

Szent István University
Postgraduate School of Veterinary Science

**The spatio-temporal pattern of malacofauna in a water-
system of the Hungarian Danube stretch**

Outline of PhD thesis

Written by:

Erika Bódis

2012

Supervisors and members of the supervisory board:

.....
Dr. Erzsébet Hornung, CSc
Szent István University
Faculty of Veterinary Science, Department of Ecology
supervisor

Dr. János Nosek, PhD
supervisor

Dr. Gábor Majoros, PhD
member of the supervisory board

Dr. Péter Sólymos, PhD
member of the supervisory board

Erika Bódis

Introduction and aims

The growing human population is making significant demands on natural resources, resulting in unprecedented habitat destruction and extensive changes in Earth systems (MEA 2005). As a general rule, a global homogenisation of community composition can be observed as a result of loss in endemic and local species and increase of wide spread generalists and invasive species (McKinney and Lockwood 1999, Mace et al. 2005). The global freshwater biodiversity is declining at far greater rates than even the most affected terrestrial ecosystems, and on average, freshwater habitats and their species are more imperilled than their terrestrial counterparts (McAllister et al. 1997). The extinction rate of freshwater biodiversity is predicted to be five times faster than all other groups of species (Ricciardi and Rasumussen 1999) and these rates of freshwater biodiversity loss may even be much higher than these estimates, since only partial data exists for most species and even less for entire freshwater communities and ecosystems.

The malacofauna including the filter-feeding mussels and detritivorous or grazing aquatic snails is an important guild in the flux of matter and energy in freshwaters (Vaughn and Hakenkamp 2001). The filter-feeding mussels have a significant function as a connecting link between the primary production and the detritus chain, and they contribute to the natural purification of water due to their organic matters filtration effect both in suspension and in the sediment layer (Strayer et al. 2004). The grazing snails have an essential role in controlling the abundance and composition of periphytic algal communities (Swamikannu and Hoagland 1989). Both benthic groups have a significant role in the aquatic food web as important food sources for fishes, birds and insects.

Despite their central ecological role in the freshwater ecosystem, the study of their spatio-temporal pattern, abundance and habitat requirements in running waters, especially in large rivers, is insufficient. Above all, data about the importance of small-sized but abundant mussels (Sphaeriidae family) is hardly known, since their identification is problematic due to their tiny body and simple shell architecture. The investigation of malacofauna is particularly important and up to date in terms of conservation biology and invasion biology, too. The freshwater mussels and certain aquatic snails are especially sensitive to anthropogenic disturbances (e.g. pollution, habitat degradation, introduction of alien species), therefore their biodiversity decrease was accelerated over the past decades, and recently the freshwater mussels are considered as the most endangered group of animals. Mussels that belong to Unionidae family are particularly vulnerable to contaminants and thus their populations are both regionally and globally in decline (Bogan 1993, Lydeard et al. 2004). Since freshwater mussels and aquatic snails influence the nutrient cycling, and they have a basic function in creating favourable water quality, their continued decline can result in cascading effects that

potentially threaten the survival of healthy freshwater ecosystem. The study of invasive species is on the agenda, because of the recently unified European water system. In the Rhine-Main-Danube water-system the mussels are expanding from both sides (*Dreissena polymorpha* and the lately occurred *Dreissena rostriformis bugensis* from the ponto-caspic region, *Corbicula fluminea* from the water-system of Rhine) and could have a great impact on native biota by changing the material cycle and energy flow in communities.

C. fluminea was first recorded in the lower section of the Hungarian Danube in 1999 (Csányi 1998-1999), and during the last decade this bivalve invaded the side-arms of River Danube and became a dominant species in benthic communities at several sites. *C. fluminea* is known as one of the most successful aquatic invertebrate invaders, which could affect all components of the freshwater ecosystem including native species composition and different ecosystem functions. The taxonomy and systematics of *Corbicula* species are confusing and raise interesting problems. The taxonomic status of *Corbicula* morphs in Europe is still unresolved. In the present study we investigated the spatio-temporal pattern, diversity and density of malacofauna along a second- and third order stream-medium-sized river-large river continuum based on seasonal samples of 2007 and 2008. Furthermore, we studied the biomass dynamics, population structure of the invasive *C. fluminea*, which is an essential new element in the Hungarian malacofauna, and we try to find a solution to the taxonomic and systematic problems of this bivalve genus. During the research we are looking for answers to the following questions:

1. What kind of changes can be identified in the diversity and density of malacofauna according to space and time?
 - a) How can the spatio-temporal pattern of malacofauna change along a second- and third order stream-medium-sized river-large river continuum?
 - b) How can the temporal pattern of malacofauna change according to years and seasons?
2. Which environmental variables influence mostly the diversity, density and spatio-temporal pattern of malacological communities?
3. What kind of natural and antropogenic stress factors influence mostly the community structure of malacofauna?
4. How much is the frequency of occurrence and abundance of protected and invasive species in the studied area?
5. What kind of biomass dynamics and population structure characterize the invasive *Corbicula fluminea*, and which environmental factors influence them?
6. Do the representatives of *Corbicula* genus in the Hungarian Danube belong to two distinct species or two morphotypes?

Material and methods

We studied the malacofauna in a second- and third order stream-medium-sized river-large river continuum in the water system of the Hungarian Danube stretch (Figure 1). In order to study the malacofauna we collected quantitative samples of bottom sediment seasonally in 2007 (April, June, August, October) and in 2008 (May, July, October). At each sampling site and occasion four replicates with an area of 0.5 m² were randomly taken along a longitudinal section of 10-15 metres. We used a hand net with an edge of 25 cm and mesh size of 500 µm, which was the best sampling method and equipment for collecting mussel and gastropod species.

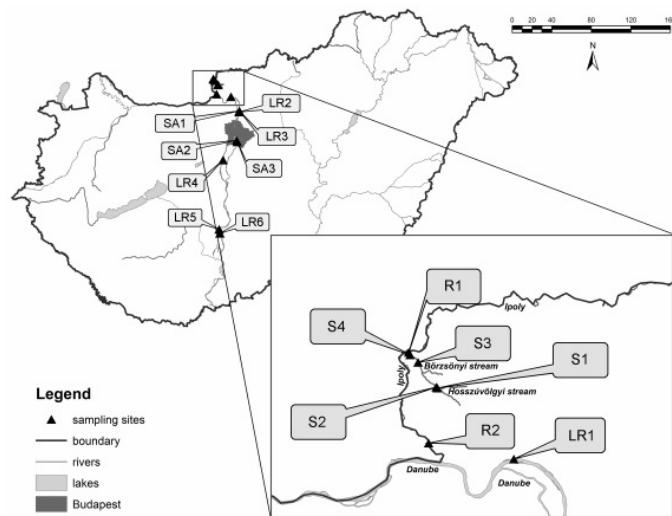


Figure 1. Map of Hungary with the 15 sampling sites (Hosszúvölgyi stream: S1, S2; Börzsönyi stream: S3, S4; River Ipoly: R1, R2; main arm of River Danube: LR1, LR2, LR3, LR4, LR5, LR6; side arms of River Danube: SA1, SA2, SA3).

