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## Diet and growth of the endemic Neretva chub, *Squalius svallize* from the Neretva river area, Bosnia and Herzegovina

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**Abstract.** The Neretva chub, *Squalius svallize* is an endemic species of the Adriatic basin of the southeastern Europe. Altogether, 60 specimens were caught by gill nets from the Neretva river area, the oldest being seven years old. The most dominant item in the diet of *S. svallize* during winter season were larvae of Trichoptera and Diptera. Diptera larvae were also dominant during spring and summer. In autumn period the largest amount in stomach content were Trichoptera larvae and Gastropoda. Plant material was present in stomach content but not dominant food item. The von Bertalanffy formula, counted from the back calculated growth in total length, appeared to be:  $L_t = 35.3 (1 - e^{-0.15(t+1.40)})$ . The phi-prime of Neretva chub (ln base) is  $\Phi' = 5.23$ . The length-weight relationship, including the fish from the entire growing period, demonstrated positive allometric growth with a b-value of 3.47. The average value for condition factor was  $CF = 0.98 \pm 0.14$  (min = 0.76; max = 1.29).

**Key words:** nutrition, condition, Croatia, Adriatic dace

### Introduction

The Neretva chub, *Squalius svallize* (Heckel & Kner, 1858) is an endemic species of the Adriatic basin of the southeastern Europe. According to Mrakovčić et al. (2006) it inhabits parts of Croatia, Bosnia and Herzegovina and Albania. These authors reported this species under the scientific name *Leuciscus svallize* and English names Adriatic dace and Balkan dace. Some *Squalius* from western Greece were reported as *S. cf. svallize* (Economou et al. 1991, Bobori et al. 2006). However, Kottelat & Freyhof (2007) indicate that *S. svallize* is restricted only to Neretva, Trebišnjica and Ljuta drainages in Croatia and Bosnia and Herzegovina. Part of the year it can live in the underground water courses (Vuković & Ivanović 1971).

On the IUCN red list the Neretva chub is registered as vulnerable species. The reason for its threat is primarily anthropogenic, down falling quality of its limited habitats (Mrakovčić et al. 2006). *S. svallize* is a common species

of the lower Neretva river and its tributaries, but the data about its biology are rare. In older literature (Kačanski et al. 1977) the first information about its food items was presented. So, the aim of the present work is to provide some biological characteristics, related to its feeding and growth.

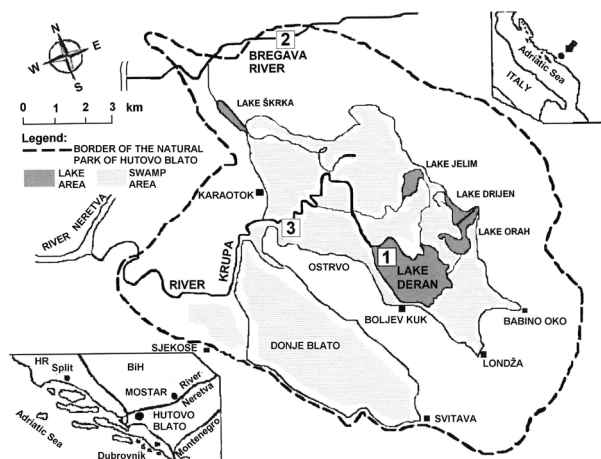
### Material and Methods

The sites under study include the area of the eutrophic Lake Deran (longitude 017 47 51 E; latitude 43 02 41 N; altitude 0), and the oligotrophic Bregava (longitude 017 45 34 E; latitude 43 06 13 N; altitude 9) and Krupa rivers (longitude 017 45 51 E; latitude 43 03 30 N; altitude 1). Deran is a part of the Hutovo Blato swamp (approximately 7 411 km<sup>2</sup> surface), and an area with abundant fish species, surrounded by three rivers: Neretva in the west, Bregava in the east and Krupa in the south, with reversible flow (Fig. 1). It is an area with Mediterranean

climate and average annual temperature 14.7°C. High quantity of oxygen, low quantities of nitrogen and phosphorus, with pH 7.2-7.8 were measured (Table 1). The bottom was gravelled and silted.

