Invasive alien species of benthic macroinvertebrates and fish in the Bulgarian sector of the Danube River – results of the Joint Danube Survey 4 (JDS4)

Teodora Trichkova[1*], Milcho Todorov[1], Marian Kenderov[2], Zdravko Hubenov[3], Ivan Botev[1], Tihomir Stefanov[3], Dilian Georgiev[4], Pavel Jurajda[5]

Supplementary Materials

Table S1. Study sites with indicated geographic coordinates and the Danube River kilometres (rkm), sorted by dates and types of water body. The sites visited more than once have the same number with the number of visits in brackets.

No.	Date	Site	Latitude	Longitude
1		Danube River		
1.1	21.07.2019	Danube River at Lom Town (744 rkm)	N 43.83110	E 23.22232
1.2	22.07.2019	Danube River at Tsibar Island (720 rkm)	N 43.81211	E 23.54578
1.3	22.07.2019	Danube River at Dolni Tsibar Village (717.5 rkm)	N 43.82018	E 23.51588
1.4	23.07.2019	Danube River upstream of Kozloduy Town, at Asparuhov Val PS (708 rkm)	N 43.78928	E 23.63410
1.5	23.07.2019	Danube River upstream of Oryahovo Town (682 rkm)	N 43.74383	E 23.91162
1.6	23.07.2019	Danube River at Esperanto Island (673 rkm)	N 43.72509	E 24.01829
1.7	23.07.2019	Danube River at Zagrazhden Village (625 rkm)	N 43.75154	E 24.57059
1.8	24.07.2019	Danube River downstream of Nikopol Town (596 rkm)	N 43.70980	E 24.91159
1.9	24.07.2019	Danube River at Lakat Island (590 rkm)	N 43.72429	E 24.97964
1.10	24.07.2019	Danube River at Belene Town, Hisarlaka Fishery Camp (577 rkm)	N 43.68143	E 25.10160
1.11	24.07.2019	Danube River at Golyama Barzina Island (572 rkm)	N 43.69450	E 25.16241
1.12	24.07.2019	Danube River downstream Svishtov Town, Pametnitsite Site (550 rkm)	N 43.61617	E 25.39962
1.13	25.07.2019	Danube River at Vardim Island (547 rkm)	N 43.625592	E 25.449188
1.14	25.07.2019	Danube River at Batin Island (526 rkm)	N 43.67141	E 25.68449
1.15(1)	25.07.2019	Danube River upstream of Ruse Town, at Prista Site (501 rkm)	N 43.81417	E 25.91844
1.16	25.07.2019	Danube River at Lyulyak Island (505 rkm)	N 43.78053	E 25.88602
1.17	25.07.2019	Danube River at Marten Village; JDS47/488 rkm	N 43.91080	E 26.06678
1.18	26.07.2019	Danube River at Aleko Island (478 rkm)	N 43.94184	E 26.08575
1.19	26.07.2019	Danube River at Ryahovo Village (464 rkm)	N 43.99608	E 26.24578
1.20	26.07.2019	Danube River at Mishka Island (461 rkm)	N 44.00839	E 26.28380
1.21	26.07.2019	Danube River at Golyam Brashlen Island (454 rkm)	N 44.03430	E 26.36030
1.22	26.07.2019	Danube River at Kalimok Island (441 rkm)	N 44.04332	E 26.50417

