Fecundity of *Sqalius orpheus* from the rivers Stryama and Chepinska

Плодноста на *Sqalius orpheus* од реките Стрјама и Чепинска

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Abstract

This paper presents the results of a study of the reproductive biology of Orpheus dace from the rivers Stryama and Chepinska of the Maritsa River basin, Bulgaria. The material is collected in the spring and autumn of 2011. Totally 312 fish were captured by electrofishing. The study found that some male fish mature sexually in their first year, while females reached sexual maturity in their second year. In the river Stryama, Orpheus dace fecundity varies from 825 to 120252 eggs; average fecundity is 18438 eggs. In the Chepinska River, fecundity is between 3552 and 52416 eggs. Average fecundity of fish from the Chepinska River is 17917 eggs. Absolute fecundity is positively correlated with an increase in fish length and weight. Orpheus dace fecundity also increases with age. For both rivers, five-year-old fish have the highest fecundity rates.

Key words: Orpheus dace, reproductive parameters, fecundity

Апстракт

Во овој труд се презентирани резултатите од студијата на репродуктивната биологија на кленот *Sqalius orpheus* во реките Стрјама и Чепинска, во сливот на реката Марица, Бугарија. Материјалот беше собиран во пролетта и есента 2011 година. Вкупно 312 риби беа уловени со електририболов. Студијата покажа дека некои машки единки полово созреваат во нивната прва година, женските единки постигнуваат полова зрелост во текот на нивната втора година. Во реката Стрјама, фекундитетот на кленот варира од 825 до 120252 јајце клетки; просечниот фекундитет е 18438 јајце клетки. Во Чепинска Река, фекундитетот варира од 3553 до 52416 јајце клетки; просечниот фекундитет е 17917 јајце клетки. Апсолутниот фекундитет е позитивно корелиран со зголемувањето на должината и тежината на рибите. Фекундитетот на кленот исто така се зголемува со староста на рибите. Во двете реки, петтодишните риби имаат највисока стапка на фекундитет.

Клучни зборови: *Sqalius orpheus*, репродуктивни параметри, фекундитет

Introduction

Orpheus dace (*Squalius orpheus* Kottelat & Economidis, 2006) is widespread in rivers of the Aegean catchment area-e.g. the Struma and Maritsa Rivers in Bulgaria, Greece and Turkey (Kottelat & Freyhof 2007). This is one of the most numerous fish in the middle zone of Maritsa River tributaries (Kolev 2013). Orpheus dace inhabits middle and lower reaches of rivers with gravel and sandy bottoms. The species also inhabits some

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lakes (Mikhailova 1964, Marinov 1989). Orpheus dace is a rheophilic species that prefers areas with moderate water flow. The fish prefers areas with alternating rapids with pools and holes. In summer, dace often remains under the branches of trees overhanging the water. In spring, the fish seeks small breeding streams. In winter, the dace hides in deep waters, but remains active for most of the cold period (Mikhailova 1964).

Orpheus dace is omnivorous. Young fish's diet consists mainly of phytoplankton and zooplankton (Conjugatae, Chlorophyceae, Phaeophyta, Dinoflagellata, Diptera). Three year old fish and older consume algae, plant seeds, as well as insect and imago larvae (Chironomidae, Ephemeroptera, *Simulium*, Coleoptera, Trichoptera), small benthic animals (Isopoda, Lumbricidae, *Planorbis*) and small fish (Mikhailova 1964).

Linear growth of Orpheus dace is relatively slow. In rivers, fish reach about 60 mm in standard length annually. Two-year-olds are between 83-119 mm long, three-year-olds reach 121-164 mm, four-year-olds: 162-240 mm and five-year-old fish: 192-223 mm (Dikov & Zhivkov 1985, Marinov 1986, Dikov et al. 1994, Stefanova et al. 2008, Kolev & Raikova 2015). The rate of mass growth is also slow. In its first year, Orpheus dace reaches about 3 g (gutted mass), at three years of age about 30 g and five-year-old fish exceed 100 g (Kolev & Raikova 2015).

