

[Research]

## Age, sex ratio, spawning season, gonadosomatic index, and fecundity of *Cobitis faridpaki* (Actinopterygii, Cobitidae) from the Siahrud River in the southeastern Caspian Sea basin

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### ABSTRACT

*Cobitis faridpaki* *Cobitis faridpaki* (Mousavi-Sabet, Vasil'eva, Vatandoust and Vasil'ev, 2011) is found in streams of the Siahrud River in the southeastern Caspian Sea basin, and this is the first report on reproductive parameters of the newly described endemic loach. Reproductive conditions are key parameters of fish populations, and their assessment is very important for endemic fishes. To study reproduction biology of this fish, sampling was done at monthly intervals throughout the year and 258 individuals of *Cobitis faridpaki*, were caught from Siahrud River in the southeastern Caspian Sea basin, in the north of Iran. Age, sex ratio, fecundity, oocytes diameter and gonadosomatic index were estimated. A regression analysis was used to find relationships between fecundity and fish size (length and weight), gonad weight and age. Sex ratio differed significantly from unity and was biased to females. The mature females and males were longer than 40 and 30 mm in total length, and +2 and +1 in age, respectively. The average egg diameter was 0.578 mm. Spawning took place from beginning of May to late July when water temperature was between 19.4 to 25.1 °C. Average GSI value at the beginning of the reproduction period was 9.12% and ranged between 2% to 20% in ripe mature females. The averages of absolute and relative fecundity were calculated as 1366 and 508, respectively. The absolute fecundity was significantly related to body weight and gonad weight. Based on the pattern of gonadosomatic index, it was concluded that this fish has a prolonged active reproduction period, which is a type of adaptation of short-lived small fishes to environmental conditions.

**Keywords:** Reproductive biology, Oocytes diameter, Ovary, Loach, Iran.

### INTRODUCTION

To date, three valid species of the genus *Cobitis* are represented in Iran. These are *Cobitis linea* (Heckel, 1849), *Cobitis faridpaki* (Mousavi-Sabet, Vasil'eva, Vatandoust and Vasil'ev, 2011) and *Cobitis keyvani* (Mousavi-Sabet, Yerli, Vatandoust, Ozeren and Moradkhani, 2012). *Cobitis faridpaki* and *Cobitis keyvani* are found in the south Caspian Sea basin. However, some researchers reported that the spined loach *Cobitis tenia* Linnaeus, 1758 is also found in the basin (Abdoli and Naderi, 2009). Some others believe that the fish can't be *C. taenia* because it is rather a northern European species and its occurrence in the southern Caspian Sea basin is unlikely (Kottelat and

Freyhof, 2007; Esmaeili *et al.*, 2010). *Cobitis linea* (Heckel, 1849) is found in the Kor River and the Hormozgan basins (Banarescu and Nalbant, 1967; Bianco and Nalbant, 1980).

*Cobitis faridpaki*, is an endemic fish, and it is distributed in the lower streams of the Siahrud River (Mousavi-Sabet *et al.*, 2011). Members of Cobitidae family are small benthic freshwater fishes with a wide distribution area covering large parts of Eurasia and Africa (Perdices and Doadrio, 1997). The loaches achieve sexual maturity in the first (males) or second (females) year of their life (Boron and Pimpicka, 2000; Marconato and Rasotto, 1989).

The main aim of the present study was to provide data on the reproduction biology

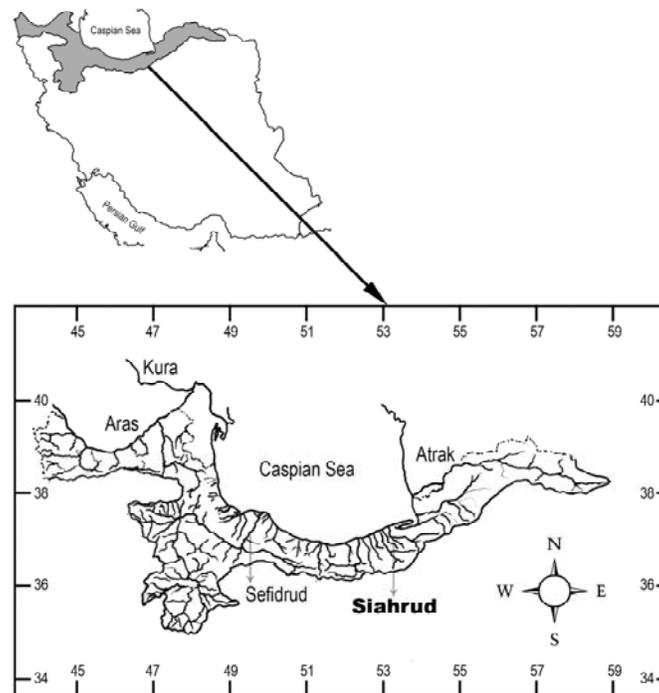
of this endemic fish, including sex ratio, gonadosomatic index, fecundity, oocytes diameter, and spawning season which are necessary for conservation programs of this fish. Information on the reproduction biology of this small and colorful loach is believed to be important due to possibility of its use in aquarium trade.

