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First Report on Growth and Reproduction of *Turcinoemacheilus Bahaii* (*Esmaeili, Sayyadzadeh, Özulug, Geiger* and *Freyhof*, 2014), in Zayandeh Roud River, Iran

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Abstract

To provide information on growth and reproductive features of recently identified *Turcinoemacheilus bahaii* from Zayandeh Roud River (Isfahan basin, Iran), sampling was performed at monthly interval throughout the year (from February 2013 to January 2014) and 868 individuals were caught by gillnet. The overall sex ratio (Male: Female) was 0.61:1 in favor of females. Based on otoliths reading, fish age was ranged from 1^+ to 4^+ and 1^+ to 5^+ for males and females, respectively. Negative allometric growth was recorded for both sexes and total populations (r^2>0.84). The von Bertalanffy growth equations were different between males and females, revealing a strong fit for both males {L_t=73.99(1-exp (-0.25(t^+2.68)} and females {L_t= 100.35(1-exp (-0.19(t+1.86)}. Sexual maturity achieved at age 1^+ and 2^+ for males and females, respectively. Based on the GSI, egg diameter, histological studies, and macroscopic characteristics of the *gonad T. bahaii* is considered asynchronous spawner with relatively long spawning season, extending from April to June. Absolute fecundity varied from 95-751 (365.75 ± 132.67) eggs and relative fecundity ranged from 94.06 to 224.85 (187.27 ± 29.75) egg/g body weights. Egg diameter ranged from 0.025 to 1.58mm.

Keywords: Loach; Age; von Bertalanffy; Growth function; Reproduction; Zayandeh Roud River

Introduction

The loaches of Iran belong to families, *Nemacheilidae* and *Cobitidae*. The members of *Nemacheilidae* are small benthic freshwater fish inhabiting running water in Asia, Europe and Ethiopia [1] and they were recently classified in seven genera [2]. Genus *Turcinoemacheilus* have been recorded scarcely [3] and added to the *nemacheilid* loach's of Iran in recent years. Nowadays, loaches of this genus are known to be very widespread and easy to distinguish from all other *Nemacheilids* [4,5].

In general, the *Nemacheilid* loaches of western Asia are poorly known due to small size and limited commercial value [1,5], recently described *Turcinoemacheilus bahaii* as a new *Nemacheilid* species from central part of Iran. *T. bahaii* is an endemic species of Zayandeh Roud River basin and its distribution and biology has not been reported in the literature so far. However, there are few studies on general biology of some other closely related species [6-9]. Hence, the principal aim of this paper was to provide data on the growth and reproductive details of *T. bahaii*, through gonad visual and histological examination, which are necessary for understanding the basic ecology of the species and also for conservation purposes. We believed that information on the growth and reproductive biology of this small and colourful Loach is important because it may enter the aquarium trade.

Materials and Methods

Present investigation was performed in Zayandeh Roud River which is the largest river in the entire interior basins of Iran. The River flows eastward from the high central part of Zagros, after passing through Esfahan province and terminates in Gavkhooni wetland.

Sampling on the basis of once per month, was carried out from February 2013 to January 2014 (in the last week of each month and before noon at two separate sites, 15 km apart, along the River (site 1: 32° 51′ 86″ E, 51° 22′ 72″ N and site 2: 32° 51′ 79″ E, 51° 37′ 33″ N) (Figure 1). To understand the habitat characteristics of the fish, besides fish sampling, some environmental factors including water temperature (C°), dissolved oxygen (mg L^{-1}), p^H and electrical conductivity (μs cm^{-1}) were measured in situ. River depth ranged from 70-170cm, width ranged from 6-15m, water temperature ranged from 2-22°C (12.98±5.45 mean±SD), dissolve oxygen ranged from 6.1-13.11mg L^{-1} (10.23±1.16 mean±SD), p^H ranged from 7.21-8.89 (7.98±0.39 mean±SD) and electrical conductivity ranged from 241-424μs cm^{-1} (302.88±39.92 mean±SD)).