Physicochemical parameters (temperature, velocity, turbidity, conductivity, redox potential, dissolved oxygen, and pH) were recorded in situ. Dissolved oxygen was measured by Winkler's method (Felföldy 1987). Chlorophyll-a was extracted from the sample by methanol, the concentration was determined according to Goodwin (1976), measuring the absorbance of the extract at 747, 666 and 653 nm. To determine the fractions of sediment and organic matter content bed sediment samples were collected by a core sampler of 4 cm diameter from the upper 5 cm layer. Samples of 63 cm³ were separated by a series of sieves to four fractions (coarse (C: 2360-750 µm), fine (F: 750-250 µm), very fine (V: 250-63 µm) and ultra fine (U: 63-0.45 µm)). The total organic matter content (TBOM) and the organic matter content of each fraction (CBOM, FBOM, VBOM and UBOM) were determined by loss at ignition (Heiri et al. 2001). Substrate types (stone, pebble, sand, silt, detritus) and vegetation (riparian vegetation, macrophytes and roots) were assessed at each site, which indicate the bottom texture.

For the population structure study of *Corbicula* genus – to gather as many individuals as possible – sampling was carried out during a period of 15 minutes. *Corbicula* samples were taken at Paks, where both morphs could be found, eight times between October 2006 and October 2007, when it was allowed by the water level. In order to examine the shell variation, we performed a morphometric study of shell characters on 728 individuals. For the analysis of height/length and width/length ratios the individuals were divided into 3 size categories to eliminate bias caused by allometric growth. The first category contains the juveniles less than 6 mm in length, the second category contains adults between 6–20 mm in length and the third category contains adults with more than 20 mm in length. In order to determine the percentage of gravid bivalves and the spawning period, 10 adult individuals of both morphs were selected at random and their gills were examined for the presence of incubating juveniles in each of the 8 sampling occasions.

To the biomass calculation of *C. fluminea* 216 specimens including 14 size categories were examined. In the laboratory, individuals were dried at 60 °C for 48 hours and compartmentalized based on shell length categories, between 2 and 22 mm. One size category generally contained 10 specimens except some categories where we did not find enough individuals. The juvenile (2-10 mm) and adult (11-22 mm) specimens of *C. fluminea* were modelled separately in order to eliminate bias caused by allometric growth.

In order to identify the genetic lineages of the two *Corbicula* morphs in Hungary, we have sequenced the mitochondrial cytochrome c oxidase subunit I (COI) gene of selected specimens. This gene was chosen because a large number of corbiculid COI sequences is already accessible in GenBank and laboratory methods are well established. As it was a preliminary barcoding study rather than a population genetic survey, only one specimen per morph per population was analyzed (morph-1: Göd (1 specimen), Paks (1 specimen); morph-2: Paks (1 specimen)). Genomic DNA extraction, amplification and sequencing have been carried out according to Glaubrecht et al. (2003). Published *Corbicula* sequences were downloaded from the GenBank and analysed together with the new sequences. Alignment was done by MEGA version 3.1 (Kumar et al. 2004). Sequences, which differed only in missing base pairs were collapsed into haplotypes and cut to 614 bp. Thus, 94 haplotypes – of which, 63 are known from the native range of the genus – were analyzed altogether. To describe general phylogenetic patterns within this genus a median network was constructed by the median-joining method (Bandelt et al. 1999) as implemented in NETWORK version 4.2.0.1 (Röhl 1997).

Environmental data were analyzed by standardized Principal Components Analysis (PCA) using the PAST program package (Hammer et al. 2001). Univariate measures including density, number of species, Shannon diversity and Pielou's evenness indices were assessed using the PAST package (Hammer et al. 2001). In order to study the spatial pattern of

malacofauna we used CLUSTER analysis by PRIMER program package (Clarke and Warwick 2001). Similarity matrix between sites was calculated using the Bray–Curtis coefficient and then analyzed using CLUSTER with average-linkage clustering algorithm based on the fourth-root-transformed density data. A two-way crossed ANOSIM2 (Clarke and Green 1988) test was used for searching differences between sites and dates. This non-parametric test compares ranked similarities between and within groups selected *a priori*. The SIMilarity PERcentages procedure (SIMPER) was used to assess the species contributing most to similarities within assemblages defined by CLUSTER analysis. To relate the bivalve and gastropod species composition to environmental variables, Canonical Correspondence Analysis (CCA) was performed using the computer program CaNOCO, version 4.5 (Ter Braak and Smilauer 2002).

Multiple linear regression models were carried out to examine the relationship between the diversity of bivalve and gastropod assemblages and the environmental parameters. Poisson regression was used to reveal the relationship between the frequency distribution of invasive and rare species. Both regression was carried out by using R program package (R Development Core Team 2007). Morphometric data of *Corbicula* were evaluated by two-way ANOVA using the R program package (R Development Core Team 2007). When ANOVA revealed a significant difference between the means, the Tukey test was performed for multiple comparisons among means. In order to calculate the biomass of *C. fluminea* a non-linear regression model was applied using the relation between the length and body dry weight. The power function ($DW = a \cdot L^b$) was used to calculate the model, where DW is the dry weight (mg) and L is the length of the shell (mm), *a* and *b* are constant parameters. Fit of the power function was judged by coefficient of determination (r^2) with the Past software version 1.36 (Hammer et al. 2001). ANOVA was performed to find differences in biomass, density and mean shell length of *C. fluminea* according to sampling sites, water types, months and years. Multiple linear regression models were carried out to examine the relationship between the environmental parameters and the biomass of *C. fluminea*. For data analysis, biomass values were log transformed to normalize and stabilize variances. For identifying the zonation valency of each sampling sites we used the work of Mouthon (1999), and the longitudinal valency of each species from Fauna Aquatica Austriaca (Moog 2002).

Results

Each bivalve and gastropod species attach to certain longitudinal zone of running waters based on their environmental demand. According to the theory of indication of zonation the sampling site can be ordered to a zonation valency on the basis of the zone preference of bivalve and gastropod species, and the species composition of the sampling site (Figure 2).