The breeding season of Orpheus dace in the Struma River basin in Bulgaria lasts from the beginning of April to the beginning of August. Data about the sexual maturation and fertility of fish from the Struma River has also been published (Mikhailova 1964).

The aim of this study was to determine a set of very important reproductive parameters (sexual structure, maturation and fertility) of Orpheus dace from the rivers Stryama and Chepinska of the Maritsa River basin. The reproductive parameters, which were obtained, were then compared with corresponding data published by other scholars, who have studied other streams of the Aegean catchment area.

Materials and Methods

Study area

The research project studies the rivers Stryama and Chepinska, both from the Maritsa River basin. The Stryama River originates in the Sredna Gora Mountains (Figure 1). It is a left tributary of the Maritsa River. In its upper reaches, the Stryama 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 river is 110 km long with a catchment area of 1789 km². The Stryama is a medium-sized mountain Bulgarian river (№ H-4 / 14.09.2012 MOSV) with an average altitude of 833 m. Declination of the river bed is small: 30%, and average flow rate is also relatively small 8 m³/s^{-1} (Stoyanov et al. 1981). In the summer, water temperature exceeds 20° C (BD-IBR 2015).

The Chepinska River is a right tributary of the Maritsa River. The river originates in the West Rhodopes Mountains; its springs are located underneath the peak Mala Siutkia (2078.7 m a.s.l.) (Figure 1). In its upper reaches, the river flows to the Northwest and is called Bistritsa. Once it reaches the town of Velingrad, the river runs northwards and near the village of Vetren it enters the Upper Thracian Valley. The Chepinska River flows into the Maritsa River near the village of Kovachevo (close to the town of Pazardzhik). The river is 81.7 km long with a catchment area of 899.6 km2. The Chepinska River is a medium sized mountain Bulgarian river (№ H-4 /14.09.2012 MOSV) with an average altitude of 1228 m. Declination of the river bed is 68%, and average flow rate is 18 m³ · s^{-1} (Stoyanov et al. 1981). In summer, water temperature reaches 20 °C (BD-IBR 2015).

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

Table 1. Sampling areas along the rivers Stryama and Chepinska.

| №	Areas	Geographic coordinates		Altitude m a.s.l.	Date of sampling
		N	E		
1	In the vicinity of the fish farm next to the Banya town, Stryama River	42°33'38,45''	24°47'54,11''	302	19.10.2011 20.11.2011 16.12.2011
2	In the vicinity of the Banya town, Stryama River	42°32'18,12''	24°49'22,78''	283	17.04.2011
3	In the vicinity of the village of Kovatchevo, Chepinska River	42°12'28,79''	24°10'54,69''	523	09.04.2011
4	In the vicinity of the village of Lozen, Chepinska River	42°11'09,62"	24°09'45,65"	240	08.04.2011 18.11.2011
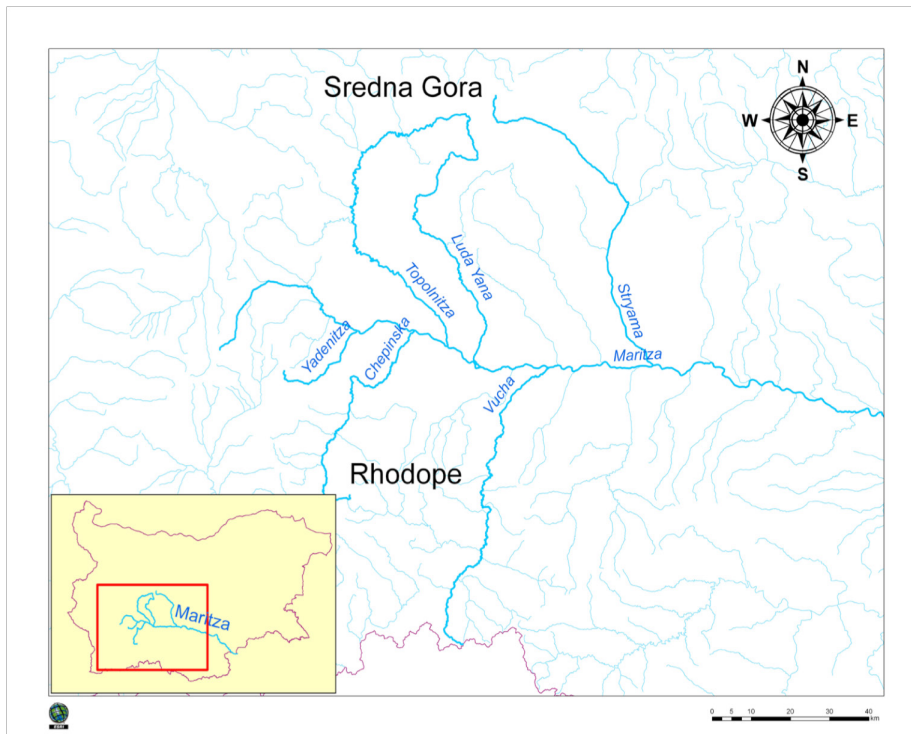
Figure. 1. Location of the rivers Stryama and Chepinska, Arc Map 10.0 (ArcGIS, ESRI 2013).

