Age, growth and reproduction of *Paracobitis malapterura* (Teleostei: Nemacheilidae) from Qom River, Iran

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Abstract: We examined 272 specimens of *Paracobitis malapterura* throughout the spawning season (February to July, 2014) from Qom River (Central Iran) to describe their age, growth, and reproduction. Sex ratio was 1:6.1 in favor of females. Among the examined specimens, the maximum age was 3$^+$ years. The specimens ranged 35-120mm in total length and 0.38-9.58g in total weight. Length-weight relationship was estimated as W=1E-05TL$^{2.94}$, W=2E-05TL$^{2.73}$, and W=1E-05TL$^{2.92}$ for females, males and combined sexes, respectively. The growth type was isometric for females and sexes combined and negative allometric for males. Based on the gonado-somatic index values, spawning occurs between late February and late April. The highest mean GSI was 1.9, and 13.85 for males and females, respectively in April. The absolute fecundity ranged from 164 to 793 with a mean of 353.3 eggs. Fecundity was positively correlated with fish size (length and weight).

Keywords: Loaches, Growth, Reproduction, Qom River, Iran.

Introduction

Most nemacheilid loaches with a high dorsal adipose crest, especially those occurring in Central Asia (Bânârescu & Nalbant 1964), Vietnam (Nguyen 2005), the Middle East (Prokofiev 2009), and China (Min et al. 2010) have been placed in the genus *Paracobitis* (Freyhof et al. 2014). The genus *Paracobitis* was appointed by Bleeker (1863) for *Cobitis malapterura*. This genus is restricted to Near East and Middle Asia, and the species of *Paracobitis* from China have been proposed to be assigned to the genera *Homatula* and *Schistura* (Nalbant & Bianco 1998). The species belonging to the genus *Paracobitis* are comparatively large-sized loaches inhabiting freshwaters of western Asia (Bănărescu & Nalbant 1995; Nalbant & Bianco 1998). There are fourteen valid species in the world, which ten valid species are reported from Iran with four others in the adjusted countries (Kottelat 2012; Esmaeili et al. 2014; Freyhof et al. 2014; Mousavi-Sabet et al.

2014). According to Esmaeili et al. (2014), Freyhof et al. (2014), Mousavi-Sabet et al. (2015) and Jouladeh-Roudbar et al. (2015a), the valid *Paracobitis* species in Iran comprises, *P. atrakensis* Esmaeili, Mousavi-Sabet, Sayyadzadeh, Vatandoust & Freyhof 2014, *P. basharensis* Freyhof, Esmaeili, Sayyadzadeh & Geiger 2014, *P. hircanica* Mousavi-Sabet, Sayyadzadeh, Esmaeili, Eagderi, Patimar & Freyhof 2015, *P. longicauda* Kessler 1872, *P. malapterura* (Valenciennes 1846), *P. molavii* Freyhof, Esmaeili, Sayyadzadeh & Geiger 2014, *P. persa* Freyhof, Esmaeili, Sayyadzadeh & Geiger 2014, *P. rhadinaea* (Regan 1906), *P. smithi* (Greenwood 1976) and *P. vignai* Nalbant and Bianco 1998.

Paracobitis malapterura is known from the Lake Namak basin and the Hable River in the Dasht-e Kavir basin (Freyhof et al. 2014; Jouladeh Roudbar et al. 2015b). There is no sufficient information available about its biological features. In addition,

among the threatened factors, drought has the most negative effect on its existence in the basin. This species has not yet been accessed for IUCN Red List (IUCN 2016). Till date, a detailed description of its life history in the Namak Lake basin has not been given in the literatures. Examination of the basic biological parameters for each species is fundamental for understanding species life history patterns for implementing implementing effective management and conservation measures of the species (Jamali et al. 2014). Therefore, the present contribution serves to describe detailed life history of *P. malapterura* from Qom River in the Namak Lake basin in Iran, a first documentation for the species biology, thereby, contributing to its future conservation.

Material and Methods
The present study was carried out in the Qom River which is located in Qom Province, Central Iran. Qom River stretches 400km and originating from the Zagros Mountain, and draining into the Namak Lake. The specimens were caught using a beach-seine with a mesh size of 2mm. The net was chosen for its simplicity and higher catch efficiency when sampling small specimens. Monthly sampling carried out from February to July 2014. In the field, all fish specimens were immediately fixed in 10% formaldehyde solution until they could be examined. A total of 272 specimens were analyzed in the laboratory. Total length (TL) was measured to the nearest 0.01mm and total weight (TW) was measured to the nearest 0.01g. Both left and right opercula were used for age estimation; banding patterns being reviewed three times (each time by a different person) using a 20-40X binocular microscope under reflected light.

