Reproductive biology of *Cobitis keyvani* (Cobitidae) from the Talar River in the southern Caspian Sea basin

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Abstract

Some aspects of the reproductive biology of *Cobitis keyvani* (Mousavi-Sabet, Yerli, Vatandoust, Ozeren and Moradkhani, 2012), an endemic Loach fish species from the Talar River in the southern Caspian Sea basin, in north of Iran was studied by regular monthly collections throughout a year. Fecundity, condition factor, gonado-somatic, modified gonado-somatic and Dobriyal indices were estimated. Regression analyses tested the dependence of fecundity on fish length, weight, gonad weight and age. Significant differences were observed between the total number of females and males, females being more abundant. The mature females and males were longer than 49 and 45 mm in total length, and +2 and +1 in age, respectively. The average egg's diameter was 0.56 mm. The spawning of *Cobitis keyvani* from Talar River took place from May to late July, when the water temperature is between 18.7 to 24.0°C. At the beginning of the reproduction period, the average GSI values were 10.09%, ranging from 6 to 25% in ripe mature females. The absolute and relative fecundity were 2211 and 586 respectively. The absolute fecundity was significantly related to body weight and gonads weight. Based on the gonado-somatic and Dobriyal indices, it was concluded that the fish has a prolonged active reproductive period. Their long reproductive activity and high range of fecundity are types of adaptation by short-lived small fishes to environmental conditions.

Keywords: Gonado-somatic index, Dobriyal index, Spawning, Condition factor, Fecundity, Iran

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Introduction

The Cobitis genius fishes are represented in Iran by three valid species. 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 southern 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). While some others believe that the fish can't be *C. taenia*, and *C. taenia* is rather a northern European species and its occurrence in the southern Caspian Sea basin is unlikely (Kottelat and Freyhof, 2007). *Cobitis linea* Heckel, 1849 is found in the Kor River basin and the upper Kul River drainage of the Hormozgan basin (Banarescu and Nalbant, 1967; Bianco and Nalbant, 1980). *Cobitis keyvani* is one of the species of the Cobitidae family and is an endemic fish from south of the Caspian Sea basin in north of Iran. This species is found in the lower streams of the Talar River (Mousavi-Sabet et al., 2012). Species of this family are small benthic freshwater fishes with a wide distribution area covering large parts of Eurasia and Africa (Perdices and Doadrio, 1997). Spined loach during the day remains buried in sand, mud or dense weed growths, being active at night, and is mostly solitary (Coad, 2012). The loaches achieve sexual maturity in the first (males) or second (females) year of their life (Boron and Pimpicka, 2000; Marconato and Rasotto, 1989). Our main aim was to provide data on the reproductive biology of the fish, including sex ratio, GSI, fecundity, spawning season and condition factor. This information is necessary for conservation programs of this endemic fish. We believed that information on the reproductive biology of this small and colorful Loach is important because it may enter the aquarium trade.

Materials and methods

The study was conducted in the Talar River (36°11.749′ N, 53°00.920′ E and altitude 473m) in the southern Caspian Sea basin (Fig. 1). Fish were collected from the river monthly from May 2009 to April 2010 by electrofishing (200-300 V), with mesh size of 0.2 cm. The water temperature of the fish catching site was measured frequently. The specimens were immediately preserved in 4% formaldehyde until they could be examined. 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). The condition factor (K) was calculated monthly by $K=10^5\times W/L^3$, where W and L are total weight and total length respectively (Biswas, 1993). To examine the monthly changes in gonads as a mean for estimating the spawning season of this Loach, three indices were used: gonado-somatic index (GSI), modified gonado-somatic index (MGSI) and Dobriyal index (D.I.). In order to determine the GSI ($GSI=Wg/W\times100$) (Nikolski, 1963), MGSI ($MGSI=Wg/W-Wg\times100$) (Nikolski, 1963) and D.I ($D.I=\sqrt[3]{Wg}$) (Dobriyal et al., 1999) ovaries were weighed (Wg) with 0.001 g accuracy.