Fish was caught by anchored gill nets (28-72 mm). All the nets were 30 meters long, while net height varied from 1 to 3 meters. Nets were set up at night and checked the next morning. All locations were studied seasonally (October 2006; February, May, August 2007). Altogether 60 specimens were caught, the oldest one being seven years old. They were measured for total length (L), from anterior-most point of the body to the posterior-most point of the caudal fin, to the nearest 0.1 cm and weighed (W) in g. All data were analyzed for the combined sexes.

Digestive tract was extracted from the analyzed fishes, after cutting out the oesophagus and anus. Thereafter, length of the digestive tract was measured. Content of the digestive tract was extracted from the front part, scaled as a wet mass by electronic balance and fixed in 4% formaldehyde. After that a quantitative and qualitative analysis of the food content in the



**Fig. 1.** Location of the sampling areas (1 – Lake Deran, 2 – Bregava River, 3 – Krupa River).

**Table 1.** Basic chemical and physical parameters obtained in the investigated area (n – number of samplings; COD – chemical oxygen demand; BOD – biological oxygen demand; evaporate rest and suspended substances – mass determination of dissolved and suspended inorganic and organic matter).

Parameters	Bregava River ( $\bar{x} \pm SD$ ) n=4	Krupa River ( $\bar{x} \pm SD$ ) n=4	Lake Deran ( $\bar{x} \pm SD$ ) n=4
Water temp. (°C)	14.92±3.98	15.43±4.32	16.35±4.53
Suspended substance (105 °C) (mg l <sup>-1</sup> )	0.41±0.26	0.64±0.33	0.80±0.42
Evaporate rest (mg l <sup>-1</sup> )	223.00±0.07	219.00±11,16	265.00±8.19
COD (O <sub>2</sub> mg l <sup>-1</sup> )	6.52±1.65	3.31±0.56	4.74±0.82
O <sub>2</sub> (mg l <sup>-1</sup> )	11.90±1.27	11.74±1.10	10.83±0.99
O <sub>2</sub> (%)	130.93±3.56	135.93±3.86	131.56±2.74
BOD (O <sub>2</sub> mg l <sup>-1</sup> )	2.79±0.25	2.99±0.35	2.51±0.18
KMnO <sub>4</sub> in mg l <sup>-1</sup> O <sub>2</sub>	8.89±0.65	9.64±0.96	10.69±0.60
NO <sub>2</sub> <sup>-</sup> (mg l <sup>-1</sup> )	0.009±0.00	0.01±0.00	0.008±0.00
NO <sub>3</sub> <sup>-</sup> (mg l <sup>-1</sup> )	0.44±0.01	0.40±0.01	0.76±0.03
Total N (mg l <sup>-1</sup> )	0.45±0,01	0,45±0.01	0.18±0.03
Total P (mg l <sup>-1</sup> )	0.01±0.01	0.00±0.00	0.02±0.00
SO <sub>4</sub> <sup>3-</sup> mg/l	3.10±0.20	4.30±0.37	1.11±0.15
Cl <sup>-</sup> (mg l <sup>-1</sup> )	9.00±0.82	24.91±1.52	25.97±1.50
Ca <sup>2+</sup> (mg l <sup>-1</sup> )	0.13±0.02	0.12±0.02	0.20±0.01
Mg <sup>2+</sup> (mg l <sup>-1</sup> )	0.59±0.02	0.57±0.01	0.36±0.02
pH	7.80±0.19	7.91±0.15	7.22±0.13

digestive tract was made where possible. The identification and the counting were done by the 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:

$$F\% = \frac{f_i}{\sum f} \cdot 100$$

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

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

where  $n_i$  = total number of one food items,  $\sum n$  = total number of food items consumed by the fish (Hyslop 1980).

Changes in feeding habits were analysed in different months and different length classes was performed using the following indices (according to Hyslop 1980).

