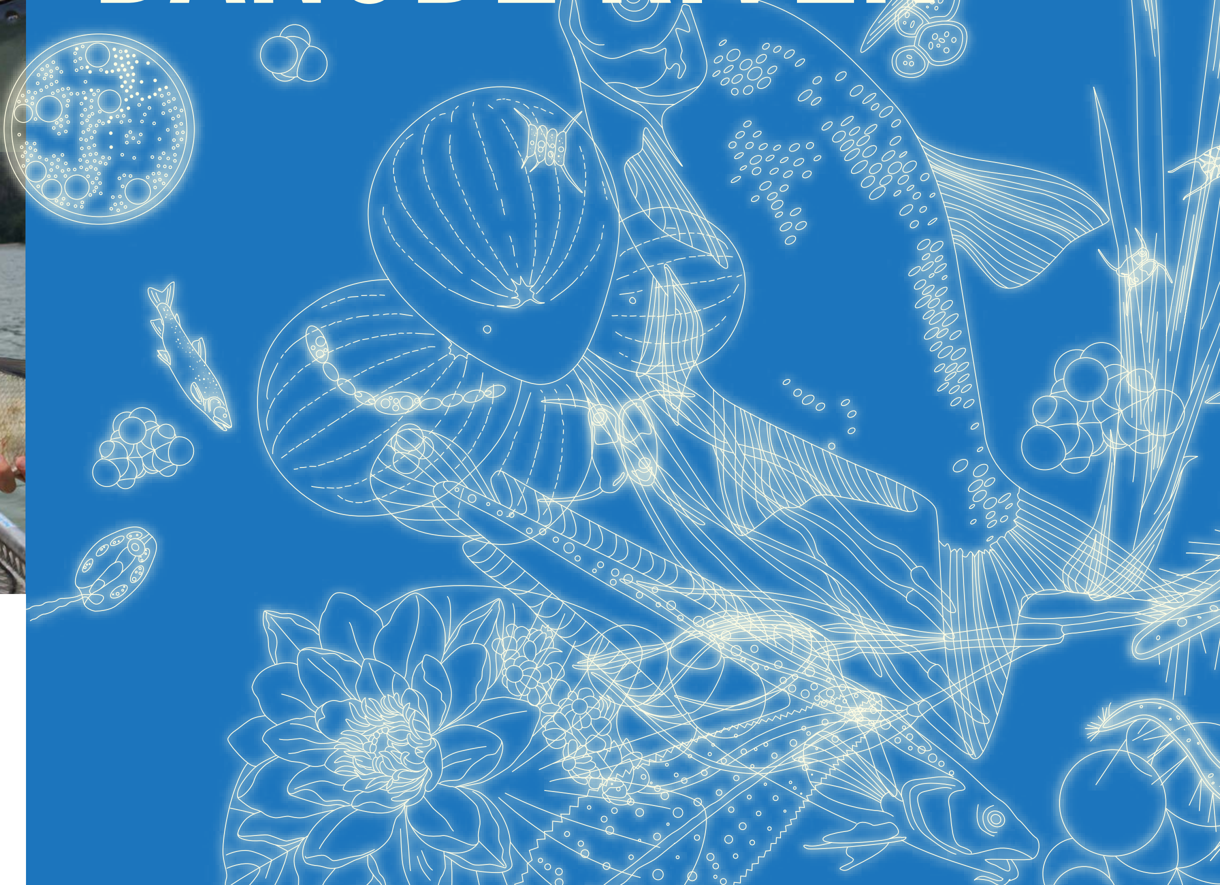
JOINT
DANUBE
SURVEY 4

JOINT DANUBE SURVEY 4 SCIENTIFIC REPORT – VOLUME 2

A SHARED ANALYSIS OF THE DANUBE RIVER



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Editors

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Microplastics in the digestive tract of two most abundant fish species in the Danube

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Abstract

The microplastic (MP) uptake by two species of fish – round goby and bleak was examined in this study. Both species belong to the most abundant fishes along the entire Danube. The round goby is a benthic species feeding predominantly on macrozoobenthos, and it is invasive in the Middle and Upper Danube, whereas bleak is a pelagic species feeding mainly on plankton, native in the entire Danube River Basin. Particles of MP were found in the digestive tract of both round goby and bleak from virtually all JDS4 sites examined. Fibres predominated considerably among the types of MP particles observed in the digestive tract of the two species of fish. Microplastics almost certainly pass through the food chains up to the top predators of the ecosystem. Nevertheless, it appears that MP particles can also be swallowed by fishes directly from the environment. The preliminary analysis of the Fulton's condition factor suggests that MP could have a negative impact on bleak. The frequency of occurrence of MP particles in the digestive tract of fishes did not follow the local quantitative parameters found in bivalves and/or sediments, though some common patterns along the longitudinal profile of the Danube seem to have emerged. In fishes, the quantity of MP appeared to have an increasing tendency downstream the Danube, especially in the tributaries, and this increase seems to culminate in the lower stretches of Middle Danube and upper stretches of Lower Danube. Digestive tracts of pelagic bleak contained significantly higher quantities of MP than those of benthic round goby. The results of this pioneer study of its kind on the Danube indicate that further, more detailed, and especially more coordinated research is necessary.

1. Introduction

Microplastics (MP) have become ubiquitous in the environment, and rivers, including the Danube, are no exception. Microplastic uptake by fish is likely to be affected by environmental MP abundance and by aspects of fish ecology, nevertheless these relationships have rarely been addressed (McNeish et al., 2018). To initiate such research in the Danube, an examination of the microplastic uptake by two species of fish – round goby *Neogobius melanostomus* (Pallas, 1814) and bleak *Alburnus alburnus* (Linnaeus, 1758) was proposed for the JDS4 project. Both species belong to the most abundant fishes along the entire Danube, and therefore, they appear to be the most suitable target species for such a pioneer study. Furthermore, the two species represent two different types in terms of their habitat preferences and feeding characteristics: round goby is a benthic species feeding predominantly on macrozoobenthos (e.g. Števove and Kováč, 2016), whereas bleak is a pelagic species feeding mainly on plankton, including crustaceans, and insects (e.g. Keckeis and Schiemer, 1990). And finally, the two species also differ in their origin – round goby is an invasive species in the Middle and Upper Danube, whereas bleak is native in the entire Danube River Basin. The main aim of this study was to evaluate the quantity and structural (shape) composition of MP in the gut content of the two target fish species from selected sites along the entire Danube and its tributaries.