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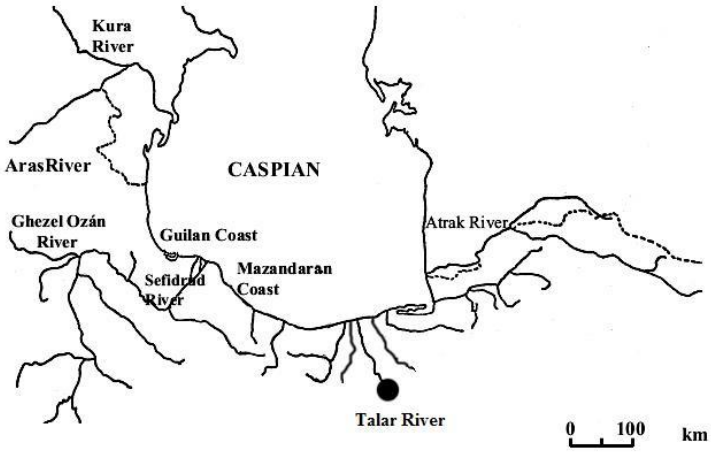

Figure 1: Map of southern Caspian Sea basin showing some of the most important rivers system, study area, the Talar River (modified from Samaee et al., 2009)

The fecundity of female Loaches from the Talar River was estimated in the fish which were caught in the beginning of May and June 2009 and those caught in May 2010. Altogether, the absolute fecundity (Fa) was estimated in 30 ovaries by calculating of the number of oocytes with a diameter greater than 0.2 mm (Kostrzewa et al., 2003; Mousavi-Sabet et al., 2011a). The relative fecundity (Fr) was expressed by dividing the absolute fecundity (Fa) by the fish body weight. The result was the number of eggs per gram of body weight (Bagenal, 1967). To determine the ovum diameter, the ovaries were preserved in 4% formaldehyde solution. The diameters of 60 ova of each female fish were measured using a Zeiss stereomicroscope model SV 6 which was fitted with an ocular micrometer. The sex was determined by using the external dimorphic characteristics which *Cobitis keyvani* exhibits, including: females were longer and more ponderous than males, a lamina circularis (Canestrini scale) was well developed at the base of the male pectoral fin, compared to females, males had much longer pectoral fins, the length of pectoral fin in males fits 16.31 percent of SL, while in females it fits 13.48 percent of SL. The Chi square test was used to assess deviation from a 50:50 sex ratio (Robards et al., 1999). The fish's age was assessed based on the annual growth of scales taken from the left side of the body, between end of the pectoral fin and the beginning of the dorsal fin. In order to compare significant differences in the GSI index between samples taken on various months and various size samples, the analysis of variance and Tukey's test were applied ($p<0.05$). The strength and significance of the relationship between the absolute fecundity (Fa) and selected individual features of the females included in the study (body length and weight, the gonad weight and the fish's age) were analyzed by determining Pearson's correlation coefficient r ($p<0.05$) and regression equations. The data were

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analyzed with the SPSS version 10.0 software package and Microsoft Excel 2010 software.

Results

This fish, *Cobitis keyvani*, is commonly found in tributary streams of the Talar River. The bottom of these water bodies is generally sandy, silt and rubble (With a 5 to 45 cm diameter), and the water is clear and slow running.

The analysis of water in two different stations in May showed that the temperature was 17.8 to 19.6 °C with pH 6.9 -7.1, O_2 5-9 mg/l and hardness 209 mg/l.During this study, 237 specimens of the Loach were caught with a total length ranging from 45.0 to 90.1 mm, standard length 37.3 to 81.5 mm and total weight from 0.5 to 6.1 g. The female specimens were longer and heavier than males and achieve maturity later than males, at the age of 2+, Tl more than 49 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 3+ and 4+ years old, some were 1+ and 2+ and only a few of them were 5+ years old. The oldest females (3.17% of total female specimens) were +5 years old but most of them (47.01%) were in their fourth year (+3) (Table 1).

