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Population Characteristics of Spirlin *Alburnoides bipunctatus* (Bloch, 1782) in Serbia (Central Balkans): Implications for Conservation

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Abstract: The aim of this study was to evaluate the population characteristics of spirlin, *Alburnoides bipunctatus*, in Serbia, since this small fish species is facing a severe decline in its abundance and its natural habitats in Europe. We investigated the spirlin population dynamics, including size, age structure, growth pattern, mortality, and exploitation rate. Additionally, we used the Uniform Manifold Approximation and Projection approach with the Decision Tree algorithm to investigate the influence of different environmental parameters on the population parameters to unveil which factors shape the abundance and distribution of spirlin. The results showed that the highest values of production, abundance, and biomass were estimated in sites with low temperature, optimal pH, and well-oxygenated water, even though we found them in heavily polluted waters with extremely high values of conductivity. Moreover, we observed a pattern of migratory behavior, in which spirlin migrate upstream to sites at a higher altitude in early summer and autumn. Despite the putative vulnerability and high sensitivity of spirlin populations, our results showed that the species was abundant, occurring in altered habitats (due to pollution, climate change, anthropogenic pressure, etc.).

Keywords: environmental quality; growth pattern; population dynamics; endangered fish; Uniform Manifold Approximation and Projection (UMAP); machine learning

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1. Introduction

Among aquatic environments, freshwater ecosystems are most sensitive to environmental disturbance [1]. We are witnessing severe ecosystem damage, the loss of suitable habitats [2–4], the damming of medium and large rivers, eutrophication, thermophication, and pollution with various substances as well as the degradation of small rivers, especially in upper watercourse stretches [5]. Moreover, climate change, within permanent anthropogenic pressure, as one of the most well-known global crises, may have forceful direct and indirect effects on freshwater biota [6,7]. It is well-known that inland water conditions are closely connected to atmospheric temperature fluctuations and weather [7], so freshwater ecosystems are highly vulnerable, and their communities are experiencing significant impacts [8], especially for fish species such as ectotherms [9]. Under such pressure, fish species with high requirements for their environmental conditions and their habitats are the first to be eliminated [5,10]. Apparently, spirlin *Alburnoides bipunctatus* (Bloch 1782) is one such species, as a rheophilic and gravel-spawning species inhabiting the upper, "salmonid", and middle parts of the river course with fast-running water [11,12]. According to Siryová [13] and Seifali [14], spirlin is sensitive to the changes caused by the degradation of small rivers, especially from dam constructions, considering its migratory behavior [15]. In addition to the disruption of connectivity for fish migration, dams also affect the sediment and water level (the water deepens the trough) [16,17]. Habitat changes due to the regulation of riverbeds and land reclamation as well as the excavation of sand and gravel could greatly reduce the spawning places for lithophilic fish species that deposit eggs on gravel, such as spirlin [18]. The increased need for the exploitation of gravel for economic improvement, especially in the construction sector in developing countries, could severely impact the local fish populations [18,19]. Moreover, anthropogenic activities such as mining, sewage discharge, agricultural discharge, and industrial toxic effluent discharge have led to an increase in organic (nutrients such as nitrates, nitrites, and phosphates) and toxic (potentially toxic elements (PTEs), pesticides, pharmaceuticals, and personal care products) pollutants in aquatic environments [20]. Some of the potentially toxic elements (PTEs) (Hg, Cd, Pb, As, Ni, and Fe) were recognized as the most critical elements in Serbia, emphasizing that the concentration of Hg and Pb was commonly above the maximum permitted concentrations by legislation [20].

Spirlin distribution ranges from eastern Europe, in nearly all of the rivers draining into the southern Baltic, North, Black, and Azov seas; to the Caspian Basin, in the upper Volga; to the Kura drainage, southward to the Iranian tributaries of the Caspian Sea [21,22]. In the past, spirlin was considered widely distributed across Europe [23,24], but in the second half of the 20th century the tendency toward the reduction in spirlin abundance was observed in many countries in Europe [5]. The spirlin's threatened status in Europe was preliminary highlighted by Lelek [23], and it was added to the list of endangered species according to the 1979 Bern Convention of the Conversation of European Wildlife and Natural Habitats (Appendix/Annex III, protected fauna) [15,25]. According to previous research, spirlin status is near-threatened in Slovakia [26–30]; endangered/extinct in Switzerland [15,31,32]; endangered in Hungary [33], Austria [34,35], Germany [36], and Poland [37,38]; and critically endangered in the Netherlands [39] and the Czech Republic [40,41], where the spirlin has a legally protected status. The last assessment at the European level was completed in 2010, and, according to the IUCN Red Book categorization, spirlin is defined as a species of "Least Concern" (LC), but it is locally threatened and endangered by river flow regulation and destruction, pollution, and the inappropriate stocking of upper river parts with trout species [42]. Brown trout is a prime example of how inappropriate stocking with this commercially important fish has influenced the changes in aquatic food webs [43]. The effect of brown trout introduction, beyond its native range, include direct predation (often very aggressive) on aquatic macroinvertebrates and native fish, such as spirlin [44]. Moreover, recent research showed that introduced brown trout tend to be more piscivorous in their introduced habitat [44].

Regarding spirlin distribution in the Balkans, Treer et al. [45] reported an increasing trend in spirlin abundance and biomass in the Sava River since the 1970s. They highlighted that suitable habitats for spirlin in this river still exist, meaning that improved water quality highly contributed to the high abundance of this species. A similar tendency toward an increase in spirlin abundance was recorded in Slovenia after heavy industry and the mining industry stopped from 1991 to 1994 [46–48].

The spirlin population is poorly investigated among the Serbian ichthyofauna. Few researchers have addressed the question of the spirlin population in this area [49,50], so it is often only cited as an accompanying species [18,51]. The status of spirlin in Serbia according to the IUCN Red Book categorization is "Least Concern" [50]. Since 1985, there have not been any records about spirlin population parameters (population characteristics' records W_{max} and L_{max}; growth parameters $L\infty$, K, and t0 as well as growth performance index φ'; length–weight relationship parameters; length and weight frequencies; mortality and exploitation rate) for Serbia reported in FishBase [52]. Šorić and Ilić [49] reported only total length and age size.