2. Material and Methods

A total of 761 specimens of round goby from 20 sites, and 1,192 specimens of bleak from 29 sites all along the Danube, including some tributaries, were used for the analyses (Tables 1 and 2). The samples were collected by national teams along with samples for the evaluation of fish communities (see Bammer et al., 2021 for details). The specimens were anesthetized and killed subsequently with a MS-222-Tricaine-S solution (0.25g 1^{-1}), and preserved immediately with ice slurry. In the laboratory, the total length (TL) of the defrosted samples was measured to the nearest 1 mm using a calliper. Both total body weight and eviscerated body weight were also taken to the nearest 0.01 g with the Kern ABJ 80-4M balance (Figure 1).

Figure 1: Samples of bleak Alburnus alburnus Linneaus, 1758 processed for examination of microplastics in their digestive track. Photo: M. Príkazská.

Table 1: Samples of round goby Neogobius melanostomus (Pallas, 1814) from the Danube and its tributaries.
TL – total length, Wt – total weight, We – eviscerated weight, n – number of individuals, Med – median, SD – standard deviation.

Site code	Site name	Date	TL (mm)				Wt (g)			We (g)		
	Danube		**n**	**Range**	**Med**	**SD**	**Range**	**Med**	**SD**	**Range**	**Med**	**SD**
JDS4-6	Jochenstein	09.07.2019	83	36-96	61.0	12.16	0.73-14.48	3.64	2.88	0.63-12.73	3.26	2.63
JDS4-6.2	Jochenstein	16.07.2019	52	41-93	67.5	10.35	1.52-13.85	4.78	2.45	1.34-12.46	4.18	2.21
JDS4-8	Oberloiben	23.07.2019	32	45-107	70.0	15.28	1.7-20.73	5.50	4.45	1.43-19.05	5.03	4.08
JDS4-10	Hainburg, upstream Morava	X.X.2019	52	54-117	76.5	16.51	2.1-25.03	6.22	6.10	1.8-22.17	5.46	5.29
JDS4-14	Bratislava	01.07.2019	49	55-118	74.0	15.63	2.39-24.1	5.12	5.42	2.03-21.7	4.35	4.93
JDS4-15	Čunovo, Gabčíkovo resevoir	02.07.2019	69	47-146	86.0	18.49	1.01-53.67	10.15	9.78	0.87-48.6	8.92	8.89
JDS4-16	Medveďov / Medve	04.07.2019	35	45-112	65.0	15.20	1.31-19.75	3.72	4.25	1.08-17.5	3.37	3.74
JDS4-23	Budapest upstream	15.07.2019	80	41-140	76.0	17.81	1.31-35.11	7.43	6.00	1.08-31.67	6.75	5.41
JDS4-24	Budapest downstream	17.07.2019	22	44-96	60.0	11.74	1.33-15.77	3.80	3.25	1.15-13.86	3.38	2.75
JDS4-28	Baja	23.07.2019	33	76-115	88.0	9.81	6.49-26.59	12.45	4.48	5.92-23.31	10.94	3.96
JDS4-31	Ilok / Backa Palanka	11.07.2019	23	51-99	62.0	12.42	1.32-17.58	3.13	3.53	1.14-15.14	2.74	3.02
JDS4-37	Downstream Pančevo	07.07.2019	5	59-86	74.0	11.65	2.35-9.09	5.27	2.81	2.12-7.67	4.82	2.23
JDS4-40	Banatska Palanka / Bazias	05.07.2019	5	50-117	63.0	23.61	1.23-20.8	2.9	7.31	1.09-19.35	2.63	6.85
JDS4-47	Downstream Ruse/Giurgiu (Marten)	13.07.2019	32	41-92	59.5	12.70	1.31-14.78	3.59	2.91	1.13-13.40	3.11	2.63
	Tributaries											
JDS4-11	Pohansko	02.07.2019	49	46-112	65.0	13.24	1.51-21.42	3.93	3.72	1.34-17.29	3.32	3.26
JDS4-12	Lanžhot	02.07.2019	51	54-120	79.0	18.33	2.34-31.28	8.55	7.50	2.01-27.63	6.82	6.71
JDS4-35	Jamena	09.07.2019	2	71-102	86.5	15.50	3.89-12.71	8.3	4.41	3.49-11.74	7.615	4.13
JDS4-39	Velika Morava mouth	06.07.2019	4	68-100	91.5	11.97	3.79-13.6	9.57	3.52	3.47-12.39	8.66	3.20
JDS4-44	Iskar mouth (rkm 0.3)	27.08.2019	41	40-90	58.0	11.13	1.18-12.28	3.44	2.50	1.00-11.17	2.89	2.26
JDS4-46	Russenski Lom mouth	28.08.2019	42	37-88	66.0	8.85	0.87-11.41	4.88	2.02	0.75-10.16	4.30	1.81

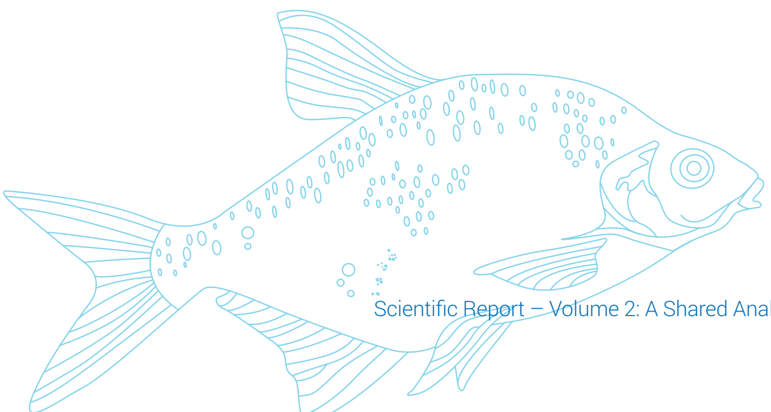

Table 2: Samples of bleak Alburnus alburnus Linneaus, 1758 from the Danube and its tributaries. Abbreviations as in Table 1.