Table 1: Standard length (Sl), total length (Tl) and body weight (W) (mean±SD) in different ages of *Cobitis keyvani* males and females from Talar River

Age	N		Sl (mm)		Tl (mm)		W (g)	
	M	F	M	F	M	F	M	F
1+	14	7	29.42±1.03	31.56±2.11	33.19±1.84	34.92±2.72	0.40±0.11	0.42±0.07
2+	28	14	40.94±3.19	38.53±3.79	47.03±3.68	46.23±4.05	1.18±0.25	1.51±0.37
3+	56	52	49.31±2.05	50.08±2.55	56.90±2.54	57.06±2.78	1.75±0.16	2.28±0.48
4+	7	55	58.84±1.91	64.17±4.27	65.04±2.13	74.14±3.85	1.97±0.19	3.39±0.64
5+	0	4	-	72.40±1.25	-	84.09±2.00	-	5.04±0.27

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

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Table 2: Absolute (Fa) and relative (Fr) fecundity in particular ranges of body length (Sl), body weight (W) and age of *Cobitis keyvani* females from Talar River

Parameters		N	Fa		Fr	
			Range	Mean±SD	Range	Mean±SD
Sl (mm)	**50.1-60**	9	755-1523	1359±402	377-892	586±211
	60.1-70	15	909-2614	2147±556	319-951	663±254
	70.1≤	6	2078-3481	2709±519	393-853	509±162
W (g)	**2.1-3**	8	755-1850	1622±547	319-769	627±204
	3.1-4	16	1025-2807	2248±650	355-951	598±227
	4.1≤	6	1856-3481	2853±761	519-770	641±109
Age	**3+**	12	755-2074	1484±593	319-762	591±166
	4+	15	997-3116	2259±720	328-951	665±214
	5+	3	2358-3481	2805±531	395-602	511±96

Table 3: Correlation coefficients r and regression equations for relationships between absolute fecundity (Fa) and body length (Sl), body weight (W), weight of ovary (Wg) and age

Relationship	N	Linear Regression	r	r^2	F	P
Fa – Tl	30	y = 62.612x – 2208.8	0.8139	0.6625	2.381	0.169
Fa – W	30	y = 333.07x + 796.57	0.4855	0.2357	4.876	0.042
Fa – Wg	30	y = 1507.7x + 783.88	0.6088	0.3707	26.664	0.001
Fa – Age	30	y = 382.78x + 592.65	0.3817	0.1457	0.780	0.700

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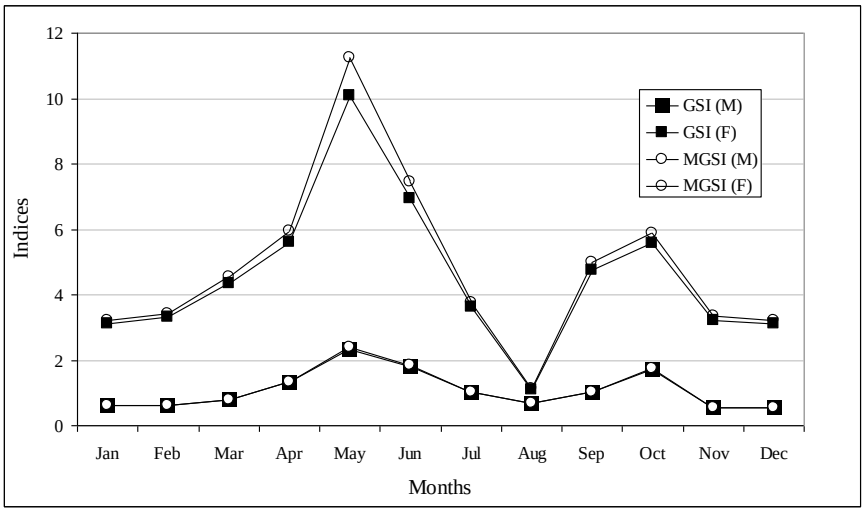

