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Source: Folia Zoologica, 60(4) : 302-307

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v60.i4.a6.2011

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Folia Zool. – 60 (4): 302–307 (2011)

Age, growth and reproductive characteristics of the Turkmenian crested loach *Metaschistura cristata* (Nemacheilidae)

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Received 22 October 2010; Accepted 26 June 2011

Abstract. *Metaschistura cristata* is a small nemacheilid loach species found at a few localities only in Turkmenistan and Iran. The present study reports, for the first time, data on its biology, based on 747 specimens collected from the River Zanglanlou in north-eastern Iran. Specimens were collected at monthly intervals over two reproductive seasons from February to June 2007 and 2008. Maximum age, based on opercula readings, was 4+ years for both sexes. Specimens ranged in size from 25 to 87 mm total length and weighed from 0.09 to 4.91 g. Length-weight relationships implied positive allometric growth for both sexes and the sex ratio was 1 : 1.2 in favour of females. The Gonadosomatic indicated that peak reproduction occurred during April and May, with highest average values of 1.473 ± 0.99 for males and 12.9 ± 7.14 for females in April. Oocyte diameter ranged from 0.53 to 1.46 mm, with a mean value of 0.943 mm. Absolute and relative fecundity ranged between 114-1246 eggs and 79.47-1285.71 eggs/g, respectively. Absolute fecundity and oocyte diameter to fish size (length and weight) were directly correlated, while the relationship between relative fecundity and fish size (length and weight) showed an inverse power function.

Key words: age, growth, reproduction, *Metaschistura cristata*, Iran

Introduction

River loach species of the genus *Metaschistura* (family Nemacheilidae) are characterised by an elongated, almost cylindrical, body, an absence of scales, no collapsible spine under the eye, a dermal crest, rounded caudal fin, and often have a distinctive pattern of bars, stripes and blotches. The Turkmenian crested loach *Metaschistura cristata is* a monotypic genus occurring in just a few streams in north-eastern Iran and Turkmenistan. Abdoli (2000) has provided some information on the morphology and biology of this species, but, in general, very little is known about its distribution and biology. As a result of this lack of data, a proper assessment of the ecological and conservation status of the species is difficult. Habitat destruction, water removal and pollution, however, appear to be severe threats to the small and isolated waters in the distribution area of *M. cristata.* The purpose of this study, therefore, was to elucidate some aspects of age, growth and reproduction in a population of Turkmenian crested loach in north-eastern Iran, thereby contributing to its future conservation.

Material and Methods

The present study was carried out on the River Zanglanlou (total length 300 km (Afshin 1994)) in Khorasan Razavi province, north-eastern Iran. Sampling took place in the last week of each month from February to June in 2007 and 2008. A total of 747 specimens were caught by electrofishing (D.C. 200-300 V., 50 Hz. and one anode) and immediately preserved in 4 % formaldehyde solution.

In the laboratory, total length was measured to the nearest 1 mm for all fish sampled. Total weight and weight of ovary (inc. sub-samples) were recorded with an electronic analytical balance to the nearest 0.01 g. Fish age was determined from banding patterns on operculae examined under a binocular microscope under reflected light at ×10-30. Growth annuli from

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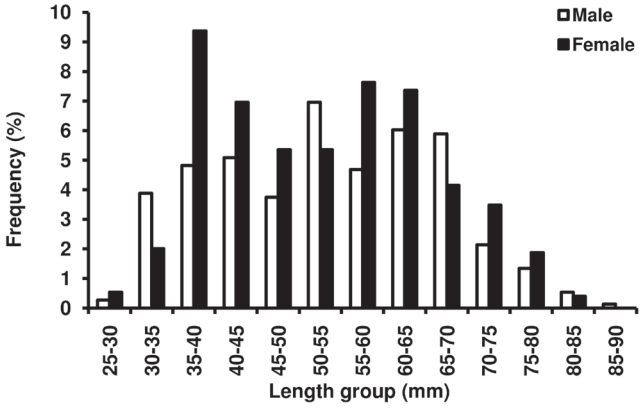

Fig. 1. *Total length (mm) frequency of males and females of* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

each operculum were counted three times, each time by a different person. The relationship between total length and total weight was determined by fitting the data in the form of: $W = aL^b$; where W is the weight in grams, L the total length in centimetres and a and b are the parameters to be estimated, with b being the coefficient of allometry based on the t-test (Pauly 1984).

