Diet and growth of spirlin, *Alburnoides bipunctatus* in the barbel zone of the Sava River

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A b s t r a c t . No spirlin have been registered in the barbel zone of the Sava River, Croatia in the late seventies of the last century. Since then, due to improved water quality the presence of spirlin gradually increased in number (23.3%) and in biomass (4.7%). The most dominant item in the diet of spirlin were *Bacillariophyceae* and *Chlorophyceae* during every monthly investigation. The diet consisted also of invertebrates which can be considered as a secondary or an accidental prey. The size related analyse of the consumed food showed *Bacillariophyceae* to be preferred food by fish of all sizes. The back calculated growth in the total length could be expressed by the following formula: $L_t = 12.0\ (1 - e^{-0.59\ (t+0.14)})$. The phi-prime of spirlin from the Sava river is $\phi' = 4.44$. The length-weight relationship, covering the fish from the entire growing period, showed an isometric growth with a b-value of 3.025 ($p > 0.05$), except of September when it was significantly allometric. That was confirmed by the non-significant relationship between condition factor and total length ($r = 0.014$; $p > 0.05$). However, CF in June, September and October (0.86 ± 0.07; 0.85 ± 0.09 and 0.87 ± 0.10, respectively) was significantly lower ($p < 0.05$) than in May and July (1.00±0.21 and 1.00±0.12, respectively). The improved water quality during the last fifteen years enabled spirlin to migrate and enlarge its population downstream, resulting in a slightly decreased condition factor.

Key words: cyprinid, Croatia, condition, feeding, threat

Introduction

Spirlin (*Alburnoides bipunctatus*) is a typical species inhabiting the barbel zone of streams (e.g. H u e t 1949, L u s k 1995). Their habitats required for spawning are of a very narrow range (M a n n 1996). Being very sensitive to human activities, the number of habitats suitable for the spawning and nursery is quite limited (P e ň á z & J u r a j d a 1993). Low tolerance to water polluted by industrial, agricultural or urban waste makes spirlin a good biological indicator of the environment quality (Č i h a ř 1999). Consequently, the spirlin is extremely threatened and nearly close to extinction in many European waters (H e r z i g - S t r a s c h i l 1991, J e d i c k e 1997, K i r c h h o f e r 1997, M u ž í k 1998) and in the Czech Republic it is protected by law (W o h l g e m u t h 1996, L u s k et al. 1998).

The susceptibility and abundance of this species in Croatia is considered as being average (M r a k o v č i ć & K e r o v e c 1997), meaning that suitable habitats for spirlin still exist. In the barbel zone of the Croatian part of the river Sava spirlin was not registered in the late seventies of the last century, but only found upstream in Slovenia (H a b e k o v i ć et al. 1990). Abundance of 2.22 %, was first recorded in 1985 (H a b e k o v i ć & P o p o v i ć 1991). After the heavy and mining industries were abandoned in Slovenia between 1991 and 1994, their relative abundance rose to 5.82 % (H a b e k o v i ć et al. 1997).

There are several papers dealing with the diet (V u k o v i ć 1968, V u k o v i ć & I v a n o v i ć 1971, S k ó r a 1972, F i l i p o v i ć & J a n k o v i ć 1978, L o s o s et al. 1980) and growth of spirlin (S k ó r a 1972, B a s t l et al. 1975, J o h a l 1979, P a p a d o p o l & C r i s t o f o r 1980, Š o r i ć & I l i ć 1985, B r e i t e n s t e i n & K i r c h h o f e r 2000). As there is a constant need to increase knowledge on fishes under threat in order to implant management and conservation actions (W o o t t o n et al. 2000, O l i v a - P a t e r n a et al. 2003) the aim of this paper was to study the existing state of the spirlin population, its feeding and growth in the barbel zone of the Sava River.