## MATERIALS and METHODS

### Study area, sampling and habitat

The study was conducted in the Siahrud River (36°26.855' N, 52°56.708' E) in southeast of the Caspian Sea basin, north of Iran (Fig. 1). Fish were collected from this river monthly from May 2009 to April

2010 by electrofishing (200-300 V). The water temperature of the catching site was measured simultaneously. This fish, *Cobitis faridpaki*, is commonly found in tributary streams of Siahrud River. The bottom of these water bodies is generally sandy, with silt and rubbles (with diameter of 5 to 30 cm), and the water is clear and slow running. The analysis of water in two different stations in May showed that the temperature was 19.4 to 25.1 °C with pH of 6.9 -7.1, dissolved oxygen (O<sub>2</sub>) concentration of 5-8 mg/l, and hardness of 361 mg/l.



**Fig 1.** Map of the southern Caspian Sea basin in north of Iran showing some of the most important rivers system and the study area, Siahrud River

### Biometry and data analysis

The fish were measured (TL: Total length, SL: Standard length to the nearest 0.1mm) and weighed (W: body weight, to the nearest 0.01 g). To examine the monthly changes in gonads as a mean for estimating the spawning season of this loach, gonadosomatic index (GSI) was used. In order to determine the GSI ( $GSI = Wg/W \times 100$ ) (Nikolski, 1963) ovaries were weighed (Wg) with an accuracy of 0.001 g. The absolute fecundity (Fa) was estimated in 30 ovaries by calculating the

number of oocytes with a diameter greater than 0.2 mm (Kostrzewa *et al.*, 2003; Mousavi-Sabet *et al.*, 2011a). To achieve accurate results, fish that were caught in the beginning of May and June 2009 and May 2010 (before spawning), were used. Relative fecundity (Fr) was expressed by dividing absolute fecundity (Fa) by the fish body weight. The result was expressed as the number of eggs per 1 g of body weight (Bagenal, 1967). To determine the oocytes diameter, the ovaries were preserved in 4% formalin solution. The diameters of 60 ova

of each female fish were measured using a Zeiss stereomicroscope (model SV 6) fitted with an ocular micrometer. The sex recognition was determined according to occurrence of the Canestrini scale (*lamina cribrosa*) and the examinations of gonad morphology were conducted after dissection. The *lamina cribrosa* (*lamina circularis*) is a platelike ossified extension of the ray and is a secondary sexual character of males (Canestrini, 1871). The Chi square test was used to assess sex ratio deviation from a 50:50 (Wootton, 1998). Age determination was done using scales taken from the left side of the body, between end of the pectoral fin and the beginning of the dorsal fin. Comparison of significant differences in the GSI index between samples taken on different months was done using analysis of variance (ANOVA I). The relationship between absolute fecundity (Fa) and body length and weight, the gonad weight and the age of females was determined by regression analysis. The data were analyzed by SPSS (version 10.0) and Microsoft Excel 2007 software.

## RESULTS

### Size, sex ratio and age

During this study, 258 specimens of *Cobitis faridpaki* were caught ranging in total length from 26.3 to 74.1 mm, standard length 21.8 to 63.4 mm and total weight from 0.4 to 4.2 g (Table 1). Females were longer, heavier and achieve maturity later

than males, at the age of 2+, TL more than 40 mm and body weight about 2 g. Results revealed that this population of the loach had a narrow age range of 1+ to 5+ years. Most of the caught fish were 2+ and 3+ years old, some were 1+ and 4+ and only a few of them were 5+ years old. The oldest females (0.70%) among specimens were +5 years old but most of them (52.55%) were in the third year (+3) of their lives (Table 1). The body length increased proportionally with their age. The older loaches were longer than those which had just reached sexual maturity (in the second and third years of life). The body weight of the older fish was heavier than the younger ones. Of the total number of 258 fish specimens caught, 114 were males and 144 were females, giving an overall sex ratio of 1:1.26 and the difference between the number of females and males was significant ( $\chi^2 = 3.4884$ ,  $p < 0.05$ ). Also, sex ratio in different months was studied (Table 2).