A total of 868 (324 males, 533 females and 11 immature) specimens were collected using two layer gillnet (with an inner mesh of 5mm and outer mesh of 1mm) (about 35 fish per month from each site). All captured specimens were immediately preserved in 4% formaldehyde solution and transferred to the laboratory for morphometric and

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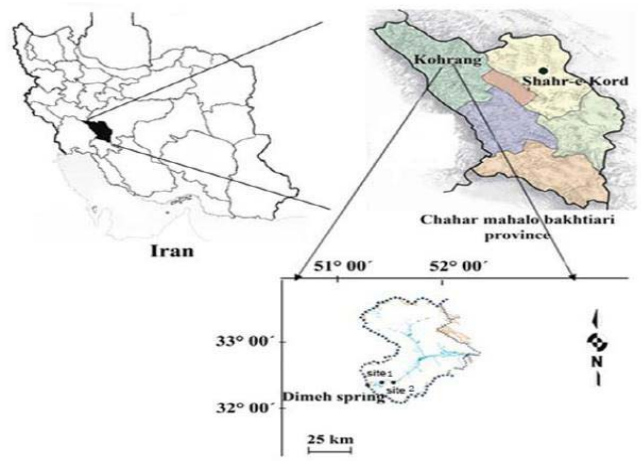

Figure 1: The study area and location of sampling sites in Zayandeh Roud River.

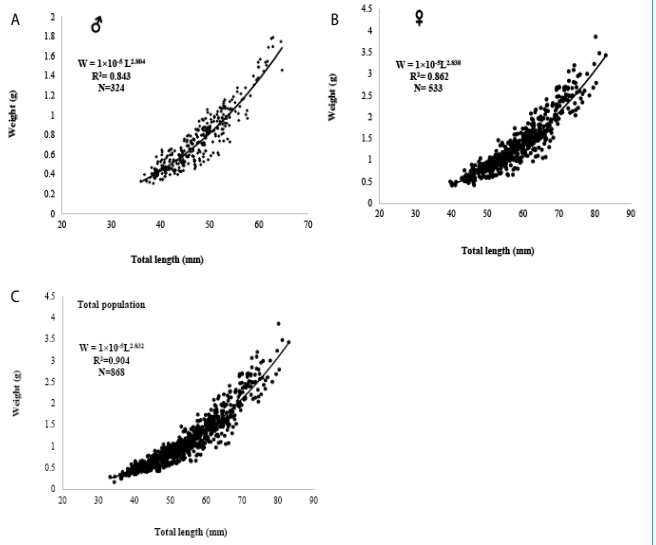

Figure 2: Relationships between length-weight for males, females and total population of *T. Bahaii* in Zayandeh Roud River.

other biological measurements. After measuring the total length of each fish to the nearest 0.01mm, the total body weight and gonads weight were measured with an electronic analytical balance to the nearest 0.01g and 0.0001g, respectively. Sex was determined by examination of the gonad tissue, either by eye for larger fish or by the aid of a microscope for small specimen. The overall ratio of males to females was tested by X^2- test. The age of fish was determined by counting otolith annual growth rings [6,8,10,11].

