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Some aspects of the life history of *Turcinoemacheilus hafezi* (Teleostei: Nemacheilidae) from Beshar River, southwestern Iran

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Abstract: We collected the specimens of *Turcinoemacheilus hafezi* throughout the spawning season (January to July, 2012) in Beshar River (south-west Iran) and investigated its age, growth, and reproductive characteristics. The maximum age was 2^+ years. Total length and weight of the specimens ranged from 29 to 61 mm and 0.17 to 1.39 g, respectively. Length-weight relationship was estimated as W = 3E-05TL$^{2.57}$ for females, W = 3E-06TL$^{3.22}$ for males and W = 1E-05TL$^{2.88}$ for the population. Sex ratio was 1:1.1 in favor of males. The growth model was isometric for males and sexes combined and negative allometric for females. Based on the gonadosomatic index (GSI) values, spawning appears to occur between late February and late April in Beshar River. The highest mean GSI was 6.73 for males and 14.50 for females in April. The absolute fecundity ranged 42–726 with a mean of 184.87 eggs. Fecundity was also positively correlated with fish size (length and weight). Egg diameter ranged from 0.4 to 1.6 mm with a mean of 0.84 mm.

Keywords: Loaches, Growth, Reproduction, Beshar River, Iran

Introduction

The family Nemacheilidae is represented in Iran by 14 species (Nalbant & Bianco 1998). Nemacheilian loaches have no commercial value but are important for the ichthyofauna (Golzarianpour et al. 2011). They inhabit a variety of inland waters, e.g. turbulent mountain streams to salty rivers in dry lowlands (Abdoli 2000). However, there is little information on their general biology in Iran (Tabiee & Abdoli 2005; Esmaeili & Ebrahimi 2006; Heydarnejad 2009). The loach is found in some rivers in the western and southwestern Iran, including Tigris, Karun and Kor rivers (Saadati 1977; Coad 1987; 2000; Abdoli 2000; Keivany et al. 2014).

Loaches of the genus *Turcinoemacheilus* are small fishes immediately distinguished from all other nemacheilids in the Middle East by the more anterior position of the anus (Banarescu & Nalbant 1964; Freyhof et al. 2011). *Turcinoemacheilus* have been recorded only a few times and records came from the Euphrates-Tigris drainage and all were identified as *T. kosswigi* (Banarescu & Nalbant 1964; Nalbant & Bianco 1998; Breil & Bohlen 2001; Golzarianpour et al. 2009).

Golzarianpour et al. (2013) recently described *Turcinoemacheilus hafezi*, as a new nemacheilid species from western Iran. *Turcinoemacheilus hafezi* (hafez loach) is known from the Karun and Dez River drainages. These rivers drain from the Zagros Mountains and flow into the lowermost part of the Tigris (Golzarianpour et al. 2013). Detailed description of its life history has not been given in the literature. In this context, examination of the basic biological parameters for each species is fundamental for understanding species life history patterns and important with respect to implementing

Table 1. The results of artificial spawning of Eurasian perch females which had the oocytes in 2nd maturity stage.

Age group	Total length ± SD	Min.–Max.	Total weight ± SD	Min.–Max.
Male				
1^+	41.22±5.60	32-50	0.52±0.0.23	0.20-0.92
2^+	48±4.24	41-55	0.86±0.24	0.58-1.23
Female				
1^+	42.23±5.68	29-55	0.54±0.18	0.17-0.99
2^+	51.32±5.09	45-61	0.92±0.20	0.68-1.39

effective management and conservation measures for the species. In light of this hypothesis, the present contribution serves to describe detailed life history of *T. hafezi* from Beshar (Bashar) River, southwestern 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 Beshar River which is located in Kohgiluyeh and Boyer Ahmad Province, southwestern Iran. Beshar River stretches 100 Km and originating from the mountains of Fars Province, and draining into the Karun River. The specimens were caught using a beach-seine with a mesh size of 2 mm. The net was chosen for its simplicity and higher catch efficiency when sampling small specimens. Monthly sampling took place from January to July 2012. In the field, all fish specimens were immediately preserved in 10% formaldehyde solution until they could be examined. A total of 189 specimens were analyzed for their life history attributes. In the laboratory, all specimens were measured for total length (TL), total body weight (TW) and gonad weight (to the nearest 0.01 g). Age was determined from both left and right opercula; banding patterns being reviewed three times (each time by a different person) using a 20-40× binocular microscope under reflected light.

