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THE APPLICATION OF SEXUAL DYMOROPHISM AND ITS BEARING IN DETERMINING THE POPULATION SEX STRUCTURE OF THE MARITSA BARBEL (*BARBUS CYCLOLEPIS* HECKEL, 1848)

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Abstract

The populations of Maritsa barbel fish (*Barbus cyclolepis* Heckel, 1848) are under increasing anthropogenic pressure, necessitating constant monitoring and assessment of the demographic indicators of this endemic and protected species. The study empirically proves the utility of a hitherto theoretically-defined method of Marinov (1964) for determining the population sex structure. It uses the difference in the height of the anal fin between male and female the Maritsa barbels in order to determine the sex of a fish specimen. The empirical investigation, undertaken in order to test the method, utilized a large sample of 518 fish specimens, caught in the Chepinska River, outside of the spawning seasons, during the time period 2006–2010. The results are then compared with those, obtained from a smaller sample, caught in the same river in the years 2010–2011, during a period in which all mature individuals will have formed gonads. The comparative analysis of the results from the two samples proves the accuracy of the method for determining juvenile fish sex and thus provides a comprehensive representation of the sex structure of Maritsa barbel populations. Empirically proving the utility of the theoretical model entails two important conclusions for assessing the reproductive potential and estimating the stock size of the fish. First, this morphological peculiarity of the Maritsa barbel permits the accurate determination of its population sex structure throughout the year, not only during the spawning season. Consequently, no killing of fish from this endemic species would be necessary in order to monitor the populations.

Key words: age groups, height of the anal fin, sexual dimorphism, sex ratio.

Introduction

Determining the sex of a fish outside of the breeding season is often a very difficult task. In general, data describing the sex structure of the sexually mature segment of the fish population is often obtained by examining the presence of caviar or semen in a fish specimen. Consequently, it is not possible to determine the sex of a juvenile fish – i.e. a fish without fully formed gonads. However, in the case of the Maritsa barbel (*Barbus cyclolepis* Heckel, 1848), the fish's morphological peculiarity may allow determining the overall sex structure of the population.

Marinov (1964) theoretically defined a method for determining the population sex

structure of the Maritsa barbel. It uses the difference in the height of the anal fin between male and female barbels, in order to determine the sex of a fish specimen. Using biometric data, Marinov found a statistically significant difference. According to Marinov (1964, 1989) it is no more than 16.6 % of the small length of the body. On the other hand, the height of the female anal fin is more than 17 % of the body length to the end of the scales cover. This morphological relationship permitted Marinov (1964) to develop a theoretical method, which uses this feature in order to determine the sex of a fish specimen.

Research investigation conducted by Kolev (2013) confirmed Marinov's biometric results and established that the average height of the anal fin of Maritsa barbel males in the Maritsa River basin was 16.3 % of the body length to the end of the scales cover. This method has not yet been applied to the task of empirically determining the population sex structure.

According to Zhivkov (1981) and Zhivkov and Yankov (1987) the average sex ratio of a population depends on the presence and duration of several periods in a fish's live. The percentage of dominance of one sex over the other during different periods, as well as the dynamics of age structure of the population, are also important. Many fish populations have been characterized by four periods, each of them described by a specific sex ratio (Nikolsky 1965; Zhivkov 1999; Yankov 1988a, 1988b; Raikova-Petrova 1992; Hamwi 2005; Rozdina 2009). Initially there are more males among the hatched fish. Therefore, the males predominate during the first period (A). In the second period (B), the number of male and female fish becomes equal. In the third period (C), the female fish predominate because of an earlier dying off of the males, which have already participated in the reproduction. The last, fourth period (D) is characterized by the survival of only female fish.

A study of barbel fecundity conducted by Kolev (2016) found similar periods in the life cycle of the Maritsa barbel from the Chepinska River. The study found that the male barbels predominate from the time of hatching, up until the fourth year of the life of a generation. The four years old fish however were mostly females, and the five years old fish were only females. The research focus of Kolev's study (2016) did not aim to provide a comprehensive description of the overall sex structure of this Maritsa barbel population. Consequently, up until now the monitoring of its population sex structure has relied on comparatively less precise estimates and by necessity these estimates have also involved the killing of sample specimens.