The biological and autecological data of spirlin are scarce, and, considering that the knowledge gap about the population trend of this small fish that is potentially under threat, the aim of this study was two-fold. The first aim was to determine the spirlin population dynamics, including size, age structure, growth pattern, mortality, and exploitation rate, as one of the important resilience traits. For the second aim, we investigated the influence of different environmental parameters on the population parameters to unveil which factors shape the abundance and distribution (occurrence of spirlin). Finally, the regional species specifics as well as the population response against environmental condition changes were analyzed in order to assess the sustainability of spirlin in the territory of Serbia.

2. Materials and Methods

2.1. Study Area and Data Collection

Almost all rivers and streams in the territory of Serbia are tributaries of the Danube River and, consequently, belong to the Black Sea basin, while a minor part of them belongs to the Aegean Sea basin and the Adriatic Sea basin [53]. In the present study, we analyzed the data of spirlin population parameters in 62 rivers; 144 sampling sites in six-river system (Great Morava, West Morava, South Morava, Drina, Timok, and Kolubara) of the Danube River drainage (the Black Sea basin); and four sampling sites in Vardar drainage of the Aegean Sea basin (Figure 1).

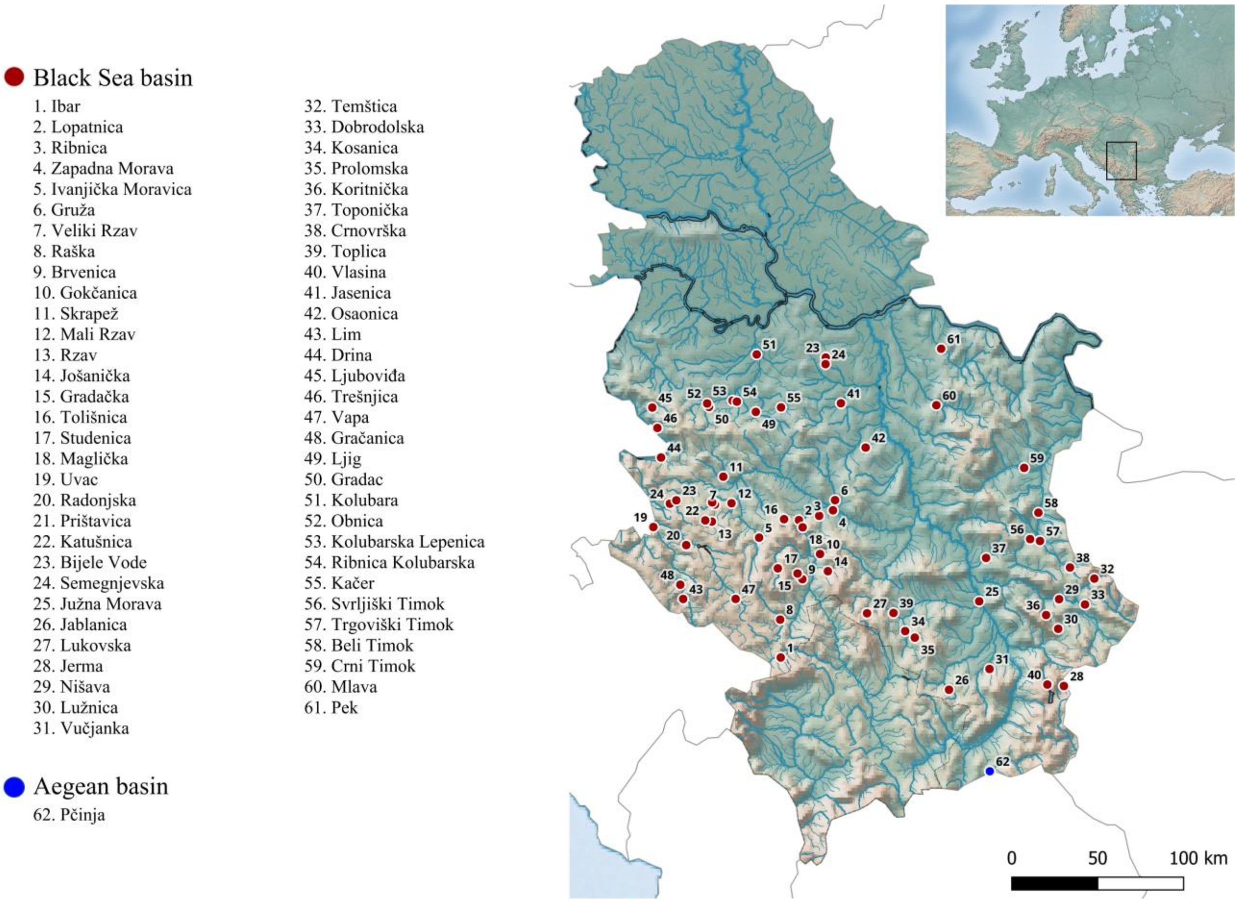

Figure 1. Map of investigated 62 rivers in Serbia (Central Balkans).

A total of 3041 specimens of spirlin were collected in 2010–2021 during three seasons: spring, summer and autumn, covering months from April to November (Supplementary Table S1). Sample sites were wadable parts of rivers at width of 3–5 m and less than 1.5 m deep. During intensive fieldwork, specimens were sampled using electrofishing equipment "AquaTech" GI 1300 (2.6 kW; 80–470 V, 70 Hz; AquaTech, Kitzbuehel, Austria). The standard electrofishing procedure was conducted using the single "zig-zag" sampling method along 50–100 m transect line (same transect was sampled twice) [51,54]. The lower and upper boundaries were settled with laser distance meter UNI-T LM50 (maximum output < 1 mW, wavelength 630–670 nm; HVAC Tools, New Zealand, Australia) and temporarily marked with flagging tape during sampling. Captured specimens were placed in an aerated container for further processing, and an effort was made to minimize the stress level to specimens. All specimens were measured for standard (SL in cm) and total length (TL in cm) as well as weighted (W, total wet weight in g) to the nearest 1 mm and 0.01 g. Additionally, the specimens were sorted into length groups, and, from the random sample, the scales were removed. After the removal, scales were cleaned with a fine brush and stored in filter paper until being taken to the laboratory. After measurement, the fish were carefully released. The fish identification was performed according to [22] and [50]. The main part of the field research was conducted within fisheries management plans. Research data up to 2011 are an integral part of the database of biodiversity in aquatic ecosystems in Serbia, ex situ conservation (BAES ex situ) http://baes.pmf.kg.ac.rs [55].