The relationship between TL and TW was determined using the equation: $TW = \alpha TL^b$; where α is the intercept and b is the slope (coefficient of allometry), as per Pauly (1984). Sex was determined by visual examination of the gonad tissue. The gonadosomatic index (GSI % = [gonad weight/TW] × 100) was calculated for each fish and mean values calculated for each sampling date. The ovaries of 58 ripe females at maturity stage IV were used to estimate absolute (AF) and relative (RF) fecundity. The ovaries were removed, weighed 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 0.05 mm using a microscope with an ocular micrometer.

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 at a significance level of $P<0.05$ using SPSS 21 software package.

Results

A total of 189 specimens of *Turcinoemacheilus hafezi* were caught during the sampling period. Males ranged from 32 to 55 mm and 0.2 to 1.23 g, while females ranged from 29 to 61 mm and 0.17 to 1.39 g. Opercula readings revealed that the majority

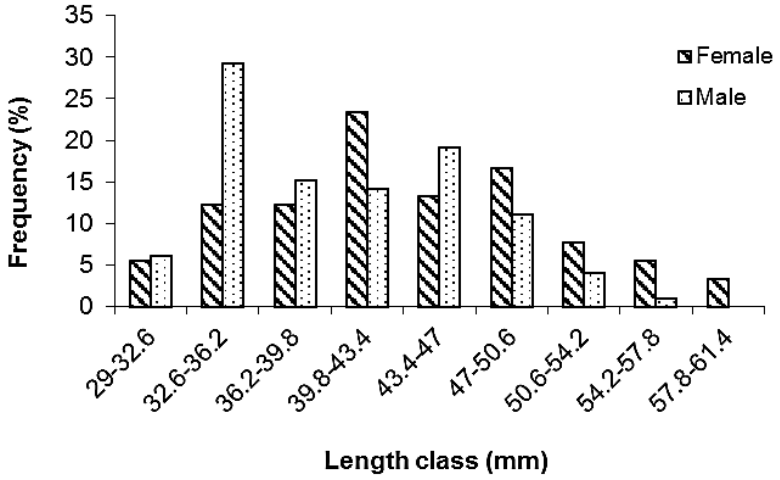

Fig.1. Total length (mm) frequency of male and female *Turcinoemacheilus hafezi* in Beshar River, southwestern Iran.

