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Long-term analysis of fish assemblage structure in the middle section of the Sava River – The impact of pollution, flood protection and dam construction

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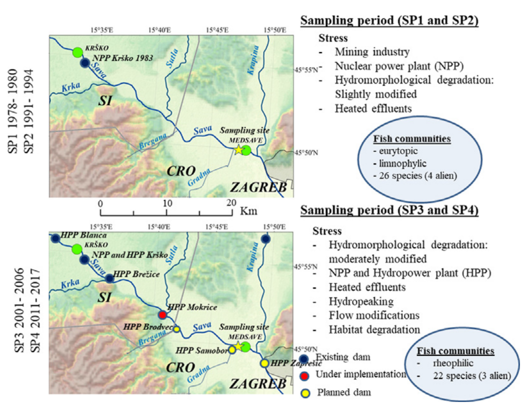
HIGHLIGHTS

- Variations in fish assemblages of the Sava River from 1978 to 2017 were analyzed.
- Limnophilic and eurytopic types of fish group were predominant from 1978 to 1980.
- Four alien fish species recorded 1978–1991, of which three remained until today.
- Changes in species composition and decline in diversity has been noted after 2001.
- Threatened *Telestes souffia* appears to be missing from the Medsave site.

GRAPHICAL ABSTRACT



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ABSTRACT

At the beginning of the 20th century, the middle section of the Sava River in Croatia was unaffected by major human activities and rich in ichthyofauna. The Sava River was important for commercial and recreational fishing for the local population, which still remains today. However, the 1920s mining industry was established in Slovenia, which emitted carbon dust into the Sava River. At the same time, the construction of embankments to mitigate flooding started in the middle section. Furthermore, in the 1980s, the Krško nuclear power plant (NPP), and in the 2010s, the Krško hydropower plant (HPP) were built in Slovenia. These activities could have an impact on the composition of fish communities downstream from the major sources of disturbances. Therefore, the main aim of this paper were to analyze the changes in fish assemblages of the Sava River from 1978 to 2017, prior to and after the construction of Krško NPP and HPP at the Medsave site on the Sava River, 20 km downstream from the major construction operations. Collected data were divided into four sampling periods (SP): SP1, from 1978 to 1980; SP2, from 1991 to 1994; SP3, from 2001 to 2006, and SP4 from 2011 to 2017.

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Hydromorphological alteration
Stressor

Besides alien fish species, water quality and hydromorphological modifications were identified as significant stressors. In SP1 and SP2 limnophilic and eurytopic fish groups were predominant, and 26 different fish species were identified, but in SP3 and SP4 rheophilic fish groups become dominant, and the diversity has declined to 21 species. Threatened species blageon, *Telestes souffia* seems to be missing from the main course of the Sava River in last 20 years. It can be concluded that disturbances in the fish assemblage pattern have coincided with the presence of multiple stressors of human origin.

1. Introduction

Human activities expose inland water ecosystems to a wide range of stressors that threaten freshwater biodiversity and ecosystem processes (Dudgeon et al., 2006; Simonović et al., 2017). Five major threat categories to freshwater biodiversity have been identified: flow modification; destruction or degradation of habitats; overexploitation; water pollution; invasion by exotic species (Dudgeon et al., 2006). The construction of nuclear (NPP) and hydropower plants (HPP) and their hydrological effects can significantly affect aquatic habitats, organisms and riverine ecosystem processes (Teixeira et al., 2012; Tonolla et al., 2017), and increase the risk of most of the threats identified by Dudgeon et al. (2006). The formation of a reservoir transforms a river into a lake, affecting turbulent river sections and causing fluctuating water levels, thus affecting flow and temperature regimes, sediment transport and species communities (Vörösmarty et al., 2010; Freyhof et al., 2015). The shift from lotic to lentic environments after dam construction often favors generalist over specialist species, and rheophilic fish species are either eliminated or severely reduced in numbers (Liermann et al., 2012); it alters assemblages of taxonomic groups and puts endemic species at increased risk of extinction, leading to biotic homogenization (Freyhof et al., 2015; Weiss et al., 2018). Furthermore, construction of HPPs, dams, and weirs cause interruption in longitudinal connectivity, upstream and downstream fish migration (Calles and Greenberg, 2009), which lead to loss of species, isolation, and decline in many fish populations (Branco et al., 2014). Additionally, depending on the amount and temperature of the discharged water, heated effluents can induce dramatic and unpredictable climatic and hydrological effects and influence the biological features of the local environment (Rong-Quen et al., 2001). This can affect fish assemblage structures by decreasing species richness and benthic cover, as an indirect impact to the fish community (Teixeira et al., 2009).

The Balkan region is a European refuge of clean and wild rivers and lakes (Vejnovic, 2017) characterized by extreme hydrographic fragmentation, with hundreds of autonomous river basins, numerous natural lakes, and artificial large and small reservoirs (Piria et al., 2018). However, the Balkan Peninsula has been under increased pressure from construction of >2500 HPPs (Schwarz and Vienna, 2015, 2017). The Adriatic Sea basin in Croatia is characterized by short and isolated karst river catchments while the Black Sea Basin represents the Danube River with vast areas of inland water network dominated by two large rivers, Sava and Drava (Piria et al., 2018). Future HPPs are planned along sensitive karst rivers as well as the large lowland rivers of the Black Sea Basin such as Drava, Sava, and Kupa (Schwarz and Vienna, 2015).

At the beginning of the 20th century, Sava River in Croatia was good water quality, rich in ichthyofauna and important inland water for commercial and recreational fishing (Habeković et al., 1990), but after 1920, carbon dust was emitted from the heavy and mining industries that started up (Simončič, 1945). In the period between 1945 and 1975, massive fish kills were observed due to heavy pollution (Herefort-Michieli, 1969; Munjko and Meštrović, 1975). Fortunately, heavy and mining industries were abandoned in Slovenia at the beginning of 1990s during the transition period to a market economy (Treer et al., 2007).

In the last 100 years, many interventions in the riverbed of the Sava for flood protection were carried out (Slukan Altić, 2010), causing habitat loss for fish spawning. The construction of HPP began in the upper part of the Sava River in Slovenia in early 1950, causing substantial changes in ichthyofaunal and other biocenose structures (Herefort-Michieli, 1969). Today, the subalpine upper Sava River in Slovenia crosses several breakthrough stretches and small basins, and is partially impounded by hydropower dams (Schwarz, 2016). In 1980s, the Krško NPP that was built on the Sava River at its middle part near the border between Croatia and Slovenia, broke upstream fish migration (Povž, 1989). Recent HPP projects along the stretch of the Sava River between the town of Krško and the city of Zagreb resulted in the construction of Krško and Brežice HPPs, which started work in 2014 and 2017, respectively (Schwarz and Vienna, 2017). In addition, due to the increasing demand for energy production, future HPP (and/or NPP) projects are planned in the Sava River Basin, despite the fact that its significant part is under the protection of Natura 2000 (Schwarz, 2016).

