Journal of Limnology and Freshwater Fisheries Research

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Maturation and Fecundity of *Barbus cyclolepis* from the Stryama River of the Maritsa River Drainage Basin in Bulgaria

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ABSTRACT

A field-based study of the reproductive aspects of Round-scaled barbel biology took place in the Stryama River, during the autumnal season of the period 2010–2011. This is the first study of the reproduction of this species. A total of 202 Round-scaled barbels were caught by electrofishing. The study found that both sexes reach sexual maturity in their second year. Females became sexually mature, once they reach 94 mm length and 13 g weight. Males became sexually mature once they reach 60 mm length and 12 g weight. Fecundity ranged from 460 to 18.420 eggs. Average fecundity was 6.944 eggs. Absolute fertility was positively correlated with the growth of fish length and mass. Consequently, five years old fish had the highest fertility rate. The average values of relative fecundity were 210 eggs per kilo. The reproductive parameters are an indicator of the growth conditions of the river, as well as the reproductive patterns and state of the fish population. The population parameters, established by this study, now represent a reference point, enabling a monitoring of the species' population dynamics.

Keywords: Round-scaled barbel, reproductive parameters, fecundity

ARTICLE INFO

RESEARCH ARTICLE

Received : 17.09.2020
Revised : 01.02.2021
Accepted : 02.02.2021
Published : 30.12.2021

DOI:10.17216/LimnoFish.796589

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How to Cite
Kolev V. 2021. Maturation and Fecundity of *Barbus cyclolepis* from the Stryama River of the Maritsa River Drainage Basin in Bulgaria. LimnoFish. 7(3): 233-240. doi: 10.17216/LimnoFish.796589

Introduction

Round-scaled barbel (*Barbus cyclolepis* Heckel, 1837) is widespread in the watercourses of the Aegean watershed as well as in some rivers of the Black Sea watershed. In the latter case, these are the rivers, flowing on the territory of the Strandzha Mountain, north of the Bosphorus Strait (Heckel 1837; Shishkov 1935; Kottelat and Freyhof 2007; Özuluğ and Saç 2019). This is the most abundant fish species in the middle zone of the Maritsa River tributaries, which include the Stryama River (Kolev 2013; Kolev 2016).

The barbel is a typical rheophilic species (Marinov 1989; Economidis 1989; Vasiliou and Economidis 2005). The fish lives in the middle course of rivers with gravel bottoms, but it is also found in the lower part of the trout zone, as well as in the upper part of the carp zone (Marinov 1989). The Round-scaled barbel's diet consists mainly of insect larvae (Chironomidae, Ephemeroptera), small crustaceans (Gamaridae) and plant detritus; it also includes other benthic animal organisms, as well as plants (Marinov 1989; Rozdina 2009). During the winter months, the species is not very active and congregates in groups, found along the riverbeds (Marinov 1989). Males reach sexual maturity in their first and second year; females - in their second. The breeding period typically takes place from mid-April to the end of July, but it can last until August (Rozdina 2009). Taking into consideration the ecological particularities of the species, Bianco (1998) includes it in the group of "medium-sized barbels adapted to life in warm waters, with moderate or standing current". The author refers to this group of barbels as a "cyclolepis group", with the Round-scaled barbel being singled out as the most typical representative.

In Bulgaria, commercial fishing in the country's inland waters is prohibited by the Bulgarian Fisheries and Aquaculture Law (ZRA 2001), so the Round-scaled barbel is not a commercial fish. However, it is one of the most popular fish for recreational fishing

in South-East Bulgaria (Kolev 2016). The fish is protected by the Bulgarian Biodiversity Conservation Law (ZBR 1992).

Analyses of the barbel's maturation and fecundity have attracted scholarly attention in both Bulgaria and Greece. The first study of the fish's breeding season in the Maritsa water basin of Bulgaria was conducted by Mihaylova (1965). Almost twenty years later Marinov (1989) published data about the barbel's spawning season and fecundity in the Aegean watershed of Bulgaria. Recent studies of the barbel's reproductive biology, sex composition, maturity and fecundity in the Bulgarian stretch of the Maritsa River were undertaken by Rozdina (2009), Raikova-Petrova and Rozdina (2012). In Northern Greece, the species was examined by Vasiliou and Economidis (2005) who described the sex structure and reproductive activity of this barbel.