2.2. Population Dynamics Parameters

The working database was divided into eight separate worksheets for each sampling year/period (2010, 2011, 2016, 2017, 2018, 2019, 2020, and 2021). The population dynamics parameters were examined separately for each sampling year, and for total sample (pooled data) for general population status assessment.

The length–weight relationship was determined according to power equation:

$$W = a \times L^b \tag{1}$$

transformed into the function [50,51]:

$$\log W = \log a + b \times \log L, \tag{2}$$

where W is the total weight (g); a is the intercept, a coefficient related to body form; L is the total length (cm); and b is a slope or regression coefficient indicating ideal isometric growth b = 3, negative allometric b < 3, and positive allometric b > 3 [56–58]. Outliers were detected by plotting log-transformed weight to log-transformed length and were omitted further analysis [59,60]. The degree of correlation among the variables was evaluated using the determination coefficient r^2 [59]. ANOVA was used to evaluate the statistical significance of the regression model detected when $p < 0.05$ [61]. To verify if b was significantly different from the expected or theoretical value (b = 3), *t*-test was performed [61,62].

The coefficient of condition (CF) was calculated following Fulton [59,63]:

$$CF = 100 \times W/L^3, \tag{3}$$

where W is the weight (g), L is the total length (cm), and 100 is a factor to bring the value of CF near unity. We calculated the CF for each specimen at each sample site, the medium value of CF for each sample site, and finally, the medium value of CF for a different year of sampling with corresponding standard deviations.

For the determination of growth model, we applied the von Bertalanffy [64] equation:

$$Lt = L\infty\ (1 - e^{(-K(t-t0))}) \tag{4}$$

The growth parameters L∞, K, and t0 were estimated using Microsoft Office 365 Excel software, in MS Addinsoft XLSTAT version 2022.5.1.1395. As we calculated the growth parameters for all sampling years and for total sample, *t*-test was performed to examine if there was statistically significant difference between obtained values for the sampling years and for the total sample. The growth performance index φ' (Phi-prime) was calculated using the formula according to Pauly and Munro [65],

$$\varphi' = \log K + 2 \log L\infty \tag{5}$$

The natural mortality coefficient (M) was estimated following empirical formula [52,58,65], linking the von Bertalanffy growth parameters, L∞ (cm), and mean annual temperature (T, °C) of water in investigated habitat:

$$M = 10^{(0.566\ -\ 0.718\ \times\ \log\ (L\infty)\ +\ 0.02\ \times\ T)} \tag{6}$$

Thus, total mortality Z was computed using linearized catch curve analysis based on age composition data [66,67], and fishing mortality F was calculated as a difference between Z and M,

$$F = Z\ 2212\ M \quad (7)$$

The value of exploitation rate E was obtained by [58,68].

$$E = F/Z \quad (8)$$

2.3. Population Parameters

In order to determine the population parameters, the mean length (L, cm), the mean weight (W, g), and the age (A) were calculated as population structure parameters. Moreover, abundance (Ab, N/km), fish biomass (B, kg/km), and production (P, kg/km^2) were estimated. The production has been calculated according to Chapman [69], and it represents biomass increase in a certain period. Biomass for age class t (Bt), mean biomass (B) between two different age classes ($\Delta t = t + 1 - t$), number of specimens in age class t (nt), and coefficient of increase in fish weight between the age class t and t + 1 (G) were calculated to obtain the Pt between two different age classes, according to formula $Pt = G \times B$. Finally, to obtain the production P for the sampling site, it was necessary to sum all previously estimated Pt. Age structure was determined by combining two methods—analyzing age (based on the determination of the number of annuli on each scale for random specimens) and length frequency distribution according to Cicek [70]. Scales used for age determination were taken from the region between the lateral line and dorsal fin. Before the examination, the scales were soaked in water, dried, and independently examined under a stereo binocular microscope (NIKON SMZ800, Nikon Corporation, Tokyo, Japan) twice by two readers without any knowledge of the length and weight of the specimens. Applying the length interval to the total number of measured specimens, we obtained the age class structure [71]. Length frequencies ranged from 3.5 to 14 cm, with interval of 2 cm. A modal class progression analysis was then applied, with a separation index of >2 used to provide meaningful differentiation and to separate the overlap [71,72]. Despite the potential overlapping in age-specific length distributions, this method is very useful in fast-growing, short-living species such as spirlin [73,74].

Simultaneously with the spirlin population data, environmental parameters were estimated for each sampling site. Water temperature T (°C), conductivity EC (µs/cm^3), pH (0–14), concentration of dissolved oxygen DO (mg/L), saturation DO% (%), and hardness H ($CaCO_3$) were measured by HANNA set instruments, and altitude ALT (m, height above mean sea level) was measured with GPS (eTrex® Legend, GarminInc., Olathe, KS, USA).

2.4. Data Analysis

As a preliminary analysis, the Spearman's rank correlation coefficient between all pairs of variables was calculated to detect statistically significant relationship between fish population parameters and environmental parameters and between environmental parameters. Correlation test was performed to act as benchmark for further analysis [75].

To examine the population parameters across temporal and spatial scales and to visualize the link with environmental parameters, we used unsupervised dimensionality reduction learning model UMAP (i.e., Uniform Manifold Approximation and Projection). There were no data on all environmental parameters for 12 sampling sites, so they were excluded from further analysis. The input matrix contained 132 rows (one row represented one sampling site) and 14 columns. Seven quantitative fish population parameters (production P, abundance Ab, fish biomass B, mean length L, mean weight W, age A, and condition factor CF) were considered in the analysis together with seven environmental parameters (water temperature T, conductivity EC, pH, concentration of dissolved oxygen DO, saturation DO%, hardness H, and altitude ALT). Since the UMAP model is based on Euclidian distances, the data were Hellinger-transformed prior to dimensional reduction modeling [76,77]. We tested the number of neighbors (n_neighbors) from 5 to 50, and set n_neighbors = 15 as the most appropriate value for achieving a balance between global and local structure during embedding of data points (virtual sampling sites). Moreover, we set the min_dust to 0.1 and spread to 1.0, which control how dense UMAP is allowed to pack data points together. Finally, an analysis of the most significant parameters for clustering was carried out through algorithms that iteratively create all possible subsets from the feature vector and then use a classification algorithm to assess which subset performs the best [75,78]. The virtual sampling sites ordinated in 2D-UMAP space were classified using a supervised Decision Tree learning algorithm. Data mining with Decision Tree algorithm was improved as a powerful, highly predictive performance for exploring complex datasets in order to discover the presentative variable for previously divided clusters [79,80].