The present scholarly contribution aims to address this research lacunae, by empirically testing the utility of Marinov's method (1964) for determining the sex of the Maritsa barbel by utilizing the difference in the height of the anal fin of male and female fish.

Study Area, Materials and Methods

The study area includes the Chepinska River, a right tributary of the Maritsa River (Fig. 1).

The Chepinska River is 81.7 km long with a catchment area of 899.6 km^2.

A total of 518 specimens of the Maritsa barbel were caught by electrofishing. It was performed according to the EN 14011:2004 instruction (Water quality – Sampling of fish with electricity).

The age of the fish was established by counting the annual rounds of fish scales.

The sex of the fish was determined by

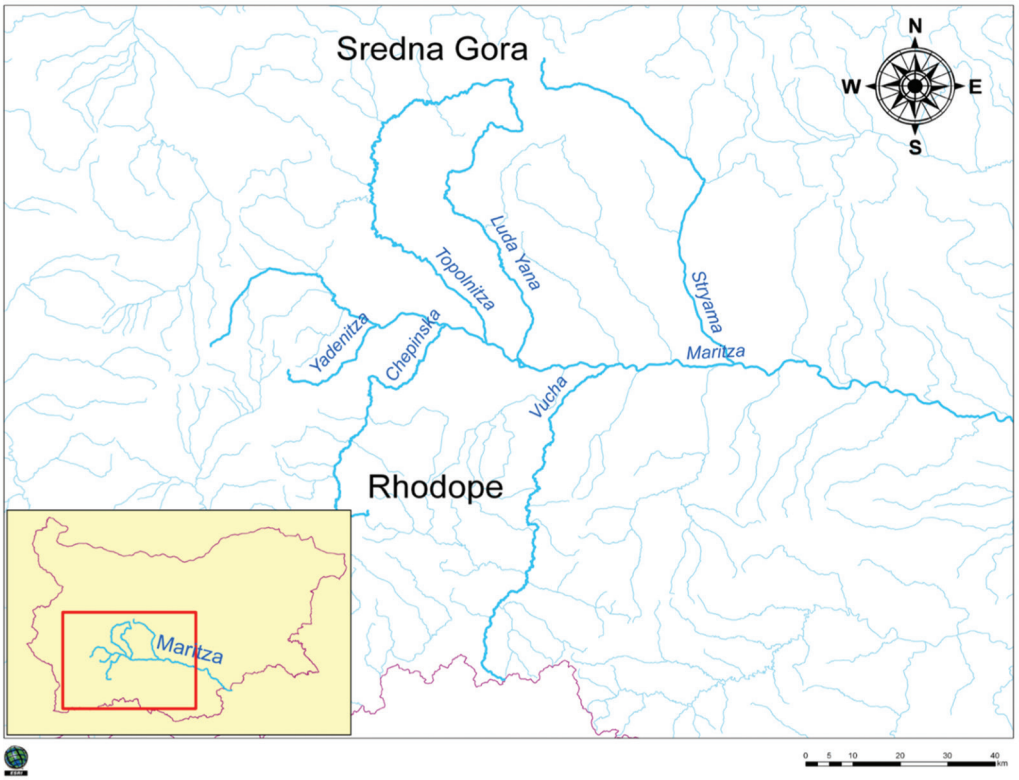

Fig. 1. Location of the Chepinska River, Arc Map 10.0 (ESRI – ArcGIS 2013).

the method proposed by Marinov (1964). Specimen with a height of the anal fin not exceeding 16.6 % of the small body length were identified as males. Specimen with a height of the anal fin equal to or exceeding 16.7 % of the small body length were identified as females.

The statistical interpretation of the results was made by using the χ^{2} criterion (Lakin 1973, Grancharova and Hristova 2006). The material was processed using Excel for Windows program.