Site code	Site name	Date	TL (mm)				Wt (g)			We (g)		
	Danube		**n**	**Range**	**Med**	**SD**	**Range**	**Med**	**SD**	**Range**	**Med**	**SD**
JDS4-6	Jochenstein	09.07.2019	67	68-130	112	12.38	2.49-18.18	11.25	3.43	2.22-15.97	9.66	3.17
JDS4-6.2	Jochenstein	16.07.2019	40	82-154	116	15.57	3.65-29.12	10.35	5.21	3.16-26.29	9.39	4.68
JDS4-8	Oberloiben	23.07.2019	35	88-140	125	10.90	5.54-20.22	15.63	3.21	4.95-18.07	13.49	2.85
JDS4-10	Hainburg, upstream Morava	X.X.2019	55	82-144	107	12.19	3.16-19.78	8.17	3.72	2.86-17.95	7.16	3.28
JDS4-14	Bratislava	1.7.2019.	53	91-172	126	15.95	5.02-32.98	13.39	6.08	4.04-27.61	11.5	5.14
JDS4-15	Čunovo, Gabčíkovo resevoir	02.07.2019	53	73-185	131	26.51	2.34-41.31	14.30	9.83	1.9-35.63	12.4	8.13
JDS4-16	Medveďov / Medve	04.07.2019	105	85-159	116	11.87	4.15-25.87	10.89	3.78	3.39-23.13	9.31	3.26
JDS4-22	Szob	03.07.2019	53	106-171	130	13.97	8.09-35.66	15.67	5.83	7.02-28.77	13.74	4.74
JDS4-23	Budapest upstream	15.07.2019	32	90-145	120	10.61	6.41-22.29	14.90	3.57	5.6-19.58	12.7	2.96
JDS4-24	Budapest downstream	17.07.2019	26	97-150	119	11.15	5.39-26.85	11.82	4.16	5.04-23.81	10.67	3.67
JDS4-28	Baja	23.07.2019	42	77-146	111,5	14.81	3.03-23.11	9.83	4.60	2.58-20.17	8.72	4.04
JDS4-29	Hercegszántó / Batina / Bezdan	09.07.2019	33	93-157	122	15.49	5.31-28.04	11.87	5.78	4.66-24.65	10.46	5.09
JDS4-31	Ilok / Backa Palanka	11.07.2019	30	94-142	114	12.74	5.57-20.6	11.12	4.29	4.8-17.90	9.74	3.70
JDS4-37	Downstream Pančevo	07.07.2019	31	71-103	83	8.26	3.7-9.87	5.07	1.65	3.34-8.33	4.51	1.36
JDS4-40	Banatska Palanka / Bazias	05.07.2019	27	75-106	89	6.60	3.01-7.91	4.55	1.18	2.58-6.87	3.99	0.99
JDS4-41	Upstream Timok	03.07.2019	40	72- 99	84	5.95	3.14-6.70	4.76	0.81	2.74-6.08	4.07	0.69
JDS4-47	Downstream Ruse/Giurgiu (Marten)	13.07.2019	59	61-113	85	11.80	1.43-11.59	4.43	2.37	1.36-10.66	4.04	2.12
	Tributaries											
JDS4-11	Pohansko	02.07.2019	51	93-134	111	9.35	5.28-16.13	9.39	2.48	4.72-14.14	8.18	2.05
JDS4-12	Lanžhot	14.07.2019	50	77-131	99	11.94	2.76-18.55	6.40	3.19	2.44-16.04	5.60	2.74
JDS4-32	Tiszasziget / Martonoš	10.07.2019	31	90-126	104	10.05	4.47-14.6	7.74	2.52	3.99-12.77	6.90	2.20
JDS4-33	Tisza mouth (rkm 1.0)	09.07.2019	30	75-103	89	6.81	3.14-7.18	5.01	1.15	2.72-6.15	4.27	1.04
JDS4-35	Jamena	08.07.2019	38	0-152	97	24.08	3.93-28.57	5.95	5.26	3.57-24.43	5.35	4.57
JDS4-36	Sava mouth (rkm 7.0)	11.07.2019	30	82-116	101	8.42	3.8-11.68	7.03	1.87	3.16-9.68	5.96	1.55
JDS4-38	Varvarin	06.07.2019	30	80-119	102	9.58	3.46-9.79	7.17	1.69	2.94-8.47	6.15	1.47
JDS4-39	Velika Morava mouth	04.07.2019	25	80-110	96	8.76	3.52-8.83	6.31	1.60	3.14-7.75	5.60	1.40
JDS4-42	Timok mouth (rkm 0.2)	27.08.2019	15	71-91	82	6.29	3.28-6.18	4.56	0.90	2.94-5.57	4.04	0.81
JDS4-44	Iskar mouth (rkm 0.3)	27.08.2019	39	65-150	105	22.78	1.96-22.46	7.84	5.50	1.72-19.82	6.91	4.83
JDS4-45	Jantra mouth (rkm 1.0)	28.08.2019	30	70-124	109,5	20.11	2.01-13.27	8.09	4.06	1.72-11.36	7.18	3.49
JDS4-46	Russenski Lom mouth	09.07.2019	42	60- 93	75	7.11	1.84-6.80	3.20	1.12	1.63-5.96	2.90	0.99

The dissected digestive tracts were fixed in 70 % ethanol and processed later using the alkaline protocol with 10% potassium hydroxide (KOH) bath for 48 h at 40 °C (Thiele et al., 2019). To eliminate the bias from possible contamination of samples from the clothing of researchers, control samples with blank filters processed with pure KOH solution were also examined for MP residues. These control samples contained a certain type (colour and shape) of fibres that were subsequently excluded from the analyses if found in samples from the digestive tracts of the examined fishes.

The solution was filtrated subsequently through a 1.6 μm mesh size glass microfiber filters, and each sample was deposited in a rinsed sterile glass Petri dish (Figure 2). The samples were finally examined visually under a Leica MZ9.5 stereomicroscope, and the MP particles were categorised according to shape, size and colour. Plastic fibres were distinguished from cotton and/or wool fibres by their smooth or regular surface (the surface of cotton fibres was irregular, woollen fibres were of scaly surface). The surface of the MP particles was double-checked under the Leica DM1000 LED microscope. In case of doubt, a hot needle was used to test the material of the fibres and other particles. The plastic particles changed their shape under the heat from the approaching needle. Particles that did not react to the needle were excluded from further examination. To express the quantity of MP fibres, the length of each fibre was measured with a calibrated ocular micrometre.

Differences between the sites as well as between the species were tested using Kruskal-Wallis test and/or Mann-Whitney test U-test (Zar, 1984).