Figure 2: Variation of mean gonado-somatic index (GSI) and Modified gonado-somatic index (MGSI) of female (F) and male (M) *Cobitis keyvani* in different months from Talar River

The body length increased proportionally to the age. The older loaches were longer than those which had just reached sexual maturity (in the second and third year of life). The body weight of the older fish was heavier than the young ones. Of the total number of 237 mature fish caught, 132 were females and 105 were males, giving an overall sex ratio of 1:1.2857. The difference between the number of females and males was significant (Chi square 3.0760, $p<0.05$). For the pooled data, the condition factor did not differ significantly between the sex (ANOVA, $p=0.412$). However, when we considered the condition factor separately for each month, we found differences in this factor from month to month (ANOVA<0.01). The condition factor of females was higher in May and July to October, and reached its lower value in June, November and December. From December to April it showed fluctuation (Fig. 3). In male specimens, the condition factor was lowest in January, and was increasing until May (Fig. 3).

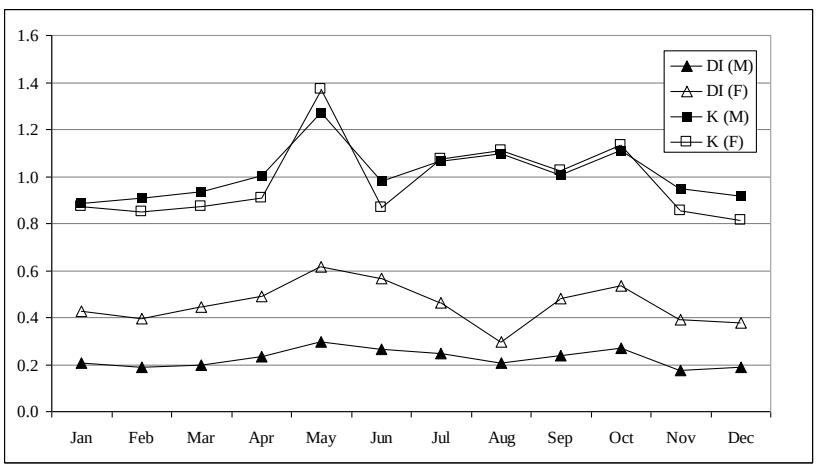

Figure 3: Variation of Dobriyal Index (D.I.) and condition factor (K) of female (F) and male (M) *Cobitis keyvani* from Talar River in different months

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Significant differences in female and male GSI and MGSI in different months were observed. There were no significant differences between GSI and MGSI. The female GSI increased during November and May, peaking at the mid of spring and decreased until August, then showed a slow increasing to October and finally decreased in November (Fig. 2). The GSI pattern showed that the reproductive period had a duration of 4 months, so this fish had a prolonged reproductive activity. In the case of males, two peaks were observed as well, in May and October. In females, D.I was 0.617 in May, falling until August (0.297) which was an indicator for the attempt to spawn by the fish in these months. An increasing trend from August to October and a decreasing trend were observed in the November values, showing a long period of spawning by this fish. In males, the Dobriyal index ranged from 0.1769 to 0.2958. D.I showed a slight increasing trend from November to May, when it was as high as 0.2958. D.I decreased gradually from May to August, which was an indicator of the first attempts to spawn by males in these months. There was an increase in October and a fall in the value during November (Fig. 3). The ovum diameters ranged from 0.02 to 1.4 mm with a mean of 0.5569 mm (SD±0.1643). They were highest in May and lowest in December (Fig. 4). The fish used to estimate fecundity with were 3+, 4+ or 5+ years old. Individual values of the absolute fecundity varied in a wide range from 694 to 3319 eggs with an average of 2211 (SD±783). The relative fecundity was 328 to 902 with a mean of 586 (SD±235) per gram body weight (Table 2). It was observed that the absolute fecundity accompanied the growth of age, weight and length of the female loaches. The absolute fecundity was significantly related to fish female total weight and also gonad weight (Table 3). The value of the r coefficient for these relationships, with the level of significance 0.05 equaled 0.3817, 0.6088, 0.8139 and 0.4855 for age, gonad weight, total length and total weight respectively.

