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THE AQUATIC MACROINVERTEBRATE COMMUNITY OF THE RIVER DANUBE BETWEEN KLOSTERNEUBURG (1942 RKM) AND CALAFAT - VIDIN (795 RKM)

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A DUNA MAKROGERINCTELEN EGYÜTTESE KLOSTERNEUBURG (1942 FKM) ÉS CALAFAT - VIDIN (795 FKM) KÖZÖTT

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KIVONAT: A vízi makrogerinctelen élőlény-együttest tanulmányozták az AQUATERRA program során a Duna Klosterneuburg (Austria, 1942 fkm) és Vidin-Calafat (Bulgária-Románia, 795 fkm) 2004. augusztus 19 és szeptember 4 között. A vizsgálatok a vízi élőlény-együttes és a Duna szerves és szervetlen mikroszennyezőinek kapcsolatára irányultak, a szárazföld felől bemosódó talaj, a folyami üledék és a víz-fázis párhuzamos felmérésével. Jelen dolgozat a vízi gerinctelenek legjellemzőbb fajainak hossz-szelvény menti elterjedését elemzi, valamint az alkalmazott három mintavételi módszer hatékonyságát is értékeli. Végül az AQEM szoftwer alkalmazásával számított néhány metrika segítségével a Duna biológiai állapotát jellemzi. Összesen 89 taxon sikerült e szakaszról kimutatni, az élőlény-együttes legdominánsabb rendszertani csoportját a puhatestűek jelentik 35 előkerült taxonnal. A vizsgálati eredmények alapján néhány jellegzetes dunai szervezet hosszirányú elterjedése alapján a Duna mentén jellegzetes szakaszokat lehet kijelölni.

ABSTRACT: Macroinvertebrate samples were collected during the AQUATERRA program on the River Danube between Klosterneuburg (Austria, 1942 river km) and Vidin-Calafat (Bulgaria-Romania, 795 river km) between 19 August and 04 September 2004. The major aim of the study was to investigate aquatic biota together with the interactions of organic and inorganic micropollutants in land originated soil, sediment and water. The present paper summarises the spatial distribution of some dominant member of the aquatic macroinvertebrate community. A comparison of different sampling methods is presented according to their efficiency. Using the AQEM software several metrics are calculated in order to evaluate the biological water quality of this Danube section. Altogether 89 taxa were detected in this Danube section. The most dominant group of the community is the Mollusca with 35 species. The distribution of some Danube specific taxa indicates different identical sections of the Danube stretch.

Key words: aquatic macroinvertebrates, taxon distribution, kicking, dredging, grabbing, biological water quality.

Introduction

The AQUATERRA Sampling Crew performed a detailed sampling program on the River Danube between Klosterneuburg (Austria, 1942 river km) and Vidin-Calafat (Bulgaria-Romania, 795 river km) in co-operation with the Secretariat of the ICPDR, Vienna. Samples from different matrices, such as water, sediment and biota were collected. The major aim of the Study was to investigate aquatic biota together with the interactions of organic and inorganic micropollutants in land originated soil, sediment and water in order to determine special hazardous hot-spots in this Danube reach. The Sampling Core Team with the support of local authorities carried out the sampling mission between 19 August – 04 September 2004. The present paper summarises the taxonomic results of the aquatic macroinvertebrate community, compares different sampling methods according to their efficiency and evaluates the biological water quality of this Danube section. Due to spatial limitations the analysis of the Danube Typology based on the macroinvertebrate community structure will be realised in another paper.

Material and method

Altogether three different sampling methods were applied for the collection of the macroinvertebrate samples during the AQUATERRA mission on the investigated Danube stretch.

Kicking with the British FBA pond net having 950 µm mesh size was used in the littoral zone of each cross section at the right and the left side, respectively. The dominant habitats were taken into consideration along an approximately 50-100 m stretch as a representative site after approaching the site by motor boat and the visual observation of the river locality. Semi-quantitative data were collected following the same amount of effort and time approach during the mission. Kicking was carried out in a rubber cloth near the shoreline in the water with 1.5 m depth. The kick and sweep technique was done at all investigated Danube stretch, between Aquaterra Danube Survey (ADS) sites 1 and 30. **Diving** was also applied during kick sampling as the most effective way of mussel collection at most of the sites, even in case of smaller depths.

Dredging in the littoral and deep water was performed from motorboat. This sampling method was applied only at the first and the second sampling cross section (ADS 1-2). The further use of the dredge was not possible due to the lacking time and the dense sampling program. The dredge had triangle shaped mouth with forked frame. The mesh size of the inside net was 500 µm. The dredge was pulled with a rope with 20 m length along approximately 10 m distance.

Grabbing was performed using the hydraulic polyp grab of the ARGUS vessel. This sampling method was done between the ADS 1 and 18 cross section of the Danube because after this stretch on the Lower Danube it was difficult to find large stones on the bank for grabbing. The grabbed stones were lifted up to the board and were washed carefully. The animals and solid debris were collected on a sieve having 500 µm mesh size.

Macroinvertebrate samples were labeled and than preserved with 70% ethanol solution. Selecting the taxa was carried out in laboratory. The taxonomic determination of *Oligochaeta* was done by M. PAUNOVIC (Belgrade), *Chironomidae* species were taken by the VUVH (Bratislava), Crustaceans were identified by professor S. KARAMAN, other groups were determined by the author.

The use of the AQEM software provided several metrics, the German lowland river type was taken into consideration for the calculation.

Results

Analysis of taxon distribution

Distribution of main taxonomic groups

The evaluation of the macroinvertebrate community is based on the results of the kick samples. Data referring to right and th left sampling locations were added, so a summarised picture is seen on Figure 1. The range of total number of macroinvertebrate taxa detected at the different 30 cross sections varies between 9 and 36. There are characteristic sections with lower and higher values on this Danube stretch.

