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Original Article

Some reproductive Features of *Cobitis* sp. from Dough River in the southern Caspian Sea basin

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Abstract: *Cobitis* sp. is an endemic spined loach species from the Dough River in the southern Caspian Sea basin, (Golestan Province, Iran). To provide some reproductive features of this fish, sampling was performed at monthly intervals throughout the year and 417 individuals were collected. The specimens ranged in total length from 27.1 to 92.9 mm and total weight from 0.11 to 6.79 g. The spawning of spined loach of Dough River occurs from March to June. The highest mean value of gonadosomatic index was observed in April as 1.96 for males and 6.61 for females. Egg diameter ranged from 0.3 to 1.3 mm, with a mean value of 0.9 mm. Absolute fecundity varied from 155.04 to 3212 eggs. Fecundity relative to total weight fluctuated from 55.41 to 634.76 eggs g^{-1}. This species is among those spawning early in spring compared to other species of this genus from southern Caspian basin.

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Introduction

The genus *Cobitis* have three valid species in Iran including *Cobitis linea* (Heckel, 1849), *C. faridpaki* (Mousavi-Sabet, Vasil'eva, Vatandoust and Vasil'ev, 2011) and *C. keyvani* (Mousavi-Sabet, Yerli, Vatandoust, Ozeren and Moradkhani, 2012). *Cobitis faridpaki* and *C. keyvani* are found in the southern Caspian Sea basin. Also, the presence of the spined loach *C. tenia* Linnaeus, 1758 has been reported in this basin (Abdoli and Naderi, 2009). Whereas others believe that *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).

The members of Cobitidae are small benthic freshwater fishes with a wide distribution area covering large parts of Eurasia and Africa (Perdices and Doadrio, 1997). Spined loach during day time 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). Since there is no information is available about reproductive biology of Dough River population of genus *Cobitis*, therefore this study was conducted to provide some reproductive features of the member of this population in southern Caspian Sea basin. The taxonomic position of the population of genus *Cobitis* from Dough River (Gorganrood River basin) is unclear, therefore it is considered as *Cobitis* sp. in the present study.

Material and Methods

A total of 417 specimens were collected monthly from March 2012 till February 2013 using electrofishing from Dough River (55°44'N, 37°27'E) (Fig. 1). In the field, all specimens were immediately preserved in 10% formaldehyde solution and transferred to the laboratory and then their total and standard lengths were measured to the nearest 0.01

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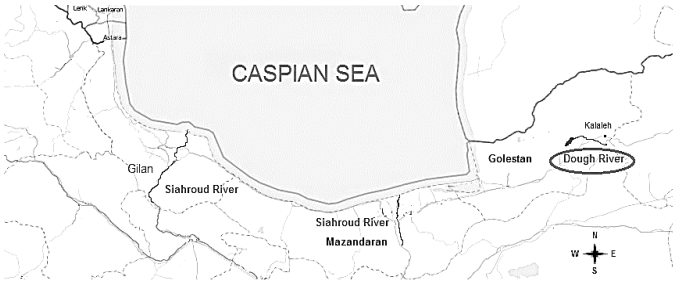

Figure 1. Map of southern Caspian basin, Dough River.

mm using calipers. Total weight and weight of gonads of both sexes were measured with an electronic analytical balance to the nearest 0.01 and 0.001 g, respectively.

The gonadosomatic index (*GSI*) was calculated as $GSI=Wg/W\times100$ (Nikolski, 1963), where *Wg* is weight of gonad and *W* total weight, to estimate the spawning season. Absolute fecundity (*AF*) was estimated in 43 ovaries. The number of eggs was estimated by the gravimetric method, using three pieces of approximately 0.02 g each from the anterior, medial and posterior positions of both ovarian lobes. The relative fecundity index was calculated as $RF = F/TW$, where *F* is absolute fecundity and *TW* total weight (Bagenal and Tesch, 1978). To determine the oocyte diameter, the ovaries were preserved in 10% formalin solution. The diameters of 30 ova of each female were measured using a microscope outfitted with an ocular micrometer.

All statistical analyses were performed with a significance level of $P<0.05$ using the SPSS 17 software package.

Results

In total, 417 specimens of *Cobitis* sp. were caught, ranging in total length from 27.1 to 92.9 mm and total weight from 0.11 to 6.79 g (Table 1).

The highest mean of *GSI* (±SD) was recorded 96 ± 1.16 for male and 6.61 ± 8.97 for female in April (Fig. 2). The female's *GSI* increased during March to April, peaking at the mid of spring and then decreased until August, then showed a slow increase in December.

