

Momir Paunović, Béla Csányi, Igor Stanković, Wolfram Graf, Patrick Leitner, Vinzenz Bammer, Thomas Huber, József Szekeres and Péter Borza



10.1 Introduction

Aquatic ecosystems are exposed to the influence of non-indigenous (non-native, alien or exotic) species. The Danube River is not an exception. Non-indigenous species were recorded among algae, aquatic macrophytes, macroinvertebrates and fish. Also, introduction of allochthonous fish species caused introduction of new fish parasites (Djikanovic et al. 2012).

The pressure caused by biological invasions has already been documented for the Danube River and its main tributaries (Literáthy et al. 2002, Csányi 2002, Csányi & Paunović 2006, Liška et al. 2008). The Danube River is a part of the Southern Invasive Corridor (Panov et al. 2009). The Southern Corridor links the Black Sea with the North Sea basin *via* the Danube-Main-Rhine waterway including the Main-Danube Canal. Thus, the Danube River is a part of one of the main routes for the migration of aquatic organisms in Europe, including the non-native species and consequently the river is exposed to high potential pressure from biological invasions.

The aim of this chapter is to present the state of the art in respect to presence of non-native aquatic species (aquatic macrophytes, aquatic macroinvertebrates and fish) in the Danube River based on Joint Danube Survey 3 (JDS3) results. Also, the present situation is compared with prior, based on previous Danube Surveys.

10.2 Methods

The study on presence and abundance of non-indigenous taxa was done based on additional analysis of datasets obtained for each biological quality element surveyed during Joint Danube Survey 3 – for details, please see Chapters 5, 7 and 9 of this report.

In addition, free diving collection was done to collect information on relative abundance of non-indigenous freshwater mussel species.

For supplementary collection of crayfish species, LiNi crayfish traps with appropriate bite (small fish, wet cat food or fresh liver, etc.) were used. At selected sites, 5-15 traps were positioned for more than 5 hours during the night at different depths and bottom types, thus covering majority of possible finding places (activity areas).

The review of neophytes along the Danube River comprised, beside non-native aquatic macrophytes, the vegetation of bank habitats.

The evaluation method presented below is not accomplished to estimate the level of biological invasion in the Danube countries and is therefore not comparable to national data on the invasion of alien species. The evaluation of invasive species is not included in the ecological assessment of surface waters according to Water Frame Directive.

In order to estimate the level of biological invasions we used the Site-specific Biological Contamination (SBC) Index (Arbačiauskas et al. 2008). SBC index estimates biological contamination (means the presence of alien species regardless of their abilities to cause negative ecological and/or socio-economic impacts) of the specific sites. It is used for comparison of biological contamination of

different locations and for estimation. Site-specific Biological Contamination (SBC) involves both the specific value of number of alien species and the specific value of an abundance of alien species in the total community by using the formula:

$$SBC = (n_a / n_{sum} + \log N_a / \log N_{sum}) / 2,$$

where n_a is a number of alien species, n_{sum} a number of all species in the sample, N_a abundance of alien species and N_{sum} total abundance of fish in the sample. For the calculation of SBC, the results of macroinvertebrate and fish JDS3 surveys were used. JDS2 datasets on macroinvertebrates (Liška et al. 2008) were additionally used to calculate SBC and compare the level of biological contamination over the time.

SBC index was calculated using ranking presented in Table 18, based on samples from the shore region only, in order to make the data comparable with JDS2 results. The index ranges from 0 to 4 and the following classification scale was used (modified original scale proposed by Arbačiauskas et al. 2008):

- 0 (no biocontamination, no pressures caused by biological invasions)
- 1 (low biocontamination, minor pressures caused by biological invasions)
- 2 (moderate biocontamination, moderate pressures caused by biological invasions)
- 3 (high biocontamination, high pressures caused by biological invasions)
- 4 (severe biocontamination, high pressures caused by biological invasions).

Table 18: Scoring scheme for SBC

Taxa Richness Contamination%	Abundance Contamination%					
	0	>0 – <10	>10-20	21-50	>50	
0	0					
>0 – <10		1	2	3	4	
>0 - <10 >10-20		2	2	3	4	
21-50		3	3	3	4	
>50		4	4	4	4	

The results were discussed in relation to basic sectioning of the Danube River to Upper, Middle and Lower Danube, as defined in Literathy et al. (2002) and Liška et al. (2008).

10.3 Results

During the JDS3 a considerable number of non-native aquatic species was recorded. Non-indigenous taxa were identified within aquatic macrophytes, macroinvertebrates and fish.