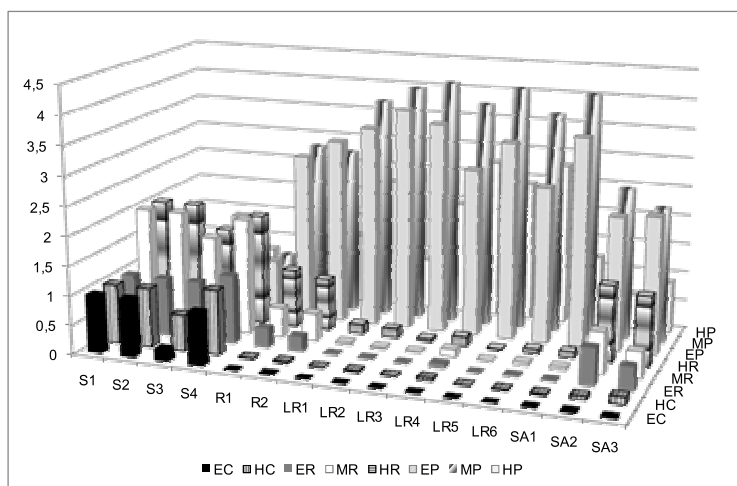


Figure 2. The zonation valency of sampling sites based on the longitudinal valency of Fauna Aquatica Austriaca (Moog 2002). Signs: EC – eucrenon, HC – hypocrenon, ER – epirhitron, MR – metarhitron, HR – hyporhitron, EP – epipotamon, MP – metapotamon, HP – hypopotamon.

During 2007 and 2008 the water level and the water discharge were different in the sampling periods, and their impact can be recorded on the composition of bivalve and gastropod assemblages. The ANOSIM2 test revealed significant differences among the bivalve and gastropod species composition according to the sampling sites and years. However, there was not significant difference among the bivalve species composition based on seasons, only the gastropod fauna showed seasonal changes in 2007.

In the studied water system on the basis of the bivalve fauna three groups can be distinguished (Figure 3). The sites represented in group 1 (S1, S2, S3 and S4) belong to streams with lower water temperature and pH, and higher total organic matter content and coarse fraction of sediment. The density and species richness were very low (mean density, Shannon diversity and Pielou's evenness of 5.1 ind. m⁻², 0.004 and 0.006 respectively), and only 2 species were present. The group was characterized by *P. casertanum*. *P. personatum* was found only at the second site of the third order Börzsönyi-stream (S4) close to the conjunction of the Börzsönyi-stream and the River Ipoly. Group 2 (LR6, SA2, SA3, R1, R2, LR3, LR1 and SA1) was located at sites of the River Ipoly, in side arms of the Danube and in the main arm of the Danube with bottom texture of silt and sand. This was the most diverse group and the density and diversity indices were high (mean density, Shannon diversity and Pielou's evenness of 109.6 ind. m⁻², 1.425 and 0.674, respectively). A total of 14 species were found and this group was dominated by *P. subtruncatum*, *P. henslowanum* and *P. supinum*. *C. fluminea*, *P. amnicum*, *S. solidum*, *S. corneum* and *P. moitessierianum* also found appropriate habitat conditions here. „*C. fluminalis*” (morph-2 under revision, Bódis et al. 2011) occurred only at site Paks (LR6) and *Anodonta woodiana* was detected only at sites Paks (LR6) and Ráckevei-(Soroksári)-Danube.

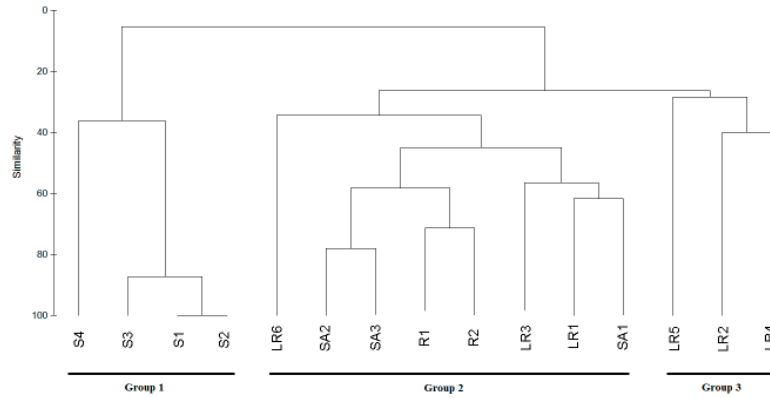


Figure 3. The hierarchical classification of sampling sites based on bivalve fauna (Bray–Curtis coefficient, average-linkage clustering algorithm).

Inside this faunal group, three smaller groups can be identified: one at LR6 sampling site with extreme high water temperature; another one at sites of the River Ipoly and the Ráckevei-(Soroksári)- Danube characterized with similar water discharge and a last one at sites of the Danube with bottom texture of silt and sand. Group 3 (LR5, LR2 and LR4) corresponded to sites of the main arm of the Danube with bottom texture of pebble, stone and high current velocity. The density and species richness were higher than in group 1, but lower than in group 2 (mean density, Shannon diversity and Pielou's evenness of 16.5 ind. m⁻², 0.651 and 0.443 respectively), and a total of 8 species were found. The group was dominated by *C. fluminea* and *S. rivicola*.

In the studied water system on the basis of the gastropod fauna six groups can be distinguished (Figure 4). The site represented in group 1 (S4) belongs to the second sampling site of Börzsönyi stream with lower water temperature and pH, and higher total organic matter content and coarse fraction of sediment. The density and species richness were very low (mean density, Shannon diversity and Pielou's evenness of 4.8 ind. m⁻², 0.5 and 0.4 respectively), and only 3 species were found. The group was characterized by *Radix labiata*. Group 2 (S3, R1b) was located at the first sampling site of Börzsönyi stream and River Ipoly. The density and species richness (mean density, Shannon diversity and Pielou's evenness of 3.3 ind. m⁻², 0.7 and 0.8 respectively) were also very low, and only 3 species were found. This group was dominated by *Haitia acuta* and *Ancylus fluviatilis*. Group 3 (LR2, LR4acd, SA2) was corresponded to sites of Ráckevei-(Soroksári)-Danube, and the main arm of the Danube with bottom texture of pebble and stone. In this group the density and species richness were higher (mean density, Shannon diversity and Pielou's evenness of 26.7 ind. m⁻², 1.0 and 0.6 respectively), and 16 species were recorded altogether. The group was dominated by *Theodoxus fluviatilis* and *Lithoglyphus naticoides*.

