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# Assessment of the ecological sustainability of river basins based on the modified the ESHIPPOfish model on the example of the Velika Morava basin (Serbia, Central Balkans)

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This study examines the ecological sustainability of the Velika Morava River Basin (Serbia, Central Balkans) under modern conditions of multiple freshwater stressors, including climate change. The impact of stressors on the ecological services of the river basin is considered, including: drinking water, irrigation, recreation, tourism, ecotourism, and fishing. In order to assess the ecological sustainability of the river basin, a modification of the ESHIPPO model was performed. The essence of the modification is a change to the ES component, which, in the basic model, assesses the degree of ecological specialization of a taxon; and in the modified assessment of the ecological stability of the ecosystem (ESE). The structure of ichthyocenosis is used as the basic biological indicator for the assessment of ESE. The assessment of river basin sustainability was obtained as the difference between ESE and the impact of multiple factors, including: habitat change, invasive species, pollution, human population growth (social factors), and the over-exploitation of resources (HIPPO). The statistical analysis (SOM—Self Organizing Map) highlights the most reliable indicators of both biotic (ichthyocenosis structure elements) and the indicators that best detect the influence of HIPPO factors. The structure of the model is based on general and easily measurable indicators, which enables its application in any river basin in the world. The results of the model indicate that 80% of the studied basin is ecologically unsustainable and that its potential environmental services are greatly reduced.

## KEYWORDS

ecological sustainability, river basin, ichthyocenosis, ESHIPPOfish model, multiple freshwater stressors

## Introduction

The ecological stability of river basins as aquatic macroecosystems in the era of the Anthropocene reflects both natural and anthropogenic influences, with their cumulative effects transmitted and accumulated downstream (Rockström et al., 2014a). Since a river basin, as a kind of “bloodstream”, permeates a land area of variable size, the ecological condition of the river basin largely reflects the state of the environment in that area.

Theoretically, the ecological stability and health of the main river basin should be a cumulative reflection of ecological stability of its tributaries and catchments. Fish communities within the river basin represent high levels of the food chain and, as such, are good indicators of long-term changes within the basin; and thus its overall stability and environmental sustainability (Canning and Death, 2018; Huang et al., 2022). The fish community structure dynamics of the Danube River basin, too, both in general and especially in its delta-sea interface area accurately reveal the present and historical impact of various natural and anthropogenic stressors on aquatic ecosystems (Simonović et al., 2010; Bănăduc et al., 2014, 2016, 2020, Simić et al., 2014a). Fish stocks offer traditional natural ecological river basin services, together with quality water for the local population (such as drinking water, crop irrigation water, and recreation) (Brugere et al., 2015).

River basin technological services, such as energy potential, river traffic, and technical water for industry and industrial agriculture, are factors that mainly reduce the quantity and quality of traditional environmental services. However, in the era of the Anthropocene, in which human populations have been growing, the need for increased use of traditional ecological services provided by aquatic ecosystems (fish resources, water resources) is constantly growing. Therefore, these activities are now unfavorable for preserving the ecological sustainability of river habitats and river basins as a whole (Petts et al., 2015).

Fish as indicators of aquatic habitat health are widely used through biotic ichthyological indices. This has been done in the USA (Karr, 1981, 1987) and, more recently, in EU countries (Jepsen and Pont, 2007; Pont et al., 2007). However, the universal application of ichthyological indices can still be hampered by regional and local differences in the structure of ichthyocenoses, complex definitions in terms of reference conditions, and different effective monitoring objectives (Stojković et al., 2011; Pitcher, 2015; Radinger et al., 2019). For the territory of Serbia, there has not been an application of the IBI index, however, the initial studies were conducted in Stojković et al. (2013).

The model that is the subject of this study is used in order to assess the overall ecological sustainability of a river basin. It generally starts from the hypothesis concerning the relationship between biodiversity, productivity, and ecosystem stability (Johnson et al., 1996). In this study, as an indicator of the ecological stability of aquatic lotic habitats, the time trend in

terms of the basic characteristics of ichthyocenoses is used, such as species diversity, abundance, biomass, body length, and age class of dominant, frequent, autochthonous, and predatory fish species. The time trend was analyzed through the spatial dimension of the entire investigated river basin. The analyzed parameters of ichthyocenoses are, for the most part, easily accessible because they are globally presented in the state programs of fisheries management plans for inland waterways. In addition, scientific institutions primarily run management programs, so they are reliable data sources. These facts enable the global use of this model assuming that there are available data regarding the continuous state of the fish stocks.

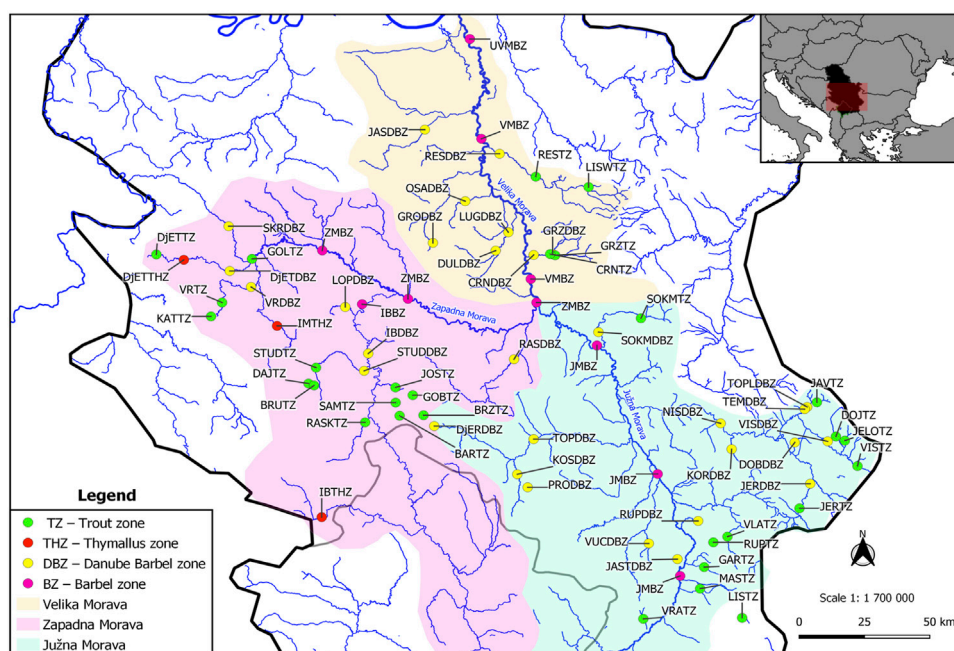
The impact of multiple stressors on inland aquatic ecosystems was obtained by analyzing the intensity of the impact of basic factors influencing biodiversity and grouped according to the acronym HIPPO (Habitat alteration, Invasive species, Pollution, Population growth, and Overexploitation) (Brennan and Withgott, 2005). This analysis of the impact of HIPPO factors has proven to be very effective as an element of the ESHIPPO fish model in assessing the environmental sustainability and conservation priorities of commercially important fish and other aquatic species in inland waterways (Simić et al., 2007; Simić et al., 2014b; Simić et al., 2015).

In our new model, the *ESE-HIPPOriver basin*, the ecological sustainability of the river basin was obtained as the difference between the mathematically expressed value of the estimated ecological stability of the basin (ESE) as a complex aquatic macro-ecosystem and the total impact of multiple stressors (HIPPO factors). The model enables water and fish resource managers to identify the most endangered habitats in the basin area, target those with conservation measures, and adjust them to effectively eliminate or mitigate the impact of endangering factors (Arlinghaus et al., 2015).

## Materials and methods

The new *ESE-HIPPOriver basin* model was designed on the example of the Morava River Basin (Serbia, Western Balkans) to assess the ecological sustainability of its river basins. The main characteristics of the Morava basin are shown in Figure 1 (Gavrilović and Dukić, 2002).

The primary data source for this study was hydrobiological and ichthyological research of the Morava river basin, conducted from 2001 to 2021. The research was conducted during the development of the fisheries management plans for the protection and the sustainable use of fish stocks (Supplementary Table S1) and it involves the monitoring of fish stocks that were done every third year in a given period. Locations for the conception of the *ESE-HIPPOriver basin* model are located on the lower, middle, and upper course of the main river (5) and the tributaries of second to fourth order



**FIGURE 1**

Map of analysed locations in the Velika Morava basin. Hydrological characteristics of the Velika Morava basin: The drainage basin of the Velika Morava is 6.126 km<sup>2</sup>, and of the whole Morava system is 38.207 km<sup>2</sup> (of that, 1.237 km<sup>2</sup> are in Bulgaria and 44 km<sup>2</sup> are in the Republic of Macedonia). This drainage basin covers 42.38% the area of Serbia. The Zapadna Morava drains an area of 15,754 km<sup>2</sup> (41.2% of the entire Velika Morava watershed). The Južna Morava has a drainage area of 15.696 km<sup>2</sup>, of which 1.237 km<sup>2</sup> is in Bulgaria (through its right tributary Nišava) (Gavrilović and Dukić, 2002).

(classification according to Horton, 1945 and Strahler, 1957) (Figure 1).

In addition to ichthyological research conducted using standard procedures (Jepsen and Pont, 2007), basic physical and chemical parameters were measured at each site, and macrozoobenthos and periphyton samples were taken. Based on abiotic and biotic parameters (macroinvertebrates, diatoms), estimates of aquatic ecological status were obtained according to Water Framework Directive (WFD, 2000). In addition to these results, this paper also used data on the ecological status of waters in the Morava basin obtained from the RS Environmental Protection (<http://www.sepa.gov.rs/>). Based on all sources, the basic working database “Morava fishbase 2001–2021” was conceived and presented in Supplementary Table S2.

The implementation of the *ESE-HIPPO* river basin model was carried out through an analysis of this database. In order to assess Ecological Stability of the Ecosystem values (an element of the ESE model), ichthyocenosis indicators were selected as indicated in Table 1. The evaluation of these indicators was performed using a three-point scale (1, 3, 5) according to the criteria shown in Table 2. In cases that there are lack of data for the evaluation of the indicators, alternative information are suggested in Table 1 (not evaluated for this study).

A partially modified evaluation system as incorporated in the ESHIPPOfish model (Simić et al., 2014b) was used to assess the impact of HIPPO factors (H-habitat alternation, I-invasive species, P-pollution, P-population growth, and O-overexploitation). The HIPPO factor evaluation system for the *ESE-HIPPO* river basin model is presented in Table 2.

To visualize the effect of each HIPPO factor on fish communities, which is represented by several ESE elements, a Kohonen artificial neural network (i.e., self-organizing map—SOM; Kohonen, 1982, 2001) was applied. The SOM technique, an unsupervised learning algorithm, is commonly used in ecological studies as a powerful tool for the clustering and visualization of large data sets (Penczak et al., 2012; Stojković et al., 2013), and was thus found to be the most convenient tool for this study.