Sex was determined by examination of gonad tissue, either by eye or with the aid of a binocular microscope (×10-40). The gonadosomatic index (GSI; gonad weight/total body weight × 100) was calculated for each fish and the mean calculated for each sampling date. To estimate fecundity, ovaries were removed from females, weighed, and then placed in Gilson's fluid for 3-4 days to harden the eggs and dissolve the ovarian membranes. The number of eggs was estimated via the gravimetric method (Bagenal & Tesch 1978) using pieces removed from both ovarian lobes of 155 ripe females caught in April. Average egg diameter was calculated by measuring 30 eggs for each female. Measurements were made to the nearest 0.05 mm using an ocular micrometer microscope.

The Pauly t-test (1984) was used to find out whether the calculated b value was significantly different from $b = 3$ (isometric growth). A comparison of GSI values during the reproductive period and of temporal variation in GSI for each sex was undertaken through analysis of variance (ANOVA). Analysis of co-variance (ANCOVA) was performed to test for significant differences in weight-length relationships between sexes. The overall sex ratio was assessed using the Chi-square test (χ^2; Zar 1984). Statistical analyses were performed using the SPSS 11.5 software package.

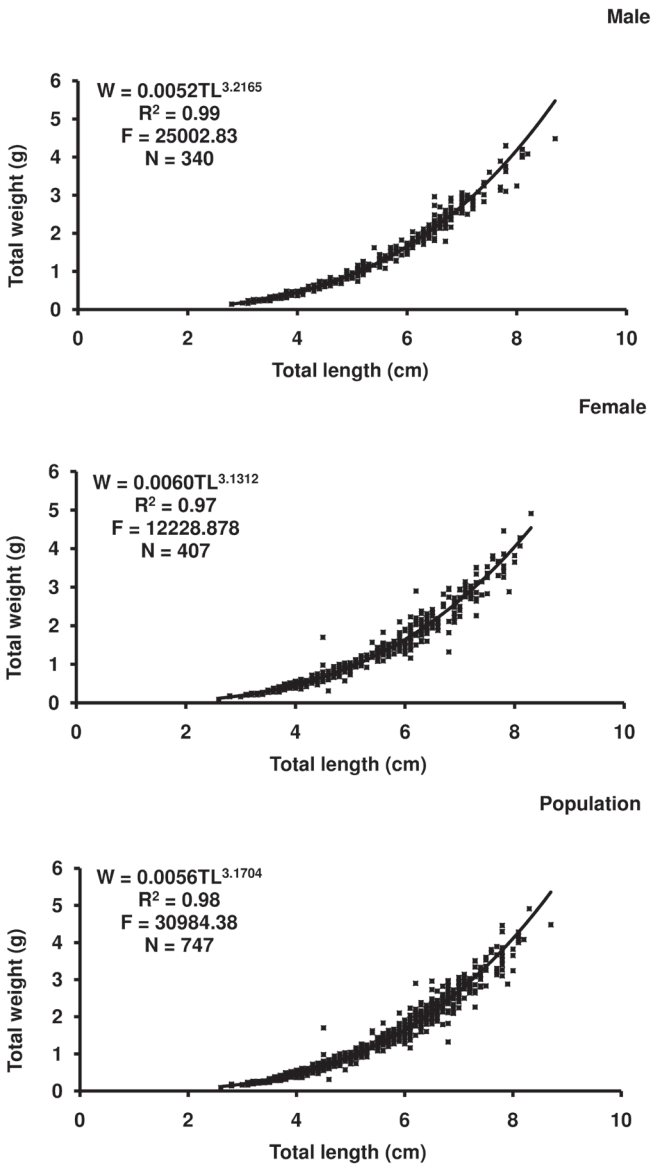

Fig. 2. *Relative growth curves (total length-total weight) for* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