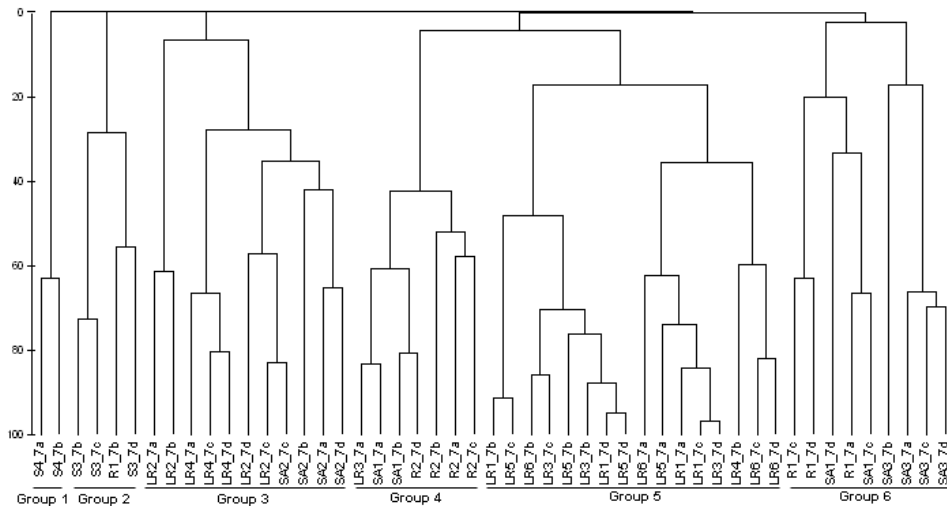


Figure 4. The hierarchical classification of sampling sites based on gastropod fauna (Bray–Curtis coefficient, average-linkage clustering algorithm). Signs: a-April, b-June, c-August, d-October.

Group 4 (R2, SA1ab, LR3a) belonged to the second site of River Ipoly, the side arm of the Danube at Göd and the main arm of the Danube with substrate type of sand. In this group the density was high (mean density, Shannon diversity and Pielou's evenness of 64.1 ind.m⁻², 0.8 and 0.5), and 13 species were found altogether. The group was dominated by *L. naticoides* and *Borysthenia naticina*. The sites of group 5 (LR1, LR3bcd, LR4b, LR5, LR6) mostly possess bottom texture of silt and sand, the density was extremely high in this group (mean density, Shannon diversity and Pielou's evenness of 583.2 ind. m⁻², 0.6 and 0.3 respectively), and 19 species were recorded altogether. The group was also dominated by *L. naticoides* and *B. naticina*. Sites of group 6 (R1acd, SA1cd, SA3) were similar in respect of the presence of vegetation, which provide favourable conditions for aquatic snails, thus in this group the species richness was high (mean density, Shannon diversity and Pielou's evenness of 45.2 ind. m⁻², 0.9 and 0.6 respectively), and 17 species were registered altogether. In this group *L. naticoides*, *Valvata piscinalis*, *Bithynia tentaculata*, *H. acuta*, and *Potamopyrgus antipodarum* were the dominant species.

Regarding the distribution of bivalve species assemblages the strongest explanatory factors were the substrate types, the current velocity, the bed sediment fractions, the organic matter content of sediment, the turbidity, the conductivity, the temperature and the chlorophyll-a content of water. The diversity of bivalve fauna was very low in streams, the diversity of bivalve communities in Börzsönyi stream significantly differed from the other water types. The very fine and ultra fine sediment fractions and the conductivity had a significant impact on the diversity of bivalve communities. Regarding the distribution of gastropod species assemblages the strongest explanatory factors were the substrate types, the bed sediment fractions, the organic matter content of sediment, and the conductivity. The gastropod assemblages had the highest diversity in the side arms of the Danube characterized by slow

current velocity. The fine, very fine and ultra fine sediment fractions, and the turbidity had a significant impact on the diversity of gastropod communities.

ANOVA analysis of *C. fluminea* data detected significant spatial variation in biomass, density and mean shell length. However, significant temporal variation in biomass was not recorded between the years, only among the months. The highest density values were detected at LR3 (August 2007: 178 ind.m⁻²). The highest biomass values were also recorded at LR3 (October 2007: 24.9697 g DW m⁻²). Multiple linear regressions were used to find the relationship between the biomass of *C. fluminea* and the environmental parameters. The high biomass values were connected with the sediment type (coarse and very fine sediment fractions) and temperature. The low biomass values were related to the organic matter content of very fine fraction of sediment.



Analysing the morphometric data by two-way ANOVA we detected that there were statistically significant differences between morphs in the height/length ratio ($p < 0.001$) and in the width/length ratio ($p < 0.001$), and among size categories in the height/length ratio ($p < 0.001$) and in the width/length ratio ($p < 0.001$). We studied the interaction between the two factors (morphs and size categories), which was significant at both ratios (height/length ratio: $p < 0.05$; width/length ratio: $p < 0.001$). The comparison of morphs within each size category was performed by Tukey test, and we found significant differences at both ratios (height/length ratio: $p < 0.001$, width/length ratio: $p < 0.05$). In summary, the conventional

morphometric measures showed that the two morphs could be distinguished by height/length) and width/length ratios. Morph-1 has more elongated shells whilst morph-2 has more spherical, rounded shells. Three different haplotypes were detected in our Danubian samples, of which “97” and “98” (both are morph-1) differs from “80” (morph-2) by 14 and 15 base pairs, respectively. The two morphs belong to distinct clades, “97” is the same haplotype, which was found in native *C. fluminea/javanica* in Thailand, in native *C. leana* in Japan, and in invasive populations in Europe and America, referred as *C. fluminea* and as “New World form A”. Danubian morph-2 bears haplotype “80”, which was hitherto found in Germany, France and Argentina and referred to as a “cryptic *Corbicula* species” and as “New World form C”. Clades of the COI network can not be matched unambiguously with species, as some clades comprise more than one *a priori* assigned species, and in the same time, some *a priori* assigned species belong to more than one clade (Figure 5).

Based on the length-frequency analysis and the determination of gravidity morph-1 had one reproductive period, from the beginning of spring, probably centred on March until October with an intensity peak in June. The analysis of morph-2 revealed two reproductive periods,

one in the beginning of winter, probably centred on December and January, and another from June to October with an intensity peak in August.