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

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

Scales for age determination were taken from above the lateral line below anterior part of the dorsal fin. Identification of scale rings was done in Scion Image program by microscope connected to the computer screen by video camera. The growth rate of Neretva chub was estimated by growth zones on scales and found from back-calculated lengths (Bagenal & Tesch 1978). The back-calculation of growth in length was studied by using Fraser-Lee formula:

$$l_n = s_n s^{-1} (l-c) + c$$

where  $l_n$  = fish length in the time of annuli formation in cm;  $s_n$  = scale radius at each annulus in mm;  $s$  = scale radius in mm;  $l$  = fish length in cm;  $c$  = correction factor from the following equation:  $L = 6.62 + 4.93 s$  ( $r = 0.869$ ;  $p < 0.01$ ). The von Bertalanffy growth function (VBGF)

was used to fit the values of growth in length (Bertalanffy von 1934) and phi-prime ( $\Phi'$ ) to study the overall growth performance (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  $t$ ,  $L_\infty$  = the ultimate total length that an average fish would achieve if it continued to live and grow,  $K$  = the growth coefficient that determines how fast the fish approaches  $L_\infty$ ,  $t_0$  = hypothetical age for  $L_t = 0$  and  $\Phi'$  = overall growth performance.

Absolute annual length increments ( $i_n$ ), average absolute length increments ( $\bar{i}_{1-7}$ ) and real growth rate ( $L_7 = 7 \bar{i}_{1-7}$ ) in cm during the first four years of life were computed, as suggested by Živkov et al. (1999).

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

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

All statistical analyses were performed in SPSS 13 program.

## Results and Discussion

The most numerous species in association with *S. svallize* in the investigated area (scientific and common names according to Kottelat & Freyhof 2007) were Dalmatian nase, *Chondrostoma knerii* Heckel, 1843, chub, *Squalius cephalus* (L., 1758), brown bullhead, *Ameiurus nebulosus* (Le Sueur, 1819), tench, *Tinca tinca* (L., 1758), Prussian carp, *Carassius gibelio* (Bloch, 1782), carp, *Cyprinus carpio* L., 1758, Neretva rudd, *Scardinius plotizza* (Heckel & Kner, 1858), plotica, *Rutilus basak* (Heckel, 1843), pumpkinseed, *Lepomis gibbosus* (L., 1758), European eel, *Anguilla anguilla* (L., 1758), rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), Atlantic trout, *Salmo trutta* L., 1758. During the research the existence

of the zubatak, *Salmo dentex* Heckel, 1852, was confirmed. It is rather unexplored, vulnerable and rare endemic species which inhabits the Cetina river in Croatia (Mrakovčić et al. 1995), the Neretva river in Bosnia and Herzegovina and the Morača river in Montenegro (Marić 1995).

Stomach content analyses were performed on 60 individuals of *S. svallize* with the total length between 15.6 and 28.0 cm. The empty stomach frequency ranged from 8 to 30%. The fullness index was lowest in February (FI=0.35) and highest in August (FI=1.71). In winter, low temperature resulted in limited feeding activity, while in summer high temperature stimulated increased food consumption (Politou et al. 1993, Haertel & Eckmann 2002).

*S. svallize* in the Neretva river, consumed 12 prey taxa, primarily as a bottom feeder.

During winter season the dominant food items were larvae of Trichoptera and Diptera. Except for autumn, Diptera larvae were also dominant during spring and summer. In autumn period the largest amount in stomach content showed Trichoptera larvae and Gastropoda. Trichoptera larvae were preferred prey during February, August and October. Consumed fish, crustaceans and gastropods were found in the guts of the Neretva chub with total lengths of 15-25 cm, 20-28 cm and 25-28 cm, respectively. Plant material was present, although not dominant food item in the stomach content (Table 2). According to Kačanski (1977) in the Neretva river the main invertebrate food were Trichoptera larvae and Chironomidae larvae (Diptera) followed. Plant material made stomach content of only few individuals.

**Table 2.** The frequency of occurrence (F%), numerical frequency (N%), vacuity coefficient (VI%), fullness index (FI%) and condition factor (CF) of *Squalius svallize* during investigated period in 2006/2007 (n – number of analyzed specimens).