The relationship between TL and TW (LWR) was determined using the equation: TW = αTL^b; where α is the intercept and b is the slope (coefficient of allometry) (Pauly 1984). Sex was determined by visual examination of the gonad tissue. The gonado-somatic index (GSI% = [gonad weight/TW] × 100) was calculated for each specimen and mean values calculated for each sampling date. The ovaries of 84

ripe females at maturity stage IV (In this stage, the ovary has been increased in size due to increasing of the yolk of the oocytes, yellow in colour, and wrinkled) were used to estimate absolute (AF) and relative (RF) fecundity. The ovaries were removed, weighed in mg and placed in Gilson's fluid for 1-3 days to harden the eggs and dissolve the ovarian membranes. Absolute fecundity was estimated using the gravimetric method, by removing three pieces from the anterior, medial and posterior of the ovary (Bagenal & Tesch 1978). Mean egg diameter was examined by measuring 25-30 eggs taken randomly from the ovaries of the females used for fecundity determination. Measurements were made to the nearest 0.05mm using a microscope with an ocular micrometer.

Fulton's condition factor (K_F) (Fulton 1904) was calculated using the equation: $K_F = 100 \times (W/L^3)$, where W is the total body weight (BW, g) and L is the total length (TL, cm). The scaling factor of 100 was used to bring the K_F close to unit. In allometric condition factor (K_A) was calculated using the equation of Tesch (1968): $K_A = W/L^b$, where W is the body weight, L is the TL and b is the LWRs parameter. Analysis of co-variance (ANCOVA) was performed to test for significant differences in weight-length relationships between sexes. Any significant difference in the overall sex ratio was assessed using the chi-square test (Zar 1984). Comparison of GSI values between sexes was carried out by analysis of variance (ANOVA). All statistical analyses were performed with a significance level of $P<0.05$ using the SPSS 21 software package.

Results
A total of 272 specimens of *P. malapterura* were caught during the sampling period. Males ranged from 35 to 95mm in length (mean=63.71±13.28) and 0.38 to 5.10g in weight (mean=2.07±1.22) and females ranged from 39 to 120mm in length (mean=66.58±14.35) and 0.44 to 9.58g in weight (mean=2.82±1.83). Opercula readings revealed that the majority of specimens were of age group 2$^+$, with

Table 1. Average total length (mm) and weight (g) at age of *Paracobitis malapterura* from Qom River, Iran.