The construction of HPPs and its associated hydrological effects can alter the fish species composition (Benejam et al., 2014). However, detailed fish assemblage analyses of the Sava River before 1978 have never been systematically undertaken. Most of the data collected in the early and mid 20th century were obtained from lists of commercial and sport fisherman's catches (Habeković and Popović, 1991). Self-sustaining population of sterlet sturgeon *Acipenser ruthenus* (Linnaeus, 1758) was extirpated from the Sava River due to the construction of the Iron Gates I and II HPPs in 1985 (Hensel and Holčík, 1997). Also, huchen *Hucho hucho* (Linnaeus, 1758), the largest European salmonid that used to be abundant in the Sava River (Simončič, 1945), was already affected by the construction of dams and its population remained self-sustaining only in the short stretch of upper Sava River (Freyhof et al., 2015). After sterlet and huchen extinction in the middle section of the Sava between Krško and Zagreb, cyprinid fish species were predominantly present in the littoral zone in the 1980s (Habeković et al., 1990, 1997), dominated by chub *Squalius cephalus* (Linnaeus, 1758) (Habeković et al., 1993, 1997).

In Croatia, three HPPs are currently under construction, while the building of 124 more is planned. Out of this number, nine planned and one under construction are located in the Sava River basin (Schwarz and Vienna, 2015, 2017). Increasing energy demand as a result of human population growth, implies the construction of new HPPs and NPPs, but consequently also increases the number and magnitude of the associated impacts (Teixeira et al., 2012; Freyhof et al., 2015; Tonolla et al., 2017; Weiss et al., 2018).

In this study data on the impact of human activities on freshwater ichthyofauna and necessity for protection of good-quality rivers as well as the conservation of endangered species will be presented. Detailed fish assemblage analyses downstream of Krško NPP before and after its construction have never been performed. Furthermore, the impact of construction of several HPPs between the town of Krško and the city of Zagreb, the pressure of hydromorphological changes in the last 10 years, and the changes in the water quality of the Sava River including how it affected the fish assemblages, remains unknown. Thus, we hypothesized that there have been changes in the structure of fish assemblages caused by multiple human impacts downstream of the main interventions in the Sava river bed. The aims of this research were to (1) analyze the variations in fish assemblages in the Sava River before and after NPP Krško started to operate; (2) investigate the responses of the fish assemblages after the construction of the HPP Krško and the HPP Brežice dams; and (3) discuss the impact of potential stressors (alien species, water quality and hydromorphological pressure) on the composition of the current ichthyofaunal structure.

2. Materials and methods

2.1. Study area

The Sava River, at the stretch between the town of Krško (Slovenia) and the city of Zagreb (Croatia), is characterized by fast flowing water (mean annual discharge of 294 m^3 s^{-1}), mean annual water temperature of 14 °C, a rip-rap river bank, and a river bed covered with gravel. Five streams and two rivers flow into the Sava River between Krško and the Medsave site: the Gradna and Bregana streams, the Sutla River, the Prilipski and Gabernica streams, the Krka River and the Molčnik stream. This study was performed at the Medsave site (latitude 45°50′04.0″N, longitude 15°46′28.3″E at an elevation of 129 m above mean sea level) near the confluence of the Gradna stream (Fig. 1). The site was chosen due to the diversity of the habitat and ease of access to all habitat types. Beside the stony rip-rap bank at the site, pebbly beaches are occasionally present. The river bank is very steep reaching 7 m in depth. At the Medsave site, in 2011, a concrete wall to mitigate flooding was constructed on the right bank of the river. On the same side of the river, in 2017, new rip-rap artificial stones were replaced and embankments were constructed. According to river classification, the stretch of the Sava River at the Medsave site belongs to the barbel zone (Hawkes, 1975).

The NPP Krško is located 30 km upstream of the Medsave site. It was connected to the power grid on 1981 and went into commercial operation in 1983. Construction work on Krško HPP, cca 10 km upstream of the NPP Krško, began in 2007, by 2012 dam construction was completed, and in 2014 it started to operate (rated head = 9.15 m). The Brežice HPP is located 20 km upstream from the sampling site. Construction work commenced in 2014 and terminated in 2017 (rated head = 11 m). The third HPP in Mokrice, which is 7 km upstream from Medsave, is under construction (http://www.he-ss.si/eng/), (rated head = 7.5 m), and up to three small HPPs are planned between the border of Slovenia and upstream of the City of Zagreb, at Brdovec, Samobor and Zaprešić (Fig. 1). The NPP Krško has none migration facilities, which block the both, upstream and downstream fish migration. At the HPP Krško and Brežice fish paths were constructed but its functionality is unknown. According to Croatian legislation every future HPPs is obligated to construct fish passes.

2.2. Ichthyofaunal data collection

2.2.1. Fish samplings

Fish samplings of the Sava River at the Medsave site in Croatia (26 in total) were performed from 2001 to 2006 and from 2010 to 2017, which represent two sampling periods (SP). In each sampling period one, two or three seasons were covered. One season were covered in 2003, 2006, 2016 and 2017, two seasons in 2010, 2012, 2013, 2014 and 2015, three seasons in 2001, 2004, 2005 and 2011. The spring season includes samplings in May, the summer season in July, and the autumn season in late September or early October (Table 1). Year 2002 and the period from