The input data matrix for the data analysis consisted of 21 ESE element (columns) and 76 samples (rows). The ESE values were log-transformed for the purposes of data normalization. As an output, a 6 × 6 two-dimensional map of hexagonal output units (neurons) was obtained, with the sites assigned to the neurons. Sites with a similar pattern of ESE elements were assigned to one particular neuron and its adjoining neurons, while sites that were significantly dissimilar in their ESE values were assigned to distant neurons, especially

**TABLE 1 ESE model indicators and criteria for their evaluation. In cases that there are lack of data for the evaluation of the indicators, alternative information for the evaluation are provided and marked with an asterisk.**

ESE indicators (code/abbreviation)	Threshold and scoring system for each ESE indicator		
	1 (low)	3 (medium)	5 (high)
Fish community composition (species diversity)			
(% Au/his): Total number of autochthonous taxa (deviation between present and historical condition) <sup>a</sup>	Deviation > 50%	Deviation < 50%	No or minor change $\pm$ 10%
The Shannon-Weaver Index (d) <sup>*</sup> (value d cannot be calculated when there is only one species except for the value 5 when in salmonid region historically occurs only <i>S. trutta</i> )	d < 1	d (1–2)	d > 2
(Al. sp%): Total number of allochthonous species <sup>b</sup>	Allochthonous species > 50%	Allochthonous species (30–50%)	Allochthonous species < 30%
The characteristics of target fish population which zonation concept is based on			
A (MF): Trend in abundance of target fish population in the past 10–20 years <sup>c</sup>	Decrease in abundance	Variation in abundance up to $\pm$ 10%	Increase in abundance
B (MF): Trend in biomass target of fish population in the past 10–20 years	Decrease in biomass	Variation in biomass up to $\pm$ 10%	Increase in biomass
Implementation of ABC (abundance biomass comparison) curves (Clarke and Warwick, 1994) <sup>*</sup>	The pattern in the abundance and biomass shows heavily disturbed assemblages	The pattern in the abundance and biomass shows moderately disturbed assemblages	The pattern in the abundance and biomass shows undisturbed assemblages
TL (MF): Trend in total length of target fish population in the past 10–20 years <sup>d</sup>	Decrease in total length	Variation in total length up to $\pm$ 10%	Increase in total length
Comparison of analyzed specimen's length with a common length of the species (FishBase) <sup>*</sup>	More than 50% of specimens TL < common length (FishBase)	% of specimens containing the common length: 50% $\pm$ 5% (FishBase)	More than 50% of specimens TL > common length (FishBase)
nAge (MF): Trend in number age classes in the past 10–20 years	Decrease in number age classes (1–2)	Stagnation in the number of age classes (3–4)	Increase in number age classes (> 4)
The characteristics of accompanying fish population in particular zone			
A, B, TL, nAge (SMF) <sup>e</sup>	The same scoring system as used for target fish species		
Predator fish species			
(nPF): Total number of predator taxa (deviation between present and historical condition) <sup>f</sup> (%)	Decrease < 50%	Stagnation or $\geq$ 1 species	Increase > 30%
(BPT): Trend in biomass predator fish population in the past 10–20 years	Decrease in biomass	Variation in biomass up to $\pm$ 10%	Increase in biomass
Total number of predator taxa <sup>*</sup>	< 50% predatory fish characteristic for the fish zone	50% predatory fish characteristic for the fish zone	All/ > 50% predatory fish characteristic for the fish zone
Biomass predator fish (BP) population in comparison to other fish populations (BO) <sup>*</sup>	BP/BO > 10%	BP/BO (10–30%)	BP/BO > 30%
Sensitive fish species			
(% SFT): Total number of sensitive fish species given in % and/or trend in the past 10–20 years <sup>g</sup>	ST $\leq$ 30% and/or decrease M $\leq$ 50% <sup>g</sup>	ST 31–60% or ST $\leq$ 30% M > 50%	ST > 60% and/or increase

<sup>a</sup>Pančić (1860); Ristić (1972, 1977), Simonović (2001).

<sup>b</sup>Modification of Site-specific Biological Contamination (SBC) (Panov et al., 2008).

<sup>c</sup>Fish river zone (Thienemann, 1928; Huet 1949, 1959), characteristics of typical zone fish species: autochthonous species, fishing species, frequency > 90%, dominant > 75%.

<sup>d</sup>TL, Total body length.

<sup>e</sup>Characteristics subtypical fish species: autochthonous species, fishing species, frequency > 75%, dominant > 50%.

<sup>f</sup>Primary and secondary piscivore fish species (excluding *Anguilla anguilla*): *Silurus glanis*, *Esox lucius*, *Sander lucioperca*, *Aspius aspius*, *Hucho hucho*, *Perca fluviatilis*, and *Salmo trutta* TL > 0.35 m.

<sup>g</sup>Fishbase data of Resilience (van Treeck et al., 2020); moderate fish species (%).

those belonging to two different clusters (Chon et al., 1996; Park et al., 2003, 2005; Penczak et al., 2012). The resolution of the SOM map was determined with respect to both rules proposed by

Vesanto et al. (2000) and Park et al. (2003). Finally, to define the boundaries between clusters on the SOM map, the k-means method was used (Jain and Dubes, 1988). Moreover, the

TABLE 2 HIPPO factor evaluation system for ESE-HIPPOriver basin model.

HIPPO factor	Indicator—percentage of the river biotopes where the particular factor is manifested					
	%	Point	%	Point	%	Point
Habitat alteration <sup>a</sup>	> 50	5	20–49.9	3	< 20	1
Changes, Fragmentation and isolation, Destruction						
Invasive species and/or inadequate stocking <sup>b</sup> (biological and/or genetic contamination)						
Pollution <sup>c</sup>						
Population growth <sup>d</sup>						
Overexploitation <sup>e</sup>						

<sup>a</sup>Stream regulation and reclamation, gravel extraction, excessive water use, including changes in most of the natural hatcheries, feeding areas and migratory corridors of the fish species, the frequency of torrents and floods in the past 20 years, the frequency of “ecological drought” in the past 20 years. Fragmentation and isolation due to the construction of dams for hydropower (HP) and small hydropower plants (SHP) and other river barriers.

<sup>b</sup>Invasive species present (%) or natural populations stocked (%) with inadequate fish juveniles (fish from commercial fishponds, genetically not compatible) within the last 10–20 years.

<sup>c</sup>Eutrophication, saprobity, toxicity, or mixed pollution (data of WFD, Supplementary Table 2).

<sup>d</sup>Population density over 150 cap/km<sup>2</sup>. Seasonal increases in the number of people and their activities (tourism, recreation, exploitation of resources).

<sup>e</sup>Statistically estimated annual catch of fish species greater than its real production within the last 10 years and/or reduction of the total biomass (data of Supplementary Table 2).

neurons were clustered according to their similarities using the unified distance matrix (U-matrix) method (Ultsch, 1993). Low intensity color areas on the U-matrix cover similar neurons, while high intensity areas divide neurons into different clusters (Park et al., 2005). In the next step of the SOM analysis, each HIPPO factor was included as a passive variable in the previously trained SOM map, which does not influence the ordination and clustering based on the active ones (Milošević et al., 2013). HIPPO factors were assigned to the SOM map as the mean values of each variable for each output neuron of the trained SOM, which was occupied by at least one input vector. We used the component planes technique to emphasize the relationship between metrics, disturbance variables, and the SOM map. The distribution of fish biomass data, metrics, and environmental and disturbance variables was presented in the form of a greyness gradient. A more detailed description of SOM methodology is presented in Stojković et al. (2013). The SOM analysis was conducted using the Matlab ver. 6.1.0.450 algorithm interface (<http://www.cis.hut.fi/projects/som-toolbox>).

The Kruskal–Wallis ANOVA and Mann–Whitney test were conducted post hoc to examine the differences between the mean values of the HIPPO factors among the clusters. The Kruskal–Wallis and Mann–Whitney tests were conducted in SPSS version 15.0 (SPSS Inc., Chicago, IL, United States).

To calculate the values of models and trends of ESE parameters, a programmed matrix was organized in the Microsoft Excel file (Supplementary Table S3). The Excel matrix is programmed to calculate ESE and HIPPO values for each investigated river basin (Rx1..n) according to the formulas:

$$ESE_{Rx1..n} = \sum(AUFT + ALSP) + (AMF + BMF + TLMF + ACMF) + (A,B,TL,AC SMF_{1,2,3 \dots n}) + (NPFS + BPFS) + S_{FT}$$

$$HIPPO_{Rx1..n} = \sum(H + I + P + P + O) \text{ or } \sum(H + I + P + P + O + Clch^1)$$

where <sup>1</sup> Clch-value was given points for climate change intensity if there is data for assessment, as follows:

- 1—direct effects of climate change on the aquatic ecosystem cannot be determined, but it can be indirectly assumed to have a weak impact (long-term average air temperature, frequency of “ecological droughts” or torrent).
- 3—moderate impact based on direct water temperature data.
- 5—strong impact based on direct water temperature data.

The level of ecological sustainability for each analyzed river “ES Rx<sup>1..n</sup> B” in a drainage is calculated based on the % of decrement of  $\sum ESE / \sum HIPPO$  according to the formula:

$$ES_{Rx^{1..n} B} = \% \ll \sum ESE / \sum HIPPO \text{ or } \sum (HIPPO + Clch)$$

and according to the scale provided in Table 3.

The total ecological sustainability for the basin (ESE-HIPPOriver basin) is assessed based on the percentage ratios of the length of river flows in relation to the total length of the basin and based on the obtained values of ESE-HIPPOriver basin according to the formula:

$$ESE-HIPPOriver\ basin = \% (Total\ L\ km - L\ HS),$$

where total L km (total basin length) is calculated as  $\sum L_{Rx1..n}$ . Therefore L HS represents the total basin length where a high degree of environmental sustainability (HS) is estimated.

It is accepted that high sustainability (HS) is a condition that corresponds to the natural state of the ecosystem, i.e., the basin is ecologically sustainable if a high sustainability (HS) value is present at more than 50% of the length of the basin (Table 3). In both economic and practical terms, the length of the basin is also very important, in situations in which model-based sustainability is assessed as moderate, as it requires less extensive and expensive protection, conservation, or improvement measures.

TABLE 3 Scale for estimating ES  $Rx^{1-n}$  B and total basin sustainability based on % HS.

Increase of $\Sigma$ ESE in %	Degree of sustainability ES $Rx^{1-n}$ B	Threshold values	Degree of sustainability ES $Rx^{1-n}$ B	The percent of HS relative to the total river length (km)	Sustainability of river basin ESE-HIPPOriver basin
> 70	Low (L)	$\pm 2\%$	M-L or L-M	HS < 50% of the total river length (km)	Unsustainable
30–70	Medium (M)		M-H or H-M		Moderately sustainable
< 30	High (H)			HS $\geq$ 50% of the total river length (km)	Sustainable

Based on the frequency of parameter values expressed in points 1, 3, and 5, the Excel matrix calculated the ratios of decline, stagnation, or growth of the populations of typical and accompanying fish species indicator values and its facilitates the selected suitable graphical representations of results.

## Results

Changes in the qualitative composition of the fish communities of the lotic habitats in the Morava basin were estimated based on available literature data from the earlier period (Pančić, 1860; Marković, 1962; Momirović, 1972; Ristić, 1972, 1977; Janković and Četković-Krpo, 1995; Simonović, 2001) and based on the results of our research over the past 20 years. The obtained results are shown in Table 4.

The data analysis from Table 4 informs the graph presented in Figure 2. In relation to the historical data, the total number of autochthonous fish species (47) in the Morava basin decreased by 21.3%. The loss of autochthonous species is greatest in the barbel zone (36.7%).

Figure 3 presents changes in the qualitative composition of the fish communities in the Morava basin due to an increase in the number of non-native fish species in the basin during the period from 2001 to 2021. *Carassius gibelio* (Pančić, 1860) is the oldest non-native species mentioned in the historical period. The real ratio of autochthonous and allochthonous fish species for the whole basin is 68.1: 31.9%, with the most prominent abundance of allochthonous species in the lower barbel zone.

Figure 3 shows the presence of non-native fish species in the entire Morava basin and fish zones (the abbreviations are the same as in Figure 2).

