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Characterization of sections of the Sava River based on fish community structure

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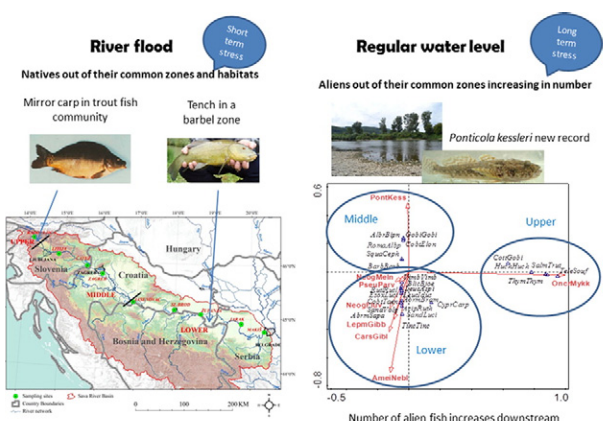
HIGHLIGHTS

- Farmed mirror carps were found in the trout fish zone of the Sava River
- Tench, a backwater fish expelled to the main river channel by flooding
- Pelagic fish species more resistant to flooding
- Most upstream finding of bighead goby in the River Sava
- The number of stressors on native ichthyofauna progressively increases downstream

GRAPHICAL ABSTRACT



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ABSTRACT

Sampling was undertaken, with the same fishing gear and along the Sava River, from its source to its confluence, in September 2014 and September 2015. In total, 44 fish species were identified, of which 37 were native species and 7 were alien. Fish samples revealed independence in terms of both species composition and their abundance under different hydrological conditions. During flooding and high water levels in 2014, pelagic fish species were sampled in greater proportion than at lower water levels in 2015 when benthic fish species were more abundant. The flood wave in 2014 was accompanied by catch of common carp, *Cyprinus carpio*, a typical lower rhithron fish species in the upper course, and of tench, *Tinca tinca*, a typical potamon fish species of backwaters, in the main channel of the lower Sava River. One specimen of bighead goby, *Ponticola kessleri*, which is common in the potamon fish community, was caught during the 2015 sampling close to the boundary between the upper and middle sections of the Sava. This is the first record of Ponto-Caspian gobies in the inland waters of Slovenia. Its finding far upstream indicates a strong effect of an as yet unidentified stress along the Sava River up to the spot where the bighead goby was sampled. Finally, these results indicate that pelagic fish species are more resistant to the stressful effect of flooding than benthic species, and that the structure of fish communities is

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influenced/affected by flooding as a short-term stressor. The progressively increasing number of alien fish species downstream in the Sava River point to the effects of long-term human-induced stressors in the area.

1. Introduction

Both taxonomic and ecological diversity and the character of fish communities in streams and rivers are strongly correlated with stream order. The transition from upper rhithron fish communities in headwater sections that usually contain streams up to the second or third order, to those belonging to the downstream, middle rhithron type of community in streams of higher (e.g., fourth and higher) orders, is usually gradual. This transition is even more gradual in fish communities along the most downstream sections in large rivers which change more slowly due to their higher complexity and greater variety of habitats. Both the ecological functioning and biodiversity patterns in large, floodplain rivers are to a great extent driven by the hydrological regime (Junk et al., 1989), which erases the distinguishing features of different habitats at low water levels. At the same time, human activities expose inland water ecosystems to a wide range of stressors that threaten the biodiversity of ecosystems and ecosystem processes (Dudgeon et al., 2006). Stress can be considered sublethal to plant and animal physiology when it leads to a decrease in food intake and fecundity (Hughes and Connell, 1999). This result in reduced biodiversity, a change in productivity, an increase in disease prevalence and the appearance/introduction of alien species, but also increases the numbers of small, opportunistic native species with a short life-span (Rapport et al., 1985).

Recent data for fish assemblages along the Sava River revealed that fish communities follow a general pattern of typology, as reported by Simonović et al. (2015a). It was apparent that middle rithron fish communities of the tributaries of the Sava River in its rithronic (middle and lower) sections extended further downstream where a gradual transition to the potamon fish community was observed. As a result, the middle and lower courses of the Sava River, where short ranges of middle rithron fish communities close to confluences with large tributaries alternate with long and stable lower rhithron type fish communities, are characterized by constantly changing fish community types. A survey of recent records (Simonović et al., 2015a) shows that fish (including lamprey) fauna of the Sava River is comprised of 74 species, 15 of which are considered alien.

The Sava River is exposed to different anthropogenic stressors, including organic and nutrient pollution and contamination with priority and other chemical substances from agriculture and local industries (Ogrinc et al., 2015., Ščančar et al., 2015), and to hydromorphological degradation of habitats (Paunović et al., 2016). A link between the in-stream physical and chemical environment and river communities provides a number of relationships across multiple spatial scales. Fish communities are considered to be an effective indicator of environmental stress (Karr, 1981), including hydromorphological degradation (Schmutz et al., 2016). The ecological status of an aquatic ecosystem is the expression of the quality of its structure and functioning, while the chemical status of an aquatic ecosystem is a reflection of its compliance with all of the quality standards established for chemical substances at the European level (national standards based on Directive 2008/105/EC – European Union, 2008, and more recently Directive 2013/39/EU – European Union, 2013).

In this study, for the first time the Sava River was sampled in two consecutive years at the same sites/locations using the same sampling strategy, from its source to its confluence with the Danube. The objectives of this study were (1) to identify the influence of flooding, elevation, physical and chemical water quality variables and habitat modifications on fish assemblages, (2) to determine co-occurrence of non-native and native fish species in fish communities and (3) to estimate the feasibility of using fish as indicators of particular stressors.

2. Materials and methods

2.1. Study area and fish sampling

Nine locations along the Sava River were sampled once in September in 2014 and 2015 (Fig. 1) to gain insight into status of fish communities on them in different hydrological conditions. Sampling details are listed in Appendix 1. Single-pass point-sample electrofishing surveys (Persat and Copp, 1989) per 1000 m of shoreline (Zalewski, 1985) were accomplished at each sampling station on various types of substrates and microhabitats (e.g., pool-riffle-glide) in the main river channel alternating in downstream direction, with the approximately same fishing effort applied in both consecutive years of sampling at different hydrological conditions. During sampling, depths ranging from 0.2 to 2 m were undertaken along the riverbank, by wading at the first locality (Radovljica) and from the boat at all others, 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 10-mm-mesh-size net was used. In order to minimize the between-operator bias, surveys were performed by the same three-person sampling team (Bain and Finn, 1990).

Fish identification was performed immediately after the sampling as described in Simonović (2001) while the newest scientific nomenclature was used according to Froese and Pauly (2016). The total length (TL, ±1 mm) and mass (M, ±0.01 g) were measured, after which the fish were released.

2.2. Statistical analysis of fish community structure and habitat stressors

Overall taxonomic diversity, as well as the diversity of fish communities at each sampling locality, was assessed by the Shannon Information Index H' (Welcomme, 1979).