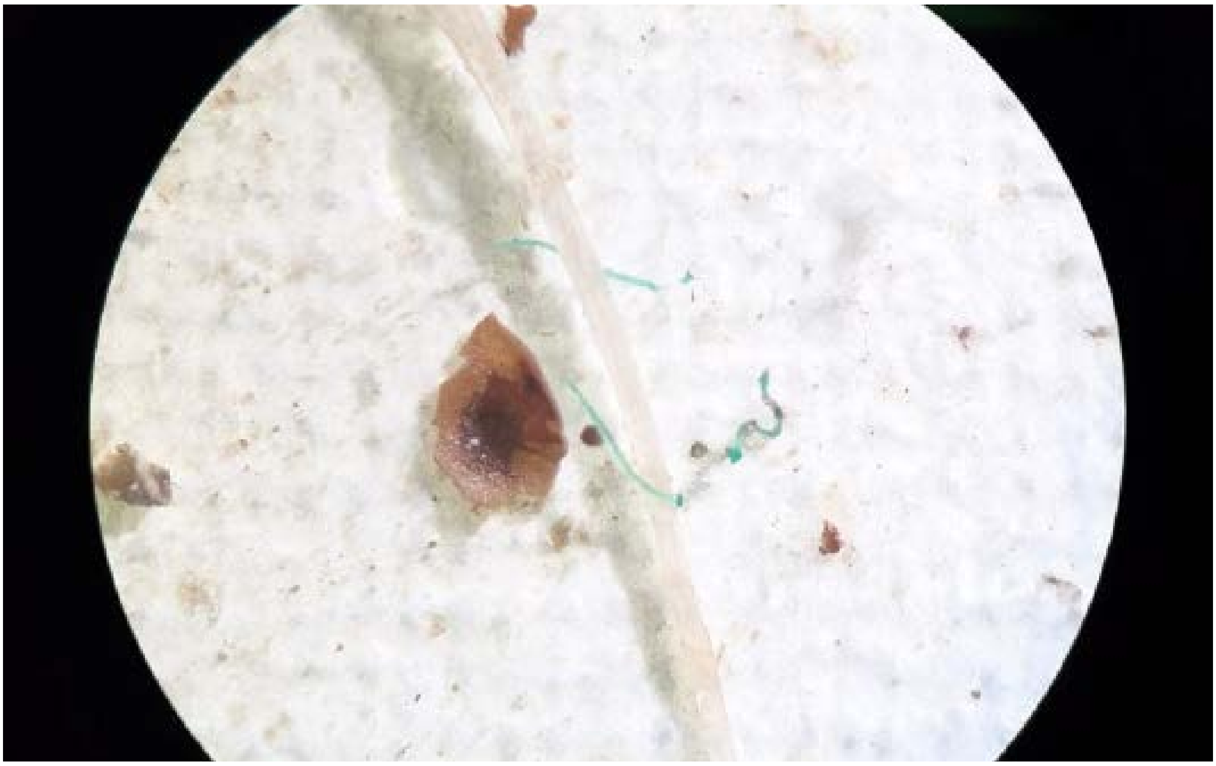

Figure 2: An example of microplastic fibre found in the digestive track of round goby Neogobius melanostomus (Pallas, 1814). Photo: A. Sabová.

3. Results and Discussion

Particles of MP were found in the samples of digestive tracts of both species (round goby and bleak) from all sites, except for the site downstream Pančevo (JDS4-37), where only 5 specimens of round goby were examined (Tables 3 and 4). The highest frequency of MP in the digestive tract of individual fishes was found in the rivers Jamena (JDS4-35) and Velika Morava (JDS4-39) for round goby (at both sites 50 % of the individuals examined contained MP), and in the river Varvarin (JDS4-38; 86,7 %) for bleak (Figure 3). In contrast, the lowest frequency was found in the Danube at Bratislava (JDS4-14; 10.2 %) for round goby, and in the Danube at Hainburg upstream Morava (JDS4-10; 14.6 %) for bleak (Figure 3).

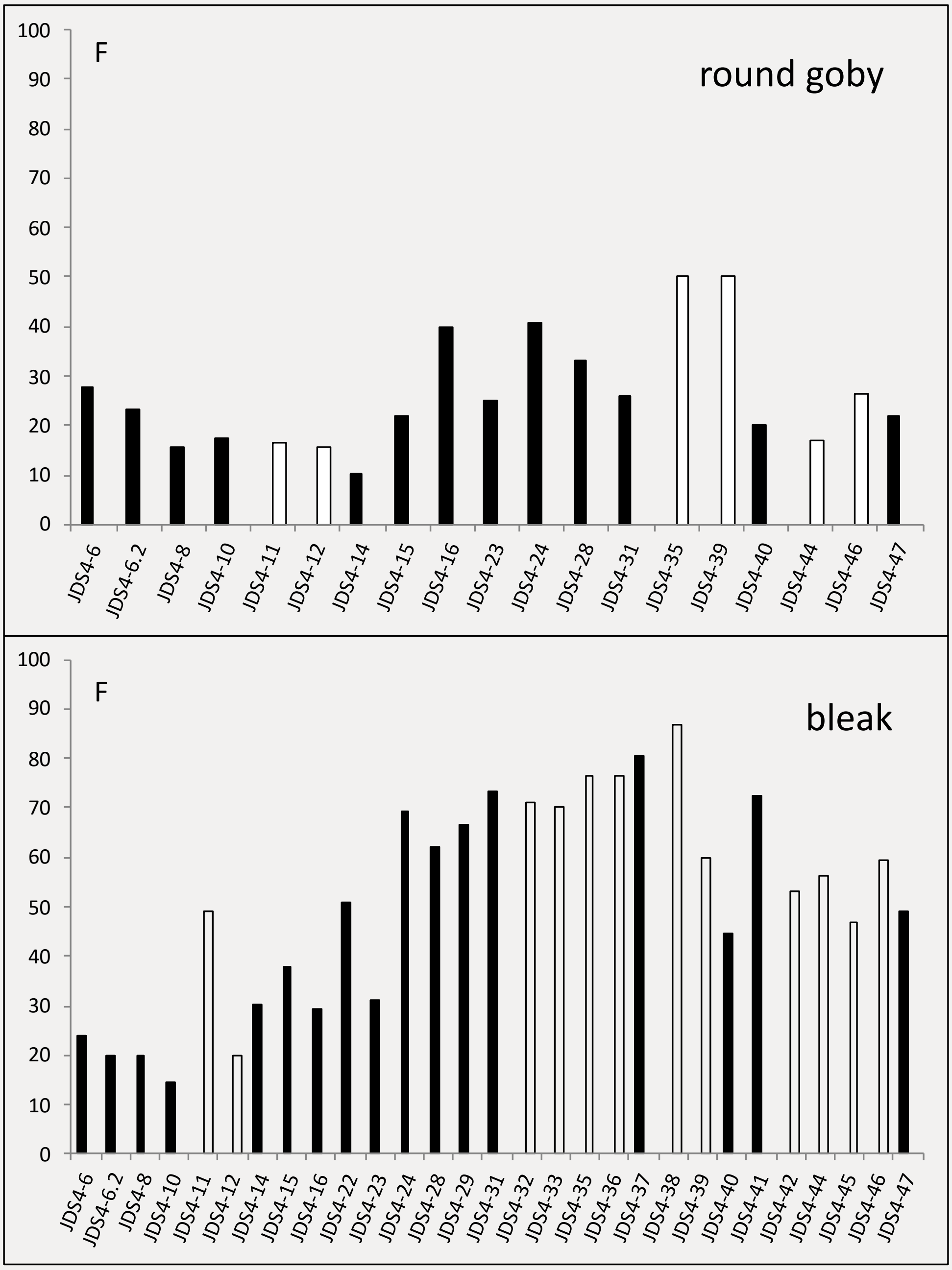

Figure 3: Frequency (F) of microplastics in the digestive tracks of round goby and bleak samples expressed as a percentage of individuals with microplastics found at the sites examined (black bars – the Danube; white bars – tributaries).