Altogether three decreasing and subsequent three increasing series of taxon numbers can be recognised on the diagram. The first continuous decrease is occurring from Klosterneuburg to the final site of Gabčikovo Reservoire. Klosterneuburg, Wildungsmauer and Hainburg are relatively rich in macroinvertebrates because the total number of taxa is above 20. The following increase can be recognised after the Gabčikovo Reservoir and lasts until the downstream section of Budapest where the highest taxon number was experienced (36). Generally it can be concluded that Szob, upstream and downstream Budapest are the most taxon rich sections of the investigated Danube. In this respect two other localities are similar to those. The site downstream Velika Morava and the other one at Banatska Palanka/Bazias, both on the lower Danube have 30 and 26 taxa, respectively.

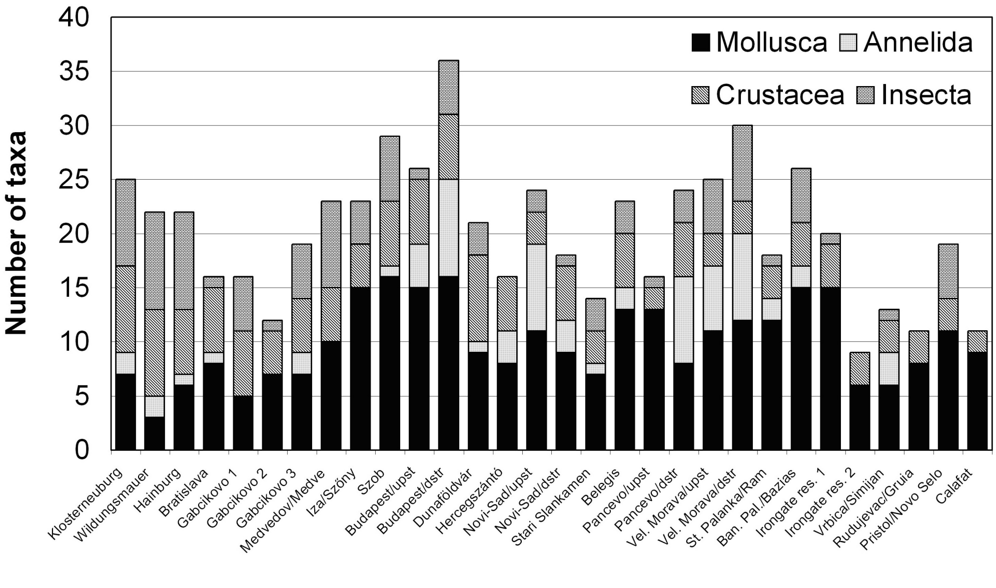


Figure 1. Number of macroinvertebrate taxa in main taxonomic groups along the Danube between 1942 and 795 rkm.

According to the diagram The relatively poorer section (taxon numbers lower than 20) is characteristic from Golubac/Koronin until Vidin/Calafat. It is very difficult to recognise direct relationship between large point pollution sources and the taxon richness/taxon depletion because the hydromorphological conditions and habitat diversity is predominantly important for the community also.

The decrease of the number of taxa is not always an instantaneous phenomenon. There was an increase below Budapest where the complete mixing occurs far away downstream of the capital. The number of taxa at Dunaföldvár (21) is not as small as at Hercegszántó (16) where more than 60 % of decrease is seen. Similarly, the numbers detected upstream Novi Sad and Beograd are bigger than at downstream where a significant decrease is evident. Above the cities 24 and 23 taxa were detected, respectively, and the number decreased to 18 and 16, in this order.

The further analysis will show an interesting consequence in the compositional change as far as different taxonomic groups are concerned. The richest Danubian macroinvertebrates belong to the Mollusca group (Figure 2). The maximum diversity of snails and mussels was observed on the section between Szőny/Iza and downstream Budapest, respectively. Another stretch with similar diversity conditions is situated between the upstream Sava section and the Golubac/Koronin section that is situated in the Iron Gate Reservoir characterised by elevated water level.

There is evidently clear that the number of Malacostraca and Insecta taxa is bigger on the Upper Danube. The number of Annelida taxa increases sharply in the vicinity of some large cities. This means that downstream Budapest, upstream Novi Sad, downstream Pancevo and around the tributary of Velika Morava the worm species play significant role in the macroinvertebrate community.

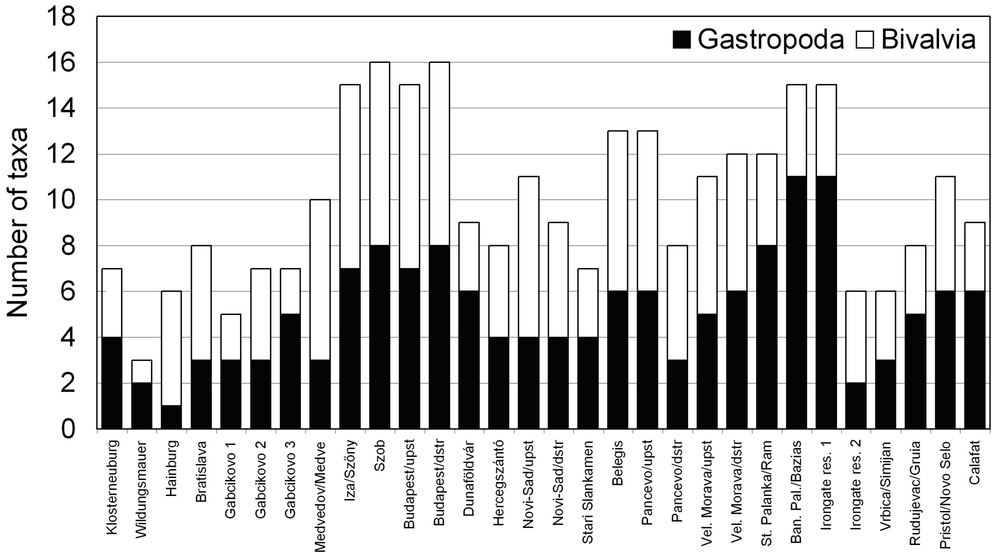


Figure 2. Number of snail and mussel taxa along the Danube between 1942 and 795 rkm.