The length-weight relationship was determined according to the equation: $W=a\ L^b$, where W is total weight; L is total length; a and b is the constants. The allometric growth index value (b) obtained was compared to the expected value by using a t-test for allometry [12]. The von Bertalaneffy growth function, $L_t = L_\infty(1-e^{-K(t-t_o)})$, was used to describe the growth of male and female of *T. bahaii*, where L_t is the total length at age t; L_∞ is the asymptotic length; K is the growth coefficient; t is the age (year from birth; t_o is the theoretical age at length zero [13]. Specific Growth Rate (SGR) was determined using the equation: $r=Ln(W_{t+1})-Ln(W_t)/\Delta t$, where r is the specific growth rate, Wt^+1 is the mean weight at age t^+1, Wt is the mean weight at age t and Δt is the time interval between age t and t+1 [14]. Sexual maturity was examined using the gonad development stages criteria and monthly variation in egg diameters [15]. Age and length at first maturity were considered as age and length at which 50% of the population was matured. To assess the monthly change in gonads as a mean for estimating the spawning season of *T. bahaii*, Gonado Somatic Index (GSI) was calculated following the formula: GSI= (gonad weight (g) / body weight (g)) × 100. The fish gonads taken for histological analyses were fixed in buffered formalin, dehydrated then paraffin – embedded. Histological sections (5-7μm) were stained with Hematoxylin and Eosin (H&E.) [16]. The developmental stages of gonads were determined as described by [17]. Fecundity was estimated for a number of fish at stage III of ovarian development (usually during April and May). Ovaries were kept in Gillson's fluid for subsequent separations of eggs. Then the total numbers of eggs was calculated. Egg diameter measurements were made by using an ocular micrometer. The data are presented as the mean ± Standard Deviation (SD). Differences were regarded as significant when $p<0.05$. The analysis was conducted using SPSS 18.0 and Excel 2013 computer software.

Results

Age and growth

The total length and weight of specimen used for estimating growth criteria ranged from 36.05 to 83.07mm and 0.31 to 3.87g, respectively. Males ranged from 36.05 to 64.78mm TL (47.52±6.03, mean±SD) and 0.31 to 1.79g W (0.75±0.31, mean±SD), while females ranged from 39.75 to 83.07mm TL (58.45±8.42, mean±SD) and 0.41 to 3.87g W (1.35±0.61, mean±SD). Comparison of the length and weight of males and females of *T. bahaii* showed that the females were significantly larger and heavier than males (t-test, $p<0.05$). Highly significant correlations ($r^2>0.82$) were found between total length and weight of both sexes (Figure 2). The b value for males, females and combined sexes indicates a negative allometric growth for *T. bahaii* (t-test, $p<0.05$). The age groups of captured fish were different for males (1^+ to 4^+) and females (1^+ to 5^+). The age group 1^+ was dominant in male population (59.88%). The largest number of females (39.77%) was at age 3^+ and the smaller percentages of population (5.04%) were at age 5^+. The average length at age (±SD) and weight at age (±SD) for males and females are presented in Table 1. Comparison of the average length and weight at age data for males and females showed that, at the age 1^+ males were longer and heavier than females but with increasing age, females become longer and heavier than males. Results of the fish specific growth rate, which was calculated based on weight length at age, are presented in Table 1. The specific growth rate of both sexes was declined with aging fish. The parameters of the von Bertalanffy growth curve based on the average length at age data were different in both sexes (L_∞=73.99, K=0.25 and t_o=-2.68 for males and L_∞=100.35, K=0.19 and t_o=- 1.86 for females).

Age and length at maturation and sex ratio

Our observations indicate that male fish matured at an earlier age and smaller length and weight than female. Age and length at first maturity were 1^+ year and 47.55mm and 2^+ year and 57.55mm total length for males and females, respectively. The specimens were

Table 1: Mean length (mm) and weight (g) at age and specific growth rate (% body weight/day) of *T. bahaii* in Zayandeh Roud River.

	Age	1	2	3	4	5
Mean length±SD	Male	44.54±3.33	51.74±1.38	55.56±2.37	60.63±2.31	-
Mean weight±SD		0.6±0.15	0.88±0.14	1.08±0.21	1.42±0.31	-
Mean length±SD	Female	42.72±3.69	52.74±2.82	60.93±3.99	68.10±2.25	73.76±3.15
Mean weight±SD		0.57±0.15	0.93±0.2	1.39±0.31	2.09±0.17	2.67±0.32
Specific growth rate	Male	-	0.39	0.27	0.28	-
	Female	-	0.5	0.4	0.4	0.24