Materials and Methods

Spirlin were caught five times during 2001 (May, June, July, September, October) by electric gear in the Sava River at Medsave, near the Slovenian border (Fig. 1). This part of the Sava River belongs to the barbel zone. The physical and chemical analyses of water were performed. The average water temperature in June was 12 °C, not exceeding 20 °C during the summer and 12 °C again in October. A high quantity of oxygen, low chemical oxygen demand (COD), low quantities of nitrogen and phosphorus, with the average pH = 7.5 were

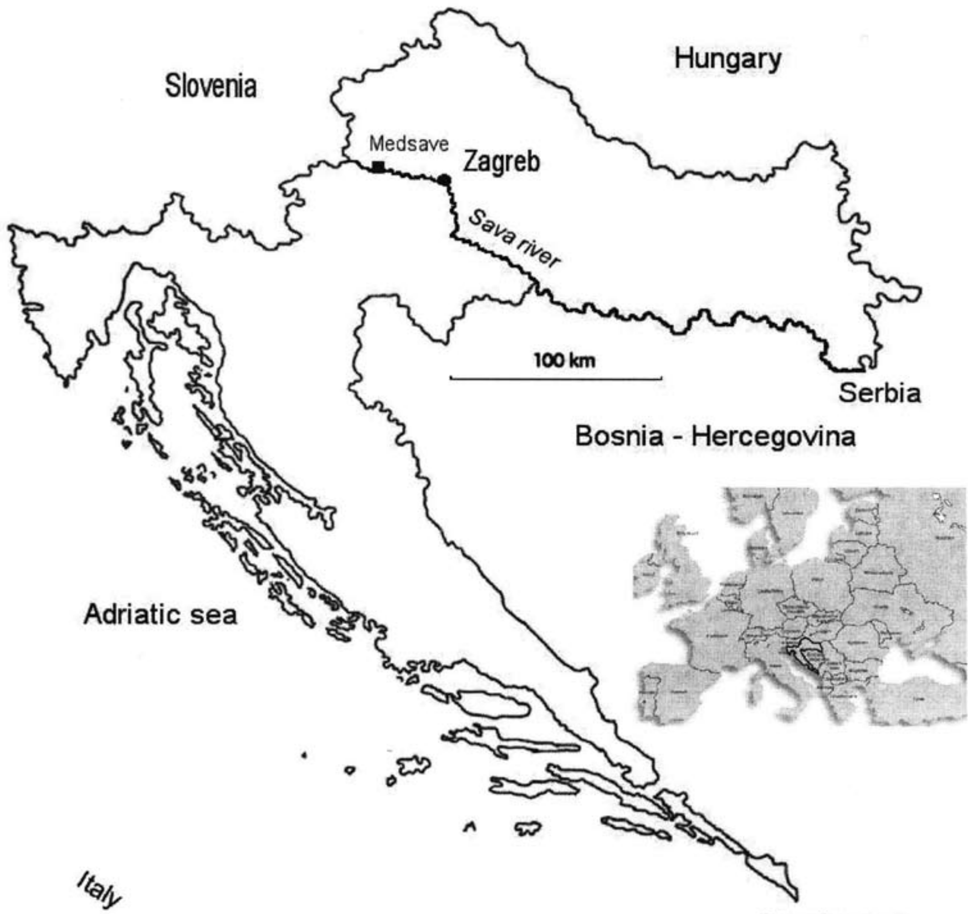

Fig 1. Location of the sampling area.

measured (Table 1). The bottom was graveled and the banks were covered by stony rip-rap. The gravel was covered by bluegreen algae and diatoms with the presence of various invertebrates (T r e e r et al. 1994).

Altogether 150 specimens were caught. They were measured for total length (L) to the nearest 0.1 cm and weight (W) in g. Scales for the age determination were taken from above the lateral line below the anterior part of the dorsal fin.

Immediately after being caught, the juveniles were preserved in 4% formaldehyde and adults were frozen to preserve their gut contents.

For the analysis of stomach contents the anterior third of the gut was used, with the food organisms still recognizable, weighed and fixed in 4% formaldehyde. When possible, the different food items in the gut were identified to the family or genus level. The identification and the counting were made using a binocular microscope.

Assessment of the diet was based on the frequency of occurrence (F%) and numerical frequency (N%) of the different diet components, using the following formulas:

$$F\% = \frac{f_i}{\sum f} x100$$

where f_i = number of stomachs containing each prey items and $\sum f$ = total number of stomachs with food;

$$N\% = \frac{n_i}{\sum n} x100$$

where n_i = total number of one food item, $\sum n$ = total number of food items consumed by the fish (H y s l o p 1980).

Table 1. Basic chemical and physical parameters of the Sava river (n= number of samplings).