Results

Age, growth and population structure

Specimens of *M. cristata* ranged from 25-87 mm total length and 0.09-4.91 g total weight. Males ranged from 28-87 mm and 0.10-4.48 g, while females ranged from 26-83 mm and 0.09-4.91 g. Length frequency distribution (Fig. 1) indicated that the most frequent size classes in samples were 50-55 mm for males and 35-40 mm for females.

Age estimates revealed that the loach has a maximum age of 4+ years in both sexes; the majority of fish in the population being 1+, representing 44 % of the total number of specimens. Observed length-at-age differed between sexes in the population, with females

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Table 1. *Mean total lengths (mm) at age (± S.D.) for* Metaschistura cristata *in River Zanglanlou – northeastern Iran.*

Age (year)	0+	1+	2+	3+	4+
Male	N = 18	N = 78	N = 148	N = 82	N = 14
Mean observed TL(mm)	29.00 ± 3.20	39.49 ± 8.21	56.15 ± 8.17	69.33 ± 8.74	82.40 ± 4.10
Female	N = 12	N = 120	N = 168	N = 94	N = 13
Mean observed TL(mm)	28.51 ± 4.11	41.01 ± 5.78	58.14 ± 6.01	72.03 ± 9.01	83.00 ± 5.05

being slightly longer than males (Table 1; ANCOVA, $F = 11.02$, $p < 0.05$).

Significant differences were observed in length-weight relationships (Fig. 2), while *b*-values implied that the body shape displays positive allometric growth (t-test, $t_{male} = 11.77$).

Sex ratio

All individuals were sexed and the overall ratio of males to females observed was 1 : 1.2. Chi-square analysis showed a significant difference from the expected ratio of 1 : 1 ($\chi^2 = 6.009$, $p < 0.05$). An unequal sex ratio was observed in most length classes (Fig. 1).

Gonadosomatic index

Significant changes were observed in temporal variation of gonad activity (ANOVA, $F_{female} = 15.08$, $F_{male} = 6.71$, $p < 0.05$). GSI values for males were significantly lower than those for females (ANOVA, $F = 59.14$, $p < 0.05$). The highest average recorded values (± standard deviation) of GSI were 1.473 ± 0.99 in April 2008 for males and 12.9 ± 7.14 in April 2007 for females (Fig. 3). According to the seasonal cycle of gonadosomatic index (Fig. 3), highest reproductive activity of this species in the River Zanglanlou, indicated by a considerable increase in GSI, extends from March to May.

Fecundity

The analysis showed a positive effect of age and size on fecundity, with a minimum number of 114 eggs from a 1+ female weighing 0.42 g and a maximum number of 1246 eggs from a 4+ fish weighing 4.46 g. The mean value of absolute fecundity was 350.80 ± 205.42 eggs/female. Fecundity-total weight and fecundity-total length correlations (Fig. 4) displayed moderate linear relationships that were statistically significant ($p < 0.05$).

Relative fecundity fluctuated between 79.47 and 1285.71 eggs/g, with a mean value of 287.78 ± 215.41. Relationships between relative fecundity (per gram) and fish size (total weight and length) showed an inverse power function (Fig. 5) and were statistically significant ($p < 0.05$).