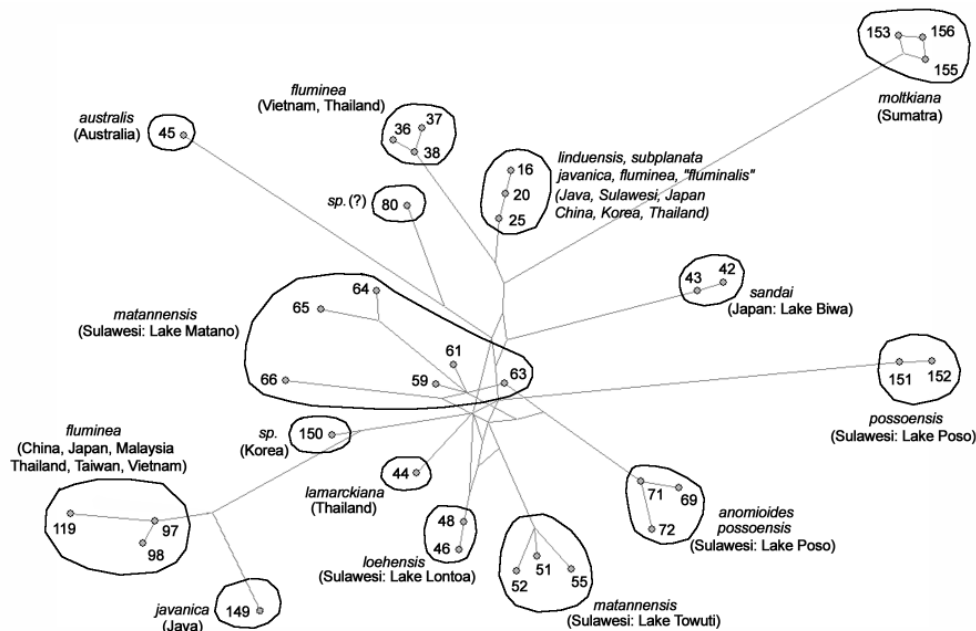


Figure 5. Median joining network of *Corbicula* COI haplotypes. The Danubian haplotypes are indicated as 97, 98 (morph-1) and 80 (morph-2).

Discussion

In the present study we investigated the spatio-temporal pattern, diversity and density of the whole malacofauna (11 gastropod and 4 bivalve families) along a second- and third order stream-medium-sized river-large river continuum based on seasonal samples of 2007 and 2008. We revealed the biomass dynamics and population structure of the invasive *Corbicula fluminea*, which is an essential new element in the Hungarian malacofauna, and tried to find solution to the taxonomic and systematic problems of this bivalve genus. During the two year-long survey 22 bivalve species and 27 gastropod species were recorded altogether. Among them 7 species are protected in Hungary and 11 species are considered as invasive. The high density presented by *P. subtruncatum*, *P. supinum*, *P. henslowanum* and *C. fluminea* suggests that these species may have a key role in the investigated ecosystem. The River Continuum Concept (Vannote et al. 1980) predicts increase in number of species and diversity downstream from springs to the middle course as a result of environmental specialization of animals living in rivers. The spatial pattern of malacofauna shows the same tendency along the second- and third order stream-medium-sized river-large river continuum. The lowest species richness was detected in streams, while the highest number of species and diversity appeared in the River Ipoly and in the side arms of the Danube. Along the studied second- and third order stream-medium-sized river-large river continuum three bivalve and six gastropod groups can be distinguished.

The least abundant and diverse groups were found in streams, which were dominated by *Pisidium casertanum*, *Pisidium personatum*, *Radix labiata*, *Haitia acuta* and *Ancylus fluviatilis*. The most abundant and diverse groups occurred in River Ipoly, in side arms of the Danube, and in the main arm of the Danube with sand and silt substrate. These groups were dominated by *Pisidium subtruncatum*, *Pisidium henslowanum*, *Pisidium supinum*, *Lithoglyphus naticoides*, *Borysthenia naticina*, *Valvata piscinalis*, *Bithynia tentaculata*, *Haitia acuta*, and *Potamopyrgus antipodarum*. The faunal groups with moderate abundance and species richness belonged to the main arm of the Danube with substrate types of pebbles and stones, where *Corbicula fluminea*, *Sphaerium rivicola*, *Pisidium supinum*, *Theodoxus fluviatilis*, *Lithoglyphus naticoides* appeared as dominant species.

The streams provided the least advantageous habitat type for most of the bivalve species, while the fine and ultra fine bed sediment fractions and the high conductivity of water had a statistically significant positive impact on the diversity of bivalve communities. The gastropod assemblages reached high diversity values in the side arms of the Danube, where the vegetation was more abundant than at other sites. The fine, very fine and ultra fine bed sediment fractions and the organic matter content of coarse bed sediment had a statistically significant positive impact on the diversity of gastropod communities, while the high turbidity had a negative impact on it. It means that the fine, very fine and ultra fine bed sediment fractions provided suitable habitat for most of the aquatic snails, and the organic matter in coarse sediment fraction is an ideal source of food for the detritivorous gastropod species. The high turbidity is in relation with the strong current velocity, which is unfavourable for the high species richness of aquatic snails, because most of the species prefer the standing water.

The spatial grouping of sites and species can be explained on a local and a landscape scale. Our results showed that landscape scale effects (e.g., water types and longitudinal gradient) determine the spatial pattern of sites and faunal assemblages along the investigated second- and third order stream-medium-sized river-large river continuum. However, the local effects (e.g., substrate types, current velocity and sedimentological characteristics (bed sediment fractions), organic matter content, conductivity) were the strongest explanatory factors. Our results are in accordance with previous studies (Pardo and Armitage 1997, Beisel et al. 1998, Brown 2003, Heino et al. 2004, Jurkiewicz-Karnkowska and Zbikowski 2004, Brooks et al. 2005, Beaty et al. 2006, Sousa 2007, 2008, Miserendino 2009) which demonstrated that substrate type, current velocity and sediment characteristics play the most important role in the distribution of benthic species, including molluscs.

During the last decade the invasive *Corbicula fluminea* became a dominant species along the River Danube, and its large biomass values can be recorded at several sites. In the studied water system significant spatial variation in biomass, density, and mean shell length

was detected among the investigated sampling sites and water types. The highest values were recorded in the main arm of the Danube and in a side arm of the Danube upstream to Budapest, where the chlorophyll-a content of water was high, and at site LR6, where the water temperature was permanently high due to the cooling water outlet of Paks Nuclear Power Plant. This site can be considered as thermal refugia for *C. fluminea*, which provides adequate conditions for the individuals to reach 40 mm shell length. The variation of biomass was determined by the sediment type, temperature and benthic organic matter content.