1.23	26.07.2019	Danube River at Tutrakan Town (431.5 rkm)	N 44.05475	E 26.62349
1.24	27.07.2019	Danube River at Kosuy Island (428 rkm)	N 44.06047	E 26.66025
1.25	27.07.2019	Danube River at Malak Preslavets Lake, upstream of the outflow canal (414 rkm)	N 44.09895	E 26.82616
1.26	27.07.2019	Danube River at Vetren Village (394 rkm)	N 44.13641	E 27.05216
1.27	12.08.2019	Danube River at Novo Selo Village (834 rkm)	N 44.16429	E 22.78660
1.28	12.08.2019	Danube River at Vrav Village (839)	N 44.19318	E 22.74367
1.29	13.08.2019	Danube River at Yasen Village (825 rkm)	N 44.12527	E 22.87619
1.30	13.08.2019	Danube River upstream of Koshava Village (811 rkm)	N 44.07990	E 23.03066
1.31	13.08.2019	Danube River at Koshava Island, southern bank (807 rkm)	N 44.05939	E 23.03489
1.32	13.08.2019	Danube River at Koshava Island, northern bank (807 rkm)	N 44.05951	E 23.03838
1.33	14.08.2019	Danube River at Ratsiaria Site (772 rkm)	N 43.82766	E 22.89036
1.34	14.08.2019	Danube River at Tsar Simeonovo Village (776 rkm)	N 43.85220	E 22.84474
1.35(1)	15.08.2019	Danube River, open canal at Vidin Town (786 rkm)	N 43.94421	E 22.85864
1.36	08.2019	Danube River at the port, upstream of Archar Village (771 rkm)	N 43.823964	E 22.901172
1.37	08.2019	Danube River at the mouth of the Archar River (769.5 rkm)	N 43.820372	E 22.930814
1.38	04.10.2019	Danube River at Ruse Town, near Riga Hotel (495 rkm)	N 43.85355	E 25.95005
1.39	04.10.2019	Danube River at Ruse Town, Port West (496 rkm)	N 43.84353	E 25.94225
1.15(2)	05.10.2019	Danube River upstream of Ruse Town, at Prista Site (501 rkm)	N 43.81430	E 25.91847
1.35(2)	23-24.10.2019	Danube River, open canal at Vidin Town (786 rkm)	N 43.94416	E 22.85888
2		Danube tributaries		
2.1	22.07.2019	Lom River at Lom Town	N 43.81881	E 23.25126
2.2	23.07.2019	Ogosta River, between Glozhene Village and Mizia Town	N 43.69156	E 23.82571
2.3	23.07.2019	Iskar River, the confluence with the Danube River	N 43.72838	E 24.44702
2.4	25.07.2019	Yantra River, 300 m upstream of the confluence with the Danube River	N 43.63867	E 25.56987
2.5	14.08.2019	Archar River upstream of Archar Village	N 43.80724	E 22.89582
2.6	14.08.2019	Archar River downstream of Darzhanitsa Village	N 43.81007	E 22.85130
2.7	15.08.2019	Topolovets River downstream of Dolni Boshnyak Village	N 44.01139	E 22.63338
2.8(1)	16.08.2019	Topolovets River, the bridge on the road Vidin – Sofia	N 43.94110	E 22.83911
2.9	16.08.2019	Voinishka River at Dunavtsi Village	N 43.92039	E 22. 82554
2.10	16.08.2019	Vidbol River at Dunavtsi Village	N 43.90360	E 22. 80586
2.11	26.08.2019	Yantra River between the villages Skalsko and Slaveikovo	N 42.98557	E 25.36796
2.12	26.08.2019	Yantra River at Pushevo Village	N 43.07445	E 25.47426
2.13	27.08.2019	Yantra River at Ledenik Village	N 43.07578	E 23.55247
2.14	27.08.2019	Belitsa River, the bridge for Plakovo Village	N 42.99263	E 25.63962