10.3.1 Neophyta

Neophytes are plant species which are non-native to a geographical region but they were introduced in recent history. They are present all around the world and the Danube is certainly not a river that neophytes would avoid. On the contrary, historical investigation shows presence of neophytes throughout the whole stretch of the Danube. They invade main channel, side channels and standing water next to the main river (Janauer and Steták 2003, Sipos et al. 2003, Valchev et al. 2006).

Besides of many individual researches of the Danube vegetation where neophytes were recorded, they were identified in both Joint Danube Survey 1 and 2 expeditions as well (Literathy et al. 2002, Liška et al. 2008).

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During Joint Danube Survey 3, out of 25 recorded neophytes, four of them belonged to aquatic macrophytes: *Azolla filiculoides* Lam., *Elodea nuttallii* (Planch.) H.St.John, *Lemna turionifera* Landolt and *Vallisneria spiralis* L. (Table 19). Other species belonged to the bank vegetation as species of open and ruderal habitats like *Xanthium strumarium* L. that was dominating on Romanian and Bulgarian banks of the Danube and sometimes pushed out other helophytes, or *Amaranthus blitum* L. dominating on the banks downstream Belgrade.

Most of the listed species are aggressive and fast spreading. Two species of neophyte trees recorded during JDS3 share the same characteristics, *Robinia pseudoacacia* L. and *Ailanthus altissima* (Mill.) Swingle. Hanover and Mebrahtu (1991) reported that *R. pseudoacacia* is adaptable to environmental extremes such as drought, air pollutants and high light intensities and that it easily propagates by seed, coppice and root suckers. It is an aggressive, thorny pioneer species and as such presents a threat to native riparian vegetation.

Among macrophytes, *V. spiralis* was the most abundant neophyte during this survey, while other species were found only occasionally. It was first recorded in Hungary upstream Budapest in Szob. Later on it was sporadically present in Danube around the tributary Velika Morava, but also very abundant downstream the Iron Gate (Derdap) Reservoir.

Table 19: List of neophyte species recorded during JDS3

Species	Origin
Abutilon theophrasti Medik.	Southern Asia
Ailanthus altissima (Mill.) Swingle	East Asia
Amaranthus blitum L.	Mediterranean region
Ambrosia artemisiifolia L.	North America
Amorpha fruticosa L.	North America
Asclepias syriaca L.	North America
Azolla filiculoides Lam.	Subtropical America
Bidens frondosa L.	North America
Buddleja davidii Franch.	East Asia
Datura stramonium L.	North and South America
Echinocystis lobata (Michx.) Torr. & A. Gray	North America
Eclipta prostrata (L.) L.	Tropics, Subtropics
Elodea nuttallii (Planch.) H.St.John	North America
Fallopia japonica (Houtt.) Ronse Decr.	East Asia
Helianthus annuus L.	North America
Impatiens glandulifera Royle	Asia
Impatiens parviflora DC.	Eurasia
Lemna turionifera Landolt	North America, East Asia
Robinia pseudoacacia L.	North America
Rudbeckia hirta L.	North America
Solidago canadensis L.	North America
Symphyotrichum lanceolatum (Willd.) G.L.Nesom	North America
Vallisneria spiralis L.	Tropics, Subtropics
Xanthium spinosum L.	South America
Xanthium strumarium L.	North America

10.3.2 Non-native aquatic macroinvertebrates

The list of non-native macroinvertebrate species recorded during the JDS3 is presented in Table 20.

Out of 34 non-native taxa recorded during the JDS3, crustaceans are the most numerous, with 19 species – Amphipoda 13, Mysida 3, Isopoda 1 and Decapoda 2. Eight alien molluscs (Bivalvia 5 and Gastropoda 3) and four annelids (Oligochaeta 2 and Polychaeta 2) were recorded.

A considerable number of alien species were recorded in the Upper and Middle section of the Danube – 24 and 27 species, respectively. Having in mind that the majority of the species identified as non-native for the Upper and Middle section of the Danube are of Ponto-Caspian origin, those species are considered as native for the Lower stretch of the Danube and thus, only seven non-native taxa were found for the Lower Danube section (marked with * – Table 20).