The mean value (± SD) of absolute fecundity was

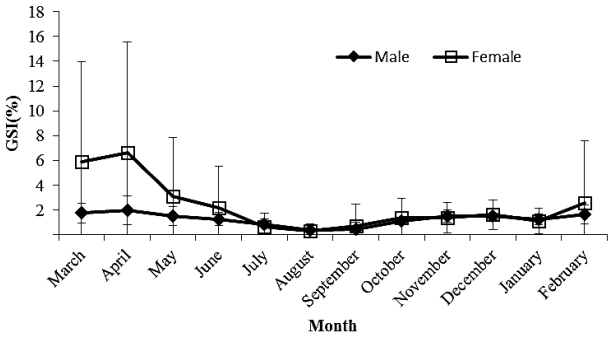

Figure 2. Monthly change of GSI in male and female of *Cobitis* sp. from the Dough River.

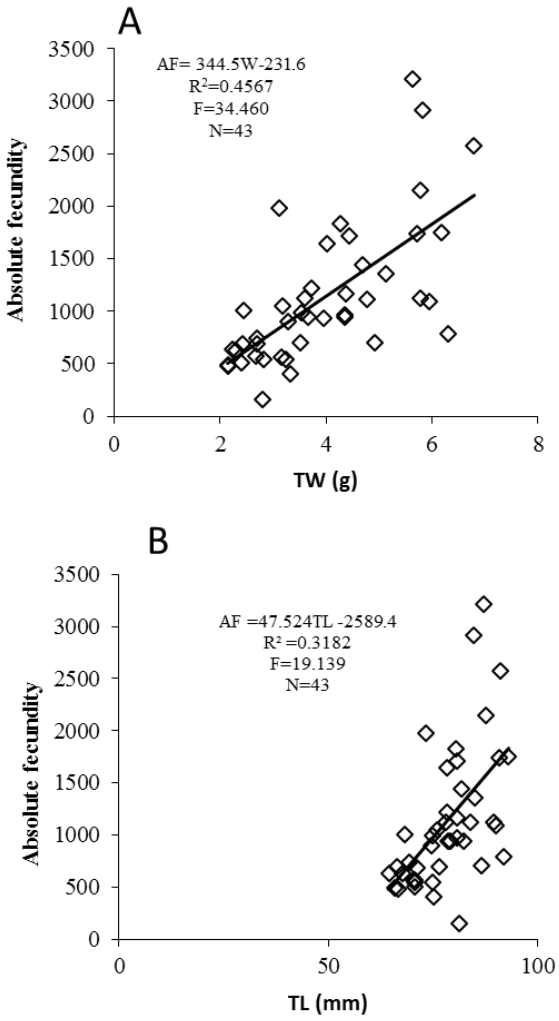

Figure 3. Relationship between absolute fecundity and (A) total length (mm) and (B) total weight (g) of female *Cobitis* sp. from the Dough River.

1132.5 ± 674.66. The absolute fecundity was significantly related to total weight and also gonad weight ($P<0.05$), (Fig. 3). The relative fecundity was 55.4 to 634.8 with a mean (±SD) of 279.5 ± 114.49 per gram body weight. There was no significant

Table 1. Length and weight (mean ± SD) of males and females of *Cobitis* sp. from the Dough River.

Sex	N	TL (mm)		TW (g)	
		Mean ± SD	Range	Mean ± SD	Range
Male	289	55.5 ± 0.7	37.7-76.8	1.29 ± 0.49	0.34-3.69
Female	128	50.4 ± 1.58	27.1-92.9	1.25 ± 1.39	0.11-6.79

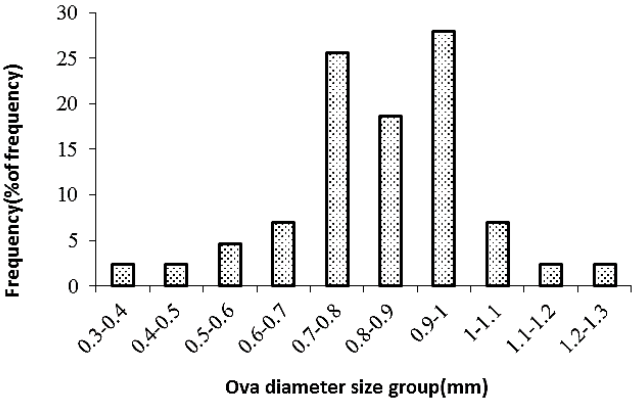

Figure 4. Oocyte diameter size frequency distribution for *Cobitis* sp. from the Dough River.

relationship between relative fecundity and length and weigh of specimens (P>0.05).