The trained SOM map (Figures 4, 5), according to the U-matrix distances, distinguish two main Clusters, X and Y, which can be further divided into two sub-clusters labeled X1 and X2, and Y1 and Y2 (Figure 4). Cluster X is generally composed of samples from the Danube barbel zone (31), which also contains several samples from the trout (5), barbel (4), and grayling (3) zone. On the other side, Cluster Y mainly contains samples from the trout zone (31) and only a few samples from the Danube

barbel zone (2). Furthermore, Cluster X differs from Cluster Y in terms of many elements of ESE (see Figure 5). However, the division into the subclusters also was the result of specific elements of ESE (autochthonous fish taxa, percentage of allochthonous species, percentage of sensitive fish taxa, standard length, abundance, biomass and age classes of accompanying fish species 2). In addition, Group X1 was characterized by high scores in terms for standard length, abundance, biomass, and age classes of accompanying fish species 3 in comparison to Group X2. On the other side, group Y1 was characterized by high scores for standard length, abundance, biomass, and age classes for accompanying fish species 1 in comparison to group X1.

In order to provide a more detailed presentation of the trend in terms of the indicator characteristics for various populations (A- abundance, B- biomass, TL - total body length, AC-age classes) of typical (main) fish species (*Barbus barbus*, *Thymallus thymallus*, *Barbus balcanicus*, and *Salmo trutta*) along the Morava basin in the period 2001–2021, Figures 6–13 graphically presented the results obtained with the % frequency of final scores (1, 3, 5) indicators.

As shown in these figures, a small part of the basin has indicator values in the rise zone (indicator value 5), the largest part is in zone 3 (indicator value 3; stagnation), and a significant part is in the decline zone (indicator value 1). The abundance of typical fish species (AMF) significantly decreases in the Danube barbel, *B. balcanicus* and trout, *S. trutta* zone. In contrast, the biomass (BMF) decreases more significantly in the barbel zone, including *B. barbus*, Danube barbel, and trout. The value of the number of age classes (ACMF) has the same trend, while the total body length of fish (TLMF) decreases significantly in the barbel zone.

The analysis of the trend of indicator characteristics of the populations of each typical (main) fish species (Figures 6, 7) shows the largest decline in *B. barbus* and a moderate decline in *S. trutta*. Populations of *T. thymallus* are in the zone of stagnation, while the increase is observed only in *B. balcanicus* populations.

The biomass of predatory fish species stagnates and decreases more than experiencing growth (Figure 8) in the barbel and grayling zones. Based on the monitored indicators, the

TABLE 4 Fish species in the Morava basin in the historical period and the period 2001–2021.

Fish taxa	Historic	Barbel zone I	Barbel zone II	Grayling zone	Danube barbel zone	Trout zone	During period 2003–2021
Fam. Petromyzontidae	+	+	+	+	+*	??	*
<i>Eudontomyzon danfordi</i> (Regan, 1911) and <i>E. mariae</i> (Berg, 1931) Ammocoetes larvae	+	+?	?	?	+*	??	*
Fam. Acipenseridae	+	+					*
<i>Acipenser ruthenus</i> Linnaeus, 1758	+	+					*
<i>Acipenser</i> spp.	?	?					?
Fam. Salmonidae	+	+(?)		+*	+*	+*	*
<i>Salmo trutta</i> Linnaeus, 1758	+			+*	??	+*	*
<i>Salmo macedonicus</i> (Karaman, 1924)					*	*	*
<i>Hucho hucho</i> (Linnaeus, 1758)	+?			?	*		*
<i>Oncorhynchus mykiss</i> (Walbaum, 1792)				*		*	*?
Fam. Thymallidae							
<i>Thymallus thymallus</i> (Linnaeus, 1758)	+			+*	(?)*	*?	*
Fam. Anguillidae	+	+	+				
<i>Anguilla anguilla</i> (Linnaeus, 1758)	+	+	?	?	?	?	?
Fam. Clupeidae	?						
Fam. Umridae	+						
Fam. Esocidae	+	+*	+*				*
<i>Esox lucius</i> Linnaeus, 1758	+	+*					*
Fam. Cyprinidae	+	+*		+*	+*	+*	*
<i>Abramis brama</i> (Linnaeus, 1758)	+	+*					*
<i>Abramis sapa</i> (Pallas, 1814)	+	+*					*
<i>Ballerus ballerus</i> (Linnaeus, 1758)	+	+*					*
<i>Blicca bjoerkna</i> (Linnaeus, 1758)	+	+*					*
<i>Alburnoides bipunctatus</i> (Bloch, 1782)	+		*	+*	+*	*	*
<i>Alburnus alburnus</i> (Linnaeus, 1758)	+	+*		+*	+*		*
<i>Alburnus chalcoides</i> (Güldenstädt 1772)	+						
<i>Leucaspis delineatus</i> (Heckel 1843)	+	+?					
<i>Leuciscus leuciscus</i> (Linnaeus, 1758)	+	?					
<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)		*					*
<i>Aspius aspius</i> (Linnaeus, 1758)	+	+*	+*				
<i>Barbus barbus</i> (Linnaeus, 1758)	+	+*	+*		+*		*
<i>Barbus balcanicus</i> Kotlík, Tsigenopoulos, Ráb and Berrebi, 2002	+	?	+*	*	+*	*	*
<i>Rutilus rutilus</i> (Linnaeus, 1758)	+	+*	+*				*
<i>Rutilus pigus</i> Lacepède, 183	+	?	*				*
<i>Scardinius erythrophthalmus</i> (Linnaeus, 1758)	+	+*?	+*?				*
<i>Leuciscus idus</i> (Linnaeus, 1758)	+	?	?				?
<i>Leuciscus leuciscus</i> (Linnaeus, 1758)	+	?	?		?		?
<i>Pellecus cultratus</i> (Linnaeus, 1758)	+	+					
<i>Phoxinus phoxinus</i> (Linnaeus, 1758)	+		+	+		+*	*
<i>Pseudorasbora parva</i> (Temminck & Schlegel, 1846)			*				*
<i>Vimba vimba</i> (Linnaeus, 1758)	+	+*	+*				*
<i>Tinca tinca</i> (Linnaeus, 1758)	+	+	+*				*
<i>Rhodeus amarus</i> (Bloch, 1782)	+	+*	+*		*		*
<i>Squalius cephalus</i> (Linnaeus, 1758)	+	+*	+*	+*	+*	*	*

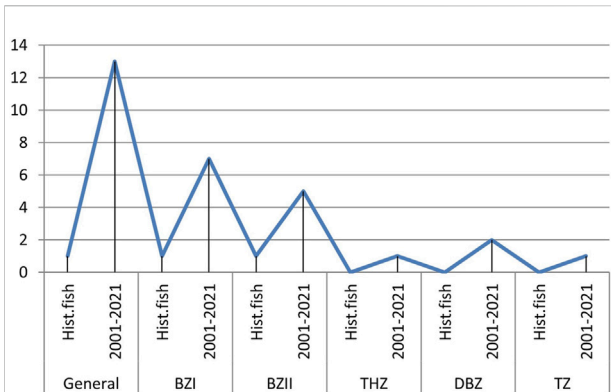
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TABLE 4 (Continued) Fish species in the Morava basin in the historical period and the period 2001–2021.

Fish taxa	Historic	Barbel zone I	Barbel zone II	Grayling zone	Danube barbel zone	Trout zone	During period 2003–2021
<i>Gobio obtusirostris</i> Valenciennes, 1842	+	+	+	?	+		*
<i>Romanogobio kessleri</i> (Dybowski, 1862)	+		+		+		+
<i>Romanogobio uranoscopus</i> (Agassiz, 1828)	+		?		+		*
<i>Romanogobio vladkovi</i> (Fang, 1943)	+				+		*
<i>Cyprinus carpio</i> Linnaeus, 1758	+	+	+				*
<i>Chondrostoma nasus</i> (Linnaeus, 1758)	+	+	+	+	*	*?	*
<i>Hypophthalmichthys molitrix</i> (Valenciennes, 1844)		*					*
<i>Arystishthys nobilis</i> (Richardson, 1845)		*					*
<i>Carassius carassius</i> (Linnaeus, 1758)	+	+	+				*?
<i>Carassius gibelio</i> (Bloch, 1782)	+	+	+	*	*		*
Fam. Percidae	+	+	+				*
<i>Perca fluviatilis</i> Linnaeus, 1758	+	+	+				*
<i>Sander lucioperca</i> (Linnaeus, 1758)	+	+	+				*
<i>Sander volgensis</i> (Gmelin, 1789)	+	+					
<i>Zingel zingel</i> (Linnaeus, 1766)	+	+	+		*		*
<i>Zingel streber</i> (Siebold, 1863)	+	+	+				
<i>Gymnocephalus baloni</i> Holcik and Hensel, 1974	?						?
<i>Gymnocephalus schraetser</i> (Linnaeus, 1758)	?						?
<i>Gymnocephalus cernuus</i> (Linnaeus, 1758)	+	+					?
Fam. Cobitidae	+	+	+		+		*
<i>Cobitis elongata</i> Heckel & Kner, 1858 and <i>C. elongatoides</i> Băcescu and Mayer, 1969	+	+	+		+		+
<i>Cobitis taenia</i> Linnaeus, 1758	+	+	+		+		+
<i>Sabanejewia balcanica</i> (Karaman, 1922)	+			*	+		+
<i>Misgurnus fossilis</i> (Linnaeus, 1758)	+	+					
Fam. Balitoridae	+				+		*
<i>Barbatula barbatula</i> (Linnaeus, 1758)	+				+		*
Fam. Siluridae	+	+	+		*		*
<i>Silurus glanis</i> Linnaeus, 1758	+	+	+		*		*
Fam. Gadidae	+						
<i>Lota lota</i> (Linnaeus, 1758)	+	+	+	+	+	+	
Fam. Ictaluridae							
<i>Ameiurus nebulosus</i> (Lesueur, 1819) and <i>A. melas</i> (Rafinesque, 1820)		*	*				*
Fam. Cottidae	+					+	*
<i>Cottus gobio</i> Linnaeus, 1758	+					+	*
Fam. Centrarchidae							*
<i>Lepomis gibbosus</i> (Linnaeus, 1758)		*	*				*
Fam. Gobiidae							*
<i>Neogobius fluviatilis</i> (Pallas, 1814)		*	*				*
<i>Neogobius gymnotrachelus</i> (Kessler, 1857)		?	?				?
<i>Neogobius kessleri</i> (Günther, 1861)		?	?				?
<i>Neogobius melanostomus</i> (Pallas, 1814)		?	?				?
<i>Proterorhinus marmoratus</i> (Pallas, 1814)		*	*				*
Fam. Odontobutidae		*					*
<i>Percottus glenii</i> Dybowski, 1877		*					*

+ present in the historical period (1860–2001). \* present in the period 2001–2021. ?—no precise data.





**FIGURE 2**  
Number of autochthonous fish species in the Morava basin in the historical and current period calculated for the entire basin and by fish zones. BbzI-lower *Barbus barbatus* zone; BbzII- *Barbus barbatus* zone, middle and upper part; Thz-*Thymallus thymallus* zone, Dbz-*Barbus balacanicus* zone, TZ-*Salmo trutta* zone.

population of *Silurus glanis* has the best characteristics in the lower part of the barbel zone (lower course of Velika Morava), *Sander lucioperca* in the middle and upper course of Velika Morava (barbel zone II), and *Aspius aspius* in the lower part of Južna Morava (barbel zone II). *Esox lucius* is found in barbel zones I and II, but with a more rapidly declining trend compared to other predators. *Hucho hucho* was introduced by restocking in all habitats and the population is currently growing only in the part of the course of the Ivanjička Moravica (IMTHZ) and covers the transition zones of Danube barbel and grayling.

Figure 8 demonstrates that the presence of sensitive fish species decreases the most in the barbel zone and somewhat less in the zone of grayling and Danube barbel.

