coefficients of parameters with the first two PCA axes. Only parameters with correlation coefficients of >0.4 and <-0.4 are listed.

Axis 1	Correlation coefficient	Axis 2	Correlation coefficient
Slope	0.760	Morphological evaluation: total	0.889
Average depth	-0.744	Morphological evaluation: channel	0.829
Average surface velocity	0.737	Morphological evaluation: floodplain	0.796
$N-NO_3$ concentration in the water	0.572	Morphological evaluation: banks	0.707
Distance from mouth	0.563	Dissolved Oxygen concentration	-0.547
Altitude	0.562	Station in impounded section	0.532
Longitude	-0.549	Naturalness of bank slope	-0.494
Station in impounded section	-0.530	Percentage of natural land use	-0.475
Width of navigation channel	-0.528	Percentage of artificial land use	0.467
Latitude	0.486	Naturalness of bank vegetation	-0.455
Average width	-0.468	Bank slope	0.453
Discharge at mean water level	-0.454	Distance from mouth	0.409
Type of substratum	0.444	Altitude	0.407
Flow regime	0.426		
$N-NO_2$ concentration in the water	-0.416		
Bars in the river bed	0.408		

The pressure profile (Figure 2.6) shows high scores of the anthropogenic pressure gradient coincide with major cities like Budapest and Vienna. The Iron Gate dams also indicate high scores. Low scores are found for sites in the Danube-Auen and Duna-Drava Natural Parks, as well as the Danube Bend, the Lower Danube Wetland System and the Danube Delta.

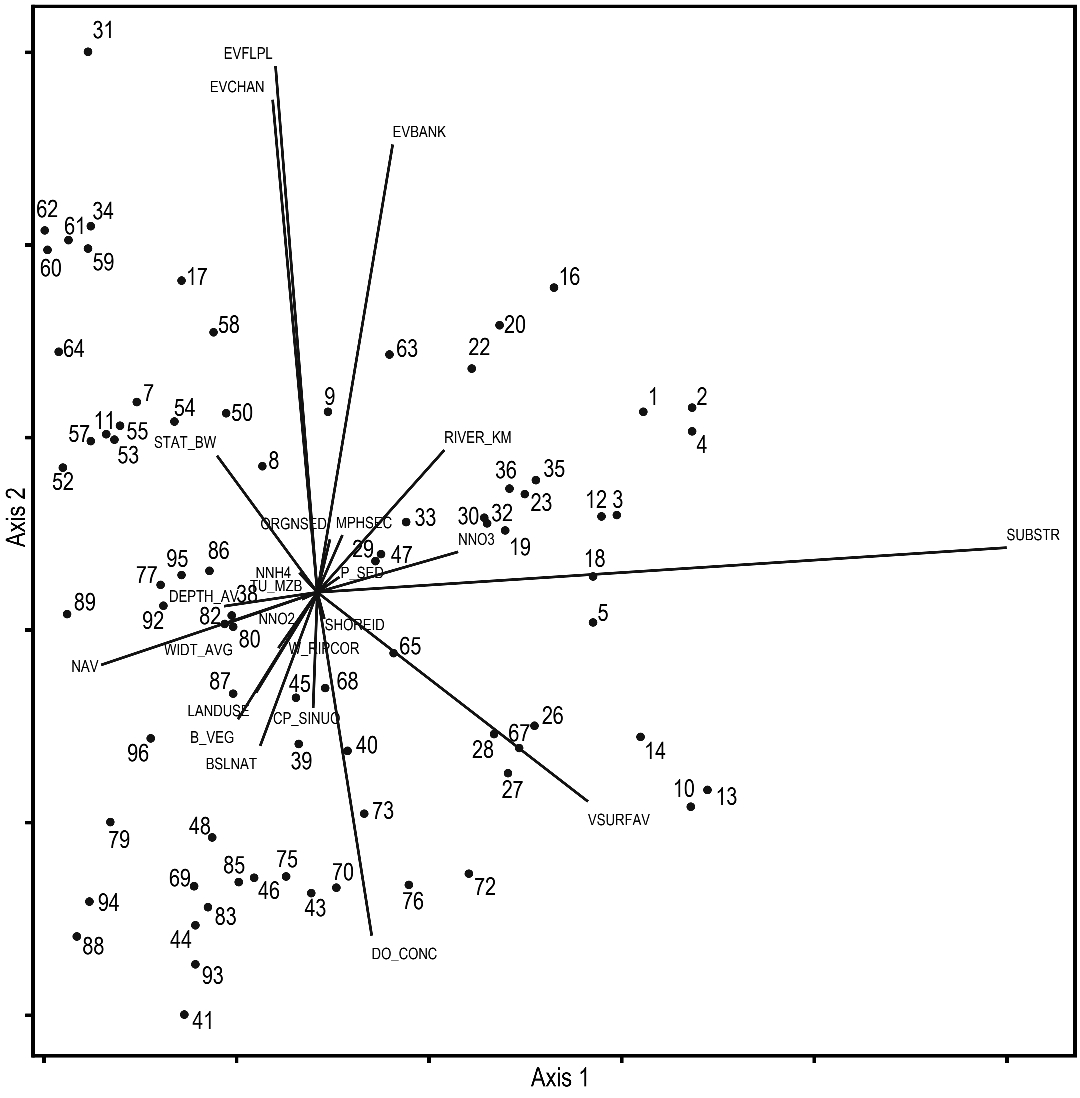

Figure 2.5: Bi-Plot of the rotated PCA for the environmental parameters and the JDS sites. Those parameters with correlation coefficients between -0.3 and 0.3 for axis 1 and 2 have been removed from the plot. R^2-values for PCA-axis 1: 0.331; PCA-axis 2: 0.325. Abbreviations are listed in Table 2.3.

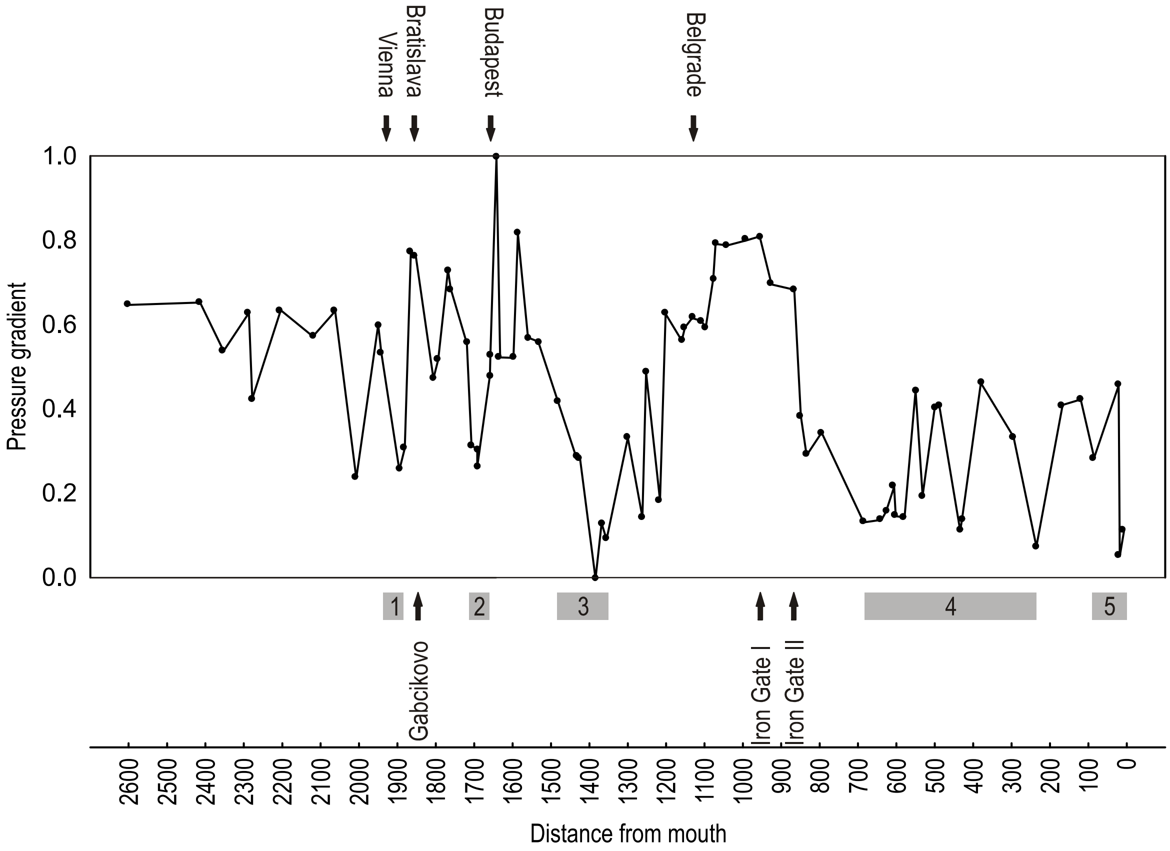

Figure 2.6: Pressure profile for the Danube River, specifying the locations of major cities, impoundments and important wetlands. The complex pressure gradient is measured in values between 0 (near-natural) to 1 (severely impaired).
1=Danube-Auen National Park; 2=Danube bend; 3=Duna Drava National Park; 4=Lower Danube Wetland System; 5=Danube Delta.

Definition of LDC sites

We identified 20 sampling sites in good and 26 in moderate abiotic status, with the majority of sites failing the class thresholds due to hydromorphological impairment. Out of in total twelve sites not meeting good abiotic status due to chemistry, ten exceeded the maximum nutrient values. None of the sites would reach a potential high status, since none of the corresponding reaches holds high hydromorphological quality. The three site groups (good, moderate, other) feature distinctly different ranges of pressure gradient scores (Figure 2.7). The maximum score among good status sites amounts to 0.317 and belongs to the sampling station at Szob (HU, rkm 1707). This value marks the upper limit of sites in least disturbed conditions (LDC sites). So, in total 27 LDC sites were allocated including all good status sites and seven sites in moderate abiotic status. Table 2.6 and Table 2.7 provide complete lists of LDC and non-LDC sites.

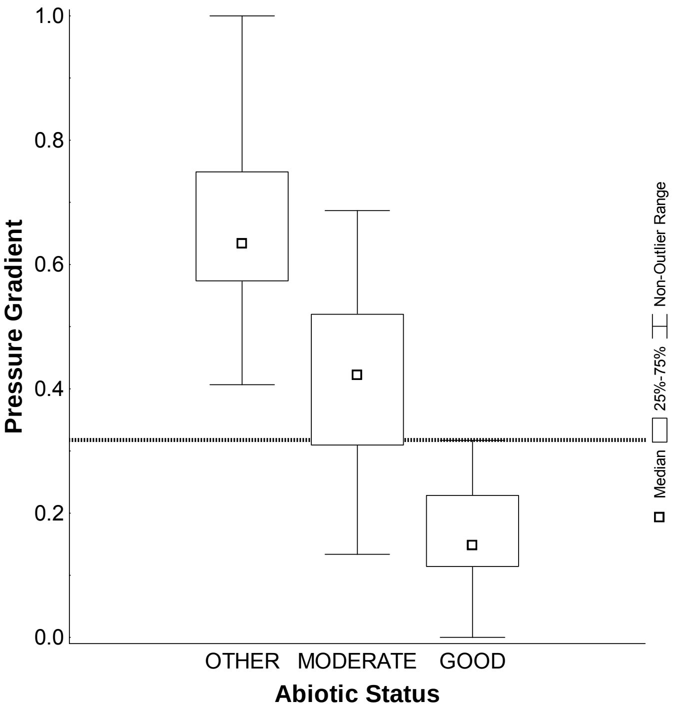

Figure 2.7: Distribution of pressure gradient among sites in different abiotic status.

Table 2.6: List of sites in least disturbed conditions (LDC sites).

JDS2 code	County	Location
JDS010	Austria	Oberloiben
JDS013	Austria	Wildungsmauer
JDS014	Austria	Upstream Morava (Hainburg)
JDS026	Hungary	Szob
JDS027	Hungary	Upstream end of Szentendre Island
JDS028	Hungary	Upstream end of Szentendre Island (arm)
JDS039	Hungary	Hercegszanto
JDS040	Herzegovina/Serbia	Batina
JDS041	Herzegovina/Serbia	Upstream from Drava
JDS043	Herzegovina/Serbia	Downstream from Drava (Erdut/Bogojevo)
JDS044	Herzegovina/Serbia	Dalj
JDS046	Serbia	Upstream from Novi Sad
JDS048	Serbia	Upstream from Tisa (Stari Slankamen)
JDS067	Romania/Bulgaria	Pristol/Novo Selo harbour
JDS069	Bulgaria/Romania	Downstream from Kozloduy
JDS070	Bulgaria/Romania	Upstream from Iskar (Bajkal)
JDS072	Bulgaria/Romania	Downstream from Iskar
JDS073	Bulgaria/Romania	Upstream from Olt
JDS075	Romania/Bulgaria	Downstream from Olt
JDS076	Romania/Bulgaria	Downstream from Turno-Magurele/Nikopol
JDS079	Romania/Bulgaria	Downstream from Jantra
JDS083	Romania/Bulgaria	Upstream from Arges
JDS085	Romania/Bulgaria	Downstream from Arges/Oltenita
JDS088	Romania	Giurgeni
JDS093	Romania/Ukraine	Vilkova Chilia arm/Kilia arm
JDS094	Romania	Bystroye canal
JDS096	Romania	Mahmudia – Saint Gheorghe arm

Table 2.7: List of sites in disturbed conditions (non-LDC sites).

JDS2 code	County	Location
JDS001	Germany	Upstream part of Iller
JDS002	Germany	Kehlheim gauging station
JDS003	Germany	Geisling power plant
JDS004	Germany	Deggendorf
JDS005	Germany	Niederalteich
JDS007	Germany/Austria	Jochenstein
JDS008	Austria	Upstream from dam Abwinden-Asten
JDS009	Austria	Upstream from dam Ybbs-Persenbeug
JDS011	Austria	Upstream from dam Greifenstein
JDS012	Austria	Klosterneuburg
JDS016	Slovakia	Bratislava
JDS017	Slovakia/Hungary	Gabcikovo reservoir
JDS018	Slovakia/Hungary	Medvedov/Medve
JDS019	Hungary	Mosoni Danube branch end
JDS020	Slovakia/Hungary	Komarno/Komarom
JDS022	Slovakia/Hungary	Iza/Szony
JDS023	Slovakia/Hungary	Sturovo/Esztergom
JDS029	Hungary	Budapest upstream
JDS030	Hungary	Budapest, end of old Danube arm
JDS031	Hungary	Rackeve-Soroksar, start of old Danube arm
JDS032	Hungary	Budapest downstream
JDS033	Hungary	Adony/Lorev
JDS034	Hungary	End of Rackeve-Soroksar arm
JDS035	Hungary	Dunaföldvar
JDS036	Hungary	Paks
JDS038	Hungary	Baja
JDS045	Herzegovina/Serbia	Ilok/Backa from Palanka
JDS047	Serbia	Downstream from Novi Sad
JDS050	Serbia	Downstream from Tisa, upstream from Sava (Belegis)
JDS052	Serbia	Upstream from Pancevo/downstream from Sava
JDS053	Serbia	Downstream from Pancevo
JDS054	Serbia	Grocka
JDS055	Serbia	Upstream from Velika Morava
JDS057	Serbia	Downstream from Velika Morava
JDS058	Serbia	Starapalanka-Ram
JDS059	Serbia/Romania	Banatska Palanka/Bazias
JDS060	Serbia/Romania	Iron Gate reservoir (Golubac/Koronin)
JDS061	Serbia/Romania	Donij Milanovac
JDS062	Serbia/Romania	Iron Gate reservoir (Tekija/Orsova)
JDS063	Serbia/Romania	Vrbica/Simijan
JDS064	Serbia/Romania	Iron Gate II
JDS065	Serbia/Romania	Upstream from Timok
JDS068	Romania/Bulgaria	Calafat
JDS077	Romania/Bulgaria	Downstream from Simnicea/Svistov
JDS080	Romania/Bulgaria	Upstream from Ruse
JDS082	Romania/Bulgaria	Downstream from Ruse/Giurgiu
JDS086	Romania/Bulgaria	Chiciu/Silistra
JDS087	Romania	Downstream from Cernavoda
JDS089	Romania	Braila
JDS092	Romania/Ukraine	Reni
JDS095	Romania	Sulina arm at Sulina

2.2.4 Discussion

The presented procedure represents a common approach to identify complex pressure gradients and fix thresholds to define reference conditions (Fereirra et al. 2005, Hering et al. 2006, Blocksom & Johnson 2009). The general aim is to discover relationships between human pressure and biological status, and then to describe the biology under defined conditions. The pressure-impact relations and the relevance of the Danube's main pressure gradient for the different biological elements are described in Chapter 0. Unlike Blocksom & Johnson (2009), we did not perform an a-priori selection of parameters related to anthropogenic pressure to allow for a comprehensive analysis of the Danube's abiotic conditions as given by the JDS2 data.

This process to identify LDC sites is generally different from "classical" reference screening applied to smaller rivers (e.g. CB GIG Rivers 2008), with regard to both the selection of screening parameters as well as the "one out-all out" principle to define reference sites. In the reference screening approach any parameter exceeding the predefined threshold value justifies the exclusion from the pool of potential reference sites. Angradi et al. (2009) highlight that for large rivers this seems an unsuitable procedure. Sampling sites are not independent since all sites except one are downstream from other sites. Therefore, autocorrelation among sites is likely to be high for some variables (Underwood 1994). Because of this autocorrelation, spatial variation in some water chemistry metrics is often minimal. Furthermore, the influence of anthropogenic land use in the catchment can hardly be quantified. Each sampling site has a vast watershed that includes hundreds of smaller catchments. This complex mosaic of different land uses does not differ significantly between adjacent sites of the river. And finally, riparian habitat criteria are not relevant for instream conditions of rivers with huge channel widths such as the Danube. Sub-catchment variables from the reach upstream of the sampling site seem to be more representative for the actual biological status.