In *Corbicula*, the plasticity of shell characteristics is considerably high and the distinction of species within this genus is still unresolved. In order to reveal the taxonomic status of the two *Corbicula* morphs inhabiting the Hungarian Danube stretch, we compared the mitochondrial COI gene (cytochrome c oxidase subunit I) sequence, the morphological characteristics, the population structure and the reproduction biology of the two *Corbicula* morphs. To study the phylogenetic relationship of the two morphs, adding own collected samples to a set of published sequences, we performed a median joining analysis instead of applying traditional tree building methods. The phylogeny, based on COI gene, which is the most frequently used barcoding sequence, does not match unambiguously with morphology based species assignments. Therefore, COI based barcoding does not provide a clue to decide whether or not the two Danubian morphs belong to distinct taxa. Nevertheless, the two morphs belong to distinct clades, morph-1 is clustered together with *C. fluminea* and morph-2 was clustered to a clade, which is hitherto found in non-native populations and its native range is undiscovered so far. With respect to the reproductive biology, clear differences were assigned for the two morphs. Morph-1 has only one reproductive period per year, while morph-2 has two reproductive periods, either of them occurred in winter. In summary the detected differences in shell morphology, mitochondrial DNA sequence, and reproductive biology indicate that it is probably correct to treat the two morphs as distinct taxa. Nevertheless, the use of the name "*C. fluminalis*" for morph-2 is probably premature, as its phylogenetic relationship with native *C. fluminalis* still needs confirmation. As long as the African and western Asian parts of the genus' range are poorly studied, origin and taxonomic position of morph-2 remain unclear. The large clade of incubating freshwater *Corbicula* includes both sexual and clonal lineages with poorly resolved relationships (GLaubrecht et al. 2003). Further molecular investigation of main freshwater *Corbicula* lineages is required to resolve the systematic and taxonomic complexities in this group. Furthermore, the discovery of convincing meiotic parental species is needed to facilitate reconstructing the evolutionary origin of clonality, and to clarify the routes of introduction in this genus.

New scientific results

1. During the two year-long survey we revealed the species richness of malacofauna along a second- and third order stream-medium-sized river-large river continuum and we recorded **22 bivalve and 27 gastropod species altogether**. Among them 2 bivalve species (*Pseudanodonta complanata* és *Unio crassus*) and 5 gastropod species (*Borysthenia naticina*, *Esperiana esperi*, *Esperiana daudebartii*, *Theodoxus danubialis*, *Theodoxus transversalis*) are protected in Hungary. From the observed **11 invasive species 7 species are wide-spread and abundant** (*Anodonta woodiana*, *Corbicula fluminea*, *Dreissena polymorpha*, *Haitia acuta*, *Lithoglyphus naticoides*, *Potamopyrgus antipodarum*, *Theodoxus fluviatilis*), whereas 4 species („*Corbicula fluminalis*”, *Dreissena rostriformis bugensis*, *Ferrissia fragilis*, *Melanoides tuberculatus*) occurred with low frequency of occurrence and abundance.
2. In 2007 the cosmopolitan *Pisidium casertanum*, in 2008 *Corbicula fluminea* was the most wide-spread bivalve species. *Corbicula fluminea* became a dominant species in the Hungarian Danube stretch in a short period of time, however, it has not colonized the tributaries including smaller rivers and streams yet. We found it in River Ipoly close to the conjunction of River Danube, but we did not record it at the sampling site situated further from the conjunction. The *Pisidium subtruncatum* was the most abundant bivalve species in 2007, whereas *Corbicula fluminea* reached the highest relative abundance in 2008. Regarding the gastropod species in both years the *Lithoglyphus naticoides* occurred with the highest frequency of occurrence and relative abundance. **The high density presented by *Pisidium subtruncatum*, *Pisidium supinum*, *Pisidium henslowanum* and *Corbicula fluminea* suggests that these species may have a key role in the investigated ecosystem.**
3. During 2007 and 2008 the **water level and the water discharge** were different in the sampling periods, and its **impact can be recorded on the composition of bivalve and gastropod assemblages**. Significant differences can be revealed among the bivalve and gastropod species composition according to the sampling sites and years. However, there was not significant difference among the bivalve species composition based on seasons, **only the gastropod fauna showed seasonal changes in 2007.**
4. The lowest species richness was detected in streams, while **the highest number of species and diversity appeared in the River Ipoly and in the side arms of the Danube.**
5. **In studied water system three bivalve groups can be distinguished.** The least abundant and diverse group (*Pisidium casertanum*, *Pisidium personatum*) was found in streams. The most abundant and diverse group (*Pisidium subtruncatum*, *Pisidium*

henslowanum and *Pisidium supinum*) occurred in River Ipoly, in side arms of the Danube, and in the main arm of the Danube with sand and silt substrate. The bivalve group with moderate abundance and species richness (*Corbicula fluminea*, *Sphaerium rivicola* and *Pisidium supinum*) adapted to the main arm of the Danube with substrate types of pebbles and stones. **The spatial pattern of bivalve species assemblages** was mainly determined by the substrate types, the current velocity, the bed sediment fractions, the organic matter content of sediment, the turbidity, the conductivity, the temperature and the chlorophyll-a content of water. The **fine and ultra fine bed sediment fractions** and the **high conductivity** of water had a statistically significant positive impact on the diversity of bivalve communities.

6. **In the studied water system six gastropod groups can be distinguished.** The least abundant and diverse two groups (*Radix labiata*, *Haitia acuta* and *Ancylus fluviatilis*) were found in the third order Börzsönyi stream and the upper section of River Ipoly. The gastropod group with moderate abundance and species richness (*Theodoxus fluviatilis* and *Lithoglyphus naticoides*) adapted to the substrate type of pebbles and stones. The group with high abundance and diversity (*Lithoglyphus naticoides* and *Borysthenia naticina*) belonged to River Ipoly, side arm of the Danube at Göd and the main arm of the Danube with substrate type of sand. The most abundant group with high species richness (*Lithoglyphus naticoides* and *Borysthenia naticina*) was mostly occurred in the main arm of the Danube with substrate type of silt and sand. The most diverse group with high abundance (*Lithoglyphus naticoides*, *Valvata piscinalis*, *Bithynia tentaculata*, *Haitia acuta*, and *Potamopyrgus antipodarum*) was characteristics to sites in River Ipoly and in the side arms of the Danube with aquatic macrovegetation. **The spatial pattern of gastropod species assemblages** was mainly determined by the substrate types, the bed sediment fractions, the organic matter content of sediment, and the conductivity. **The gastropod assemblages reached high diversity values in the side arms of the Danube.** The **fine, very fine and ultra fine bed sediment fractions** and the **organic matter content of coarse bed sediment** had a statistically significant positive impact on the diversity of gastropod communities, while the high **turbidity** had a negative impact on it.
7. In summary the **spatial pattern of malacofauna can be explained both on a local and a landscape scale.** Our results show that in respect to the spatial scale the studied second- and third order stream-medium-sized river-large river continuum can be separated into two sections. Along the second- and third order stream-medium-sized river-large river continuum (S1-LR1) the phenomenon of continuity can be revealed, whereas in the further section of the large river the local natural (current velocity and bottom texture) and the antropogenic effects form or enhance the mosaic spatial pattern of the large river.