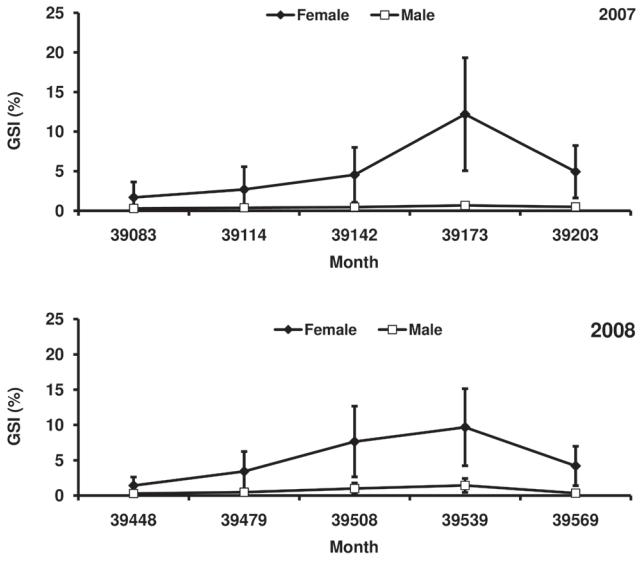

Fig. 3. *Monthly variation of GSI in* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

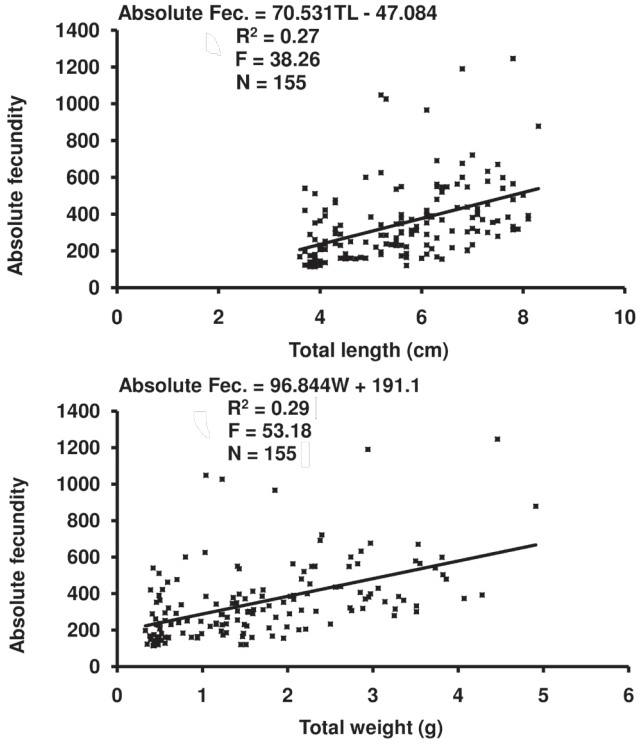

Fig. 4. *Relationship between absolute fecundity and fish size (total length mm and total weight g) of female* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

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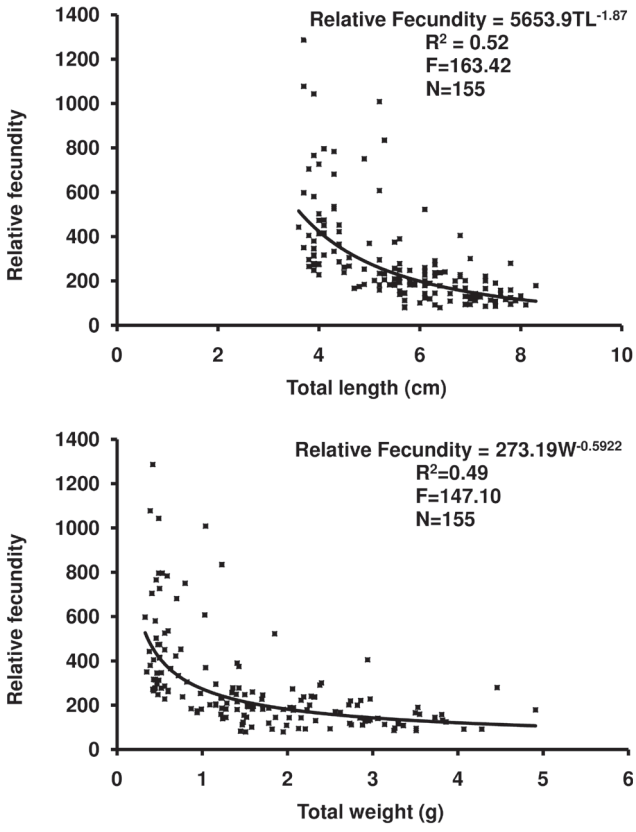