Against this background, we fixed the actual LDC threshold on the pressure gradient that represents a complex combination of individual pressures at the sampling site. The abiotic classification scheme only had a supportive character, validating the qualitative significance of the gradient. The LDC sites can generally be described by the following features: On average, the sites are located in reaches that show good hydromorphological quality of the channel, the banks and the floodplain. None of these reaches feature worse than moderate hydromorphological quality, and no monitoring site is situated in an impounded section. The water at LDC sites shows average oxygen concentrations of 8.6 mg/l, and the means of the nutrient values measured in the water fall within good status of the TNMN classification scheme (see Table 2.4). The mean width of the riparian corridor amounts to approximately 2.1 km, and at least some large woody debris is present at most of the sites. On average, both banks feature natural slopes and the immediate vicinity of most sites is dominated by natural land use.

The LDC sites are unequally distributed over the course of the Danube. The positive correlations with the variables *distance from mouth* and *altitude* (see Table 2.5) reveal that the pressure gradient is related to the river length: increasing distance from source means less impacted river reaches. However, most of the environmental variables varying along the river course are determining the first abiotic PCA gradient reflecting longitudinal change. The pressure gradient represented by the second PCA-axis is uncorrelated to this longitudinal gradient given by the first PCA-axis (Jongman et al. 1995). We assume that the composition of the pressure gradient is almost unbiased by natural variation due to longitudinal shift. Thus, the LDC site threshold is valid for the entire river course and needs not to be defined for specific river sections. The relation of the pressure gradient to longitudinal river features is actually caused by the different degree of man-made disturbance, i.e. mainly due to pronounced river modification (cascade of dams, channelisation) and intensive agricultural land use (elevated nitrate concentrations) in the upper river section.

The data availability for this analysis was generally constraint to the parameters sampled during JDS2. With regard to the relevance of LDC site allocation we considered the data on hydromorphological evaluation and impoundment most suitable. The spot measurements of the physico-chemical parameters are problematic. Their comparison with the averaged TNMN values at selected sites show significant discrepancies, especially for the ammonium and orthophosphate concentrations. Single measurements do not allow for an adequate record of the usually high parameter variability and are thus inappropriate to determine relationships with the biological status. Further refinement of this approach to define LDC sites needs to integrate physico-chemical data measured on a regular monitoring basis as done in the TNMN.

2.3 Revision of the WFD typology of the Danube River: The proposal of four Danube Intercalibration Stretches

2.3.1 Abstract

We recommend no revision to the existing WFD typology of the Danube River as specified by Moog et al. (2008). For the intercalibration exercise of the Danube River we propose the delineation of four Danube Intercalibration Stretches considering the original Danube Section Types and outcomes of the JDS 2 data analyses.

2.3.2 Introduction

Surface water body typologies are the basis for ecological status assessment. The benchmark for this assessment, the near-natural reference state, is defined on a water body type-specific basis. A stream, for instance, features biological communities that are determined by its geographical, hydromorphological and physico-chemical framework. The national methods to assess the ecological quality of surface waters are adapted to these environmental conditions. The intercalibration exercise is covering large geographical areas including diverse environmental conditions. The process requires the allocation of distinct abiotic water body types present in these areas. Comparison and harmonization of quality classifications between Member States have to consider the specific adaptations of the national methods. The most effective procedure to elaborate intercalibration solutions is the collation of common datasets to which all national methods can be applied. A strict definition of common types allows for an analysis of similar ecological features that cover the broad geographical gradients and thus enable the harmonization of quality classifications between the various countries.

The common intercalibration types defined by ECOSTAT (2004) are based on abiotic descriptors. For rivers, these descriptors cover catchment size, altitude, geology, channel width and substrate. However, none of these types includes rivers larger than 10,000 km^2 catchment area. The international working group on large river intercalibration was set up in 2009, and has not yet produced a common typological framework for the intercalibration of large rivers. We thus had to establish a specific intercalibration typology for the Danube River that covers different biological quality elements. This task was based on the work of Moog et al. (2008) that subdivided the entire course of the river into ten Danube Section Types. These sections primarily differentiate between the main morphological units that the Danube is forming on its course from the Black Forest to the Black Sea. Moog and colleagues used macrozoobenthos data sampled during the first Joint Danube Survey in 2001 (Literáthy et al. 2002) to show that most these section types are inhabited by distinct communities. The Danube Section Typology represents a meaningful improvement of the former separation of the river into upper, middle and lower course. Furthermore, it is used in the international management of the Danube (ICPDR 2005).

For the purpose of intercalibration we aimed at defining typologically homogeneous Danube stretches that are shared by at least two Member States. Our allocation considered the existing Danube Section Types and additionally accounted for the analytical outcomes of data on phytoplankton, macrophytes, benthic diatoms and benthic invertebrates sampled during the second Joint Danube Survey (Liska et al. 2008). From this report, we took the chapters written by Graf et al. (macrozoobenthos), Makovinska et al. (benthic diatoms), Dokulil & Kaiblinger (phytobenthos), and Janauer et al. (macrophytes) into consideration. Our own analyses concentrated on additional classification analyses of biological data and the validation of the proposed intercalibration types. In this chapter we define four Danube Intercalibration Stretches (DIS) featuring a homogeneous abiotic framework and similar biological communities: the Upper DIS from Neu Ulm (DE) to the Morava confluence (AT/SK), the Northern Pannonian DIS to Paks (HU), the Southern Pannonian DIS to Bazias (RO) and the DIS from Turnu Severin (RO) to Isaccea (RO). The Danube reaches below 10,000 km^2 catchment area and the Danube Section Types 7 (Iron Gates Danube) and 10 (Danube Delta) were excluded.

2.3.3 Methods

To establish the basis for the definition of common intercalibration stretches of the Danube we reviewed the studies on the Danube Section Types presented in Moog et al. (2008) and Sommerhäuser et al. (2003). Due to their coherent designation and high relevance in the international river basin management the adaptation of these section types was a prerequisite for our work. Furthermore, we regarded the recent studies of the colleagues that worked with the JDS2 data. Graf and colleagues (in Liska et al. 2008) conducted cluster and ordination analyses based on the complete macrozoobenthos assemblages, and the composition and abundance of mussel and crustacean species only. Makovinska and colleagues (in Liska et al. 2008) applied cluster analysis to the diatom data. Dokulil & Kaiblinger (in Liska et al. 2008) evaluated the distribution of phytoplankton chlorophyll-a and biomass along the entire course of the Danube. Janauer and colleagues (in Liska et al. 2008) investigated whether the macrophyte communities within each Danube Section Type were significantly different from each other.

In addition to these studies we carried out constraint cluster analysis applying the “valley segment affinity Search Technique” (VAST) according to Brenden et al. (2008). This technique identified groups of spatially adjacent sampling sites with similar biological communities. We employed this approach to the samples from either the complete dataset or only sites meeting the good or moderate abiotic quality (see Chapter 2.2). By combining the information derived from these various sources we delineated the Danube Intercalibration Stretches (DIS). The biological assemblages of these stretches were processed by a Multi Response Permutation Procedure (MRPP, McCune & Grace 2002) to test for significant differences between the biological communities. To characterize the macrophyte and macrozoobenthos communities occurring in these stretches we applied an IndVal analysis (Dufrene & Legendre 1997) using sampling sites in good or moderate abiotic quality. This analysis combines data on relative frequency and abundance of a taxon within each stretch to calculate a species indicator value that reveals the taxon’s steadiness and dominance.

2.3.4 Results

Table 2.8 gives an overview of the proposed four DIS. Their boundaries coincide with the major changes of the Danube typology according to Moog et al. (2008). Three section types are not included in the intercalibration typology (1: Upper Course of the Danube, 7: Iron Gate Danube, 10: Danube Delta). These reaches are either parts of the Danube parts with only one adjacent country (Section 1), or feature specific Danube sections that are not sufficiently covered by the JDS2 data (Section 7 and Section 10; only four JDS sampling stations each). Furthermore, the DIS are in line with the delineation of the upper, middle and lower course of the Danube (Literáthy et al. 2002). Figure 2.8 depicts the location of the stretches on the Danube River Basin District map.

Table 2.8: Danube Intercalibration Stretches (DIS).

No.	Danube Intercalibration Stretch (DIS)	Riparian States	RKM		Danube Section Type (Moog et al. 2008)	Danube course (Literáthy et al. 2002)
			UP	DWN		
					1 Upper Course of the Danube	Upper
I	Upper	DE, AT	2414	1880	2 Western Alpine Foothills Danube	
					3 Eastern Alpine Foothills Danube	
					4.1 Lower Alpine Foothills Danube Sub-Section 1	
II	Northern Pannonian	AT, SK, HU	1880	1533	4.2 Lower Alpine Foothills Danube Sub-Section 2	Middle
					5 Hungarian Danube Bend	
III	Southern Pannonian	HU, HR, CS, RO	1533	1071	6 Pannonian Plain Danube	
					7 Iron Gate Danube	
IV	Lower	RO, BG, MD, UA	931	100	8 Western Pontic Danube	Lower
					9 Eastern Wallachian Danube	
					10 Danube Delta	

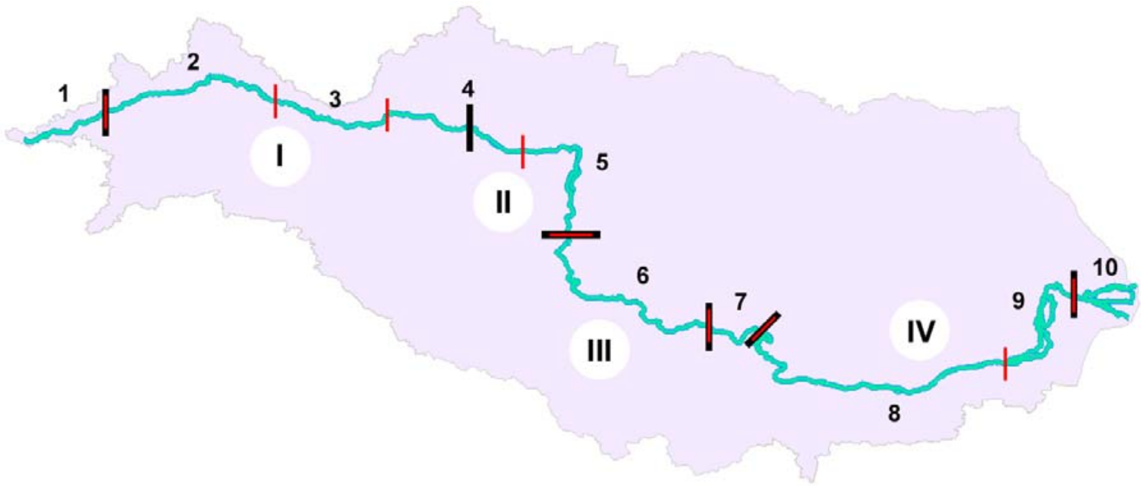

Figure 2.8: The location of the Danube Section Types (Moog et al. 2008) (Arabic numerals, red borders) and the Danube Intercalibration Stretches (DIS) (Roman numerals, black borders) on the River Basin District map. Danube Sections Types 1, 7 and 10 are not covered by the DIS delineation.

The review of the BQE-specific reports confirms the selection of the individual stretch boundaries: The cluster and ordination analyses based on the complete macrozoobenthos assemblage reflect both the ten section types and the three main courses of the Danube. The classification based on mussel and crustacean assemblages reveals five distinct types that back the intercalibration stretch boundaries at the Morava confluence (AT/SK, rkm 1880), Paks (HU, rkm 1533) and Turnu Severin, (RO, rkm 931) (Graf et al. in Liska et al. 2008).

The cluster analysis presented by Makovinska et al. (in Liska et al. 2008) supports the definition of the stretch boundary at the Morava confluence. The upper boundaries of the Northern Pannonian and the Lower DIS correspond roughly to distinct diatom clusters. It has to be noted that these analyses were conducted on the complete set of sampling sites including JDS stations impacted by pollution, hydromorphological degradation or impoundment. The chlorophyll-a data derived from phytobenthos samples distinguish between three Danube reaches (Dokulil & Kaiblinger in Liska et al. 2008) that subdivide the Southern Pannonian DIS with chlorophyll-a values exceeding 10 µg/l from the Northern Pannonian DIS upstream and the Lower DIS downstream. The macrophyte communities of the individual Danube Section Types show significant differences, thus supporting the allocation of each DIS. Janauer et al. (in Liska et al. 2008) recommend to subdivide the Danube Section Type 4 close to Bratislava to account for the heterogeneous character of this type. This confirms our DIS boundary at the Morava confluence. The results of the constraint clustering approve the boundary between the first and second DIS for macrozoobenthos and macrophytes, and the boundary between the third and forth DIS for macrozoobenthos, benthic diatoms and phytoplankton. According to the MRPP analysis all four intercalibration stretches feature significantly different biological communities. The diatom assemblages of the first and second stretch show no significant differences.

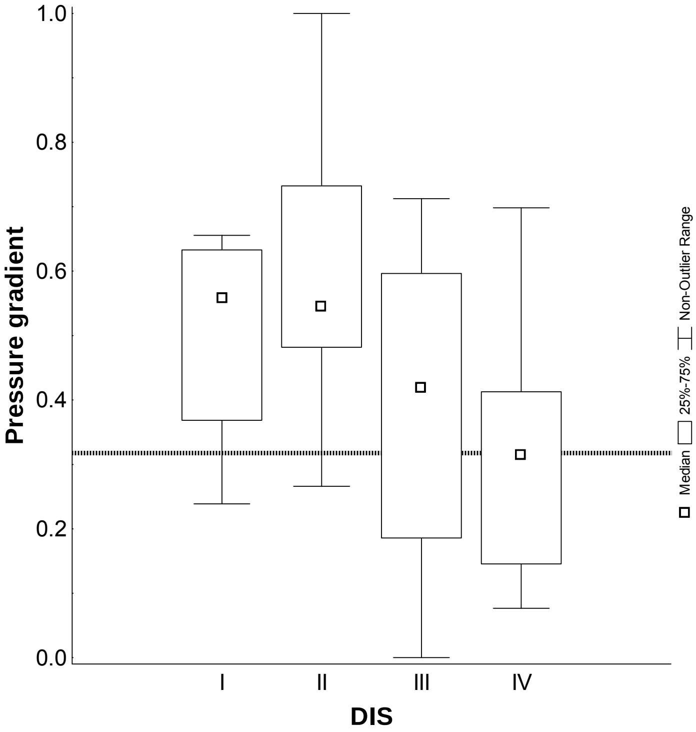

Figure 2.9: Distribution of abiotic pressure gradient among Danube Intercalibration Stretches (DIS). The dotted line marks the Least Disturbed Condition threshold derived in Chapter 2.2.

The values of the abiotic pressure gradient derived in Chapter 2.2 show a distinct distribution among the four DIS (Figure 2.9). The environmental quality of the two upstream Danube stretches is generally lower with in total six sites meeting the LDC criteria (Table 2.9). The more downstream stretches feature conditions of higher environmental quality.

Table 2.9: Number of JDS sampling sites located at each Danube Intercalibration Stretch (DIS) including the number of sites meeting the Least Disturbed Condition criteria (LDC sites).

DIS	I - Upper	II - Northern Pannonian	III - Southern Pannonian	IV - Lower
# sites	12	18	17	22
# LDC sites	3	3	7	11

The IndVal analysis of the macrophyte data shows that mosses are clearly dominating the Upper DIS (Table 2.10). In addition, this stretch holds typical river helophytes such as *Phalaris arundinacea*, *Rorippa amphibia*, *Lycopus europaeus* or *Veronica anagallis-aquatica*. Compared to the other stretches DIS I shows highest values of the Austrian Index for Macrophytes, a specific assessment method for the Danube River (Figure 2.10). The second stretch features submerged and floating hydrophytes that are generally indicative of eutrophic conditions (e.g. *Potamogeton pectinatus*, *Lemna* sp.). Only three taxa were identified in the IndVal analysis of the Southern Pannonian stretch: The two free-floating species *Spirodela polyrhiza* and *Salvinia natans* and the common helophyte *Polygonum hydropiper*. The Lower DIS is characterized by the occurrence of *Ceratophyllum demersum*, *Najas minor* and *Potamogeton gramineus* alongside helophytes of the genera *Bidens* and *Polygonum*.

The analysis of macrozoobenthic indicator species reveals on average 15 taxa per DIS with significant indicator values above 20 percent (Table 2.11). Several of these taxa are either common large river organisms or specific to the entire course of the Danube. Their listing in only one stretch does generally not reflect its relevance for the other stretches. These biases are probably due to specific features of the JDS2 dataset.

2.3.5 Discussion

In the DIS allocation emphasis was place on the agreement with the existing Danube Section Typology described by Moog et al. (2008). Our study focused on the establishment of practical intercalibration units that reflect homogeneous entities with regard to biological populations. The results of the statistical analyses supported our proposal of the four DIS. Based on these stretches the intercalibration exercise shall be carried out. In a follow-up action between-stretch comparisons will be necessary, also comprising the harmonization with national classifications applied at other large rivers such as the Rhine or the Odra. However, the distinct biological features of each DIS (and those of other rivers) require to define biological benchmarks for intercalibration on a stretch-specific basis.

Typological analyses on biotic communities require data from near-natural reference sites. Most results from the multivariate classifications are of limited suitability because also samples from impaired stations were taken into account. Graf and colleagues (in Liska et al. 2008) partly used dataset excluding samples from dammed sections and saprobically impacted stations. We based our analysis on samples taken from stations complying with the good and moderate abiotic status presented in Chapter 2.2. However, these sites are not in near-natural reference state, thus cluster and ordination analysis in many cases did not match exactly with the proposed DIS boundaries. Although, type allocation and setting of natural borders is generally artificial and influenced by the limited coverage of the Danube River by the JDS monitoring stations, we are confident that the DIS represent practicable units for the further intercalibration process.

Table 2.10: Characteristic macrophyte taxa of the four DIS (based on JDS2 sampling stations in at least moderate abiotic status) – DIS = Danube Intercalibration Stretch; IndVal = Indicator Value (p<0.1; Dufrene & Legendre 1997).