8. ***C. fluminea* is one of the most successful invasive bivalve species**, in the last decade it became a dominant element in the benthic communities of River Danube. This species shares the same habitat with the rare *S. rivicola* and *P. amnicum*, and *C. fluminea* has a statistically significant impact on the two rare bivalve species. At those sites (R2, LR1, LR5), where the abundance of *C. fluminea* was low, the *S. rivicola* and *P. amnicum* could reach high abundance. **Significant spatial variation in biomass, density, and mean shell length was detected** among the investigated sampling sites and water types. The highest values were recorded in the main arm of the River Danube and in a side arm of the Danube upstream to Budapest, where the chlorophyll-a content of water was high, and at site LR6, where the water temperature was permanently high due to the cooling water outlet of Paks Nuclear Power Plant. This site can be considered as thermal refugia for *C. fluminea*, which provides adequate conditions for the individuals to reach 40 mm shell length. **The variation of biomass was mainly determined by the sediment type, temperature and benthic organic matter content.**
9. The taxonomy and systematics of *Corbicula* species is still unresolved. In the studied water system the **COI gene sequence analysis of the two *Corbicula* morphs** does not show an unambiguously match with morphology based species assignments. Therefore, COI based barcoding does not provide a clue to decide whether or not the two Danubian morphs belong to distinct taxa. Nevertheless, the **two morphs belong to distinct clades, morph-1 is clustered together with *C. fluminea* and morph-2 was clustered to a clade, which is hitherto found in non-native populations** and its native range is undiscovered so far. **With respect to the reproductive biology, clear differences were assigned for the two morphs.** Morph-1 has only one reproductive period per year, while morph-2 has two reproductive periods, either of them occurred in winter. In summary the **detected differences in shell morphology, mitochondrial DNA sequence, and reproductive biology indicate that it is probably correct to treat the two morphs as distinct taxa.** Nevertheless, **the use of the name “*C. fluminalis*” for morph-2 is probably premature**, as its phylogenetic relationship with native *C. fluminalis* still needs confirmation. As long as the African and western Asian parts of the genus' range are poorly studied, origin and taxonomic position of morph-2 remain unclear.

List of publications

Publications in peer-reviewed journals with impact factor

Bódis E., Nosek J., Oertel N., Tóth B., Hornung E., Sousa R.: **Spatial distribution of bivalves in relation to environmental conditions (Middle Danube catchment, Hungary)**. *Community Ecology*, 12(2). 210-219, 2011. IF: 0,974

Bódis E., Nosek J., Oertel N., Tóth B., Fehér Z.: **A Comparative Study of Two *Corbicula* Morphs (*Bivalvia*, *Corbiculidae*) Inhabiting River Danube**. *Internat. Rev. Hydrobiol.*, 96(3). 257-273, 2011. IF: 1,48

Publications in peer-reviewed journals without impact factor

Bódis E., Nosek J., Oertel N., Tóth B.: **A bentál strukturális és funkcionális elemei a Duna vízrendszerében 3. A kagylófauna térbeli mintázata a környezeti változók függvényében**. *Hidrológiai Közlöny*, 29-32, 2011.

Oertel N., Nosek J., Bódis E., Borza P., Tóth B.: **A bentál strukturális és funkcionális elemei a Duna vízrendszerében 2. A meio- és makrogerinctelen társulások**. *Hidrológiai Közlöny*, 65-68, 2011.

Oertel N., Nosek J., Bódis E., Borza P., Tóth B.: **Dunai makrogerinctelen-mintavételek tanulságai a Gödi-sziget térségében**. *Acta Biol. Debr. Oecol. Hung.*, 21. 139-153, 2010.

Nosek J., Oertel N., Bódis E., Tóth B.: **A bentikus szervesanyag és a makrogerinctelen társulások tér- és időbeli változása a Duna Kismaros (1688 fkm) és Göd (1668 fkm) közötti szakaszán**. *Acta Biol. Debr. Oecol. Hung.*, 20. 165-179, 2009.

Bódis E., Nosek J., Oertel N.: **Spatio-temporal pattern of mussels (*Corbiculidae*, *Dreissenidae*, *Sphaeriidae*) in the water-system of the Hungarian Danube**. *Arch. Hydrobiol. Suppl. Large Rivers*, 18(1-2). 293-308, 2008.

Bódis E.: **Contribution to the macroinvertebrate fauna of the Hungarian Danube IV. Mussels (*Bivalvia*: *Corbiculidae*, *Dreissenidae*, *Sphaeriidae*, *Unionidae*)**. *Folia Historico Naturalia Musei Matraensis*, 32. 57-68, 2008.

Bódis E., Nosek J., Oertel N., Tóth B.: **A kagylófauna longitudinális eloszlása a Duna vízrendszerében**. *Acta Biol. Debr. Oecol. Hung.*, 18. 09-20, 2008.

Bódis E.: **Spatio-temporal pattern of the small-sized mussel fauna in the Danube above Budapest**. *Acta Biol. Debr. Oecol. Hung.*, 16. 21-32, 2007.

Bódis E.: **The biomass dynamics of *Corbicula fluminea* invasive mussel**. *Acta Biol. Debr. Oecol. Hung.*, 16. 9-20, 2007.

Book chapter

Puky M., Ács É., Bódis E., Borza P., Kiss K.T., Tóth A.: **Biológiai inváziók a magyarországi Duna-szakaszon.** Válogatás az MTA Ökológiai és Botanikai Kutatóintézet kutatási eredményeiből, 2009. ÖBKI Műhelyfüzetek 2. pp. 99-103, 2010. ISBN: 978-963-8391-47-6.

Conference proceedings

Bódis E., Nosek J., Oertel N., Tóth B.: **Longitudinal dispersion of mussel fauna in a second order stream - Danube continuum in North Hungary.** In: Proceedings of the 37th International Conference of IAD, Chisinau, Moldova, pp. 97-102, 2008. ISBN 978-86-911997-0-8.

Puky M., Ács É., Bódis E., Borza P., Kiss K. T., Tóth A.: **Invasive algae, plant, bivalve and crustacean species along the Hungarian Danube section: arrival time, colonisation characteristics, relative importance.** In: Proceedings of the 37th International Conference of IAD, Chisinau, Moldova, pp. 76-81, 2008. ISBN 978-86-911997-0-8.

Conference presentations

Bódis E., Pohner Zs., Tóth B., Oertel N., Ács É., Bíró P.: **Két veszélyeztetett kagylófaj intraspecifikus diverzitásának vizsgálata.** LIII Hidrobiológus napok, Tihany, 2011.

Bódis E., Nosek J., Oertel N., Tóth B.: **Effects of environmental variables on mussel assemblages along a second order stream – large river continuum in North Hungary.** 38th International Conference of IAD, Dresden, Germany, 2010.

Oertel N., Nosek J., Bódis E., Borza P., Tóth B.: **Spatio-temporal changes of benthic organic matter and macroinvertebrate communities in the Danube Bend (Hungary).** 38th International Conference of IAD, Dresden, Germany, 2010.