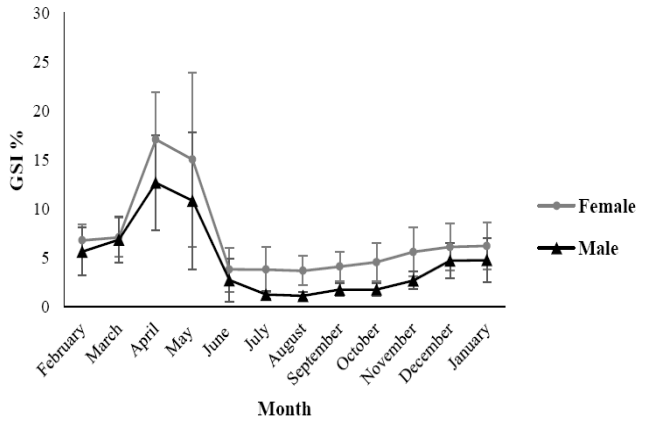

Figure 3: Monthly variations in the Gonado Somatic Index (GSI) of male and female *T. Bahaii.*

composed of 37.81% males, 62.19% females, the sex ratio of 0.61:1 (male : female), which was significantly different from the expected 1:1 ratio (X^2- test, $p<0.05$).

Gonad cycle

The monthly changes in GSI of both sexes followed almost similar pattern but the GSI values of males were lower than those of females (Figure 3). Significant differences were found in the males and females GSI in different months ($p<0.05$; One-way ANOVA). The GSI of males and females fish peaked at April and May and then started to decline, reaching the lowest value in June. This month considered to be the start of the resting period. Thus the reproductive period for this species seems to occur between April and June when water temperature is between 8.7-11.5°C. The gonadal developmental stages of *T. bahaii* are described in five stages following [17].

Stage I **(Immature):** The immature stage was characterized by presence of Primary Growth (PG) oocytes, with nucleoli located in the peripheral part of the nucleus and no atretic oocytes (Figure 4a). Ovarian walls were thin and there was little space between oocytes. There was no evidence of oil droplets in Primary Growth oocytes (PG). The diameter of oocytes ranged from 1-10μm. In the testes, only Spermatogonia (Sg) were present (Figure 5a).

Stage II **(Developing):** In this stage Primary Growth (PG), Cortical Alveolar (CA), primary Vitello genic (Vtg 1) And secondary Vitellogenic (Vgt 2) oocytes were present (Figure 4b, c). At the primary Vitellogenic (Vgt 1), small yolk globules appear and accumulate in the peripheral 1cytoplasm and in the secondary Vitellogenic (Vgt 2) Become larger in size and number. The diameter of oocytes ranged from 6 to 33μm. At this stage, oocytes in several developmental stages were present in the ovary. In testes spermatocytes were evident (Figure