All statistical analysis were performed using Microsoft Office 365 Excel software, in MS Addinsoft XLSTAT version 2022.5.1.1395. UMAP model with Decision Tree was carried out using BioVinci software (BioTuring Inc., San Diego, CA, USA) version 3.0.9.

3. Results

3.1. Population Dynamics Parameters

Based on the extensive dataset and following the presented methodology, we derived the results of the population dynamics parameters for spirlin for the period from 2010 to 2021. The results of the length and weight frequency, LWR, and the condition factor for each sampling year and total sample are summarized and presented in Table 1.

Table 1. Length–weight relationship parameters, Fulton condition factor, and type of growth of spirlin in Serbia, Central Balkans (N—number of sampling sites per year; n—total number of sampled specimens; L and W range—minimum and maximum size of total length and weight, respectively; a—the intercept; b—the regression coefficient; SEb—standard error of b; r^2—the coefficient of determination; CF—the medium value of Fulton condition factor for each sampling year; SEcf—standard error for CF; A—allometry (A− and A+ negative and positive allometric growth).

Year	N	n		Range L (cm)	Range W (g)	b	SEb	a	r^2	CF	SEcf	A
2010	10	118	Sexes combined	5.2–11.5	1.5–15.0	2.533	0.249	0.025	0.745	0.98	0.08	A−
2011	14	277		3.5–11.5	1.2–16.0	2.848	0.221	0.013	0.967	0.89	0.05	A+
2016	17	344		5.4–12.0	1.0–17.1	3.184	0.216	0.005	0.818	0.91	0.03	A+
2017	53	1322		5.0–13.7	2.0–29.2	2.780	0.119	0.015	0.841	1.00	0.03	A−
2018	14	450		6.3–12.1	2.0–17.2	2.873	0.327	0.011	0.762	0.89	0.05	A−
2019	4	76		8.0–10.5	4.0–12.0	3.740	0.096	0.001	0.953	0.93	0.01	A+
2020	29	315		5.5–12.5	1.0–21.0	3.384	0.080	0.003	0.832	0.79	0.01	A+
2021	7	139		6.5–12.5	2.0–16.0	3.438	0.123	0.002	0.849	0.78	0.02	A+
Total sample	∑148	∑3041		3.5–13.7	1.0–21.0	3.258	0.053	0.004	0.889	0.94	0.02	A+

In total, 3041 specimens of spirlin analyzed in this study were in the range from the minimum 3.5 to the maximum 13.7 (mean length 8.44 ± 1.55 SD) cm in total length and from the minimum 1.0 to the maximum 29.2 (mean weight 6.73 ± 3.78 SD) g in weight. The calculated values of parameter b were in the expected range of 2–4, varying from 2.533 to 3.740, and were 3.258 for the total sample (Table 1). However, a *t*-test indicated that there was a statistically significant difference between the obtained values of coefficient b for all sampling years, compared to the isometric growth and ideal value of b, b = 3 ($p < 0.05$). The coefficient of determination r^2 for all the sampling years varied from 0.745 to 0.967 (Table 1). The linear regression was significant for the sampling years (ANOVA, $p < 0.05$, $df = 1$). The estimated mean value of the Fulton condition factor (CF) for the total sample (± SD) was estimated as 0.94 ± 0.16 (Table 1).

Age estimation showed the maximum age of 5, and the most frequent group was the 2+ age group (48.69%) for the total sample (Figure 2).

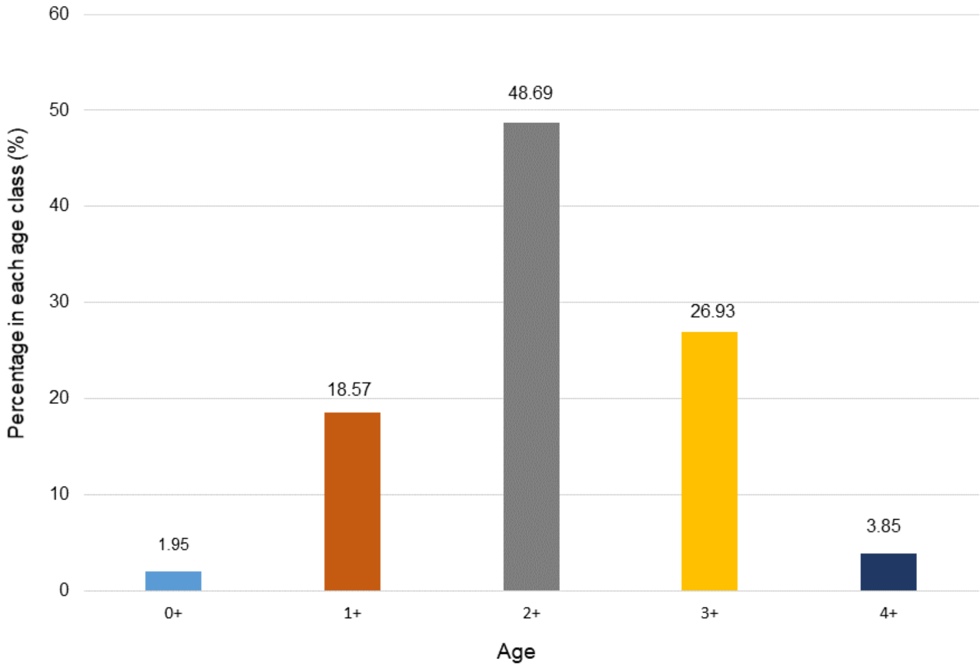

Figure 2. Age class structure of spirlin populations for total sample.