The analysis of the trend of indicator characteristics (A, B, TL, AC) of subtypical (accompanying) fish species by river basin zones are shown in Figures 9–11. In the barbel zone, *C. nasus* has the strongest indicator characteristics of populations in relation to other fish species (Figure 9).

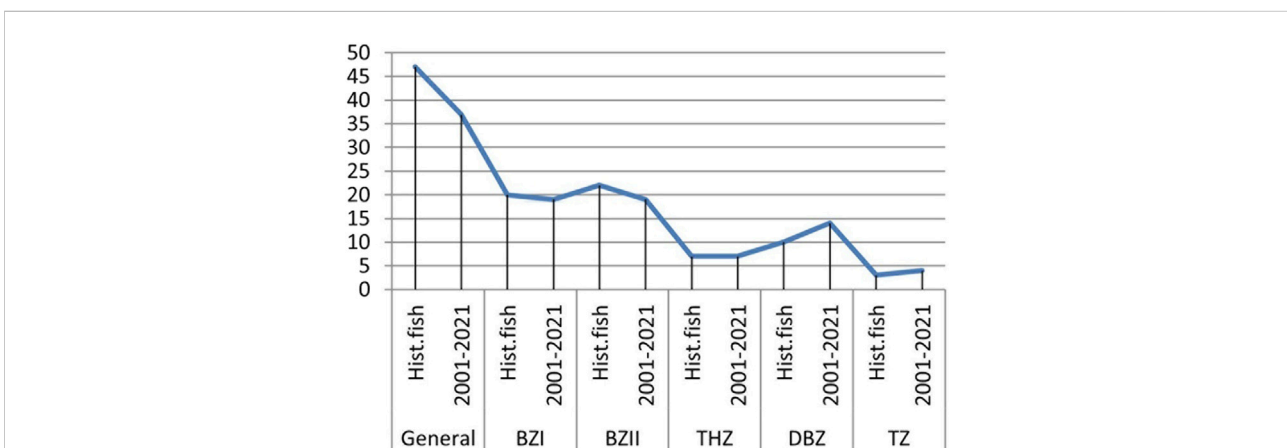
Significant frequencies of *Squalius cephalus* and *Alburnoides bipunctatus* can be seen in the Danube barbel zone (Figure 10), as well as the overall declining trend of indicator characteristics of *S. cephalus* populations (Figure 10C), and increasing trend of *A. bipunctatus* (Figure 10B).

In the brown trout zone (Figure 11), an expected significant frequency of *Cottus gobio* and *B. balcanicus* was detected (Figure 11A). Moreover, *Phoxinus phoxinus* has a declining trend (Figure 11C) compared to the increasing trend for *B. balcanicus* (Figure 11B).

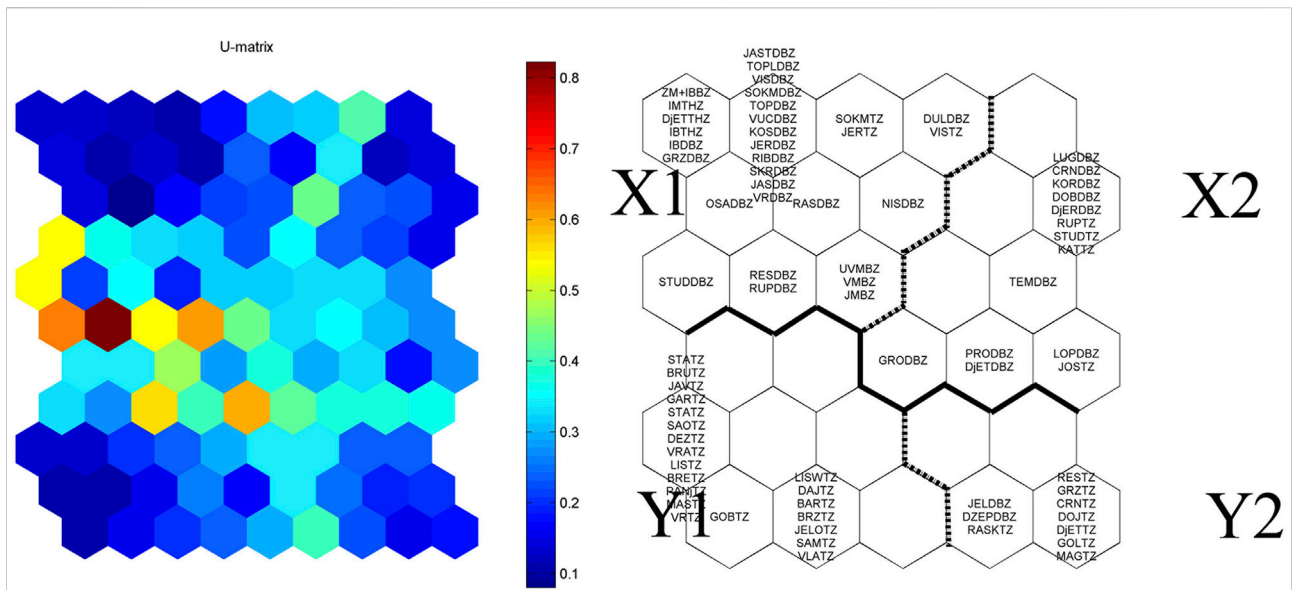
In the SOM network, from Cluster X to Cluster Y, the mean values of scores for all HIPPO factors decrease (Figure 12). The Kruskal–Wallis ANOVA test confirms significant differences among the clusters in all estimated HIPPO parameters, but the Mann–Whitney post hoc test reveals which clusters/subclusters are significantly different for each estimated parameter (Table 5).

The influence of the HIPPO factor is also provided as a graphical representation of the frequency of given points awarded (Figure 13).

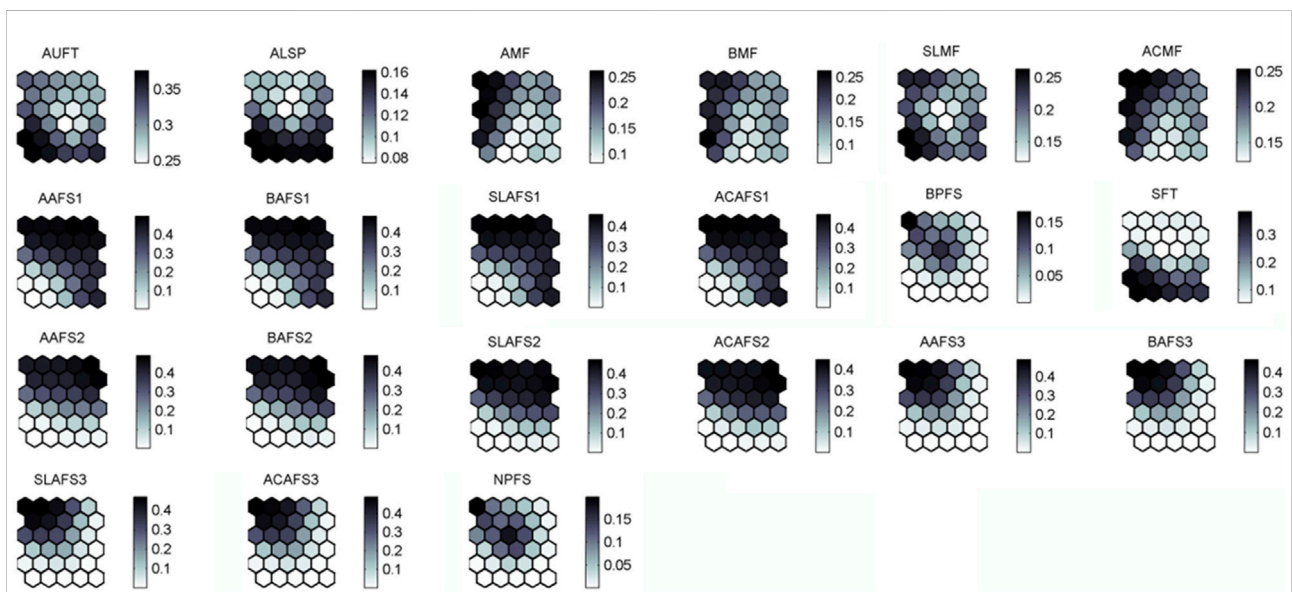
Based on these analyses, we find that habitat changes have a significantly strong impact in the Danube barbel and trout zones, while the invasive species impact is the strongest in the lower barbel zone and more moderate in the Danube barbel and trout zones (in the trout zone due to genetic contamination). Pollution significantly impacts the barbel zone and part of the Danube barbel zone (lower parts of the fourth-order tributaries). The presence of humans and their permanent or seasonal activities are significant in the Danube barbel zone and some parts of the brown trout zone. According to these results, over-exploitation of



**FIGURE 3**  
Presence of non-native fish species in the entire Morava basin and in fish zones (the meaning of abbreviations is the same as in Figure 2).



**FIGURE 4**  
A SOM map formed by 30 hexagons representing neurons. Clusters of neurons (X1, X2, Y1, Y2) are distinguished on the basis of the U-matrix. The color intensity indicates the degree of dissimilarity.