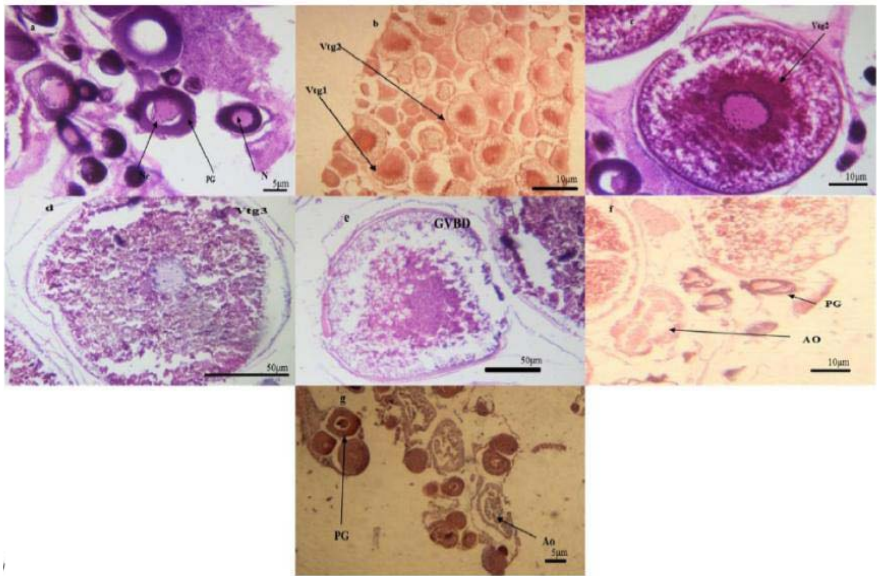

Figure 4: Histological sections of *T. bahaii* ovarian development stained with H&E. a) Stage I (Immature), b,c) Stage II (Developing), d,e) Stage III (Spawning capable), f) Stage IV (Regressing), g) Stage V (Resting). PG: Primary Growth oocyte, N: Nucleus, Ne: Nucleoli, Vgt 1: primary vitellogenic oocyte, Vgt 2: secondary vitellogenic oocyte, Vgt 3: tertiary vitellogenic oocyte, Ao: Atretic oocyte, GVBD: Germinal Vesicle Break Down.

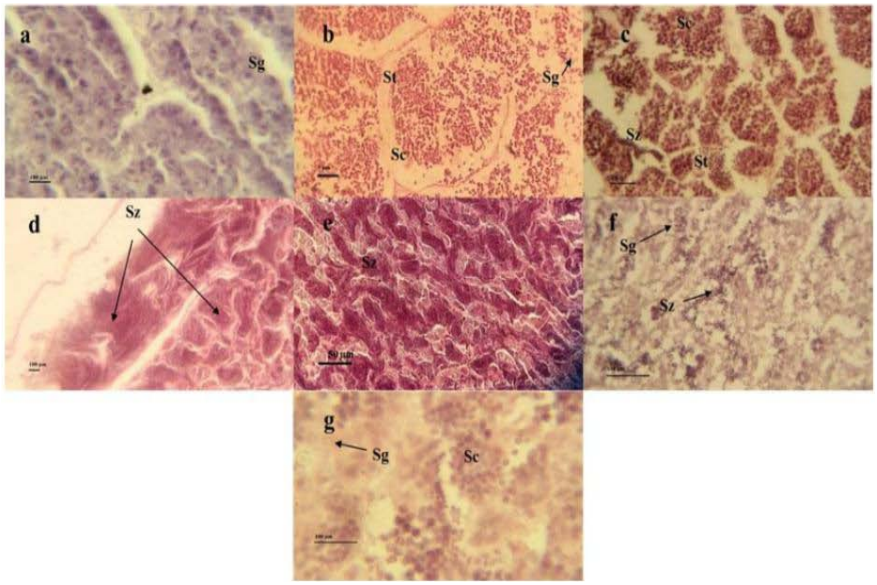

Figure 5: Histological sections of *T. bahaii* testicular development stained with H&E. a) Stage I (Immature), b,c) Stage II (Developing), d,e) Stage III (Spawning capable), f) Stage IV (Regressing), g) Stage V (Resting), Sg: Spermatogonia, Sc: Spermatocyte, St: Spermatid, Sz: Spermatozoa.

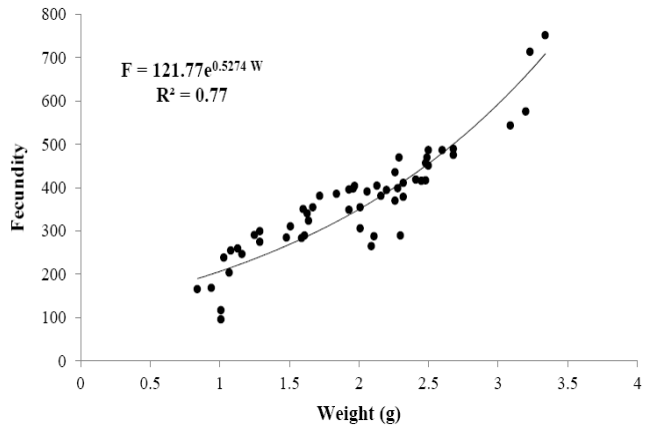

Figure 6: Fecundity-weight relationship in *T. Bahaii* females.