The distribution of aquatic insects has different picture (Figure 3). Most of them belong to the Chironomidae group. The upper Danube is relatively rich in insects, Medve, Szob and below Budapest have the biggest taxon numbers. Smaller values were detected in the Gabčikovo Reservoir and from Dunaföldvár only few species are detected on the middle and lower Danube, as well. The fauna of the Iron Gate Reservoir contains several stagnant water taxa. The free flowing Pristol/Novo Selo section is characterised by Chironomidae only.

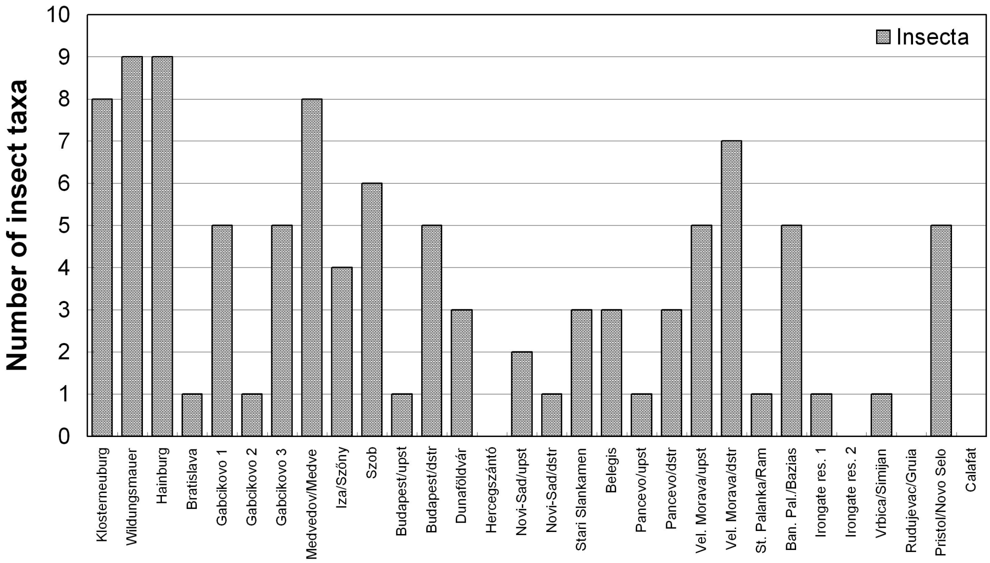
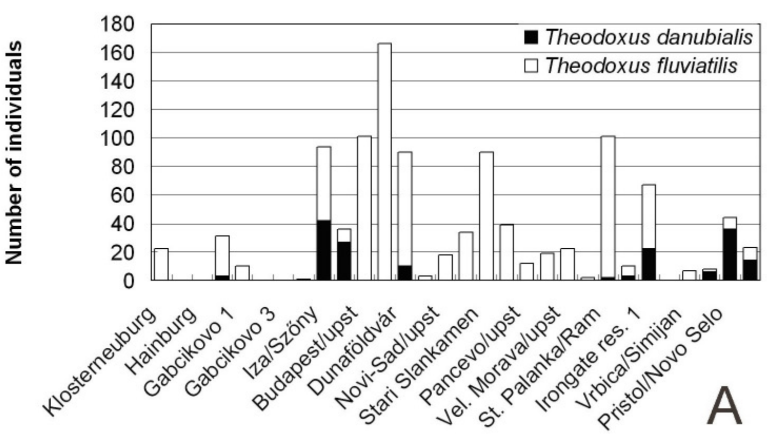


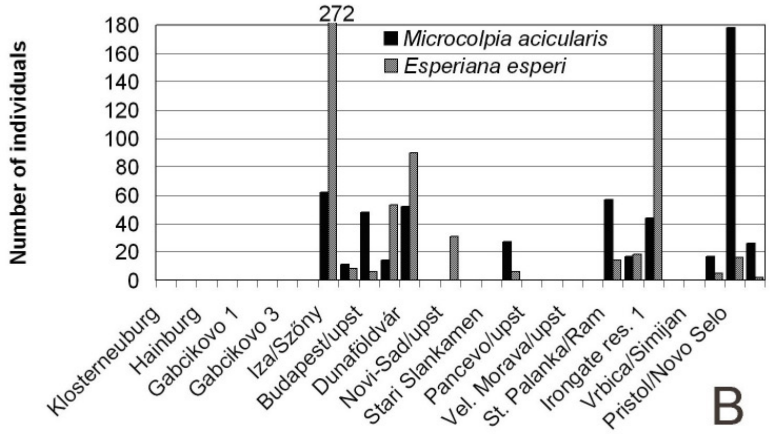
Figure 3. Number of Insecta taxa along the Danube between 1942 and 795 rkm.

Species distribution along the Danube

The most frequent species of the Danube River are coming from the group of Mollusca and the Crustacea, especially Malacostraca. First the distribution of the snail taxa has to be discussed and after that the data on the Malacostraca species will be introduced.

Regarding to the most characteristic snails of the Danube River (Figure 4) the two *Theodoxus* species have to be mentioned from this water body. The most common one is *Theodoxus fluviatilis* that was detected first in the Danube River in the middle of 80ies. At that time this aquatic Neritidae species was known in the Hungarian water shed only in few locations along the River Tisza. A considerable population was discovered in the Danube in 1987 living just around the Budapest section. New localities were revealed in the next years in Gönyü (1991), in Rajka (1992, dredging in the middle river bed) (CSÁNYI 1996). The species was distributed along the entire Budapest stretch of the river in the beginning of the 90ies, and it occurred in Hungary at Százhalombatta, Adony and Dunaföldvár, as well. Comparing to the previous distribution data *Theodoxus fluviatilis* seems to be very common along the investigated Danube stretch according to our observation nowadays. There are peaks in the individual number in the vicinity of Budapest, at the Tisza confluence and around Bazias. This snail species tolerates well the organic pollution of the Danube River. Abundant populations are registered at the lower stretch from Novi Sad and around Belgrade, too.