Table 20: Non-native macroinvertebrates recorded during the JDS3

Species Bryozoa Pectinatella magnifica (Leidy 1851)* Turbellaria Dendrocoelum romanodanubiale (Codreanu 1949) Polychaeta Hypania invalida (Grube, 1860) Manayunkia caspica (Annenkova, 1928) Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902) Bivalvia	Ponto-Caspian Ponto-Caspian Ponto-Caspian Ponto-Caspian Indo-Pacific	
Pectinatella magnifica (Leidy 1851)* Turbellaria Dendrocoelum romanodanubiale (Codreanu 1949) Polychaeta Hypania invalida (Grube, 1860) Manayunkia caspica (Annenkova, 1928) Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)	Ponto-Caspian Ponto-Caspian Ponto-Caspian Indo-Pacific	
Turbellaria Dendrocoelum romanodanubiale (Codreanu 1949) Polychaeta Hypania invalida (Grube, 1860) Manayunkia caspica (Annenkova, 1928) Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)	Ponto-Caspian Ponto-Caspian Indo-Pacific	
Polychaeta Hypania invalida (Grube, 1860) Manayunkia caspica (Annenkova, 1928) Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)	Ponto-Caspian Ponto-Caspian Indo-Pacific	
Hypania invalida (Grube, 1860) Manayunkia caspica (Annenkova, 1928) Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)	Ponto-Caspian Indo-Pacific	
Manayunkia caspica (Annenkova, 1928) Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)	Ponto-Caspian Indo-Pacific	
Oligochaeta Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)	Indo-Pacific	
Branchiura sowerbyi (Beddard, 1892)* Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)		
Potamothrix moldaviensis (Vejdovsky and Mrazek, 1902)		
	Dente Occaion	
Rivalvia	Ponto-Caspian	
Corbicula fluminea (O. F. Müller, 1774)*	East Asia	
Corbicula fluminalis (O. F. Müller, 1774)*	East Asia	
Sinanodonta woodiana (Lea, 1834)*	East Asia	
Dreissena polymorpha (Pallas, 1771)	Ponto-Caspian	
Dreissena bugensis (Andrusov, 1897)*	Pontic	
Gastropoda		
Physella acuta (Draparnaud, 1805)*	North America	
Potamopyrgus antipodarum (J. E. Gray, 1853)*	New Zealand	
Potamopyrgus antipodarum f. carinata (J. T. Marshall, 1889)	New Zealand	
Decapoda		
Orconectes limosus (Rafinesque, 1817)	North America	
Pacifastacus leniusculus (Dana, 1852)	North America	
Amphipoda		
Chelicorophium robustum (G. O. Sars, 1895)	Ponto-Caspian	
Chelicorophium curvispinum (G. O. Sars, 1895)	Ponto-Caspian	
Chelicorophium sowinskyi (Martynov, 1924)	Ponto-Caspian	
Echinogammarus ischnus (Stebbing, 1899	Ponto-Caspian	
Echinogammarus trichiatus (Martynov, 1932)	Ponto-Caspian	
Gammarus roeseli (Gervais, 1835)		
Obesogammarus obesus (G. O. Sars, 1894)	Ponto-Caspian	
Obesogammarus crassus (G. O. Sars, 1894)	Ponto-Caspian	
Pontogammarus sarsi (G. O. Sars, 1894)	Ponto-Caspian	
Dikerogammarus villosus (Sowinsky, 1894)	Ponto-Caspian	
Dikerogammarus haemobaphes (Eichwald, 1841)	Ponto-Caspian	
Dikerogammarus bispinosus (Martynov, 1925)	Ponto-Caspian	
Mysida		
Limnomysis benedenii (Czerniavsky, 1882)	Ponto-Caspian	
Katamysis warpachowsky (G. O. Sars, 1877)	Ponto-Caspian	
Paramysis lacustris (Czerniavsky, 1882)	Ponto-Caspian	
Niphargus hrabei (S. Karaman, 1932)	Ponto-Caspian	
Isopoda		
Jaera istri (Vieuille, 1979)	Ponto-Caspian	
Trichoptera		
Cladotanytarsus conversus (Johannsen, 1932)	Southeast Asia	

Among non-native macroinvertebrates, taxa of North American (4), Asian (4) of New Zealand (2) and Indo-Pacific (1) origin were identified, but spreading of Ponto-Caspian species from the Lower to the Middle and Upper Danube was found to be the most frequent case – 22 taxa of Ponto-Caspian original distribution were identified during the JDS3.