Table 3: Microplastics (MP) in the digestive tracks of round goby Neogobius melanostomus (Pallas, 1814) from the Danube and its tributaries. N fish – number of individuals with (Pozit) and without (Negat) MP, N MP particles – number of MP particles found in the digestive track, Mean – the mean number of MP particles per individual from a sample, Fil – filaments, Otp – other type of particles.

Site code	Site name	N fish		N MP particles					Total length of filaments (μm)		
	Danube	**Pozit**	**Negat**	**Range**	**Total**	**Mean**	**Fil**	**Opt**	**Range**	**Med**	**SD**
JDS4-6	Jochenstein	23	60	0-2	24	0.29	17	7	200-16000	1630	3832
JDS4-6.2	Jochenstein	12	40	0-2	13	0.25	10	3	300-4250	2250	1483
JDS4-8	Oberloiben	5	27	0-1	5	0.16	4	1	600-2250	1600	590
JDS4-10	Hainburg, upstream Morava	9	43	0-3	11	0.21	8	3	400-4400	1400	1420
JDS4-14	Bratislava	5	44	0-1	5	0.1	3	2	500-1800	1400	544
JDS4-15	Čunovo, Gabčíkovo resevoir	15	54	0-2	17	0.25	11	6	500-50000	6000	13575
JDS4-16	Medveďov / Medve	14	21	0-2	16	0.46	13	3	500-7500	1200	1985
JDS4-23	Budapest upstream	20	60	0-2	25	0.31	22	3	200-16600	2775	3708
JDS4-24	Budapest downstream	9	13	0-2	11	0.5	9	2	300-4500	675	1455
JDS4-28	Baja	11	22	0-2	13	0.39	12	1	300-5000	2375	1584
JDS4-31	Ilok / Backa Palanka	6	17	0-3	12	0.52	12	0	800-13500	5700	4743
JDS4-37	Downstream Pančevo	0	5	0	0	0	0	0	0	0	0
JDS4-40	Banatska Palanka / Bazias	1	4	0-1	1	0.2	1	0	3500-3500	3500	0
JDS4-47	Downstream Ruse/Giurgiu (Marten)	7	25	0-3	10	0.31	10	0	1000-21000	2500	6837
	Tributaries										
JDS4-11	Pohansko	8	41	0-3	10	0.2	3	7	800-10000	4000	3813
JDS4-12	Lanžhot	8	43	0-2	9	0.18	6	3	100-3100	1750	911
JDS4-35	Jamena	1	1	0-1	1	0.5	1	0	200-200	200	0
JDS4-39	Velika Morava mouth	2	2	0-1	2	0.5	2	0	4500-6500	5500	1000
JDS4-44	Iskar mouth (rkm 0.3)	7	34	0-1	7	0.17	5	2	250-3500	1250	1084
JDS4-46	Russenski Lom mouth	11	31	0-2	13	0.31	11	2	1000-10500	5000	2820
Total		**174**	**582**	**0-3**	**205**	**0.27**	**160**	**45**	**100-50000**	**2125**	**5521**

Table 4: Microplastics (MP) in the digestive tracks of bleak Alburnus alburnus Linneaus, 1758 from the Danube and its tributaries. Abbreviations as in Table 3.

Site code	Site name	N fish		N MP particles					Total length of filaments (µm)		
	Danube	**Pozit**	**Negat**	**Range**	**Total**	**Mean**	**Fil**	**Opt**	**Range**	**Med**	**SD**
JDS4-6	Jochenstein	16	51	1-4	22	0.33	22	0	450-17250	2250	5479
JDS4-6.2	Jochenstein	8	32	1-2	12	0.30	11	1	600-2500	1250	582
JDS4-8	Oberloiben	7	28	1-2	9	0.26	8	1	1950-16500	5700	4991
JDS4-10	Hainburg, upstream Morava	8	47	1-3	10	0.18	9	1	1200-17600	2200	6172
JDS4-14	Bratislava	16	37	1-7	28	0.53	11	17	200-4000	1350	1061
JDS4-15	Čunovo, Gabčíkovo resevoir	20	33	1-5	35	0.66	18	17	200-7800	2375	2457
JDS4-16	Medveďov / Medve	31	74	1-4	42	0.40	19	23	500-8000	1800	2211
JDS4-22	Szob	27	26	1-3	38	0.72	25	13	500-5680	1300	1484
JDS4-23	Budapest upstream	10	22	1-3	15	0.47	12	3	200-9000	2660	2831
JDS4-24	Budapest downstream	18	8	1-3	27	1.04	23	4	250-8500	1650	2700
JDS4-28	Baja	26	16	1-7	52	1.24	49	3	200-12840	2440	3333
JDS4-29	Hercegszántó / Batina / Bezdan	22	11	1-6	52	1.58	42	10	300-10980	2750	2912
JDS4-31	Ilok / Backa Palanka	22	8	1-5	47	1.57	45	2	200-10690	2780	2769
JDS4-37	Downstream Pančevo	25	6	1-13	66	2.13	62	4	680-11780	2700	3183
JDS4-40	Banatska Palanka / Bazias	12	15	1-16	40	1.48	37	3	280-18900	2210	4845
JDS4-41	Upstream Timok	29	11	1-13	58	1.45	55	3	350-12600	2250	2910
JDS4-47	Downstream Ruse/Giurgiu (Marten)	29	30	1-7	52	0.88	50	2	500-41500	3380	7511
	Tributaries										
JDS4-11	Pohansko	25	26	1-6	49	0.96	44	5	100-9800	1500	2274
JDS4-12	Lanžhot	10	40	1-2	16	0.32	15	1	350-9000	2450	2835
JDS4-32	Tiszasziget / Martonoš	22	9	1-7	61	1.97	51	10	240-14680	2800	4589
JDS4-33	Tisza mouth (rkm 1.0)	21	9	1-4	43	1.43	41	2	520-9000	2880	2322
JDS4-35	Jamena	29	9	1-12	90	2.37	88	2	200-16700	3200	4179
JDS4-36	Sava mouth (rkm 7.0)	23	7	1-8	58	1.93	57	1	300-9520	2620	2536
JDS4-38	Varvarin	26	4	1-9	76	2.53	73	3	360-16980	2860	4981
JDS4-39	Velika Morava mouth	15	10	1-5	28	1.12	21	7	420-7060	2200	2118
JDS4-42	Timok mouth (rkm 0.2)	8	7	1-2	9	0.60	7	2	460-3980	1600	1385
JDS4-44	Iskar mouth (rkm 0.3)	22	17	1-10	41	1.05	21	20	460-4050	1100	1013
JDS4-45	Jantra mouth (rkm 1.0)	14	16	1-3	20	0.67	19	1	400-8200	1520	2740
JDS4-46	Russenski Lom mouth	25	17	1-4	36	0.86	36	0	400-9000	2000	2177
Total		**566**	**626**	**1-16**	**1132**	**0.95**	**971**	**161**	**100-41500**	**2200**	**3815**