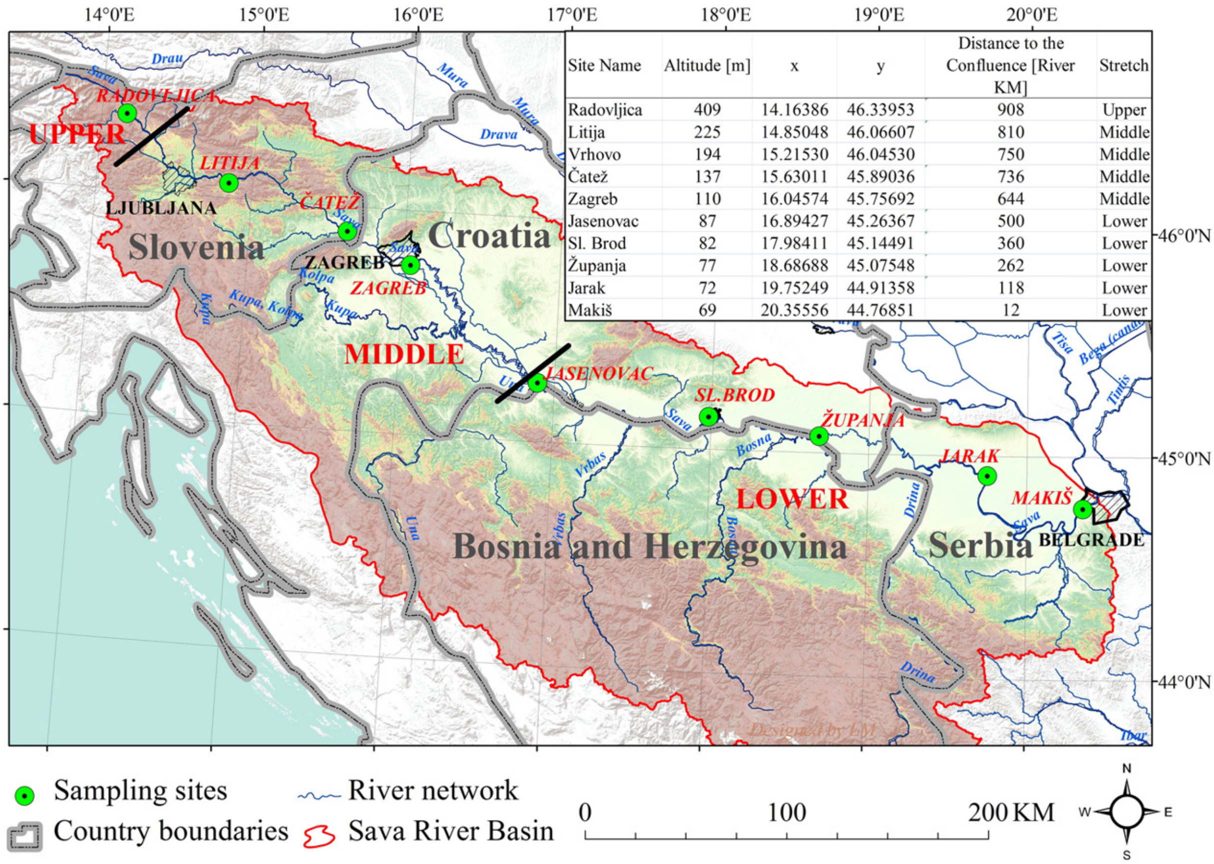
The additional measure that complements the ecological component of diversity was estimated using the Evenness Index (J) (Legendre and Legendre, 1983) for the fish community at each sampling locality.

The fish communities were characterized by calculating the Ecological Index Ei that Šorić (1998) introduced for fish species in inland waters of the Danube River system in Serbia and adjacent regions. The index uses the rank f (mass) of each fish species in the sample according to its relative abundance ($f(<1\%) = 1; f(1\text{–}3\%) = 2; f(3\text{–}10\%) = 3; f(10\text{–}20\%) = 4; f(20\text{–}40\%) = 7; f(>40\%) = 9$) , and K indicator values for each type of aquatic habitat (1 for upper rhithron, 2 for middle rhithron, 3 for lower rhithron, and 4 for potamon habitats) that are shared by a particular fish species. It is calculated using the expression:

$$E_i = \frac{\sum K_i f_i}{\sum f_{i.}}$$

Fish communities with an E_i value lower than 1.5 are of the upper rhithron type, those with E_i up to 2.5 are of the middle rhithron type, those with E_i up to 3.5 are of the lower rhithron type, and those over 3.5 belong to the potamon fish community type.

The independence between fish community structures at each sampled locality during both years of sampling under different hydrological conditions was tested by Contingency Analysis, using the χ^2 test of goodness-of-fit (Sokal and Rohlf, 1981).



Site Name	Altitude [m]	x	y	Distance to the Confluence [River KM]	Stretch
Radovljica	409	14.16386	46.33953	908	Upper
Litija	225	14.85048	46.06607	810	Middle
Vrhovo	194	15.21530	46.04530	750	Middle
Čatež	137	15.63011	45.89036	736	Middle
Zagreb	110	16.04574	45.75692	644	Middle
Jasenovac	87	16.89427	45.26367	500	Lower
Sl. Brod	82	17.98411	45.14491	360	Lower
Županja	77	18.68688	45.07548	262	Lower
Jarak	72	19.75249	44.91358	118	Lower
Makiš	69	20.35556	44.76851	12	Lower

Fig. 1. Sampling localities at the River Sava during 2014 and 2015.

To determine the gradient length of the response data, unimodal unconstrained DCA (Detrended Correspondence Analysis) was used. Collected fish species were chosen as response variables to analyze the relationships between fish assemblages and water level (1 – high; 2 – low), with alien fish species as environmental (explanatory) variables and locations as nominal explanatory variables. High water level refers to the flood event that occurred in September 2014, whereas the low water level refers to the period of low and stagnant water level in September 2015 (Appendix 1).

To determine the relationship between fish species and habitat stressors, several physical-chemical parameters were chosen: the concentrations of As, Cd, Pb, Cr (μg L^{-1}) in the water (AsH_2O, CdH_2O, PbH_2O and CrH_2O), pH and water temperature (t) and four habitat variables: the hydromorphological status, bottom substrate (1 – rocks, large stones and boulders; 2 – pebbles, gravel and coarse sand; 3 – fine sand and mud), the ecological status (1 – High; 2 – Good; 3 – Moderate; 4 – Poor; 5 – Bad); chemical status (Che1 – satisfactory and Che2 – unsatisfactory), according to the Water Framework Directive (2013). The data used for the classification according to the chemical and ecological status was taken from the Sava River Basin Management Plan (ISRBC, 2013). Assessment of the hydromorphological status was done for each site using the simple descriptive criteria developed for large fluvial rivers as follows: 1 – High status (undisturbed – no visible hydromorphological degradation); 2 – Slightly modified (visible/measurable consequences to biota 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). Relationships between stress variables and the structure of fish communities were analyzed by constrained Canonical Correspondence Analysis (CCA) with response data log transformations and forward selection only for habitat variables, and by the constrained axis test and unrestricted permutations, using the CANOCO 5 software package (Ter Braak and Šmilauer, 2012).