Taxa	February FI=0.35 VI=30 CF ± Sd=0.89 ± 0.08 n=20		May FI=0.46 VI=8 CF ± Sd=1.09 ± 0.09 n=13		August FI=1.71 VI=21 CF ± Sd=1.05 ± 0.15 n=14		October FI=0.40 VI=8 CF ± Sd=0.94 ± 0.16 n=13	
	F %	N %	F %	N %	F %	N %	F %	N %
	Plant material	+	+	+	+	+	+	+
Turbellaria	-	-	13.23	11.90	1.33	2.12	3.80	6.15
Nematodes	6.55	5.47	5.41	5.45	-	-	-	-
Gastropoda	9.44	7.53	-	-	3.95	1.93	30.55	25.95
Oligochaeta	4.22	3.31	1.65	4.47	-	-	-	-
Crustacea	10.58	8.27	9.00	21.22	1.63	6.87	2.77	6.38
Ephemeroptera	8.68	4.91	-	-	9.21	4.98	6.25	11.10
Plecoptera	-	-	3.71	3.21	6.32	5.3	-	-
Trichoptera	23.50	32.13	3.71	2.99	11.28	15.53	45.15	31.22
Coleoptera	7.35	15.16	3.71	2.63	-	-	-	-
Hymenoptera	-	-	40.43	31.75	11.28	13.77	-	-
Diptera	22.46	19.10	19.15	18.38	25.00	24.66	3.89	7.31
Fishes	7.22	4.12	-	-	30.00	24.87	7.59	11.89

Out of 60 specimens caught, the majority (23) were 4+ that can be attributed to the mesh size. The back calculated growth in total length of *S. svallize*, presented in Table 3 could be

expressed by the following formula:

$$L_t = 35.3 (1 - e^{-0.15(t+1.40)})$$

**Table 3.** Age structure (years in Roman numerals), mean length at age ( $L_1$ - $L_7$ , in cm) and absolute annual length increments ( $i_n$  in cm) of the *S. svallize* from the Neretva tributary based on back-calculated data (n – number of fish studied).

Age group	n	$L_1$	$L_2$	$L_3$	$L_4$	$L_5$	$L_6$	$L_7$
III	13	10.8	13.5	15.8				
IV	23	11.7	14.2	16.6	18.8			
V	9	11.4	14.6	18.2	20.3	22.1		
VI	8	12.5	16.0	18.6	20.6	23.0	24.9	
VII	7	11.4	14.5	18.1	21.7	23.6	25.3	25.7
Total	60	60	60	60	47	24	15	7
Mean	L	11.6	14.6	17.5	20.4	22.9	25.1	25.7
$i_n$		11.6	3.0	2.9	2.9	2.5	2.2	0.6

Average absolute length increments during the first seven years were:  $\bar{i}_{1-7} = 3.67$  cm. These data are closer to the ones obtained for chub (*Squalius cephalus*) in Croatia (Treer et al. 1997) that lives in the similar environments and is closely related to *S. svallize*. The phi-prime of the Neretva chub from the Neretva tributary (ln base) is  $\Phi' = 5.23$ . This value is also close to the ones obtained for *S. cephalus* in Croatia ( $\Phi' = 5.69$ ), (Treer et al. 1997) and British isles ( $\Phi' = 5.90$ ), (Hickley & Dexter 1979). The phi-prime of *S. svallize* from the Neretva tributary presented with the log base ( $\Phi' = 2.27$ ) fits within the values obtained for the ones for *Squalius* species in Greek Kremasta and Tavropos reservoir (2.23 and 2.18, respectively) (Bobori et al. 2006). As the overall growth performance ( $\Phi'$ ) has minimum variance within the same and related species,

and does not depend on the different growth rate (Moreau et al. 1986), this again shows the similar growth pattern of these *Squalius* species.

The average value for condition factor was  $CF = 0.98 \pm 0.14$  (min = 0.76; max = 1.29). In autumn and winter it was significantly lower ( $p < 0.05$ ), than in spring and summer (Table 2). The length-weight relationship, including the fish from the entire growing period ( $W = 0.0023 L^{3.47}$ ;  $r^2 = 0.952$ ;  $p < 0.01$ ;  $n = 60$ ), demonstrated positive allometric growth with a high b-value of 3.47. The area of Lake Deran is eutrophic, what together with the Mediterranean climate allows longer feeding period, and so could explain considerable high consumption of animal food and higher growth in both, length and weight, of *S. svallize* from this habitat.

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