Value	Taxon	Group	IndVal (%)
I	*Cinclidotus riparius*	Moss	93
I	*Phalaris arundinacea*	Helophyte	76
I	*Polygonum lapathifolium*	Helophyte	55
I	*Fontinalis antipyretica*	Moss	40
I	*Impatiens glandulifera*	Helophyte	40
I	*Leskea polycarpa*	Moss	40
I	*Rorippa amphibia*	Helophyte	40
I	*Cinclidotus fontinaloides*	Moss	39
I	*Rorippa sylvestris*	Helophyte	28
I	*Rumex hydrolapathum*	Helophyte	26
I	*Alopecurus geniculatus*	Helophyte	20
I	*Funaria hygrometrica*	Moss	20
I	*Glyceria fluitans*	Helophyte	20
I	*Juncus sp.*	Helophyte	20
I	*Lycopus europaeus*	Helophyte	20
I	*Veronica anagallis-aquatica*	Helophyte	20
II	*Potamogeton pectinatus*	Submerged hydrophyte	63
II	*Lemna gibba*	Floating hydrophyte	61
II	*Lemna minor*	Floating hydrophyte	50
II	*Potamogeton nodosus*	Floating hydrophyte	47
II	*Zannichellia palustris*	Submerged hydrophyte	44
II	*Elodea nuttallii*	Submerged hydrophyte	39
II	*Najas marina (N. major)*	Submerged hydrophyte	32
III	*Spirodela polyrhiza*	Floating hydrophyte	79
III	*Salvinia natans*	Floating hydrophyte	50
III	*Polygonum hydropiper*	Helophyte	22
IV	*Ceratophyllum demersum*	Submerged hydrophyte	54
IV	*Bidens sp.*	Helophyte	51
IV	*Polygonum sp.*	Helophyte	42
IV	*Najas minor*	Submerged hydrophyte	36
IV	*Potamogeton gramineus*	Submerged hydrophyte	32
IV	*Xanthium strumarium*	Helophyte	27

Table 2.11: Characteristic macrozoobenthos taxa of the four DIS (based on JDS2 sampling stations in at least moderate abiotic status) – DIS = Danube Intercalibration Stretch; IndVal = Indicator Value ($p<0.05$; Dufrene & Legendre 1997); L = large river taxon, D = Danube specific taxon, U = Upper Danube, M = Middle Danube, Lo = Lower Danube; Number = Danube Section Type.

DIS	Taxon	Group	IndVal (%)	Characterisation acc. to Sommerhäuser et al. (2003)
I	*Dina sp.*	Hirudinea	80	L
I	*Corophium curvispinum*	Crustacea	74	D
I	*Stylodrilus heringianus*	Oligochaeta	72	-
I	*Jaera istri*	Crustacea	64	D
I	*Caenis luctuosa/macrura*	Ephemeroptera	60	-
I	*Potamopyrgus antipodarum*	Gastropoda	57	-
I	*Psychomyia pusilla*	Trichoptera	40	U, 3+4
I	*Polypedilum (Polypedilum) laetum-Gr.*	Diptera	40	-
I	*Microtendipes pedellus-Gr.*	Diptera	40	-
I	*Ephoron virgo*	Ephemeroptera	40	3+4+5
I	*Pisidium (Odhneripisidium) moitessierianum*	Bivalvia	39	-
I	*Chironomus (Chironomus) riparius-Agg.*	Diptera	38	-
I	*Lumbriculidae Gen. sp.*	Oligochaeta	38	-
I	*Harnischia sp.*	Diptera	36	-
I	*Pisidium (Euglesa) casertanum ponderosum*	Bivalvia	34	-
I	*Dikerogammarus haemobaphes*	Crustacea	31	D,8
I	*Hypania invalida*	Polychaeta	22	-
II	*Pisidium (Henslowiana) supinum*	Bivalvia	84	L
II	*Pisidium (Pisidium) amnicum*	Bivalvia	82	-
II	*Sphaerium solidum*	Bivalvia	73	-
II	*Borysthenia naticina*	Gastropoda	72	-
II	*Obesogammarus obesus*	Crustacea	70	D
II	*Unio tumidus*	Bivalvia	60	-
II	*Hypania invalida*	Polychaeta	52	-
II	*Unio pictorum ssp.*	Bivalvia	47	Lo,10
II	*Sphaerium rivicola*	Bivalvia	42	L
II	*Anodonta anatina*	Bivalvia	42	-
II	*Sinanodonta woodiana*	Bivalvia	41	-
II	*Pisidium (Henslowiana) henslowanum*	Bivalvia	41	-
II	*Hydropsyche contubernalis*	Trichoptera	36	L,2+3+4
II	*Haplotaxis gordioides*	Oligochaeta	34	-
II	*Stylodrilus heringianus*	Oligochaeta	28	-
II	*Psammoryctides sp.*	Oligochaeta	27	-
III	*Dreissena polymorpha*	Bivalvia	76	D,8
III	*Dikerogammarus villosus*	Crustacea	73	D,5
III	*Chernovskiia cf. orbicus*	Diptera	71	-
III	*Chironomus (Chironomus) nudiventris*	Diptera	50	-
III	*Beckidia zabolotzkyi*	Diptera	49	-
III	*Plumatella fungosa*	Bryozoa	44	-
III	*Paratendipes intermedius*	Diptera	43	-
III	*Cladotanytarsus sexdentatus*	Diptera	33	-
III	*Corbicula fluminalis*	Bivalvia	33	-
III	*Sinanodonta woodiana*	Bivalvia	26	-
III	*Anodonta anatina*	Bivalvia	25	-
III	*Unio pictorum*	Bivalvia	24	Lo,10
III	*Parachironomus frequens-Gr.*	Diptera	22	-
III	*Dicrotendipes modestus*	Diptera	22	-
IV	*Dreissena bugensis Lv. gr*	Bivalvia	82	-
IV	*Microcolpia acicularis*	Gastropoda	64	-
IV	*Theodoxus danubialis*	Gastropoda	58	D,M+Lo
IV	*Dikerogammarus haemobaphes*	Crustacea	55	D,8
IV	*Isochaetides michaelseni*	Oligochaeta	50	8
IV	*Pontogammarus sarsi*	Crustacea	50	8
IV	*Polypedilum (Polypedilum) nubifer*	Diptera	46	-
IV	*Viviparus viviparus*	Gastropoda	46	-
IV	*Polypedilum (Tripodura) scalaenum-Gr.*	Diptera	42	-
IV	*Theodoxus transversalis*	Gastropoda	41	D
IV	*Chironomus (Chironomus) acutiventris*	Diptera	36	-
IV	*Holandriana holandrii*	Gastropoda	32	-
IV	*Paratendipes connectens*	Diptera	32	-

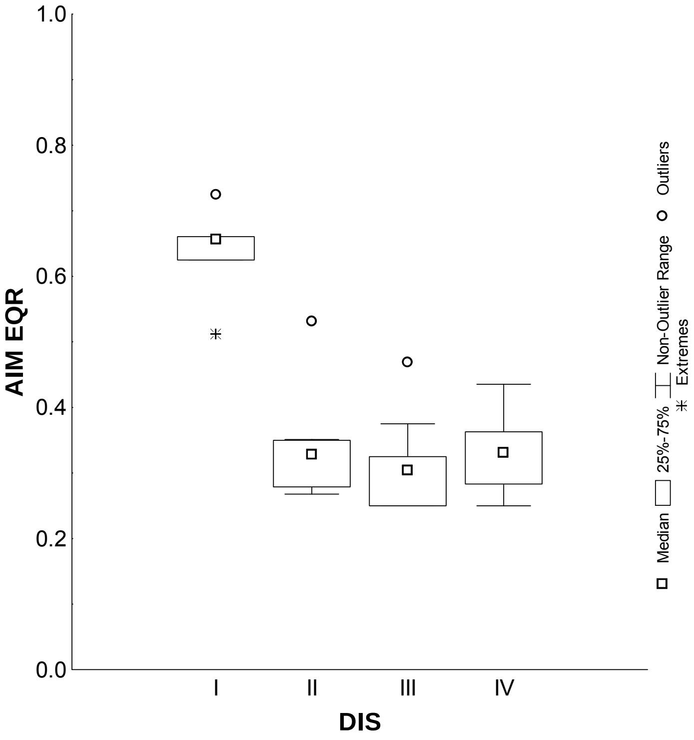

Figure 2.10: Distribution of the Austrian Index for Macrophytes scores (AIM EQR) among sites in good and moderate abiotic status at the different Danube Intercalibration Stretches (DIS).

2.4 Pressure-response analysis

2.4.1 Introduction

The intercalibration exercise aims at harmonising the national definitions of good ecological status. Ideally, this harmonisation is based on objective criteria that establish transnational baselines for the comparison of national classifications. This process has to be recorded in the so-called “Boundary Setting Protocol” (Pollard & van de Bund 2005). The protocol requires to demonstrate the relationship between ecological status and anthropogenic pressure. The good status of the national classifications shall represent similar deviations from undisturbed conditions. Provided that the biological assessment is responsive to stress, specific levels of anthropogenic pressure can define a harmonised notion of good status (Birk & Hering 2009). For the Danube these levels are specified in the criteria for least disturbed conditions, and represented by the 27 LDC sites described in Chapter 2.2. In this chapter we are testing the response of the biological communities and selected assessment metrics to pressure parameters. First we investigate by indirect gradient analysis (Jongman et al. 1995) which pressure variables show significant effects on the different biological assemblages (macrozoobenthos, macrophytes, benthic diatoms and phytoplankton). The outcomes give an indication about the relevance of the abiotic pressure gradient for the ecological status derived from the BQEs. For those elements, that show distinct response patterns (i.e. absolute Spearman correlation coefficient of pressure gradient with any of the assemblage descriptors > 0.5), we explore suitable biological metrics reflecting the impact of the pressure gradient.

2.4.2 Methods

Description of data basis

We used the biological data sampled during the second Joint Danube Survey to describe the assemblage patterns via multivariate ordination analysis (Detrended Correspondence Analysis, DCA). For the pressure-response analysis we combined the macrozoobenthos data gained by the different sampling techniques and lumped together the information of the individual sampling locations to a single dataset per station. The macrophyte and benthic diatom data were treated in the same way. For details on the acquisition of the field data refer to Liska et al. (2008).

Pressure-response analysis: Community level

The DCA was employed for all BQEs separately using CANOCO for Windows Version 4.51 (Ter Braak & Šmilauer 2003). DCA is an ordination technique used for unimodal data. Biological data often reflects such a distribution (Jongman et al. 1995). For the analysis, down weighting of rare species and detrending by segments were selected. In a following step, an indirect gradient analysis of the PCA- and DCA-axis was performed by Spearman correlation. For those BQE assemblages that showed clear relationships with the pressure gradient (Spearman correlations >0.5), we identified the single key variables driving community change. The ranges of DCA axis scores correlating with the pressure gradient were compared between LDC and non-LDC sites at each Danube Intercalibration Stretch.

Pressure-response analysis: Biological metric level

From the macrozoobenthos samples per JDS station we calculated 363 biological metrics using the ASTERICS software (Meier et al. 2006). These also comprised the indices used in ecological status assessment of Germany (Potamon-Type-Index, Schöll et al. 2005), Austria (Z&M Saprobic Index, Zelinka & Marvan 1961) and the Slovak Republic (Z&M Saprobic Index, % oligosaprobic indicators, BMWP score, Rhithron Type Index, % metarhithral, Index of Biocoenotic Region, % Type Aka+Lit+Psa, Margalef Diversity Index, EPT-Taxa, Number of Families). We identified the metrics that revealed obvious and meaningful differences between LDC and non-LDC sites. The analysis was carried out for all JDS sites combined, or specific for each Danube Intercalibration Stretch.

20 biological metrics were derived from the macrophyte data. These included trophic indices (e.g. IBMR, Haury et al. 1998; ITEM, Birk et al. 2007), richness measures (e.g. total number of sampled macrophyte taxa; ratio of moss or hydrophyte taxa) and metrics to assess the ecological status (Austrian Index for Macrophytes, Pall & Mayerhofer 2008; Slovak Macrophyte Assessment Method). Furthermore, we defined stretch-specific macrophyte indicators, whose absolute abundance was significantly correlated to the abiotic pressure gradient (Spearman correlation). For the Southern Pannonian DIS the average indicator score weighted by the taxon’s relative abundance was calculated per JDS sampling site using the Spearman correlation coefficient as

species-specific indicator scores (Birk 2009). At this stretch the metric values were compared for LDC and non-LDC sites.

2.4.3 Results

Pressure-response analysis: Community level

The DCA results for the BQEs are shown in Table 2.12. The first axis of the extracted macrozoobenthos gradient has a Spearman correlation coefficient of -0.764 to the longitudinal gradient. The second axis correlates most strongly with the pressure gradient, showing a Spearman correlation coefficient of -0.705. The second axis of the DCA from the macrophyte data is also significantly correlated to the pressure gradient (Spearman correlation coefficient of -0.512). Neither the phytoplankton nor the benthic diatom assemblages show clear relationships with the pressure gradient. Thus, we did not include these biological elements into further analyses.

Axis 1 of the macrozoobenthos DCA (Table 2.13) is mainly related to longitudinal variables (*catchment size, distance from mouth, altitude, average depth, discharge at mean water level* and *slope*) whilst axis 2 shows correlation to variables indicating pressure (*morphological evaluation: total and channel, hydromorphological alterations, naturalness of bank slope, station in impounded section* and *average surface velocity*).

Table 2.12: DCA results for the BQEs macrozoobenthos, macrophytes, benthic diatoms and phytoplankton, including the total variance (inertia), eigenvalues, gradient lengths, correlations to the pressure and longitudinal PCA-gradient (see Chapter 2.2) and the number of sites and species.

	DCA Specifications				Spearman correlation coefficients		Remarks
	DCA Axis	Total Variance (inertia)	Eigenvalue	Length of gradient	Pressure gradient	Longitudinal gradient	
Macrozoobenthos	First	3.774	0.391	3.308	0.062	-0.764	JDS001 removed (different taxonomical composition) (77 sites, 311 species)
	Second		0.244	2.766	-0.705	0.154	
	Third		0.168	2.236	-0.078	0.077	
Macrophytes	First	3.965	0.561	4.080	-0.046	-0.371	(78 sites, 124 species)
	Second		0.246	3.389	-0.512	-0.189	
	Third		0.159	2.714	-0.217	-0.300	
Benthic diatoms	First	2.548	0.228	3.612	-0.238	-0.380	5 sites missing: JDS019, JDS072, JDS086, JDS088, JDS096 (73 sites, 343 species)
	Second		0.142	1.809	0.014	-0.303	
	Third		0.093	1.480	0.357	-0.446	
Phytoplankton	First	2.271	0.494	2.841	-0.221	-0.464	(78 sites, 98 species)
	Second		0.238	2.172	-0.304	-0.266	
	Third		0.151	3.165	-0.277	-0.152	

Table 2.13: Spearman correlation coefficients of variables with the first two DCA-axes of the macrozoobenthos gradient. Only variables with coefficients of > 0.4 and < -0.4 have been listed.

Axis 1	Correlation coefficient	Axis 2	Correlation coefficient
N-NO$_3$ concentration in the water	-0.641	Morphological evaluation: total	-0.663
Catchment size	0.637	Hydromorphological alterations	-0.640
Distance from mouth	-0.637	Morphological evaluation: channel	-0.623
Altitude	-0.635	Naturalness of bank slope	0.559
Latitude	-0.630	Morphological evaluation: bank	-0.534
Average depth	0.618	Bank slope	-0.518
Longitude	0.616	Station in impounded section	-0.464
Discharge at mean water level	0.594	Average surface velocity	0.450
Slope	-0.584	Percentage of artificial land use	-0.437
Average surface velocity	-0.569	Flow regime	0.433
N-NO$_2$ concentration in the water	0.549	Amount of large woody debris	0.425
Average width	0.466	Bank stabilization	-0.423
Naturalness of bank vegetation	0.437	Dissolved oxygen concentration	0.419
Type of substratum	-0.430	Percentage of natural land use	0.417
N-NH$_4$ concentration	0.428	Macrophyte Secchi depth	-0.414
Bank stabilization	-0.423	Morphological evaluation: floodplain	-0.411
		Discharge at mean water level	0.406
		Width of riparian corridor	0.406

Table 2.14: Spearman correlation coefficients of variables with the first two DCA-axes of the macrophyte gradient. Only variables with coefficients of > 0.4 and < -0.4 have been listed.

Axis 1	Correlation coefficient	Axis 2	Correlation coefficient
Average width	0.679	Catchment size	0.590
Discharge at mean water level	0.584	Distance from mouth	-0.589
Toxic unit from phytoplankton data	0.563	Longitude	0.531
Toxic unit from macrozoobenthos data	0.561	Latitude	-0.485
N-NO$_3$ concentration in the water	-0.552	Percentage of natural land use	0.460
Distance from mouth	-0.462	Percentage of artificial land use	-0.455
Catchment size	0.459	Macrophyte Secchi depth	-0.446
Slope	-0.455	Width of riparian corridor	0.445
Width of riparian corridor	0.432	Discharge at mean water level	0.445
		N-NO$_2$ concentration in the water	0.434
		Morphological evaluation: floodplain	-0.429
		Bank stabilization	-0.414

The first axis of the macrophyte gradient (Table 2.14) is most strongly correlated with longitudinal variables such as *average width, discharge at mean water level* and *distance from mouth.* The second axis is correlated to longitudinal variables (*catchment* *size and distance from mouth*) but also to variables indicating pressure, which is consistent with the correlation to the pressure gradient (*percentage of natural land use* and *morphological evaluation: floodplain*).

The second axes of the macrozoobenthos and macrophyte DCAs, both explaining about 25 percent of the total community variance, cover different value ranges at LDC versus non-LDC sites. The stretch-specific analyses reveal pronounced differences for all stretches expect the Lower Danube Intercalibration Stretch (Figure 2.11 and Figure 2.12).