Age group	Total length±SD	Min.–Max.	Total weight±SD	Min.–Max.
Male				
1+	62.38±12.51	35-67	1.85±0.53	0.38-2.46
2+	65.13±11.19	52-82	2.06±0.98	1.04-3.69
3+	90±5.00	85-95	4.74±0.37	4.37-5.10
Female				
1+	65.74±10.59	39-90	2.60±1.22	0.44-5.51
2+	75.63±11.04	53-98	3.92±1.86	1.40-9.02
3+	85.80±15.12	70-120	4.66±2.05	2.25-9.58
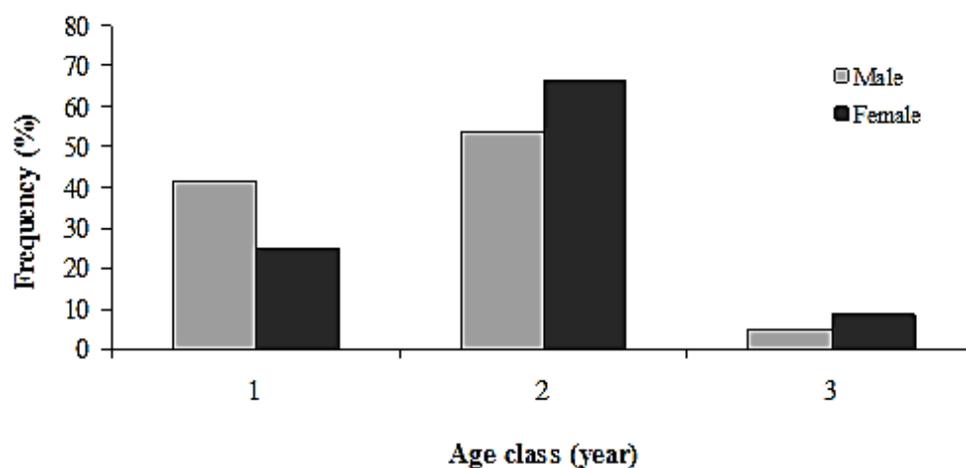
Fig.1. Age (year) frequency of male and female specimens of *Paracobitis malapterura* from Qom River, Iran.
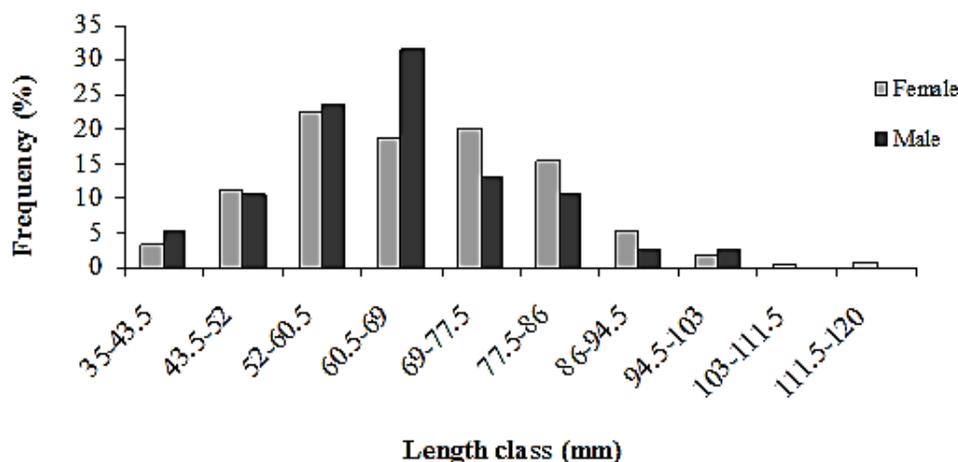
Fig.2. The length- frequency distribution of male and female *Paracobitis malapterura* from Qom River, Iran.

3+ being the oldest age recorded for both sexes. Observed length-at-age in the population was different between sexes, females being longer and heavier than males (Table 1).

Age frequency distribution of the fish (Fig. 1)