Materials and methods

Specimens were collected in the spring and autumn of 2011. A total of 312 Orpheus dace specimens were caught in the rivers Stryama and Chepinska. The material was collected 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 were collected from each Orpheus dace 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. Scales were then examined with a microscope Olympus CX 31, at 40× magnification. Fish age was determined by counting the annual rings of a scale. For this purpose, a diagonal caudal radius of the scales was used.

The gonad, collected from each mature female Orpheus dace, 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× mag-

nification. The sex of a specimen was determined by dissecting fish, caught in spring and early winter.

The level of gonads' maturity was determined by a 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).

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

where:
g-gonads' mass
W-mass of a fish without its entrails (*g*)

Relative fecundity (*RF*) was assessed directly by a ratio of absolute fertility and fish without intestines (*F/W*) (Spanovskaya & 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).

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

where:
F-absolute fertility (n)
W -mass of a fish without its entrails (*g*)

An analysis of the absolute fertility of Orpheus dace from different water sources was made by comparing

absolute fertility calculated for the same fish weight (Zhivkov 1999).

A degree of association between F and L, as well as between F and W was determined by a correlation coefficient (r). Pearson's χ^2- test was also performed to evaluate the hypotheses (Lakin 1968, Grancharova & Hristova 2006). All calculations were performed in MS-Excel 2010.

Results

Age-sex composition, age and length of first maturity

The age and sexual composition of the Orpheus dace population from the Stryama River consists of five age groups. It is presented in Table 2.

The average sex ratio of the Orpheus dace's populations from the Stryama River is estimated as: ♂:♀ = 1.81:1. This difference is statistically significant (χ^2=3.858, χ^2critical=3.8; p<0.05). The age and sexual composition of the Orpheus dace population from the Chepinska River is presented in Table 3.

The sex ratio of the Orpheus dace's population from the Chepinska River is 38:27 ♂:♀. This difference is not statistically significant (d=1; χ^2=1.43, χ^2critical=3.8; p<0.05) that is, the sex ratio does not differ from the normal (♂:♀ = 1:1).

The youngest sexually mature specimen is from the Stryama River. It is a one-year-old male, which is 78 mm long and weighs 5 g. The youngest sexually mature female is also captured in the Stryama River: a two-year-old fish, which is 105 mm long and weighs 14 g.