The aim of this study was to determine a set of very important reproductive parameters (sexual structure, maturation and fertility) of the Round-scaled barbel from the Stryama River. The so-obtained set of reproductive parameters was then compared with corresponding data published by other scholars, who have studied other streams in the Aegean catchment area.

Materials and Methods

Study Area

The research project studies the Stryama River, which is a left tributary of the Maritsa River, originating in the Sredna Gora Mountains (Figure 1). In its upper reaches, the Maritsa tributary flows between the Stara Planina and the Sredna Gora mountains. Once it reaches the town of Banya, the river runs to the south. Near the village of Rajevo Konare, the Stryama enters the Upper Thracian Valley. The tributary flows into the Maritsa River near the village of Manole (East of the city of Plovdiv). The Stryama River is 110 km long with a catchment area of 1789 km^2.

The Stryama is a medium sized mountain Bulgarian river (NO: H-4 /14.09.2012 MOSV) with an average altitude of 833 m. Declination of the river bed is small, so the Stryama River's average flow rate is relatively small - 8 m/s (Stoyanov et al. 1981). In the summer, water temperature exceeds 20° C (BD-IBR 2015).

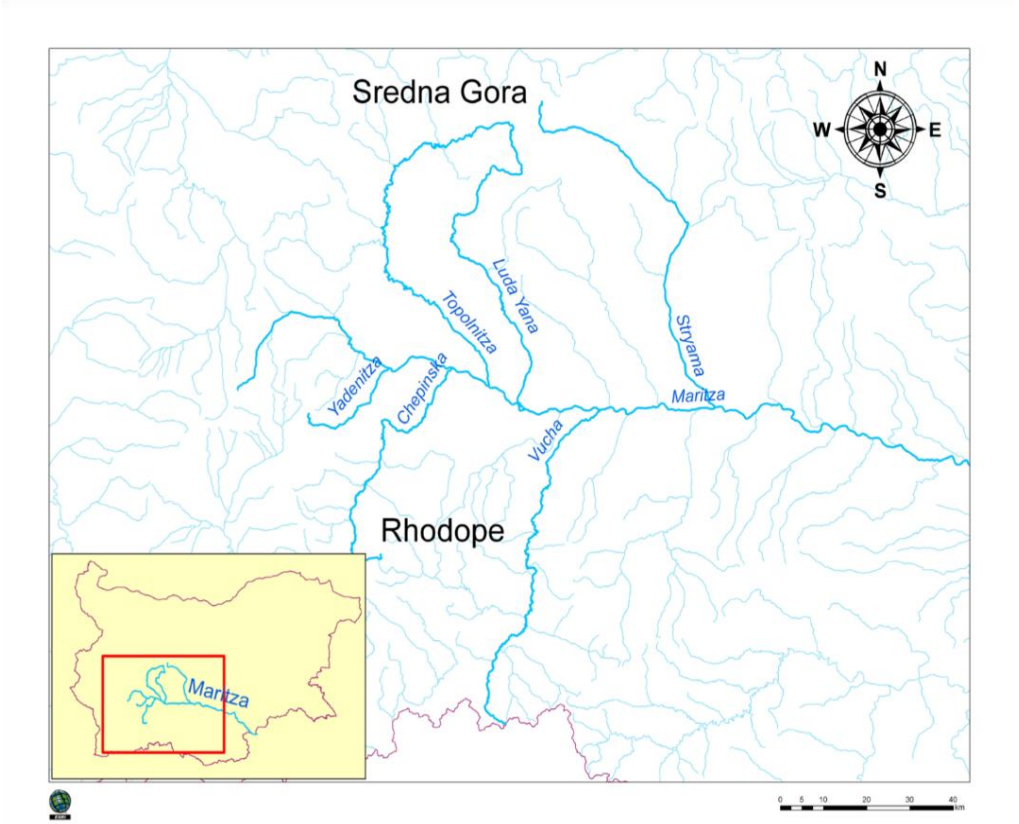

Figure 1. Location of the Stryama River (Bulgaria), Arc Map 10.0 (ESRI – ArcGIS 2013).