Parameters	n	$\bar{X}$±SD
Water temp. °C	10	15,3±4,38
O_2 (mg l^{-1})	10	9,16±1,38
Free CO_2 (mg l^{-1})	1	0,726
$KMnO_4$ in mg $O_2 l^{-1}$	10	9,293±3,93
NH_3 (mg l^{-1})	8	0,056±0,043
Hardness ($CaCO_3$ mg l^{-1})	9	0,45±0,26
Ca^{2+} (mg l^{-1})	9	0,181±0,102
Mg^{2+} (mg l^{-1})	10	0,267±0,114
NO_3^- (mg l^{-1})	5	2,75±1,48
NO_2^- (mg l^{-1})	10	0,135±0,048
$NaNO_2$ (mg l^{-1})	10	0,202±0,071
PO_4^{3-} (mg l^{-1})	10	0,201±0,095
P_2O_5 (mg l^{-1})	10	0,15±0,071
Free Cl (mg l^{-1})	7	0,033±0,024
pH	10	7,5±0,19

The analysis of changes in feeding habits in different months and different length classes was performed using the following indexes (according to H y s l o p 1980):

$$\text{Fulness index (FI \%)} = \frac{\text{Total stomach contents weight}}{\text{Fish weight}} \text{ x } 100$$

$$\text{Vacuity coefficient (VI\%)} = \frac{\text{Number of empty stomachs}}{\text{Total number of guts analysed}} \times 100$$

The food items were classified into three categories based on Almeida et al. (1993): accidental, F% < 10; secondary, 10≤ F% < 50; preferential, F≥ 50.

Reading of scale rings was performed using the microscope connected through video camera into the computer screen (Scion Image Program). The back-calculation of the growth in length was studied using the Fraser-Lee formula, taking into account that the relationship between the scale radius (s) in mm and the total length in cm appeared to be L = 2.53 + 40.06 s (r = 0.989; p<0.01). The von Bertalanffy growth function (VBGF) was used to study the overall growth performance using the values of growth in length and phi-prime (Φ') (Bertalanffy 1934; Sparre & Venema 1992):

$$L_t = L_\infty (1-e^{-K(t-t_0)})$$
$$\Phi' = \ln K + 2 \ln L_\infty$$

where L_t = total length at age t, L_∞ = the ultimate total length of an average fish should achieve if it continue to live and grow, K= the growth coefficient that determines how fast the fish approaches L_∞, t_0= hypothetical age for L_t=0 and Φ'= overall growth performance.

The absolute annual (i_n) and the average absolute length increments ($\bar{i}_{1-4}$) and real growth rate ($L_4 = 4\ \bar{i}_{1-4}$) in cm during the first four years of life were computed, as suggested by Živkov et al. (1999).

To establish the length-weight relationship the commonly used formula $W = aL^b$ was applied (Ricker 1975), where W = weight in grams, L = total length in cm, and a and b are the constants. The condition factor (CF) was calculated as:

$$CF = W \cdot L^{-3} \cdot 100$$

Results and Discussion

In 2001, the trend of increasing the abundance of spirlin was continuing in this part of the Sava River. The fish catches during the late seventies, when this species was not registered among the 1396 specimens of the fish caught (Habeković et al. 1990), were followed by the first record of one specimen (2.22 % of the overall abundance) in 1985 (Habeković & Popović 1991) and 5.82 % (129 specimens out of 2217 fish caught) at the beginning of nineties (Habeković et al. 1997). In 2001, spirlin reached 23.3 % in of the total abundance and 4.7 % of the total mass (this paper). The highest relative abundance (34.3%) occurred in October and the lowest one (12.2%) in July. These data coincide with observations made by Breitenstein & Kirchhofer (2000) who detected significantly higher quantities of spirlin in autumn and winter than in summer, as a result of extended summer migration phases. Kainz & Gollmann (1990) found this proportion of spirlin in different Austrian rivers to be generally less than 20% (maximum 34%). The most abundant species of the fish community, except of *A. bipunctatus* in the river Sava were *Leuciscus cephalus*, *Alburnus alburnus*, *Barbus barbus*, *Barbus peloponnesius* and *Cobitis* spp.

Stomach content analyses were performed on 86 individual of spirlin with the total length between 5.6 and 11.2 cm. The frequency of empty stomachs ranged 0–67 %. The fullness index was the lowest in September (FI = 0.594) and the highest in June (FI = 2.506)

Table 2. The frequency of occurrence (F%), numerical frequency (N%), vacuity coefficient (VI%), fulness index (FI%) and food item importance (I): preferential (P), secondary (S) and accidental (A) of stomach content of spirlin caught at Medsave, 2001 (n= number of analyzed specimens).