Relations between absolute fertility and body length, as well as absolute fertility and body weight. Relative fecundity

Figures 2 and 3 show a relationship between total fecundity and standard body length (to the end of the scale cover). In order to study the relationship between length and fecundity, fish are grouped into size classes

Table 2. Size and sexual composition of the Orpheus dace's population from the Stryma River

| Size class	Age (years)	1			2			3				4		5		total
(L, mm)	Sex	j	♂	♀	j	♂	♀	j	♂	♀	j	♂	♀	♂	♀	total
51 - 60		1														1
61-70		3														3
71-80		7	1								4					12
81-90		1			2	1										4
91-100		9			6	1										16
101-110		1			7	7	1									16
111-120					10	7			2							19
121-130					11	15	3		4							33
131-140					3	7	1	3	7	3						24
141-150					5	5	2	2	5	3						22
151-160					1	1	5		2	5	1			1		16
161-170						1	1	1	2	9	1	1	1			17
171-180						1			6	3	1					11
181-190					1	1			5	4		2	2			15
191-200									3			1	1		1	6
201 - 210									3				2			5
211-220													1			1
221-300													3		1	4
Σ fish of all size classes		22	1	0	46	47	13	6	39	27	7	4	10	1	2	**225**

Legend: j- juvenile specimens

Table 3. Size and sexual composition of the Orpheus dace's population from the Chepinska River

| Size class (L, mm)	Age (years) / Sex	1			2			3			4			Total
		j	♂	♀	j	♂	♀	j	♂	♀	j	♂	♀	Total
101-110					3									3
111-120					3	2								5
121-130					3	8								11
131-140						13			4					17
141-150					1	8	2		2					13
151-160						6	1		2	1				10
161-170							3		4	2				9
171-180						1	3							4
181-190						1				2				3
191-200										1			3	4
201 - 210										3				3
211-220													1	1
241 - 270										1		1	2	4
Σ fish of all size classes		0	0	0	10	39	9	0	12	10	0	1	6	**87**