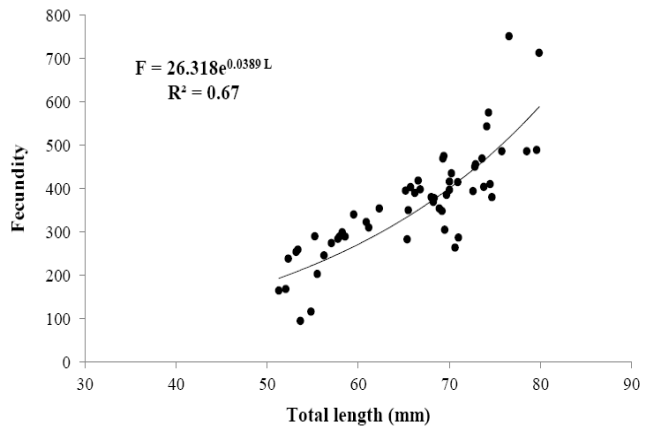

Figure 7: Fecundity-total length relationship in *T. Bahaii* females.

5b, c). Primary Spermatocyte (Sc1), Secondary Spermatocyte (Sc2), Spermatid (St) and Spermatozoa (Sz) were present in histological sections.

Stage III (Spawning capable): This stage was characterized by the appearance of tertiary Vitellogenic (Vtg 3) oocytes in which yolk globules filled more than two third of the cytoplasm and lipid droplets fuse with one another around the nucleus (Figure 4d,e). In this stage most of oocytes had reached the Vgt 3 stage and nucleus began to leave central position and migrate toward animal pole. The oocytes diameter had reached to the maximum value in this phase and ranged from 17-60 μm and the oocytes of last stages were observed. In testes Spermatozoa (Sz) were observed in lumen of lobules (Figure 5d, e). Spermatocytes were present throughout the testes at this stage.

Stage IV **(Regressing):** As the reproduction cycle ends, fish move in to the regressing stage, which is recognized by the presence of oocytes atresia, a reduced number of vitellogenic oocytes (Figure 4f). In the ovary cross sections atretic oocytes and some Vitellogenic (Vgt 1, Vgt 2 and Vgt 3) oocytes were present. Testes in this stage were histologically characterized by depletion of spermatozoa (Sz) in lumen of lobules (Figure 5f). The amount of Spermatozoa (Sz) present was noticeably reduced from that seen in spawning capable phase.

Stage V **(Resting):** This stage was characterized by ovaries containing atretic and Primary Growth (PG) oocytes similar to the immature phase but can be differentiated from immature phase by presence of more space between oocytes in ovary and atretic follicles Figure 4g. In testes resting stage was characterized by Spermatogonia (Sg) in lobules throughout testes and also some possible Spermatozoa (Sz) remain in sperm ducts (Figure 5g).

Fecundity and egg diameter

Absolute fecundity was determined in 56 females during the spawning period. The mean value (±SD) of absolute fecundity was 365.75±132.67 egg; ranging from 95 to 751 egg/fish. The relationship between absolute Fecundity (F) and Fish weight (W) and Total Length (TL) are shown in Figure 6,7. There were positive correlation

Table 2: Monthly variations in the egg diameter (mm) of *T. bahaii* in Zayandeh Roud River (2013-2014).