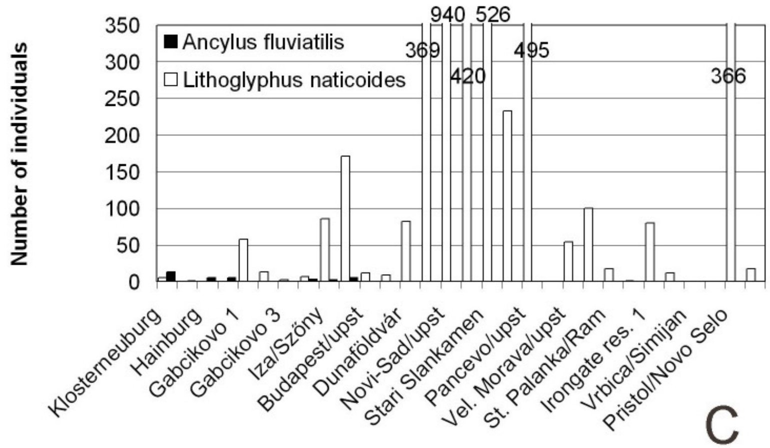


Figure 4. Abundance of aquatic snails along the Danube between 1942 and 795 rkm. (A: *Theodoxus danubialis, T. fluviatilis;* B: *Microcolpia acicularis, Esperiana esperi;* C: *Lithoglyphus naticoides, Ancylus fluviatilis*)

Figure 4A suggests that the strictly Danubian *T. danubialis* is not so tolerant species as *T. fluviatilis* because it is more rare in the whole Danube section. Although it was detected at Bratislava, the taxon is rare in the Upper Danube. The Danube between Iza/Szőny and Szob is the most characteristic section of the distribution of *T. danubialis* in the investigated Danube stretch. Significant populations are present in Dunaföldvár, in the Iron Gate (especially at Golubac/Koronin) and the lower Danube at Pristol/Novo Selo where this species is again one of the most abundant member of the Danubian macroinvertebrate community.

An interesting morphological problem has to be mentioned in case of this two Theodoxus species. Several *Theodoxus fluviatilis* specimens collected during the AQUATERRA mission have very similar zick-zack pattern on their shells to the well known drawing pattern of *T. danubialis*. Therefore it is difficult to make the exact taxonomic determination in several occasions. The solution for that problem is to look for the opercular coloration of the snails. In case of the *T. danubialis* the operculum has light yellow colour whereas the *T. fluviatilis* is characterised by stronger coloured lamellae by black and reddish-brownish pattern in the middle part. Another possible key for the distinguishing of the two species is that the zick-zack drawing is a little bit denser and the black lines are narrower in case of *T. fluviatilis*.

There are two other snails that both are strictly Danubian species (*Esperiana esperi, Microcolpia acicularis*) and that were found almost in all cases (at twelve sampling sections) together (Figure 4B). Both of them are missing upstream Iza/Szőny (downstream Vagh River) but starting from this location to downstream direction they are frequently found along the river. *Esperiana esperi* seems to be more tolerant species than the other one because it is very common along the sections that have significant and regular organic pollution. This kind of sites is Iza/Szőny that is influenced by the cities of Komarno/Komárom. The overall maximum of the individual number of *Esperiana esperi* was detected here (272 ind./sample). Similar high value was found in the middle Iron Gate section (Golubac/Koronin) that is situated upstream the Kazan Pass. Finally, the sections just below Budapest and Dunaföldvár characterised also by a strong *E. esperi* population.

Microcolpia acicularis has a similar distribution, being absent in the upstream part of the study section. Its massive occurrence was detected at several sites along the study area (Iza/Szőny, upstream Budapest, Dunaföldvár, Stara Palanka/Ram, Golubac/Koronin). The larges amount of this Tiaridae species was registered in the lower end of the stretch (Pristol/Novo Selo). This is that site where the *Theodoxus danubialis* is the other character species of the Danube River.

Interesting behavioural differences during flooding were observed between the two species in the Komárom section in June 1996 (unpublished data of CSÁNYI). Large amount of dead shells of *Esperiana esperi* was collected that time well above the actual small water level. The large number of dead snails indicated that this species moved up to higher places very fast during higher water levels such as flooding. In contrast, *Microcolpia acicularis* specimens were present only 80 cm below the water level demonstrating that this species does not change its position like the previous one. Beside of morphological similarities this observation on behavioural differences indicates that there might be well recognisable differences in the life strategy of these animals.

The result of the distribution pattern of the third pair of snails is presented. Two species having sharply different character are illustrated in Figure 4C. *Ancylus fluviatilis* as a specific rheophilous species is found only on the upper Danube between Klosterneuburg and Szob in relatively very low individual number. *Lithoglyphus naticoides* is the snail that prefers to inhabit the fine sediment surface of the slow flowing sections. Therefore it is clear why the maximum sized populations are distributed between Hercegszántó and upstream Pancevo. The number of individuals of the snails was considerable high on this section. It varies generally between 200 and 400 per sample, having the two maximums in the lowest Hungarian section (940 ssp. at Hercegszántó) and at the Tisza confluence (526 ssp. at Stary Slankamen). Upstream Pancevo this value was still high (almost 500 ssp.).

The number of specimens in the Iron Gate section never reached those high values mentioned at upstream. The free flowing, relatively faster Pristol/Novo Selo stretch has high individual numbers again.

It is very interesting that this species was totally absent from the section downstream Pancevo during the AQUATERRA mission. The vicinity of the large pollution sources of the petrochemical industry is heavily effected by oil spills that can be recognised in the thick sediment layer at several places.

The Crustacea group, especially Malacostraca taxa are the most abundant macroinvertebrates in several places along the Danube, as far as their biomass and frequencies are concerned (ICPDR (2002). Altogether there were twelve taxa collected during the AQUATERRA mission. Seven Amphipoda were determined among the Crustacean group members that are the most common organisms along the Danube River.