Legend: j- juvenile specimens

(Tab. 3). Absolute fecundity is calculated as a weighted average for each size class. In both cases, the difference is well-expressed by a linear function with a relatively high degree of reliability. As length was increasing, absolute fertility was also increasing.
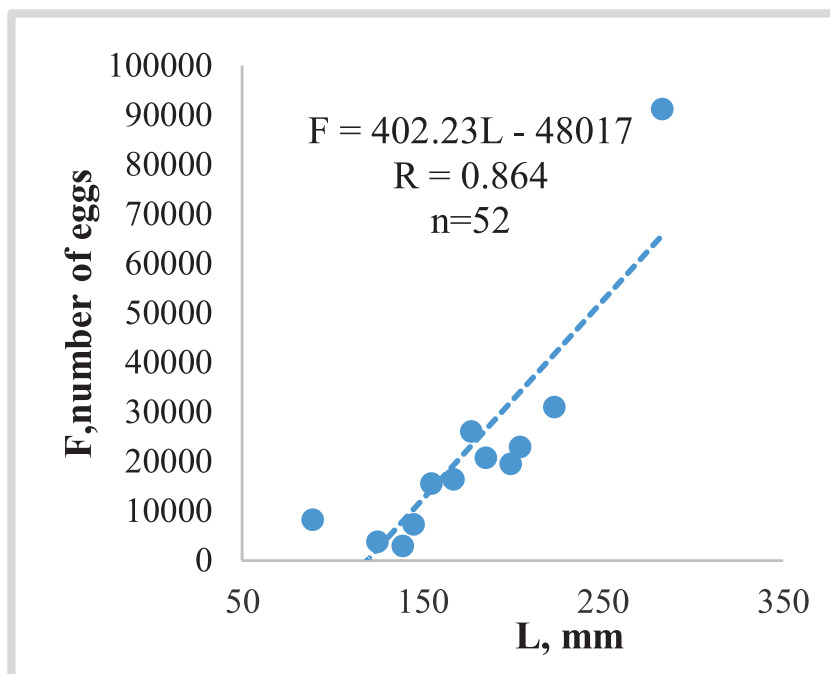
Figure. 2. Relationship between total fecundity (F) and body-length (L) to the end of the scale cover, the Stryama River
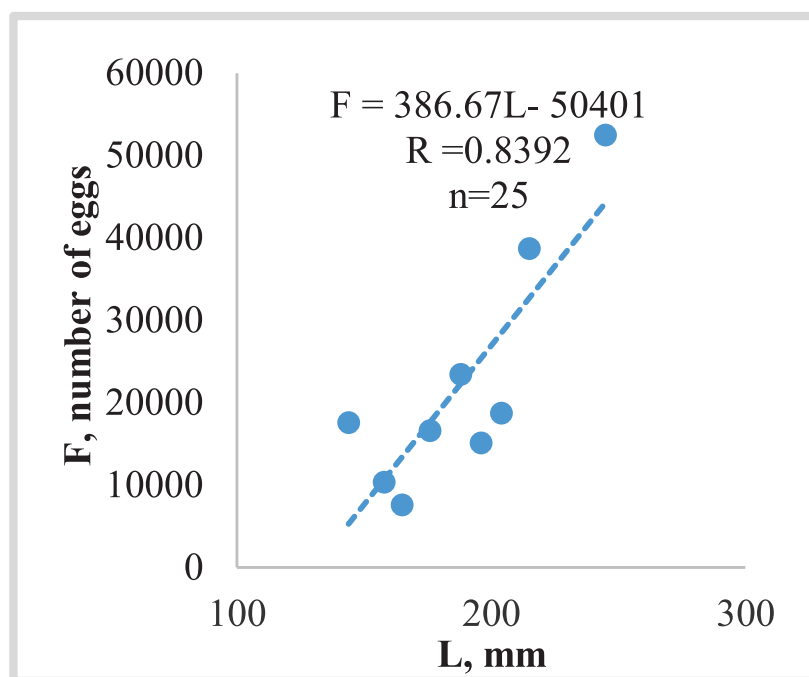
Figure. 3. Relationship between total fecundity (F) and body-length (L) to the end of the scale cover, the Chepinska River
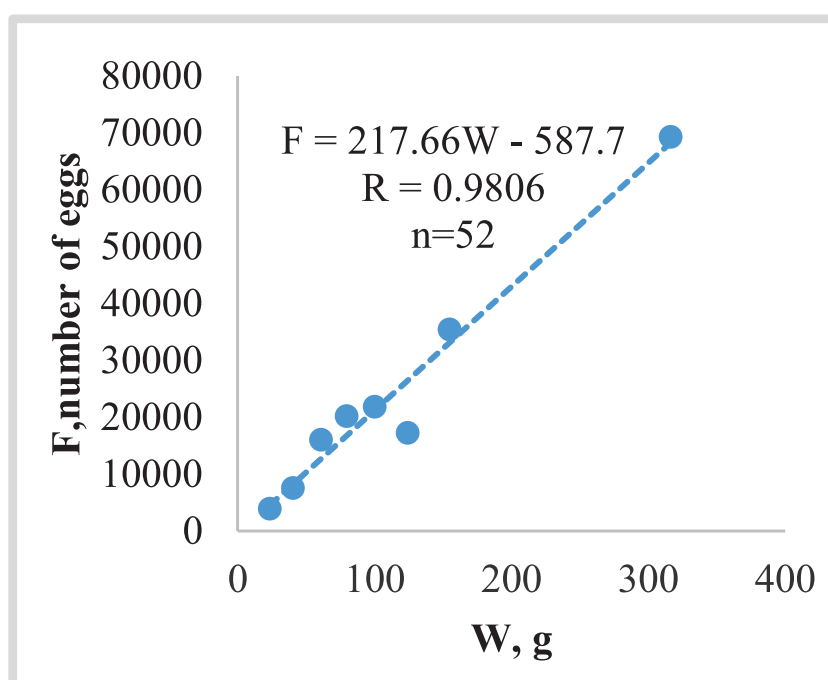
Figure 4. Relationship between total fecundity (F) and average weight (W) for Orpheus dace from the Stryama River

Average absolute fecundity of Orpheus dace by size classes (12 size classes) from the rivers Stryama and Chepinska is presented in Table 4 and, respectively, Table 5. Average individual fecundity of Orpheus dace from both rivers increases for the larger size classes, albeit unevenly.