indicated that the most frequent age classes in the samples were 2 year for males and females. Length and weight frequency distribution of *P. malapterura* (Figs. 2 and 3) indicated that the most frequent size classes in the samples were 60.5-69mm and 0.38-
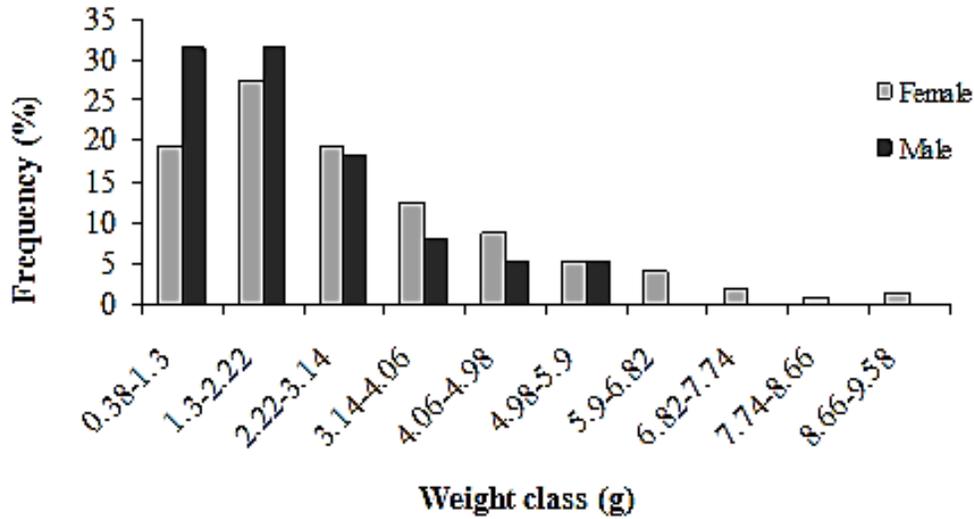
Fig.3. The weight-frequency of male and female *Paracobitis malapterura* from Qom River, Iran.
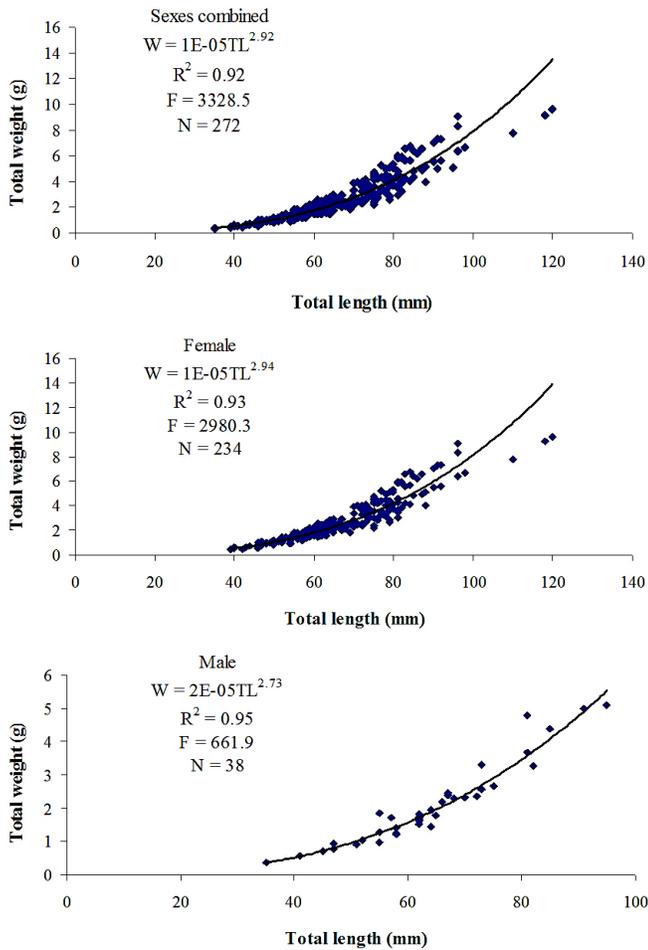

Fig.4. Relative growth curves (total length-total weight) for males, females, and sexes combined of *Paracobitis malapterura* from Qom River, Iran.

2.2g for males and 52-60.5mm and 1.3-2.2g for females. Females were rare in length classes greater than 111.5-120mm.

The overall ratio of males to females was 1:6.1 and Chi-square analysis indicated a significant difference from the expected ratio of 1:1 ($\chi 2 = 337.98$, $P>0.01$). Also, an unequal sex ratio was observed among the length classes (Fig. 2). The total length-weight relationships were evaluated for males, females and sexes combined. A significant relationship with the high regression coefficient ($r^2 >0.96$) was found between the length and weight of the loach. Length-weight relationships were found as $W=2E-06TL^{2.73}$ for males, $W=1E-05TL^{2.94}$ for females, and $W=1E-05TL^{2.92}$ for both sexes combined (Fig. 4).

The growth type was isometric for females and sexes combined, because the b value was not significantly different from 3 (Pauly's Test, $t_{female}=1.12$, $t_{sexes\ combined}=1.47$, and $t_{pooled}=1.96$, $P>0.05$) while growth model was negatively allometric for males (Pauly's Test, $t_{male}=2.58$ and $t_{pooled}=1.96$, $P<0.05$).