Specimens for this study were collected from two sampling sites (Table 1).

Table 1. Sampling areas along the Stryama River

№	Areas	Geographic coordinates		Alti-tude	Date of sampling
		N	**E**	m.a.s.l.	
1	In the vicinity of a fish farm near the Banya town	42°33‘38,45’’	24°47‘54,11’’	302	19.11.2010
2	In the vicinity of the bridge of the Banya town	42°32‘18,12’’	24°49‘22,78’’	283	17.04.2011
3	In the vicinity of a fish farm near the Banya town	42°33‘38,45’’	24°47‘54,11’’	302	20.10.2011
4	In the vicinity of a fish farm near the Banya town	42°33‘38,45’’	24°47‘54,11’’	302	20.11.2011

Specimens were mainly collected in the autumn of 2010–2011. A total of 202 Round-scaled barbel specimens were caught in the Stryama River by using the method of electrofishing. A SAMUS 725G converter was used, providing up to 640 V direct current (DC), with a frequency of 50 Hz and output power reaching up to 200 W. The catch was performed according to the EN 14011:2004 instruction (Water quality – Sampling of fish with electricity).

Standard length (*L*) was measured with a 1 mm precision, while weight (*W*) was measured with a 1 g precision.

More than ten scales ware collected from each barbel specimen. They were taken from underneath the dorsal fin; an equal number of scales was taken from the left and right side of the dorsal fin. Next, the scales were dried up and stored in small papers bags. The scales were then examined with a microscope Olympus CX 31, at 40× magnification. Each scale was sandwiched between two microscope slides. Fish age was then determined by counting the annual rings of a scale. For this purpose, the diagonal caudal radius of the scales was used.

The gonads, collected from each female barbel, was stored in a test tube in a fridge.

The number of fish eggs was determined by using the weighting method. First, 1 g of gonads, was placed between two microscope slides. Next, caviar was counted by using a counting chamber. This operation was performed with a microscope Olympus CX 31, at 40× magnification. The sex of a specimen was determined by dissecting the fish, caught in early winter.

The level of gonads’ maturity was determined by the coefficient *b* from equation 1, which describes the relationship between gonads’ mass and the mass of a fish without its entrails (Morozov 1964; Zhivkov 1985; Yankov 1988).

$$g = a + bW \quad (1)$$

where:

g – gonads’ mass (g)

W – mass of a fish without its entrails (g)

Relative fecundity (*RF*) was assessed directly by the ratio of absolute fertility and fish without gut (*F*/*W*) (Spanovskaya and Grigorash 1976). Another method to determine relative fertility (*RF*) was also used. The coefficients a and b were calculated from equation 2 (the equation reveals the dependence between absolute fertility – *F* and the mass of fish without intestines – *W*) (Zotin 1961; Zhivkov 1999).

$$F = a + bW \quad (2)$$

where:

F – absolute fertility (n)

W – mass of a fish without its entrails (g)

An analysis of the absolute fertility of Round-scaled barbel from different water sources was made by comparing the absolute fertility calculated for the same fish weight (Zhivkov 1999).

Results

Sex composition, age and length of first maturity

The age and sexual composition of the Round-scaled barbel population from the Stryama River are presented in Table 2. The sample includes six age groups. The first age group is solely comprised of juvenile specimens. The second age group encompasses sexually mature barbels, but many of fish are still juveniles. Three and four years old barbels were the most numerous (groups 3 and 4). In the fifth and sixth age groups, only females were found.

