# Defining biological benchmarks for the intercalibration of the Danube River

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## 1 Introduction

One of the key elements of the European Water Framework Directive 2000/60/EC (WFD) is to ensure "good status" for all surface waters by 2015. The first step towards this aim was 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 International Commission for the Protection of the Danube River (ICPDR) recently published the first management plan (ICPDR 2009) according to the requirements of the WFD. To classify the ecological status of surface waters countries are using different biological assessment methods (Birk & Schmedtje 2005). Although these methods are based on common principles such as the use of Biological Quality Elements (BQE) and assessment against near-natural reference conditions, they differ in how the biological data are sampled, processed and evaluated. This poses the problem that status classification between countries in international basin management may be incomparable.

According to the WFD status classification of a water body depends on how much the biological community is deviating from undisturbed (reference) conditions. Obviously, in Europe's very large rivers undisturbed conditions no longer exist (Buijse et al. 2005; Van Looy et al. 2008). In this regard Birk & Hering (2009) developed an 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 reference as the result of transnational harmonization.

This paper is mainly based on data sampled during the second Joint Danube Survey (JDS2, Liska et al. 2008). Following an approach similar to Birk & Hering (2009), biological benchmarks are established for the Danube River. Secondly, we derive Danube Intercalibration Stretches based on existing typologies and biological data. Within each stretch we identify biological metrics that describe the macrophyte and macrozoobenthos communities and which can be used for the purpose of intercalibration.

## 2 Setting biological benchmarks and global definition of least disturbed conditions

The basis for our analysis were selected environmental parameters sampled at 78 sites in the main channel of the Danube River. The data included basic physico-chemical parameters, chemical quality elements and hydromorphological descriptors. We conducted a Principal Component Analysis (PCA) and extracted a complex gradient. We then identified sites in Least Disturbed Conditions (LDC; Stoddard et al. 2006), which serve as a basis for setting biological benchmarks.

Parameters reflecting the longitudinal river gradient (e.g. slope, average channel depth, average surface velocity and distance from mouth) appeared to be mostly correlated to PCA-axis 1. Parameters that are related to abiotic pressure (e.g. morphological evaluation scores, dissolved oxygen concentration, impoundment, naturalness of bank slope) determined PCA-axis 2. This second component was thus identified as the anthropogenic pressure gradient. It covers the whole length of the Danube River and reflects impairments, for instance, from major riparian cities or impoundments, as well as National Parks and other near-natural reaches (Figure 1).

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We then set a threshold value on the pressure gradient based on the TNMN water quality classification (e.g. ICPDR 2007), supplemented by including the overall hydromorphological quality of each site (Table 1). We identified 20 sampling sites in good and 26 in moderate abiotic status, with the majority of sites failing the class thresholds for hydromorphological quality. None of the sites would reach high abiotic status since none held high hydromorphological quality. The worst gradient score for sites in good abiotic status was taken as a threshold for the definition of LDC sites: all good status and seven moderate status sites were identified as such.