The GSI values of males were significantly lower than those of females. The maximum recorded values of GSI were 1.90 ± 0.52 and 13.85 ± 4.70 in April for males and females, respectively. The GSI of
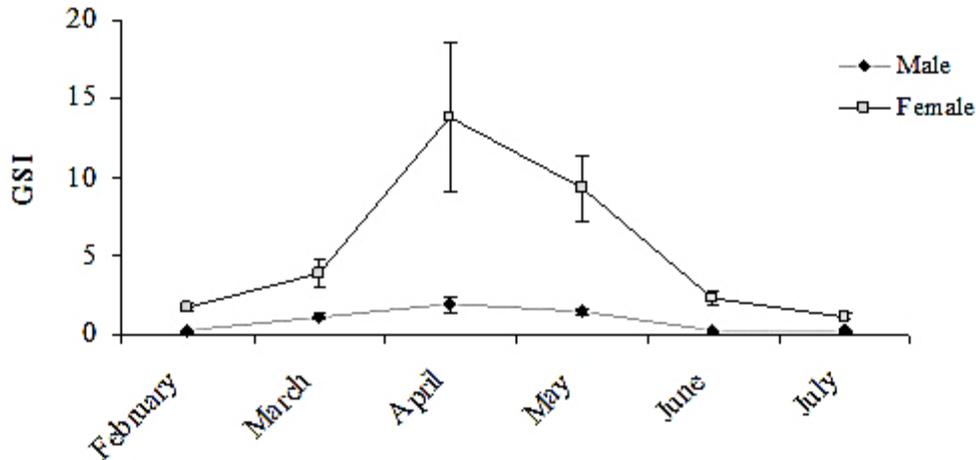
Fig.5. Monthly distribution of GSI for *Paracobitis malapterura* from Qom River, Iran.
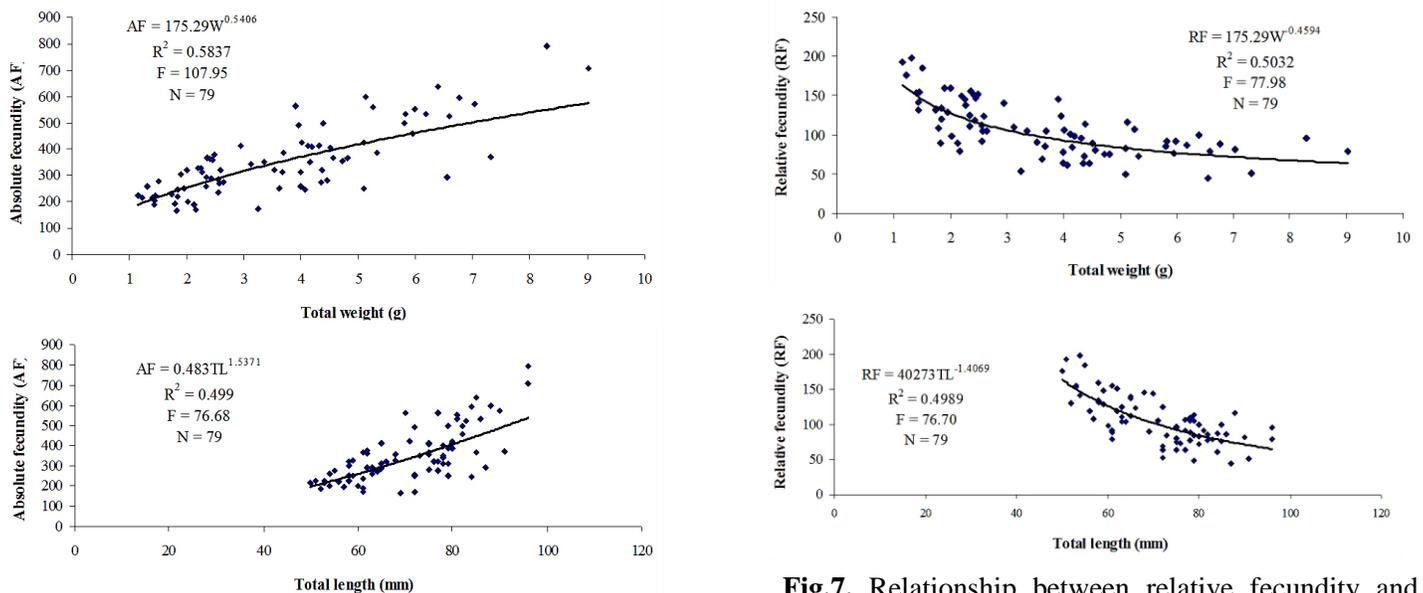

$AF = 175.29W^{0.5406}$
$R^2 = 0.5837$
$F = 107.95$
$N = 79$

$AF = 0.483TL^{1.5371}$
$R^2 = 0.499$
$F = 76.68$
$N = 79$

Fig.6. Relationship between absolute fecundity and total weight and total and length of female *Paracobitis malapterura* from Qom River, Iran.

$RF = 175.29W^{-0.4594}$
$R^2 = 0.5032$
$F = 77.98$
$N = 79$

$RF = 40273TL^{-1.4069}$
$R^2 = 0.4989$
$F = 76.70$
$N = 79$

Fig.7. Relationship between relative fecundity and total weight and total length of female *Paracobitis malapterura* from Qom River, Iran.

both sexes followed almost the same pattern (Fig. 5). Spawning occurred between March and April, when higher GSI values were observed. It thereafter decreases in May showing start of the resting period. Absolute fecundity ranged from 164 to 793 eggs/female with the mean of 353.3±132.7. There was significant relationship between absolute fecundity and fish size (in total length and weight) and the correlation coefficients calculated between fecundity and each of independent variables (length

and weight) were judged to be much moderate and statistically significant (Fig. 6).