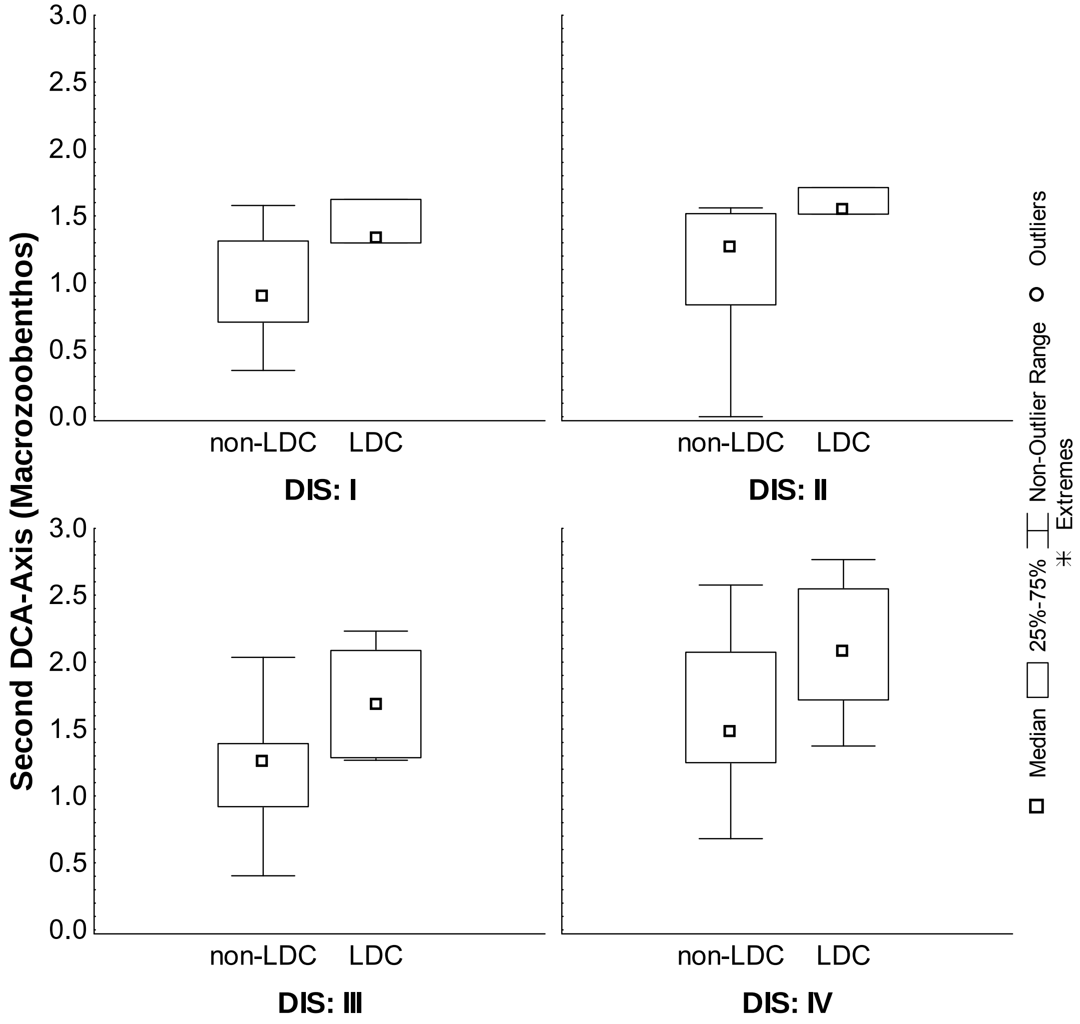

Figure 2.11: Distribution of the second DCA axis for macrozoobenthos (related to the pressure gradient) among LDC and non-LDC sites for each Danube Intercalibration Stretch.

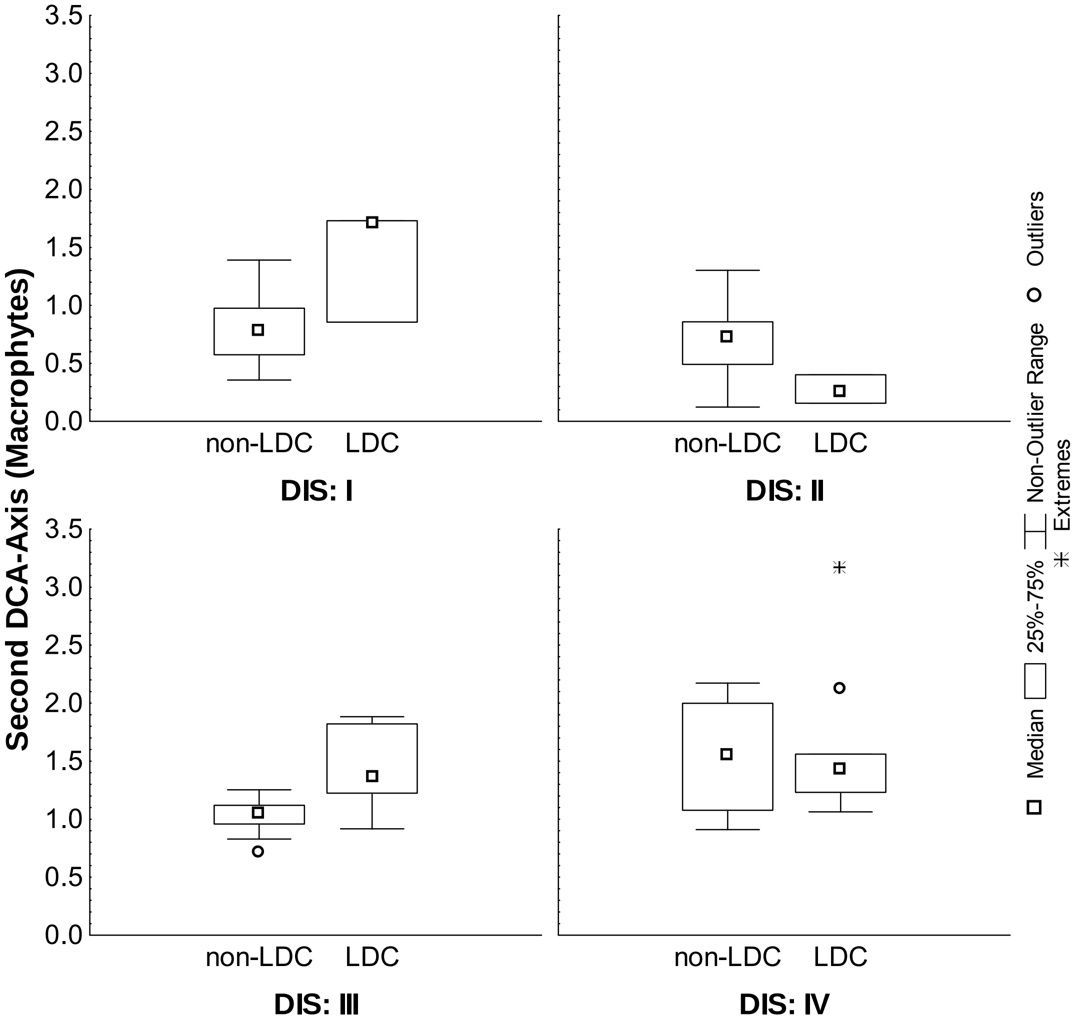

Figure 2.12: Distribution of the second DCA axis for macrophytes (related to the pressure gradient) among LDC and non-LDC sites for each Danube Intercalibration Stretch.

Pressure-response analysis: Biological metric level

Macrozoobenthos

Complete Danube: Sites in least disturbed conditions generally feature lower values of the German Saprobic Index (new version). The scores of the Lotic-Invertebrate Flow Evaluation (LIFE; Extence et al. 1999) are elevated, indicating higher current velocities at less disturbed sites (Figure 2.13).

DIS I: The macrozoobenthos fauna of the Upper Danube Intercalibration Stretch shows obvious differences between LDC and non-LDC sites for several biological metrics

(Figure 2.14). LDC sites are dominated by crustaceans such as *Jaera istri* and species of the genus *Dikerogammarus*. The entire biocoenosis is characterised by shredders and scrapers, and the amount of passive filter feeders is significantly higher than at non-LCD sites. Vice versa, active filter feeders are more frequent at the impacted sites. The least disturbed conditions of this stretch feature a higher ratio of taxa preferring gravely substrate. The impacted sites show larger shares of organisms living in littoral habitats. Taxa indicating oligosaprobic conditions are pronounced at LDC sites, and non-LDC sites feature significantly higher percentages of alpha-mesosaprobic indicators. Thus, LDC sites show low Z&M Saprobic Index values at around 1.85.

DIS II: Similar to DIS I crustaceans are dominating the least disturbed sites, that are additionally characterised by higher percentages of rheophilous taxa. This corresponds with the elevated scores of the LIFE index. At some LDC sites, Trichoptera taxa are abundant. In contrast, non-LDC sites feature higher ratios of Oligochaet-taxa. The Portuguese GOLD index, that decreases with higher proportions of Gastropod, Oligochaet and Diptera abundances, shows lower values at non-LDC sites. Values for the Potamon-Type-Index (PTI; Schöll et al. 2005) and the Z&M Saprobic Index are by trend higher at impacted sites. Figure 2.15 depicts the relevant plots for this intercalibration stretch.

DIS III: Only three macrozoobenthos metrics show distinct differences between LDC and non-LDC sites in the Southern Pannonian DIS (Figure 2.16). Impacted sites feature higher taxa numbers and abundances of Oligochaets. Furthermore, the Czech Average Score Per Taxon and the new version of the German Saprobic Index, both metrics to indicate the water quality, assign higher status to the sites in least disturbed conditions.

DIS IV: At sites in least disturbed conditions of the Lower DIS the invertebrate biocoenosis is characterised by a higher proportion of taxa colonizing sandy, gravely or stony substrate. In contrast, most taxa at non-LDC preferring fine sediments. Similar to DIS II the two site groups feature different ranges of LIFE and Portuguese GOLD scores. LDC sites show a broad range of Trichoptera abundance. The distributions of these four metrics are presented in Figure 2.17.

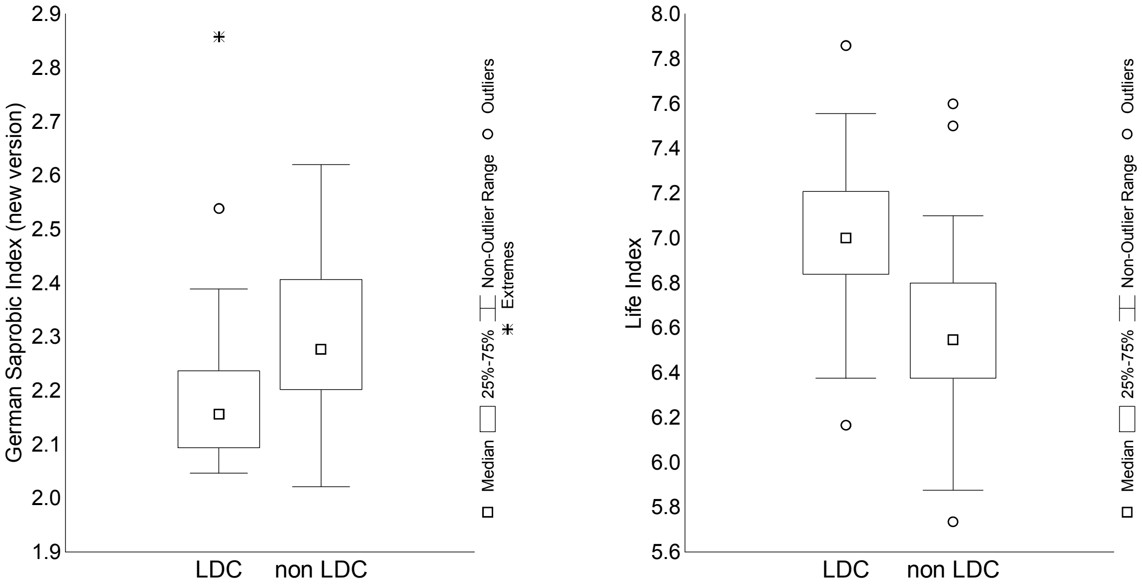

Figure 2.13: Box-plots for differences in macrozoobenthos metrics **across the Danube River** between LDC and non-LDC sites.

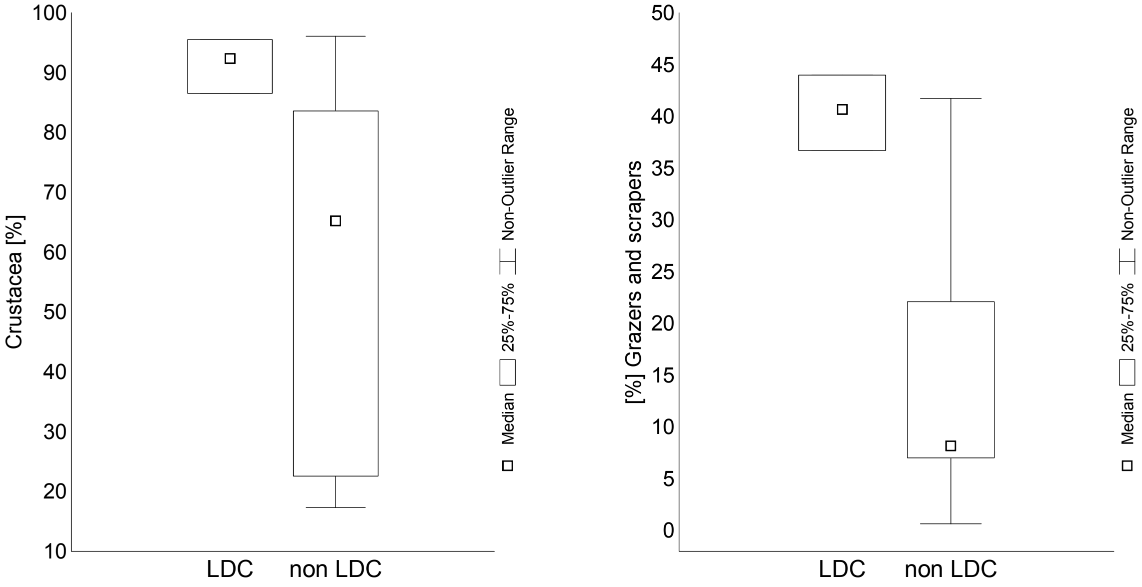

Figure 2.14: Box-plots for differences in macrozoobenthos metrics at **DIS I** between LDC and non-LDC sites.

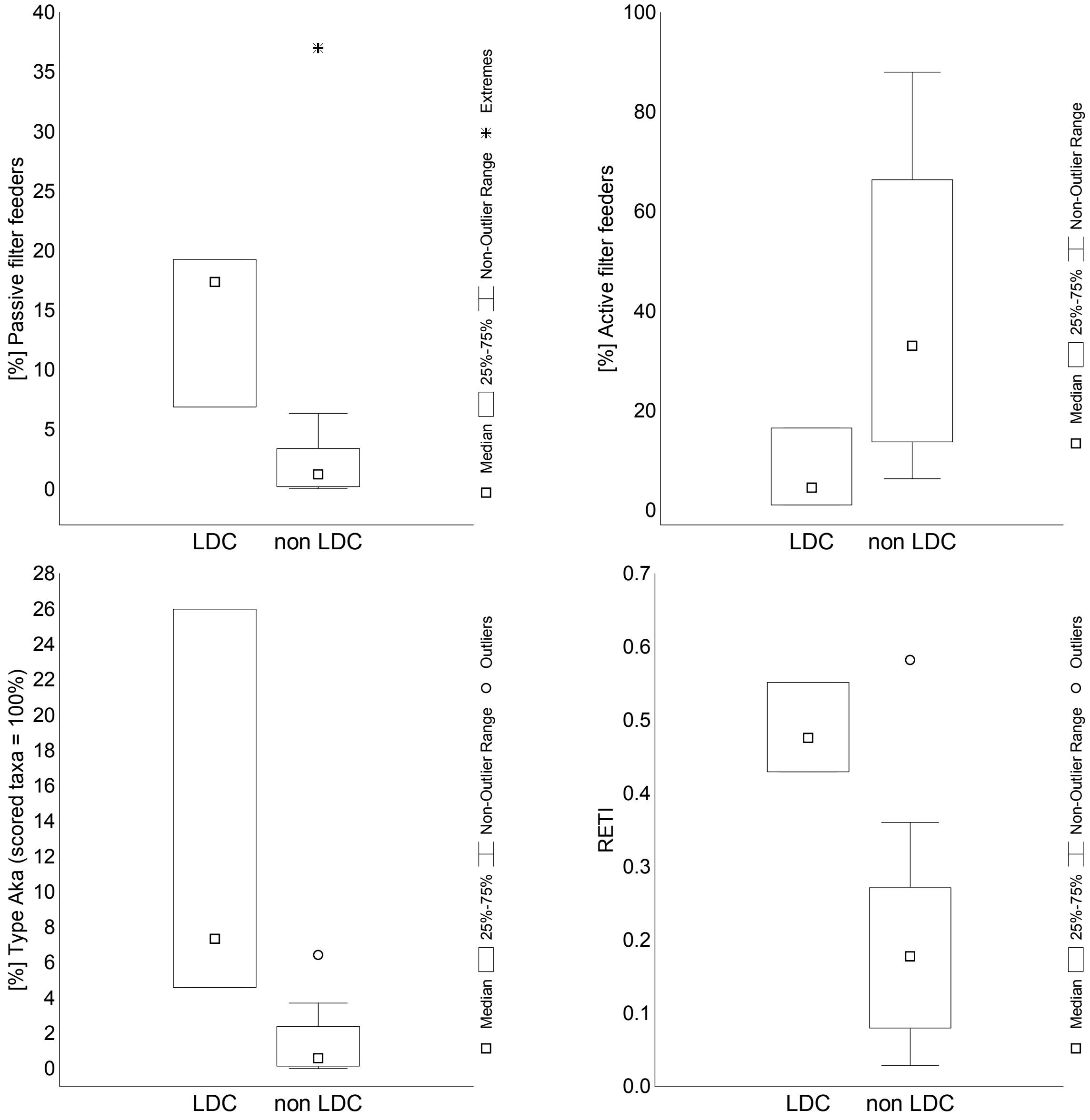

Figure 2.13 (continued): Box-plots for differences in macrozoobenthos metrics at **DIS I** between LDC and non-LDC sites.