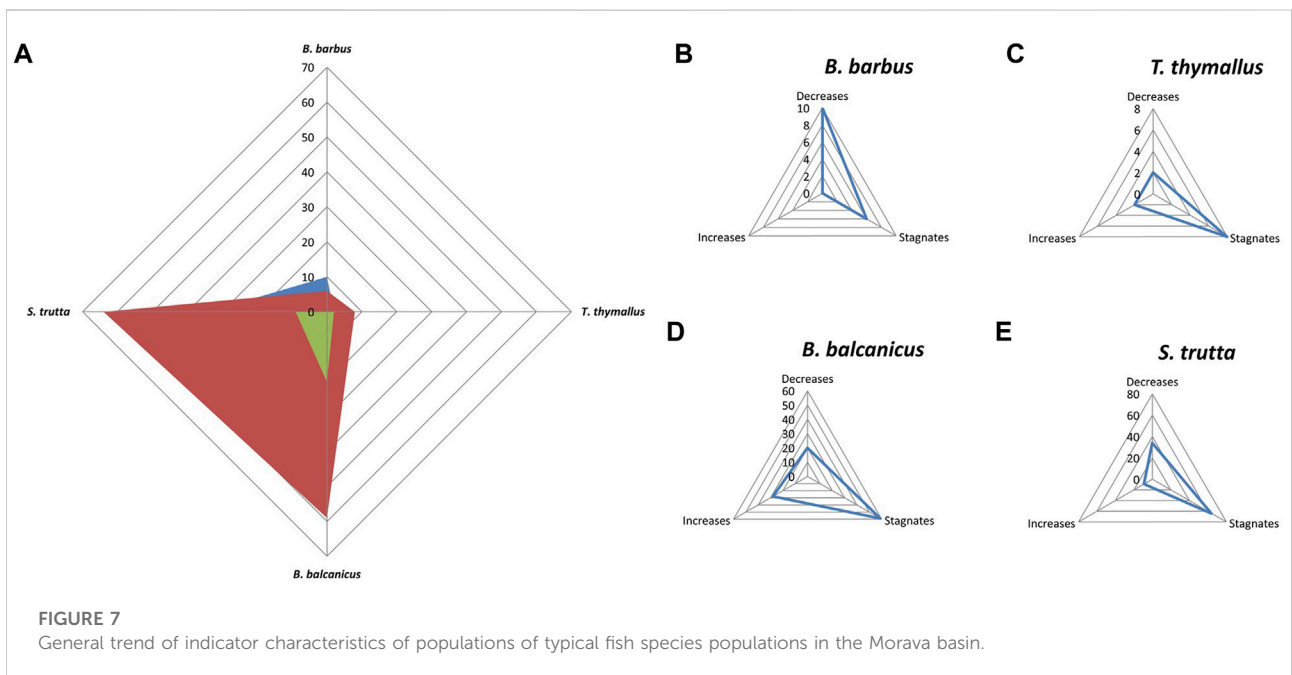
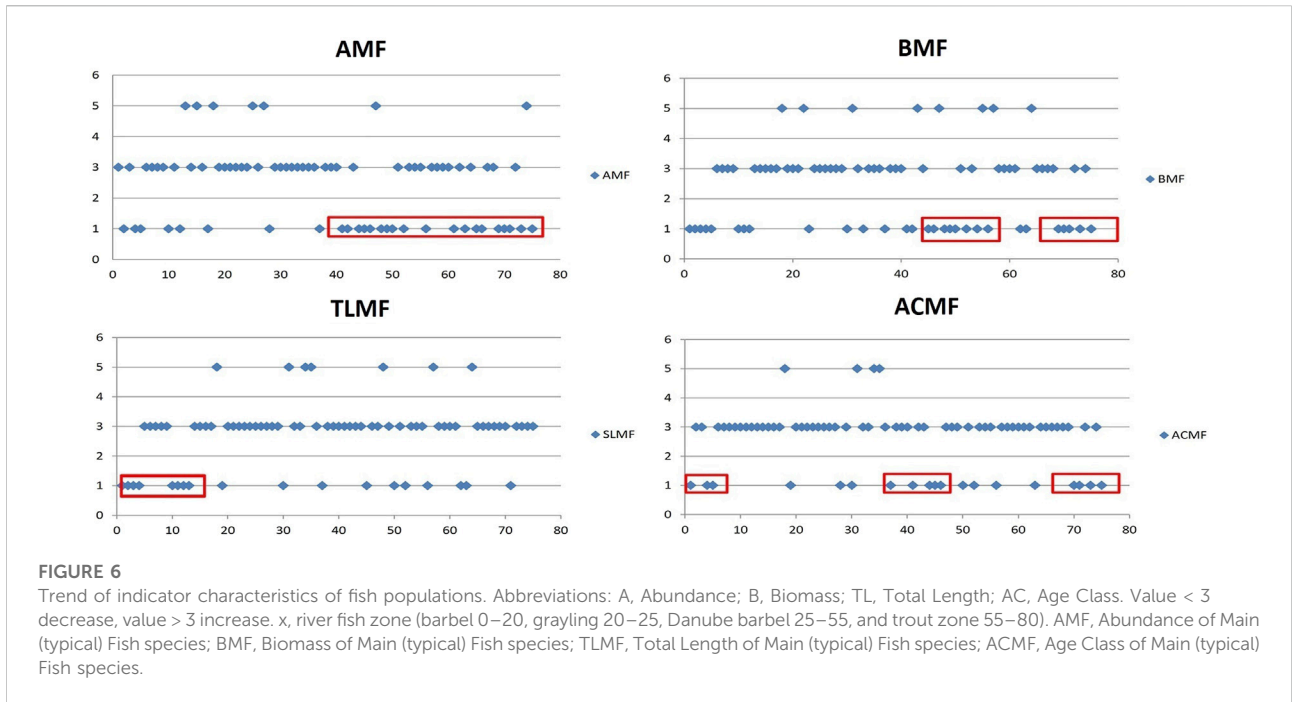


**FIGURE 5**  
Visualization of a distribution patterns of 21 ESE elements in the previously trained SOM. The intensity of the gray color indicates species previously assigned scores.

fish resources has a predominantly moderate effect in all basin zones.

The final values produced by the *ESE-HIPPOriver basin* model for the Morava river basin are shown in Figure 14. If, for the purposes of sustainability reference values, it is considered

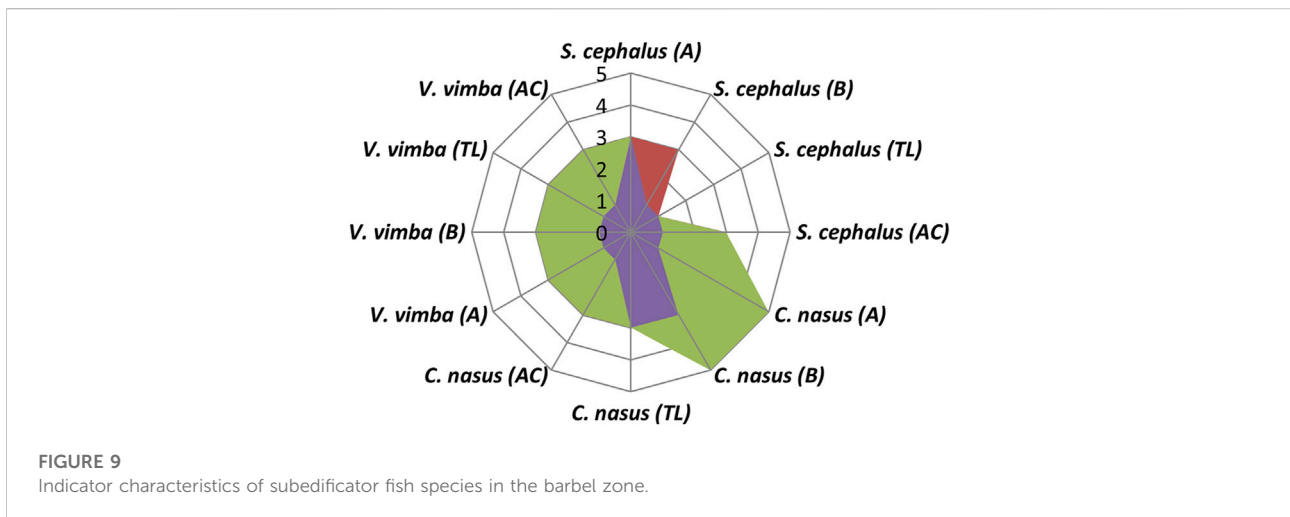
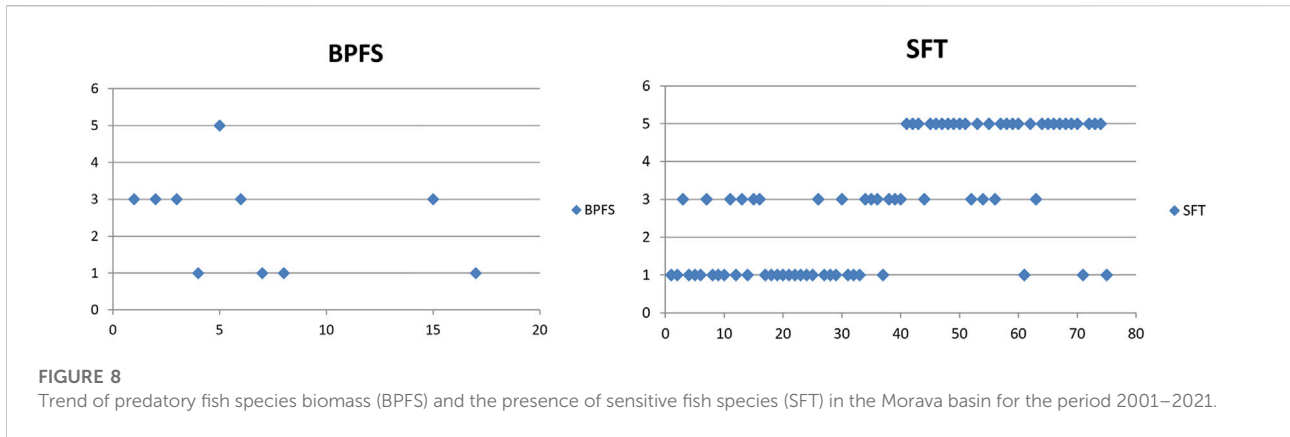
that the theoretical assumption that aquatic ecosystems in their natural state are ecologically sustainable, then according to the model, the Morava river basin is sustainable along only 19.6 km of the length of the river. This percentage decreases if the climate change factor is introduced into the model (in this case, if the



value is 1, which means that the factor impact is weak, primarily due to lack of data), and then the sustainability percentage decreases by about 5%. Analyzing the values produced by the *ESE-HIPPO* river basin model by fish river zones (Figure 14), the highest level of sustainability was obtained for the grayling zone (50%), followed by brown trout (31.1%), slightly lower for the Danube barbel zone (21.8%), and lowest for the barbel zone (0%).

## Discussion

Human activities in the Anthropocene era rapidly and efficiently reduce the natural ecological sustainability of inland water bodies in many ways, both directly and indirectly; including, for example, air pollution, soil pollution, deforestation, and accelerating climate change (Rockström et al., 2014b; Gozlan and Britton, 2015; Falkenmark et al.,

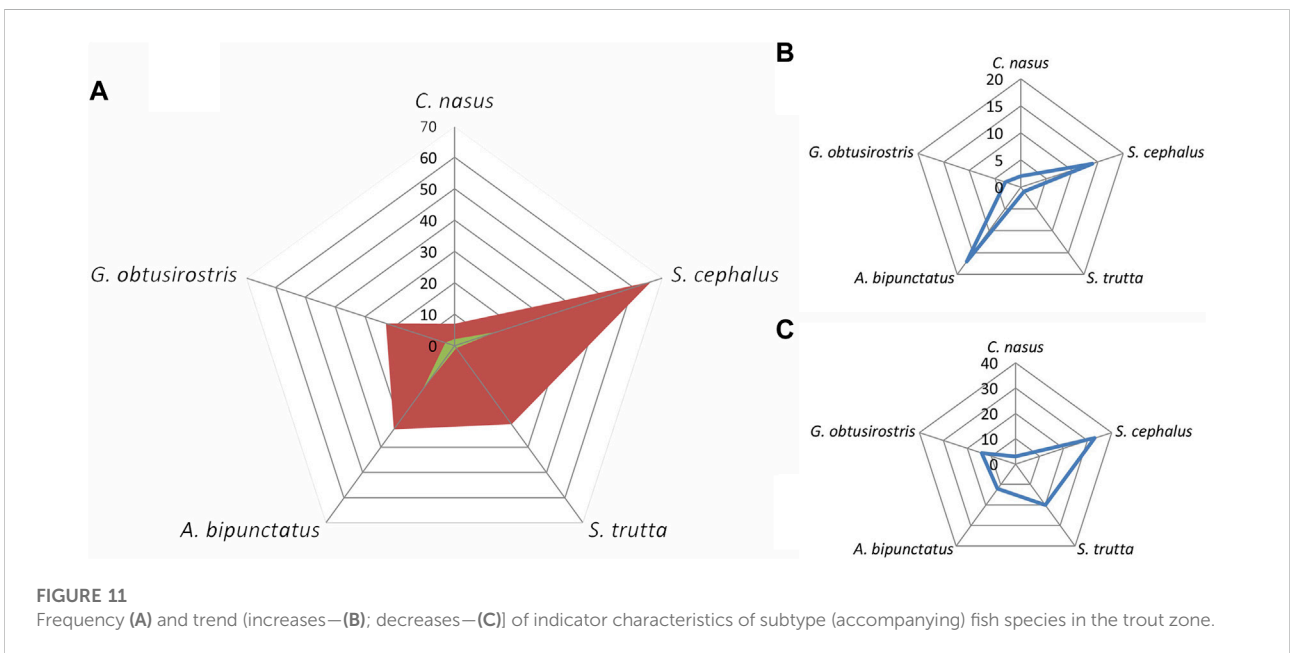
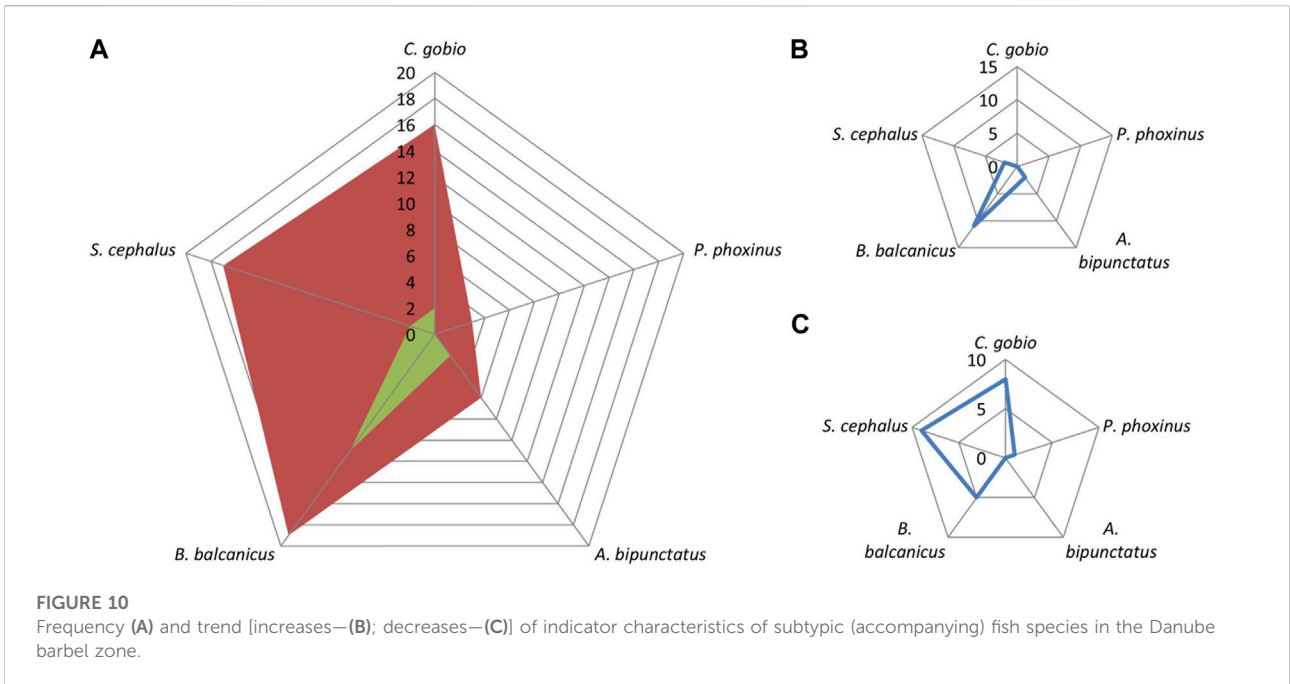