Taxa	May VI=0 FI=2.120 n=3			June VI=17 FI=2.506 n=6			July VI=50 FI=1.170 n=12			September VI=67 FI=0.594 n=15			October VI=40 FI=1.970 n=50		
	F %	N %	I	F %	N %	I	F %	N %	I	F %	N %	I	F %	N %	I
Cyanobacteria	-	-		5.00	60.33	A	-	-		7.69	0.27	A	3.07	0.97	A
Xantophyceae	-	-		-	-		15.38	0.32	S	23.08	1.85	S	0.53	>0.01	A
Bacillariophyceae	88.88	98.40	P	75.00	34.80	P	15.38	35.08	S	38.47	38.41	S	89.73	97.56	P
Chlorophyceae	11.12	1.60	S	-	-		53.86	64.50	P	23.07	59.20	S	6.16	1.44	A
Ephemeroptera	-	-		5.00	0.16	A	7.69	0.05	A	7.69	0.27	A	-	-	
Other Insecta	-	-		5.00	0.16	A	-	-		-	-		-	-	
Asellus aquaticus	-	-		-	-		7.69	0.05	A	-	-		-	-	
Oligochaeta	-	-		10.00	4.55	S	-	-		-	-		0.51	0.03	A

Table 5. Absolute annual total length increments (i_n), average absolute total length increments ($\bar{\imath}_{1\text{-}4}$) and real growth rate ($L_4$=4 $\bar{\imath}_{1\text{-}4}$) in cm during first four years for spirlin growth in the Sava and other rivers (standard lengths in the Ogosta, Rokytná, Eliseva and Radimna rivers were transformed into total lengths according to Papadopol & Cristofor 1980 and Johal 1979).

Location	Source	i_1	i_2	i_3	i_4	i_5	i_6	i_7	$\bar{\imath}_{1\text{-}4}$	L_4
Sava, Croatia	this paper	5.9	2.8	1.3	1.0				2.75	11.0
Ogosta, Bulgaria	Johal 1979	5.2	1.1	1.1	-	-	-	-	-	-
Rokytná, Czech Republic	Johal 1979	4.1	1.6	1.5	1.1	1.1	1.5	1.1	2.08	12.0
Eliseva, Romania	Papadopol & Cristofor 1980	4.4	1.7	2.3	1.1	-	-	-	2.38	9.5
Radimna, Romania	Papadopol & Cristofor 1980	3.5	2.8	2.0	1.5	-	-	-	2.45	9.8
Morava, Serbia	Šorić 1985	3.2	1.4	1.0	1.0	0.7	0.5	0.8	1.65	6.6
Aare, Switzerland	Breitenstein & Kirchhofer 2000	2.9	4.3	3.5	1.3	-	-	-	3.00	12.0

(Table 2). In different length classes the vacuity index ranged 12.5–56 %, the fullness index ranged from 1.143 in length class 9.1–10 cm and 1.874 in length class 7.1–9 cm (Table 3).

During the monthly investigations the dominant diet components were Bacillariophyceae and Chlorophyceae. The diet also consisted of invertebrates which can be qualified as a secondary or an accidental prey. Their presence varied from month to month. The largest amount of invertebrates was in June (Table 2). The size related variation of the consumed prey indicated that fish of all sizes preferred Bacillariophyceae. The most abundant classes 7.1–9 cm had also the largest amount of prey (Table 3). Results from other authors vary. According to Vuković (1968) spirlin are zoophagous. These results are based only on the gut length. Vuković & Ivanović (1971) noted that the main food of spirlin were planktonic and nektobenthic organisms. Filipović & Janković (1978) analysed the diet of spirlin from the Serbian Mirovštica River and found mainly taxa of Trichoptera and Chironomidae, however no trace of plant food. More detailed report was given by Skora (1972) and Losos et al. (1980). According to Skóra (1972) the main items of the stomach content of spirlin based only on food mass were invertebrates (~60%), followed by algae (~30%). Losos et al. (1980) concluded that the main food of spirlin in the Jihlava River in the Czech Republic were the zoobenthic organisms while, especially in spring, a great amount of filamentous algae, diatoms and detritus were found in their guts.