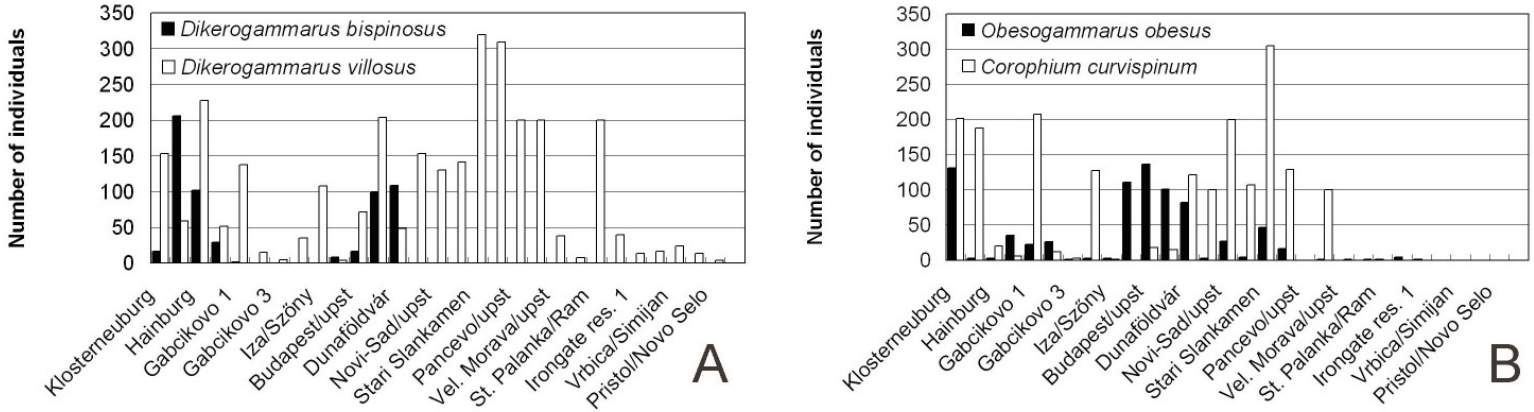
The species *Dikerogammarus villosus* is the example of that taxon that is wide spread, living on both the Middle and the Lower Danube section (Figure 5A). There are two well recognisable sections where they are abundant: at the Upper stretch above Gabčikovo Reservoir, and, from the Hungarian Danube until the Iron Gate Reservoir. *Dikerogammarus bispinosus* is sharply restricted to the Upper Danube except the Danube stretch of the Slovakian Water Barrage System. *Obesogammarus obesus* distributes mainly the upper Danube also (Figure 5B). There are only sporadic presence data of the species form the lower stretch.

Corophium curvispinum inhabits the same Danube section but in a bigger individual number. The lowermost section of the detection was at Pancevo (downstream). No occurrence data were registered in the Iron Gate Reservoir and below that.

The Danubian distribution of two Crustaceans species having entirely different ecological requirements is shown on Figure 5C. The lotic *Jaera istri* is strictly limited to the upper half of the river stretch until the lowest Hungarian site, Hercegszántó. Large individual numbers exceeding 100 i/sample were registered on the Austrian section and at Iza/Szőny. This animal was missing from the Gabčikovo stretch.

The Mysididae species, *Limnomysis benedeni* is a typical lenitic taxon that was found in large numbers in the Gabčikovo Reservoir and on the entire Lower Danube impounded by the Iron Gate Reservoir. Small individual numbers were registered between the Slovakian reservoir and Calafat at many sites but these large abiundance conditions are typical to the slow flowing/stagnant water stretches.

Due to the limited extension of this paper the spatial distribution of other taxa is not discussed her but further discussion is given concerning the comparison of the efficiencies relating to different sampling methods.



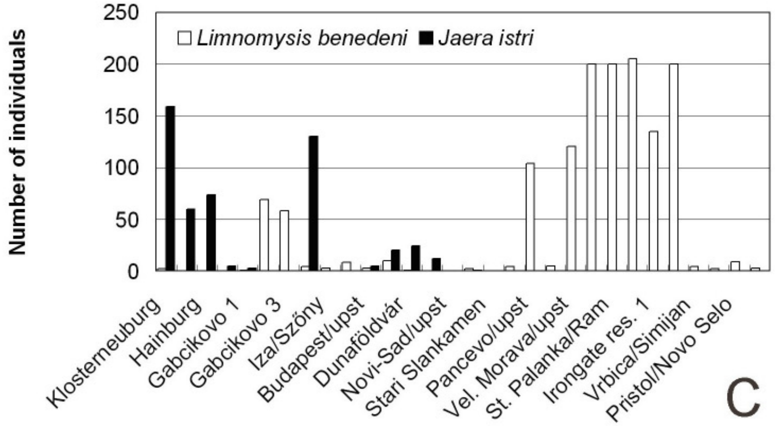


Figure 5. Abundance of Malacostraca species along the Danube between 1942 and 795 rkm. (A: *Dikerogammarus bispinosus, D. villosus*; B: *Obesogammarus obesus, Corophium curvispinum*; C: *Limnomysis benedeni, Jaera istri*)

Comparison of sampling methods

In the beginning section of the AQUATERRA sampling mission all the three sampling methods were tried out. Grabbing from the ARGUS vessel and kicking in the littoral zone were done simultaneously; dredging took extra time after finishing the other two procedures. It turned out very soon that this prolonged time necessary for the dredging influenced the whole duration of the given sampling, thus, dredging was not continued from *ADS 3* anymore. Kicking and grabbing were performed on the Danube section of *ADS 1* and *18*. Finally, only kicking was continued after this stretch along the whole AQUATERRA mission. Therefore, at first the results all of this three sampling methods referring to the first two sites are shown, later the grabbing and kicking samples collected at the upper 18 sites will be compared in terms of taxon and individual numbers, as well.

As far as the comparison of the three efficiencies is concerned, results of the upper two sampling sites are analysed only. Figure 6 A and B illustrates that dredging was not very effective concerning these groups at these locations. Crustaceans and Insecta, especially Chironomidae are the most abundant and taxon rich groups on the uppermost section of the Danube (*ADS 1-ADS 2*). Regarding to the kicking and grabbing methods, both the numbers of detected taxa and detected individuals were bigger in case of these dominant groups.

Kicking caught altogether 10 Crustacean species, whereas by dredging and grabbing only 7 taxa were detected. Kicking resulted in 12 insect taxa, grabbing collected 14 and dredging provided only 3 of them. Taking the individual numbers into consideration similar phenomenon is seen. Over 500 and over 700 specimens of Crsutaceans were counted in the kick and the grabbed samples, respectively. Dredged sample contained less than 100 individuals. The amount of insects was not significant in case of dredging. Considerable number of individuals was identified both in the kick and in the grabbed samples. The dominant insect groups are Caddis and mayfly taxa in this upper section. Dredging was the most effective way for collecting snails and mussels only at the first two sampling sites the but these animals represent not as rich group of macroinvertebrates as on the lower stretch.