2019). Long-term hydroecological and ichthyological research on streams in the Morava river basin (Serbia, Central Balkans) has shown that river health reflects the health of terrestrial ecosystems, air quality, and indirectly, the social status of human communities. In this study, the central indicator of the health of inland waters and the vast land area around them is the river basin. Here, the Morava basin study area covers 45% of the Serbian territory and therefore indicates the state of the environment of a significant part of the country.

The *ESE-HIPPOriver basin* model estimates the multiple impacts of stressors on the ecological sustainability of the river basin based on the fish communities. Fish are the top members of the food chain, so the structure and production of their populations reflects the ecological sustainability of the aquatic ecosystem (Arthington et al., 2004; Tejerina-Garro et al., 2005). For marine ecosystems, models assessing the sustainability of fish stocks and/or marine habitats are generally more developed than for inland waterways (Cooke et al., 2016). To assess the ecological status of inland waterways in

the United States, IBIs were developed (Karr, 1981; 1987) and later modified for Europe and included in the WFD methodology (Jepsen and Pont, 2007; Pont et al., 2007). However, models assessing the ecological sustainability (stability, ecological efficiency, and production) of inland waterways and/or river basins are less well represented. In the context of our study, the work of Yang et al. (2021) is significant. The authors use elements of this approach to assess the structure of ichthyocenosis, such as alpha and beta diversity indices, and ABC (Abundance/Biomass Comparison) curves to assess community stability.

Based on field data, these authors presented the possible impacts of various factors on the fish community and river habitats, such as dams, pollution, and erosion. The *ESE-HIPPOriver basin* model is designed not just for scientific usage but also for practitioners (technicians, water and fish resources managers). For the latter, the developed system of three-point scoring of ichthyocenosis structure indicators (A- Abundance, B- Biomass, TL—Total body Length, and AC - Age Classes) significantly simplifies obtaining the final results of sustainability assessment, i.e., without the necessary

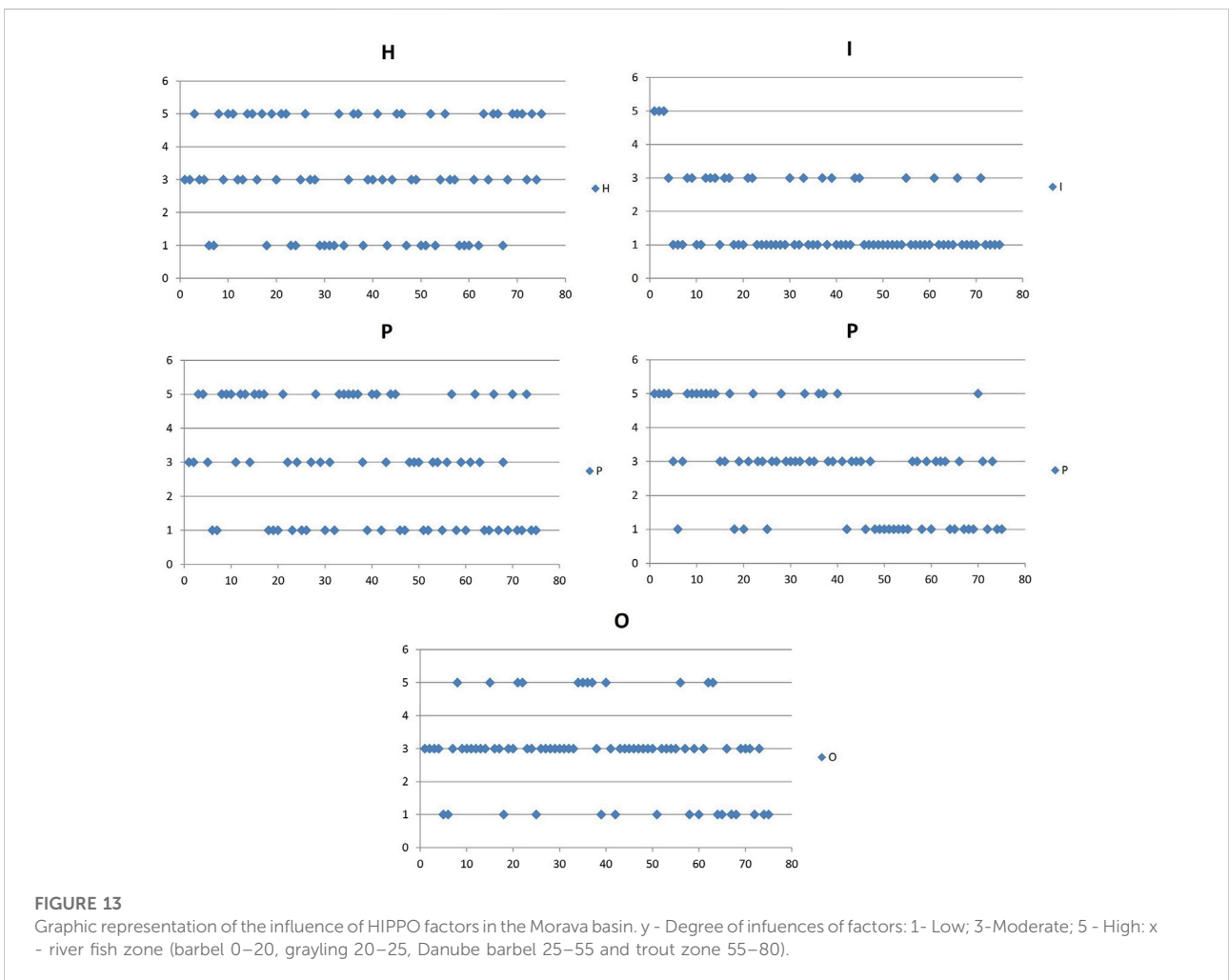
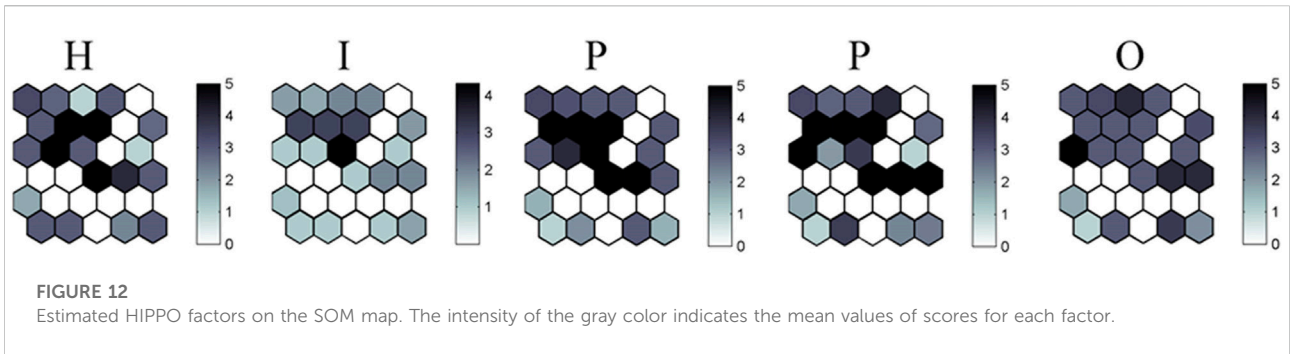


use of complex mathematical and statistical methods. Therefore, in this work we compared the results obtained using SOM analysis and the results based on the simple addition of numerical values of indicators (points) in the active Excel matrix (Table 1; Supplementary Table S3) as a graphic representation.

The main specificity of the model on which the ecological sustainability of the basin is assessed, is the temporal and spatial trend of ichthyocenosis structure indicators. These indicators define the ecological stability of the ecosystem

(ESE) as the first element of the model. The second set of indicators defines the impact of individual and cumulative effects of HIPPO factors, which reduce the ecological stability of ecosystems. The overall ecological stability of the basin estimated by the model results from the strength of the reduction of the ecological stability of the ecosystem by endangering factors.

However, one of the main problems of freshwater fisheries, the frequent lack of long-term catch statistics and



other parameters important for the state of the fish stock, may cause the problem for the appropriate usage of the model. On the one hand, the concept of the model based on the trend of ichthyocenosis indicators should force users of the fisheries areas to introduce regular monitoring of fish stocks so that the results obtained on the basis of the model are reliable and

useful. However, if such data are not available for any reason or the regular monitoring of fish stocks is not performed, the secondary parameters shown in [Table 1](#) could be applied. These parameters refer to the results obtained on the basis of standard scientific methods of analysis of fish stocks.

TABLE 5 Mean values  $\pm$  standard deviation of HIPPO factors in each cluster of sites.