The relative fecundity varied from 44 to 198 eggs (mean=107.5±34.6). The negative relationship between relative fecundity and body size was statistically significant (Fig. 7).

The different condition factors including allometric and Fulton's of *P. malapterura* from Qom River are shown in Table 2. The allometric condition factor values ranged from 0.0092 to 0.018 for males, and 0.0059 to 0.013 for females, with the mean of

Table 2. Fulton's Condition factors for males, females, and sexes combined for *Paracobitis malapterura* from Qom River, Iran.

	n	Min	Max	Mean ± SD	CL95%
Male	38				
K_F		0.56	1.11	0.73±0.11	0.69-0.77
K_A		0.0092	0.018	0.0119±0.0017	0.0113-0.0124
Female	233				
K_F		0.52	1.15	0.85±0.14	0.83-0.87
K_A		0.0059	0.013	0.0095±0.0016	0.0093-0.0097
Combined sex	271				
K_F		0.52	1.15	0.83±0.15	0.81-0.85
K_A		0.0061	0.014	0.0096±0.0017	0.0094-0.0098

N: Number; Min: Minimum; Max: Maximum; SD: Standard Deviation; CL: Confidence Limit for mean values;
KF: Fulton's condition factor; KA: Allometric condition factor.

0.0119±0.0017 and 0.0095±0.0016 for males and females, respectively. The Fulton's Condition Factor values ranged from 0.56 to 1.11 for males and 0.52 to 1.15 for females, with the mean of 0.73±0.11 and 0.85±0.14 for males and females, respectively.

Discussion

There is scarce data on the biology of *P. malapterura* from Iranian freshwaters. Average total length and weight (12.0cm and 9.58g) was observed in this study, was slightly less than length of 13.3 and 13.0cm, weight of 16.40 and 30.55g in *P. malapterura* reported by Tabiee & Abdoli (2005) and Patimar (2009), respectively. Compared to the other species, *P. malapterura* has a medium size (in total length and weight), and females exhibit a much wider range in length and a higher maximum length than males.

Variation in size (length and weight) in different populations of a species could be explained due to different exploitation patterns and/or ecological conditions (Jamali et al. 2015). In this sense, while the loach is not subject to commercial exploitation, variations in the environmental conditions seem to be the main factors affecting Iranian loach populations. The maximum age of *P. malapterura* was less than of those reported by Patimar et al. (2009) for *P. malapterura* (now *P. hircanica*) and Patimar et al. (2010) for *P. kessleri*. Patimar et al. (2009) and Patimar et al. (2010) found a maximum age of 4[+] years for *P. malapterura* (now *P. hircanica*) in Zarrin-

Gol River and *P. kessleri* in Zanglanlou River, respectively.

Weight-length relationships produced good fits and biologically sound results and could be used for comparison proposes (Jamali et al. 2015). The total length-somatic weight relationship showed that growth model was different in males and females, suggesting an apparent difference in fitness condition between the sexes. Analysis of the data available in the literatures (Przybylski & Valladolid 2000; Slavik & Rab 1996; Patimar et al. 2011a, b; Alavi Yeganeh et al. 2011; Daneshvar et al. 2013) shows that the value of *b* in loach species can vary considerably, indicating a change in body form with species, itself probably an effect of different environmental habitat conditions and species characteristics (i.e. morphological characteristics of the species).

Sex ratio of *P. malapterura* was 1:6.1 in favor of females. Nikolsky (1980) reported that sex ratio varied considerably from species to species; but in the majority of species, it is close to one. However, subsequent changes in this ratio may be explained by a number of hypotheses, including differences in habitat preference according to the season or sex, sampling errors, or selective mortality (Fernandez & Rossomanno 1997). Sex ratio was also reported significantly different for some species belonging to Nemacheilidae (e.g. *P. malapterura*, (1:1.27 in favor of females) (Patimar et al. 2009); and *Turcinoemacheilus hafezi*, (1:1.1 in favor of females)

(Jamali et al. 2014)).