Month	Egg diameter (max-min)mm (mean±SD)	Number of egg
February	0.18-0.73 (0.49±0.12)	252
March	0.18-0.98 (0.62±0.25)	220
April	0.43-1.58 (0.92±0.29)	402
May	0.2-1.45 (0.85±0.34)	274
June	0.05-0.65 (0.31±0.18)	190
July	0.08-0.28 (0.15±0.05)	196
August	0.025-0.23 (0.14±0.04)	194
September	0.05-0.48 (0.21±0.1)	388
October	0.05-0.58 (0.25±0.13)	400
November	0.05-0.63 (0.30±0.15	400
December	0.13-0.65 (0.43±0.14)	252
January	0.15-0.75 (0.45±0.16)	402

between absolute fecundity and each of the variables. The relative fecundity was ranged from 94.06 to 224.85 with mean value (±SD) of 187.27±29.75 egg/g body weight. No correlations were found between relative fecundity and fish size (weight or length). The mean egg diameter was significantly different during the year ($p<0.05$; One-way ANOVA). The diameter of egg ranged from 0.025 to 1.58mm and the largest mean of egg diameter was observed in April and May (Table 2) (according to the Table 2, the finding of egg diameter during the year is in agreement with the diameter of oocytes in histological analysis). The egg diameter like the GSI, confirmed that the spawning period occurred during April and June (Table 2). Also a large heterogeneity was observed in egg diameter among females during the spawning period, ranging from 0.2 to 1.58mm.

Discussion

This work represents the first attempt to study growth and the key reproductive characteristics of *T. bahaii* in Zayandeh Roud River. The regression coefficients (b) obtained from length-weight relationship shows negative allometric growth for both sexes of *T. bahaii* and lies within the range reported by [18] who specified b between 2.5 and 3.5. This type of growth very well suited with habitat and the life style of adult *T. bahaii*, where they utilized a faster flow rate and gravelly, pebble stone beds, as like other species of this genus (*T. kosswigi*) which has elongated body, allowing fish to avoid damage from high flow velocity by hiding among bedrocks [19].

The highest age classes recorded for males and females population were different, being 4^+ and 5^+, respectively. Maximum age in populations of a species is usually controlled by existing environmental conditions which are defined as the variation in biological parameters of population [20]. In general, loaches are short lived and fast growing fishes (Table 3). Specific growth rate of males and females declined as they aged, the highest rate recorded at age 1^+. This could be due to the fact that the fish allocates less and less energy to growth as approaches their sexual maturity and thereafter [21]. Similar findings reported in the literatures for *Barbatula barbatulus* [10], *Cobitis taenia* [22,23], *Cobitis paludica* [24] and *Cobitis narentana* [25].

According to growth parameters of von Bertalanffy, females of *T. bahaii* achieved a higher infinite length than males, possibly due to the longer life span, which is supported by other researchers [22,25]. However, genetically determined characteristic, such as theoretical maximum length (L_∞) can be affected by the varying environmental conditions [21,26]. The growth constant K suggests a relatively faster approach of males to asymptotic length than females, as reported in other studies [21,23,25].

A significant departure of sex ratios from 1:1 (X^2-test, $p<0.05$) was found with an excess of females (0.61: 1; M:F). Similar inequalities of sex ratios in other species of fish have also been reported by others [27,28]. Nikolsky [15] reported that sex ratio varied considerably from species to species, but in the majority of species, it is close to one. However dominance of one sex to the other may be related to a number of factors including the differences in reproductive strategy, habitat preference according to the season or sex, sampling errors or selective mortality [29].

In this study females of *T. bahaii* reached maturity later than males, the maturity age was estimated at 1^+ and 2^+ for males and females, respectively. Although no data are available for other populations of this species but there are some reports from other loach species [7,30]. Vinyoles [8] reported that the first time maturation of females *Barbatula barbatula* were not occurred before 1^+ age. The youngest mature male and female of *T. bahaii* were 1^+ year old, similar to that reported for *Noemacheilus barbatulus* [31]. The first spawning age in fish is affected by species, size of fish and environmental factors (for example: temperature and feeding) [32]. Nonetheless, females of *T. bahaii* were mature at greater length (57.55mm) than males (47.55mm). According to our finding and those reported by other researchers males of *T. bahaii* reached the maturity at smaller size compare to females, confirming that the general sexual dimorphism for species of the family *Nemacheilidae* [7-9,30,31].