2.15	27.08.2019	Zlatarishka River upstream of Zlataritsa Village	N 43.05019	E 25.86643
2.16	27.08.2019	Stara Reka River at Slivovitsa Village	N 43.08403	E 26.00662
2.17	28.08.2019	Rositsa River at Lesicheri Village	N 43.20986	E 25.42173
2.18	28.08.2019	Rositsa River at Dichin Village	N 43.20985	E 25.48206
2.19	28.08.2019	Rositsa River at Vodoley Village	N 43.20757	E 25.50103
2.20	28.08.2019	Rositsa River at Resen Village	N 43.20870	E 25.55727
2.21	29.08.2019	Yantra River at Dolna Oryahovitsa Town	N 43.15312	E 25.73070
2.22	29.08.2019	Stara Reka River downstream of Gorski Dolen Trambesh Village	N 43.20131	E 25.83354
2.23	29.08.2019	Yantra River at Draganovo Village	N 43.20678	E 25.73699
2.24	30.08.2019	Yantra River, the bridge near Krivina Village	N 43.61295	E 25.59129
2.25	30.08.2019	Yantra River at Belyanovo Village	N 43.61444	E 25.61160
2.26	30.08.2019	Yantra River at Byala Town, the bridge of Master Kolyu Ficheto	N 43.46976	E 25.72474
2.8(2)	23.10.2019	Topolovets River, the bridge on the road Vidin – Sofia	N 43.94110	E 22.83911
2.27	24.10.2019	Archar River downstream of Archar Village	N 43.81326	E 22.92151
2.28	24.10.2019	Archar River upstream of the confluence with the Danube River	N 43.82011	E 22.92597
3		Lakes and canals		
3.1	22.07.2019	Canal at Dolni Tsibar Village	N 43.81918	E 23.51713
3.2	26.07.2019	Canal at Brashlen Village	N 44.00691	E 26.33039
3.3	26.07.2019	Canal at Kalimok Village	N 44.02653	E 26.51704
3.4	27.07.2019	Outflow canal of Malak Preslavets Lake	N 44.09932	E 26.82974
3.5	27.07.2019	Malak Preslavets Lake, northern shore	N 44.09963	E 26.83108
3.6	28.07.2019	Srebarna Lake, at the pier (Site 1)	N 44.10321	E 27.06375
3.7	28.07.2019	Srebarna Lake, at the road (Site 2)	N 44.08977	E 27.07323
3.8	22.10.2019	Canal at Dobri Dol Village	N 43.77578	E 23.00478
4		Reservoirs		
4.1	22.07.2019	Kovachitsa Reservoir	N 43.78812	E 23.35488
4.2	15.08.2019	Farfun Reservoir	N 43.81228	E 22.66023
4.3	15.08.2019	Boinitsa Reservoir	N 43.92091	E 22.52078
4.4	15.08.2019	Kalina Reservoir	N 44.07045	E 22.76708
4.5	26.08.2019	Bilyakovets Lake	N 42.96346	E 25.22409
4.6	25.10.2019	Ogosta Reservoir, Site 1 (at the road)	N 43.39169	E 23.17000
4.7	26.10.2019	Ogosta Reservoir, Site 2 (Rasnika)	N 43.38527	E 23.18206

1. Danube River

Table S2. Benthic macroinvertebrates: crayfish and molluscs (Gastropoda and Bivalvia) recorded in the Bulgarian sector of the Danube River during JDS4 (2019). dF (%) – frequency of occurrence; RA (%) – relative abundance.

Species	dF (%) (up to 2 m depth)	RA (%) Crayfish (up to 2 m depth)	RA (%) Molluscs (up to 2 m depth)	dF (%) (2–4.5 m depth)	RA (%) Molluscs (2–4.5 m depth)
Decapoda					

Pontastacus leptodactylus	10.71	57.14			
Faxonius limosus	3.57	42.86			
Gastropoda					
Theodoxus danubialis	71.43		5.69	18.18	0.91
Theodoxus fluviatilis	78.57		8.07	18.18	0.52
Theodoxus transversalis	3.57		0.02	9.09	0.13
Viviparus acerosus	21.43		1.19		
Viviparus sphaeridius	25.00		2.98		
Viviparus viviparus	10.71		0.08		
Esperiana esperi	64.29		13.94	18.18	0.65
Microcolpia daudebartii	46.43		6.78	9.09	0.13
Holandriana holandrii	50.00		2.69	9.09	0.39
Bithynia danubialis	3.57		0.12		
Bithynia tentaculata	21.43		1.01	27.27	2.21
Lithoglyphus naticoides	32.14		49.82	9.09	0.26
Radix auricularia	7.14		0.66		
Physella acuta	3.57		0.10		
Planorbarius corneus	3.57		0.02		
Bivalvia					
Pseudanodonta complanata	3.57		0.02		
Sinanodonta woodiana	17.86		0.56		
Unio crassus	7.14		0.04		
Unio pictorum	28.57		0.47	9.09	0.13
Unio tumidus	10.71		0.14	9.09	0.26
Dreissena rostriformis bugensis	14.29		0.12	27.27	14.57
Dreissena polymorpha	25.00		1.13	36.36	3.38
Corbicula fluminea	53.57		4.35	90.91	76.46
		100	100		100

Table S3. Fish species recorded in the Bulgarian sector of the Danube River during JDS4 (2019). dF (%) – frequency of occurrence; RA (%) – relative abundance; CPUE – catch-per-unit effort 100 m beach seining.