### Gonadosomatic index

Significant differences were observed in female and male GSI in different months (ANOVA,  $p < 0.05$ ). The female gonadosomatic index increased during November to May, peaked at mid spring and then decreased until August, showed a slight increase in October and finally declined in November (Fig. 2).

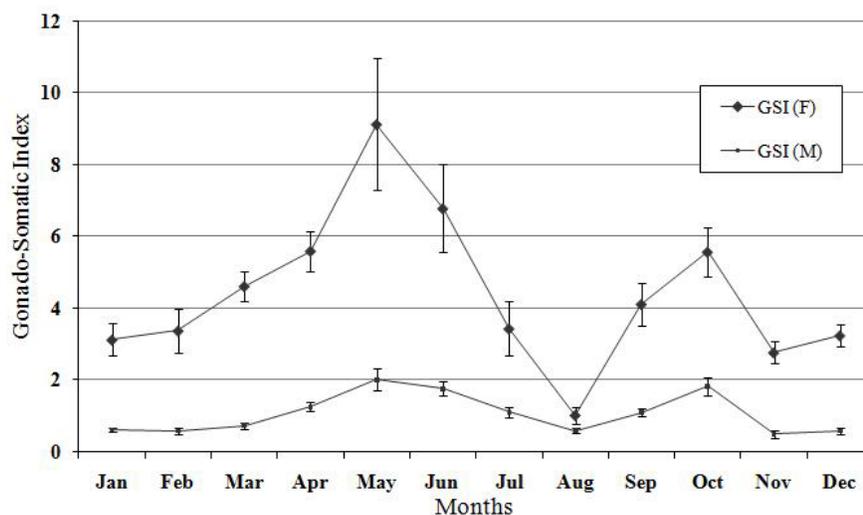


Fig 2. Variation of mean ( $\pm$ S.E) gonadosomatic index (GSI) of female (F) and male (M) for *Cobitis faridpaki* from the Siahrud river in different months

### Oocytes diameter and fecundity

The oocytes diameter ranged from 0.02 to 1.4 mm with a mean of 0.5777 mm (SD±0.1602). The maximum mean oocyte diameter was observed in May and

minimum in December. There was a peak in the number of yellowish-yolk oocytes (0.8-1.4 mm diameter) in May (Fig. 3).

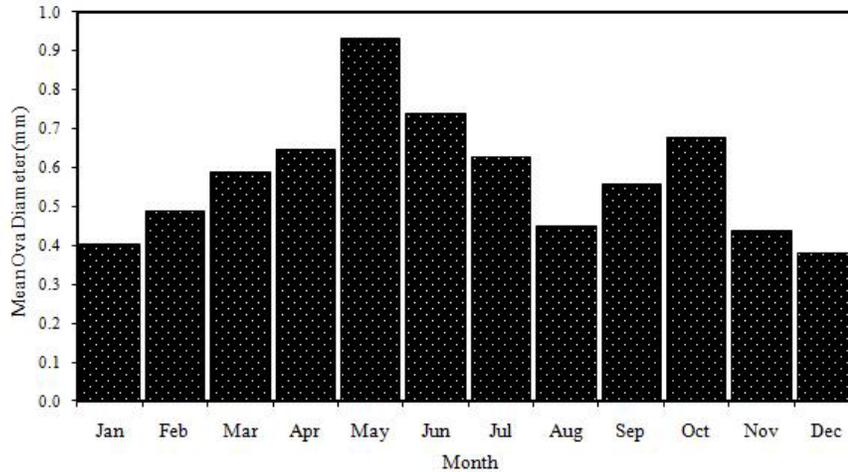


Fig 3. The mean diameter of oocytes (mm) of *Cobitis faridpaki* from the Siahrud River

The fish used to estimate fecundity were 3+, 4+ or 5+ years old. Individual values of the absolute fecundity varied in a wide range from 558 to 2849 eggs with an average of 1366.7 eggs (SD±593). Relative fecundity was 263 to 851 eggs with a mean of 508.23 eggs (SD±197) per gram body weight (Table 3).