The highest mean number of MP particles in the digestive tract of individuals was recorded in the samples of round goby from the Danube at Ilok (JDS4-31; 0.52) followed closely by the samples from the tributaries Jamena (JDS4-35; 0.5) and Velika Morava (JDS4-39; 0.5; Table 3). Concerning bleak, the highest mean number of MP particles was recorded in the samples from the tributaries Varvarin (JDS4-38; 2.53) and Jamena (JDS4-35; 2.37), followed by the Danube downstream Pančevo (JDS4-37; 2.13; Table 4). On the other hand, the lowest contamination rate of the digestive tract (i.e. the mean number of MP particles) was found in the Danube at Bratislava (JDS4-14; 0.1) in case of round goby (Table 3), and in the Danube at Hainburg upstream Morava (JDS4-10; 0.18) in case of bleak (Table 4).

Whereas the variability in the number of MP particles found in the digestive tract of round goby individuals was rather low all along the Danube including its tributaries (0 – 3 particles), it was found to be considerably higher in case of bleak, in which it ranged from 1 – 2 to 1 – 16 MP particles (Tables 3 and 4). Indeed, no statistically significant differences between the sampling sites all along the Danube, including its tributaries, were found in the number of MP particles in the digestive tract of round goby (Kruskal-Wallis test: H (15, N= 745) =24.53, p =0.057)). In contrast, multiple significant differences between the sampling sites appeared in the number of MP particles in the digestive tract of bleak (Kruskal-Wallis test: H (28, N= 1192) =240.05, p =0.00). Most of these differences were found between the sites in the Upper and upper Middle Danube, i.e. from Jochenstein (JDS4-6) to Budapest upstream (JDS4-23) and the sites in the lower Middle Danube and Lower Danube, i.e. from Budapest downstream (JDS4-24) to the Danube upstream Timok (JDS4-41), including some tributaries (Figure 4).

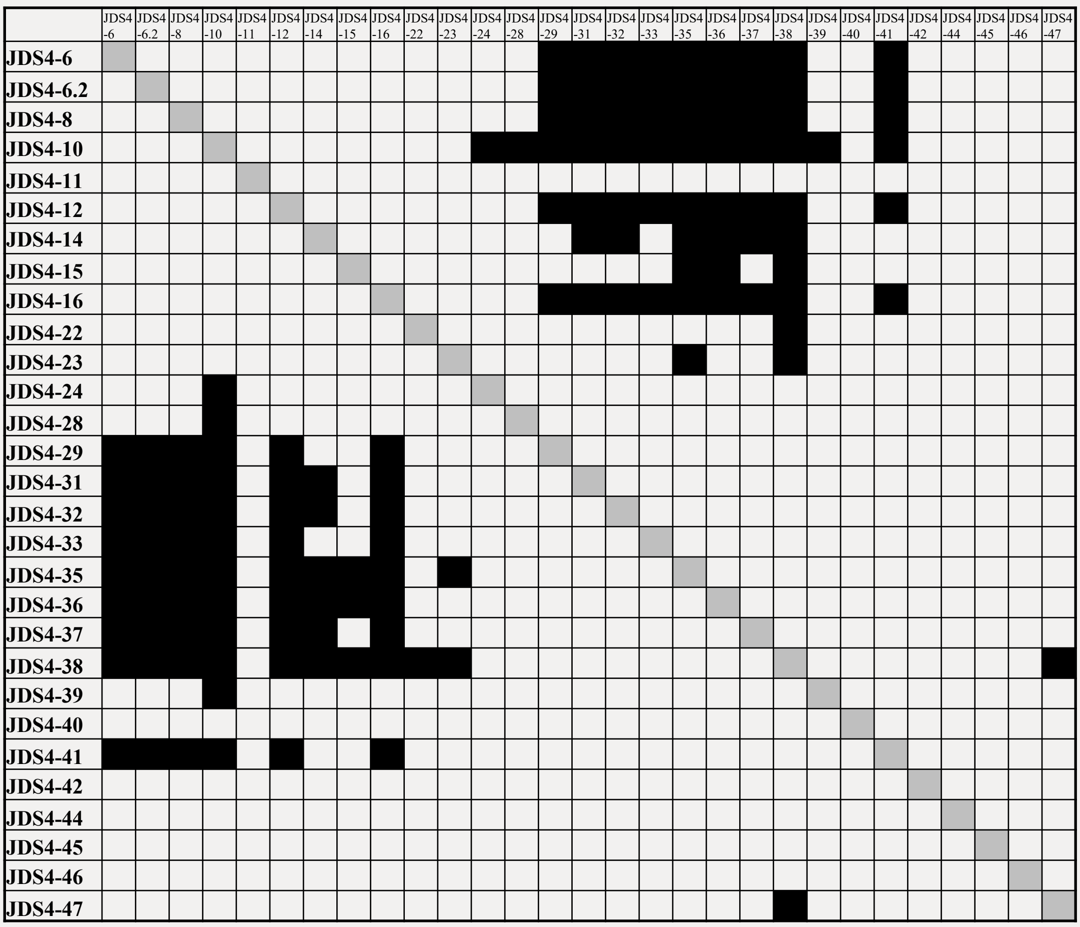

Figure 4: Significant differences (black squares) between the respective sampling sites concerning the number of microplastic particles found in the digestive tracks of bleak.

Concerning the type of MP particles observed in the digestive tract of both round goby and bleak, fibres predominated considerably. A total of 160 and 971 fibres were found in the round goby and bleak samples, respectively, compared to 45 and 161 particles of other types. These other types of MP particles had the form of microscopic balls, square fragments or fibre clumps.

The maximum quantity of MP recorded in the digestive tract of round goby is expressed in terms of the total length of fibres found in the Danube at Čunovo, in the Gabčíkovo reservoir (JDS4-15; 50 000 µm), and was more than 20-times higher than the median of all samples (Table 3). In bleak, the largest amount of fibres was observed in the samples from the Danube downstream Ruse/Giurgiu (JDS4-47; 41 500 µm), almost 20-times more than the median of all samples (Table 4).