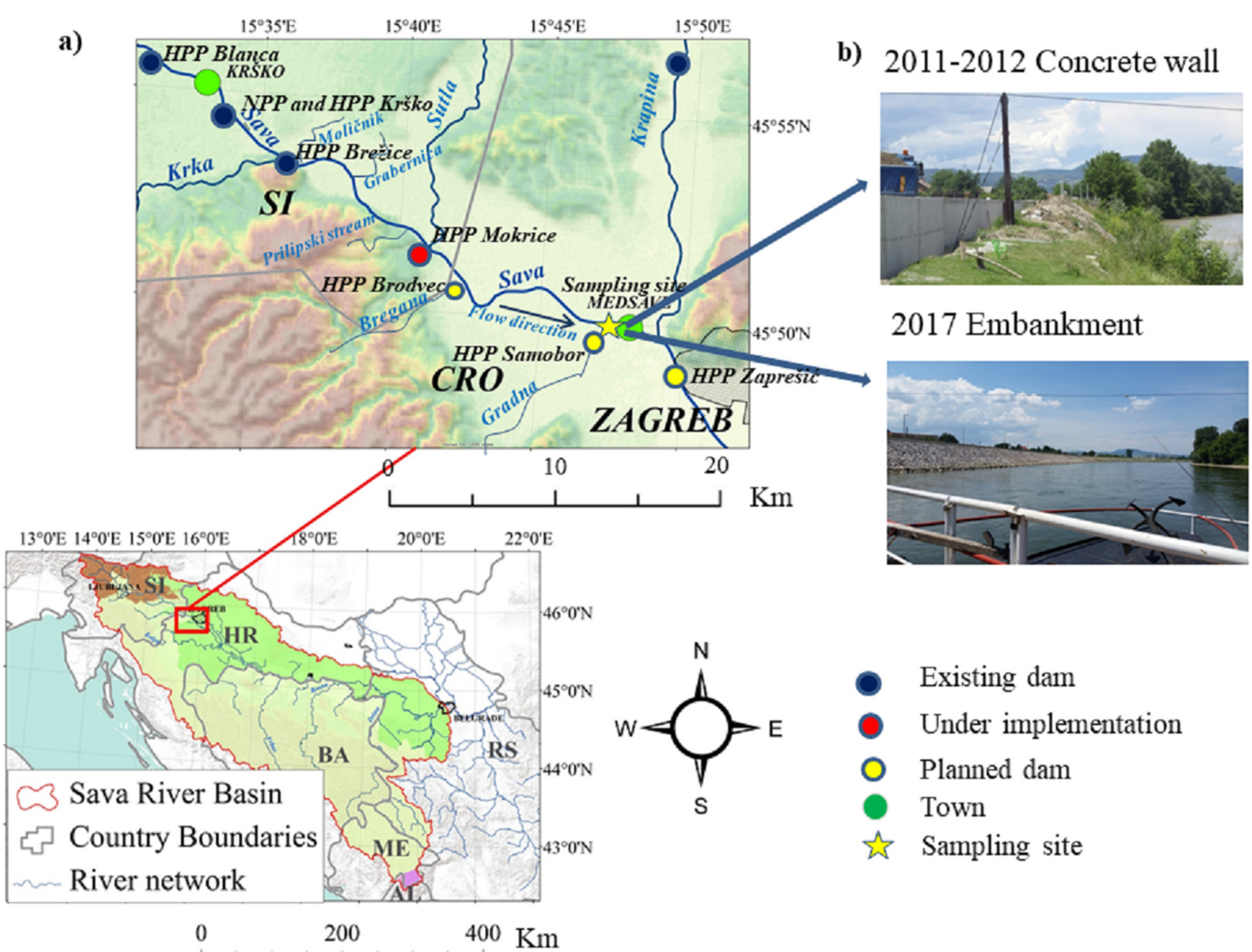

Fig. 1. Medsave sampling locality at the Sava River: a) distribution map of existing and planned HPP and main tributaries at the stretch between the Krško and Medsave towns; b) recent hydromorphological changes at the sampling site.

Table 1
List of the 32 sampled fish species, their scientific and common names, abbreviations, diet strategy, ecological requirements, the percentages in total catch numbers and total number of fish catch by each year at the Medsave site during four sampling periods (SP) in the River Sava (*alien fish species, n = number of samplings; A = spring, B = summer, C = autumn; E = eurytopic, R = rheophilic, L = limnophilic; Inse/inve = insectivore/invertivore, Inve/Pisci = invertivore/piscivore, Herbi/Inve = herbivore/invertivore).

Scientific name and authority	Abbreviation	Common name	Diet strategy	Ecological requirements	Year/percentage in total catch numbers																			
					SP 1 n = 8			SP 2 n = 12				SP 3 n = 11					SP 4 n = 15							
					1978	1979	1980	1991	1992	1993	1994	2001	2003	2004	2005	2006	2010	2011	2012	2013	2014	2015	2016	2017
					A, C	A,B,C	A,B,C	A,B,C	A,B,C	A,B,C	A,B,C	A,B,C	C	A, B, C	A, B, C	A	B, C	A, B, C	A, C	A, C	A, C	A, C	C	C
Abramis brama (Linnaeus, 1758)	AbraBra	Common bream	Inse/inve	E	1.0					0.3				0.1	0.2									
Ameiurus sp.*	Ameiurus	North American bullhead	Piscivore	E	0.6																			
Alburnoides bipunctatus (Bloch, 1782)	AlbBip	Spirlin	Inse/inve	R		6.7	0.9	4.8	5.7	3.0	9.9	22.7	18.1	3.4	29.2	29.4	88.7	26.6	10.9	56.1	22.4	57.9	43.5	57.8
Alburnus alburnus (Linnaeus, 1758)	AlbAlb	Bleak	Inse/inve	E	46.0	19.3	4.4	3.2	13.0	14.2		9.1	37.9	9.9	9.9	4.2	2.2	10.4	40.2	4.1	32.2	1.3	1.6	6.2
Barbatula barbatula (Linnaeus, 1758)	Barbatul	Stone loach	Inse/inve	R				0,3	0.5	0.5	1.0					2.9								
Barbus balcanicus Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002	BarbBalc	Danube barbel	Inse/inve	R		5.9			0.9	0.5	3.0	16.5		0.4	0.4	0.4							0.6	8.3
Barbus barbus (Linnaeus, 1758)	BarbBarb	Barbel	Inse/inve	R	1.6	1.8		5.1	1.1	2.5	6.9	4.9	0.6	2.3	7.4	3.4	0.3	20.8	30.5	25.6	12.5	19.4	32.6	8.7
Carassius carassius (Linnaeus, 1758)	CarasCar	Crucian carp	Omnivore	E		2.0								0.2	0.2		0.3							
Carassius gibelio (Bloch, 1782)*	CarasGib	Gibel carp	Omnivore	E		0.8	0.9			2.0			1.7	1.3							1.3			0.4
Chondrostoma nasus (Linnaeus, 1758)	ChonNasu	Common nase	Herbivore	R	1.0	1.6			1.1			0.7	0.6	1.2	6.8		0.6		0.9		2.6	0.8		
Cobitis sp.	Cobitis	Loach	Inse/inve	R	0.6	3.9	4.4	31.8	23.4	25.9	45.7	7.9	9.2	8.4	4.0	8.0	1.2	1.2	1.1	1.3	3.9	3.8	6.5	9.6
Cyprinus carpio Linnaeus, 1758	CyprCarp	Common carp	Omnivore	E	0.3		0.9		0.2															
Esox lucius Linnaeus, 1758	EsoxLuci	Northern pike	Piscivore	E	1.0	0.6																		
Gobio obtusirostris Valenciennes, 1842	GobGob	Gudgeon	Inse/inve	R				5.1	0.2	3.6	5.4	2.0	2.2	1.7	8.4		0.7		1.3		0.7	0.4		0.4
Gymnocephalus cernua (Linnaeus, 1758)	GymnCern	Ruffe	Inse/inve	E	1.9	0.2																		
Lepomis gibbosus (Linnaeus, 1758)*	LepGib	Pumpkinseed	Inse/inve	L	0.3	0.6	6.2	0.6	0.2	0.3	1.5			0.2		0.4						0.4		0.2
Leuciscus aspius (Linnaeus, 1758)	LeucAsp	Asp	Piscivore	R										0.1				0,1						