Absolute fecundity was significantly related to female body weight and also gonad weight (Table 4). The regression coefficient values were 0.4214 and 0.8182 for gonad weight and total body weight, respectively.

Table 1. Total length (TL), standard length (SL), and body weight (W) (mean±SD) in different ages of the *Cobitis faridpaki* males and females from the Siahrud River

Age	N		TL (mm)		SL (mm)		W (g)	
	M	F	M	F	M	F	M	F
1+	14	4	27.14±1.75	28.66±3.09	23.26±2.03	24.07±3.15	0.40±0.17	0.40±0.19
2+	29	41	40.11±3.86	38.87±4.76	34.72±3.95	31.49±3.62	0.73±0.32	1.02±0.27
3+	66	68	48.05±1.83	49.03±3.41	42.08±2.17	42.88±3.12	1.32±0.20	1.69±0.54
4+	5	29	61.52±1.75	64.50±3.63	53.12±1.65	55.74±3.24	1.88±0.36	2.73±0.69
5+	0	2	-	73.85±0.21	-	62.15±0.49	-	4.13±0.32

N: number of specimens, SD: standard deviation, M: male specimens, F: female specimens

Table 2. Sex ratio of *Cobitis faridpaki* from the Siahrud River in different months

Month	Total No.	Male No.	Female No.	Male percent	Female percent	M:F	Expected No.	Chi-Square
Jan	16	7	9	43.750	56.250	1:1.286	8	0.2500
Feb	21	8	13	38.095	61.905	1:1.625	10.5	1.1904
Mar	20	9	11	45.000	55.000	1:1.222	10	0.2000
Apr	20	8	12	40.000	60.000	1:1.500	10	0.8000
May	27	12	15	44.444	55.556	1:1.250	13.5	0.3334
Jun	15	9	6	60.000	40.000	1:0.667	7.5	0.6000
Jul	31	15	16	48.387	51.613	1:1.067	16.5	0.0304
Aug	28	9	19	32.143	67.857	1:2.111	14	3.5714
Sep	15	7	8	46.667	53.333	1:1.143	7.5	0.0666
Oct	22	8	14	36.364	63.636	1:1.750	11	1.6364
Nov	25	13	12	52.000	48.000	1:0.923	12.5	0.0400
Dec	18	9	9	50.000	50.000	1:1.000	9	0.0000

**Table 3.** Absolute (Fa) and relative (Fr) fecundity in particular ranges of body length (Sl), body weight (W) and age of *Cobitis faridpaki* females from the Siahrud River

Parameters	N	Fa		Fr		
		Range	Mean±SD	Range	Mean±SD	
Sl (mm)	50.1-60	21	558-1244	1114±503	281-796	468±216
	60.1≤	9	735-2849	1814±752	263-851	556±207
	2.1-3	22	558-1392	1282±519	279-725	539±241
W (g)	3.1-4	6	586-2234	1639±743	263-851	479±188
	4.1≤	2	1526-2849	2368±403	448-689	568±170
	3+	19	618-1563	1101±609	263-643	461±190
Age	4+	9	558-2427	1647±784	295-851	575±184
	5+	2	1886-2849	2368±403	448-689	568±170

**Table 4.** Correlation coefficients r and regression equations for relationships between absolute fecundity (Fa) and: body length (TL), body weight (W), weight of ovary (Wg) and age

Relationship	N	Linear Regression	r	r <sup>2</sup>	F	P
Fa - TL	30	y = 47.973x - 940.11	0.7645	0.5844	1.802	0.365
Fa - W	30	y = 551.46x + 480.39	0.8182	0.6694	6.615	0.038
Fa - Wg	30	y = 1421.7x + 986.36	0.4214	0.1776	30.403	0.007
Fa - Age	30	y = 718.81x - 835.03	0.5933	0.3520	1.759	0.413

## DISCUSSION

### Age and size of fish

Females achieve maturity, later than the males, at the age of 2+, when Sl is about 56 mm and body weight about 2 g (Boron and Pimpicka, 2000; Robotham, 1981). The spined loach is a short-lived species. Most of the caught fish were 2+ and 3+ years old, some were 1+ and 4+ and only a few of them were 5+ years old (Robotham, 1981), while in the present study the oldest females (0.70%) were 5+ years old but most of them (52.55%) were in the fourth year of their lives.