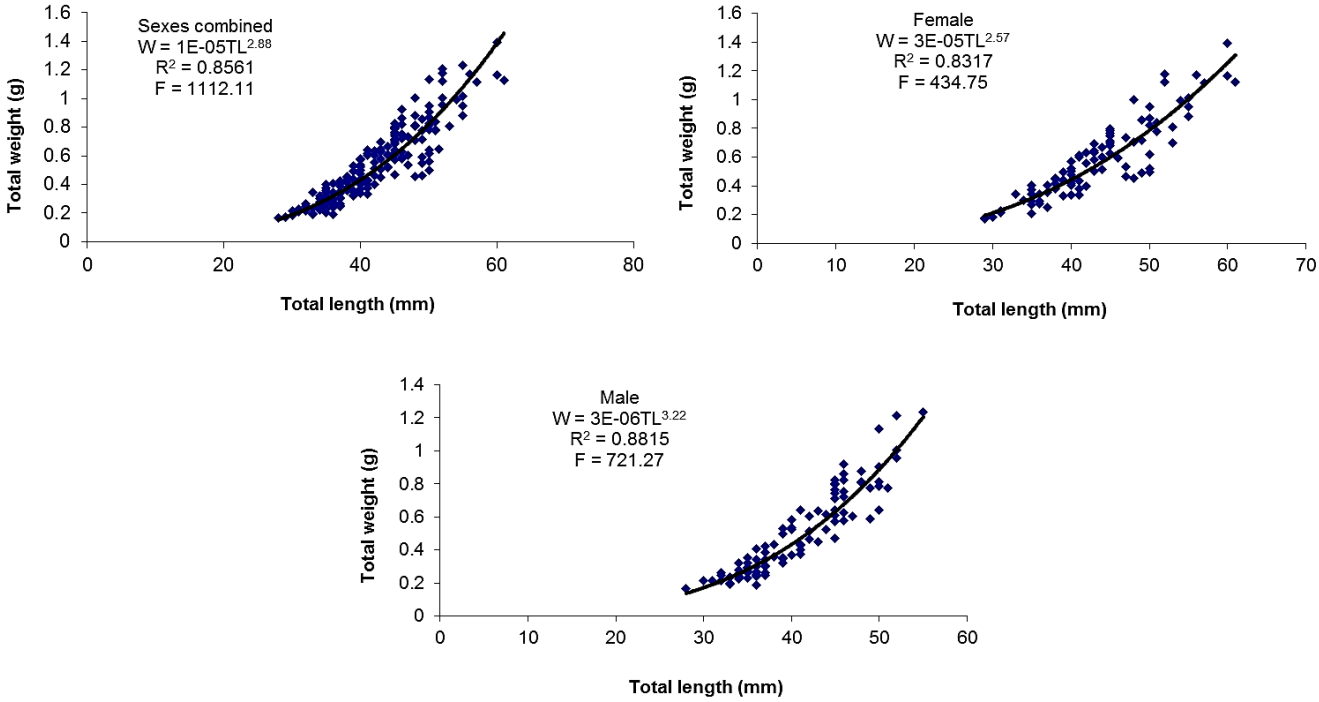

Fig.2. Relative growth curves (total length vs. total weight) for females, males and sexes combined of *Turcinoemacheilus hafezi* in Beshar River, southwestern Iran.

of specimens were of age group 1+, with 2+ 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). Length frequency distribution of the fish (Fig. 1) indicated that the most frequent size classes in the samples were 32.6-36.2 mm for the males and 39.8-43.4 for the females. Males were rare in length classes greater than 54.2-57.8 mm.

The growth model was isometric for males and sexes combined, because the *b* value was not significantly different from 3 (Pauly's Test, t_{male}=1.80, $t_{sexes\ combined}$=1.48, t_{pooled}=1.96, P>0.05) while growth model was negatively allometric for females (Pauly's Test, t_{female}=3.44, t_{pooled}=1.96, P<0.05).

The overall ratio of females to males was 1 : 1.1 and Chi-square analysis indicated a significant difference from the expected ratio of 1 : 1 ($\chi 2$ =

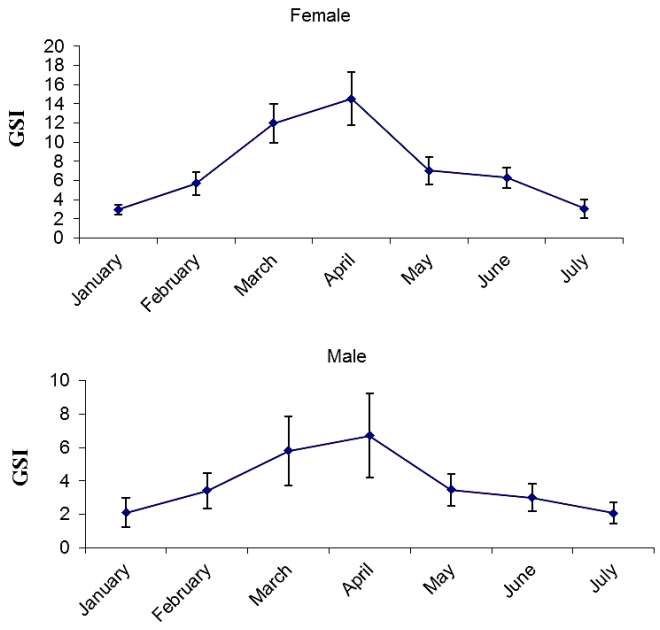

Fig.3. Monthly distribution of GSI in hafez loach in the Beshar River. Top, females; below, males.

17.36, P<0.05). Also, an unequal sex ratio was observed amongst the length classes (Fig. 1). The total length-weight relationships were evaluated for males, females and sexes combined. A significant relationship with the high regression coefficient (r >0.91) was found between the length and weight of the loach. Length-weight relationships were found as $W = 3E\text{-}06TL^{3.22}$ for males, $W = 3E\text{-}05TL^{2.57}$ for females, and $W = 1E\text{-}05TL^{2.88}$ for sexes combined (Fig. 2).

The GSI values of males were significantly lower than those of females. The maximum recorded values of GSI were 6.73 ± 2.52 and 14.50 ± 2.78 in April for males and females, respectively. The GSI of both sexes followed almost the same pattern (Fig. 3). The reproductive period for this species in the river is thus March and April when GSI is considerably higher. It thereafter decreases in May showing start of the resting period.