Fig. 5. *Relationship between relative fecundity and fish size (total length cm and total weight g) of female* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

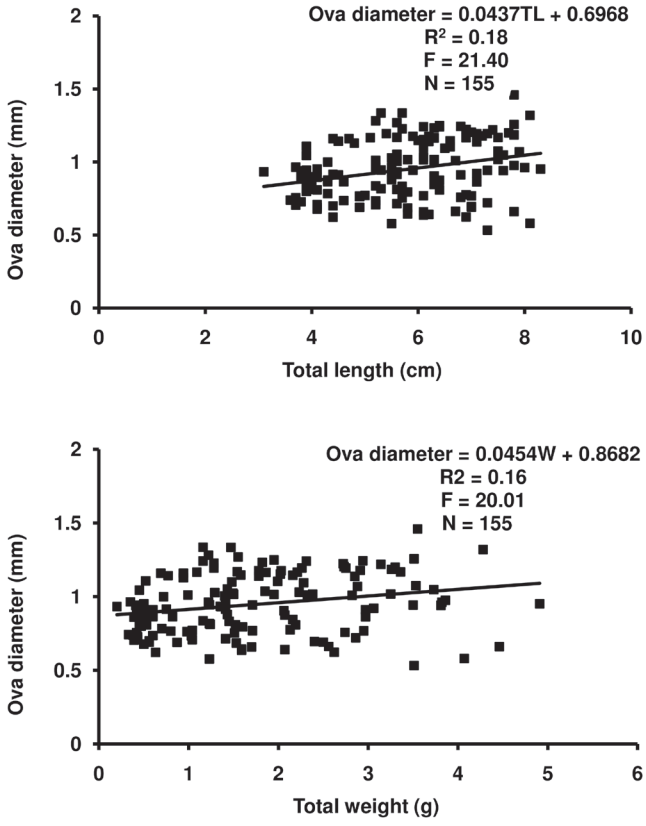

Fig. 7. *Relationship between egg diameter and fish size (total length cm and total weight g) of female* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

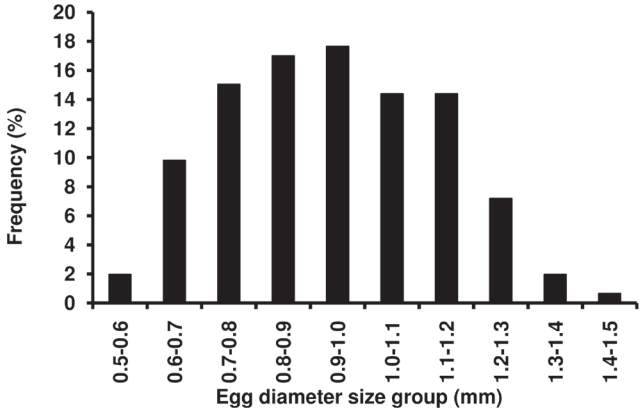

Fig. 6. *Size frequency distribution of egg diameters of* Metaschistura cristata *in the River Zanglanlou – northeastern Iran.*

Egg diameter

Egg diameters ranged from 0.53 to 1.46 mm, with a mean value of 0.943 ± 0.196. Size distribution of the eggs indicated that the majority ranged from 0.7 to 1.2 mm (Fig. 6). Egg diameter was significantly correlated ($p < 0.05$) with both female total length and weight, though with a moderate correlation coefficient (Fig. 7).

Discussion

During sampling we observed that *M. cristata* occurred in high numbers at the investigated locality, indicating that local conditions were highly suitable for this species.