Species	**dF%**	**RA (%) beach seine**	**Mean CPUE 100 m beach seine**	**RA (%) gill nets**
Cyprinidae				
Abramis brama	25.93	0.46	7.78	4.20
Alburnus alburnus	100.00	57.56	239.94	1.47
Ballerus ballerus	11.11	0.13	3.89	
Ballerus sapa	11.11	0.38	20.42	0.84
Barbus barbus	3.70	0.02	1.67	
Blicca bjoerkna	55.56	6.24	52.32	75.00
Carassius gibelio	37.04	0.64	7.87	0.42
Chondrostoma nasus	37.04	2.79	27.17	
Ctenopharyngodon idella				
Cyprinus carpio	44.44	2.75	22.22	

Hypophthalmichthys molitrix	3.70			0.84
Leucaspius delineatus	7.41	0.04	1.67	
Leuciscus aspius	70.37	1.45	7.37	
Leuciscus idus	44.44	0.88	8.49	0.63
Pelecus cultratus	3.70			0.21
Pseudorasbora parva	7.41	0.05	2.50	
Rhodeus amarus	18.52	0.32	7.50	
Romanogobio vladikovii	11.11	0.27	8.33	
Rutilus rutilus	62.96	5.91	41.93	12.61
Scardinius erythrophthalmus	3.70	0.05	7.50	
Squalius cephalus	11.11	0.16	7.50	0.21
Vimba vimba	22.22	0.28	5.67	1.47
Cobitidae				
Cobitis elongatoides	33.33	1.11	11.85	
Cobitis strumicae	3.70	0.02	1.67	
Siluridae				
Silurus glanis	3.70	0.02	1.67	
Esocidae				
Esox lucius	7.41	0.02	1.67	0.42
Syngnathidae				
Syngnathus abaster	18.52	0.09	1.67	
Centrarchidae				
Lepomis gibbosus	7.41	0.13	8.34	
Percidae				
Perca fluviatilis	22.22	0.13	2.33	1.68
Sander lucioperca	18.52	0.19	3.84	
Gobiidae				
Babka gymnotrachelus	29.63	0.91	10.63	
Neogobius fluviatilis	81.48	14.49	61.44	
Neogobius melanostomus	48.15	1.30	9.72	
Ponticola kessleri	44.44	1.19	9.31	
Proterorhinus semilunaris	3.70	0.02	1.67	

Table S4. Biocantamination and ecological status of studied sites in the Bulgarian sector of the Danube River during JDS4 (2019). SBCI – site-specific biocontamination index; IBCI – integrated biocontamination index of particular site or taxonomic group of animals according to the sampling method; N/A – not assessed. Ecological status is classified according to the EU Water Framework Directive 2000/60/EC Classification scheme, including the following categories: High ecological status (indicated with a blue colour); Good ecological status (green colour); Moderate ecological status (yellow colour); Poor ecological status (orange colour); and Bad ecological status (red colour).

Site	SBCI Crayfish	SBCI Molluscs (up to 2 m)	SBCI Molluscs (2–4.5 m)	SBCI Fish Beach seine 100 m	SBCI Fish Gill nets	IBCI	Ecological status

1.1	0	1	3	1		**1**	Good
1.2			4			**4**	Bad
1.3		2		0		**1**	Good
1.4		2	4	0		**2**	Moderate
1.5		0	4	0		**0**	High
1.6		4				**4**	Bad
1.7		4		0		**2**	Moderate
1.8		0		0		**0**	High
1.9			4			**4**	Bad
1.10		2		0		**1**	Good
1.11		3				**3**	Poor
1.12		1		1		**1**	Good
1.13			4			**4**	Bad
1.14	0	1		0		**0**	High
1.15	0	2		0		**0**	High
1.16			4			**4**	Bad
1.17		2	4	0		**2**	Moderate
1.18		4				**4**	Bad
1.19		3		0		**1/2**	Moderate
1.20			4	0		**2**	Moderate
1.21		3		0		**1/2**	Moderate
1.22		3		0		**1/2**	Moderate
1.23		2		2		**2**	Moderate
1.24			4	0		**2**	Moderate
1.25		0		2		**1**	Good
1.26		0	4	0		**0**	High
1.27		2		2		**2**	Moderate
1.28		0		0		**0**	High
1.29		0		0		**0**	High
1.30		0		1		**0/1**	Good
1.31				2		**2**	Moderate
1.32						N/A	N/A
1.33		0		3		**1/2**	Moderate
1.34		0		2		**1**	Good
1.35	4	3			2	**3**	Poor
1.36						N/A	N/A
1.37						N/A	N/A
1.38		3				**3**	Poor
1.39						N/A	N/A
IBCI	1	2	4	1	2	2	Moderate

2. Danube Tributaries

Table S5. Benthic macroinvertebrates: crayfish and molluscs recorded in the Danube tributaries during JDS4. dF (%) – frequency of occurrence; RA (%) – relative abundance.