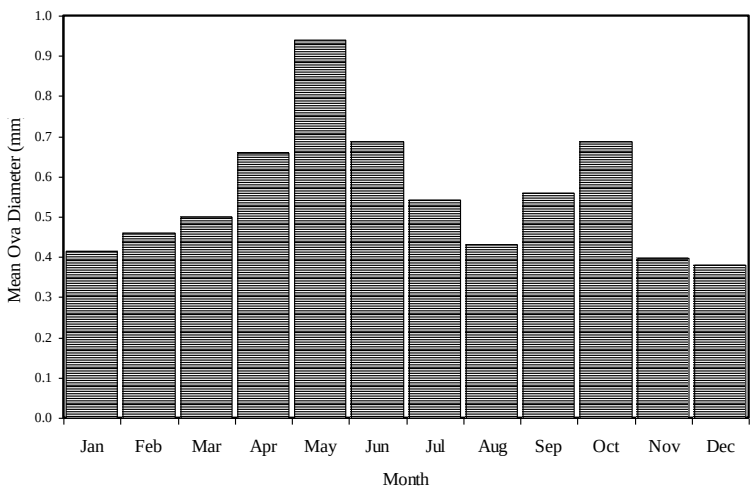

Figure 4: Mean oocyte diameter (mm) by month for *Cobitis keyvani* in Talar River

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Discussion

Females achieve maturity later than the males; at the age of 2+, Sl is about 56 mm and body weight about 2 g (Boron and Pimpicka, 2000; Robotham, 1981). The loach is a short-living 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). The oldest females (3.17%) among specimens in the present study were 5+ years old but most of them (47.01%) were in the fourth year of their lives. This is the first study concerning reproduction process of *Cobitis keyvani*. Spawning of *C. taenia* takes place from May to July, usually in shallow littorals, 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* Canestrini 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° and finishes in May. The spawning period in Talar River lasted until late July. A similar time is reported for *Cobitis* sp. from Babolrud River in the same basin (Mousavi-Sabet et al., 2011a). As late as in late-July, in some females ovaries filled with oocytes were observed ready to be laid. However, in August, the ovaries of most of the loaches under study indicated the end of the reproductive period. In Lake Zegrze, female loach's ovaries were filled with mature eggs in August (Boron and Pimpicka, 2000). The development stages of gonads did not correspond with the age and/or size of the examined females.

The process of accumulating reserve substances in the ovaries of the females can be obtained partly by tracing the changes in the gonado-somatic 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 the spawn (Marconato and Rasotto, 1989; Rinchard 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). Changes in the gonado-somatic 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 the time, the values of GSI vary widely; from 4 to 26% for *Cobitis* sp. in Iran (Mousavi-Sabet et al., 2011a), from 2 to 20% for *C. elongatoides* in the Czech Republic (Halacka et al., 2000), from 12 to 26% for *C. taenia* in Italy (Marconato and Rasotto, 1989), but also lower from 7 to 17% for *C. simplicispina* in Turkey (Ekmekci and Erk'akan, 2003) or from 5 to 18% for *Cobitis* sp. in Lake Lucien