Egg diameter ranged from 0.3 to 1.3 mm, with a mean value (± SD) of 0.9 ± 0.88. Size distribution of eggs indicated that the majority of oocytes ranged from 0.9 to 1 mm (Fig. 4).

Discussion

Gonadosomatic Index is an indirect method for estimation of spawning time in fishes (Biswas, 1993). In spined loaches, this index increases in spring and summer, which are spawning seasons, and low in winter (Wootton, 1979). In this study, *GSI* reached its peak in April in both sexes, which indicates concurrency of sexual maturity. As compared with other species of this genus from southern Caspian Sea basin, this species is among those spawning early in spring (early spring species). Concurrency of the gonadosomatic index peak in males and females is among the characteristic of the population or species of studied southern Caspian Sea fishes. Typically peaks of this index are observed in the fish with time difference (although little), while in this species, peak of the gonad growth occurs concurrently. Single peak gonad growth indicates that this species spawns once a year. As Borone et al. (2008) reported in *C. taenia* from Klavoj Lake, Poland from May to July when water temperature is higher than 18.5ºC. A similar time was reported for *C. bilineata* (Marconato and Rasotto, 1989) in northern Italy and for *C. faridpaki* and *C. keyvani* (Mousavi Sabet et al., 2012) for southern Caspian Sea basin. In the study conducted by Patimar et al. (2011) in Siahroud Stream on *C.* cf. *satunini* species, spawning time were reported to be between April and June, which is similar to spawning time of European loaches i.e. *C. elongatoides* and *C. trichonica*. Spawning time for eastern Caspian Sea species of the genus *Cobitis* was from March to June (Mousavi Sabet et al., 2012)

Spawning time depends on various factors such as temperature and food supply. Among various factors, temperature is the most important determinant of spawning time. In southern Caspian Sea basin, spawning was reported to occur during a period in which the largest food resources were supplied in the environment for the fish species living in the streams and rivers (Abdoli and Naderi, 2009). Overall gonadosomatic index pattern is similar to that of other species and various populations of loaches, and there is no special difference in this regard. The only difference may be peak time of this index, which is in turn occurs in different months depending on environmental conditions, especially temperature.

Fecundity is among the important biological indices that indicate broad changes under diverse environmental conditions in different populations. In this study, absolute fecundity has a significant relationship with fish size (length and weight), but there was no significant relationship between

relative fecundity and length and weigh, which is consistent with the studies conducted by Oliva et al. (2002) on *C. paludica* and Patimar et al. (2011) on *C.* cf. *satunini*. Direct and significant relationship of absolute fecundity with fish sizes in the studied population indicate that the energy allocated to reproduction is directly related to the size (length and weight) of the fish; i.e. by increasing age and consequently length and weight, overall energy allocated to reproduction increases. But lack of a significant relationship between relative fecundity and fish size indicates that increased energetic investment in reproduction per weight or length unit does not follow a special rule.

One of the most important cases in examination of reproduction activities and fecundity of the populations is examination of ovum diameter. It seems that increasing food availability in the living environment of fishes in pre-spawning periods has evident effect on increasing weight and ova sizes. In this study, observed ovum varied in size from 0.4 to 1.3 mm. In *C.* cf. *satunini* in southern Caspian Sea basin, ovum diameter was reported in the range of 0.44 to 1.02 mm (Patimar et al., 2011), which is similar to the results of our study.

The highest absolute fecundity rate in our study (3212) in 5 years old female fish was higher than 1400 ova (Lobon et al., 1984), 1235 ova (Soriguer et al., 2000) and 1984 ova (Oliva et al., 2002) for *C. paludica*, and 1366 ova for C*. faridpaki*, and 2211 ova for *C. keyvani* (Mousavi Sabet et al., 2012), but lower than 4282 ova for *C. taenia* (Bohlen, 1986) and 4666 ova for *C.* cf. *satunini* (Patimar et al., 2011). If combined with increased ovum diameter, high fecundity in the populations indicates the increased energetic investment in reproduction. But if combined with decreased ovum diameter, the increased absolute fecundity indicates high energy allocation to reproduction per weight unit. Since relative fecundity had no significant relationship, the latter cannot be easily concluded. But overall increase in reproduction energy in *C. faridpaki* and *C. keyvani* can be attributed to the studied population. However, high absolute fecundity in the population studied by Patimar et al. (2011) and *C. taenia* (Bohlen, 1986) also indicates that the studied population is in lower rank for allocation of reproduction energy as compared to some populations and/or species.

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