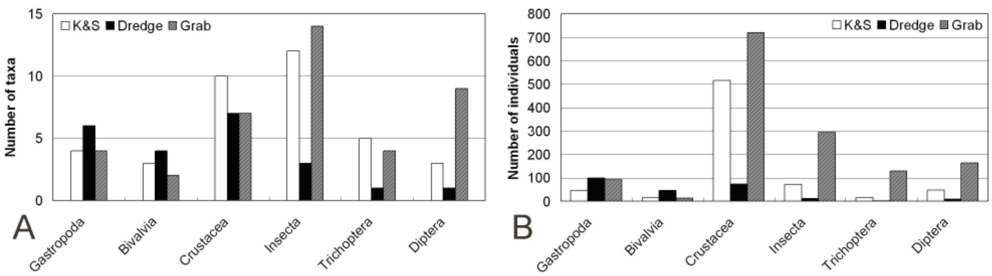


Figure 6. Number of taxa (A) and number of individuals (B) of macroinvertebrates collected by kicking, dredging and grabbing on ADS section 1-2

Grabbing and kicking was continuously applied on the Danube River between *ADS 1* and *ADS 18*. Results of this two sampling methods has to be compared in order to analyse the efficiencies of them. Detailed results of the number of taxa are seen on Figure 7A and B.

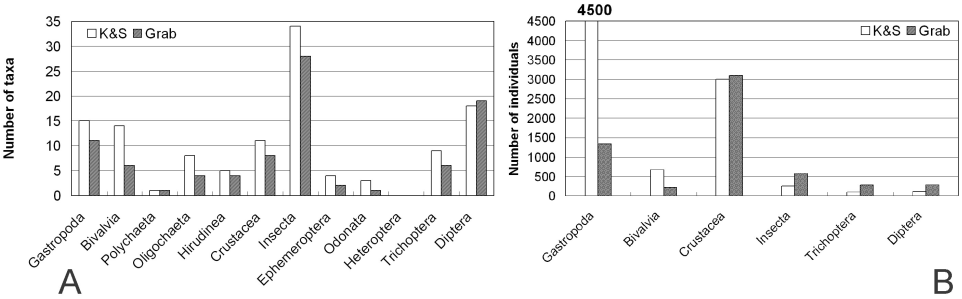


Figure 7. Number of taxa (A) and number of individuals (B) of macroinvertebrates collected by kicking and grabbing on ADS section 1-18

The diagram indicates that the kicking method was more effective in terms of the detection of the number of macroinvertebrate taxa in all main taxonomic groups. There is a relatively large difference in the total number of taxa among the two sampling methods. Altogether 74 taxa were found by the kick and sweep method. Grabbing resulted in 53 taxa.

Generally it can be concluded that approximately 10-20 % difference exists between the efficiencies of the two methods (Figure 7A) except mussel species because the efficiency of kicking is double than the grabbing. The explanation of this phenomenon is that the additional search (i.e. diving) during kick sampling for the sporadically distributed mussel species is very important part of any Danubian sampling program.

Figure 7B contains the results of the abundance conditions in the main taxonomic groups. It is clearly indicated by the diagram that only small differences were identified in the main taxonomic groups except the aquatic snails. Altogether three times more specimens were present in the kicked samples than in the grabbed ones. The explanation of this phenomenon is that the most abundant snail species in the Danube river is *Lithoglyphus naticoides* living on soft sediment surface. The grab contains usually limited number of large sized stones of solid surface where other, less abundant taxa are common (*Theodoxus* species). The sediment substrate represents only smaller part of grabbed samples, so, in this case kick samples contain the most abundant group of organisms in larger amount.

Beside of the snail group Crustaceans represent the dominant Danubian taxa. There is no significant difference among the two investigated sampling methods in the efficiency of the detection of that group at all.

It is very difficult to have significant conclusion about the comparison of these three sampling methods due to the fact that there is big difference in the spatial extension of each procedure. There are data for all of the three methods only from two cross sections that are situated on the uppermost end of the study area, and where all of them were applied. However, it should be emphasised that more precise and detailed dredging activity is necessary in future for studying the possibility of the application of that sampling method. In certain conditions (i.e. flooding) this way of approach could be the only available practical method for the investigation of such a large river as the Danube.

The length of the Danube stretch where grabbing and kicking were carried out is more significant. Therefore the results of the analysis and comparison of these sampling methods seems to be more reliable. Besides of these difficulties it should be emphasised that in future programs dealing with the ecological status of large European rivers the detailed dredging exercise has to be implemented in order to help in the development of new methodological development. There are several former experiences in this field like the Investigation of the Tisza River (2001) when more effort and time was given to this specific topic.

Biological water quality of the sampling locations

Metrics of taxon richness: BMWP and ASPT

Several metrics were calculated using the AQEM software to illustrate the ecological status and the biological water quality of the investigated Danube River stretch. One classification of sampling sites was carried out according to the modified Hungarian BMWP/ASPT method that is the current national method during the macroinvertebrate monitoring in Hungary. The family taxa are ordered into modified score group and both the total BMWP score and, the ASPT value are taken into consideration during the classification procedure. Figure 8 indicates the quality classes (A) and sub-classes (B) experienced in the Danube during the sampling mission.