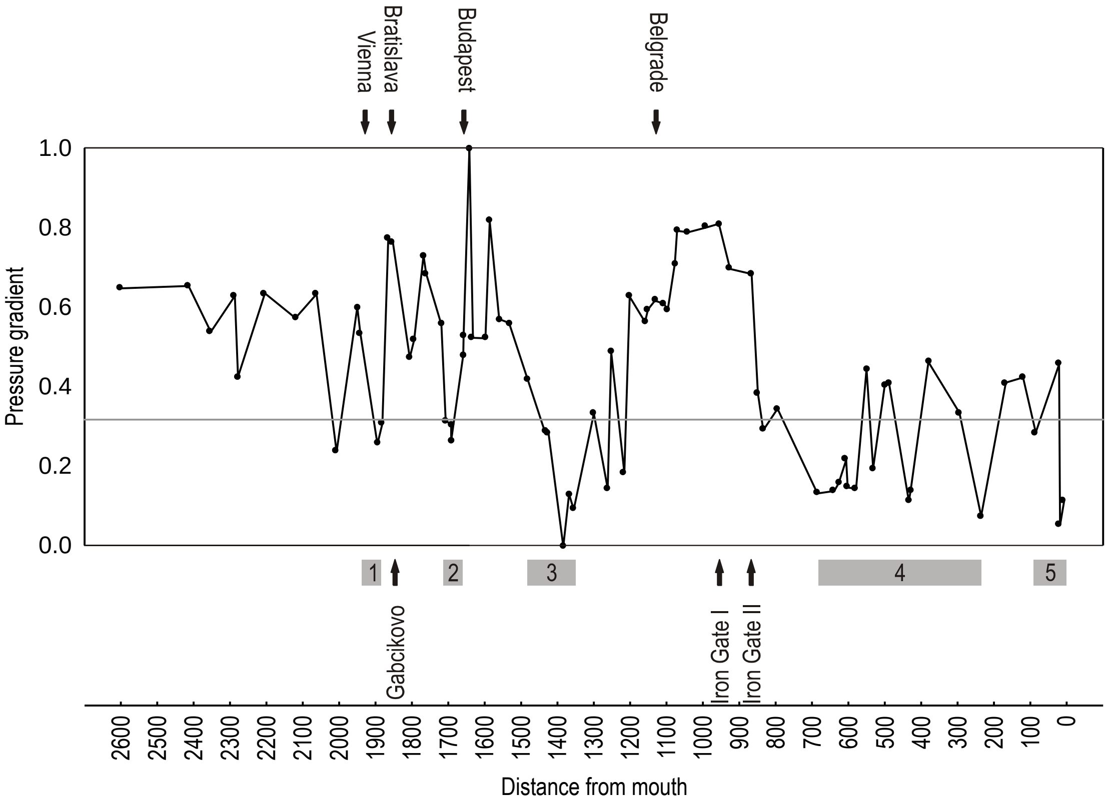


***Figure 1.*** *Pressure profile for the Danube River, specifying the locations of major cities, impoundments and important wetlands. The complex pressure gradient (including hydromorphology, impoundments, bank slope and $O_2$ concentration) is measured in values between 0 (near-natural) to 1 (severely impaired). The gray line represents the LDC-threshold, the worst gradient score for sites in good abiotic status. 1=Danube-Auen National Park; 2=Danube bend; 3=Duna Drava National Park; 4=Lower Danube Wetland System; 5=Danube Delta. Points indicate the sampling stations of JDS2 (Liska et al. 2008).*

***Table 1.*** *Abiotic classification scheme to identify sampling sites in good and moderate status ($N\text{-}NH_4$=Ammonium, $N\text{-}NO_2$ = Nitrite, $N\text{-}NO_3$ = Nitrate, TP = Total Phosphorus, $P\text{-}PO_4$ = Orthophosphate, DO = Dissolved Oxygen, HYMO = Overall hydromorphological quality class; all concentrations given in mg/l).*

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

Good abiotic status represents TNMN classes I and II, whilst moderate ecological status comprises class III. Sites failing these thresholds fall in classes IV and V (ICPDR 2007).

# 3 Delineating Danube Intercalibration Stretches

To establish the basis for the definition of Danube Intercalibration Stretches (DIS) we reviewed the studies on the Danube Section Types presented in Moog et al. (2008) and Litheráthy et al. (2002). 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 respected the JDS data (Liska et al. 2008), especially the cluster and ordination analyses of macrozoobenthos, macrophyte and diatom data, and the distribution of phytoplankton chlorophyll-a and biomass along the entire course of the Danube.

In addition, we carried out constraint cluster analysis for the biological data. By combining the information derived from these various sources we delineated the four intercalibration stretches shown in Figure 2. Three section types from Moog et al. (2008) are not included in the intercalibration typology (1: Upper Course of the Danube, 7: Iron Gate Danube, 10: Danube Delta). These reaches were either non-international parts of the Danube (Section 1), or featured 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).