Species	dF (%)	RA (%) Crayfish 100 m	RA (%) Molluscs 10 m²
Decapoda			
Pontastacus leptodactylus	10	36.36	
Faxonius limosus	20	63.64	
Gastropoda			
Lithoglyphus naticoides	5		1.77
Radix auricularia	5		0.04
Physa fontinalis	5		0.02
Physella acuta	5		0.02
Bivalvia			
Anodonta anatina	20		0.93
Sinanodonta woodiana	45		0.77
Unio crassus	30		25.14
Unio pictorum	25		0.63
Dreissena polymorpha	5		0.02
Corbicula fluminea	85		70.66
		100	100

Table S6. Fish species recorded in the Danube tributaries during JDS4. dF (%) – frequency of occurrence; RA (%) – relative abundance; CPUE – catch-per-unit effort 100 m dip net.

Species	dF%	RA (%)	CPUE dip net 100 m
Cyprinidae			
Abramis brama	5	0.10	1.00
Alburnoides bipunctatus	35	6.23	8.86
Alburnus alburnus	35	2.21	3.14
Barbus barbus	10	0.50	2.50
Barbus petenyi	55	2.91	2.64
Blicca bjoerkna	15	6.63	22.00
Carassius gibelio	35	14.94	21.29
Chondrostoma nasus	10	0.20	1.00
Cyprinus carpio	35	3.81	5.43
Gobio gobio	15	0.30	1.00
Leucaspius delineatus	10	0.80	4.00
Leuciscus aspius	5	0.30	3.00
Leuciscus idus	15	1.40	4.67

Pseudorasbora parva	15	1.30	4.33
Rhodeus amarus	70	37.71	26.86
Rutilus rutilus	30	0.70	1.17
Squalius cephalus	90	11.03	6.11
Vimba vimba	15	0.70	2.33
Cobitidae			
Barbatula barbatula	5	0.20	2.00
Cobitis elongatoides	20	0.70	1.75
Cobitis strumicae	15	0.30	1.00
Sabanejewia balcanica	15	0.40	1.33
Centrarchidae			
Lepomis gibbosus	5	0.10	1.00
Percidae			
Perca fluviatilis	10	0.20	1.00
Gobiidae			
Babka gymnotrachelus	25	1.51	3.00
Neogobius fluviatilis	20	0.70	1.75
Neogobius melanostomus	35	1.81	2.57
Ponticola kessleri	10	0.30	1.50
Proterorhinus semilunaris	40	2.01	2.50
		100	

Table S7. Biocantamination and ecological status of studied sites in the Danube tributaries during JDS4. SBCI – site-specific biocontamination index; IBCI – integrated biocontamination index of particular site or taxonomic group of animals; N/A – not assessed. Ecological status is classified according to the EU Water Framework Directive 2000/60/EC Classification scheme, including the following categories: High ecological status (indicated with a blue colour); Good ecological status (green colour); Moderate ecological status (yellow colour); Poor ecological status (orange colour); and Bad ecological status (red colour).

Site	SBCI Crayfish Dip net 100 m	SBCI Mollu scs 10 m²	SBCI Fish Dip net 100 m	IBCI	Ecological status
2.1	0	4	4	4	Bad
2.2		4	0	2	Moderate
2.3		4		4	Bad
2.4		4		4	Bad
2.5	4	4	0	4	Bad
2.6			0	0	High