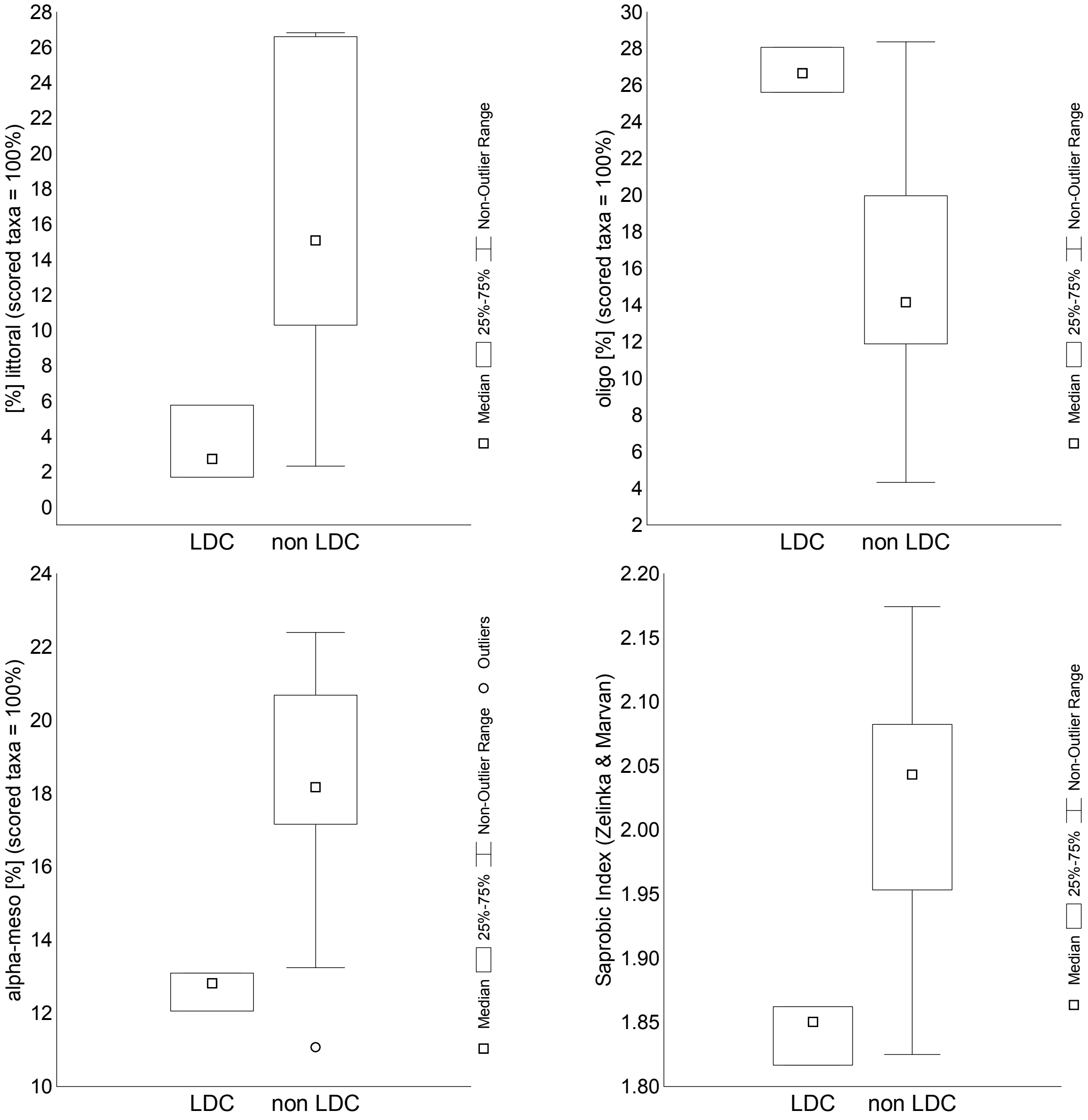

Figure 2.13 (continued): Box-plots for differences in macrozoobenthos metrics at **DIS I** between LDC and non-LDC sites.

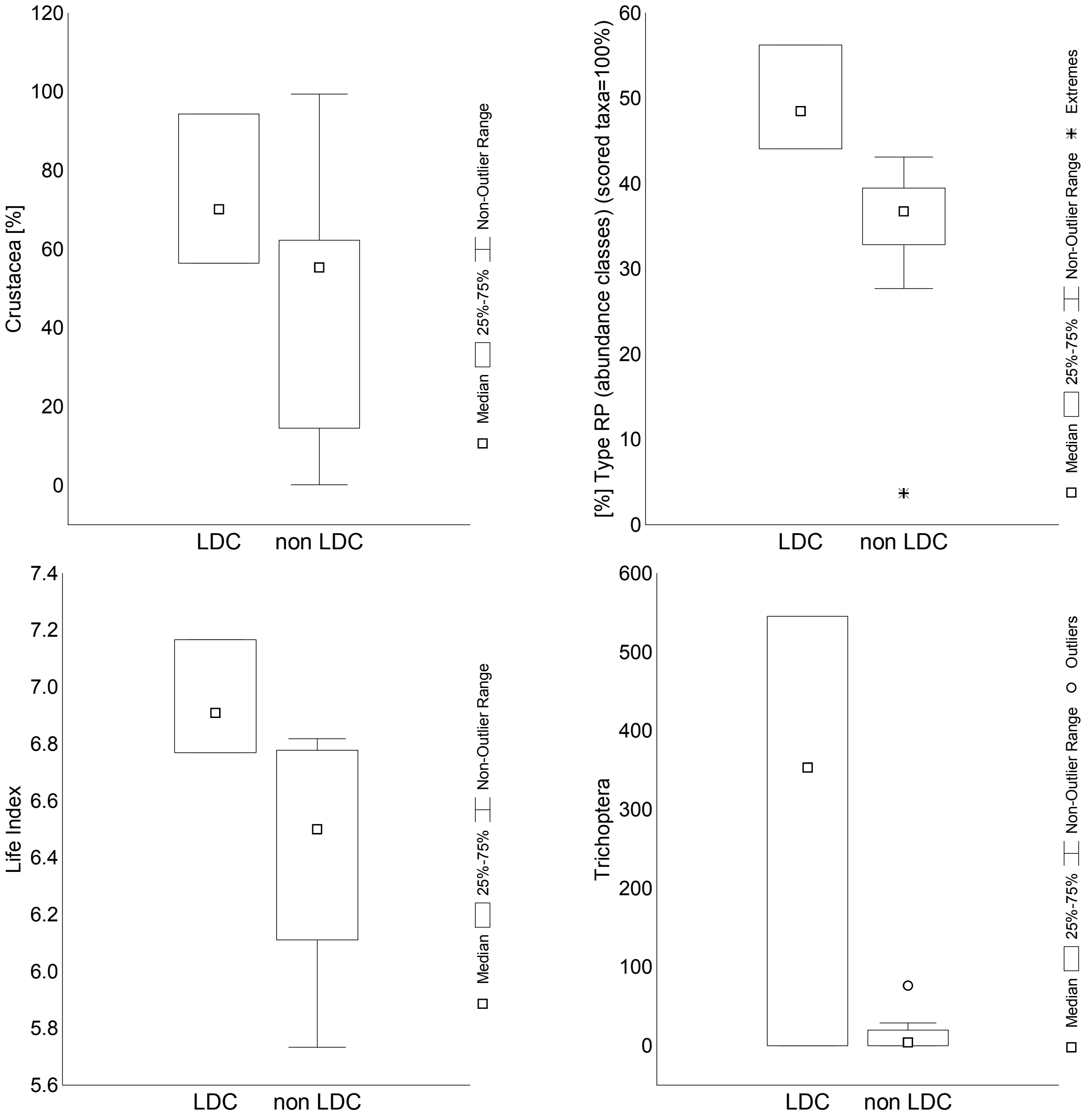

Figure 2.15: Box-plots for differences in macrozoobenthos metrics at **DIS II** between LDC and non-LDC sites.

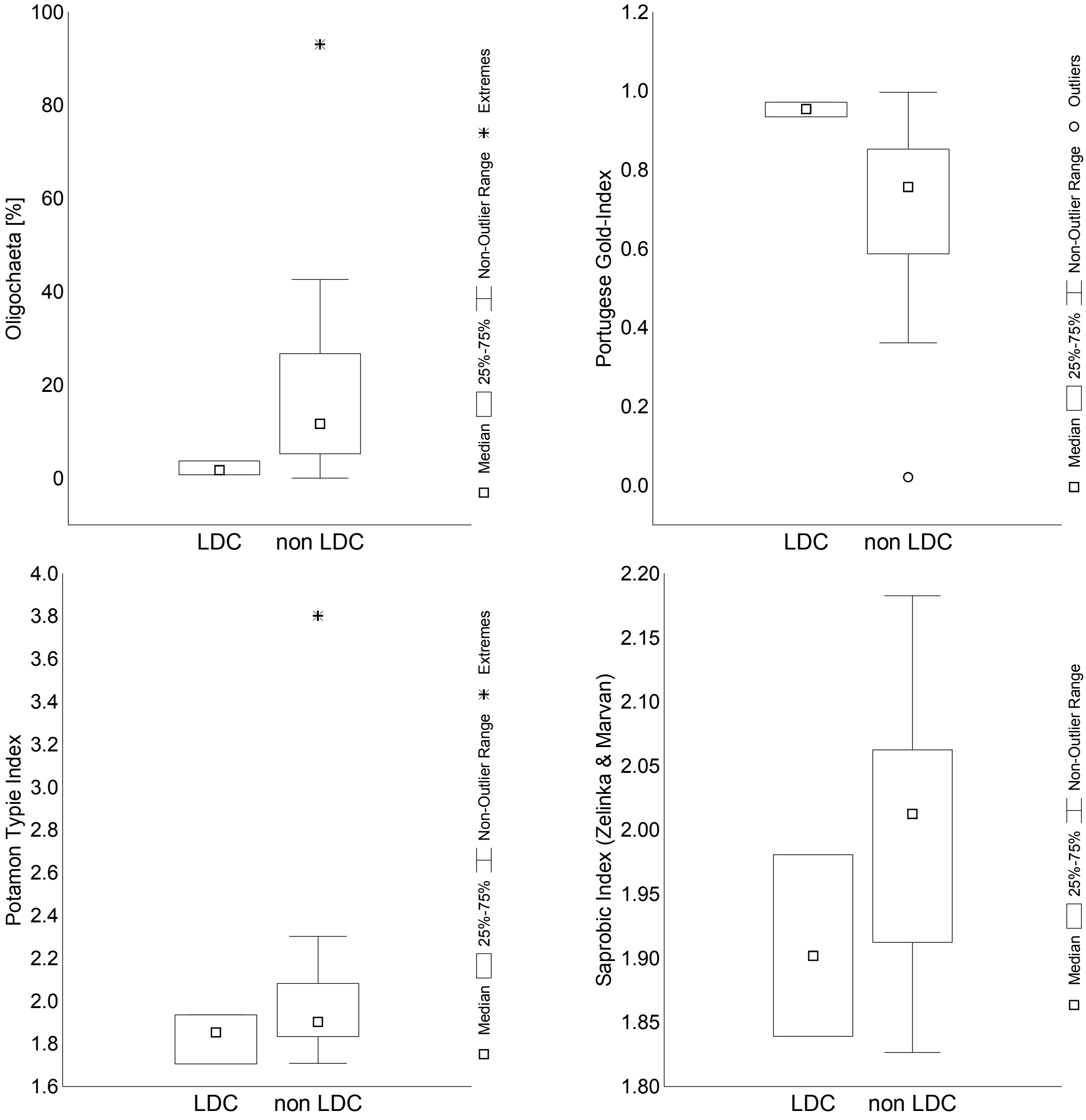

Figure 2.14 (continued): Box-plots for differences in macrozoobenthos metrics at **DIS II** between LDC and non-LDC sites.

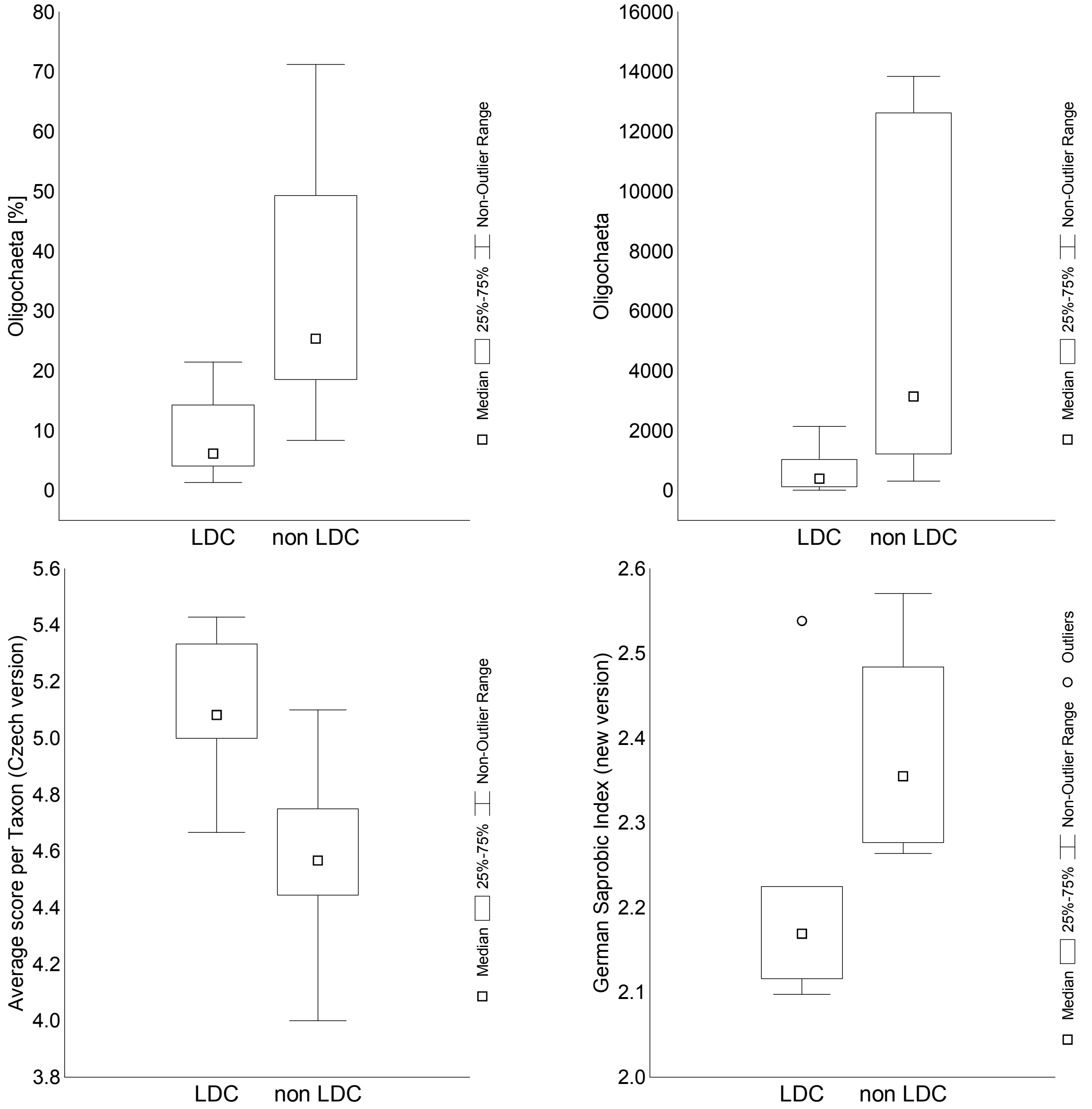

Figure 2.16: Box-plots for differences in macrozoobenthos metrics at **DIS III** between LDC and non-LDC sites.

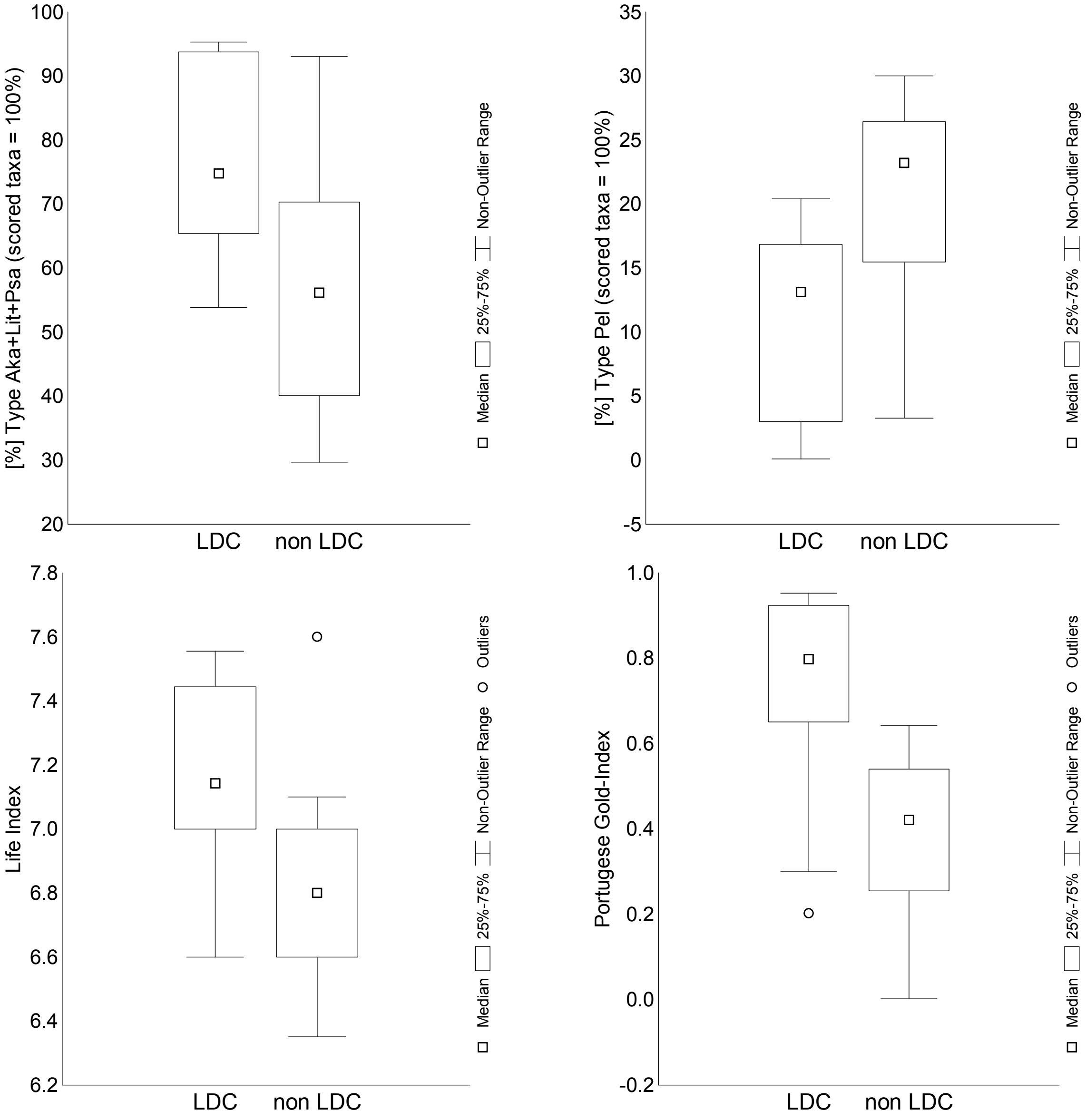

Figure 2.17: Box-plots for differences in macrozoobenthos metrics at **DIS IV** between LDC and non-LDC sites.