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(Kostrzewa et al., 2003) and from 5.2% to 12.2% for *C. elongatoides* in Hungary (Eros, 2000). In *Cobitis keyvani* from Talar River, the highest average value of GSI (calculated in late April, before spawning) was about 10.09%, ranging from 6 to 25%. In order to estimate the absolute fecundity of the loach from Talar River, all the oocytes with the limiting diameter of 0.2 mm were counted. A similar limiting value was adopted for *Cobitis* genus from Lake Lucien (Kostrzewa et al., 2003) and the Babolrud River (Mousavi-Sabet et al., 2011a). 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 Talar River was about 2211 eggs, with the number ranging from 694 to 3319 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) were comparable to the estimated value for the fish in Talar River and equaled 2180 and 2078 eggs on average respectively. The absolute fecundity of the 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), the fecundity of the largest female *C. taenia* equaled 1400 eggs, whereas according to Bohlen (1998) the 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 keyvani* from Talar River. The 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 the relative fecundity ranged from 28 to 204 eggs per gram 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). The relative fecundity in the loach from Talar River was higher and equaled 328 to 902 with a mean of 586 eggs per gram of a female body weight. According to the present study, the relative fecundity in the loach from Lake Klawój equaled 629 eggs (Juchno and Boron, 2006).

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زیست شناسی تولیدمثل ماهی *Cobitis keyvani* (Cobitidae) در رودخانه تالار، حوضه جنوبی دریای خزر

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چکیده

در تحقیق حاضر برخی جنبه‌های زیست‌شناختی یک چرخه کامل تولیدمثلی سگ‌ماهی جویباری بومزاد ایران، سگ‌ماهی کیوان *Cobitis keyvani* (Mousavi-Sabet, Yerli, Vatandoust, Ozeren and Moradkhani, 2012) در رودخانه تالار، حوضه جنوبی دریای خزر، شمال ایران، با نمونه‌برداریهای ماهیانه به مدت یکسال مورد بررسی قرار گرفت. هماوری، شاخص وضعیت، شاخص گنادی- بدنی، شاخص اصلاح شده گنادی- بدنی و شاخص دابریال مورد بررسی قرار گرفتند. آزمون رگرسیون میان میزان هماوری و طول کل، وزن بدن، وزن گناد و سن ماهیان ماده مورد آزمایش قرار گرفت. تفاوت معنی‌داری بین تعداد کل ماهیان نر با تعداد ماهیان ماده در جمعیت مورد آزمایش مشاهده شد، در حالی که تعداد ماده‌ها بیشتر بود. ماهیان نر و ماده به ترتیب در طول کل بیش از ۴۵ و ۴۹ میلی‌متر و سنین ۱+ و ۲+ بالغ بودند. میانگین قطر تخمکها ۰/۵۶ میلی‌متر بود. این ماهیان در رودخانه تالار از اواسط اردیبهشت تا اواخر تیر ماه، زمانی که دمای آب ۱۸/۷ تا ۲۴ درجه سانتی‌گراد بود، تخم‌ریزی کردند. میانگین شاخص گنادی- بدنی ماهیان ماده بالغ در ابتدای دوره تولیدمثل ۱۰/۰۹ درصد و با دامنه تغییرات از ۶ تا ۲۵ درصد محاسبه گردید. میانگین هماوری مطلق و نسبی به ترتیب ۲۲۱۱ و ۵۸۶ بودند. میزان هماوری مطلق به صورت معنی‌داری به وزن بدن و وزن گناد وابسته بود. بر اساس روند سالیانه شاخص‌های گنادی- بدنی و دابریال می‌توان بیان نمود که این ماهی دارای دوره تولیدمثلی طولانی مدتی می‌باشد. دوره طولانی تولیدمثل و میزان بالای هماوری در این ماهیان سازگاریهایی هستند که ماهیان کوچک و کوتاه عمر، با شرایط محیط زیست خود ایجاد نموده‌اند.

واژگان کلیدی: شاخص گنادی- بدنی، شاخص دابریال، هماوری، تخمریزی، فاکتور وضعیت، ایران

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