2.7			0	0	High
2.8	4	4	4	4	Bad
2.9	4	4	2	4	Bad
2.10		4	3	3/4	Bad
2.11			0	0	High
2.12				N/A	N/A
2.13		4	0	2	Moderate
2.14		0	0	0	High
2.15		0	0	0	High
2.16			0	0	High
2.17			0	0	High
2.18				N/A	N/A
2.19				N/A	N/A
2.20	0	4		2	Moderate
2.21		4	2	3	Poor
2.22		4	0	2	Moderate
2.23		3	0	1/2	Moderate
2.24		4		4	Bad
2.25		4		4	Bad
2.26		4	2	3	Poor
2.8				N/A	N/A
2.27	4	4	2	4	Bad
2.28		4	3	3/4	Bad
IBCI	4	4	2	4	Bad

3. Lakes and Canals

Table S8. Benthic macroinvertebrates (Gastropoda) recorded in the standing water bodies (lakes and canals) adjacent to the Danube River during JDS4. dF (%) – frequency of occurrence; RA (%) – relative abundance.

Species	dF (%)	RA (%)
Gastropoda		
Viviparus contectus	16.67	2.27
Lymnaea stagnalis	33.33	6.81
Physa fontinalis	16.67	1.14
Physella acuta	16.67	12.5
Gyraulus laevis	16.67	26.14
Planorbarius corneus	83.33	50.00
Planorbis planorbis	16.67	1.14

		100

Table S9. Fish species recorded in the standing water bodies (lakes and canals) adjacent to the Danube River during JDS4. dF (%) – frequency of occurrence; RA (%) – relative abundance.

Species	dF (%)	RA (%)
Cyprinidae		
Alburnus alburnus	33.33	1.16
Blicca bjoerkna	33.33	4.04
Carassius gibelio	66.67	39.31
Chondrostoma nasus	16.67	2.89
Leucaspius delineatus	16.67	1.16
Pseudorasbora parva	16.67	0.58
Rhodeus amarus	33.33	10.4
Rutilus rutilus	33.33	5.2
Scardinius erythrophthalmus	16.67	1.16
Squalius cephalus	16.67	0.58
Cobitidae		
Cobitis elongatoides	16.67	7.51
Misgurnus fossilis	50.00	6.36
Ictaluridae		
Ameiurus melas	16.67	2.89
Siluridae		
Silurus glanis	16.67	3.47
Gasterosteidae		
Pungitius platygaster	16.67	5.78
Centrarchidae		
Lepomis gibbosus	33.33	1.16
Odontobutidae		
Perccottus glenii	66.67	4.04
Gobiidae		
Neogobius fluviatilis	16.67	0.58
Proterorhinus semilunaris	16.67	1.73
		100

Table S10. Biocantamination of studied sites in the standing water bodies (lakes and canals) adjacent to the Danube River during JDS4 (2019). SBCI – site-specific biocontamination index; IBCI – integrated biocontamination index of particular site or taxonomic group of animals.

Site	SBCI Molluscs (Gastropoda) Dip net 100 m	SBCI Fish Dip net 100 m	IBCI
3.1	0	3	1/2
3.2	0	3	1/2
3.3	0	4	2
3.4	0	4	2
3.5	3		3
3.6		4	4
3.7		4	4
3.8	0		0
IBCI	1	4	2/3

4. Reservoirs

Table S11. Fish species recorded in the studied reservoirs in the Danube River Basin during JDS4 (2019). dF (%) – frequency of occurrence; RA (%) – relative abundance.

Species	dF (%)	RA (%)
Cyprinidae		
Carassius gibelio	40	7.69
Squalius cephalus	20	2.80
Cobitidae		
Cobitis elongatoides	40	6.29
Ictaluridae		
Ameiurus melas	20	1.40
Syngnathidae		
Syngnathus abaster	20	9.09
Centrarchidae		
Lepomis gibbosus	80	11.89
Percidae		
Perca fluviatilis	20	5.59
Gobiidae		
Neogobius fluviatilis	60	53.15
Proterorhinus semilunaris	20	2.10
		100

Table S12. Biocantamination of studied reservoirs in the Danube River Basin during JDS4 (2019). SBCI – site-specific biocontamination index; IBCI – integrated biocontamination index of particular site or taxonomic group of animals; N/A – not assessed.

Site	**SBCI Crayfish Dip net 100 м**	**SBCI Molluscs 10 м2**	**SBCI Fish Dip net 100 м**	**IBCI**
4.1	0		3	1/2
4.2				N/A
4.3			4	4
4.4			4	4
4.5				N/A
4.6		4	3	3/4
4.7	0	4		2
IBCI	0	4	4	4