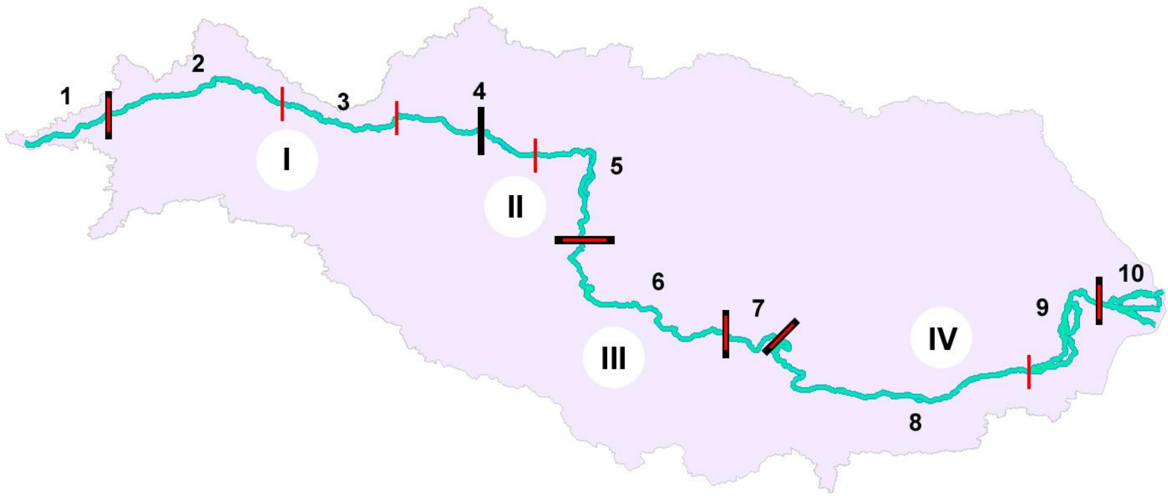


***Figure 2.*** *The location of the Danube Section Types (Moog et al. 2008) (Arabic numerals, red borders) and the Danube Intercalibration Stretches (DIS) (Roman numerals, black borders) on the River Basin District map. I = Upper DIS; II = Northern Pannonian DIS; III = Souther Pannonian DIS; IV = Lower Pannonian DIS. Danube Sections Types 1, 7 and 10 are not covered by the DIS delineation.*

# 4 Testing the biological response to the pressure gradient

We used the macrophyte and macrozoobenthos data sampled during JDS2 to describe the assemblage patterns via Detrended Correspondence Analysis (DCA) and applied 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 are significantly different between LDC and non-LCD sites for each stretch.

***Table 2.*** *Selected macrozoobenthos metrics for the different Danube Intercalibration Stretches, the groups for which they are indicative (highest median value) and the number of sites (LDC and non-LDC) within the stretch. All median values between groups are significantly different (U-test, p<0.05).*

| DIS | Biological Metric | Indicative for | Number of sites |
|---|---|---|---|
| All stretches combined | LIFE (Lotic-Invertebrate Flow Evaluation) | LDC | 24 LDC<br>45 non-LDC |
| | German Saprobic Index | non-LDC | |
| I | % Grazers and Scrapers | LDC | 3 LDC<br>9 non-LDC |
| | % Gravel-prefering | LDC | |
| | % Littoral-prefering | non-LDC | |
| | Austrian Saprobic Index | non-LDC | |
| II | % Rheophilous | LDC | 3 LDC<br>15 non-LDC |
| | LIFE (Lotic-Invertebrate Flow Evaluation) | LDC | |
| | GOLD (Gastropoda-OLigochaeta and Diptera) | LDC | |
| III | Czech ASPT (Average Score Per Taxon; Armitage et al. 1983) | LDC | 7 LDC<br>10 non-LDC |
| | % Oligochaeta | non-LDC | |
| | Oligochaeta (number of individuals) | non-LDC | |
| | German Saprobic Index | non-LDC | |
| IV | LIFE (Lotic-Invertebrate Flow Evaluation) | LDC | 11 LDC<br>11 non-LDC |
| | GOLD (Gastropoda-OLigochaeta and Diptera) | LDC | |
| | % Mud-preferring | non-LDC | |