3.2. Growth Pattern

The von Bertalanffy growth equation with parameters L∞, K, and t0 and the growth performance index φ′, mortality, and exploitation rate are presented in Table 2. The lowest value of L∞ and the highest value of K were observed in 2017 (L∞ = 12.48 cm, K = 0.41 $year^{-1}$), contrary to 2010, where we reported the highest value of L∞ and the lowest value of (L∞ = 17.30 cm, K = 0.22 $year^{-1}$). Finally, we reported the value for the total sample as L∞ = 17.11 cm, K = 0.22 $year^{-1}$. Based on the *t*-test, no significant difference was found among the L∞, K, and t0 values for the different sampling years or the total sample ($p = 0.190$, $p > 0.05$). The growth performance index φ′ was 1.81, while the natural (M), fishing (F), and total (Z) mortality rates were 1.06, 0.03, and 1.09, respectively, and the exploitation rate was 0.01 for the total sample.

Table 2. The von Bertalanffy growth model equation (±1.89 SD of L∞ for total sample), the growth performance index φ′, mortality rates (natural mortality M, fishing mortality F, and total mortality Z), and the exploitation rate E.

Year	Von Bertalanffy Equation		φ′	M	F	Z	E
2010 **	Lt = 17.30 $[1-e^{(-0.22(t+1.70))}]$		1.82	0.93	0.08	1.01	0.07
2011	Lt = 14.79 $[1-e^{(-0.29(t+0.37))}]$		1.80	1.21	0.01	1.22	0.008
2016	Lt = 17.04 $[1-e^{(-0.22(t+0.76))}]$		1.80	1.07	0.06	1.13	0.05
2017 *	Lt = 12.48 $[1-e^{(-0.41(t+0.35))}]$		1.80	1.41	0.02	1.43	0.01
2018	Lt = 16.99 $[1-e^{(-0.23(t+0.67))}]$		1.82	0.94	0.08	1.02	0.07
2019	Lt = 13.34 $[1-e^{(-0.37(t+0.56))}]$		1.82	1.15	0.02	1.17	0.01
2020	Lt = 13.37 $[1-e^{(-0.36(t+0.57))}]$		1.81	1.40	0.01	1.41	0.007
2021	Lt = 13.70 $[1-e^{(-0.34(t+0.60))}]$		1.81	1.26	0.06	1.32	0.04
Total sample	Lt = 17.11 $[1-e^{(-0.28(t+0.93))}]$	±1.89	1.81	1.06	0.03	1.09	0.01

* minimum value of L∞ and maximum value of K; ** the opposite.

3.3. Population Parameters and Environmental Parameters

The main descriptive statistics of the seven fish population parameters and seven environmental parameters for the total sample and the minimum and maximum values of each parameter as well as the mean value with the corresponding standard deviation are reported in Table 3.

Table 3. Descriptive statistics with standard deviations of seven fish population parameters and seven environmental parameters ± standard deviations.

Parameters	Abbreviation	Measuring Unit	Minimum	Maximum	Mean	±SD
Production	P	kg/km^2	0.02	32.88	1.39	3.19
Abundance	Ab	N/km	28.00	2240.00	419.87	430.93
Biomass	B	kg/km	0.08	23.90	2.72	3.38
Mean length	L	cm	3.51	13.7	8.44	1.55
Mean weight	W	g	1.00	29.20	6.73	3.78
Age	A	/	1.00	5.00	2.34	0.60
Condition factor	CF	/	0.74	1.15	0.94	0.16
Temperature	T	°C	8.40	27.10	17.79	4.33
Conductivity	EC	µs/cm^3	60.00	1300.00	364.27	149.29
pH	pH	0–14	6.70	10.33	8.15	0.66
Dissolved oxygen	DO	mg/L	4.75	13.70	9.32	5.51
Saturation	DO%	%	52.50	173.70	103.58	9.71
Hardness	H	($CaCO_3$) (mg/L)	80.00	650.00	186.85	77.51
Altitude	ALT	m	34.00	970.00	341.44	176.84

Table 4 shows the Spearman's coefficients between the fish population parameters and environmental parameters as well as between the environmental parameters. The non-statistically significant correlations between pairs were marked with *nss*, using a *p* threshold of 0.001. The fish population parameters appear correlated with the other fish population parameters, i.e., P, B, and Ab, and negatively correlated with the environmental parameters, i.e., EC, H, pH, and ALT. The three parameters (EC, pH, and ALT) pointed to a strong and moderate negative correlation. A strong negative correlation was estimated between EC and Ab. Moreover, T pointed the weak negative correlation to the fish population parameters. DO and DO% were instead positively correlated with the fish population parameters.

If we consider the environmental parameters, EC appears correlated with pH, T, and H (especially to H) and negatively correlated with ALT. ALT is negatively correlated with almost all the fish population parameters as well as the environmental parameters and positively correlated with DO and DO%.

3.4. UMAP with Decision Tree

The virtual sampling sites were ordinated with a UMAP model in two-dimensional space (Figure 3) and classified with the Decision Tree algorithm into seven clusters (Figure 4). The ordination mainly reflected both population parameters and environmental parameters' gradients, with the exception of altitude (ALT). A primary gradient for ordination reflected the conductivity (EC) of the sampling sites, where a gradient is shown from left (sampling sites with a lower value of EC as well as hardness H and pH, contrary to the high concentrations of dissolved oxygen DO) to right (sampling sites with a higher value of EC, H, and pH, opposite to the low value of DO) side of UMAP space (Figure 3).

The sampling sites with high abundance, production, and biomass were distributed in clusters along the lower end of the left side on the UMAP space, contrary to the right side, where they were detected the lower values of abundance, production, and biomass (Figure 3). Moreover, the altitude (ALT) was pointed to as one of the parameters that shape the distribution of the species, clearly separate from cluster 0 (sampling sites at a high altitude) (Figure 4).

Table 4. Rho Spearman's correlation coefficients between all pairs of variables (population parameters and environmental parameters). Positive and negative correlations are highlighted in brown and blue shades, with a linear scale of four intervals with colors darkening with higher values.