Leuciscus idus (Linnaeus, 1758)	LeuIdu	Ide	Omnivore	R									1.9											
Leuciscus leuciscus (Linnaeus, 1758)	LeucLeuc	Common dace	Omnivore	R				2.6	6.4			0.5	7.2	0.1	0.4			0.1		2.1				
Perca fluviatilis Linnaeus, 1758	PerFlu	European perch	Inve/Pisci	E	7.7	3.9	2.7													0.3		1.3	0.5	
Pseudorasbora parva (Temminck & Schlegel, 1846)*	PseParv	Topmouth gudgeon	Inse/inve	E				0.6		15.2			1.4	0.9	2.3						0.7	0.4		
Rhodeus sericeus (Pallas, 1776)	RhodSer	Bitterling	Inse/inve	E	0.3	5.3	39.8		1.4	1.3			1.1	2.7	0.6				2.2	0.3		1.7		3.0
Romanogobio uranoscopus (*Agassiz, 1828*)	RomUran	Danubian longbarbel gudgeon	Inse/inve	R		0.4																0.4		
Rutilus rutilus (Linnaeus, 1758)	RutilRut	Roach	Herbi/Inve	E		8.4	8.8	9.9	4.5	8.1				0.9	0.8							0.4		
Rutilus virgo (Heckel, 1852)	RutilVirg	Cactus roach	Inse/inve	R			0.9				1.0			1.8	0.2			3.9	0.9	0.7	1.3	0.4		
Scardinius erythrophthalmus (Linnaeus, 1758)	ScaEryt	Rudd	Herbi/Inve	L	0.3	0.2				0.5	1.0													
Salmo trutta Linnaeus, 1758	SalTrut	Brown trout	Inve/Pisci	R		0.2		0.3																
Silurus glanis Linnaeus, 1758	SilGlan	Wels catfish	Piscivore	E	0.3		0.9						1.4	0.3				0.1		0.3	2.0		1.1	0.2
Squalius cephalus (Linnaeus, 1758)	SquaCeph	Chub	Omnivore	R	35.8	36.6	29.2	35.7	39.1	22.1	24.6	35.7	15.0	39.6	27.3	51.3	6.0	33.7	11.6	8.2	20.4	11.0	13.6	4.8
Telestes souffia (Risso, 1827)	TelSouf	Blageon	Inse/inve	R		0.2			1.4															
Tinca tinca (Linnaeus, 1758)	TincTinc	Tench	Omnivore	L		0.2																		
Vimba vimba (Linnaeus, 1758)	VimVim	Vimba bream	Inse/inve	R	1.3	1.2			0.9				1.7	24.5	1.9			3.1	0.4	1.0		0.4		0.4
Total number of fish catch (number of species) in sampling season A					168 (12)	156 (13)	21 (7)	92 (9)	114 (13)	123 (8)	58 (5)	124 (7)		227 (11)	303 (13)	238 (8)		388 (8)	1550 (4)	807 (11)	89 (9)	53 (9)		
Total number of fish catch (number of species) in sampling season B					–	165 (13)	47 (9)	103 (9)	228 (9)	129 (9)	56 (6)	108 (7)		468 (16)	71 (7)		646 (5)	2025 (5)	–					
Total number of fish catch (number of species) in sampling season C					145 (10)	169 (17)	45 (6)	119 (7)	98 (7)	142 (8)	89 (7)	174 (6)	359 (14)	236 (19)	100 (7)		39 (6)	378 (6)	740 (9)	62 (4)	63 (8)	184 (11)	184 (8)	519 (12)
Total number of fish catch (number of species) in all sampling seasons					313 (16)	490 (21)	113 (12)	314 (12)	440 (15)	394 (16)	203 (10)	406 (9)	359 (14)	931 (20)	474 (16)	238 (8)	685 (8)	2791 (10)	2290 (10)	869 (11)	152 (11)	237 (15)	184 (8)	519 (12)
Total number of fish catch (number of species) in each SP					916 (26)			1351 (22)				2408 (22)					7727 (21)							

2007 to 2009 were not included in further analysis because samplings were not conducted at the investigated site. Single-pass point-sample electrofishing surveys (Persat and Copp, 1989) per 100 m of shoreline (Zalewski, 1985) were carried out at one sampling point at the Medsave site. Sampling efforts were measured as the number of fish caught per 100 m of shoreline. Various types of substrates were covered in the littoral zone (shallow zone near the river bank) of the river in the upstream direction, with approximately the same fishing effort applied under different hydrological conditions.

Sampling was performed in depths ranging from 0.2 to 1 m along the riverbank during daylight hours. Electric gear (Hans Grassl EL 63 II, 220/440 V, 17.8/8.9 A) with a ∅50-cm rounded stainless-steel anode and a 10-mm-mesh-size net was used to limit the catch predominantly to adult specimens. To minimize between-operator bias, surveys were performed by the same three-person sampling team (Bain and Finn, 1990).

Fish identification was done immediately after the sampling. For SP from 2001 to 2006 Vuković and Ivanović (1971) and for SP from 2010 to 2017 Kottelat and Freyhof (2007) identification key was used. The latest scientific nomenclature was used according to Froese and Pauly (2018). The genus *Cobitis* sp. for Balkan loach *Cobitis elongata* Heckel & Kner, 1858 and spined loach *Cobitis elongatoides* Bcescu & Mayer, 1969, and the genus *Ameiurus* sp. for brown bullhead *Ameiurus nebulosus* (Lesueur, 1819) and black bullhead *Ameiurus melas* (Rafinesque, 1820) were used to avoid the possibility of misidentification. Namely, in Vuković and Ivanović (1971) only one loach species were described, and in the data collected until 2006, the total number of both loach species were recorded. The collected fish specimens were counted, after which the fish were released.

2.2.2. Historical ichthyofaunal data

Historical ichthyofaunal data at the Medsave site were also divided into two SP. One SP represents ichthyofauna collected in 1978, 1979, and 1980, before the NPP Krško started to operate (Habeković et al., 1990 and references within) in spring (May), summer (late June or early July), and autumn (late September or early October). Only during 1978 fish samplings were performed in two seasons, spring and autumn (Table 1). The same team performed second ichthyofaunal survey after several years of NPP Krško activity in the four-year period from 1991 to 1994, also in three seasons (spring, summer and autumn), (Habeković et al., 1997 and references within). Fish identification in both SP was performed according to Vuković and Ivanović (1971) identification key. The same electrofishing method in 1978–1980 and 1991–1994 was used as samplings taken for the purpose of this study.