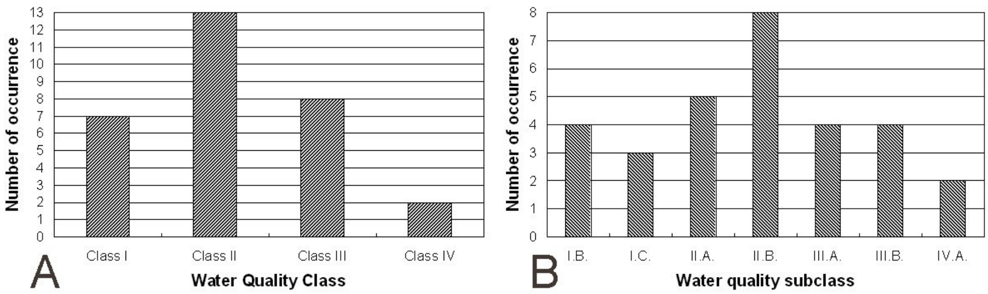


Figure 8. Water quality classes (A) and subclasses (B) of the sampling locations based on the modified BMWP/ASPT values of aquatic macroinvertebrate community

It is clear that the majority of sites belong to the second class. The number of sites having first and third classes is relatively big also. There are only few sites receiving worst quality than third.

Analysing the BMWP and ASPT values it is shown that the Total BMWP score has specific minimum values at the two big reservoir sections (Figure 9). The reason of that is that the relatively poor habitat types have certain poor faunal elements, too. The lowest score is experienced at Tekija/Orsova section where the homogenous sloppy bed forms a uniform habitat without any special richness of biota. The same situation was found at Hercegszántó that represents the first section of the Lower Danube. It is very difficult to take representative sample from such kind of aquatic environment. It is obviously indicated that in deep waters the sampling methodology concerning the macroinvertebrates requires further development and introduction of new procedures.

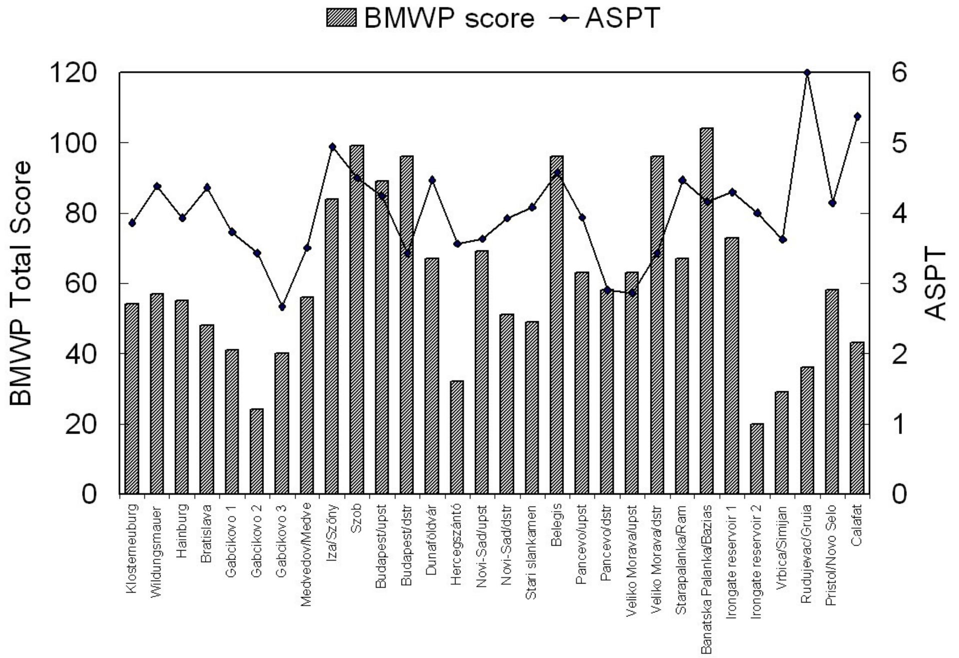


Figure 9. The change of the Hungarian BMWP and ASPT values along the Danube

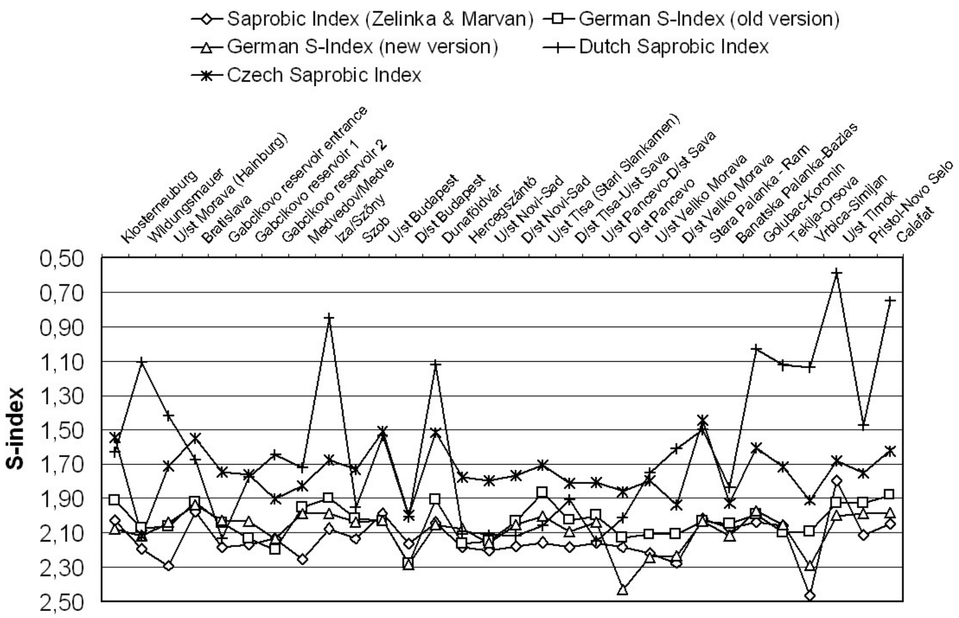


Figure 10. The change of several Saprobic indices along the Danube River

The formulation of the ASPT value is a bit different. The first minimum is situated at the Gabčikovo Reservoir, too. The second one is seen downstream Pancevo that probably indicates the frequent oil spill of the petrochemical industry. Finally there is no such low value anywhere else in the downstream stretch.

Metrics of organic pollution: saprobity indices

There are different Saprobic indices illustrated along the investigated Danube stretch in the Figure 10. Although the use of the Dutch Saprobic index probably has limited relevance it is interesting that the clearest sections are the Middle Hungarian and the Lower Serbian/Romanian/Bulgarian stretches. This index is very sensitive to the effect of Budapest that has to be emphasised, too. The worst section is registered in the Serbian Danube between Novi Sad and the Iron Gate Reservoir.