	P	Ab	B	L	W	A	CF	T	EC	pH	DO	DO%	H	ALT
P	1.000	0.850	0.890	0.168	0.213	0.382	0.111	−0.038	−0.404	−0.450	0.038	0.015	−0.348	−0.451
Ab		1.000	0.870	*nss*	*nss*	0.265	0.051	−0.183	−0.875	−0.574	0.117	0.084	−0.271	−0.481
B			1.000	0.247	0.310	0.344	0.216	−0.117	−0.614	−0.524	0.066	*nss*	−0.166	−0.452
L				1.000	0.790	0.017	0.050	−0.037	−0.107	−0.080	*nss*	−0.051	−0.112	−0.470
W					1.000	0.092	0.454	−0.038	−0.052	−0.024	−0.037	−0.092	−0.018	−0.205
A						1.000	0.105	*nss*	−0.208	0.020	−0.064	−0.116	−0.107	−0.104
CF							1.000	*nss*	0.017	−0.011	−0.110	−0.111	*nss*	0.099
T								1.000	0.177	−0.060	−0.317	0.108	0.142	−0.448
EC									1.000	0.474	−0.098	0.035	0.886	−0.280
pH										1.000	0.164	0.229	−0.073	0.118
DO											1.000	0.655	−0.172	0.218
DO%												1.000	−0.059	0.100
H													1.000	−0.316
ALT														1.000

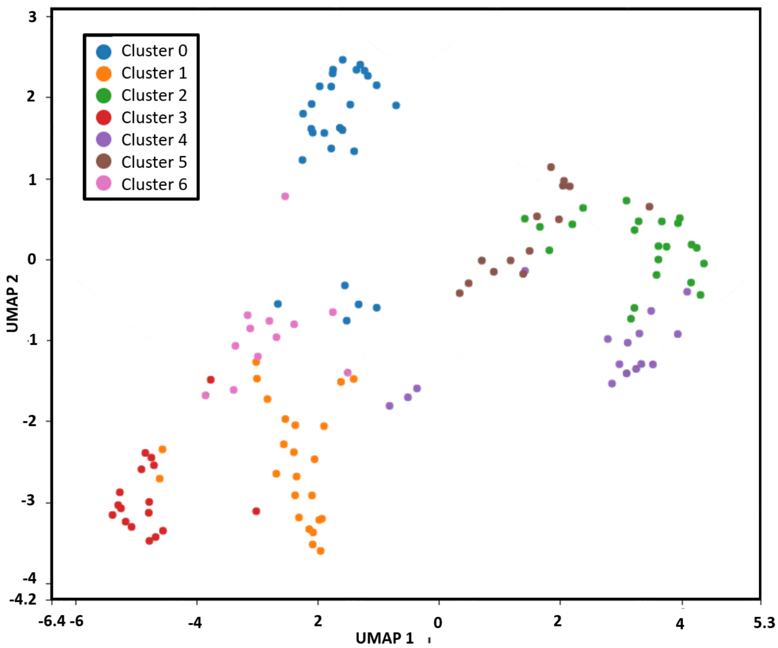

Figure 3. Ordination of sampling sites using UMAP model. Colors stand for different seven clusters of sites identified by Decision Tree algorithm. Each point on the 2D UMAP space presents different sampling sites. The clusters along the lower left part of the UMAP space (colored in red, orange, and rose) were characterized by higher values of population parameters, reflected by lower values of conductivity, temperature, and hardness as well as a higher oxygen ratio, contrary to the clusters observed in the upper-right side (colored in green, brown, and lilac).

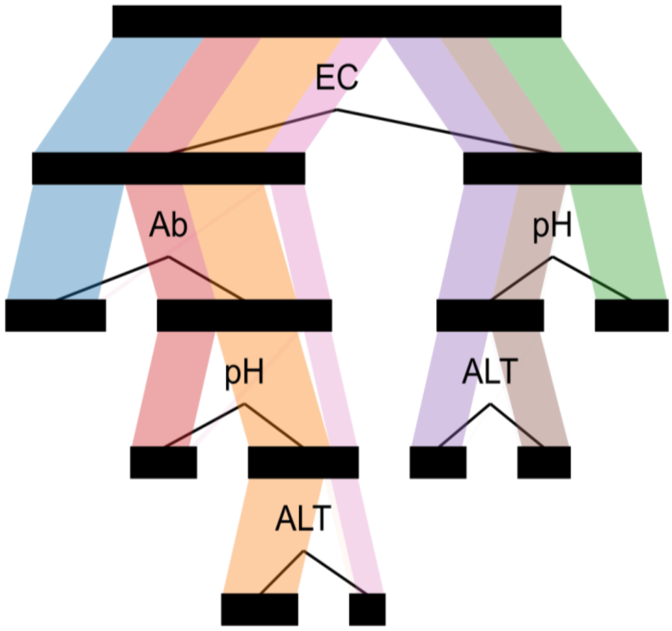

Figure 4. The Decision Tree algorithm was used to detect the most significant parameters for clustering.

4. Discussion

The potential recovery of spirlin populations in some regions is supposed to be related to the combination of various factors, small body size, short life expectations, rapid growth, reproductive strategy (early maturation, already from year 2, and several spawning episodes), high mortality rates, and migratory behavior [5,81], which allows the spirlin to recolonize formerly abandoned habitats. Therefore, efforts should be made to collect more data on the local/regional level, and conservation must focus on habitat improvements and migration facilities. Kováč et al. [82] considered that implementing cost-effective conservation strategies and the management of spirlin populations on a global level depends on locally collected data. More recently, most scientists have been studying the population genetics of fish species, while the primary ecological aspect has been greatly neglected. Recognizing the importance of understanding the species habitat requirements of commercially insignificant fish species [15], which are still of great importance in the food webs of predatory and commercially important fish species, we obtained the detailed spirlin population dynamics, including size, age structure, growth pattern, mortality, and exploitation rate. Moreover, to our knowledge, this is the first study that has applied a UMAP model with the Decision Tree algorithm for classification, in order to link the environmental parameters and population parameters. Milošević et al. [77] used a UMAP model, but with the Louvain algorithm for the ecological dataset, including the fish dataset. The results from both studies confirmed that the ordinations obtained by the UMAP model were ecologically meaningful.

We found that the maximum value of the length of spirlin was 13.7 cm, and the maximum value of weight was 29.2 g. Age estimation showed the maximum age was 5, and the most frequent was 2+ (48.62%), then 3+ (26.93%), indicating the vital populations (Figure 2). The obtained results correlated with Breitenstein and Kirchhofer [15], since they reported similar results for spirlin in the Aare River in Switzerland, emphasizing that spirlin, like most other fish, has an allometric type of growth, reaching a weight of about 20 g and a length of about 13 cm. Comparing the obtained results in our study with some previous reports on the studied spirlin populations inhabiting the Caspian Basin and European regions shows that the maximum age and size (length and weight, respectively) vary a quite a lot among the regions (we compared our results with those reported in the FishBase database) [52]. This characteristic could be interpreted as an interpopulation pattern specifically related to the different environmental conditions, the growth rate, and natural selection [83,84].