Very little ecological data exists for Nemacheilidae species. Smyly (1955) and, more recently, Vinyoles et al. (2010) have published comprehensive studies on European *Barbatula barbatula*; however, due to completely different habitat preferences, this species cannot be used for comparison with *M. cristata*. In comparison with other nemacheilid loach species from Iran (Abdoli 2000), *M. cristata* has a medium life-span not exceeding 4-years and reaches a comparatively large size (by both length and weight). Only the crested loach *Paracobitis malapterura*, which can reach a maximum length and weight of 130 mm and 30.55 g, respectively (Patimar et al. 2009), is larger than *M. cristata*.

The *b*-values observed in this study conform to the suggestion of Carlander (1969) that *b* normally falls between 2.5 and 3.5. The weight-length relationship exponent indicated that growth is positively allometric

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in this species, with a *b*-value > 3 indicating that the fish becomes more rotund as length increases with age. A weight-length relationship has not previously been established for Turkmenian crested loach and, therefore, the level of variation in the allometry coefficient remains unclear. The functional regression *b* value is directly related to weight and is affected by ecological factors such as temperature, food supply, spawning conditions and habitat characteristics within each year (Ismen 2005). Differences observed in allometry coefficient suggest an apparent difference in fitness condition between sexes. In comparing species, the *b*-values estimated for the studied population of *M. cristata* differ from those found for *P. malapterura* (Patimar et al. 2009), reflecting a change in body-form between species (i.e. morphological characteristics of the species) and, possibly, the effect of differing environmental conditions acting as a local selective pressure on the species.

The sex ratio of *M. cristata* was 1 : 1.2 in favour of females. Nikolsky (1963) pointed out that the ratio of males is higher during early life stages, but that the female ratio becomes higher during later stages. In accordance with this, the slight dominance of females in *M. cristata* could be the consequence of a higher survival rate of adult females. A similar female biased sex ratio has also been observed in other species of Nemacheilidae (e.g. *P. malapterura*, Patimar et al. 2009; *Barbatula barbatula*, Vinyoles et al. 2010).

In north-eastern Iran, spawning of *M. cristata* occurs during April and, potentially, May. This does not correspond with duration of spawning period for some other species of loache in Iran (e.g. the reproductive season of *P. malapterura* in northern Iran extends from April to June). This rather short period of reproduction may be a result of the unstable river environment in the sample area. The GSI values for *M. cristata* are commonly higher than those for *P. malapterura* (Patimar et al. 2009), which could be interpreted through a higher energetic investment in reproduction.

There is a widespread trend for fecundity in fishes to be positively correlated with length (Peters 1983) as the amount of energy available for egg production and the egg accommodation capacity of the body cavity increases with fish size (Jonsson & Jonsson 1999). In the present study, absolute fecundity was positively correlated with fish size (length and weight), indicating that total energetic investment in reproduction tends to increase with fish size. The relationship between relative fecundity and both fish weight and length was negatively correlated, i.e. the quantity of eggs per unit somatic weight did not increase proportionally in larger spawners and the quantity of eggs showed a relative decrease with increase in fish size. This means that total energetic investment in reproduction tends to be higher in larger fish and the proportional energetic investment in reproduction (as energy allocation per unit of fish size) tends to be decreased with increase in fish size. For *M. cristata*, estimated absolute and relative fecundities differed from those previously reported for *P. malapterura* (see Patimar et al. 2009). For example, absolute fecundity for *M. cristata* is lower than that for *P. malapterura*, with mean values of 350.80 eggs/female and 456 eggs/female, respectively. The largest recorded eggs of *M. cristata* (1.46 mm) were considerably smaller than those recorded for *P. malapterura* (2.80 mm) by Patimar et al. (2009). Additionally, the range in egg size was wider for *P. malapterura* (0.11-2.80 mm), implying that *M. cristata* has a lower relative variation in egg size. Inter-loach species variations in egg size suggest that the two species follow different reproduction strategies.

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