3. Results

3.1. Taxonomic and ecological diversity of fish species along the Sava River

During both years of sampling, a total of 44 fish species were collected at nine localities along the Sava River, of which 37 were native and 7 were alien (Appendix 1). In 2014 and 2015, there were 34 and 43 fish species in the samples, respectively. Sampling in 2014 was undertaken exactly during the period of flood wave (see Appendix 1). Overall Diversity (H_i) was greater in 2014 than in 2015 for samples from the first three localities (Radovljica, Litija and Čatež) situated in Slovenia, and for one of two localities (Jarak) in the lower section of the Sava in Serbia (Table 1). The Evenness Index (J_i) only partially followed this trend, as it increased or was similar in samples at localities in the middle and lower sections of the Sava (Table 1). Fish community structure assessed at particular localities (e.g., Radovljica, Litija, Jasenovac, Županja, Jarak and Makiš) in the two years, each of them being with very different hydrological conditions, were sufficiently similar to match well by ecological characterization (E_i), whereas those from other localities (e.g., Čatež, Zagreb and Slavonski Brod) were very different for their fish community structure in two years of investigation (Table 1).

Certain fish species characteristic for the type of the fish community at particular sections were missing from the 2014 samples in the upper

Table 1
Fish samples from the Sava River collected in 2014 and 2015 at nine localities (U = upper, M = middle and L = lower section), with the number of species (*S*), the Shannon Diversity Index (*H'*), the Evenness Index (*J*) and the Ecological index (E_i) for each sample and with the test of independence (χ^2 test) for the structure of fish communities in two years of sampling; df denotes the degrees of freedom and p denotes the significance of the results at 95% (<0.05, i.e. *) and 99% (<0.01, i.e. ***) probability levels.

Locality	Country	2014				2015				χ^2	df	p <
		S	*H'*	*J*	E_i	*S*	*H'*	*J*	E_i			
Radovljica (U)	Slovenia	6	1.536	0.857	1.40	5	1.214	0.755	1.000	31.236	6	***
Litija (M)		6	1.626	0.908	1.96	7	1.323	0.680	1.875	24.967	8	*
Čatež (M)		8	1.563	0.752	2.63	13	1.308	0.510	2.343	125.748	15	***
Zagreb (M)	Croatia	6	1.379	0.770	2.46	16	2.126	0.767	3.023	104.818	16	***
Jasenovac (L)		5	0.927	0.576	3.18	9	1.784	0.812	3.265	25.014	11	*
S. Brod (L)		3	0.284	0.258	3.40	12	1.517	0.610	3.529	60.272	12	***
Županja (L)		2	0.637	0.918	3.00	14	1.603	0.607	3.275	63.528	14	***
Jarak (L)	Serbia	20	2.547	0.850	3.58	16	2.231	0.805	3.565	144.972	24	***
Makiš (L)		11	1.812	0.756	3.57	21	2.415	0.793	3.500	94.216	21	***

(e.g., huchen *Hucho hucho*), middle (gudgeon *Gobio gobio* and white-finned gudgeon *Romanogobio albipinnatus*) and lower courses (starlet *Acipenser ruthenus*, burbot *Lota lota*, topmouth gudgeon *Pseudorasbora parva*, Volga pikeperch *Sander volgensis*, pumpkinseed *Lepomis gibbosus* and three Ponto-Caspian goby species, monkey *Neogobius fluviatilis*, round *N. melanostomus* and bighead *Ponticola kessleri* goby) of the river. Compared to 2014, in 2015 in the upper course only blageon *Telestes souffia* was absent, and in the middle course only the rare Danube bleak *Alburnus chalcoides* was not found. Of the species that were absent in the 2014 samples, the majority were either riverbank ambush predators (huchen, burbot, Volga pikeperch), and/or bottom-dwelling benthivorous (starlet, common and white-finned gudgeon, monkey, round and racer goby and pumpkinseed). Two species (blageon and Danube bleak) that were not observed in the 2015 samples are pelagic and schooling, mainly insectivorous fish (Fig. 2).

Comparison of all fish samples collected in the two years revealed significant independence of species composition and abundance (Table 1).

3.2. Temporal fish assemblages and identified stressors

DCA analysis of the first axis revealed a long gradient length (7.11) of response data and justified further use of unimodal constrained multivariate methods.

During the 2014 flood, pelagic fish species were sampled in greater proportion than in 2015 when fish characteristic of benthic communities were more abundant. The flood wave in 2014 was also accompanied by a catch of tench *Tinca tinca*, a typical potamon fish species, in the main channel of the lower Sava River where it is not usually found (Fig. 2).

According to the structures of fish communities, three large sections were distinguished by CCA analysis: the upper (Radovljica), middle (Litija, Čatež and Zagreb) and lower sections (Jasenovac, Županja, Slavonski Brod, Jarak and Makiš). The first four eigenvectors (λ) of the CCA explained almost 85% of the total variability of fish samples, with the first two explaining over 60% of variability ($\lambda_1 = 0.894$, 43.72%; $\lambda_2 = 0.4402$; 65.60%, $\lambda_3 = 0.2328$, 77.17%, $\lambda_4 = 0.1561$, 84.93%).

Members of the fish community in the upper section that was rhithronic in character were benthivorous brown trout, rainbow trout and bullhead *Cottus gobio*; the presence of grayling *Thymallus thymallus*, blageon and predatory huchen pointed to the transition of the fish community to the middle rhithronic type. Only common carp *Cyprinus carpio* Linnaeus, 1758, a typical lower rhithron fish species, was located between the upper and lower rhithron groups possibly as a result of the escape of mirror carps, two were sampled, from the nearby rearing fish pond at Radovljica during the high water level (Fig. 3A; Appendix 1). The middle section was characterized by a rhithron fish community, represented by benthivorous barbel *Barbus barbus*, insectivorous spirlin *Alburnoides bipunctatus* and omnivorous chub *Squalius cephalus*. Lower rhithron fish communities downstream of Jasenovac contained species typical for watercourses with a low flow velocity. The most frequently caught fish were common carp, northern pike *Esox lucius*, pike perch *Sander lucioperca*, common bream *Abramis brama* and roach *Rutilus rutilus* (Fig. 3A). In 2015, the difference between the numbers of fish