2.2.3. Fish data treatment

SP of ichthyofaunal survey were classified into four groups. The first group (SP1) covered the period from 1978 to 1980 (before NPP Krško construction); the second group (SP2) represented samplings from 1991 to 1994 (after NPP Krško construction and during HPP Vrhovo construction); the third group (SP3) included samples from 2001 to 2006 (before the HPPs Blanca, Boštanj and Krško were constructed), and the fourth group (SP4) contained samplings from 2011 to 2017 (after the HPPs Blanca, Boštanj and Krško were constructed).

Collected ichthyofauna were also classified by the feeding strategy (omnivore, insectivore/invertivore, piscivore, herbivore, invertivore/piscivore, herbivore/invertivore) and by three ecological groups (limnophilic, rheophilic and eurytopic).

2.3. Habitat stressors

At the same time, while fish samplings were conducted in SP2 and SP3, physical and chemical analyses of water were performed. Temperature °C, conductivity μS cm^{-1}, O_2 mg L^{-1}, pH and O_2% were measured by a handheld multi-parameter instrument Multi 340i. Parameters NH_3, NO_2^-, NO_3^-, PO_4^3 and free Cl^- (mg L^{-1}), were measured by Hanna HI 83200 multiparameter photometer. For COD_{Mn} (mg L^{-1}) assessment potassium permanganate method was used (Appendix Table S1).

Historical water chemistry data for SP1 were used from Munjko and Meštrović (1975) which were not performed at the same time as fish samplings but were considered as relevant for the late 1970s. Water chemistry data in SP2 were performed at the same time when fish samplings were conducted and, are documented in Treer et al. (1994) and Habeković et al. (1997), (Appendix Table S2).

Based on historical water quality parameters and data collected from 2001 to 2017, the chemical status (Che1 – satisfactory; Che2 – unsatisfactory) was determined according to the Official Gazette (2013) that was created based on Water Framework Directive (European Union, 2001, 2013).

Hydromorphological characteristics for SP1 and SP2 were based on the description taken from Habeković et al. (1990, 1997) and Treer et al. (1994) and the hydromorphological description collected in SP3 and SP4 were categorized using criteria developed for large fluvial rivers according to Simonović et al. (2017), as follows: 1 – high status (undisturbed – no visible hydromorphological degradation); 2 – slightly modified (visible/measurable consequences on biota are not visible; modification of banks and/or bottom recorded only locally, in short stretches extending <20% of the surveyed length of the river, thus not influencing aquatic biota); 3 – moderately modified (the modification has measurable consequences on aquatic biota and riparian vegetation; visible hydromorphological changes extend along >20% of the surveyed length of the river; longitudinal connectivity of the river is uninterrupted; flood protection dikes are at a distance from the river banks); 4 – highly modified (the majority of the surveyed stretch is regulated; longitudinal connectivity is violated; flood protection dikes are near to the river bank; hydrological features of the river are changed).

2.4. Statistical analysis

The number of fish species caught each year and historical ichthyofaunal data were presented as numerical frequency N%, using the following formula:

$$N\% = \frac{n_i}{\sum n} \, 100$$

where n_i is the total number of a particular fish species and Σn is the total number of fish species caught each year.

To determine the gradient length of the response data, unimodal unconstrained Detrended Correspondence Analysis (DCA) was used to determine which multivariate method, unimodal or linear, is the better choice to use in further analyses. The gradient length measures the beta diversity in community composition (the extent of species turnover) along the individual independent gradients (ordination axes), (Lepš and Šmilauer, 2003). If the longest gradient is larger than 4.0, unimodal constrained methods should be used. Use of a linear constrained method would not be appropriate, since the data are too heterogeneous and too many species deviate from the assumed model of linear response. However, if the longest gradient is shorter than 3.0, the linear method is probably a better choice, but unimodal methods also could be used (Lepš and Šmilauer, 2003).

Three types of response data (i.e. primary data set, with fish species or/and specimens as individual response variables) were used: (1) the number of fish species and their specimens sampled per 100 m shoreline, in each sampling season of each year in order to analyze the relationships between fish assemblages, its variation during different season, year, water quality, hydromorphology (nominal explanatory variables) and the presence of alien fish species (explanatory variables), and their ratio in relation to each SP (nominal explanatory variables), (2) the number of fish species and their specimens sampled per

100 m shoreline within each SP in order to determine the most prevalent ecological groups and diet strategy (nominal explanatory variables) per SP; and (3) the third response data set was represented by the number of fish species of each ecological group and their feeding strategy in each sampling season of each year, in order to analyze how they responded to different water quality status (nominal explanatory variables) within each SP.

Alien fish species represented the environmental (explanatory) variables and habitat stressors (water quality and hydromorphology), year, SP, feeding strategy and ecological groups nominal explanatory variables.

To test the most prevalent ecological fish groups and diet strategy, their response to different water quality status and hydromorphology and the variation in fish assemblages per each SP, unimodal constrained Canonical Correspondence Analysis (CCA) with response data log transformations (to dampen the effects of dominant species), the constrained axis test and unrestricted Monte Carlo permutation test (999 permutation; $p < 0.05$) of fist axis using the CANOCO 5 software package were used (Ter Braak and Šmilauer, 2012). CCA allows the explanatory variables to be selected that contributed significantly to the explanation of the variations in response variables, using a forward selection procedure, estimates the contribution of explanatory variables, and assesses the statistical significance of these relations (Lepš and Šmilauer, 2003). This option uses a partial Monte Carlo permutation test to assess the usefulness of each potential predictor variable for extending the subset of explanatory variables used in the ordination model (Lepš and Šmilauer, 2003). Therefore, CCA interactive forward selection was chosen to test the significance of explanatory variables: sampling year, sampling seasons, SP, alien fish species, hydromorphological parameters and water quality status (Che) to explain variations in the relation to (1) fish species assemblage and (2) each ecological and feeding strategy groups assemblage. Fish species diversity diagram were derived from CCA with interactive forward selection where response variables were presented as count of species within the samples. Summary results of CCA were given as eigenvalue (eigenvector) of the estimated axis, measuring its relative importance in summarizing the response data. Values of eigenvectors are in the range from 0 to 1, with the maximum value (1), representing the contributions of the variables to the canonical axes (Ter Braak and Šmilauer, 2012).

3. Results

3.1. Fish composition

In total, 32 fish species were identified site between 1978 and 2017, of which 28 were native and 4 were alien. In SP1 26 species, in SP2 and SP3 22 species, and in SP4 21 species were caught. In the Sava River, for the first time three alien fish species were recorded in SP1; these were the North American bullhead (*Ameiurus* sp.), pumpkinseed *Lepomis gibbosus* (Linnaeus, 1758) in 1978 and gibel carp *Carassius gibelio* (Bloch, 1782) in 1979. In SP2 (in 1991), the topmouth gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846), was found. During the SP3 and SP4, the North American bullhead was not recorded again, but three other alien species regularly appeared in the catches (Table 1).