During the JDS3, the North American freshwater bryozoans species *Pectinatella magnifica* (Leidy 1851) (Bryozoa: Phylactolaemata: Plumatellida; common names: magnificent bryozoan or moss animal) was recorded for the first time in the main course of the Danube (Zorić et al. 2014). After the initial detection of the magnificent bryozoan in the Rackeve-Soroksar Danube side Arm in 2011 (Szekeres et al. 2013), the species rapidly colonized a 900 km long stretch of the Danube, between river kilometres 1586 (Hungary, downstream Budapest) and 685 (Romanian-Bulgarian stretch of the Danube). Beside, spreading of *Manayunkia caspica* Annenkova, 1929, a Ponto-Caspian species, was recorded in the Middle Danube – sites 16-21.

Crustaceans of Ponto-Caspian origin *C. curvispinum*, *D. villosus* (Amphipoda) and *J. istri* (Isopoda), as well as molluscs species of Asian origin *C. fluminea* (Bivalvia) were found to be the most abundant and frequent non-native macroinvertebrate taxa along the entire Danube. Thus, mean abundance of *D. villosus* was 529 ind./m² for the Upper Danube and 431 ind./m² for the Middle Danube, while the abundance of *C. curvispinum* was 247 ind./m² for the Upper Danube and 310 ind./m² for the Middle Danube (both species native for the Lower Danube).

The significant influence of non-native taxa to the Danube ecosystems is well illustrated by mean percentage participation of alien macroinvertebrates within the three main Danube sections (Figure 76).

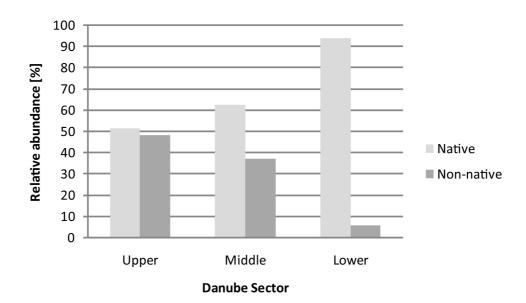


Figure 76: Mean percentage participation of native and non-native aquatic macroinvertebrate species within the three main Danube sections

10.3.3 Non-native fish species

During the JDS3, a total of 12 non-native fish species were recorded (Table 21).

Eight alien taxa were recorded for the Upper Danube, 9 for the Middle, while only 4 species that are non-native were identified in the Lower section of the Danube (marked with * – Table 21).

As in the case of aquatic macroinvertebrates, fish species that are non-native for the Middle and Upper Danube of the Ponto-Caspian origin were the most numerous – 5 species. Beside, species of Asian (4 taxa) and North American origin (3 taxa) were recorded.

Based on the share of non-native species in total fish community abundance (Figure 77), the Upper stretch of the Danube is exposed to higher pressure of biological invasions.

Table 21: Non-native fish species recorded during the JDS3

Species	Origin	
Carassius gibelio (Bloch, 1783)*	Asia	
Gasterosteus aculeatus (Linnaeus, 1758)	North America, Europe	
Hypophthalmichthys molitrix (Valenciennes, 1844)	Asia	
Lepomis gibbosus(Linnaeus, 1758)*	North America	
Neogobius fluviatilis (Pallas, 1814)	Ponto-Caspian	
Babka gymnotrachelus (Kessler, 1857)	Ponto-Caspian	
Neogobius kessleri (Günther, 1861)	Ponto-Caspian	
Neogobius melanostomus (Pallas, 1814)	Ponto-Caspian	
Oncorhynchus mykiss (Walbaum, 1792)	North America	
Perccottus glenii (Dybowski, 1877)*	Asia	
Proterorhinus semilunaris (Heckel, 1837)	Ponto-Caspian	
Pseudorasbora parva (Temminck et Schlaegel, 1842)*	Asia	

10.3.4 Assessment of the level of biocontamination

Data on aquatic macroinvertebrates and fish from the JDS3 were used to evaluate the present situation, while results of macroinvertebrate survey from the JDS2 were also employed in order to compare the situation over the time – six year period, 2007-2013.

The results of calculation of SBC were presented in Table 22.

Table 22: SBC Index results

	SBC MZB 2013 (JDS3)	SBC Index fish 2013 (JDS3)	SBC MZB 2007 (JDS2)
Overall Range	0-4	1-2	0-4
Overall mean	3	2	4
Upper Danube Range	2-4	2-4	0-4
Upper Danube Mean	3	4	4
Middle Danube Range	1-4	1-3	3-4
Middle Danube Mean	3	2	4
Lower Danube Range	0-4	1-4	2-4
Lower Danube Mean	1	1	3

Based on the SBC calculation for macroinvertebrate dataset, the index ranged from 0 (no biocontamination) to 4 (severe biocontamination).