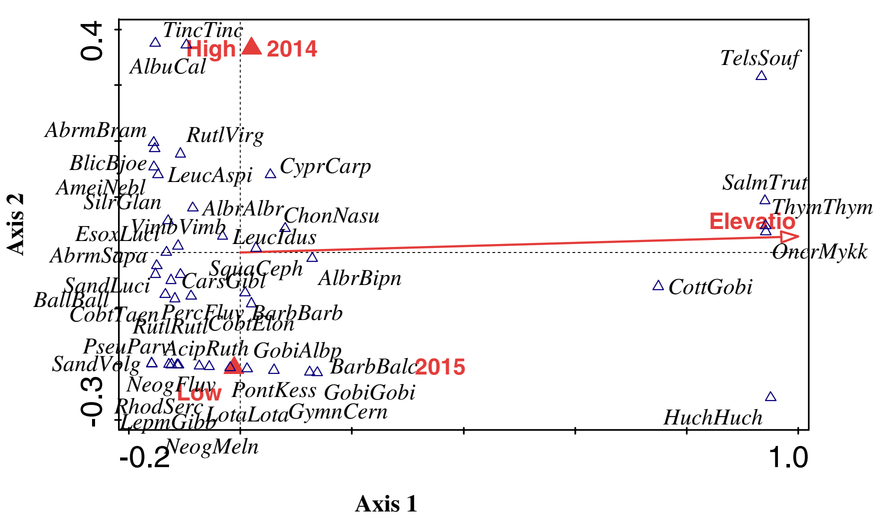


Fig. 2. Canonical correspondence analysis ordination diagram with the main fish species, in samples collected in 2014 and 2015 at nine localities, corresponding to high and low water levels and river elevation; ▲ – nominal explanatory variables, Δ – fish species, → – explanatory/response variables. Total variation is 2.80454, explanatory variables account for 33.3% of the variation; Monte Carlo permutation test results on all axes: pseudo-F = 3.7, p = 0.002.

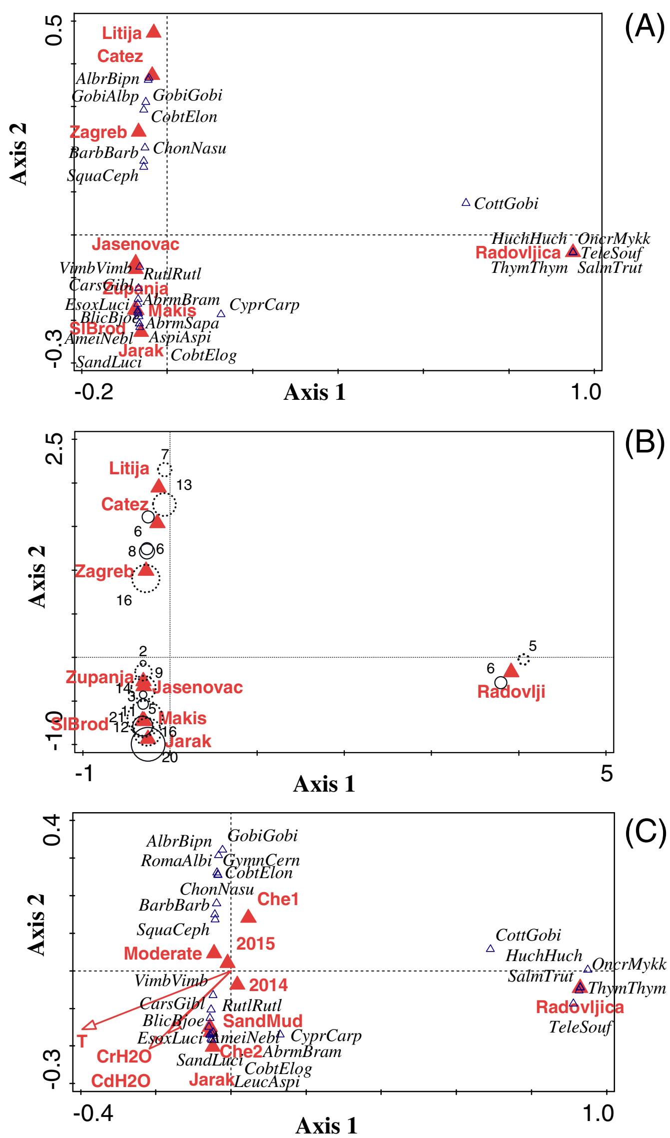


Fig. 3. Canonical correspondence analysis ordination diagram representing (A) the main fish species in samples collected in 2014 and 2015 at nine localities. Total variation is 2.804, explanatory variables account for 71.1% of the variation; Monte Carlo permutation test results on all axes: pseudo- F = 2.9, P = 0.002. (B) Species diversity diagram. (C) Constrained analysis with forward selection of hydromorphological parameters and sites; total variation is 2.545, explanatory variables account for 80.7% of the variation (▲ –nominal explanatory variables, Δ – fish species, ○ – fish diversity (number of fish species in the sample; dotted circle lines denote 2015 sampling year), → – explanatory/response variables, T = temperature).

species at each locality increased progressively in a downstream direction (Table 1) when compared to 2014. At upstream locations, where the number of species in fish communities was smaller, these differences were not as prominent (at Radovljica, 5 and 6 fish species were sampled in 2014 and 2015, respectively). However, differences were more obvious at the more downstream localities (excluding Jarak): at Županja, 2 and 14 fish species were caught in 2014 and 2015, respectively, and at Makiš, 11 and 21 fish species were caught in 2014 and 2015, respectively (Fig. 3B).

Using CCA analysis of the physical and chemical parameters and habitat variables of the structures of fish communities, several significant stressors were determined; these were the chemical status, cadmium and chromium concentrations and hydromorphological modifications [($p < 0.01$, the first four eigenvectors of the CCA explained 85% of the total variability of fish samples, with the first two explaining over 60% ($\lambda_1 = 0.85$, 41.37%; $\lambda_2 = 0.4244$, 62.02%; $\lambda_3 = 0.256$, 74.49%, $\lambda_4 = 0.1795$, 83.22%)]. The water quality of the upper region was satisfactory; however, the downstream sections were significantly polluted, due to the presence of high concentrations of cadmium and chromium, especially at the Jarak site. The middle and lower sections of the Sava are moderately modified, characterized by a sandy and muddy bed and increasing water temperature downstream (Fig. 3C). Only significant variables and sites in Fig. 3C were plotted ($p < 0.01$ and $p < 0.05$, respectively), while Fig. 3A presents the distribution of other non-significant locations.

One bighead goby was caught in September 2015 at the locality of Čatež (725 rkm), close to the boundary between the upper and middle sections of the Sava River, and a few kilometers upstream of the border with Croatia. This is the first record of Ponto-Caspian goby for the inland waters of Slovenia (Appendix 1).

Of the samples caught in both years of sampling, the number of alien fish species in the Sava River increased progressively downstream, with 1 (rainbow trout *Oncorhynchus mykiss*) alien fish species caught in the upper section, 2 (gibel carp *Carassius gibelio* and bighead goby) in the middle, and 7 (gibel carp, topmouth gudgeon, brown bullhead *Ameiurus nebulosus*, pumpkinseed, round goby and racer goby) in the lower section (Fig. 4).