3.2. Fish ecology, assemblage and stressors

The initial examination of fish species as a response data with unconstrained DCA analysis indicated a gradient length of 2.3 (Fig. 2A) and 1.3 for the feeding strategy and ecological groups (Fig. 2B) were determined, suggested linear multivariate methods as a better choice, but unimodal CCA method also could have been used. Gradient length of 4 for SP as a response data (Fig. 3A) showed the data unimodally distributed, and indicated that constrained CCA is the only appropriate multivariate analysis.

Using CCA with an interactive forward selection, water quality ($p = 0.002$), hydromorphological modifications ($p = 0.004$), and three alien fish species, except gibel carp ($p = 0.016$), were identified as significant stressors. It appears that gibel carp has no impact on fish assemblages ($p > 0.05$). Eigenvectors (λ) of the CCA explained 69.7% of the total variability of fish samples, with the first four explaining over 50% of the variability (λ1 = 0.2247, 18.04%; λ2 = 0.1558 30.55%; λ3 = 0.1381, 41.64%; λ4 = 0.1093, 50.41%). Significant differences were found in all three years of SP1 ($p = 0.004$), in 1992 of SP2 ($p = 0.004$), in 2003 and 2004 of SP3 ($p = 0.014$; 0.004, respectively), and in 2011 in SP4 ($p = 0.040$), (Table 2), which suggested important turning points for fish assemblages in each SP. Changes in fish assemblage between sampling seasons were also tested but significant differences were not found ($p > 0.05$). During SP1 and SP2, unsatisfactory water quality was observed, which significantly affected fish assemblages ($p = 0.002$). Moderate hydromorphological modifications and significant changes ($p = 0.004$) in fish assemblages were detected for SP4 (Fig. 2A, Table 2).

Changes in fish diversity were observed during all four sampling periods and sampled years. Even in SP1, when water quality was unsatisfactory, the number of fish species within samples was higher in each successive year (between 10 and 13). The count of fish species within samples after 1991 decreased and in SP3 and SP4 was in the range of 6–10 (Fig. 2B). The North American bullhead was associated with a larger number of species and a lower water quality. It seems that

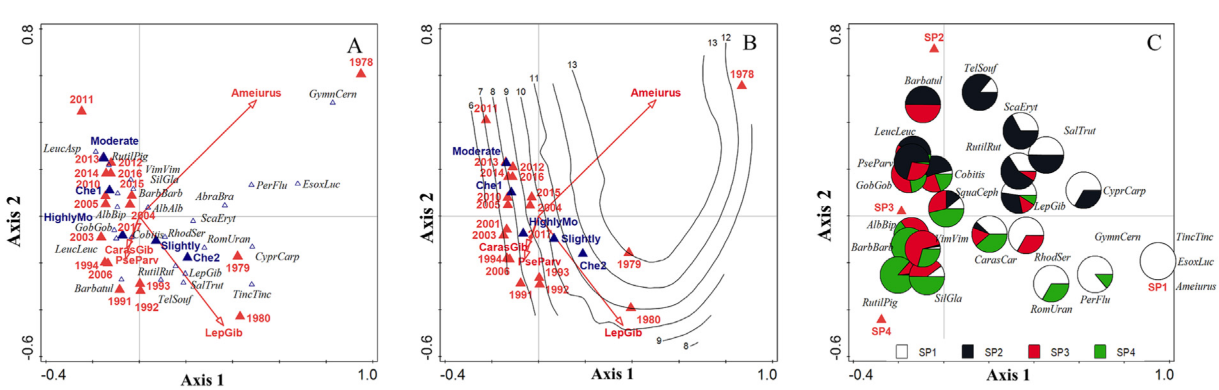

Fig. 2. CCA ordination diagram representing (A) Constrained analysis of the main fish species in samples collected from 1978 to 2017, hydromorphological and water quality status with the correspondence of alien fish species at the Medsave site; gradient length 2.3, total variation is 1.78, explanatory variables account for 69.7% of the variation; Monte Carlo permutation test results on all axes: pseudo-F = 2.2, $p = 0.002$ (see Table 2 for results of interactive forward selection of environmental variables) (B) Species diversity diagram. (C) Distribution of species defined by the sampling period; gradient length 2.3, total variation is 1.78, explanatory variables account for 19.5% of the variation; Monte Carlo permutation test results on all axes: pseudo-F = 3.4, $p = 0.002$ (▲ – nominal explanatory variables, Δ – fish species, → – explanatory/response variables, SP – sampling period) (see Table 1 for abbreviations).

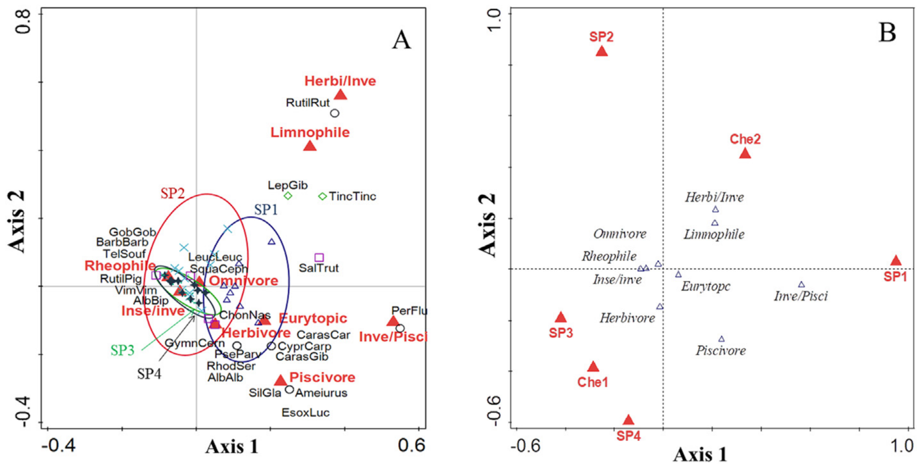

Fig. 3. Constrained CCA of the diet strategy and ecological groups (A) of the fish species represented in each of the four sampling periods; gradient length 4, total variation is 1.78, explanatory variables account for 26.3% of the variation; Monte Carlo permutation test results on all axes: pseudo-F = 1.2, p = 0.026. (B) The correspondence of water quality and sampling period; gradient length 1.3; total variation is 0.35, explanatory variables account for 27.8% of the variation Monte Carlo permutation test results on all axes: pseudo-F = 5.4, p = 0.002. (▲ – nominal explanatory variables, Δ – fish species, SP – sampling period,△ — SP1, × — SP2, ✦ — SP3, — SP4, ◯ Eurytopic, □ Rheophile, ◇ Limnophile), (see Table 1 for abbreviations).