Based on calculated SBC Index for 2013 (JDS3) for macroinvertebrates and fish, the level of biocontamination of entire section of the Danube River covered by the investigation could be assessed as moderate to high. Both communities that were used for calculation (macroinvertebrates and fish) showed higher level of biocontamination for the Upper (high to severe biocontamination) and Middle Danube (moderate to high biocontamination), in compare to the Lower Danube (low biocontamination).

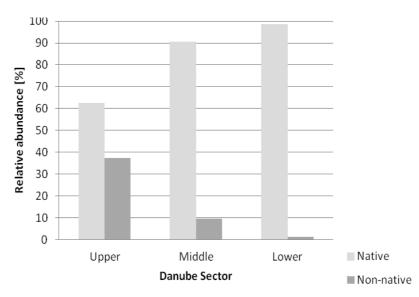


Figure 77: Mean percentage participation of native and non-native fish species within the three main Danube sections

10.3.5 Comparison with previous results

The number of non-native species recorded during the previous Danube Surveys is presented in Table 23. As it could be seen from Table 23, considerable number of non-indigenous taxa is recorded at each sampling occasion. The rise of number of identified alien macroinvertebrate taxa over the period 2001-2013 is evident. Having in mind that Danube Surveys have been planned with aim to provide comparable datasets, the recorded pattern concerning the number of non-indigenous macroinvertebrate taxa over the time realistically illustrates the situation.

The number of non-native taxa within other biological quality elements is relatively constant over the period covered.

Although the number of recorded alien MZB taxa is higher in 2013 (JDS3) when compared to those recorded in 2007 (JDS2), the comparison of the level of biocontamination based on the results of JDS3 (2013) and JDS2 (2007) surveys (Table 22) indicated lower level of biocontamination in 2013, mostly due to reduced participation of non-native taxa in total abundance of macroinvertebrate community.

Table 23: Review of number of non-native taxa recorded during the previous Danube Surveys:

JDS1 (Literáthy et al. 2002), Aquaterra Danube Survey (ADS – Csányi & Paunović 2006)

and JDS2 (Liška et al., 2008) and JDS3. Number in brackets denote additional
criptogenic species

Quality element	JDS1 (2001)	ADS (2004)	JDS2 (2007)	JDS3 (2013)
Aquatic macrophytes	3	-	6	4
Macroinvertebrates	12	13	20	34
Fish	-	-	14 (1)	12 (1)

The results of comparison of the results of the Danube expeditions in period 2001-2013 showed that there is a continuous influence caused by biological invasions. New species of alien aquatic macroinvertebrates have been recorded over the time. Incomers sometime occupy considerable area over a short time period – e.g. in the case of *P. magnifica*. Bioinvasion process is complex and presented results pointed to variation of relative participation of non-indigenous taxa in total community, which is illustrated by the values of SBC index.

10.4 Conclusions

Based on the results of JDS3, the Danube River is significantly exposed to influence of non-native species.

Twenty five neophytes (4 aquatic), 34 non-native aquatic macroinvertebrates and 12 non-native fish species were recorded during the JDS3 survey.

The level of biocontamination of the section of the Danube River covered by the investigation was estimated as moderate to high, with higher level of biocontamination for the Upper (high to severe biocontamination) and Middle Danube (moderate to high biocontamination), in comparison to the Lower Danube (low biocontamination).

The overview of the situation of bioinvasions over the period 2001-2013, based on the results of four Danube Surveys (JDS1 – 2001, ADS – 2004, JDS2 – 2007 and JDS3 – 2013), clearly showed constant influence of alien species to native biota and considerable rise of number of non-native aquatic macroinvertebrate species. Thus, during the JDS3 (2013), 22 more alien macroinvertebrate species were recorded when compared with JDS1 (2001). Although the number of recorded alien species raised over the time, non-native species are found to be less dominant in 2013 (JDS3) when compared to 2007 (JDS2), which resulted in lower level of biocontamination in 2013.

The results of JDS3 show that biological invasions within the Danube River Basin should be properly managed. This implies that further work has to be done in the fields of collecting of basic information on the distribution of alien species and their influence on native biota, developing effective tools for the assessment of the level of pressures caused by the bioinvasions, as well as designing the appropriate mitigation measures.

It is important to evaluate accurately and rationally the real pressure of each invader to native ecosystems, because its influence on the native biota should not be considered *a priori* as negative.

10.5 References

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