4. Discussion

The structure of fish communities that was determined in the two sampling campaigns at specific sections of the Sava River strongly resembles the structure described by Simonović et al. (2015a), who analyzed the records that were available from various sources for the last decade. Fish communities revealed a strong upstream-to-downstream gradient, as defined by Askeyev et al. (2014), along the Sava River. The significant differences between (i.e. the independence of) the samples acquired in the two years of sampling with prominent changes in the water regime (Marušić, 2014) are observed as differences in structure and abundance, especially when particular ecological types of fish (pelagic and benthic) were considered. Sampling of fish in two consecutive years at the same stations did not affect the results of the structure of fish communities on them for several reasons: 1) all fish sampled, identified and measured for mass and length were returned alive into the

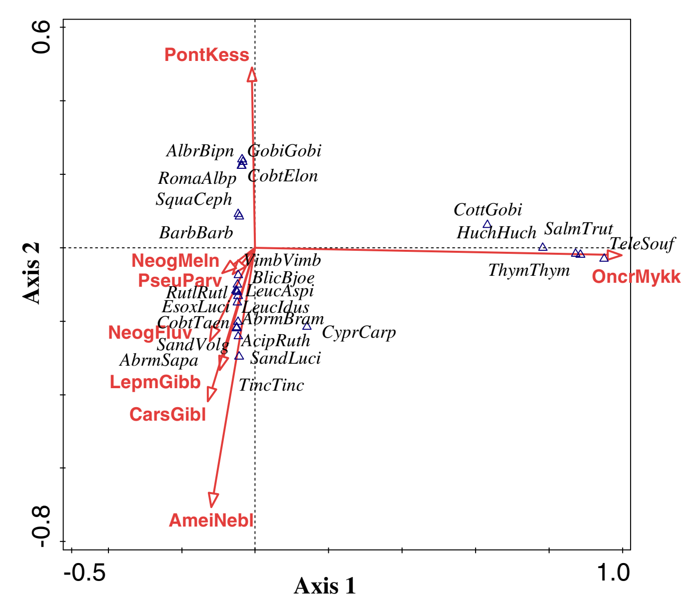


Fig. 4. Canonical correspondence analysis ordination diagram with the correspondence of alien fish species to the native ones in particular types of fish community, where Δ represents native fish species and → explanatory/response variables. Total variation is 2.584, explanatory variables account for 66.6% of variation, Monte Carlo permutation test results on all axes: pseudo- F = 2.2, p = 0.002.

River Sava at the same locality where they were sampled.; 2) the time period between two sampling campaigns was a whole year, and 3) hydrological conditions that varied greatly in a year period between two samplings (Appendix 1) also impacted on the fish community at the sampling sites. In addition, the Chisquare testing performed (Table 1) also revealed independence between the samples taken in two consecutive years. The relative efficiency of single-pass electrofishing of longer stream sections vs. double-pass electrofishing of shorter reaches gives approximately the same level of accuracy and precision for any assemblage level variable, e.g., richness, composition, relative abundance, etc. (Sály et al., 2009). Thus, single-pass electrofishing can be used to detect spatial and temporal trends in abundance and species richness given standardized effort (Bertrand et al., 2006). In addition to that, considering that sampling of the smallest size samples as possible ensures the effectiveness in sampling of an aggregate population (Gerard and Berthet, 1971), the results presented here can be considered a simulation of continuum of the riverscape approach introduced by Fausch et al. (2002) that provides collecting of continuous data at a coarser spatial resolution. Our findings are in agreement with Rogers et al. (2005), who concluded that differences in both abundance and diversity were more common at sites with the a high degree of river water-level variability. Nevertheless, the main character of a given fish community was not substantially affected by the flooding event, since at the majority of sampling localities (in 6 out of 9 cases), the type of fish community that was assessed in both years of the sampling using the Ecological Index E_i was the same. In addition to this, the characterization of the fish communities as determined by the Ecological Index (Table 1) completely corresponded to the results of the CCA (Fig. 3A). The robustness of the Ecological Index is self-recommending for its use to characterize fish communities in this specific area of investigation.

Apart from the characterization of fish communities along the Sava River, CCA served to validate the downstream increase in fish community diversity (Fig. 3B), and the association of particular alien species to particular fish community types (Fig. 4). The only deviation from expectation was the position of the bighead goby (Fig. 4), which is commonly associated with a potamon type of fish community (Ćaleta, 2007). A single bighead goby was unexpectedly observed far upstream on the boundary section between Slovenia and Croatia, in a typical middle-rhithronic fish community. The presence of bighead goby there implies an occurrence of the yet unknown stress in this section of the Sava River.

Taking into account the motility of fishes and great variety with regard to their ecological preferences for particular habitats, water quality, current velocity, food, etc., the study of fish can expose short-term, acute, and long-term stress events. The results of sampling in 2014 compared to those obtained in 2015 revealed that flooding as a short-term stressor impacted significantly either certain site-specific factors, such as benthic ambush predators and bottom-dwelling benthivorous fish species of fish communities, or whole fish communities (Table 1) that revealed different character at particular sampling locations (e.g., at localities Čatež and Zagreb). Thus, at the high water level sampling in 2014, despite of the same fish-community characterization, at several localities pelagic, insectivorous fish species (e.g., bladgeon at Radovljica and Danube bleak at Jasenovac) were less affected by that stress than benthic (e.g., brook barbel, common barbel and nase at Radovljica and gibel carp, spined loach *Cobitis elongatoides*, pumpkinseed *Lepomis gibbosus* and monkey goby at Jasenovac) and predators (e.g., huchen at Radovljica and pike, chub, asp and burbot at Jasenovac). The functioning of large river systems with adjacent floodplains, as those occurring along the Sava River, is strongly influenced by the flood pulse (Sousa and de Freitas, 2008) usually occurring in winter or spring in the continental climate of Eastern Europe. Although the impacts of winter and spring floods on lotic ecosystems have been well studied, the effects of summer floods are less well known (George et al., 2015). An unexpected and extreme hydrological event occurred in the late summer of 2014 in the Sava River during the sampling, whereas a year later, the hydrological conditions were stable. Given that the structures of fish communities were tested under different hydrological conditions, the obtained results suggested that at least in part the abrupt and considerable increase in water level exerted a strong impact on them (Table 1). In addition, the two unexpected events (the capture of 2 carp in the uppermost section of the Sava River and tench in the main river bed) were valuable indicators of the action of the flood as a stressor. The carp were most likely washed from the nearby fish-rearing pond (e.g. Vošče) to the main river bed in the uppermost section of the Sava River by the sudden and substantial rise in water level while tench were most likely washed by the flood wave from the nearby dead arm to the main river bed in the lower section of the Sava River. Their presence in uncommon habitats revealed the effect of short-term stress on the fish community due to flooding. Results in this study also showed that fish species belonging to particular ecological groups (e.g. pelagic) are probably more resistant to the effect of flooding than those belonging to other groups (e.g. benthic). This is probably because pelagic fish have very limited opportunities to avoid a flood wave, unlike benthic fish species, which have at their disposal many inshore shelters (riprap embankments, sunken trees, small bays and oxbows and counter-current pockets of water), which are available at high water levels at increased depths along the river banks. This effect of reduced spatial variability during flooding in large rivers in both tropical and temperate areas has already been described by Thomaz et al. (2007). Such redistribution can also remarkably affect the sampling, since neither common electrofishing nor netting gear can reach these fish in these circumstances. One possible solution for increased effectiveness of sampling in extremely high water levels would be to apply specially designed fishing gear (e.g., demersal trapping gears). However, this is not feasible for many reasons, such as the high demand for human resources, equipment and transport gears, time- and money-consumption, the differential fishing success of a variety of fishing gears, and danger to the fishermen working in such extreme sampling conditions. A far more realistic and effective way to examine the structure of fish community is to perform sampling at medium-to-high water levels with standardized fishing gear and a standardized fishing effort (Flotemersch and Blocksom, 2005). This procedure, which is also common in investigations of fisheries (Achleitner et al., 2012), provides comparable samples and is much more reliable in assessing the effect of stressors acting on an ecosystem (Branco et al., 2016). Furthermore, sampling during high waters, particularly on the Sava River, presents its own challenges and shortcomings and the same catch effort probably does not give the same catch results, which consequently make difficulties to distinguish true changes in the population parameters (Ricker, 1975). However, for the first time the Sava River was sampled in two consecutive years at the same sites in the same period of the year using the same sampling strategy, from its source to its confluence and presented results can give insight into fish community changes during unexpected summer flood event, as a short term stress, throughout the whole river course. Finally, differences in the habitat preference of particular fish species can also be used to detect the nature of sudden and short-term stressors, such as chemical or physicochemical pollutants, in a river ecosystem. If pelagic fish would be more affected by pollution than benthic fish, that would help to narrow the search for the pollutants to those of specific properties, e.g., of the low specific weight.