pumpkinseed tolerates poorer water quality than gibel carp and topmouth gudgeon, all three species were recorded during lower diversity. (Fig. 2B). Only in SP1, a native potamal, tench *Tinca tinca* (Linnaeus, 1758), and lower rithron species, northern pike *Esox lucius* (Linnaeus, 1758) and ruffe *Gymnocephalus cernua* (Linnaeus, 1758), were caught. The common carp *Cyprinus carpio* (Linnaeus, 1758), rudd *Scardinius erythrophthalmus* (Linnaeus, 1758), lower rithron species, brown trout *Salmo trutta* (Linnaeus, 1758), upper rithron species and the threatened blageon *Telestes souffia* (Risso, 1827) were recorded only in SP1 and SP2. Of these seven species, only blageon was a typical middle-rithron species at the investigated site, which seems to have disappeared in the last 20 years.

After 2001, in SP3 and SP4, the spirlin *Alburnoides bipunctatus* (Bloch, 1782), barbel *Barbus barbus* (Linnaeus, 1758), gudgeon *Gobio obtusirostris* (Valenciennes, 1842), vimba bream *Vimba vimba* (Linnaeus, 1758) and chub *Squalius cephalus* (Linnaeus, 1758), all typical middle rithronic species, have become more numerous. The only top predator, Wels catfish *Silurus glanis* (Linnaeus, 1758), was caught after 2001, its number of individuals increasing since then. The Danube barbel, *Barbus balcanicus* (Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002) and asp *Leuciscus aspius* (Linnaeus, 1758) were represented only in SP3 and SP4 (Fig. 2C).

Table 2
Results of interactive-forward-selection of explanatory variables in relation to variation of fish species: only significant values of the sampling year, the sampling period (SP), alien fish species, hydromorphological and the water quality status (Che) are presented. Total variation is 1.78, explanatory variables account for 55.3%of the variation; Monte Carlo permutation test results on all axes: pseudo-F = 3.4, p = 0.002 (see Fig. 3A and B for ordination diagram).

Variables	Explains %	Contribution %	Pseudo-F	p
Sampling year				
1978	6.8	9.8	3.6	0.002
1979	3.6	5.1	2.3	0.002
1980	3.6	5.1	2.3	0.004
1992	3.2	4.7	2.2	0.004
2003	4.8	6.9	2.9	0.014
2004	2.9	4.2	2.0	0.008
2011	2.3	3.3	1.7	0.040
Sampling period				
SP1	10.8	15.4	5.3	0.002
SP2	5.5	7.9	3.2	0.002
Alien fish species				
Pseudorasbora parva	5.6	8.1	3.1	0.002
Ameiurus sp.	3.9	5.6	2.4	0.016
Lepomis gibbosus	2.9	4.2	2.1	0.002
Hydromorphological status				
Moderately modification	3.0	4.3	2.0	0.004
Water quality status				
Che1	5.5	7.9	3.2	0.002
Che2	5.5	7.9	3.2	0.002

CCA of fish ecological groups and diet strategy shows that limnophilic and eurytopic, piscivore, invertivore/piscivore and herbivore/invertivore were mostly represented in the SP1 (p = 0.002). Eigenvectors (λ) of the CCA explained 26.3% of the total variability of fish samples, with the first four explaining over 80% of the cumulative fitted variability (λ1 = 0.1768, 37.76%; λ2 = 0.1076; 60.76%, λ3 = 0.0670, 75.07%, λ4 = 0.0436, 84.38%). (Fig. 3A). Concurrent with improved water quality (p = 0.004), already in the SP2 (p = 0.002), as well as in the SP3 (p = 0.040) and SP4, rheophilic and insectivore/invertivore fish species dominated. SP1 was significantly different from SP2 and SP3 (p = 0.002), while there were no significant differences between SP3 and SP4 and they overlapped (Fig. 3A, 3B). Eigenvectors (λ) of the CCA explained 27.8% of the total variability of fish samples, with the first three explaining 100% of the cumulative fitted variability (λ1 = 0.0628, 64.77%; λ2 = 0.0235; 88.96%, λ3 = 0.0107, 100%) (Fig. 3B).

4. Discussion

The importance of the Sava River in history has been focused on commercial and recreational fishing as a basic source of good quality food for local inhabitants, but the number of catch rapidly declined in the second half of the 20th century and available resources remarkably deteriorated (Habeković et al., 1990). The Sava River, from its source until the place where Krka River flows into it, was a typical trout zone until the 1950s, and downstream, from the mouth of the Sutla River up to the town of Sisak, a barbel zone suitable for middle rithron ichthyofauna (Povž, 1989). This has been changed during the second half of the 20th century and the trout zone left only in the first 100 km of the upper reach of Sava River (Simonović et al., 2017) probably caused by the construction of several HPPs and NPP (Herefort-Michieli, 1969; Schwarz, 2016).

Declining in fish diversity was recorded in the Slovenian stretch of the Sava River, where 44 fish species were recorded before the 1980s, and 30 species after (Povž, 1989). Therefore, it was expected that at the beginning of the Croatian stretch of the River Sava, at the Medsave site, a similar pattern would occur. In this research, in total 26 species were recorded before the 1980s, with diversity at present reduced to 21 species (Table 1; Fig. 2C).

In experiments using fish marking, Povž (1989) have argued that NPP Krško is a barrier, preventing fish from migrating upstream in the Sava River due to non-functional fishways. The consequence of this was that in the middle course of the River Sava, downstream of Krško, species migrated only to the rivers Sutla and Krka. Also, the NPP Krško emits warm water and causes heating of the Sava by several degrees Centigrade (Jukić, 2018), which probably represents another barrier to fish migration. In present research, fish migration behavior was not studied, but planned construction of HPP Mokrice represents a threat for loosing migration routes of species identified in the current research, for example, for cactus roach *Rutilus virgo* (Heckel, 1852) or the Danube barbel.

It should not be forgotten that until the 1990s, the mining industry in Slovenia was active (Treer et al., 2007). Research into the water quality in the 1970s and 1980s of the Sava River, upstream of Zagreb, revealed the presence of fine coal particles, a high content of organic materials, oil and phenols (Munjko, 1977), which were probably responsible for fish kills in the mid 1970s in the main course of the river (Munjko and Meštrović, 1975). The dominance of eurytopic and limnophilic fish groups during SP1 (1978–1980) was the result of unsatisfactory water quality. In such water conditions, huchen could not migrate downstream despite the absence of a physical barrier at the investigated site before the 1980s. Only during SP1 were several specimens of northern pike and tench, typically limnophilic species characteristic of lower and calmer sections of rivers and surrounding floodplains and wetland habitats, recorded. The occurrence of these species confirmed the eutrophic environment and pointed to the historical existence of backwaters in nearby surroundings, of well-developed aquatic vegetation and a soft substrate (Froese and Pauly, 2018).