Like other teleosts, reproductive activity in *T. bahaii* follows an annual cycle of gonad growth and reproduction which peaks during certain seasons of the year to favor the survival of off springs [33,34]. GSI of *T. bahaii* peaked in April and May during spring when the favorable conditions in environmental variables such as food supply and temperature for gonad development and reproduction are exist. Similar spawning pattern also reported for other loach species in

Table 3: Range of total length (mm) and weight (g) recorded for different species of *Nemacheilid*, loaches from different areas.

Species	Number	Range of length (mm)	Range of weight (g)	Age	Study area	Source
Noemacheilus barbatulus	475	-	-	0^+-3^+	Dorset chalk stream(England)	Mills at al. 1983
Paracobitis malapterura	370	38-130	0.68-30.55	0^+-4^+	Zaringol River(Iran)	Patimar et al., 2009
Metaschistura cristata	475	25-87	0.09-4.91	0^+-4^+	Zanglanlou River(Iran)	Patimar et al., 2011
Turcinoemacheilus hafezi	189	32-61	0.17-1.39	1^+-2^+	Beshar River(Iran)	Jamali et al., 2014
Turcinoemacheilus bahaii	857	36.05-83.07	0.31-3.87	1^+-4^+(♂) 1^+-5^+(♀)	Zayandeh Roud River(Iran)	Present study

Iran for example, in *P. malapterura* [6], *M. cristata* [35] and *Cobitis satunini* [36]. Gonad growth in fish is accompanied by the progression of gamete development stages which terminate in the production of viable oocytes and sperm. All stages of oogenesis and spermatogenesis were observed in the gonads of *T. bahaii* throughout the annual cycle, although the abundance of certain oocyte stages varied during the season. Interestingly, mature and atretic oocytes were observed almost throughout the year in the ovary. However, mature oocytes were particularly abundant in April and May, declining in June when females may have spawned as suggested by a reduction in GSI values. Similar conditions were observed in male fish. These observations suggest that *T. bahaii* undergoes asynchronous gonad development, starting ovulation and spermeation in April and continued through May then lasted till June. All the stages reported here were comparable to those identified by [37,38].

Although our study showed a positive correlation between absolute fecundity and fish size (weight and length) (Figure 6, 7) but relative fecundity failed to support such a correlation, which is consistent with the literature reports on *P. malapterura, M. cristata and Cobitis sp.* [6,7,35,36,39]. Direct relationship of absolute fecundity with fish weight and length implies that the energy allocated to reproduction is directly related to the fish size. The absolute fecundity range of *T. bahaii* (95-751) was different from values reported for other loaches [6,40]. So in comparison with other closely related loaches, *T. bahaiiis* is not considered a highly fecund species. Temporal variation in egg diameter was observed, so that the highest mean recorded in April and May and then decreased. In *T. hafezi* egg diameter was ranged from 0.4 to 1.6mm in spawning season [9], which is close to *T. bahaii* (0.2 to 1.58mm). These values are much smaller than the one reported for *P. malapterura*, up to 2.8mm [6], which could be perhaps due to vary and many reasons among them, various stages of maturity of fish and environmental conditions [15]. In conclusion, growth and reproductive pattern of *T. bahaii*, inhabiting in Zayandeh Roud River, is characterized by a low number of age groups, fast growth in first year leading to sexual maturity early in life, a short life span and low fecundity. Many of these life-history characteristics are typical of species inhabiting unstable environments. Histological investigation showed that ovary usually contained oocytes at different developmental stages, hence *T. bahaii* can be considered as an asynchronous fish like the other loach species [8,31,37].

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