Results and Discussion

Table 1 presents the results obtained. By the use of the morphological feature, the sex of the youngest age groups of the Maritsa barbel was determined. These were the juvenile fish.

For the one, two and three years old barbel fish the sex ratio was one to one (B period). The females were more than the males in the group of the four years old fish (C period). In the group of the five years old barbel the predomination of the females was considerable (Table 1).

The average sex ratio calculated using the height of the anal fin, was characterized by the predomination of female fish ♂:♀ = 1:1.3 (d = 1; χ^{2} = 9.5; χ^{2} stand. = 3.8; P > 0.05).

The sex ratio in different size classes, obtained with the help of the height of the anal fin, is shown in Table 2. There

Table 1. Sex ratio of the Maritsa barbel population from the Chepinska River.

Age group, years	Females ♀, %	Sex ratio (♂:♀)	*d*	χ^2	$\chi^{2\ stand.}$	*P*	*n*
One-year-olds	49	1:1	1	0.08	3.84	<0.05	109
Two year-olds	50	1:1	1	-	-	-	44
Three-year-olds	52	1:1	1	0.58	3.84	<0.05	246
Four-year-olds	68	1:2.1	1	11.64	3.84	>0.05	88
Five-year-olds	97	1:30	1	27.13	3.84	>0.05	31
Sum							518

Note: *d* – degree of freedom; *n* – number of fish.

Table 2. Sex ratio of the size classes, describing the Maritsa barbel population, established using the percentage of the height of the anal fin of the small body length.

Size class (*L*), mm	Females ♀, %	Sex ratio (♂:♀)	*d*	χ^2	$\chi^{2\ stand.}$	*P*	*N*
21–30	0	1:0	-	-	-	-	4
31–40	42	1:1	1	0.47	3.84	<0.05	19
41–50	39	1:0.63	1	2.08	3.84	>0.05	39
51–60	66	1:0.82	1	4.12	3.84	>0.05	41
61–70	46	1:1	1	0.31	3.84	<0.05	52
71–80	49	1:1	1	0.04	3.84	<0.05	110
81–90	55	1:1	1	0.85	3.84	<0.05	95
91–100	64	1:1.8	1	6.21	3.84	>0.05	78
101–110	61	1:1.6	1	1.09	3.84	>0.05	23
111–120	83	1:5	1	8.00	3.84	>0.05	18
121–130	89	1:8	1	5.44	3.84	>0.05	9
131–140	86	1:1	1	3.57	3.84	<0.05	7
141–150	100	0:1	-	-	-	-	12
151–210	100	0:1	-	-	-	-	11
Sum							518

were more male fish in the first four size classes 21–60 mm (A period). Fish with the length to the end of the scale cover between 61 mm and 91 mm had a normal sex ratio 1:1 (B period). Female fish dominated the size classes of fish longer than 91 mm (C period). The only exception was the size class 131–140 mm, because the difference between the two sexes was not statistically significant. However, the value of the χ^2 was much closer to the standard value ($\chi^{2\ stand.} = 3.84$) and the uncertainty in this difference was due to the small number of fish in this size class. There were only female fish in the size class of length greater than 141 mm (D period).

Sex ratio of Maritsa barbel population size classes, established by using the height of the anal fin by an age group, was closer to the results obtained by studying sexually mature fish (Kolev 2016) (Table 3). A study of fecundity by Kolev (2016) determined that the presence of only female fish in the fifth and last age group was due to the small number of fish in the sample.

Table 3. Sex structure of the age groups, describing the Maritsa barbel population, established during the study of fish fecundity (Kolev 2016).

Age structure, years	Juveniles, %	Males ♂, %	Females ♀, %	Sex ratio (♂:♀)	*d*	χ^2	$\chi^{2\ stand.}$	*P*	*n*
One-year-olds	100	-	-		-	-	-	-	1
Two year-olds	64	36	0	1:0	1	-	-	-	14
Three-year-olds	60	40	0	1:0	1	-	-	-	48
Four-year-olds	0	85	15	1:0.2	1	6.23	3.84	>0.05	13
Five-year-olds	0	0	100	0:1	1	-	-	-	21
Sum									97

The results of the sex determination of the Maritsa barbel, by using the two methods cited (by the height of the anal fin and by the sex products of the fish) were comparatively evaluated to be very different (Kolev 2016) (tables 2 and 4).