Table 2. Size and sexual composition of the Round-scaled barbel's population from the Stryma River

Size class (L, mm)	Number of fish																
	Age (years)	1			2			3			4			5		6		total
	Sex	j	♂	♀	j	♂	♀	j	♂	♀	♂	♀	♂	♀	♂	♀	total
61 – 70		2			1	5			1								9
71 – 80					8	7		4	2								21
81 – 90					3	1		10	21								35
91 – 100								7	26		3						36
101 – 110								5	11	5	4	2					27
111 – 120							1		7	4	2	5					19
121 – 130							1			3		12					16
131 – 140										3		17					20
141 – 150										2	1	4					7
151 – 160												1		2			3
161 – 170												1		4		1	6
171 – 180												1		1			2
181 – 190														1			1
∑ fish of all size classes		2			12	13	2	26	68	17	10	43	0	8	0	1	202

Legend: j- juvenile specimens, all fish aged four, five and six years in the sample are sexually mature

The barbel population from the Stryama River was relatively young. Seven- and eight-year-old barbels were absent. In the first age group, there were only two specimens, which had not yet reached sexual maturity. Six-year-old fish were very few. Among the one-year-old fish, there were no mature specimens. The first sexually mature specimens were found to be a two-year-old male over 60 mm in length. Female fish mature at a length of more than 90 mm. Female fish from the Stryama River also developed sexually at the earliest in the second year. The smallest sexually mature female was 94 mm in length. Most of the two-year-old barbels from the Stryama River were already mature. Males predominated in the second and third age groups, but the sex ratio of the Round-scaled barbel population from the Stryama River did not change.

The average sex ratio of the Round-scaled barbel's populations from the Stryama River is estimate as: ♂: ♀ 1.28:1. This difference was not statistically significant (d=1; χ^2=2.47, χ^2critical=3.83; P<0.005) that is, the sex ratio did not differ from the normal – ♂:♀ 1:1.

Relationships: absolute fertility-body length, fertility-body weight. Relative fecundity

Figure 2 shows the relationship between total fertility and the standard length of the body to the end of the scale cover.

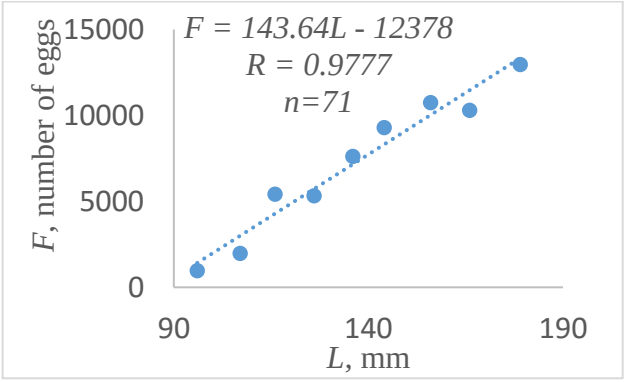

Figure 2. Relationship between total fecundity (F) and standart length (L) of the body to the end of scale cover of Round-scaled barbel from the Stryama River

Fish are grouped into nine size classes (Table 3). Absolute fecundity is calculated as the weighted average for each size class. This relationship is described by a linear equation with a high degree of reliability. As the length was increasing, absolute fertility was also increasing.

The average absolute fertility of the Round-scaled barbel in different size classes is presented in Table 3.

Table 3. Change of relative fecundity by length classes

	Length class (*L*, mm)	Average length (mm)	Range (number of eggs)	Average absolute fecundity (*F*) (number of eggs)	*n*
1	91-100	96	624-1330	977	2
2	101-110	107	1038-3245	1986	5
3	111-120	116	2408-9486	5430	10
4	121-130	126	1042-8928	5333	16
5	131-140	136	4560-13134	7632	20
6	141-150	144	5700-15840	9304	6
7	151-160	156	9950-11720	10754	3
8	161-170	166	7839-15680	10315	6
9	171-190	179	4600-18720	12975	3
∑					71

Average fertility increased evenly in the higher length classes. Increasing the length by 50 mm led to an approximate doubling of fish's average absolute fertility. Individual fertility of the Maritsa barrel from the Stryama River was between 460 and 18.720 eggs. The average individual fertility rate of the Round-scaled barbel was 6.944 eggs. These results have been obtained from 71 specimens; the length of each of these ranged between 91 mm 185 mm. The most fertile female barbel, found in the sample, was a five years old fish, 185 mm in length and weighing 78 g. Its fertility was 18.720 eggs. The specimen with the lowest fertility of 624 eggs was a two-year-old female 94 mm long and weighed 13 g.