An additional indicator for the presence of stressors, as inferred from the described sampling, was the number of alien species. During the assessment of the invasive potential of alien fish species in inland waters of the Balkans, Simonović et al. (2013, 2015b) and Piria et al. (2016a) assigned high invasiveness to rainbow trout, gibel carp and brown bullhead. A progressive increase in the number of alien fish species in certain downstream sections of the Sava River implied that the action of long-term stressors of an as yet undetermined nature was greater in the lower section. It is indicative that none of alien Ponto-Caspian goby species was recorded in the Sava River before 1970, i.e., prior to the construction of the Iron Gate One dam at the rkm 943 that created a huge reservoir (upstream to the rkm) on the Danube River. The dam has tremendously slowed down the water flow of the Danube River even upstream of the confluence of the Sava River to it (rkm 1170), up to the Slankamen village (rkm 1215). The first finding of monkey goby in the section of the Danube River upstream of the Iron Gate One reservoir dates from the Spring 1984 (PS, unpublished), whereas the first published finding in the Sava River in the Belgrade area (rkm 5) dates from 15 May 1994, and the next one at the city of Šabac (rkm 121) dates from 24 September 1998 (Simonović et al., 2001). Bighead goby dispersed upstream of the Danube and Sava Rivers simultaneously with monkey goby, whereas racer *Babka gymnotrachelus* and round goby followed them with about a decade in delay (PS, unpublished). In addition to the construction of the huge Iron Gate One dam and hydrological consequences it has caused, there are also other kinds of human impact that might act as long-term stressors. The decrease in water quality that accompanies hydromorphological alterations, as detected by CCA (Fig. 3C), could be an additional reason for the increase in the number of alien species (Tejerina-Garro et al., 2005) at downstream locations. However, considering that the downstream increase in size and habitat complexity was accompanied by an increase in the number of species in the fish communities, the increase in the number of alien species in the communities is a reflection of the availability of the appropriate resources for their acclimatization and naturalization (Richardson et al., 2000). The increase in size and complexity of resources along the Sava River also favors increased human activities, with the floodplains providing a rich array of ecosystems (Tockner et al., 2010). Increased human activities, responsible for nutrient loading from intensive agriculture, river regulation, water use, etc. modify the floodplain ecosystem and expose native fish communities to stress and vectors of alien species introduction (through fish farming, bait-fishing, ornamental pet trading). Various human activities act as stressors in various types of interactions (in terms of Piggott et al., 2015), with the increase in resource availability, rendering the Sava River ecosystem a specific type of ecological receptor, with the number of alien fish species serving as an indicator of the type and the magnitude of the stress. Acquiring knowledge about the components of stress and relating them to the number of alien species is important for identifying/creating a quantifiable indicator of stress. Likewise, it is also worth examining how the effect of invasive species on native fish communities can be used to estimate the intensity and/or type of stress. Thus, the abundance (dominance) of non-native rainbow trout in both sampling years at the uppermost Sava River Radovljica locality (Appendix 1, Fig. 4) is an obvious indicator of both/either recreational fishing and/or trout fish farming that can act as stressors in fish. In contrast to that, the great complexity of ecosystems in downstream sections of the Sava renders the elucidation of the impact on fish community structure from different stressors much more difficult. Likewise, the intensity of ecosystem stress due to the presence of alien species is difficult to assess, since it depends on the number of alien species acclimatized and naturalized in habitats in particular sections of the Sava River, and on their invasive potential. Jackson et al. (2016) found that biota in freshwater ecosystems, in contrast to those in marine ones, response to multiple stressors much more in antagonistic manner, the most probably due to greater environmental variability inherent to them that fosters greater potential for acclimation and co-adaptation to multiple stressors. It would be interesting to correlate the number of alien fish species in certain sections of the Sava River with social variables (settlement, population and hydrotechnical constructions) in order to obtain better insights into their interdependence.