It is remarkable that the other four indices did not determine such big differences along the river at all. This calls for our attention that the Saprobic index analysis has important limitations because not only the organic pollution should be taken into consideration.

Diversity indices

Altogether three diversity metrics are compared during the analysis. It is very difficult to explain the spatial variation of these indices (Figure 11). Such inconsistencies like:

1. the values are increasing in the Gabčikovo Reservoir;
2. There is a maximum downstream Budapest;
3. There is a serious increase downstream Pancevo;
4. The sites in the Iron Gate Reservoir have big diversity index values.

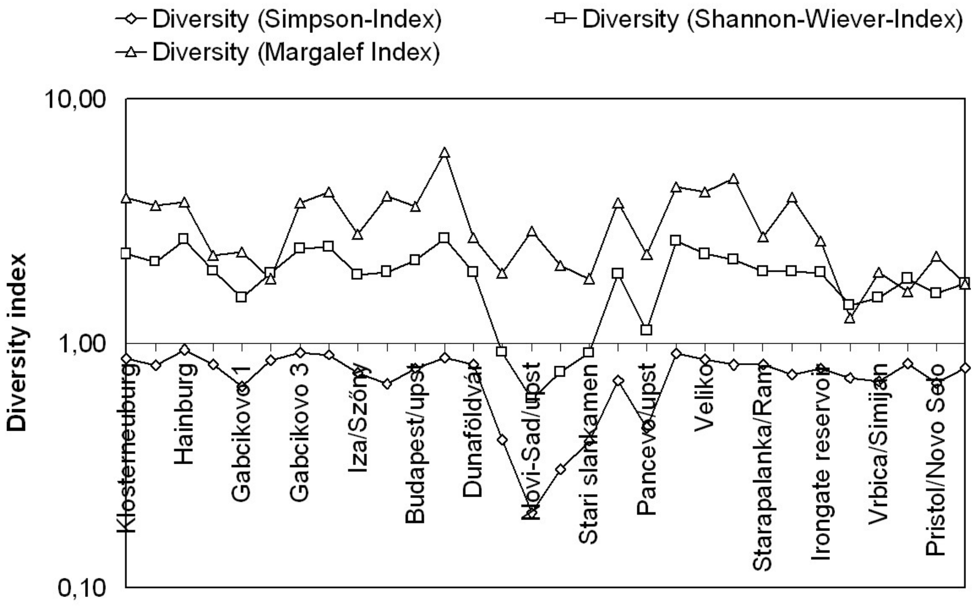


Figure 11. Diversity indices along the long section of the Danube River

At this moment the interpretation problems concerning the Diversity indices can not be solved yet. It has to be concluded that in case of the ecological research of large European rivers there are several methodological problems existing. Further research will probably help to solve these questions.

Conclusions

Based on these on-site investigations several general statements taking the distribution and coexistence of specific taxa into consideration can be established for the Danube River. These statements concerning the bioindication phenomenon are referring to the general pollution situation and the naturalness of the given sampling section, as follows:

1. Significant individual numbers of *Theodoxus danubialis* refer to hydromorphologically natural sections and relatively low organic load because this snail species is a sensitive and sporadically present, but very characteristic taxon of the Danube River.
2. Such kind of sites is characterised by large number of *Microcolpia acicularis*, as well. So, the coexistence of *T.danubialis* and *M. acicularis* is a well recognisable indication of the good ecological situation of the given river stretch.
3. In these sites the amount of *M. acicularis* always exceeds the amount of *Esperiana esperi*.

Summarising the field experiences gained during the AQUATERRA mission we have to conclude that

1. The same amount of sampling effort was applied during the long section sampling program;
2. Therefore the data set is homogenous due to the same methodology;
3. The relative differences from this homogenous data set could be illustrative for the ecological characterisation of the Danube.
4. This program covered only one season. It should be emphasised that the Danube River flows through several ecoregions, so, there might be considerable phenological differences in the fauna existing between different sections of the river that would required more attention and more detailed sampling program.

On one hand, the analysis of the species distribution and the abundance conditions of taxa are useful tools of the ecological characterisation of the Danube River. There are many cases when this comparison provides clearly understandable phenomena because several data explicitly interpret the environmental pressure and impact, as well (i.e. downstream Pancevo there are no *Lithoglyphus naticoides* snail, etc.).

On the other hand, it should be pointed out that the available habitat types are homogenous at certain sections, but they can be more diverse on other site. Obviously the application of multihabitat sampling methodology would be the best solution in case of the Danube River, too. Unfortunately, in case of large rivers like the Danube there are not enough international experiences by which an appropriate yard stick (like the AQEM protocol) could be clearly referred to, until now.

Therefore the evaluation of the individual numbers of macroinvertebrate taxa has a limitation: in certain cases when the sampled habitat was representative to the locality, the individual numbers could be compared to each other. In other cases, when it was not possible to involve all available habitat types in the sampling program due to the large dimensions of the river, this way of evaluation might be misleading.

According to our experiences there are several sampling locations on the Upper and Middle Danube that have well definable habitat composition. The data collected at these localities are representative enough to compare them to each other (between the Gabčikovo Reservoir and Dunaföldvár, the Upper and Middle Hungarian Danube section).

The lower stretch of the study area is more complicated because the impounded area of the Danube by the Iron Gate I and II are extending up to Belgrade. Both the limnetic and lotic sections can occur at this section near to and far from each other. The representative sampling is difficult in these circumstances because the complicated problem of the hydromorphological alterations.

The central problem of sampling in large rivers is how to quantify the amount of different organisms having different size, different life strategy, different occurrence characters, different environmental requirements, etc. Representative sampling needs careful estimation of the habitat structure in a large river water body where the gaps of the spatial and the temporal dimensions are going to be successfully solved. Hopefully, the present project had several useful outcomes to solve the problem of the methodology of ecological survey in case of large rivers. Further data analysis and comparison of the data to the other data sets (JDS, TNMN, national programs, etc.) might provide useful tools in that respect.

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