JDS4: Biology and indication of ecological status

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With the data from Joint Danube Survey 4 (JDS4) for biological quality elements values for the **indication of the ecological status for the sampling sites** were estimated. Those results are not approved ecological status assessments for water bodies on national level as not all required WFD criteria could be met by the JDS design (e.g. not considering aspects regarding representative site selection, choice of sampling time in relation to season and discharge, selection of assessment indices). Additionally many water bodies of the Danube are designated as Heavily Modified Water Bodies – for them, on a binding national level other legal objectives, summarized in the ecological potential, come into place and replace the ecological status.

Aquatic macroinvertebrates

The sediment inhabiting animals of the biological quality element macrozoobenthos, the aquatic macroinvertebrates, are indicators for oxygen depletion due to pollution by degradable organic substances (Index: SI, saprobic index) as well as for general habitat degradation (index: SK MMI, multi-metric index used in Slovakia). The results of saprobic index analyses show that organic pollution is a local problem, because 81% of sites (67% of samples) show an indication of good or high status. As also known from past surveys and TNMN (Transnational Monitoring Network of the ICPDR) data the indication of good and high status decreases downstream – 91% of sites in the Upper Danube, 80% in the Middle Danube and 67% in the Lower Danube. The multi-metric index shows a different picture: only 37% of the sites reach an indication of good status, pointing at hydromorphological deficits caused by a variety of pressures.

Figure 1: MZB sampling at site with gravel in Upper Danube (© F. Wagner)

Fish

Most species of the reference communities can still be found at nearly all sites, even at hydromorphologically strongly altered stretches. Hence, the diversity of aquatic habitats is still present in an extent to allow species to survive. However, the indication of ecological status for fish is pointing towards a failing status for a majority of the sites in the Danube. Several indices were used by the experts and all of them show the deficits of the fish community caused by hydromorphological pressures (good status according to FIS (Fish Index Slovakia): 11% of sites, EFI (European Fish Index): 23%, FIA (Fish Index Austria): 25%, see contribution of Pont et al. this issue). Those indices were not developed and are not suitable for the whole length of the Danube, however, the national assessments with the same data also show corresponding low 17% of the sites reaching the objective of good status.

Phytobenthos

The indicative status of benthic diatoms (index: Slovakian IPS - Indice de Polluosensibilité Specifique, Specific Pol-

Figure 2: MZB sampling in Lower Danube (© M. Paunovic)

lution Sensitivity Index) decreased from the Upper Danube towards the mouth. In the Upper Danube 61% of the sites indicate good status, in the middle section of the Danube 20% of the sites and in the Lower Danube none. However, it should be noted that results from national assessment of the JDS4 data differ essentially from this indicative assessment, especially for the Lower Danube. Additionally, nutrient levels do not reflect the differences in phytobenthos assessment – diatoms are used particularly as indicators for nutrient pollution.

Macrophytes

Water plants are well known indicators for hydromorphological alterations. The abundance of floating macrophytes in the middle and lower reach of the Danube River suggests good lateral connectivity to backwaters. Just like three Joint Danube Surveys before, the results demonstrate that in certain river stretches there is naturally a lack of microhabitats with proper conditions for the successful growth of macrophytes. This causes almost plant-free river parts without macrophytes or with insignificant abundance – making the assessment difficult to impossible. Based on the comparison of outcomes of previous Joint Danube Surveys, the composition of macrophytes is stable in terms of richness and diversity over several years.

Phytoplankton

In contrast to previous Joint Danube Surveys, when only one sample per site was taken, during JDS4 samples were collected monthly from April to September enabling an assessment of the ecological status according to the methodology guidelines of the member states. Thus, instead of Chlorophyll a (after TNMN methodology) this time the national indication of the status was used. However, the results are similar to previous investigations – 92% of sites show high or good ecological status, only two sites were classified as indication for moderate status.

Is the ecological status of the Danube improving?

From the biological results of JDS4 we have the impression that the ecological status of the Danube is at least at some locations improving, which might be a consequence of mitigation measures of the past years. However, also deterioration can be observed. This is in line with the findings of hydromorphology experts who pointed out that both improvements but also slight deteriorations took place in recent years. Details can be found in the contribution of Schwarz (this issue) and in the final scientific JDS4 report.

Invasive alien species

The Danube River and the main tributaries are under considerable influence of biological invasions. Data from the biological groups demonstrate that the number of recorded

Figure 3: Dikerogammarus villosus in the Upper Danube (© W. Graf)

alien species revealed is lower in the Lower Danube in comparison to Upper and Middle Danube, since the Lower Danube can be considered as native habitat of some animals and plants that are classified as aliens in the more upstream located areas. The comparison with JDS3 data reveals that the rise of the invasive alien species is progressing.