A relationship between total fecundity and weight of the Orpheus dace in the rivers Stryama and Chepins-

ka is shown in Figures 4 and 5. The total number of fish from each of the studied rivers was grouped into several size classes (g), by using an increment of 20 g. Size classes of fish heavier than 130 g have a wider range due to the smaller number of fish. A relationship between absolute fertility and fish weight is described by a linear equation with a high degree of reliability in both cas-

Table 4. Change of individual fecundity by length classes of Orpheus dace from the Stryma River.

	Length class (*L*, mm)	Average length (mm)	Range (number of eggs)	Average absolute fecundity (*F*) (number of eggs)	n
1	71-110	89	5940-10 320	8130	2
2	121-130	125	825-5232	3701	4
3	131-140	139	1656-5770	2821	4
4	141-150	145	4120-12 078	7246	4
5	151-160	155	3300-39 600	15 433	9
6	161-170	167	5520-35 630	16 362	10
7	171-180	177	475-35 061	25 991	3
8	181-190	185	9633-40 579	20 696	6
9	191-200	199	16 112-22 876	19 494	2
10	201-210	204	14 895-38 720	22 897	3
11	211-230	223	25 410-33 880	30 921	3
12	261-300	283	62 100-120 252	91 176	2
Σ					52

Table 5. Change of individual fecundity by length classes of Orpheus dace from the Chepinska River.

	Length class (*L*, mm)	Average length (mm)	Range (number of eggs)	Average absolute fecundity (*F*) (number of eggs)	n
1	141-150	144	6672-28438	17555	2
2	151-160	158	9264-11285	10303	3
3	161-170	165	3552-14664	7577	5
4	171-180	176	9027-25688	16570	3
5	181-190	188	7888-41208	23407	5
6	191-200	196	4893-25284	15088	2
7	201-210	204	12928-24975	18669	3
8	211-220	215	38665-38665	38665	1
9	241-250	245	52416-52416	52416	1
Σ					25

$$F = 209.2W - 404.31$$
$$R = 0.9511$$
$$n = 25$$
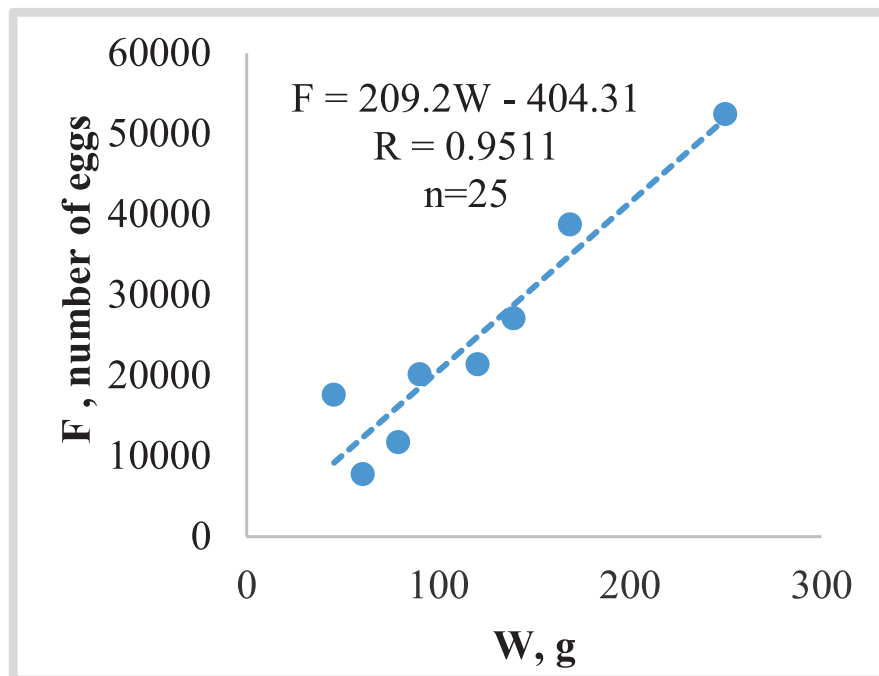
Figure 5. Relationship between total fecundity (F) and average weight (W) for Orpheus dace from the Chepinska River

Table 6. Change of individual fecundity by age classes of Orpheus dace from the Stryama River.