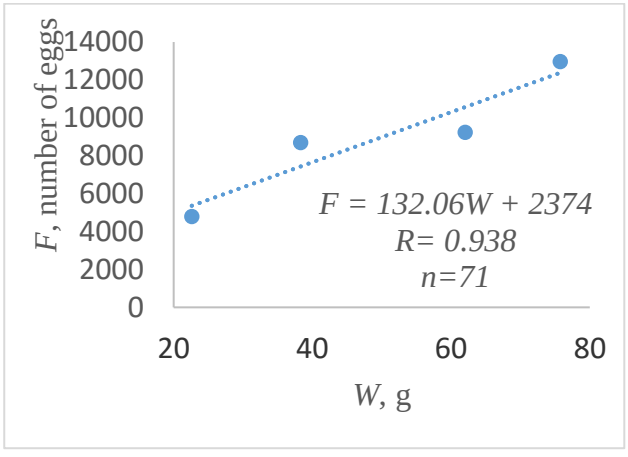

Figure 3. Relationship between total fecundity (F) and averge weight (W) for Round-scaled barbel from the Stryama River

Figure 3 presents the relationship between total fecundity and weight. The fish specimens are divided into four classes of weight (g), as follows: I- *11.0-30.9*; II-*31.0-50,9*; III-*51.0- 70.9*; IV-*71.0-90.9*. Absolute fecundity is calculated as the weighted average for each weight class.

As fish age increases, total fecundity also increases. This strong correlation is confirmed by a linear equation, with a high degree of reliability, which has been derived from the data.

Absolute fertility of Round-scaled barbel with the Stryama River increases with age. The relationship between absolute fertility and age is presented in Figure. 4.

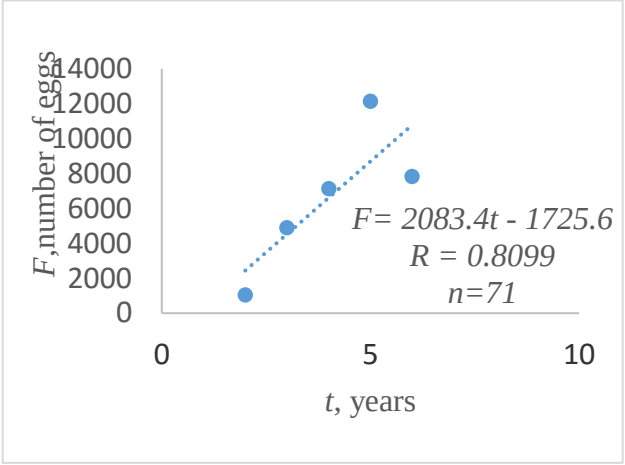

Figure 4. Relationship between total fecundity (F) and age (t, years) for Round-scaled barbel from the Stryama River

The study also estimates a frequently used parameter in fishery analyses: relative fish fertility, in order to perform a comparison among the size classes. Table 4 presents the changes of relative fertility of Round-scaled barbel with weight increase.

Table 4. Relative fecundity (*RF*, eggs) of Round-scaled barbel from the Stryama River

Size classes (*W*, g)	Weight (g)	Relative fertility (number of eggs)	N
11.0-30.9	22.6	213	36
31.0-50.9	38.3	227	27
51.0-70.9	62	149	5
71.0-90.9	75.7	171	3
Average and sum	-	190	∑ 71

The tables summarize an important finding: the number of eggs per unit weight tends to be inversely related to fish mass – i.e. as fish mass increases, relative fecundity decreases.

Discussion

The population of Round-scaled barbel from the Stryama River is characterized by three age groups, which differ by their sex ratio. The first age group includes two- and three-year-old specimens and is characterized by a predominance of male fish. Females are more numerous in the second age group, which includes four-year-old fish. In the last age group, there are only female five- and six-year-old fish. This data confirms results obtained by Zhivkov and Yankov (1987) and Zhivkov (1999) about the sex ratio of populations of other freshwater fish. Five- and six-year-old specimens, with body-length greater than 150 mm, are females. As is the case with other fishes (Nikolsky 1974; Kottelat and Freyhof 2007), Round-scaled barbel females have longer lives and reach greater body size.