The amount of fibres in the digestive tract of round goby was rather similar in most sites all along the Danube, including the tributaries (Table 3). Significant differences between the respective sites were found only in 8 out of 221 combinations of the sites compared (Kruskal-Wallis test: H (15, N= 745) =29.46, p =0.014; Figure 5). On the other hand, in bleak, multiple significant differences between the sampling sites were found in the amount of fibres in the fishes' digestive tract (Kruskal-Wallis test: H (28, N= 1192) =251.55, p =0.00). Most of the differences could be seen between the sites from the Upper Danube and upper Middle Danube, i.e. from Jochenstein (JDS4-6) to Budapest upstream (JDS4-23) and the sites in the lower Middle Danube and Lower Danube, i.e. from Ilok/Backa Palanka (JDS4-31) to Varvarin (JDS4-38), the Danube upstream Timok (JDS4-41), Ruselski Lom (JDS4-46) and the Danube downstream Ruse/Giurgiu (JDS4-47; Figure 6).

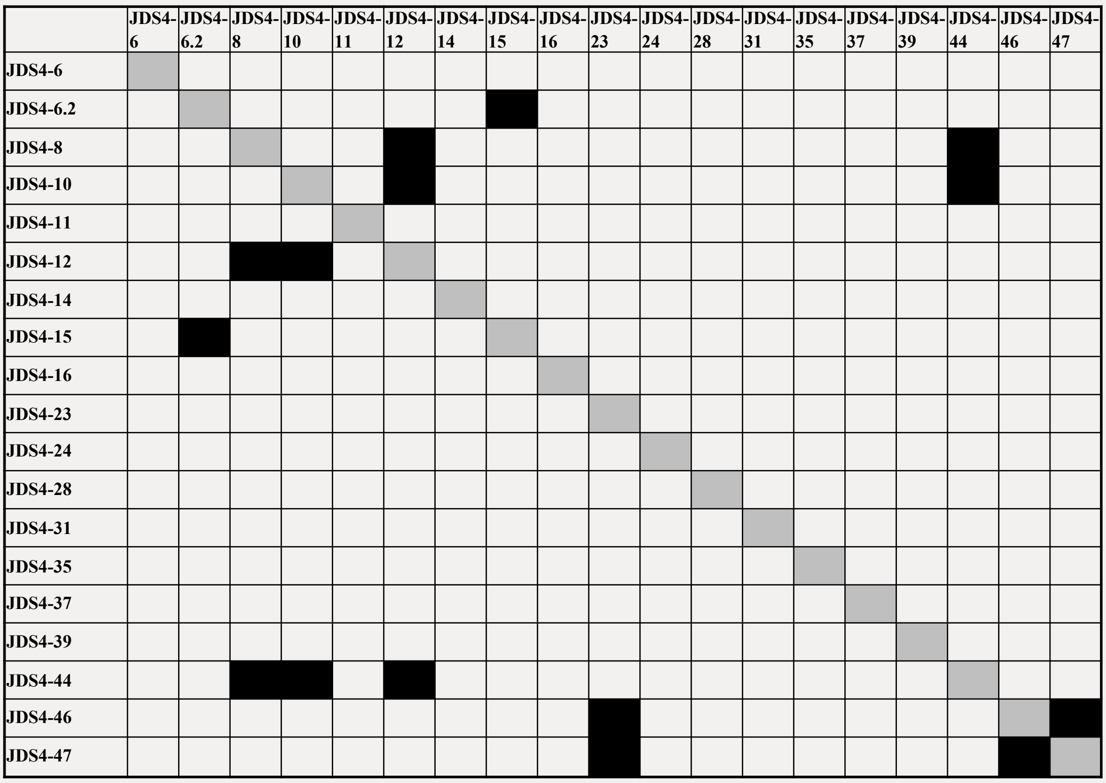

Figure 5: Significant differences (black squares) between the respective sampling sites concerning the quantity (total length) of microplastic fibres found in the digestive tracks of round goby