Age groups	Range (number of eggs)	Average absolute fecundity F (number of eggs)	n
1	10320-10320	10320	1
2	825-39600	9800	12
3	1656-40579	14939	27
4	14985-62100	29114	10
5	16112-120252	68182	2

Table 7. Change of individual fecundity by age classes of the Orpheus dace from the Chepinska River.

Age groups	Range (number of eggs)	Average absolute fecundity F (number of eggs)	n
2	3552-28438	11891	10
3	4893-24975	13802	10
4	25284-52416	38198	5

Table 8. Relative fecundity (RF, eggs) of Orpheus dace from the Stryama River

Size classes (W, g)	Weight (g)	Relative fecundity (number of eggs)	n
11-30	23	166	4
31-50	41	184	12
51-70	61	263	14
71-90	80	253	9
91-110	100	218	3
111-130	124	139	4
131-170	155	228	3
171-430	316	219	3
Average and sum	-	209	Σ 52

Table 9. Relative fecundity (RF, eggs) of Orpheus dace from the Chepinska River

Size classes (W, g)	Weight (g)	Relative fecundity (number of eggs)	n
31– 50	46	385	2
51– 70	61	127	7
71-90	79	147	4
91-110	91	221	3
111-130	121	177	5
131-150	139	194	2
151-250	209	219	2
Average and sum	-	210	Σ 25

es. This clearly shows that fish with greater mass produce more caviar.

Fecundity naturally increases for older fish in both rivers (Tables 6 and 7). Our sample did not include enough adult specimens for which fertility decreased.

In this study, a parameter frequently used in fishing analyses was calculated: relative fertility of the fish (Tables 8 and 9).

Discussion

The sample of Orpheus dace from the Stryama River consists of five age groups. The first age group includes mainly young specimens; only one mature male is found. The second age group is the most numerous and includes more sexually mature individuals. The number of young fish in this group is still considerable. The number of

juveniles in the other age groups is decreasing. Five-year-old fish are very few. The population age-sex structure is simplified: there are only two periods. The first is dominated by male fish. Fish at the age of one, two and three years fall into this period. The second period is characterized by female dominance and includes fish from the fourth and fifth age groups. The sample from the Chepinska River includes four age groups. One-year-old fish have not been caught. In the second age group, there are juvenile specimens. This is the most numerous groups. Age groups, consisting only of females were not found. The fourth age group is less numerous. The age-sex structure of the Chepinska River population is similar to that from the Stryama River. The first period, characterized by predominantly male specimens of two and three-year-old fish. Females dominate in the second period, which includes four- and five-year-old fish.

Orpheus dace populations from the rivers Stryama and Chepinska are characterized by the predomination of young fish. The fifth age group in the sample from the Stryama River includes only three specimens; this age group is absent from the sample from the Chepinska River. For both rivers, two year old fish are the most numerous, 67% of the catch in the Stryama River and respectively 47% in Chepinska River. This data shows that the populations are under a strong pressure from sport fishing and predators and probably influenced by water pollution. This fact is confirmed by the early maturation of males and their predominance in the Stryama River population. The oldest and largest fish are mostly females and they are not abundant in the populations from both rivers. Individual fecundity increases with an increase of body length. This trend is better expressed in the Stryama River. The data about the fecundity of Orpheus dace from the Stryama River makes it clear that fertility increases about two times for fish longer than 150 mm and about four times for fish longer than 210 mm.

An increase in absolute fecundity as mass increases is very well represented by the study. This finding confirms a claim by many authors (Nikolsky 1974, Zhivkov 1999) that individual fecundity is directly dependent on the weight of female fish. Fish increase their length and weight with age. Quite naturally, with increasing age, fecundity of Orpheus dace from the rivers Stryama and Chepinska also increases.