Out of the 150 specimens caught, 123 were 1+, 13 were 2+, seven 3+ and only one 4+ (Fig. 2). In October there were caught also 6 bigger YOY (exceeding 6 cm in total length). Such rapid and great losses of this small fish were detected also by other authors. Breitenstein & Kirchhofer (2000) found most of the fish caught to be 0+ and 1+ (only one individual was 5+), while Skóra (1972) concluded that the majority of spirlin died off after 5th and 6th year.

The back-calculated growth of spirlin in total length (Table 4) was expressed by the following formula:

$$L_t = 12.0\ (1\text{-}e^{-0.59\ (t+0.14)})$$

The phi-prime of spirlin from the Sava River is ϕ'=4.44. This value is very close to the ones obtained from the data at other localities: Dunajec basin (ϕ'=4.10), (Skóra 1972),

Table 3. Size related variation of the frequency of occurrence (F%), numerical frequency (N%), vacuity coefficient (VI%), fulness index (FI%) and food item importance (I): preferential (P), secondary (S) and accidental (A) of the main food categories consumed by spirlin (n= number of analyzed specimens).

Taxa	5–7 cm VI=40%, FI=1.671 n=5			7.1–9 cm VI=44%, FI=1.874 n=55			9.1–11 cm VI=56%, FI=1.143 n=23			11.1–13 cm VI=13%, FI=0.890 n=3		
	F %	N %	I	F %	N %	I	F %	N %	I	F %	N %	I
Cyanobacteria				4.07	2.93	A	1.72	0.09	A			
Xantophyceae	7.15	0.53	A	2.33	0.03	A	1.72	0.04	A			
Bacillariophyceae	71.42	79.61	P	80.82	89.30	P	87.93	98.82	P	83.34	91.12	P
Chlorophyceae	21.43	19.86	S	9.30	7.57	A	5.17	0.89	A	16.66	8.88	S
Ephemeroptera				1.16	0.01	A	1.73	0.02	A			
Other Insecta				0.58	>0.01	A						
Asellus aquaticus				0.58	>0.01	A						
Oligochaeta				1.16	0.16	A	1.73	0.14	A			