The first finding of bighead goby in Slovenia could be interpreted as a warning of the presence of an yet undefined stressor (Simonović et al., 2001; Jurajda et al., 2005; Piria et al., 2016b). The record for bighead goby so far upstream of the Sava River is reliable, as it was easily identified by its obvious pelvic disc with its frontal membrane with strong lateral lobes protruding. This feature distinguishes the bighead goby from the otherwise very similar bullhead (Froese and Pauly, 2016), a common benthic fish in fish communities occurring at that locality (Jelić, 2012). The history of the upstream spread of Ponto-Caspian gobies along the Sava River started in 1994, when monkey and bighead goby were recorded at the Novi Beograd (rkm 4) and Šabac (rkm 121) localities in Serbia (Simonović et al., 2001). Mustafić (2005) recorded monkey goby at Jasenovac (rkm 516) and bighead goby at Slavonski Šamac (rkm 370), whereas Jakovlić et al. (2015) recorded bighead goby at Davor (rkm 400); close to this locality, the most recent finding of bighead goby was at Čatež (rkm 725). The absence of bighead goby between Davor and Čatež may be due either to the limitations of the electrofishing method (Polačik et al., 2008), especially of fish without a swim bladder, to the presence of substrate dominated by fine particles (sand, fine sand and sediment), which is a habitat unsuited for this species (Erős et al., 2005), or due to a stepping-stone pattern of upstream invasion (Cerwenka et al., 2014). The spreading of Ponto-Caspian gobies along a 725-km stretch in less than twenty years is a serious development. The introduction of bighead goby could affect the native fish community by competing with bullhead, stone loach *Barbatula barbatula* and white-finned gudgeon, as Jurajda et al. (2005) have reported. In addition, adult bighead goby forage the fry of other fish species (Simonović et al., 2001), and their predatory activity can also cause a decline in the abundance of native fish species (Piria et al., 2016b). Furthermore, more occurrences of carp and possibly other cyprinids as well as non-native salmonids could be expected in the upper section of the Sava due intensive angling and corresponding fishery management activities (e.g., fish stocking) in the region, which are a common driver of environmental change (Bain, 1993). This could lead to a disturbance in native fish communities and decline, or extinction of native species, leaving resources they utilized at disposal and making them more susceptible to the successful introduction of other alien species. Strayer (2010) also stated that such disturbances generally favor invasions, as a stressed ecosystem is more susceptible to invasions.

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References

Achleitner, D., Gassner, H., Luger, M., 2012. Comparison of three standardised fish sampling methods in 14 alpine lakes in Austria. Fish. Manag. Ecol. 19, 352–361.
Askeyev, O., Askeyev, I., Askeyev, A., Monakhov, S., Yanybaev, N., 2014. River fish assemblages in relation to environmental factors in the Eastern extremity of Europe (Tatarstan Republic, Russia). Environ. Biol. Fish 97 (12), 1277–1293.
Bain, M.B., 1993. Assessing impacts of introduced aquatic species – grass carp in large systems. Environ. Manage. 17, 211–224.
Bain, M.B., Finn, J.T., 1990. Analysis of microhabitat use by fish: investigator effect and investigator bias. Rivers 2, 57–65.
Bertrand, K.N., Gido, K.B., Guy, C.S., 2006. An evaluation of single-pass versus multiple-pass backpack electrofishing to estimate trends in species abundance and richness in prairie streams. Trans. Kans. Acad. Sci. 109, 131–138.
Branco, P., Santos, J.M., Amaral, S., Romão, F., Pinheiro, A.N., Ferreira, M.T., 2016. Potamodromous fish movements under multiple stressors: connectivity reduction and oxygen depletion. Sci. Total Environ. 572, 520–525.
Ćaleta, M., 2007. Ekološke znacajke ihtiofaune nizinskog dijela rijeke Save (Ecological characteristic of the ichthyofauna of the Sava River lowlands) PhD Thesis Faculty of Science, University of Zagreb, Zagreb, Croatia, p. 232.
Cerwenka, A.F., Alibert, P., Brandner, J., Geist, J., Schliewen, U.K., 2014. Phenotypic differentiation of Ponto-Caspian gobies during a contemporary invasion of the upper Danube River. Hydrobiologia 721, 269–284.
Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182.
Erős, T., Sevcsik, A., Tóth, B., 2005. Abundance and night-time habitat use patterns of Ponto-Caspian gobiid species (Pisces, Gobiidae) in the littoral zone of the River Danube, Hungary. J. Appl. Ichthyol. 21, 350–357.

European Union, 2008. Directive 2008/105/EC of the European Parliament and the Council on Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and Amending Directive 2000/60/EC (was published in the Official Journal on 24 December 2008).

European Union, 2013. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy.

Fausch, K.D., Torgensen, C.E., Baxter, C.V., Li, H.W., 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. Bioscience 52, 483–498.

Flotemersch, J.E., Blocksom, K.A., 2005. Electrofishing in boatable rivers: does sampling design affect bioassessment metrics? Environ. Monit. Assess. 102, 263–283.

Froese, R., Pauly, D., 2016. FishBase. World Wide Web Electronic Publication. www.fishbase.org (version (01/2016)).

George, S.D., Baldigo, B.P., Smith, A.J., Robinson, G.R., 2015. Effects of extreme floods on trout populations and fish communities in a Catskill Mountain river. Freshw. Biol. 60, 2511–2522.

Gerard, G., Berthet, P., 1971. Sampling strategy in censusing patchy populations. In: Patril, G.P., Pielou, E.C., Waters, W.E. (Eds.), Statistical Ecology. Penn State University Press, pp. 59–67.

Hughes, T.P., Connell, J.H., 1999. Multiple stresses on coral reefs. Limnol. Oceanogr. 44, 932–940.

ISRBC, 2013. Sava River Basin Management Plan. International Sava River Basin Commission 235 p http://www.savacommission.org/.

Jackson, M.C., Leoewen, C.J.G., Winebrooke, R.D., Chimimba, C.T., 2016. Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob. Chang. Biol. 22, 180–189.

Jakovlić, I., Piria, M., Šprem, N., Tomljanović, T., Matulić, D., Treer, T., 2015. Distribution, abundance and condition of invasive Ponto-Caspian gobies *Ponticola kessleri* (Gunther, 1861), *Neogobius fluviatilis* (Pallas, 1814), and *Neogobius melanostomus* (Pallas, 1814) in the River Sava basin, Croatia. J. Appl. Ichthyol. 31 (5), 888–894.

Jelić, D., 2012. New data on the distribution of *Cottus gobio* (Scorpeniformes; Cottidae) in Croatia with special overview of the Adriatic basin. Ribarstvo 70 (1), 1–8.

Junk, W.J., Bayley, P.B., Sparks, R.E., 1989. The flood pulse concept in river-floodplain systems. Can. Spec. Publ. Fish. Aquat. Sci. 106, 110–127.

Jurajda, P., Černý, J., Polačik, M., Valová, Z., Janáč, M., Blažek, R., Ondračková, M., 2005. The recent distribution and abundance of non-native *Neogobius* fishes in the Slovak section of the River Danube. J. Appl. Ichthyol. 21, 319–323.

Karr, J.R., 1981. Assessment of biotic integrity using fish communities. Fisheries 6 (6), 21–27.

Legendre, L., Legendre, P., 1983. Numerical Ecology. Elsevier, Amsterdam, Netherlands (410 pp).

Marušić, J., 2014. Uvodnik: 2014. – godina s rekordnim brojem poplava u Republici Hrvatskoj (Editorial: 2014 - year with a record number of floods in the Republic of Croatia. Hrvatske vode, 22, 90, 287 (In Croatian).

Mustafić, P., 2005. Indeks biotičkog integriteta riblje zajednice velikih rijeka Hrvatske. (Index of Biotic Integrity of the Fish Community in the Large Croatian Rivers) Doctoral thesis Faculty of Science, University of Zagreb, Zagreb, Croatia, p. 185 In Croatian with English summary.