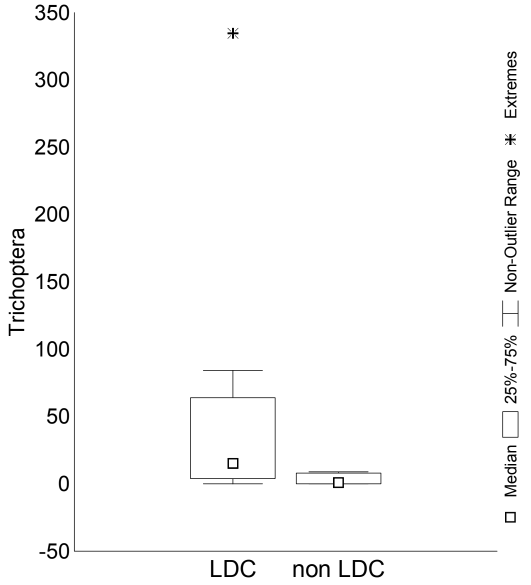

Figure 2.16 (continued): Box-plots for differences in macrozoobenthos metrics at **DIS IV** between LDC and non-LDC sites.

Macrophytes

The macrophyte metrics sensitive to trophic conditions such as the Austrian Index for Macrophytes distinguish LDC sites from impaired sites rather well (Figure 2.18) in the upper Danube Intercalibration Stretch. The metrics calculated for the data of other stretches indicate no site differences. The correlation analysis of the abundance of each taxon with the site’s pressure gradient value revealed a different number of significantly related macrophyte taxa for each DIS (Table 2.15). Most taxa are positively correlated with increasing pressure, and could thus be regarded as stretch-specific disturbance indicators. Figure 2.19 depicts the differences between LDC and non-LDC sites using the defined indicator scores in a weighted average metric. This metric almost perfectly distinguishes least disturbed from impacted sites.

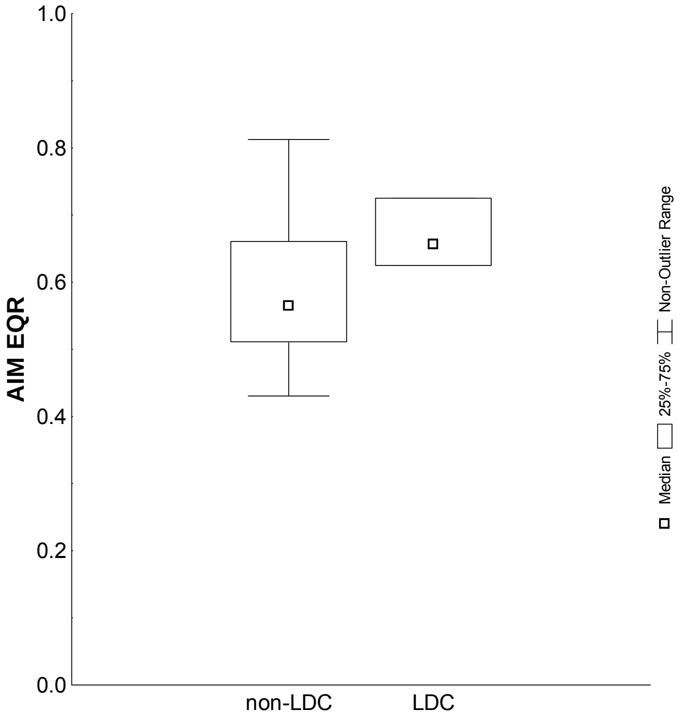

Figure 2.18: Distribution of the EQR values of the Austrian Index for Macrophytes (AIM) among LDC and non-LDC sites for the first Danube Intercalibration Stretch.

Table 2.15: Macrophyte taxa significantly correlated to the pressure gradient (p<0.05), including the Danube Intercalibration Stretch (DIS), number of occurrences within LDC and non-LCD sites, and Spearman correlation coefficient (CorrCoef) of the taxon's abundance with the abiotic pressure gradient site values.

DIS	Taxon	# occurrences per DIS			CorrCoef
		LDC	non-LDC	sum	
I	*Cinclidotus fontinaloides*	2	0	2	-0.650
I	*Fontinalis antipyretica*	1	6	7	0.655
I	*Rhynchostegium riparioides*	0	4	4	0.744
II	*Phragmites australis*	0	5	5	0.759
III	*Azolla filiculoides*	0	6	6	0.645
III	*Ceratophyllum demersum*	5	9	14	0.765
III	*Lemna gibba*	0	3	3	0.635
III	*Lemna minor*	5	10	15	0.700
III	*Myriophyllum spicatum*	3	7	10	0.510
III	*Najas marina (N. major)*	0	8	8	0.799
III	*Phragmites australis*	0	3	3	0.504
III	*Potamogeton gramineus*	0	7	7	0.793
III	*Potamogeton nodosus*	3	8	11	0.637
III	*Potamogeton perfoliatus*	0	7	7	0.805
III	*Sagittaria sagittifolia*	1	5	6	0.491
III	*Salvinia natans*	4	10	14	0.583
III	*Vallisneria spiralis*	1	6	7	0.666
IV	*Bidens sp.*	3	7	10	0.488
IV	*Scirpus lacustris*	0	2	2	0.499
IV	*Tamarix ramosissima*	2	0	2	-0.424

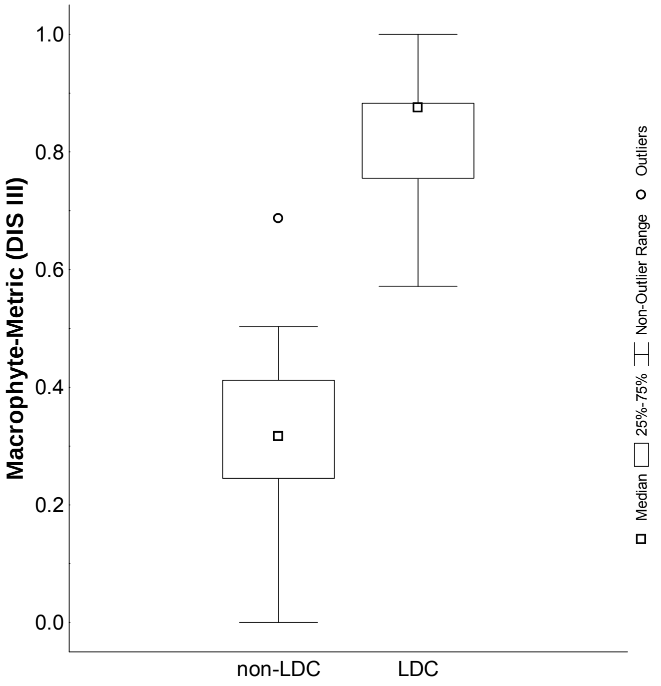

Figure 2.19: Distribution of Macrophyte-Metric for the third Danube Intercalibration Stretch among LDC and non-LDC sites.

2.4.4 Discussion

The pressure-response analysis reveals an obvious relation of the macrozoobenthos and macrophyte communities to the abiotic pressure gradient. The signal for the phytoplankton and benthic diatom assemblages is less clear. Why is this the case? In the first line both algae elements are responsive to the nutrient status of the river. However, the pressure gradient is most strongly reflecting hydromorphological quality, impoundment and oxygen conditions. The nutrient concentrations show comparatively small gradients, with only 2 sites failing the moderate status for orthophosphate, and 3 sites for total phosphate, both in the lower Danube. The discrepancies between averaged TNMN samples and single JDS measurements additionally blur potential relations.

The strong correlations of the macrozoobenthos fauna with the pressure gradient can be reviewed by various aspects of the community. This becomes evident in the different ranges for least disturbed and impacted sites of selected biological metrics. The stretch-specific analysis reveals that each stretch represents a peculiar entity and underlines the importance of setting stretch-specific biological benchmarks against the globally derived pressure gradient. In addition, LIFE index and Saprobic Index show significant differences for the entire river course.

The presented metrics best reflect the response of the macrozoobenthos community to the main anthropogenic pressure gradient of the Danube River. Following Birk & Hering (2009) biological benchmarks can be set using selected summary statistics for the metric distributions at LDC sites. For example, the global distribution of the German Saprobic Index values shows good status for the majority of LDC sites. The Austrian Saprobic Index classifies the LDC sites of DIS I and II in high or good status. However, ranges are generally small and partly overlapping with the non-LDC signal. For the two upper Danube stretches the relevance of this approach could particularly be increased by expanding the data basis. Although an inclusion of additional reaches in least disturbed conditions is not feasible (since no more such reaches exist at the Upper Danube), the number of samples from LDC sites could be enlarged.

3 Technical support of the Eastern Continental Geographical Intercalibration Group

Seven European Member States are participating in the Eastern Continental Intercalibration Group (Austria, Czech Republic, Slovak Republic, Hungary, Slovenia, Bulgaria, Romania), with Slovenia joining the group for the second round of intercalibration (Figure 3.1). The group aims at intercalibrating the national assessment methods used in river monitoring according to WFD demands. Relevant quality elements comprise benthic invertebrates, macrophyte and phytobenthos, and phytoplankton. Methods evaluating the fish fauna are compared in a separate activity (Jepsen & Pont 2007).

Figure 3.1: Map of Europe showing countries participating in the Eastern Continental Intercalibration Group (AT: Austria, CZ: Czech Republic, SK: Slovak Republic, SI: Slovenia, HU: Hungary, RO: Romania, BG: Bulgaria).

By end of 2007 the first round of intercalibration was accomplished. In the EC GIG results were achieved for the Austrian and Slovak assessment methods using benthic invertebrates (Birk 2007). Furthermore, the outcomes of this first exercise delivered guidelines for boundary setting for the non-WFD compliant methods of Czech Republic, Hungary, Bulgaria and Romania. The ongoing development of national assessment methods complying with the WFD requirements did not allow for the intercalibration of further Member States.

For the second round of intercalibration lead by the Czech Republic technical support was given on various issues related to the practical intercalibration work. First, the national data requirements for the intercalibration of river assessment methods in the EC GIG were specified (Chapter 3.1). To gain information about the national methods to be intercalibrated, an overview on ecological assessment schemes was generated (Chapter 3.2). Finally, an amendment to the current list of common intercalibration stream types used in the EC GIG was proposed (Chapter 3.3)

3.1 National data requirements for the intercalibration of river assessment methods in the Eastern Continental GIG – Second round of intercalibration

General requirements

- For the intercalibration exercise biological data and supportive environmental data are required. The collation of the latter is generally more time-consuming since different data sources need to be addressed (including expert judgement).
- Data need to originate from sites belonging to relevant intercalibration river types.
- Data shall cover the whole gradient of anthropogenic degradation (high to bad status) of the respective river type.
- Data need to be equally distributed among quality classes. At least 30 sites in high and good status are required per intercalibration stream type and country, resulting in a total number of approximately 75 sites that cover the whole quality gradient.
- Data shall be sampled by a standard procedure that is generally applied for WFD monitoring purposes in your country; sampling seasons must not vary significantly (homogeneous dataset).
- Physico-chemical measurements need to be related to the biological samples; therefore they shall cover the period of one year (half year for nutrients) prior to the biological sampling date. The mean of monthly measurements is an appropriate summary statistic.

Specific requirements - Biological data

- taxonomical composition
- abundance
- any other relevant biological data (e.g. macrophyte growth form)

Specific Requirements - Environmental data

- The collation of environmental data aims at either defining reference sites (unimpacted by anthropogenic activity) or identifying sites in at least good environmental status (in case few/no near-natural sites are available).
- The definition of reference sites follows the Central-Baltic GIG criteria modified for the Eastern Continental GIG.
- The identification of sites in at least good environmental status follows the procedure applied in the intercalibration of macrozoobenthos and diatom assessment methods of Austria and the Slovak Republic undertaken in the first round of intercalibration (see Birk & Hering 2009). In addition, some criteria derived from the fish intercalibration exercise have been added. The required parameters comprise:
    - Mean values of Biological Oxygen Demand (5 days), Total Phosphorus, Ortho-phosphate, Conductivity (see also “general requirements”),
    - Hydromorphological quality status of the site (see Table 3.1),
    - Catchment land use parameters (% urban land use, % intensive agriculture, % non-intensive agriculture),
    - Data about several other pressures and their modalities (according to the fish intercalibration exercise) (see Table 3.2).

Table 3.1: Classification scheme to assess the hydromorphological quality status of invertebrate sampling sites.

Class 1 - near-natural hydromorphological conditions
- Stream type specific variability of channel depth and channel width, shallow profile, close connectivity of the stream and the floodplain
- Natural channel substrate conditions (composition and variability), presence of dead wood
- Bank profile and bank structure unmodified
- Presence of natural riparian vegetation
- Natural hydromorphological dynamic is maintained
- Low degree of anthropogenic land use in the floodplain
Class 2 - moderately altered hydromorphological conditions
- Decreased variability of channel depth and channel width
- Minor changes to bank morphologies, or only one bank is fixed with "soft works"
- Riparian vegetation altered
- Loss of stream length, longitudinal profile is altered by man
Class 3 - severely altered hydromorphological conditions
- Obvious presence of hard engineering
- Severe modifications of instream structures, bed and bank fixation and artificial substrates
- No or only minor variability of channel substrate
- No riparian zone between river and land use
- Channelised, straightened and/or deep-cut river
- Disconnection of river and floodplain

Table 3.2: Data requirements on other pressures and their modalities.

Pressure type	Scale	Pressure modality 1	Pressure modality 2	Pressure modality 3	Pressure modality 4
Impoundment	site	no impoundment at the site scale	impoundment at the site scale but slight flow velocity reduction	impoundment at the site scale and strong flow velocity reduction	-
Hydropeaking	site	no hydropeaking, no alteration of the hydrograph	hydropeaking, slight alteration of the hydrograph	hydropeaking, alteration of the hydrograph, potentially affecting the fish fauna	-
Water abstraction	site	site not affected by water flow alteration	site slightly affected by water abstraction (less than 10% of the median annual flow and the median monthly flow during a critical period, e.g. low flow period)	site significantly affected by water abstraction (more than 10% of the median annual flow and the median monthly flow during a critical period, e.g. low flow period)	site strongly affected by water abstraction (more than 50% of the median annual flow and the median monthly flow during a critical period, e.g. low flow period)
Upstream dams influence	site	no influence of dam located upstream on the site itself (flow regulation, temperature, sedimentation, reservoir flushing, …)	slight influence of dam located upstream on the segment itself (flow regulation, temperature, sedimentation, reservoir flushing, …). No clear potential effect on the fish fauna at the site	strong influence of dam located upstream on the reach itself (flow regulation, temperature, sedimentation, reservoir flushing,…)	-
Water temperature modification (excluding dam effect)	site	no alteration of the temperature regime at the site scale due to other pressures than a dam located upstream (cooling water release, …)	alteration of the temperature regime at the site scale due to cooling water release (more than 1°C)	-	-
Canalisation / Cross section alteration (segment scale)	segment	no canalisation, no alteration of the "natural" cross section (no "hard works" affecting the whole river). No flow velocity increase	slight alteration (less than 10% of the segment affected by "hard works"). No flow velocity increase	significant alteration (a main part of the segment is affected by "hard work"). Flow velocity increase	strong alteration (straitened river, Technical-U-profile section, …). Flow velocity increase
Riparian vegetation	site	no direct alteration of the riparian vegetation (i.e. adjacent natural vegetation appropriate to the type and geographical location of the river)	slight alteration of the riparian vegetation	strong alteration of the riparian vegetation	no more riparian vegetation (due to human activities)
Local Habitat alteration (site scale)	site	no alteration of instream habitats, no "soft work" (bank protection, …), no significant sedimentation, no important degradation of the river bed (incision, deepening, …).	slight alterations. < 20% of the site is affected by "soft works"	significant alterations	strong alterations

Pressure type	Scale	Pressure modality 1	Pressure modality 2	Pressure modality 3	Pressure modality 4
Dykes (flood protection)	segment	no dykes for flood protection	presence of dykes for flood protection; distance from main channel still provides significant lateral connectivity	presence of dykes for flood protection; distance from main channel still allows some lateral connectivity	presence of dykes for flood protection; no lateral connectivity
Alteration of the former floodplain (when present) Segment scale	segment	no alteration	more than 50% of the former floodplain remaining connected to the river	10- 50% of the former floodplain remaining connected to the river	only some waterbodies remaining connected
Toxic Risk. Priority substances list	segment	no or very minor (e.g. only atmospheric input, no input in the segment itself)	weak risk, linked to one/two particular substances of the official list, or to known, but limited toxic input	high risk (a clearly known point source which can affect the segment, upstream from site in general)	-
Water acidification	segment	no present acidification, no liming, no previous acidification	slight acidification (slight liming, slight previous acidification)	present acidification	-
National water quality index (segment scale)	segment	class 1 (no alteration)	class 2 (minor alteration)	class 3	> class 3 (depending on the number of classes in the national index)
Water quality alteration (local scale)	site	no visible sign of eutrophication (algae or macrophyte proliferation), no visible sign of organic pollution, no visible sign of organic sedimentation	slight signs ($BOD_5 < 3$ mg/l for salmonid rivers, $BOD_5 < 5$ mg/l for non salmonid rivers)	clear signs (occurrence of green algae, …)	strong eutrophication (important O_2 depletion, …)
Navigation	segment	no or only low navigation intensity (commercial transport, large ship)	low intensity	high intensity	-
Recreational use with high intensity (angling, boating, ...)	site	no intensive use associated with a clear effect on the river biota	intensive use associated with a clear effect on the river biota	-	-

3.2 Design of an overview on national assessment methods to be intercalibrated in the second round of intercalibration

Basic element of any intercalibration exercise is comprehensive information about the schemes that countries are applying to evaluate their water courses. To gain an overview of national assessment methods used by the participants in the intercalibration exercise, a questionnaire was designed. This questionnaire included 33 questions and requests separated into the four main parts: country, sampling/survey procedures, typology and reference conditions, assessment and classification (Table 3.3).

Table 3.3: List of questions/requests included in the questionnaire on national assessment methods.