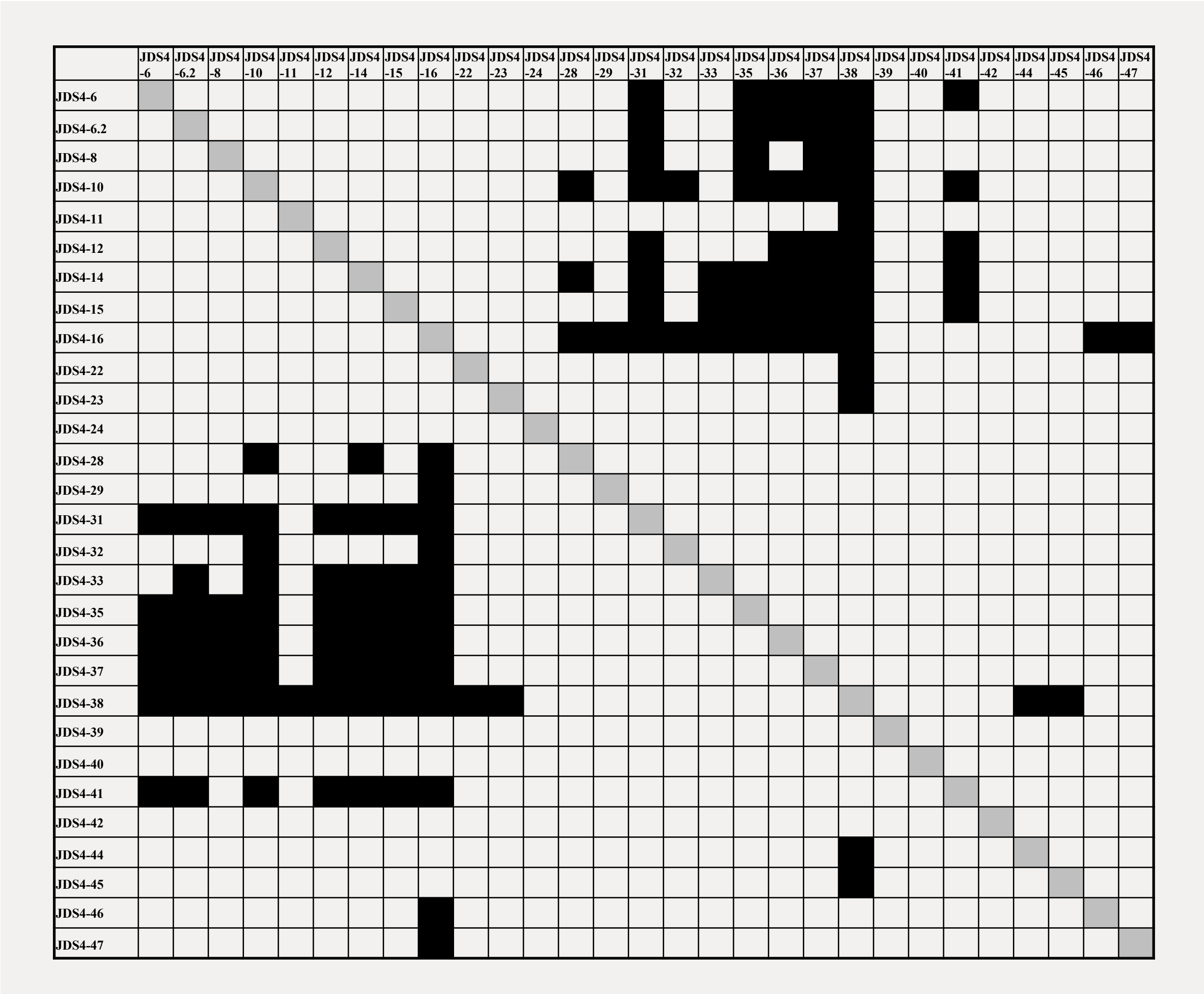

Figure 6: Significant differences (black squares) between the respective sampling sites concerning the quantity (total length) of microplastic fibres found in the digestive tracks of bleak.

As expected following the findings of MP in sediments (Asenova et al., 2021) as well as in bivalves (Raković et al., 2021), MP particles were also found in the digestive tract of the fish species examined. Microplastics are simply present all along the Danube including its tributaries, and they almost certainly pass from the environment through the food chain up to the top predators in the ecosystem, though this should be the subject of further studies. Although the composition and origin of MP were not analysed in this study, the predominance of fibres in the guts of fishes (Tables 3 and 4) reveals differences between animals from different trophic levels (Raković et al., 2021), which suggests that MP particles can also be swallowed by fishes directly from the environment.

One of the most important questions that remains is the possible impact of the presence of MP on individual organisms, populations or even entire ecosystems. Indeed, MP in aquatic environments may adversely affect predation abilities (de Sá et al., 2015), health (Derraik, 2002; Boerger et al., 2010) and survival of fishes (Lönnstedt and Eklöv, 2016). To get a very first picture about the potential impact on fish individuals from the Danube, Fulton's condition factor (Ricker, 1975) – i.e. an index of the apparent health of fish –was calculated for each specimen examined in this study. Preliminary analyses did not reveal any impact on round goby, as no significant difference in the Fulton's condition factor was found between the individuals that had MP particles in their digestive tract and those that did not (Mann-Whitney test: $U = 47914.5$, $p = 0.23$). However, in bleak the difference was statistically significant (Mann-Whitney test: $U = 162830$, $p = 0.016$) which suggests that MP could have a negative impact on the condition of this pelagic species. Of course, further data and analyses are necessary to reach more reliable conclusions.

The frequency of occurrence of MP particles in the digestive tract of fishes did not copy the local quantitative parameters found in bivalves and/or sediments (Figure 3, Raković et al., 2021, Asenova et al., 2021), though some common patterns along the longitudinal profile of the Danube seem to have emerged. In fishes it appears that the quantity of MP has a tendency to increase downstream the Danube, especially in the tributaries, and this increase culminates in the lower stretches of the Middle Danube and upper stretches of Lower Danube (Figure 3). The similarity between the quantities of MP in sediments, bivalves and fishes can be seen in the higher levels found in most (though not all) tributaries, as well as in case of a few Danubian sites, for example in the Danube at Budapest (JDS4-23 and JDS4-24) with elevated quantities found in all the three elements (Figure 3, Raković et al., 2021, Asenova et al., 2021). Nevertheless, further and especially more coordinated research is necessary to reach reliable conclusions.

The type of MP particles found in the digestive tract of the two species of fishes differed considerably from those found in bivalves. Both in round goby and bleak fibres highly predominated, whereas clams contained mainly various other fragments of MP (Raković et al., 2021). Fibres have been known to carry a risk of secondary contamination of samples from the clothing of researchers, however, careful processing of the samples in the laboratory as well as the conservative approach to the evaluation of samples should eliminate such possible bias (see Material and Methods). The predominance of fibres in the digestive tract of riverine fishes has also been reported in other studies (e.g. McNeish et al., 2018; Hellquist, 2019; Bosshart et al., 2020). For example, in the tributaries of Lake Michigan, fibres represented as much as 97 % of inorganic material found in the digestive tract of fishes (McNeish et al., 2018). What the reason for this is, should be the subject of further research.

Round goby and bleak differed in the number of MP particles found in their respective digestive tract significantly (Mann-Whitney test: $U = 325585.5$, $p = 0.00$). Unexpectedly, significantly higher quantities of MP were not found in round goby, which is a benthic and zoobenthivorous species, but in bleak, which is pelagic. Indeed, round goby samples from the Danube reached a considerably lower frequency of MP in their digestive tract (10 – 50 %) than those from the tributaries of Lake Michigan or Lake Ontario (97 – 100 %; McNeish et al., 2018; Hellquist, 2019). Whether this suggests a lower contamination of the Danube by MP is rather questionable due to the lack of further data. The diet of bleak also contains a component from outside the aquatic environment – flying insects. Larvae of many of these insects are aquatic, being a part of zoobenthos, and bleak are a frequent prey of predatory fishes which makes the questions about how the MP travels through food-webs in freshwater ecosystems even more complex.

4. Conclusions

Particles of MP were found in the digestive tracts of both round goby and bleak from virtually all JDS4 sites examined. Fibres predominated considerably among the types of MP particles observed in the digestive tract of the two fish species. Microplastics are present all along the Danube including its tributaries, and they almost certainly pass through the food chains up to the top predators of the ecosystem. Nevertheless, it appears that MP particles can also be swallowed by fishes directly from the environment. The possible impacts of MP on individual organisms, populations or even whole ecosystems remain questionable, though the preliminary analysis of the Fulton's condition factor suggests that MP could have a negative impact on bleak. The frequency of occurrence of MP particles in the digestive tract of fishes did not follow the local quantitative parameters found in bivalves and/or sediments, though some common patterns along the longitudinal profile of the Danube seem to have emerged. In fishes the quantity of MP appeared to have an increasing tendency downstream the Danube, especially in the tributaries, and this increase seems to culminate in the lower stretches of Middle Danube and upper stretches of Lower Danube. The digestive tracts of pelagic bleak contained significantly higher quantities of MP than those of benthic round goby. The findings of this study provide only the very first picture about the question examined; thus further, more detailed, and especially more coordinated research is necessary.

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