In the material collected for a study of fish fecundity, there were only a few specimens with a body length between 21 mm and 60 mm, because the catch was made in the late autumn, and it was mostly targeting the bigger fish (Kolev 2016) (Table 4). No female fish with formed caviar and with a body length less than 120 mm were found in the sample. The sex structure was characterized by the presence of only three periods: a period with a presence of only male fish (A period), a period with a normal sex ratio 1:1 (C period) and a period in which only female fish were found (D period).

The sexual dimorphism of the height of the anal fin was very well expressed in the young age groups, unlike the development of gonads (tables 2 and 3). This morphological particularity made it

Table 4. Sex ratio of size classes, describing the Maritsa barbell population, established during the study of its fecundity (Kolev 2016).

Size class (*L*), mm	Juveniles, %	Males ♂, %	Females ♀, %	Sex ratio (♂:♀)	*d*	χ^2	$\chi^{2\ stand.}$	*P*	*n*
21–30	-	-	-	-	-	-	-	-	-
31–40	-	-	-	-	-	-	-	-	-
41–50	-	-	-	-	-	-	-	-	-
51–60	-	-	-	-	-	-	-	-	-
61–70	1	0	0	-	-	-	-	-	1
71–80	82	18	0	1:0	-	-	-	-	17
81–90	53	47	0	1:0	-	-	-	-	15
91–100	52	48	0	1:0	-	-	-	-	29
101–110	11	89	0	1:0	-	-	-	-	9
111–120	0	100	0	1:0	-	-	-	-	2
121–130	0	33	67	1:1	1	0.33	3.84	<0.05	3
131–140	0	0	100	0:1	-	-	-	-	2
141–150	0	0	100	0:1	-	-	-	-	11
151–210	0	0	100	0:1	-	-	-	-	8
Sum									97

possible to obtain a comprehensive representation of the sex structure of Maritsa barbel populations.

The study found only one male fish in the five years old group, so the dominance of the females in this age group was very high (Table 3). It was clear that the males gradually die during this age period. This finding has been confirmed by Rozdina (2009), who found out that the seven and eight years old barbels were only females. Obviously the fourth period (D), when only females survive, can only be identified for older barbels. Although in this sample no fish of such age was identified, such barbels had been recorded by other authors (Dikov and Zhivkov 1985, Vasiliou and Economidis 2005).

The average sex ratio of the Maritsa barbel population from the Chepinska River estimated by this research (♂:♀ = 1.3:1) is very different from the value (♂:♀ = 1:1) obtained by the study of Maritsa barbel fertility in the same river (Kolev 2016). These results can be explained by the larger sample size, utilized in the present study, as well as by the ability to determine the sex of the juvenile fish.

Conclusions

Based on the empirical analysis and verification, discussed in the body of this article, the study concludes that the method proposed by Marinov (1964), for determining the sex of The Maritsa barbel by using the height of the anal fin, is indeed accurate. Consequently, the method allows including the youngest age groups in describing barbel population sex structure. Determining the sex of the juvenile fishes is indispensable for achieving a more comprehensive understanding of the populations sex structure.

The difference in the height of the anal fin between the male and female of the Maritsa barbels allows utilizing much larger sample sizes, because in this case it will also enable the inclusion of juvenile fish. This, in turn, allows obtaining higher population monitoring accuracy and greater statistical significance of the research analyses. Moreover the method, proposed by Marinov (1964) and empirically verified by the present study, is fast and easy to use.

In conclusion, the main contribution of this publication is that it presents and verifies a method, which allows determining the sex structure of a Maritsa barbel population without killing any fish and even outside the breeding period. Minimizing fish losses is very important, especially since the Maritsa barbel is an endemic species for the Balkan Peninsula and it is also protected by the Bulgarian Biodiversity Conservation Act (applications 2 and 4 of the Law).

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