Bódis E., Nosek J., Oertel N., Tóth B.: **A bentál strukturális és funkcionális elemei a Duna vízrendszerében 3. A kagylófauna térbeli mintázata a környezeti változók függvényében.** LII. Hidrobiológus napok, Tihany, 2010.

Oertel N., Nosek J., Bódis E., Borza P., Tóth B.: **A bentál strukturális és funkcionális elemei a Duna vízrendszerében 2. A meio- és makrogerinctelen társulások.** LII. Hidrobiológus napok, Tihany, 2010.

Bódis E., Nosek J., Oertel N., Tóth B.: **A kagylóegyüttesek térbeli mintázatát befolyásoló környezeti változók vizsgálata egy másodrendű patak-folyam kontinuum mentén.** VII. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Sümeg, 2010.

- Oertel N., Nosek J., Bódis E., Borza P., Tóth B.: **Dunai makrogerinctelen mintavételek tanulságai a Gödi-sziget térségében.** VII. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Sümeg, 2010.
- Puky M., Ács É., Bódis E., Borza P., Erős T., Kiss K., Tóth A.: **Aquatic invasions along the Danube accelerated around the millenium in Hungary: colonisation characteristics, taxonomical differences, relative importance.** World Conference on Biological Invasions and Ecosystem Functioning, Porto, Portugal, 2009.
- Bódis E., Nosek J., Oertel N., Tóth B.: **Spatio-temporal dynamics of malacofauna along a second order stream - large river continuum.** 2nd European Congress of Conservation Biology, Prague, Czech Republic, 2009.
- Bódis E., Nosek J., Oertel N., Tóth A., Tóth B.: **Investigation of longitudinal pattern of malacofauna and sediment characteristics with ArcInfo 9.3.** GISDATA Users Conference 2009, Opatija, 2009.
- Bódis E., Nosek J., Oertel N., Tóth B.: **A malakofauna tér-időbeli dinamikája egy kontinuum rendszer mentén.** VI. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Villány, 2009.
- Nosek J., Oertel N., Bódis E., Tóth B.: **A bentikus szervesanyag és a makrogerinctelen társulások tér- és időbeli változása a Duna Kismaros (1688 fkm) és Göd (1668 fkm) közötti szakaszán.** VI. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Villány, 2009.
- Puky M., Ács É., Bódis E., Borza P., Kiss K., Tóth, A.: **Invázív fajok a Duna magyarországi szakaszán.** 8. Magyar Ökológus Kongresszus, Szeged, 2009.
- Bódis E., Hornung E.: **A malakofauna longitudinális eloszlása egy patak-folyam kontinuum mentén.** MTA ÁTB és az ÁODI közös januári éves konferenciája, Budapest, 2009.
- Bódis E., Nosek J., Oertel N., Tóth B.: **Longitudinal distribution of malacofauna in the water system of River Danube.** Molluscan Forum, Natural History Museum, London, 2008.
- Bódis E., Nosek J., Oertel N., Tóth B.: **Longitudinal dispersion of mussel fauna in a second order stream - Danube continuum in North Hungary.** 37th International Conference of IAD, Chisinau, Moldova. 2008.
- Puky M., Ács É., Bódis E., Borza P., Kiss K. T., Tóth A.: **Invasive algae, plant, bivalve and crustacean species along the Hungarian Danube section: arrival time, colonisation characteristics, relative importance.** 37th International Conference of IAD, Chisinau, Moldova, 2008.

- Bódis E., Nosek J., Oertel N., Tóth B.: **Biomass and population dynamics in genus *Corbicula* in the Hungarian Danube section.** Neobiota: 5th European Conference on Biological Invasions, Prague, Czech Republic, 2008.
- Bódis E., Nosek J., Oertel N., Tóth B.: **A kagylófauna longitudinális eloszlása a Duna vízrendszerében.** V. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Nyíregyháza, 2008.
- Bódis E., Hornung E.: **A *Corbicula fluminea* invazív kagylófaj tér-időbeli és biomassza dinamikája a magyar Duna-szakaszon.** MTA ÁTB és az ÁODI közös januári éves konferenciája, Budapest, 2008.
- Bódis E., Hornung E., Nosek J., Oertel N.: **Spatio-temporal and biomass dynamics of *Corbicula fluminea*, an invasive mussel in the Hungarian Danube section.** World Congress of Malacology, Antwerp, 2007.
- Bódis E., Nosek J., Oertel N.: **A magyarországi Duna-szakasz kagyló faunájának vizsgálata az elmúlt évtizedben.** XLIX. Hidrobiológus Napok, Tihany, 2007.
- Bódis E.: **A *Corbicula fluminea* invazív kagylófaj biomassza dinamikája.** IV. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Tihany, 2007.
- Bódis E.: **A kistestű kagylófauna tér- és időbeli mintázata a Budapest feletti Duna-szakaszon.** IV. „MaViGe” Makroszkopikus Vízi Gerinctelenek Kutatási Konferencia, Tihany, 2007.
- Bódis E., Hornung E.: **Az invazív kagylófajok életmenet jellemzői.** III. Szünzoológiai Szimpózium, Budapest, 2007.
- Bódis E., Nosek J., Oertel N.: **Mussel fauna (*Corbiculidae*, *Dreissenidae*, *Sphaeriidae*) in the water-system of the Hungarian Danube.** 36th International Conference of the International Association for Danube Research (IAD), Klosterneuburg & Vienna, 2006.

Acknowledgement

I would like to express my gratitude to my supervisors, to Dr. Erzsébet Hornung, and to Dr. Nosek János for their personal and professional support.

I am grateful to Dr. Gyula Huszenyicza and Dr. Miklós Rusvai, the former and the current head of SZIE Postgraduate School of Veterinary Science for the opportunity to fulfil my PhD

I would like to express my thanks to Dr. Nándor Oertel and Dr. Tihamér Keve Kiss, the former head of Hungarian Danube Research Station, and to Dr. Gábor Guti, the current head of the Danube Research Institute to provide the infrastructural conditions of my PhD work.

Special thanks to Dr. Oertel Nándor, Dr. Fehér Zoltán, Dr. Ronaldo Gomes de Sousa, Dr. Majoros Gábor, and Dr. Sólymos Péter for their valuable help and advices.

I would like to acknowledge Dr. Thomas von Rintelen for the genetic analysis of *Corbicula* samples, and Tóth Bence for analysing the distribution of bed sediment fractions and the organic matter content of bed sediment.

Furthermore thanks to Bodrogi Berta, Borza Péter, Pohner Zsuzsanna, Sipkay Csaba, Tóth Adrien, Tóth Bence, Tóth Balázs and Tumbász Anett who provided help in the field or laboratory work.

This work was supported by a grant (OTKA T 046180) from the Hungarian Scientific Research Fund.