The mean value of absolute fecundity was 184.87 ± 54.27 (SD) eggs/female, it ranged from a minimum value of 42 eggs to a maximum value of 726 eggs. There was no significant relationship between absolute fecundity and fish size (total length and

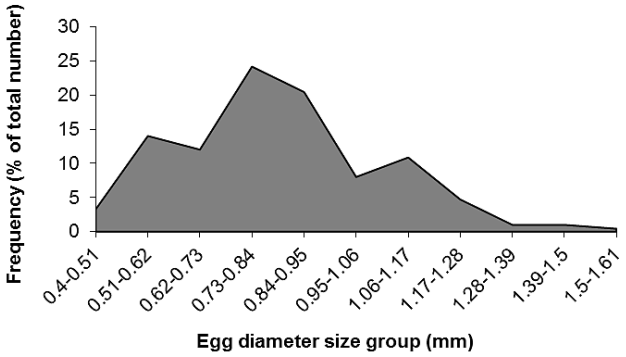

Fig.4. Size frequency distribution of oocyte diameter in female hafez loach in Beshar River.

weight) and the correlation coefficients calculated between fecundity and each of independent variables (length and weight) were low and were not statistically significant. The relative fecundity relative fluctuated from 43 to 765 eggs (236.7 ± 165.5). The relationship between relative fecundity and fish body size (either to the length or to the weight) was not statistically significant.

The ovaries of mature females contained large yolk-filled eggs that ranged in size from 0.4 to 1.6 mm (mean: 0.84 ± 0.21). The majority of oocytes were between 0.73 to 0.84 mm in diameter (Fig. 4). The relationship between egg diameter and fish body size (either to the length or to the weight) was not statistically significant.

Discussion

Because of lack of data on the *Turcinoemacheilus hafezi*, it is rather difficult to describe the current position of biology and ecology of the species. The sample size clearly indicates that the species has a population distributed in the river with low numbers, indicating that the species may be at risk because of ecological threats. Unfortunately, very little ecological data exists for Nemacheilid species. The population displays an age structure in which the younger age-classes are underrepresented in the overall sample. Longevity was 2 years, which is in line with observations made in other loach species, indicating that the species has a relatively short life span, a trend common to the loaches. Our analyses

revealed no sexual dimorphism in growth, though females have occasionally been found to grow more rapidly than males. Males *T. hafezi* have a smaller body size compared to females, confirming the general sexual dimorphism for species of the family Nemacheilidae. The narrow size range of age groups indicates a single short spawning season producing single cohort.

Weight-length relationships produced good fits and biologically sound results and could be used for comparison proposes. 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 literature (Przybylski & Valladolid 2000; Slavik & Rab 1996; Patimar et al. 2011a, b) shows that the value of *b* in loach species as in other species (Alavi Yeganeh et al. 2011; Daneshvar et al. 2013) 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).

Turcineomacheilus hafezi provides the first record of a strongly male-biased sex ratio in population of Nemacheilid fishes in Iran. 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 different with species of Nemacheilidae (e.g. *Paracobitis malapterura*, Patimar et al. 2009; *Barbatula barbatula*, Vinyoles et al. 2010).

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 on fertility. The absolute fecundity of the species was different with range values reported elsewhere for other loaches. The maximum absolute fecundity value of 765 eggs from a 2^+ years old *T. hafezi* female is lower than the 1400 eggs (Lobon-Cervia & Zabala 1984) and 1986 eggs (Oliva-Paterna et al. 2002) observed for *Cobitis paludica*, and the 4282 eggs for *C. taenia* (Bohlen 1999) and 4666 for *C.* cf. *satunini* (Patimar et al. 2011a), and higher than 1180 for *P. malapterura* (Patimar et al. 2009) and 1246 for *Metaschistura cristata* (Patimar et al. 2011b). Compared to these loach species, therefore, the species is characterized by low fecundity.

The spawning of *T. hafezi* 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 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) and *M. cristata* (April) (Patimar et al. 2011b). The mean maximum GSI values for females *T. hafezi* are commonly higher than those for *C.* cf. *satunini* (Patimar et al. 2011a), *M. cristata* (Patimar et al. 2011b), and *P. malapterura* (Patimar et al. 2009).

In summation, the present study reports the first documentation on *T. hafezi*, indicating age, growth and reproduction. Although the Karun River Basin has priority areas for ichthyobiodiversity conservation in south-west 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.

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