Several biological metrics are reflecting these differences on the stretch-specific level for the macrozoobenthos community (Table 2). These include the Lotic-Invertebrate Flow Evaluation (LIFE; Extence et al. 1999) for all stretches, DIS II and IV, and the percentage of rheophilous taxa in DIS II, both indicating higher current velocities at less disturbed sites. Two saprobic indices (German and Austrian), scoring higher with increasing organic pollution, show higher values at non-LDC sites in several stretches. The Portuguese GOLD index (Buffagni et al. 2005) that decreases with higher proportions of Gastropoda, Oligochaeta and Diptera abundances, shows lower values at non-LDC sites for DIS II and IV. A similar indication is given by the Oligochaeta abundance and number of taxa in DIS III.

For the macrophytes only the Austrian Index for Macrophytes AIM (Pall & Mayerhofer 2009) was responsive to the pressure gradient in the Upper DIS. We deduced a number macrophyte indicators from a correlation analysis of taxa abundance with the pressure gradient, 13 of which are in DIS III (Table 3).

***Table 3.*** *Macrophyte taxa significantly correlated to the pressure gradient (p<0.05), including the Danube Intercalibration Stretch (DIS) and the groups for which they are indicative (negative correlation to the pressure gradient = for LDC; positive = non-LDC).*

| DIS | Taxon | Indicative for | DIS | Taxon | Indicative for |
|---|---|---|---|---|---|
| I | *Cinclidotus fontinaloides* | LDC | III | *Phragmites australis* | non-LDC |
| I | *Fontinalis antipyretica* | non-LDC | III | *Potamogeton gramineus* | non-LDC |
| I | *Rhynchostegium riparioides* | non-LDC | III | *Potamogeton nodosus* | non-LDC |
| II | *Phragmites australis* | non-LDC | III | *Potamogeton perfoliatus* | non-LDC |
| III | *Azolla filiculoides* | non-LDC | III | *Sagittaria sagittifolia* | non-LDC |
| III | *Ceratophyllum demersum* | non-LDC | III | *Salvinia natans* | non-LDC |
| III | *Lemna gibba* | non-LDC | III | *Vallisneria spiralis* | non-LDC |
| III | *Lemna minor* | non-LDC | IV | *Bidens sp.* | non-LDC |
| III | *Myriophyllum spicatum* | non-LDC | IV | *Scirpus lacustris* | non-LDC |
| III | *Najas marina (N. major)* | non-LDC | IV | *Tamarix ramosissima* | LDC |

# 5 Conclusions and outlook

The homogenous nature of the JDS-data allowed for the development of a common approach to identify complex pressure gradients and fix thresholds to define least disturbed conditions. The general aim is to discover relationships between human pressure and biological status, and then to describe the biology under defined conditions. It sets the basis for intercalibration of the Danube River and the methodology can be extrapolated to other large rivers.

We fixed the LDC threshold on the pressure gradient that represents a complex combination of individual pressures at the sampling sites. 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 (Table 1). The mean width of the riparian corridor amounts to approximately 2.1 km, and at least some large woody debris is present at most sites. On average, both banks feature natural slopes and the immediate vicinity of most sites is dominated by natural land cover (riparian vegetation, floodplain forest).

Our DIS 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. The distinct biological features of each DIS and those of other rivers seem to require defining biological benchmarks for intercalibration on a stretch- and river-specific basis.

The pressure-response analysis reveals an obvious relation of the macrozoobenthos and macrophyte communities to the abiotic pressure gradient. The strong correlations of the macrozoobenthos fauna with the pressure gradient can be reviewed by various aspects of, and metrics that describe the community. The stretch-specific analysis reveals that each stretch represents a particular 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. Following Birk & Hering (2009) biological benchmarks can be set using selected summary statistics for the metric distributions at LDC sites. For example, the distribution of the German Saprobic Index values shows good status for the majority of LDC sites.

For the macrophyte community only the Austrian Index for Macrophytes AIM was found to be significant for the upper DIS. However, we deduced a number of indicator species, which can serve as a basis for further development of metrics for the Southern Pannonian DIS. 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), for a more detailed description of least disturbed conditions the number of samples from LDC sites could be enlarged.


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