For the species, reproductive investment is not homogeneous throughout the age-classes; fecundity is low in 1^+ females, but steadily rises in the subsequent age-classes, indicating positive effect of age (because of size) on fertility. The absolute fecundity of the species was different with range values reported elsewhere for other loaches. The maximum absolute fecundity calculated as 793 eggs from 3^+ years old specimens of *P. malapterura* female that is lower than the 1400 eggs (Lobon-Cervia & Zabala 1984) and higher than 1180 reported for *P. malapterura* (now *P. hircanica*) (Patimar et al. 2009). Compared to these loach species, therefore, the species is characterized by low fecundity.

The spawning of *P. malapterura* in the studied locality occurred once according to our observations and lasted 4 weeks, starting from late February, potentially in April. This rather short period of reproduction period may be a result of the unstable river environment in the sampling area. The course of spawning is similar to those of other loach species: *C.* cf. *satunini* (April-May) (Patimar et al. 2011a); *P. malapterura* (April-May) (Patimar et al. 2009); *M. cristata* (April) (Patimar et al. 2011b) and *T. hafezi* (April) (Jamali et al. 2014).

There is no published information on the Fulton's condition factor of *P. malapterura* which is comparable with the present results. The maximum Allometric and Fulton's condition factor of *P. malapterura* (0.014 and 1.15) was more than of that of *Oxynoemacheilus bergianus* (0.01 and 1.0) (Jamali et al. 2015. The condition factor reflects, through its variations, information on the physiological state of the fish in relation to its welfare. From a nutritional point of view, there is the accumulation of fat and gonad development (LeCren 1951). From a reproductive point of view, the highest *K* values are reached in some species (Angelescu et al. 1958). The condition factor also gives information when comparing two populations living in certain feeding, density, climate, and other conditions; when determining the period of gonad maturation; and when following up the degree of feeding activity of a species to verify whether it is making good use of its feeding source (Weatherley 1972).

In summation, the present study reports the first documentation on *P. malapterura* from the Namak lake basin, indicating its age, growth and reproduction. Although the Namak Lake basin has priority areas for ichthyodiversity conservation in Central Iran, there is no officially protected area for the fishes. In this sense, understanding the geographical distribution and spawning of the species are essential to promote strategies and select priority areas for conservation of the species. In this sense, this species must be considered as vulnerable in regard to its future survival.

Acknowledgments

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سن، رشد و تولیدمثل لوچماهی تاجدار *Paracobitis malapterura* (ماهیان استخوانی عالی: سگماهیان جویباری) از رودخانه قم، ایران

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چکیده: در این مطالعه ۲۷۲ نمونه لوچماهی تاجدار *Paracobitis malapterura* از رودخانه قم، حوضه نمک (مرکز ایران) برای تعیین سن، رشد و تولیدمثل در طی فصل تولیدمثل (از بهمن ماه ۱۳۹۲ تا تیرماه سال ۱۳۹۳) مورد بررسی قرار گرفت. نسبت جنسی نر به ماده ۱ نر به ۶/۱ ماده بود. در میان نمونههای بررسی شده حداکثر سن ⁺۳ سال بود. نمونهها در دامنه طولی ۳۵ تا ۱۲۰ میلیمتر و دامنه وزنی ۰/۳۸ تا ۹/۵۸ گرم قرار داشتند. رابطه رگرسیونی طول و وزن برای مادهها، نرها و هر دو جنس ترکیبی بهترتیب $W=1E-05TL^{2.94}$، $W=2E-05TL^{2.73}$ و $W=1E-05TL^{2.92}$ بود. الگوی رشد برای جنس ماده و هر دو جنس ترکیبی ایزومتریک و برای جنس نر آلومتریک منفی بود. بر اساس شاخص گنادی (GSI)، تخمریزی در اواخر بهمن ماه تا اواخر فروردین ماه صورت میگیرد. بزرگترین متوسط شاخص گنادی ۱/۹ برای نرها و ۱۳/۸۵ برای مادهها در فروردین ماه بود. دامنه همآوری مطلق از ۱۶۴ تا ۷۹۳ تخم با میانگین ۳۵۳/۳ تخم به ازای هر ماده بود. همآوری دارای همبستگی مثبت با اندازه ماهی (طول و وزن) بود.
کلماتکلیدی: لوچماهی تاجدار، رشد، تولیدمثل، حوضه نمک، ایران.