The estimation of LWR is one of the most-used methods in fisheries science for authentic biological and ecological data assessment [85], improving the knowledge about a species' life history during a certain period [86]. Based on the LWR estimated from a regression of log weight on log length (Table 2), we registered a positive allometric type of growth (t-test, b = 3, $p < 0.05$, $df = 8$), which may be related to spirlin's specific habitat requirements and, therefore, its specific phenotype [87]. These findings are in good agreement with Kováč et al. [82], who characterized the growth rate of spirlin in the Rudava River with two periods of isometric growth and one short period of allometric growth. The LWR results showed the relatively good status of the spirlin populations in the studied sites, indicating the existence of adequate food and suitable conditions for growth and development, since the availability of food leads to a faster growth rate. These findings improve the sustainability of spirlin in the investigated area.

Treer et al. [88] investigated the LWR of spirlin in Croatia, and, in four of the five sites examined, spirlin had positive allometric growth, which was in concordance with our results, considering that spirlin had positive allometric growth during five sampling periods as well as for the total sample. Moreover, Torcu-Koç et al. [89] reported similar values of b for spirlin in Turkey. In contrast, we found much higher values for b in contrast to those reported in northern Iran by Patimar and Dowlati [90]. We supposed that the effect of different feeding habits (diet), food availability, and environmental quality could be the possible reason for such a variation in b value. For example, Treer et al. [45,88] found that the algae Bacillariophyceae and Chlorophyceae were the most dominant items in the diet of spirlin, and the invertebrates could be considered as accidental prey. Conversely, Filipović and Janković [91] analyzed the diet of spirlin from the Serbian Mirovštica River and mainly found the taxa of Trichoptera and Chironomidae. It appears that species have the availability to adapt their diet based on the prey availability. On FishBase's official website, the average value of coefficient b for spirlin is 3.10, indicating positive allometric growth. The given models in our study determined up to 96.7% of the dependent variable. The obtained values of r^2 could be affected by using combined sexes, considering that it is very difficult to distinguish the sexes in the field [45].

From the aspect of understanding the general conditions for the growth and development of fish during a season and/or year, besides LWR, the condition factor is also important [85]. Since the mean values of the condition factor in the multiannual period (2010–2021) for spirlin in our study ranged from 0.78 to 1.00 (Table 1), our findings lend support to previous findings in the Polish San and Dunajec basins, where it was stated that the values of the condition factor for spirlin range from 0.82–0.94 [83]. Generally, the value of CF aspires one [82], so the results from this study indicated that spirlin was in a good body form. The maximum observed CF value in this study (CF = 1.15) (Table 3) within the observed positive allometric growth could indicate the existence of favorable environmental conditions in this area [45,92,93]. Although CF varies between specimens, generally the average value of CF for a season and year is not so variable. Until recently, it was considered that CF represents an ideal reflection of the food, growth, and, therefore, general health of fish [92,94]. Novel studies pointed to LWR as a better indicator of the above-mentioned attributes of fish species, expressing the correlation of fish condition with environmental conditions [95]; therefore, the results should be comparatively interpreted.

The growth parameters can significantly reflect upon the conditions of the niche of this species and can be used as a comparison to different habitats [88]. The values calculated in this study, where $L\infty$ was 17.11 cm and K was 0.22 year^{-1}, were similar to those reported in Croatia (Bednja River $L\infty$ = 15.5 cm and K = 0.33 year^{-1}; middle Korana river $L\infty$ = 15.4 cm and K = 0.23 year^{-1}) [88] as well as in Iran (Uzineh Qanat $L\infty$ = 15.3 cm and K = 0.23 year^{-1}) [84]. In contrast, our results were higher than those reported in Estonia ($L\infty$ = 13.1 cm and K = 0.49 year^{-1}) and Bosnia and Herzegovina ($L\infty$ = 12.0 cm and K = 0.59 year^{-1}) but lower than those reported in Czechia ($L\infty$ = 20.1 cm and K = 0.15 year^{-1}) and Turkey (L∞ = 22.9 cm and K = 0.09 year^{-1}) [89]. The comparison of the von Bertalanffy growth parameters L∞ and K between the different populations of spirlin confirms the theory that the faster the growth rate is, the smaller the length of the fish is, and vice versa. Since almost 50% of the specimens (48.62%) in the total sample were in the 2+ age class, and, considering that spirlin has a faster growth rate especially in the first year, lower values for the growth rate were expected.

The growth performance index φ′ calculated in this study was in the range of 0.83 and 1.83. Therefore, the φ′ calculated for the sampling years as well as for the total sample in this study was close to that previously reported for spirlin in FishBase database. These data confirm the reliability of spirlin growth curves, as the overall growth performance φ′ has a minimum variance within the same species [96]. Additionally, the high natural and low fishing mortality rate and, thus, the low exploitation rate were expected, considering that spirlin is not a commercially interesting fish in this region. A high mortality rate is common for spirlin in the waters of the Pannonian valley [97].

Small-bodied fish species such as spirlin often experience high natural mortality rates corresponding to low resistance, which is balanced by early maturation and high fecundity to support a quick recovery from a disturbance [81]. As a result of its fast growth rate, especially observed in the first year, and the regulatory mechanisms of populations [15], it appears that spirlin displays a wide capacity for population plasticity [84,98]. Its spatial distribution, environmental requirements, and migratory behavior are of particular interest. Therefore, we used a UMAP as the best-fitting model for our dataset, to visualize the spirlin distribution over the past 10 years, linking the spirlin population parameters and environmental parameters (Figures 3 and 4). According to our results, spirlin was widespread in the investigated area (Figure 1) and could be found in the rivers with different ecological statuses and environmental conditions, given the observed wide range of all the environmental parameters (T, EC, pH, DO, DO%, H, and ALT) (Table 3). The Decision Tree algorithm pointed to EC, pH, and ALT as good predictors for population parameters (i.e., abundance) (Figures 3 and 4). In spite of the observed wide range of the environmental parameters, we registered the highest values of abundance, biomass, and production in Clusters 3, 1, and 6, respectively, where there were similar observed environmental conditions (i.e., a lower value of T, pH, and EC), contrary to the low abundance in Clusters 2, 4, and 5, where there were high values detected of EC and H and unfavorable values of pH. In addition, the results of the Spearman's correlation coefficients confirmed the ordination and classification obtained by the UMAP model, meaning that the population parameters were negatively correlated with environmental parameters, i.e., EC, H, pH, T, and ALT, and positively correlated with DO and DO% (Table 4).