Brown trout was recorded at the Medsave site only in SP1 and SP2, although only a few individuals, as this species typically inhabits upstream reaches (trout zone) that are characterized by a faster flow and higher oxygen concentrations (Aarts and Nienhuis, 2003). It is likely that the occasional brown trout specimens migrated to the Sava River from the surrounding streams, the Gradna and Bregana, which are typical trout zone.

However, several years ago, the construction work undertaken by the Croatian Waters Company along the entire lower course of the Bregana stream to channelize and cement parts of the riverbed, completely altered the mouth of the stream into the Sava. Plans are also in place to construct a small hydroelectric plant at Bregana, which will laterally block the river course and alter the flow regime (http://zagrebnasavi.hr/item/mhe-brdovec/). The Bregana stream is the only known spawning site for blageon in Croatia. Therefore, alterations in the natural flow regimes of rivers, indicates a strong negative anthropogenic impact on the blageon population at the Medsave. Indeed, in present research blageon was not recorded in SP2 and SP3, despite improved water quality, which could refer to disturbances in hydrological regimes (Vucić et al., 2017) and possible on going deterioration processes in fish assemblages (Kruk et al., 2017). Additionally, the section of the Sava River upstream of Zagreb is the only site where blageon has been recorded in Croatia (Vucić et al., 2017).

Water quality of the Sava River in SP3 and SP4 was significantly improved (Treer et al., 2006), resulting in a change from a limnophilic to a rheophilic fish assemblage. However, an embankment against flooding was constructed in the 1960s and serious hydromorphological changes began in 2010 with the construction of a concrete wall that was followed by the erection of a new embankment in 2017. Thus, in SP4, when the year 2011 was a turning point with regard to changes in fish assemblages (Fig. 3. Table 1), hydromorphological modifications were the primary reasons for the disturbances in fish assemblages rather than the construction of HPP Krško in that period. Construction works on the HPP Krško started in 2011 and it became fully operational in 2014. Nonetheless, fish migration was not possible after completion of the NPP Krško (Povž, 1989), and from the overlapping patterns of fish assemblages in SP3 and SP4 (Fig. 2A) we concluded that the dam most probably did not have a great impact on downstream ichthyofauna. However, the HPP Brežice that began operation in 2017 raises concern because of its potential impact. Also, construction work on HPP Mokrice, 7 km upstream from the Medsave site, is planned to start soon, and it represents a new threat to fish assemblages downstream due to the loss of connections with the Krka and Sutla rivers, the main migration routes for remaining rheophilic fish species (Povž, 1989).

The disadvantage of this research represents the lack of summer season sampling in data collection after year 2001 in SP3 and SP4. Additionally, during 2003, 2006 in SP3, and 2016 and 2017 in SP4 only one season was covered, which could have caused underestimation in the number of species. Furthermore, in SP1 and SP2, different sampling team collected ichthyofaunal data and bias has not been minimized (Bain and Finn, 1990), which reveals another disadvantage of this study. However, significant differences between sampling seasons were not found in this research, suggesting that the final result was not particularly affected.

In the Medsave area upstream from Zagreb, three small HPPs are planned, at Brdovec, Samobor and Zaprešić (Program Sava, 2014). It is important to note that these planned power plants will affect three NATURA 2000 areas important for the protection of species and habitats: Sutla (HR2001070), the Sava upstream from Zagreb (HR2001506), and Medvednica (HR2000583); all include fish as the target species for protection (Weiss et al., 2018).

Although the planned power plants will be designed to produce minimal differences in elevation, they will have a negative impact on certain native fish species that are highly migratory, in particular the Danubian brook lamprey *Eudontomyzon vladykovi* (Oliva & Zanandrea, 1959), which currently uses this stretch of the river to migrate to suitable spawning sites upstream in the Sutla River, the cactus roach and the Danube barbel as strongly migratory species, as well as blageon, about which little is known of its actual presence in this stretch of the Sava River, making it difficult to predict possible impacts (Bănăduc and Curtean Bănăduc, 2015; Vucić et al., 2017).

The cumulative impacts of these three planned HPP should also not be ignored, particularly in tandem with the existing structures that are already in operation upstream in Slovenia and the additional seven HPP planned for construction in and downstream of Zagreb. The cumulative impacts of changes to the flow regime over this large section of the Sava River will ultimately alter the specific habitats currently inhabited by native ichthyofauna, opening up new opportunities for invasion and competition by alien species.

The number of alien species is an additional indicator for the presence of stressors in aquatic environments (Simonović et al., 2017). At the Medsave site, gibel carp, pumpkinseed and North American bullhead were recorded for the first time in 1978 and 1979, although these species were probably present earlier in the River Sava (Piria et al., 2018). The topmouth gudgeon recorded in 1991 indicated that during the six years of its upstream spread from its first record in the River Sava (Habeković and Popović, 1991), this species utilized the available resources for its acclimatization and naturalization (Richardson et al., 2000). In 1993, the topmouth gudgeon appeared in huge abundance, subsequently declining over several years and it is successfully naturalized today. Two more findings of alien species were identified near the confluence of the Sutla River: the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Povž, 1989) and the bighead goby *Ponticola kessleri* (Günther, 1861) (Simonović et al., 2017) but was not recorded in present research at Medsave. Also, three new alien species: the round goby *Neogobius melanostomus* (Pallas, 1814), racer goby *Babka gymnotrachelus* (Kessler, 1857) and monkey goby *Neogobius* *fluviatilis* (Pallas, 1814), may occur in near future at the investigated site (Piria et al., 2018).

Results from this research reveal that the disturbances in fish assemblage patterns in the past and recent periods coincided with the presence of multiple stressors; however, a completely satisfactory conclusion is still not possible without further research. Processes which cause disturbances in fish assemblages during the time is complex, particularly if ongoing processes are still influencing the area. Furthermore, fish assemblage dynamics is a function of synergic and antagonistic effects of natural and anthropogenic abiotic factors which are difficult to separate (Kruk et al., 2016). Migration barriers determine fish distribution at a scale much larger than the sampling site (Kruk et al., 2017) and that is why we suggest that future research should be more exhaustive and planned for similar periods of the year taking in the account more parameters that can give more inside into fish assemblage structures. We expect a dire future scenario with new planned HPP dams downstream (Schwarz, 2016): the Medsave region could be converted into a slow-flowing waterbody, optionally connected with Sutla River, suitable for lower rithronic and potamal species as well as for new fish invasions (Strayer, 2010).

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