Absolute fecundity of Round-scaled barbel from the Stryama River increases with fish's growth in length and mass. Total body size growth leads to an increase of fish's reproductive potential. Similar results have been reported by numerous other scholars (Raikova-Petrova 1992; Vasiliou and Economidis 2005; Rozdina 2009; Raikova-Petrova and Rozdina 2012). The positive correlation, found between fecundity and weight, confirms the suggestion of several scholars (Nikolsky 1965, 1974; Zhivkov 1999) that the main factor, enhancing fertility, is an increase in fish's weight. This finding is the reason for an increase in fertility with age. Our data confirms the findings of some authors (Raikova-Petrova 1992; Vasiliou and Economidis 2005; Rozdina 2009; Raikova-Petrova and Rozdina 2012) that, as it ages, the barbel increases its ability to produce eggs due to its increased body weight.

Table 5 presents the total fecundity data. Using a method, recomended by Zhivkov (1999), the fecundity of barbels from the rivers Maritsa, Chepinska and Stryma are calculated, at weights of 12, 25, 50, 100 and 200 grams.

Table 5. Absolute fecundity (*F*, eggs) of Round-scaled barbel, calculated at the same weight (*W*,g)

Author and River	*F/W* Relationship	Height *W* (g). Absolute fecundity (number of eggs)				
		12	25	50	100	200
Rozdina 2009, (Maritsa River)	$F = 81.33W + 2203.3$; $r = 0.99$, $n = 61$	3179	4237	6270	10 336	18 469
Kolev and Raikova 2019 (Chepinska River)	$F = 149.92W - 1453.7$; $r = 0.9746$, $n = 23$	345	2294	6042	13538	28530
Present data, 2015 (Kolev 2016) (Stryama River)	$F = 132.06W + 2374$; $r = 0.938$, $n = 71$	3959	5676	6603	15 580	28 786

Absolute barbel fertility is greater for the Stryama River than for the Maritsa and the Chepinska Rivers (Table 5). This finding is valid for all size groups. Smaller fish from the Chepinska River are less fecund than fish from the other two rivers. With an increase in mass over 100 g, the individual fecundity of the Chepinska River barbel approaches that of the Stryama River barbel. Given that the fattening of barbel from the Maritsa River (Rozdina 2009) is greater than that of fish from the rivers Stryama and Chepinska (Raikova-Petrova and Kolev 2015; Kolev 2016), the higher fertility of fish from these two rivers is probably related to some abiotic factors that negatively affect the survival of caviar and larvae. These factors can be a shorter vegetation period, lower water temperatures in spring and early summer and higher current speed in these two rivers. Apart from these factors, the reason for the smaller reproductive capacity of small-sized specimens from the Chepinska River is probably the poorer food base.

Barbel from this river has slower length growth than fish from the rivers Maritsa and Stryama (Kolev 2016).

Relative fertility increased only as it concerned small fish. Larger Round-scaled barbel had less relative fertility. The probable cause is that as fish ages, the following changes take place: dietary changes, food shortage for the larger size classes and food competition between individuals (Nikolsky 1965). Fertility data for the Stryama River barbel leads to the following conclusions: male fish become sexually mature once they are two years old. Females reach sexual maturity once they reach their second year. In October and November, the barbel has already spawned. At that time, the fish has already formed new caviar for the next year. The set of reproductive parameter estimates, determined by this study, provides the first quantitative estimates for the Round-scaled barbel population from the Stryama River and will substantially improve scientific knowledge about the reproductive potential of the fish. A better understanding of the population status will also hopefully enhance the monitoring capabilities of the relevant authorities, and thus improve the prospects for sustainable management of this endemic and protected fish.

Acknowledgments

I am grateful to Prof. Dr. G. Raikova for her help with the development of the study's methodology. I am also grateful to the reviewers and editors of the Journal of Limnology and Freshwater Fisheries Research for their valuable comments and suggestions on the manuscript.

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