Spirlin was very abundant, reaching an abundance of up to 2240 specimens per km (Table 3 and Supplementary Table S1), sometimes accounting for up to 65% of the total catch. It occurred in catches mostly together with brown trout, Danube barbel, *Gobio* sp., chub, and minnow, but it also occurred in small amounts together with eurytopic fish such as perch, even with wels. According to Stojković et al. [51], spirlin is one of the three most-representative rheophilic fish for the upper and mid-upper sectors of the largest tributaries of the Morava River system, which is characterized by low temperature and a high oxygen ratio, so our results partly correlated with these findings.

It has been assumed that spirlin demands quite high environmental requirements and river sections that are not polluted but are well-supplied with oxygen [99]. During our field research, we noticed that spirlin dies very quickly after being sampled, if it is not released back into the habitat in a short period of time; even so, some individuals do not survive the oxidative stress, though we registered spirlin's occurrence in highly polluted water with extremely high values of conductivity (Pek River at an altitude of 34 m and a 1300 μs/cm^3 value for EC in this study). Potentially, a non-invasive method, such as environmental DNA, or visual methods, could be used for future fish monitoring to minimize the stress level of the specimens. Vetemaa [100] registered small amounts of spirlin specimens in the streams of Estonia that had a certain level of pollution. Similar results were reported in Lithuania, where spirlin occurred in water courses with a higher degree of eutrophication [101]. Korte et al. [102] also registered spirlin in high numbers, even in organically polluted rivers, as did Breitenstein and Kirchofer [15] in the Aare River in Switzerland. In addition, Simić et al. [18] reported a trend toward an increase in spirlin occurrence in the Danube barbel zone, although this zone is enriched with organic nutrients (nitrates and phosphates).

Even if the spirlin avoids eutrophic waters and rivers with a slow current and poor water quality [5,25,100,103], it appears that it shows a fairly high level of pollution. High values of EC (up to 1300 µs/cm^3 in Pek River) are usually an indirect indicator of pollution, because they present a close relationship with the dissolved salt content, which is often associated with anthropogenic activities (i.e., urban sewage) [104–107]. Moreover, agriculture and urbanization growth led to increased water enrichment, causing a discernable decrease in water quality [81]. The Pek River is considered to be very polluted (due to the mining industry) with toxic elements Cu, Pb, As, and Mn, which were often above the maximum permitted concentrations, as well as nitrates, nitrites, and phosphates. EC is also affected by temperature: as water temperature increases, the conductivity increases (a weak positive correlation in this study, as shown in Table 4). A weak negative correlation of abundance to temperature (Table 4), with the observed water temperature up to 27 °C (Table 3), corroborates the issues considered above. Kirchhofer and Breitenstein [15] reported 27 °C as the maximum observed water temperature value in which spirlin still endures.

Spirlin is a potamodromous species, and it has a tendency to migrate upstream in two periods, in early summer (from mid-June to mid-July) and more so in autumn (from mid-August to mid-October) [15], and this corresponds well with our results (Cluster 0 mostly includes localities with high values of altitude, in which it was sampled during the early summer and autumn months) (Figure 3 and Supplementary Table S1). The spirlin altitudinal shifts are connected with a decrease in the stream flow rate as well as with temperature. Global warming will continue to progress, and it is expected to rise by 1.5 °C above preindustrial levels until the 2050s, according to the International Panel on Climate Change [108]. Mountain streams are considered to remain suitable habitats for rheophilic fish species, but the IPCC expects that warming in the mountain segments of rivers will be the same as the European average or even above it [108,109]. Some works considered the altitude as a proxy of anthropization [75,110], since the higher density of point infrastructure (such as dams, run-of-river hydropower plants, human-made waterworks, etc.) is in the mountains [111]. The question arises about whether migration behavior will be a limiting factor for the occurrence of this small fish species, or whether it will adapt to the environmental changes. Moreover, the question that arises is whether spirlin's tolerance of different environmental conditions is related to its acclimatization, due to its occurrences in poor quality water with high temperature.

5. Conclusions

The obtained results showed that spirlin is widespread in Serbia, and it is common in rivers with different ecological statuses and conditions. We registered its occurrence in both types of river sections: those that are not polluted and are well-supplied with oxygen as well as in those with poor water quality that are highly polluted. This species is representative of the rheophilic fish complex, but it may also occur in lowlands. However, high values of abundance were estimated in rivers with low temperature and a high oxygen ratio. The UMAP was divided seven clusters, reflecting both the population parameters and environmental parameters' gradients, with the exception of altitude. No clear gradient was visible for altitude like the other parameters, but Cluster 0 is clearly distinguished. Considering spirlin's tendency to migrate upstream to find suitable places for spawning, Cluster 0 includes localities with mostly high values of altitude, in which spirlin was sampled during the early summer and autumn months. These findings demonstrate that habitats with favorable conditions for spirlin spawning still exist.

All the considered results suggest that the status of spirlin populations is still satisfactory, emphasizing the sustainability of the populations in the investigated area. It appears that spirlin has the ability to acclimate, given the observed wide range of all the environmental parameters in the investigated habitats. Although the impacts of climate change will overwhelmingly be negative, spirlin could in fact be the "winners" of climate change. Our study provides the framework for new investigations of spirlin in the Central Balkans, and considerable progress has been made concerning the decreasing trend of spirlin in many European rivers. Thus, conservation should focus on the protection and improvement of suitable habitats. The situation is, thus, still incomplete, and future studies on the current topic are, therefore, needed to prevent the further global decline in the spirlin population.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d15050616/s1. Table S1: A list of investigated sampling sites for period 2010–2021. The estimated fish population parameters were presented as well as the number of clusters previously defined by UMAP. The standard deviations for each parameter were estimated and are presented in Table 3.

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Data Availability Statement: The datasets generated and analyzed during the current study are presented in this paper and the Supplementary Materials. One part of the data is available at http://baes.pmf.kg.ac.rs.

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