Ogrinc, N., Kanduč, T., Kocman, D., 2015. Integrated approach to the evaluation of chemical dynamics and anthropogenic pollution sources in the Sava River Basin. In: Milačič, R., et al. (Eds.), The Sava RiverThe Handbook of Environmental Chemistry Vol. 31. Springer-Verlag, Berlin Heidelberg, pp. 75–94.

Paunović, M., Grošelj, S., Milačić, R., Grđan, S., Zuliani, T., Vidaković, I., Vučković, I., Vićanović, J., Ščančar, J., Makovinska, J., Mišíková Elexová, E., Očadlík, M., 2016. Steps towards integrated water management in the Sava River Basin. Water Res. Manag. 6 (1), 3–10.

Persat, H., Copp, G.H., 1989. Electrofishing and point abundance sampling for the ichthyology of large rivers. In: Cowx, I. (Ed.), Developments in Electrofishing. Fishing News Books, Oxford, pp. 203–215.

Piggott, J.J., Townsend, C.R., Matthaei, C.D., 2015. Reconceptualizing synergism and antagonism among multiple stressors. Ecol. Evol. 5 (7), 1538–1547.

Piria, M., Povž, M., Vilizzi, L., Zanella, D., Simonović, P., Copp, G.H., 2016a. Risk screening of non-native freshwater fishes in Croatia and Slovenia using FISK (Fish Invasiveness Screening Kit). Fish. Manag. Ecol. 23, 21–31.

Piria, M., Jakšić, G., Jakovlić, I., Treer, T., 2016b. Dietary habits of invasive Ponto-Caspian gobies in the Croatian part of the Danube River basin and their potential impact on benthic fish communities. Sci. Total Environ. 540 (1), 386–395.

Polačik, M., Janáč, M., Jurajda, P., Vassilev, M., Trichkova, T., 2008. The sampling efficiency of electrofishing for *Neogobius* species in a riprap habitat: a field experiment. J. Appl. Ichthyol. 24, 601–604.

Rapport, D.J., Regier, H.A., Hutchinson, T.C., 1985. Ecosystem behavior under stress. Am. Nat. 125, 617–640.

Richardson, D.M., Pyšek, P., Rejmànek, M., Barbour, M.G., Dane Panneta, F., West, C.J., 2000. Naturalization and invasion of alien plants: concepts and definitions. Divers. Distrib. 6, 93–107.

Ricker, W.E., 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Board Can. 191 (382 pp).

Rogers, M.W., Allen, M.S., Dale Jones, M., 2005. Relationship between river surface level and fish assemblage in the Ocklawaha River. Florida. River. Res. Appl. 21, 501–511.

Sály, P., Erős, T., Takács, P., Specziár, A., Kiss, I., Bíró, P., 2009. Assemblage level monitoring of stream fishes: the relative efficiency of single-pass vs. double-pass electrofishing. Fish. Res. 99, 226–233.

Ščančar, J., Heath, E., Zuliani, T., Horvat, M., Kotnik, J., Perko, S., Milačić, R., 2015. Elements and persistent organic pollutants in the sediments of the Sava River. In: Milačič, R., et al. (Eds.), The Sava RiverThe Handbook of Environmental Chemistry Vol. 31. Springer-Verlag, Berlin Heidelberg, pp. 95–122.

Schmutz, S., Jurajda, P., Kaufmann, S., Lorenz, A., Muhar, S., Paillex, A., Poppe, M., Wolter, C., 2016. Response of fish assemblages to hydromorphological restoration in central and northern European Rivers. Hydrobiologia 769, 67–78.

Simonović, P., 2001. Ribe Srbije [Fishes of Serbia]. NNK International, Zavod za zaštitu prirode Srbije. Belgrade, Biološki fakultet (248 pp).

Simonović, P., Paunović, M., Popović, S., 2001. Morphology, feeding, and reproduction of the round goby, *Neogobius melanostomus* (Pallas), in the Danube River Basin, Yugoslavia. J. Great Lakes Res. 27 (3), 281–289.

Simonović, P., Tošić, A., Vassilev, M., Apostolou, A., Mrdak, D., Ristovska, M., Kostov, V., Nikolić, V., Škraba, D., Vilizzi, L., Copp, G.H., 2013. Risk assessment of non-native freshwater fishes in four countries of the Balkans region using FISK, the invasiveness screening tool for non-native freshwater fishes. Mediterr. Mar. Sci. 14/2, 369–376.

Simonović, P., Povž, M., Piria, M., Treer, T., Adrović, A., Škrijelj, R., Nikolić, V., Simić, V., 2015a. Ichthyofauna of the River Sava System. In: Milačič, R., et al. (Eds.), The Sava RiverThe Handbook of Environmental Chemistry Vol. 31. Springer-Verlag, Berlin Heidelberg, pp. 361–400.

Simonović, P., Vidović, Z., Tošić, A., Škraba, D., Čanak-Atlagić, J., Nikolić, V., 2015b. Risks to stocks of native trout of the genus *Salmo* (Actinopterygii: Salmoniformes: Salmonidae) of Serbia and management for their recovery. Acta Ichthyol. Piscat. 45 (2), 161–173.

Sokal, R.R., Rohlf, F.J., 1981. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman. New York, USA, Oxford 778 pp.

Šorić, M.V., 1998. Pollution of the Morava River (Danube basin, Serbia) and changes of the ichthyofauna under its influence. Acta Biologica Iugoslavica - Ichthyologia 30, 51–70.

Sousa, R.G.C., de Freitas, C.E.C., 2008. The influence of flood pulse on fish communities of floodplain canals in the Middle Solimões River. Brazil. Neotrop. Ichthyol. 6 (2), 249–255.

Strayer, D.L., 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshw. Biol. 55 (Suppl. 1), 152–174.

Tejerina-Garro, F.L., Maldonado, M., Ibañez, C., Pont, D., Roset, N., Oberdorff, T., 2005. Effects of natural and anthropogenic environmental changes on riverine fish assemblages: a framework for ecological assessment of rivers. Braz. Arch. Biol. Technol. 48, 91–108.

Ter Braak, C.J.F., Šmilauer, P., 2012. Canoco reference manual and user's guide: software for ordination, version 5.0. Microcomputer Power, Ithaca, USA 496 pp.

Thomaz, S.M., Bini, L.M., Bozelli, R.L., 2007. Floods increase similarity among aquatic habitats in river-floodplain system. Hydrobiologia 579, 1–13.

Tockner, K., Pusch, M., Borchardt, D., Lorang, M.S., 2010. Multiple stressors in coupled river-floodplain ecosystems. Freshw. Biol. 55 (Suppl. 1), 135–151.

Welcomme, R.L., 1979. Fisheries Ecology of Floodplain Rivers. Longman, New York (317 pp).

Zalewski, M., 1985. The estimate of fish density and biomass in rivers on the basis of relationship between specimen size and efficiency of electrofishing. Fish. Res. 3, 147–155.