COUNTRY
Biological Quality Element (BQE)
Name of Method
Literature Reference (complete method)
Contact Person's Email
SAMPLING/SURVEY PROCEDURE
Sampling/Survey Month(s)
Short description of sampling/survey procedure
Sampling/Survey tools used (e.g. Surber sampler, grapnel etc.)
Characterisation of representative sampling/survey site (length, width, features)
Which abiotic data are recorded?
Recorded Taxonomic Groups (e.g. only diatoms; hydrophytes; Insecta)
Level of Identification (e.g. species, sub-species etc.)
Record of Abundance (e.g. individuals per square-metre; abundance classes)
Specification of Abundance Scheme
Which taxon codes are used to store the data (e.g. AQEM shortcodes; OMNIDIA shortcodes)?
Literature Reference (survey procedure)
TYPOLOGY AND REFERENCE CONDITIONS
Is the assessment method based on a specific national stream typology for the BQE?
Which national stream types (relevant for the BQE assessment) are covered by the common intercalibration types?
Have reference conditions been defined for your national types for this BQE?
How is the stream type specific reference expressed (e.g. exact definition of the reference community, reference value of selected metrics, group of species occurring in reference state)?
Do you think that an important stream type is missing in the list of common IC types (see Table 3.4 for details)?
Literature Reference (typology and reference conditions)
ASSESSMENT AND CLASSIFICATION
Assessment Category (e.g. biotic index, multimetric index etc.)
Which metrics are used?
How are these metrics combined to achieve the ecological quality class?
Do the numeric results from which the (overall) quality classes are directly derived show continuous scale (e.g. metric values ranging from 0 to 1 which are then classified by quality boundaries)?
Please specify the range of numeric results which can possibly be reached by your method.
Please specify all quality class boundary values.
Which rules did you follow to define the high-good and good-moderate boundary? Please give a precise description of this boundary setting.
Aim of the Assessment (e.g. evaluation of general degradation, appraisal of the trophic state, assessment of substrate quality)
Which criteria must be met to consider assessment results valid (e.g. minimum number of taxa found at site etc.)?
Literature Reference (assessment and classification)
Which features make your assessment method WFD-compliant?
Which software are you using to calculate the assessment index?

3.3 Proposal for an amendment in the delineation of common intercalibration types in the Eastern Continental GIG

In this section an amendment in the delineation of common intercalibration stream types for the EC GIG is proposed. Common intercalibration types are the basis for the comparison of the national classification of good ecological status.

The amendments are recommended in the light of the experiences gained in the first round of intercalibration (2004 – 2007). Furthermore, they are given with regard to the official results obtained in this first round, i.e. the full intercalibration of invertebrate classification schemes of Austria (type R-E4) and Slovak Republic (types R-E1, R-E2 and R-E4). The proposed additions allow for the intercalibration between the EU Member States, accession countries and additional countries in the Danube River Basin.

The proposal retains the general type descriptions based on ecoregion, catchment area, altitude, geology and substrate. Using this broad typological framework it will be necessary that the common types are specified more precisely with regard to the biological quality elements to be intercalibrated.

Table 3.4 lists all common intercalibration types including the five types already defined for the first round of intercalibration and five additional types proposed for the second round of intercalibration. To each type several countries have been assigned.

It is required to discuss this proposal among national experts participating in the EC GIG. All amendments can be subject to further improvement and revision.

Table 3.4: Proposal for an amendment in the delineation of Common Intercalibration Types in the Eastern Continental GIG.

Stream type abbreviation	Common intercalibration type	Participating countries	Ecoregion (Illies, 1967)	Catchment area [km^2]	Altitude [m]	Geology	Channel substrate	Comments
R-E1	Carpathians: small to medium, mid-altitude	CZ, SK, RO, UA	10 (The Carpathians)	10 - 1,000	500 - 800	siliceous	gravel and boulder	
R-E2	Plains: medium-sized, lowland	Ecoregion 11: HU, SK, RO, HR, CS, SI Ecoregion 12: RO, MD, UA, BG	11 (Hungarian Lowlands) and 12 (Pontic Province)	100 - 1,000	< 200	mixed	sand and silt	#separate types per ecoregion ? #sub-types necessary for aquatic flora (based on alkalinity)
R-E3a	Plains: large, lowland	Ecoregion 11: HU, SK, RO, HR, CS, SI Ecoregion 12: RO, MD, UA, BG	11 (Hungarian Lowlands) and 12 (Pontic Province)	1,000 - 10,000	< 200	mixed	sand, silt and gravel	#separate types per ecoregion ? #sub-types necessary for aquatic flora (based on alkalinity)
R-E3b	Plains: very large, lowland	Ecoregion 11: HU, SK, RO, HR, CS, SI Ecoregion 12: RO, MD, UA, BG	11 (Hungarian Lowlands) and 12 (Pontic Province)	> 10,000	< 200	mixed	sand, silt and gravel	#separate types per ecoregion ? #sub-types necessary for aquatic flora (based on alkalinity)
R-E4	Plains: medium-sized, mid-altitude	Ecoregion 11: AT, HU, SK, RO, HR, CS, SI Ecoregion 12: RO, MD, UA, BG	11 (Hungarian Lowlands) and 12 (Pontic Province)	100 - 1,000	200-500	mixed	sand and gravel	#separate types per ecoregion ? #sub-types necessary for aquatic flora (based on alkalinity)
R-E6	Danube River: middle and downstream	Ecoregion 11: AT, SK, HU, HR, CS Ecoregion 12: RO, BG, MD, UA	11 (Hungarian Lowlands) and 12 (Pontic Province)	> 131,000	< 134	mixed	gravel and sand	to be separated into sub-types
R-EX$_1$	Balkan: medium-sized, calcareous, mid-altitude	BA, HR, SI, CS	5 (Dinaric Western Balkan)	100 - 1,000	200 - 500	calcareous	gravel	
R-EX$_2$	Balkan: large, calcareous, mid-altitude	BA, HR, SI, CS	5 (Dinaric Western Balkan)	> 1,000	200 - 500	calcareous	sand and gravel	
R-EX$_3$	Balkan: large, calcareous, lowland	BA, HR, SI, CS	5 (Dinaric Western Balkan)	> 1,000	< 200	calcareous	sand and gravel	
R-EX$_4$	Carpathians: large, mid-altitude	CZ, SK, RO, UA	10 (The Carpathians)	> 1,000	200 - 500	siliceous	gravel and boulder	

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5 Appendices

5.1 National methods to assess the ecological status of the Danube River

Table 5.1 provides an overview of the countries that apply WFD compliant assessment methods at the Danube River. Hungary, Bulgaria and Romania have not yet developed any classification scheme. Phytoplankton is only assessed by Germany, including the evaluation of biomass and taxonomical composition (Mischke & Behrendt 2007). This multi-metric scheme combines the appraisal of chlorophyll-a concentrations, the type-specific phytoplankton index based on indicator taxa and the percentage of Chlorophyceae biomass. Austria is not assessing phytoplankton for the Danube, and the Slovak method is under development.

Table 5.1: Overview of national assessment methods of the Danube River (X=method in use, -=no method, u.d.=method under development, SI=Saprobic Index)

Country	BQE			
	PP	MA	PB	MZB
DE	X	-	X	X
AT	-	X	X	X (only SI)
SK	u.d.	X	X	X
HU	u.d.			
BG	u.d.			
RO	u.d.			

Macrophytes are used for ecological status assessment by Austria and the Slovak Republic. The Austrian method is based on Danube-specific indicator taxa whose values are combined with the abundance estimates of plant species at a site to yield an Ecological Quality Ratio (Pall & Mayerhofer 2008). The Slovak scheme uses several metrics (German Reference Index adopted to Slovak conditions, French IBMR, Shannon diversity and a conservation index) to assess the site quality (Livia Tothova, pers. comm.). Germany does not use aquatic plants to evaluate the Danube River.

Phytobenthos is used by Germany, Austria and the Slovak Republic to assess the ecological status of the Danube. Germany employs two diatom indices (German Reference Index and Trophic Index, Schaumburg et al. 2006), furthermore the non-diatom algae are evaluated in a separate module considering four indicative groups (sensitive and tolerant taxa). The Austrian method features a multi-metric module for the phytobenthos assessment (Austrian Reference Index, Saprobic and Trophic

Indices) that integrates non-diatom taxa in the index calculations (Pfister & Pipp 2009). The Slovak Republic has a method that combines individual assessment metrics for diatoms to an overall index (see Birk & Vogel 2007).

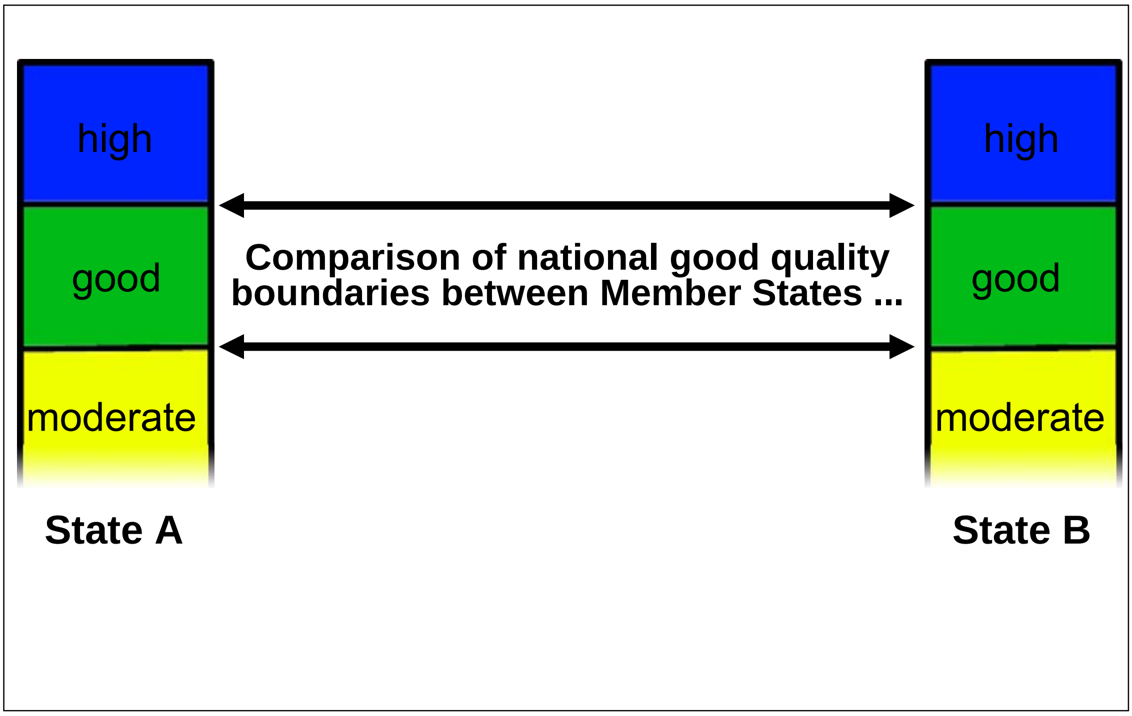
The German method to assess the ecological status using macrozoobenthos is a specific large river adaptation of the multi-metric scheme PERLODES (Meier et al. 2006). Core metric of the assessment is the Potamon-Typie-Index (Schöll et al. 2005). This index is based on the evaluation of typical large river species. Austria has not yet developed a complete macrozoobenthos method for the Danube but uses the Saprobic Index for the evaluation of organic pollution (Moog et al. 1999). The Slovak method is a multi-metric index derived from ten individual scores (Saprobic Index, % oligosaprobic indicators, BMWP score, Rhithron Type Index, % metarhithral, Index of Biocoenotic Region, % Type Aka+Lit+Psa, Margalef Diversity Index, EPT-Taxa, Number of Families).

5.2 Talk given at the “JDS 2 Report Writing Meeting”, Senec (March 2008)

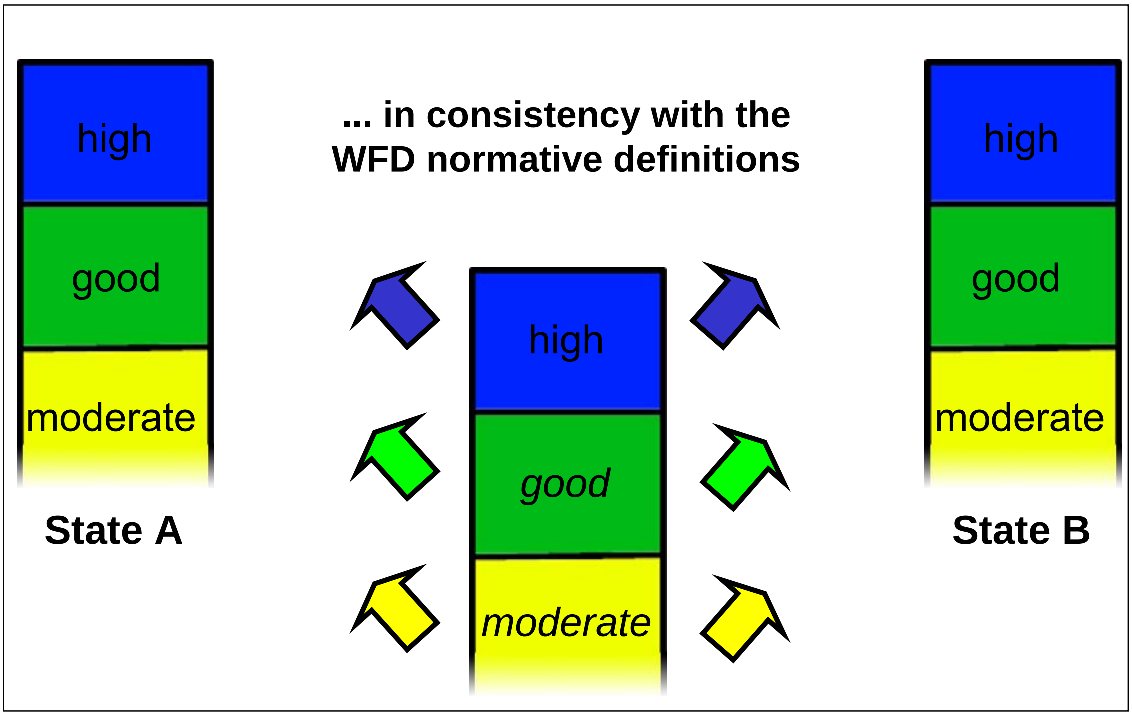
Towards Danube Intercalibration using JDS data

Outline of background and proposed procedure

Sebastian Birk
University of Duisburg-Essen, Germany

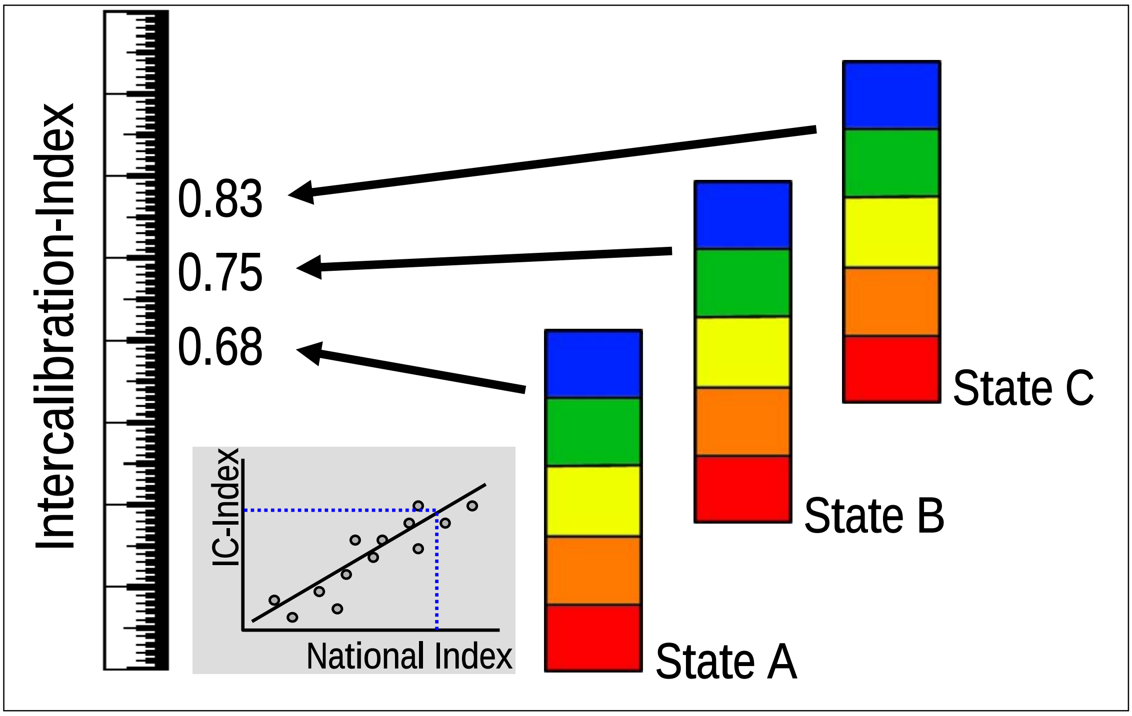


Aim of intercalibration



Aim of intercalibration

I. COMPARISON



Intercalibration method
„common metric“

II. CONSISTENCY WITH WFD NORMATIVE DEFINITIONS

WFD NORMATIVE DEFINITIONS (ANNEX V)