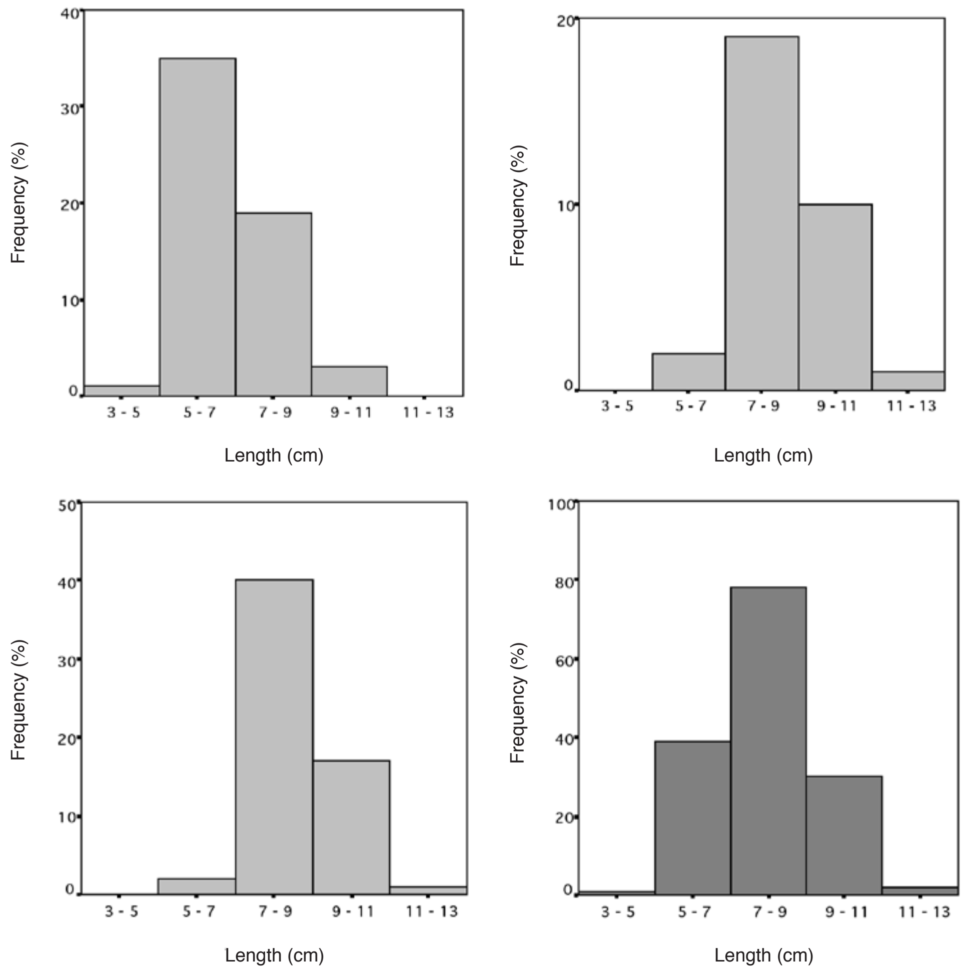

Fig 2. Length frequency distributions of spirlin (n=150) caught in Sava river in 2001 (spring up left, summer up right, autmn down left, total down right).

river Turiec (ϕ'=4.22), (B a s t l et al. 1975, the standard lengths transformed to total ones according to Johal 1979), river Radimna (ϕ'=4,13), (P a p a d o p o l & C r i s t o f o r 1980, standard lengths transformed to total ones according to Johal 1979), river Aare (ϕ'=4.70), (B r e i t e n s t e i n & K i r c h h o f e r 2000) and the Croatian average for this species (ϕ'=4.24), (T r e e r et al. 2000). This data confirm the reliability of spirlin growth curve, as the overall growth performance (ϕ') has a minimum variation within the same species and does not depend on their different growth rate (M o r e a u et al. 1986).

Compared to other rivers the growth in length of spirlin from the Sava River is quicker during the first two years of their life (Table 5), the K parameter being 0.59. However, taking into account the first four age groups to which belongs most of the all populations, the average absolute length increments ($\bar{\imath}_{1-4}$) were much higher than those registered in the Serbian and Czech rivers – closer to the values in the Romanian rivers, but lower than those in Switzerland.

Table 4. Spirlin in the river Sava. Age structure (years in Roman numerals) and mean total length at age (L_1–L_4, in cm) of the population based on back-calculated data taken from fish caught in 2001 (n = number of fish studied).

Age group	n	L_1	L_2	L_3	L_4
I	123	6.0			
II	13	5.7	8.5		
III	7	6.1	8.9	10.1	
IV	1	5.8	8.7	9.9	11.0
Total	144	144	21	8	1
Mean		5.9	8.7	10.0	11.0

The length-weight relationship expressed for the fish of the entire growing period, demonstrated isometric growth with a b-value of 3.025 (S.E. = 0.1078), which was not significantly different from 3 ($p>0.05$). However, in September a significant negative allometric growth was determined (Table 6) which is related to the non-significant relationship between condition factor and total length (CF = 0.8738 + 0.0016 TL; r = 0.014; $p>0.05$). The dispersion of data for older fish was primarily due to the changes of CF over the year. In June, September and October (0.86±0.07; 0.85±0.09 and 0.87±0.10, respectively) it was significantly lower ($p<0.05$) than in May and July (1.00±0.21 and 1.00±0.12, respectively). Such CF changes, although not statistically significant, in this multiple spawning species were also detected by B r e i t e n s t e i n & K i r c h h o f e r (2000) in the Aare River. These values of condition factor were similar to those found in Polish San and Dunajec basins (0.82–0.94), which never exceeded 1 (S k ó r a 1972). The CF of spirlin found only upstream in Slovenia twenty five years ago, was quite high (CF=1.06), (T r e e r et al. 2000), in comparison with the lower values found nowadays. Possible reasons are the competition for food and relatively high growth in length, as a result of adaptation to the new habitat (D a d i k y a n 1972). This shows that the improvement of the water quality in this area over the last fifteen years enabled spirlin to migrate and enlarge its population downstream, but consequently slightly lowered its condition factor.

Table 6. Parameters for the regression ($W=aL^b$) between the total length (L, cm) and weight (W, g) of spirlin from the Sava river.

Parameters	May	June	July	September	October	All seasons
a	0.0550	0.0199	0.0198	0.0297	0.0031	0.0083
b	3.255	2.597	2.674	2.428*	3.485	3.025
S. E. (b)	0.1669	0.2539	0.1861	0.2589	0.1603	0.1078
r	0.974	0.872	0.977	0.911	0.943	0.941
r^2	0.948	0.760	0.954	0.830	0.891	0.885
n	23	35	12	20	60	150

* b= significantly different from 3 (p<0,05)

A c k n o w l e d g e m e n t s

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