Regarding macrozoobenthos at some sampling sites invasive alien species reach extremely high abundances. For example, in the upstream reaches of the Danube the genera Dikerogammarus sp. and Echinogammarus sp. accounted for 99% of species diversity and biomass. The invasive crayfish *Faxonius limosus* was present along the entire Danube, with larger abundance in Lower Danube. For the future, a critical adaptation of indicator values for some of those species is therefore necessary.

However, like all biological systems, the distribution and abundance patterns of alien species are also highly dynamic. For example, the Asian clam *Corbicula fluminea,* first found in the lower Hungarian Danube in 1998, was detected in high densities during JDS3, but was detected only in low densities during JDS4.

Figure 4: Sampling fine sediment for DNA analysis of sediment inhabiting invertebrates (© F. Wagner)

Future of ecological assessment: (e)DNA-based tools

Within the scientific program of JDS4 molecular methods using DNA and environmental DNA (eDNA) for the identification of species (and higher taxonomical groups) were applied for the first time at the scale of an international river basin. A variety of different sample types was used for testing scientific approaches and to evaluate the applied performance of the molecular methods, but also a comparison concerning the applicability of (e)DNA methods for WFD status assessment was done.

Fish experts used intercalibration common metrics for ecological assessment of sites with data from classical fish survey and from eDNA analysis. For 46% of the sites they found the same status class and for 70% of the sites the final classification of reaching or failing the WFD objective of good status was identical.

For benthic invertebrates, the sites were compared by using the Austrian SI (saprobic index) and MMI (multi-metric-index). Both indices were calculated with species data originating from classical MHS sampling (multi-habitat-sampling), DNA from bulk samples (like classical samples – all material mixed together) and DNA from preservation liquid (alcohol extracted from the bulk samples). A comparison was done by using abundance data but also presence and absence of species for classical samples (DNA methods did not deliver abundance estimates but presence/absence-values). Accordance of the status class assessment is high for the SI between classical samples and preservation liquid (62%) and even higher between classical samples and bulk samples (66%). The accordance increases to over 80% when using presence/absence data for classical samples. This difference shows that the use of exact abundance data may account for information that is not given when using presence/absence information. For the MMI the identical status classes identified by the three different methods is few percent lower but follows the same pattern as described above for the SI.

For the information if the site reaches or fails the quality objective of the WFD – the good ecological status – the accordance between classical sampling and molecular methods is even higher and reaches up to 93%.

For three sampling sites the indicative status for benthic invertebrates based on the Austrian indices SI and MMI was calculated for the above mentioned sample types and additionally for eDNA from water samples. The results are astonishingly close together and when looking at the index values they are even closer.

These results demonstrate the high potential of DNA-methods for ecological assessment – especially taking into consideration that this was a test only and for sound status assessment adaptations of the assessment method would be necessary (e.g. reference values, performance of metrics).

For more details see the final scientific JDS4-report at: http://www.danubesurvey.org/jds4/publications/scientific-report

Invasive alien species of macroinvertebrates along the Danube River – JDS4 screening

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Introduction

Several international Danube surveys have proven that invasive alien species (IAS) have a profound influence on native biodiversity of the Danube River Basin (DRB) (Zorić et al. 2014, 2015, Borza et al. 2015, ICPDR 2015, Csányi et al. 2021, Trichkova et al. 2021). The Danube River connects the Black Sea Basin to Western Europe as dominant water route of the 'Southern Invasion Corridor', forming the European Invasion Network (Panov et al. 2009). The spread and expansion of IAS can happen in both directions: upstream and downstream. According to the origin of invasive species, some of them are alien to Europe, others are native to Europe (outside the Ponto-Caspian region), while significant share of these taxa has Ponto-Caspian origin. The latter are in immediate hydrological connection with their native area. Several species of macroinvertebrates (mainly belonging to the crustaceans) and fish (Gobiidae) expanded their range from the Black Sea area and the Lower Danube to the Middle and Upper Danube River during the last decades and appeared in new habitats, even as far as Western European rivers (Bij de Vaate et al. 2002). Considering the importance of IAS in terms of the implementation of the Water Framework Directive, a specific IAS program has been developed and implemented during Joint Danube Survey 4 (JDS4) at regional and national levels (Csányi et al. 2021, Trichkova et al. 2021). The evaluation of the dataset collected during the survey is described here with special attention to the distribution of macroinvertebrate species alien to the Danube River Basin.