### Reproduction

This is the first study concerning reproduction biology of *Cobitis faridpaki* in Siahrud River from the southeast Caspian Sea basin. However, there are some reports of some aspects of reproduction of other spined loach species (genus *Cobitis*); Spawning of *C. taenia* takes place from May to July, usually in shallow littoral waters, among the submerged vegetation where the water temperature is at least 16-18°C (Boron and Pimpicka, 2000; Bialokoz, 1986). A similar time of spawning was observed for *C. bilineata* from northern Italy (Marconato and Rasotto, 1989). A later spawning period (June-July) was observed for *C. taenia* from the United Kingdom (Robotham, 1981). Ekmekci and Erk'akan (2003) showed that the *C. simplicispina* from Turkey begins to spawn in April, when the water temperature is

15°C and ends in May. The spawning period in the Siahrud River lasted until the middle of July. In the present study some ripe female specimens were found in mid July. Similar times are reported for *Cobitis* sp. from the Babolrud River and *Cobitis keyvani* from the Talar River in the same basin (Mousavi-Sabet et al., 2011a; Mousavi-Sabet et al., 2012b). However all the studied fish were in the spent stage in the month of August showing the end of their reproduction period. Then a secondary increasing in ovaries development was observed from August to October, when some of the fish were ready to spawn. In Lake Zegrze, female loach ovaries were filled with mature eggs in August (Boron and Pimpicka, 2000).

The process of accumulating reserve substances in the ovaries of the females can be obtained partly by tracing the changes in the gonadosomatic index. In species which spawn in late spring and in summer such as loach, the index remains low in winter and then rises sharply just before spawning (Marconato and Rasotto, 1989; Rinchar and Kestemont, 1996; Wootton, 1979). A rapid increase in the weight of ovaries takes place when the temperature rises and increasing amounts of food are consumed (Wootton, 1979). The same pattern was observed in *Cobitis faridpaki* from the southern Caspian Sea basin. Changes in the gonadosomatic index, calculated for the population in which females spawn in batches, must not be

used as the only credible indicator of the number of batches laid. The highest GSI values from 18% for the fish from Lake Lucien (Kostrzewa *et al.*, 2003) to 26% in the loach from Italy (Marconato and Rasotto, 1989) were observed during the reproduction period. At this time, the values of GSI vary widely; from 4 to 26% for *Cobitis* sp. from Iran (Mousavi-Sabet *et al.*, 2011a), from 6 to 25% (10.09% in average) for *Cobitis keyvani* from Iran (Mousavi-Sabet *et al.*, 2012b), from 2 to 20% for *C. elongatoides* from the Czech Republic (Halacka *et al.*, 2000), from 12 to 26% for *C. taenia* from Italy (Marconato and Rasotto, 1989), but were also lower from 7 to 17% for *C. simplicispina* from Turkey (Ekmekci and Erk'akan, 2003) or from 5 to 18% for *Cobitis* sp. from Lake Lucien (Kostrzewa *et al.*, 2003) and from 5.2% to 12.2% for *C. elongatoides* from Hungary (Eros, 2000), while, in *Cobitis faridpaki* from the Siahруд River, the highest average value of GSI (calculated in late April, before spawning) was more than 9%, ranging from 2 to 20%.

### Fecundity

In order to estimate the absolute fecundity of the loach from the Siahруд River, all the oocytes with the limiting diameter of 0.2 mm were counted. A similar limiting value was adopted for genus *Cobitis* from Lake Lucien, Babolrud River and Talar River (Kostrzewa *et al.*, 2003; Mousavi-Sabet *et al.*, 2011a; Mousavi-Sabet *et al.*, 2012b). Some authors included larger oocytes, over 0.3 mm (Boron and Pimpicka, 2000) or over 1 mm (Bialokoz, 1986). In this study, the average absolute fecundity of female loach from the Siahруд River was about 1366 eggs, ranging from 558 to 2849 eggs. The absolute fecundity of species from the genus *Cobitis* caught in Lake Lucien and Lake Klawoj (Kostrzewa *et al.*, 2003; Juchno and Boron, 2006) equaled 2180 and 2078 eggs on average, respectively. Also in similar ranges, the absolute fecundity of *Cobitis* sp. and *Cobitis keyvani* caught in the Babolrud and Talar Rivers equaled 2172 and 2211 eggs on average, respectively (Mousavi-Sabet *et al.*, 2011a; Mousavi-Sabet *et al.*, 2012b). The absolute fecundity of the spined loach from Lake Dgal Wielki was low and ranged from 175 to 452 eggs, as only the oocytes larger than 1 mm were counted (Bialokoz, 1986). The number of