Variable	Group			
	X1	X2	Y1	Y2
H	3.00 $\pm$ 1.63 <sup>a</sup>	3.29 $\pm$ 1.54 <sup>a</sup>	2.24 $\pm$ 1.48 <sup>a</sup>	2.80 $\pm$ 1.47 <sup>a</sup>
I	1.97 $\pm$ 1.25 <sup>a</sup>	1.71 $\pm$ 1.17 <sup>ab</sup>	1.10 $\pm$ 0.43 <sup>b</sup>	1.40 $\pm$ 0.84 <sup>ab</sup>
P	3.52 $\pm$ 1.45 <sup>a</sup>	3.57 $\pm$ 1.22 <sup>a</sup>	1.76 $\pm$ 1.17 <sup>b</sup>	2.00 $\pm$ 1.05 <sup>b</sup>
P	3.19 $\pm$ 1.58 <sup>ab</sup>	3.71 $\pm$ 1.68 <sup>a</sup>	2.33 $\pm$ 1.59 <sup>b</sup>	2.40 $\pm$ 1.89 <sup>ab</sup>
O	3.19 $\pm$ 1.19 <sup>ab</sup>	3.57 $\pm$ 0.93 <sup>b</sup>	2.14 $\pm$ 1.08 <sup>c</sup>	2.60 $\pm$ 1.28 <sup>abc</sup>
HIPPO	14.87 $\pm$ 5.41 <sup>a</sup>	15.86 $\pm$ 4.13 <sup>a</sup>	9.57 $\pm$ 4.15 <sup>b</sup>	11.20 $\pm$ 3.93 <sup>b</sup>

Values not sharing a common letter are significantly different:<sup>abc</sup>  $p < 0.05$ .

## The qualitative composition of fish in the Morava basin

There are not enough scientific and professional data to determine the exact historical qualitative composition of the Morava basin fish communities. From the oldest publication of Pančić (1860), the presence of fish species in the Morava basin can only be partially reconstructed. More precise information is provided in later publications (Ristić, 1972; Simonović, 2001; Stojković et al., 2013), with the useful data provided in professional publications on fishing and fishing waters of Serbia and the Morava basin (Marković, 1962; Momirović, 1972).

Based on the available data, it is possible to state that the loss of autochthonous species is about 20% in relation to the historical composition. Of the fishery-important species from the basin, the Crucian carp (*Carassius carassius*) and the tench (*Tinca tinca*) (Simić et al., 2013; Lujčić et al., 2017) have disappeared or are very rare. There are also no new and reliable findings of species such as *Alburnus chalcoides*, *Leucaspius delineatus*, *Leuciscus leuciscus*, *Pelecus cultratus*, *Zingel streber*, *Gymnocephalus baloni*, *Misgurnus fossilis*, and *Lota lota*. In addition, the findings and catch of *Acipenser ruthenus* are extremely rare. Furthermore, the most significant changes and loss of autochthonous fish species are located in the lower parts of the Velika Morava.

Along with the loss of autochthonous species, the qualitative composition of the Morava basin ichthyofauna is changing with the expansion of allochthonous species, mainly from the Danube. The more substantial breach of autochthonous species occurred from 1960 to 1970 and 1990 to 2000 (Zorić et al., 2014). However, there is generally no detailed examination of non-native fish species in the Morava Basin. In Stojković et al. (2013), *Lepomis gibbosus* and *Pseudorasbora parva* are listed as frequent species. The results presented here confirm the frequency of these species, along with the frequent findings of the invasive *Neogobius fluviatilis* (Djikanović et al., 2013; Smederevac-Lalić et al., 2019). It is assumed that most allochthonous and allochthonous-invasive species in the lower course of the Velika Morava may also be found in the Danube. The Prussian carp (*C. gibelio*) is the only naturalized

allochthonous species in the Morava basin previously mentioned in Pančić (1860).

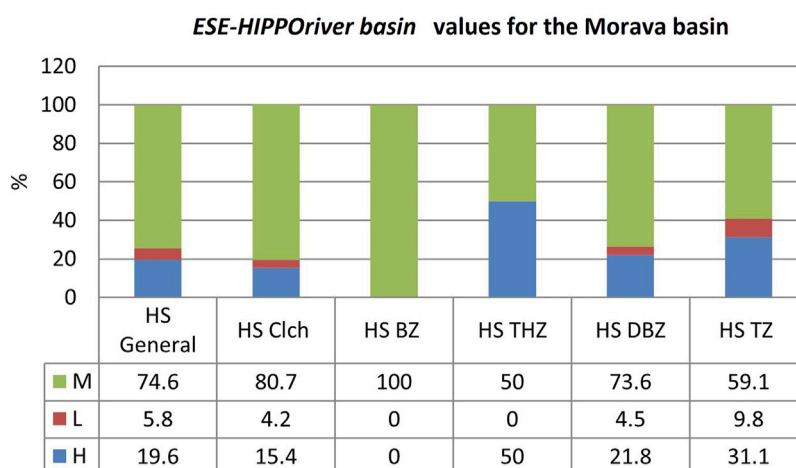
## Analysis of key population structure indicators of typical and accompanying fish species in the ichthyocenosis of the Morava basin and their significance for the ESE-HIPPO river basin model

Results from this study are similar to those of Stojković et al. (2013). In their work, SOM analysis, which included the abiotic characteristics of habitats and fish communities in the Morava basin, detected three clusters. Clusters differed in their abiotic parameters and ichthyocenosis composition; and coincided with barbel, Danube barbel, and trout zones.

Our analysis revealed several unfavorable phenomena in the structure of populations of typical and accompanying fish species in the basin. In the barbel zone, the population of *B. barbus* as a typical species for this zone declined. At the same time, the population of *Chondrostoma nasus* remains stable and dominates this zone. According to van Treeck et al. (2020), the general sensitivity of *B. barbus* is higher (High) compared to *C. nasus* (Moderate). However, the sensitivity of both species to the spawning substrate is high. The estimated susceptibility is undoubtedly important for the population status of these species in our research. In the barbel zone, the influence of various endangering factors is present, but the exploitation of gravel and sand from the riverbed is the dominant phenomenon here, which reduces the suitable places for spawning *B. barbus*. In contrast, *C. nasus* has an advantage in terms of spawning because it migrates upstream and can still find suitable places for spawning at the mouths of tributaries. For a successful spawning, *C. nasus* especially requires access to the mouths of tributaries of the Zapadna Morava and Ibar [Ribnica (RIBDBZ), Studenica (STUDBZ), and Lopatnica (LOPDBZ)].

The grayling zone is the shortest in the Morava basin since its significant shortening occurred after 1970, when the grayling population disappeared from the river Studenica (left tributary of the Ibar River) (Janković, 2010). Marić et al. (2011) have shown that the populations of *T. thymallus* in the Ibar are the only autochthonous ones for the Morava basin. Therefore, they are especially important in terms of conservation issues. According to these authors, *T. thymallus* populations from Veliki Ržav (IBTHZ) and Ivanjička Moravica (IMTHZ) are allochthonous and originate from the waterways of Slovenia. *Thymallus thymallus* populations stagnate in all habitats except the river Djetinja (DjETTZ), where a slight increase has been recorded. However, there are no data on the genetic structure of grayling from the Djetinja river, which was also introduced by restocking.

The Danube barbel zone in the Morava basin is very heterogeneous, so, in addition to *B. balcanicus* as a typical species, there is also the frequent occurrence of *S. cephalus*,



**FIGURE 14**

Final values of the ESE-HIPPOriver basin model for the Morava river basin (Serbia, Central Balkans). Abbreviations: H, high level of sustainability; M, medium level of sustainability; L, low level of sustainability. HS -% of the length of the catchment stream which has a high sustainability in relation to the total length. HS Clch -% HS under the influence of climate change. Th.zone - grayling zone, *Thymallus thymallus* zone; D.b.z—Danube barbel zone with: THZ - grayling zone, TZ - trout zone, DBZ - Danube barbel zone.

and brown trout (*S. trutta*). In this zone, range expansion and population growth are evident for *Alburnoides bipunctatus*, which, according to the classification system of van Treeck et al. (2020), is considered a resistant species.

In the trout zone, the typical species *S. trutta* has a smaller decrease in the indicator characteristics of the populations. At the same time, an increased presence of *B. balcanicus* is noted. Additionally, in this zone, the indicator characteristics of *P. phoxinus* populations are significantly declining.

### Impact of multiple stressors on the ecological sustainability of the Morava basin

The ESE-HIPPOriver basin model allows the assessment of single and cumulative stressors on river basins. SOM analysis and graphical presentation of the influence of HIPPO factors on the Morava river basin show the unequal intensity of various factors' influences on different river basin zones. Habitat changes and/or destruction vary in shape and extent throughout the basin. In the barbel zone, which includes the largest rivers in basins, regulation of the riverbed and land reclamation, as well as excavation of sand and gravel, are the main factors that change and degrade the river habitat. At the beginning of the 1960s, Velika Morava River flow was regulated to prevent floods, and the river was shortened by about 80 km. This greatly reduced the flood zone and lost natural spawning places for a large number of phytophilous fish species (Janković et al., 1972; Šorić, 1996; Vlahović et al., 2006). The consequences of this are the extinction of fish species such as

*T. tinca* (Lujčić et al., 2017) and *C. carassius*, and a significant population decline for *Cyprinus carpio* and *Abramis brama*. The flow of the Morava has been regulated for the past 15 years due to the construction of highways along the Južna and Zapadna Morava.

The exploitation of gravel from the Morava riverbed is an ongoing process that has accelerated since 2000. The increased need for this material is due to the economic improvement of Serbia, especially in the construction sector. The consequences of sand and gravel exploitation on river habitats and fish has been moderately studied, so Gavriletea (2017) and Koehnken and Rintoul (2018) pointed out that sand exploitation in the Missouri River (Kansas, USA) increases the population of lotic fish species and decreases rheophilic populations such as sturgeon. Similar results are reported by Paukert et al. (2008) and Freedman et al. (2013) for US rivers. However, they also point out the higher occurrence of non-native species in river areas affected by sand and gravel exploitation. Mingist and Gebremedhin (2016) in Ethiopian rivers (Arno-Garno, Pib) point to severe negative impacts of sediment mining on local fish populations due to the destruction of hatcheries and disruption of migratory routes. The study was not supported by quantitative data, but they were obtained by surveying quarry workers. Our research shows a quantitative increase in the number and biomass of resistant allochthonous fish species in areas where sand exploitation has been conducted, such as *C. gibelio* and *L. gibbosus*.

In the Morava basin, habitat fragmentation due to the construction of dams is most pronounced in the Danube barbel zone (tributaries of the third and fourth order). In the



past 70 years, 225 accumulations of various purposes and capacities have been built. The smallest number of built dams (one) are on the main streams of the Velika, Zapadna, and Južna Morava in the barbel zone. In contrast, the number of barriers due to the construction of small hydropower plants in the trout zone has increased sharply in the last 10 years, and 46 derivation-type small hydropower plants have been built. The consequences of small hydropower plants on trout populations are the greatest. This is a significant cause of the declining trend of populations of this species in the Morava basin, which was obtained as a result of this study. Numerous studies both worldwide and in Serbia have demonstrated the negative consequences of dams on rivers for fish biodiversity and river ecosystems in general (Radojković et al., 2019; Ganassin et al., 2021; Khatun and Pal, 2021; Sousa et al., 2021). During our research, the impact of hydro-accumulations (especially large ones) on the downstream parts of rivers is stated, which can be called the “habitat inversion effect”. It is reflected in the permanent discharge of cold water from the accumulation downstream and the formation of areas with average colder water in relation to the average temperature of the river. These colder parts of the river are inhabited by trouts (*S. trutta*, *Salmo macedonicus*) and in some rivers form stable populations. Examples are the Sokobanjska Moravica River (SOKMTZ) under the Bovan reservoir, where the population was formed by stocking, and the Nišava River (NISDBZ) where, due to the influence of the Zavoj reservoir, the trout population is formed spontaneously from tributaries.

The influence of allochthonous and allochthonous-invasive species is most significant in the barbel zone in the lower part of the Velika Morava. This is because most non-native species that inhabit the Danube can be found in this part of the basin (Simonović et al., 2010). There are no stable populations of non-native species in other basin zones; however, they are located in reservoirs. Therefore, there is a real danger that some rheophilic invasive species, such as *N. fluviatilis*, will eventually settle in parts of rivers upstream of reservoirs. The penetration of invasive fish species into new habitats by Panov et al. (2008) is called biological contamination, and the penetration of allochthonous haplotypes of grayling and brown trout into the trout zone of the Morava basin can be described as genetic contamination.