HIGH STATUS

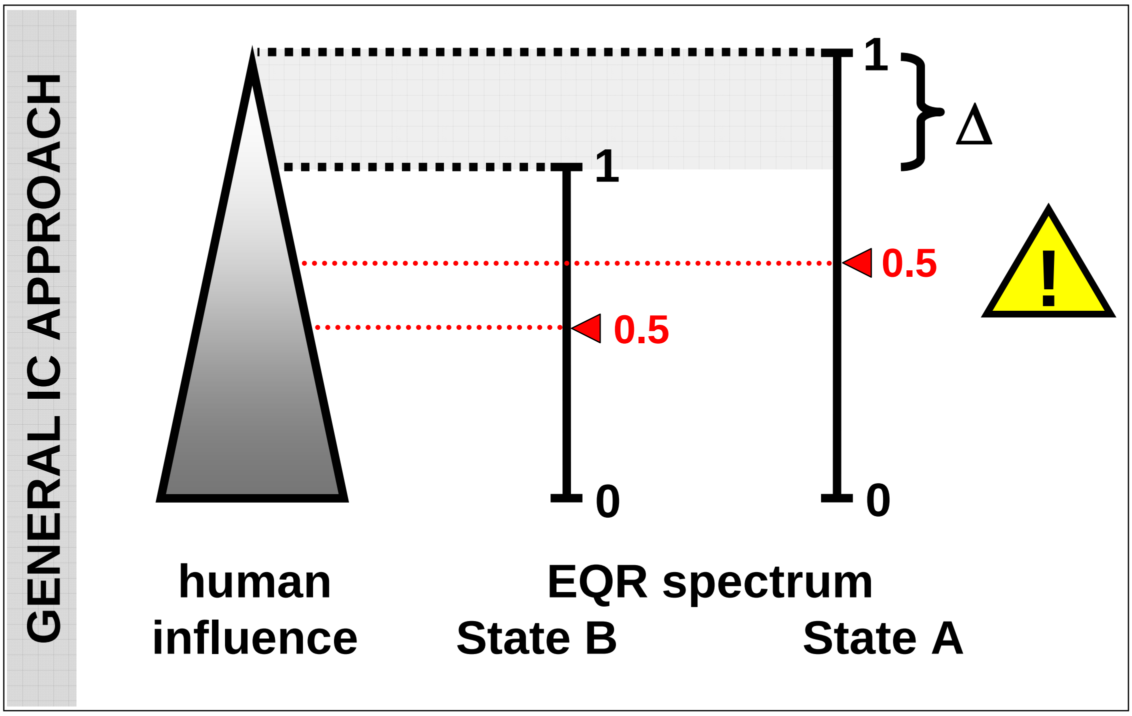
„BQEs [...] show no or very minor evidence of distortion“

GOOD STATUS

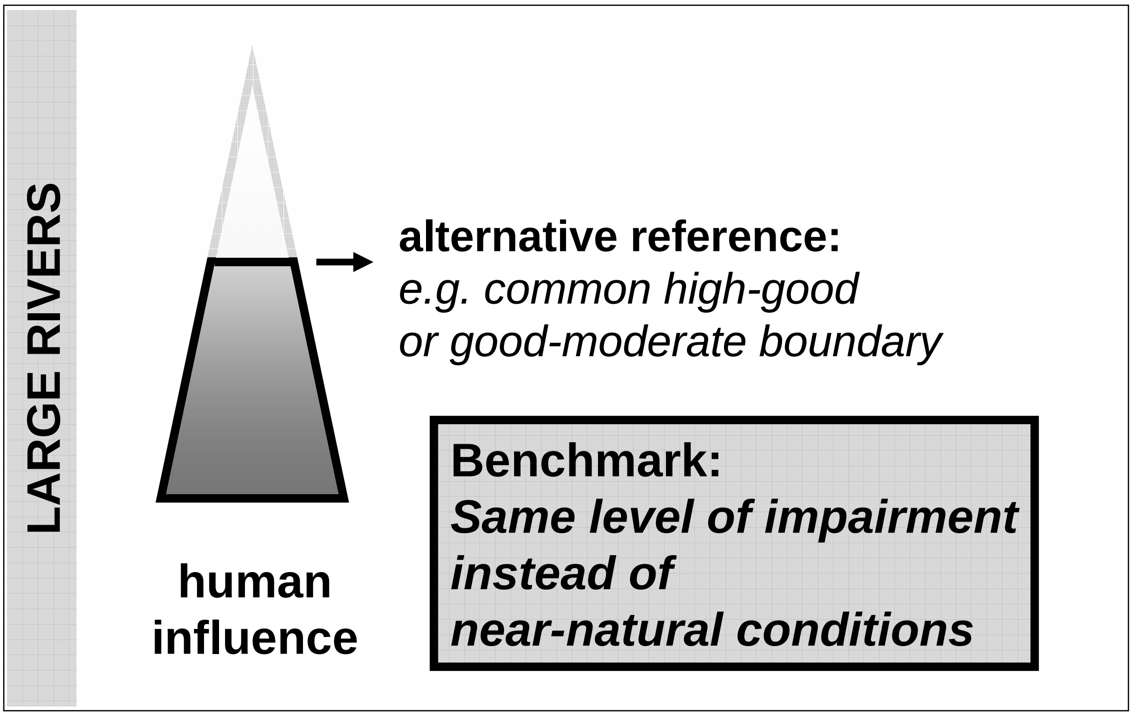
„BQEs [...] deviate only slightly [...] from undisturbed conditions“

MODERATE STATUS

„BQEs [...] are significantly more disturbed than under [...] good status“



Common definition of high status using near-natural reference sites



alternative reference:
e.g. common high-good
or good-moderate boundary

Benchmark:
Same level of impairment
instead of
near-natural conditions

Alternative reference

JDS2 intercalibration analysis

Further issues

- Revision of Danube Section Typology (BQE-specific types)

- Availability of national assessment methods

- Relation to ongoing JDS2 data analyses, esp. concerning
    - Development of common assessment metrics
    - Gradient analysis
    - Pressure-impact analysis
    - Definition of abiotic thresholds related to ecological quality

JDS2 intercalibration analysis

Principal steps

I. Gradient analysis: identification of (complex) stressor gradients

II. Pressure-impact analysis:
which (complex) stressors affect which BQE?

III. Definition of abiotic thresholds:
which sites meet criteria of high or good environmental status?

IV. Evaluation of sites using
common metrics/national assessment methods
→ definition of high-good or good-moderate boundary

5.3 Talk given at the “Large River Intercalibration Meeting”, Koblenz (April 2009)

Towards Danube Intercalibration using JDS data

Outline of background and first results

Sebastian Birk

I. NATIONAL ASSESSMENT

National assesment methods for the Danube River

Country	BQE				
	PP	MA	PB	MZB	FI
DE	X	X	X	X	X
AT	-	X	X	X (only SI)	X
SK	u.d.	X	X	X	u.d.
HU	u.d.				
BG	u.d.				
RO	u.d.				

u.d. = under development
SI = Saprobic Index

II. PRESSURE GRADIENT & LDC

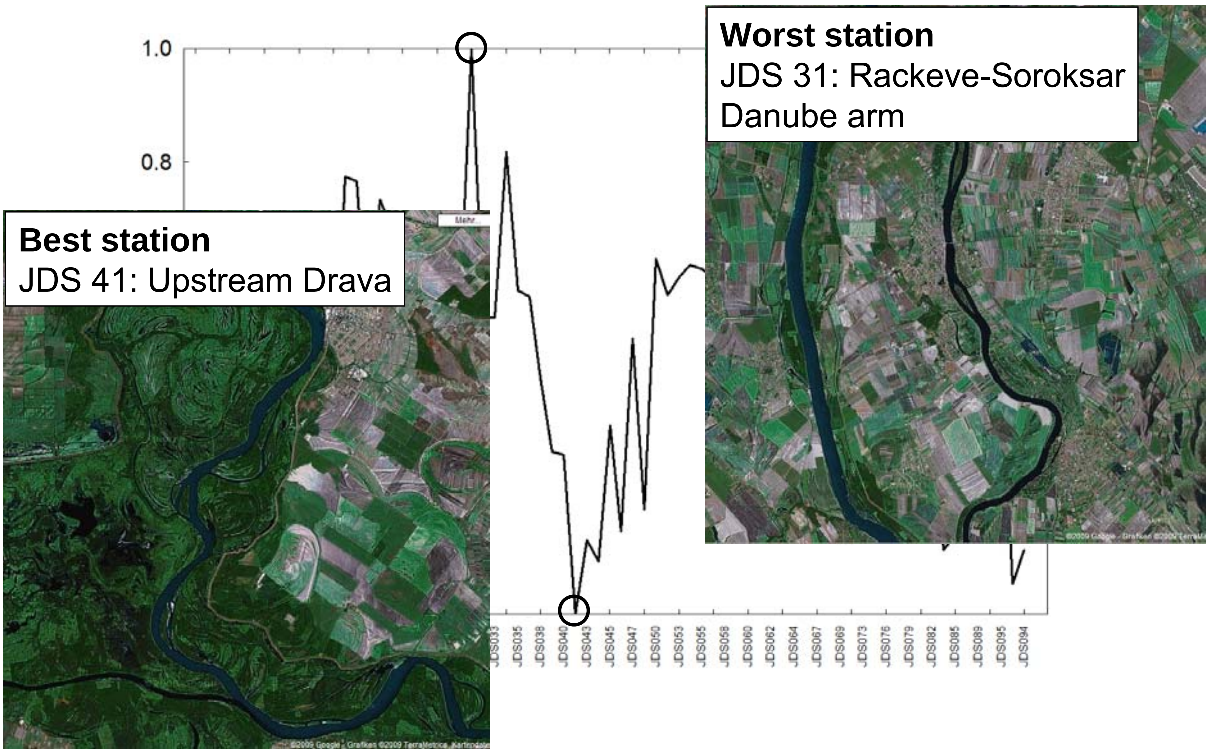
Danube‘s main pressure gradient

- RKM 2600 to RKM 0
- based on JDS2 abiotic data and TNMN nutrient data
- Method: Principal Component Analysis

- Hydromorphological reach quality (channel, banks, floodplain)
- Dissolved oxygen concentration at site
- Site in backwater
- Naturalness of bank slope at site
- Naturalness of bank vegetation at site

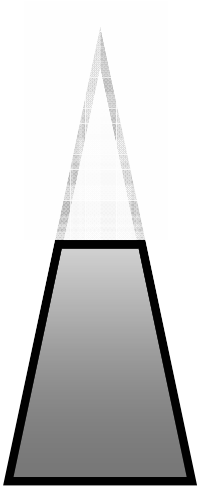
→ **Hydromorphology, impoundment, organic pollution**

“Pressure profile“



Worst station
JDS 31: Rackeve-Soroksar Danube arm

Best station
JDS 41: Upstream Drava



LCD = Least Disturbed Conditions

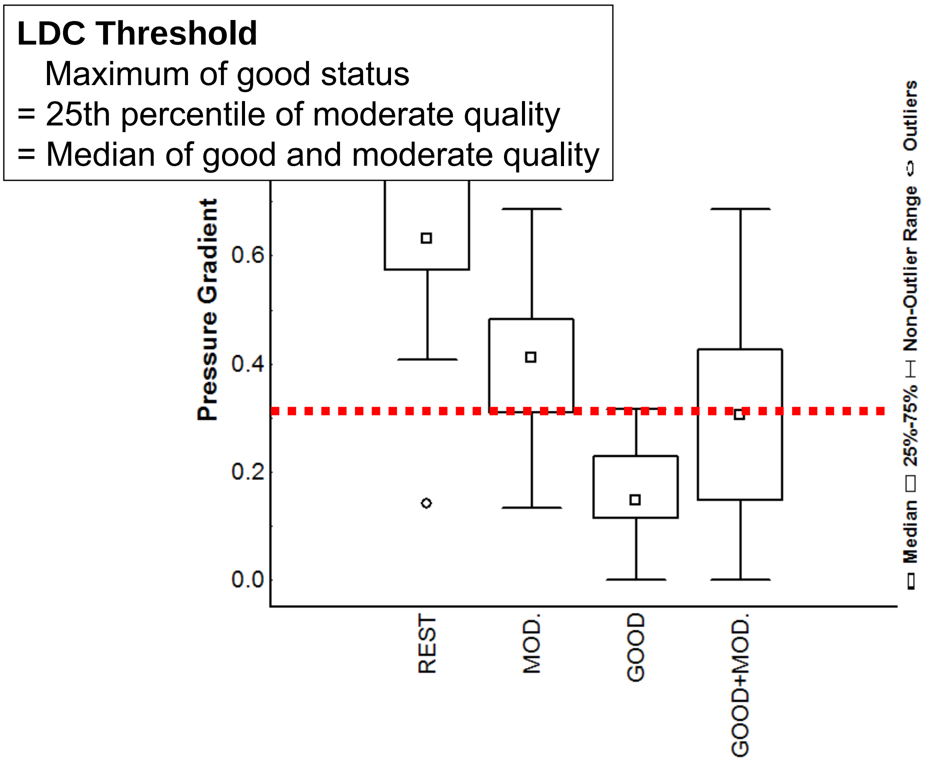
(Stoddard et al. 2006)

Concept to define large river reference used by
Blocksom, K. & B. R. Johnson, 2009. Development of a regional macroinvertebrate index for large river bioassessment. Ecological Indicators 9: 313-328.

TNMN classification including Hydromorph. Quality

	NH_4-N	NO_2-N	NO_3-N	TP	PO_4-P	DO	HYMO (mean)
GOOD	≤ 0.3	≤ 0.06	≤ 3	≤ 0.2	≤ 0.1	≥ 6	≤ 2
MOD.	≤ 0.6	≤ 0.12	≤ 6	≤ 0.4	≤ 0.2	≥ 5	≤ 3

18 sites of good quality,
28 sites of moderate quality



LDC Threshold
Maximum of good status
= 25th percentile of moderate quality
= Median of good and moderate quality

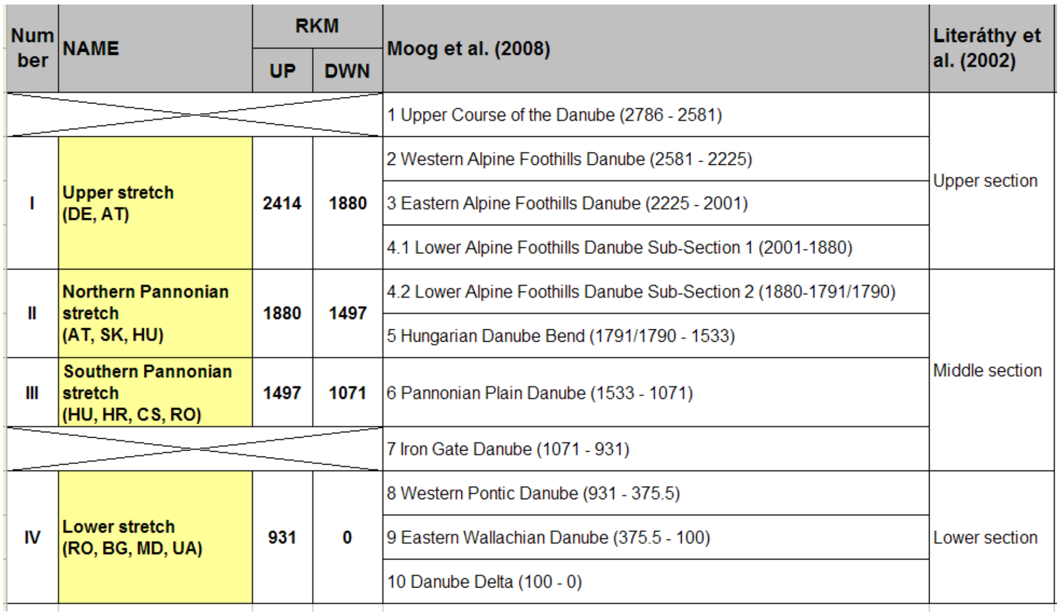
Distribution of pressure gradient among sites of different quality

III. TYPOLOGY

Danube Intercalibration Typology

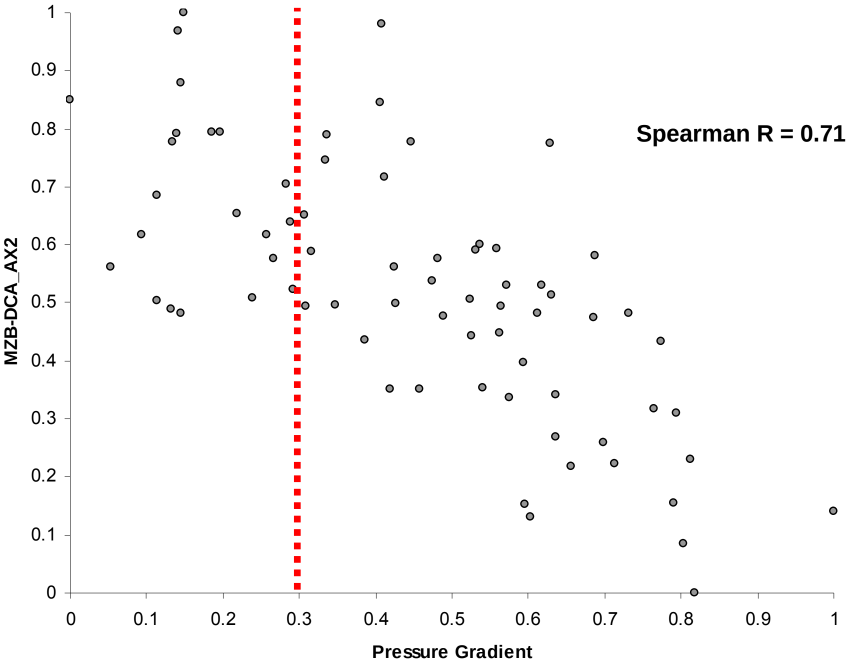
- Based on Danube Section Typology (Moog et al. 2008)
- Established for the intercalibration of the BQEs macrozoobenthos, macrophytes and diatoms, phytoplankton
- Investigation of JDS2 data (clustering, ordination, group difference testing (MRPP))
- Identification of characteristic species at sites of good and moderate quality (IndVal Analysis)

Danube Intercalibration Typology



Number	NAME	RKM		Moog et al. (2008)	Literáthy et al. (2002)
		UP	DWN		
				1 Upper Course of the Danube (2786 - 2581)	Upper section
I	Upper stretch (DE, AT)	2414	1880	2 Western Alpine Foothills Danube (2581 - 2225)	
				3 Eastern Alpine Foothills Danube (2225 - 2001)	
				4.1 Lower Alpine Foothills Danube Sub-Section 1 (2001-1880)	
II	Northern Pannonian stretch (AT, SK, HU)	1880	1497	4.2 Lower Alpine Foothills Danube Sub-Section 2 (1880-1791/1790)	Middle section
				5 Hungarian Danube Bend (1791/1790 - 1533)	
III	Southern Pannonian stretch (HU, HR, CS, RO)	1497	1071	6 Pannonian Plain Danube (1533 - 1071)	
				7 Iron Gate Danube (1071 - 931)	
IV	Lower stretch (RO, BG, MD, UA)	931	0	8 Western Pontic Danube (931 - 375.5)	Lower section
				9 Eastern Wallachian Danube (375.5 - 100)	
				10 Danube Delta (100 - 0)	

IV. MACROZOOBENTHOS



Pressure – Impact – Relationship