oocytes obtained in this way referred probably only to the first batch. Only the largest mature eggs were counted in order to estimate the fecundity of the loach in northern Italy (Marconato and Rasotto, 1989). In this case, the number of eggs equaled 1012. According to Lobon-Cervia and Zabala (1984), fecundity of the largest female *C. taenia* equaled 1400 eggs, whereas according to Bohlen (1998) fecundity of the females of this species was much higher and ranged from 2905 to 4258 eggs (3618 on average). Statistically significant relationships were found between the absolute fecundity and body weight and gonad weight in *Cobitis faridpaki* from the Siahруд River. Absolute fecundity of the fish from Lake Zegrze ranged from 112 to 1520 eggs and positively correlated with the body size, length and the age of the females (Boron and Pimpicka, 2000) and relative fecundity ranged from 28 to 204 eggs per 1 g of the body weight, 108 on average. The relative fecundity in a related species of *C. elongatoides* ranged from 35 to 105 eggs (Eros, 2000). Relative fecundity in the loach from the Siahруд River was higher and equaled 263 to 851 with a mean of 508 eggs per 1 g of a female body weight. According to the present study, the relative fecundity in the spined loach from Lake Klawoj, the Babolrud and Talar Rivers equaled 629, 590 and 586 eggs, respectively (Juchno and Boron, 2006; Mousavi-Sabet *et al.*, 2011a; Mousavi-Sabet *et al.*, 2012b).

Based on reproduction parameters, it was concluded that this fish has a prolonged active reproduction period, which is a type of adaptation of short-lived small fishes to environmental conditions.

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سن، نسبت جنسی، فصل تخم‌ریزی، شاخص گنادی-بدنی و هم‌آوری ماهی *Cobitis faridpaki*(Actinopterygii, Cobitidae) در رودخانه سیاهرود از جنوب شرقی حوضه آبریز دریای خزر

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چکیده

ماهی (*Cobitis faridpaki* (Mousavi-Sabet, Vasil'eva, Vatandoust and Vasil'ev, 2011) در جویبارهای رودخانه سیاهرود در حوضه جنوبی دریای خزر یافت می‌شود، و این اولین گزارش از شاخص‌های تولیدمثلی این ماهی بوم‌زاد تازه توصیف شده، می‌باشد. شرایط تولیدمثل از شاخص‌های کلیدی شناخت جمعیت‌های ماهیان محسوب شده و شناخت آنها به‌خصوص برای ماهیان بوم‌زاد از اهمیت ویژه‌ای برخوردار است. در این مطالعه، نمونه‌برداری به صورت ماهیانه و به مدت یکسال انجام شد و در مجموع 258 عدد ماهی *Cobitis faridpaki* از رودخانه سیاهرود (حوضه جنوبی دریای خزر) در شمال ایران صید شد. سن، نسبت جنسی، هم‌آوری، قطر تخمک‌ها و شاخص گنادی-بدنی مورد بررسی قرار گرفتند. همچنین وجود رابطه میان هم‌آوری و اندازه ماهی (طول و وزن)، وزن گناد و سن مورد محاسبه و بررسی قرار گرفت. نسبت جنسی به صورت معنی‌داری متفاوت و تعداد ماهیان ماده بیشتر از نرها بود. ماهیان ماده و نر به ترتیب با اندازه بیش از 40 و 30 میلی‌متر و سن 2+ و 1+ بالغ بودند. میانگین قطر تخمک‌ها 0/578 میلی‌متر محاسبه شد. تخم‌ریزی در اردیبهشت ماه تا اواخر تیر ماه، زمانی که حرارت آب بین 19/4 تا 25/1 درجه سانتی‌گراد بود، انجام شد. میانگین شاخص گنادی-بدنی (GSI) در ماهیان ماده بالغ رسیده، 9/12 درصد با دامنه بین 2 تا 20 درصد محاسبه شد. میانگین هم‌آوری مطلق و نسبی به ترتیب 1366 و 508 عدد تخم بودند. ارتباط مستقیم معنی‌داری بین هم‌آوری مطلق با وزن بدن و وزن گناد مشاهده شد. بر اساس روند سالانه نمودار شاخص گنادی-بدنی مشخص شد که ماهی مورد مطالعه دارای دوره تولید مثل نسبتاً طولانی می‌باشد که این یکی از سازگاری‌های ماهیان کوچک (با عمر کوتاه) با شرایط محیطی محسوب می‌شود.