Generally, absolute fecundity of Orpheus dace from the Stryama River is between 475 and 120252 eggs and weighted average individual fecundity is 18438 eggs. In the Chepinska River, absolute fecundity of varies from 7577 to 52416 eggs, and weighted average individual fecundity is 17917 eggs. The study finds that the maximum fecundity of Orpheus dace is similar to the results obtained by Mikhailova (1964), who indicates about 145000 eggs maximum fecundity of this species of fish in the Struma River.

Table 10 presents the total fecundity data. Using the method recommended by Zhivkov (1999), fecundity of Orpheus dace from the rivers Struma, Chepinska, Stryama and the Batak Dam are calculated, at weights of 100, 150, 200 and 250 grams. Data for chub (*Squalius cephalus* Linnaeus 1758) from the Iskar River is also shown in the Table 10.

Fecundity values of Orpheus dace from the rivers Struma, Stryama, Chepinska and the Batak dam (from the Aegean watershed) are close. The observed differences in species fecundity are a result of differences in the living conditions in different water bodies. The Batak Dam is located at the highest altitude (1108 m) and vegetation period there is the shortest. In addition, standing water bodies are not the most preferred place for an Orpheus dace. As a result, species fertility there is the lowest, despite a rich nutritional base.

Of the three mentioned rivers, the Struma flows in the southernmost part of the Bulgarian territory and the warmest period of the year is the longest there. This river is one of the longest Bulgarian rivers and is also characterized by greater water quantities compared to the Stryama and the Chepinska rivers (Hristova 2012) and probably better living conditions for Orpheus dace. The highest fish fecundity is found in the Struma River. Usually fish weighing 200 g, which are mostly 5 year olds, have an absolute fertility of approximately 41000-43000

Table 10. Absolute fecundity (*F*, eggs) of Orpheus dace, calculated at the same weight (*W*, g).

Author (River)	F/W Relationship	Height *W* (g). Absolute fecundity (number of eggs)			
		100	150	200	250
Mikhailova 1964 (Struma River)	F = 342W - 17405; r =0.85, n =19	16795	31195	50995	68095
Zhivkov & Raikova-Petrova 1983 (Batak Rezervoir)	F = 133W + 7380; r=0.98, n=57	20680	27330	33980	40630
Hamwi et al. 2009 (*Squalius cephalus*, Iskar River)	F = 78W-5868; r =0.98, n = 64	1932	5832	9732	13632
Present data (Stryama River)	F = 218W-588; r = 0.98, n = 52	21212	32112	43012	53912
Present data, (Chepinska River)	F = 209W-404; r =0.95, n = 25	20496	30946	41396	51846

eggs (the rivers Chepinska and Stryama), approximately 34000 eggs (the Batak dam) and approximately 51000 eggs (the Struma River).

In Table 10 there is data about chub from the Iskar River. This is a different fish species, inhabiting another watershed and living in different conditions. Therefore, there is a clearly visible difference in its fertility compared to that of Orpheus dace from the other water bodies, discussed so far.

For many fish, relative fecundity decreases with an increase of weight (Raikova-Petrova 1992, Hamwi et al. 2009, Raikova-Petrova and Rozdina 2012) but such a strict tendency has not been established for Orpheus dace populations from the rivers Stryama and Chepinska. The weighted average relative fecundity of fish from the studied rivers is 208 eggs per gram.

Fecundity data for Orpheus dace from the rivers Stryama and Chepinska leads to a following conclusions: male fish become sexually mature during their first and second year. Females reach sexual maturity after their second year. The maximum fecundity of Orpheus dace from middle zone tributaries of the Maritsa River reaches approximately 120000 eggs and the average fecundity value is approximately 18000 eggs.

The set of reproductive parameter estimates, determined by this study, provides the first quantitative estimates of Orpheus dace populations from the rivers Stryama and Chepinska 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 relevant authorities, and thus improve the prospects for a sustainable management of this species.

Acknowledgements

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