The harmful effect of allochthonous haplotypes on indigenous populations of brown trout and grayling in the territory of Serbia, especially on their adaptive value and autochthonous genetic diversity, is indicated by previous studies (Marić et al., 2006, 2011, 2022; Tošić et al., 2016; Simonović et al., 2017; Kanjuh et al., 2021). Results show that around 7% of rivers are genetically contaminated in the Morava basin. In addition, trout populations in the Morava basin have autochthonous Danubian haplotypes contaminated with Atlantic and Adriatic haplotypes. Allochthonous haplotypes were mainly introduced by the stocking practices that took place intensively after the Second World War. During that period, scientific

knowledge about the influence of foreign genes on the genetic diversity of indigenous species was not developed, so the material for restocking the rivers of the Morava basin, especially trout zones, was brought from more or less remote geographical areas (Lake Ohrid and rivers from Slovenia and Bosnia and Herzegovina). After that period, the rainbow trout (*Oncorhynchus mykiss*) was also used for restocking (Janković and Raspopović, 1960).

Pollution as a stressor is present in various forms and intensities in the entire Morava basin. The influence of this factor is most pronounced in the Danube barbel and the barbel zones. Organic pollution from human settlements and industry dominates in the Danube barbel zone, while mixed pollution (organic and toxic) is mainly present in the barbel zone. In addition to municipal and industrial wastewater pollution, the Morava basin is also significantly affected by water leached from agricultural areas because 2/3 of the basin is used for agricultural purposes. The process of eutrophication is manifested not only in the reservoirs of the basin but also in the river flows, especially in the Danube barbel and barbel zones. The phenomenon of “algae bloom” due to the excessive development of Cyanobacteria is represented in the reservoirs of the basin (Djordjević et al., 2015; Simić et al., 2017), but this phenomenon and its impact on fish has not been studied in the rivers of the basin. Results from the Australian rivers (Bowling and Baker, 1996; Bowling et al., 2013) have demonstrated that new cyanobacterial blooms in slow-flowing rivers occur during dry years and emphasize the fact that blooms are intensifying due to climate change. In the future, scientific research should be dedicated to the process of eutrophication and flowering of cyanobacteria, especially in the lower slow-flowing part of the Velika Morava.

In researching the impact of heavy metals on fish in the Morava basin (Milošković et al., 2016, 2022), barbel, bream, and catfish stand out as the species in which the highest concentration of heavy metals have been found. This study found an increased concentration of heavy metals in fish from Velika and Zapadna Morava. The study by Jovanović et al. (2018), based on genotoxicity tests, estimates that the total contamination by pollutants of the largest rivers of the Morava basin (Velika, Zapadna, and Južna Morava) is significantly correlated with results obtained through the use of the ESE-HIPPOriver basin model. Thus, the most substantial impact of pollution is found in Velika Morava, the lower part of the Južna, and the middle course of the Zapadna Morava (Figures 12, 13). The model detects that pollution as a factor has the strongest impact in the Morava basin on tributaries of the fourth order in zones that can be marked as the lower barbel zone. This zone has the highest percentage of habitats with a low level of environmental sustainability. During the research on the entire Morava basin, a significant presence of plastic waste was ascertained. Research on the impact of plastics, especially microplastics in marine aquatic ecosystems, is very relevant today but is relatively less well represented among freshwater studies (Parker et al., 2021). Moreover, no studies

have been conducted on the impact of microplastics in the Morava basin.

The intensity of pollution as a factor for the ecological sustainability of the Morava basin is correlated with the population density. About two million people live in the basin area; the highest population density is in the Velika Morava basin (101 people/km<sup>2</sup>); slightly lower in the Zapadna Morava basin (87 people/km<sup>2</sup>), and the lowest in the Južna Morava basin (78 people/km<sup>2</sup>) (Vlahović et al., 2006). The general demographic trend in the basin area is depopulation. In contrast, in the last 10 years, economic growth in all sectors of the economy has increased (Manić and Lutovac, 2022). Agriculture is intensifying in areas that coincide with the barbel and lower Danube barbel zones, while the electric power industry and tourism coincide with the trout zone. The *ESE-HIPPO* river basin detects a greater influence on the population through the quantity and quality of human activities than the peak of the average population density. Such an impact is indicative of the trout zone being located in the mountainous basin area. In the mountainous areas of the basin, the population density is the lowest, and the impact comes from mass tourism and the construction of small hydropower plants. Hydropower plants are automated and do not require large increases in the number of people for their operation, but the effect of their work on mountain river basins is very strong (Zhong and Power, 1996; Bunn and Arthington, 2002; Kemp, 2015). Studies of the impact of mass tourism on mountainous areas are mainly based on the global impact on the environment or the social aspect of the population and less on aquatic ecosystems, fish resources and fisheries (Demirović et al., 2017; Miller 2022). This study shows a strong influence of mass tourism in the Morava basin on visually attractive rivers, such as Lisine waterfall (LISWTZ).

The excessive exploitation of resources in this study mostly refers to the data on the exploitation of fish resources in the Morava basin. In contrast, the use of water resources for water supply and irrigation is shown as a total effect. Until Second World War, fishing in the Morava basin was carried out in the form of commercial fishing on the Velika Morava and recreational fishing in the rest of the basin. Following this period, fishing was legally limited to recreational fishing, which is still the only form of fishing in the entire Morava basin. There are no data on fishing pressure in the Morava basin before the 1960s; however, if we compare the number of recreational fishermen from before 2003 of about 17,000 and after 2003 at around 28,000 (Janković et al., 1972; Supplementary Table S1), a significant increase is evident, theoretically leading to higher fishing pressure. However, these data cannot be taken as relevant because of the lack of accurate fishing statistics on annual catches for recreational fishermen by fisheries managers, which is a chronic problem for freshwater fisheries in Serbia and most of the world (Cooke et al., 2016; Burgin, 2017). Based on the analysis of indirect data

such as biomass trends, the total length of fish stocks, age structure, and sensitivity of fish species to fishing (Supplementary Table S3), the model indicates moderate fishing pressure in the entire Morava basin. If this factor is viewed independent of other factors, it could be considered relatively acceptable and sustainable if we suppose that it is analyzed in conjunction with the generally declining values of indicators A, B, TL, and AC populations of fish species, the intensity of HIPPO factors, and climate change. In that case, it acts as an unfavorable factor that reduces the overall environmental sustainability of the basin. This cumulative effect of factors, including recreational and commercial fishing on river ecosystems, is also indicated in the previous work (Arlinghaus, 2005; Cooke and Cowx, 2006; Lewin et al., 2006; Carpenter et al., 2011). There is a weaker impact of recreational fishing in the trout zone than in the Danube barbel zone, and especially the barbel zone. We believe that the “catch and release” fishing regime in the Morava basin, the only permitted form of fishing in the trout zone, which has been around since 2013, has also contributed to this trend (Simić et al., 2016; Supplementary Table S1).

Excessive exploitation of fish stocks in the Morava basin is also affected by some provisions of the law (the Law on Protection and Sustainable Use of Fish Stocks, Official Gazette of RS, No. 128/2014) that define the organization of fishing. Namely, in the territory of Serbia, the large fisheries areas are managed by just one fisheries stakeholder. Such a system makes it difficult to manage (provide a sufficient number of fish keepers, control the prescribed fishing quotas) and effectively combat poaching, as a significant factor in over-exploitation, especially the fish species of commercial importance (catfish, pike, perch) (Mickiewicz et al., 2020). Therefore, we believe that poaching is a significant factor in disrupting the sustainability of inland water ecosystems. This problem is unfairly neglected in relation to illegal, unreported, and unregulated (IUU) seafood on in marine contexts (Vince et al., 2021; Bartolo, 2022). Also, recreational fishermen buy a single fishing license that is valid for the entire territory of Serbia. This enables the fishing waters with the best and most attractive fish stock to be visited and engaged in recreational fishing by most fishermen. Additionally, it significantly increases the allowable fishing pressure, which cannot be controlled. In the Morava basin, this phenomenon was constant at the largest reactors, especially the Velika and Zapadna Morava.

In this paper, due to the lack of long-term data, only the general impact of climate change on the ecological sustainability of the Morava River Basin is presented. The Republic Hydrometeorological service of Serbia (<https://www.hidmet.gov.rs>) reports shows an increase in the average air temperature for Serbia from 1981 to 2021, i.e., the climate is becoming warmer and with less precipitation on average (web: <http://www.hidmet.gov.rs>). This climate data was used in the model and quantified for the whole basin as existing but with a

low impact (Impact Level 1). The obtained values of basin sustainability are about 5% lower than those without climate change factors, and more in the Danube barbel and trout zone. This result indicates the need for more extensive research in the future into the impact of climate change on the Morava basin. This research aims to assess the sensitivity of the basin to climate change in order to take mitigation measures (Harrod, 2015; Marković et al., 2017; Morales-Marin et al., 2019; Jarić et al., 2020).

## Conclusion

River basins are the most widespread form of inland waterway but, despite their undoubted ecological and economic importance, comprehensive data on the state and sustainability are still significantly under-represented in relation to marine ecosystems.

The *ESE-HIPPO* river basin model provides an assessment of the ecological stability of the lotic parts of the basin based on the trends of key indicators of fish populations within ichthyocenoses and the impact of multiple modern anthropogenic stressors. Our results essentially represent the degree of weakening of the ecological stability of the basin under the influence of multiple stressors in time and space.

The Morava basin occupies about 45% of the territory of Serbia and, according to the model, 80% of the basin has a low or medium level of sustainability in relation to the high level of sustainability, which is considered to be the natural state. The model indicates that the parts of the basin that belong to the trout zone and the Danube barbel zone are more sensitive and that, in a higher percentage and in a shorter period of time, they decline into a state of low sustainability. On the other hand, large river basins belonging to the barbel zone have greater resistance to multiple stressors and can maintain a moderate level of sustainability for a long time.

In terms of basin environmental services, only parts with a high level of sustainability also have economic viability. From these areas, it is possible to sustainably use healthy drinking water (without expensive purification treatments), healthy water for irrigation and fish farming, and healthy fish (fish without contamination of meat with various toxic substances). Also, these areas provide conditions for quality recreation and ecotourism.

Our model can assess the sustainability of each part of the basin and the basin as a whole. In this way, protection and/or conservation measures can be taken according to their priority, i.e. the first of all of the parts of the basin that are most endangered or most sensitive but under threat, or immediately on the entire basin.

In order to apply this model, it is necessary to establish continuous monitoring of indicators of ichthyocenosis structure and the assessment of stressor type and intensity. This necessity strongly relates to the managers of companies that use inland water resources to modernize and improve their management in order to preserve, restore or improve environmental services of inland waterways.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Materials](#), further inquiries can be directed to the corresponding authors.

## Ethics statement

The animal study was reviewed and approved by the Branka Ognjanović, Faculty of Science, University of Kragujevac, Serbia; Ana Petrović, Faculty of Science, University of Kragujevac, Serbia; Miloš Matić, Faculty of Science, University of Kragujevac, Serbia; Vitomir Anđelković, Faculty of Science, University of Kragujevac, Serbia; Milanko Šekler, Veterinary Specialized Institute Kraljevo, Kraljevo, Serbia; Miloš Radaković, Institute for Nature conservation of Serbia.

## Author contributions

VS wrote the original draft, AP, TV, SS, DB, AC-B, writing—review and editing. MS-P performed statistical analysis. All authors have read and agreed to the published version of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

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