Int. J. Aquat. Biol. (2018) 6(4): 189-197
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Reproductive biology of two sympatric species of tooth-carps: *Aphanius hormuzensis* and *Aphanius furcatus*, from south of Iran (Teleostei: Aphaniidae)

Mojtaba Masoudi[1], Hamid Reza Esmaeili[*1], Mehrgan Ebrahimi[1,4], Azad Teimori[2], Mahvash Seifali[3]

[1]Developmental Biosystematics Research Laboratory, Zoology Section, Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran.
[2]Department of Biology, Faculty of Sciences, Shahid-Bahonar University of Kerman, Kerman, Iran.
[3]Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran.
[4]School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia.

Abstract: Some aspects of the reproductive biology of two endemic tooth-carps, *Aphanius hormuzensis* and *A. furcatus*, from southern Iran, were studied by regular monthly collections throughout one year. Significant differences were observed between the total number of females and males, females being more abundant. Based on the pattern of reproductive indices including the gonado-somatic index and Dobriyal Index, it was concluded that these fishes spawn in April and May. The estimated absolute fecundity of *A. hormuzensis* ranged from 78 (TL = 32.2 mm) to 730 (TL = 51.1 mm), with a mean value of 219.78±66.50 oocytes per fish based on 15 females. The relative fecundity ranged from 68.45 to 518.54 oocytes/g body mass (Mean±S.D: 237.67±96.87 oocytes/g). For *A. furcatus*, the estimated absolute fecundity ranged from 53 (TL = 26.9 mm) to 102 (TL = 32.04 mm), with a mean value of 93.73±45.37 oocytes per fish based on 15 females. The relative fecundity ranged from 22.41 to 123.65 oocytes/g body mass (Mean±S.D: 64.98±23.37 oocytes/g). Due to overlapping of spawning season in these two sympatric species, it seems that other pre- and post-zygotic factors are responsible for absence of natural hybrids in the studies tooth-carps in the Mehran River.

Article history:
Received 5 September 2018
Accepted 6 November 2018
Available online 25 August 2018

Keywords:
Reproductive indices
Fecundity
Spawning season
Sex ratio
Sexual dimorphism

Introduction

The tooth-carps genus *Aphanius* is the sole native representative of the family Aphaniidae in the Old World and represents a relic of the ancient ichthyofauna of the Tethys (Kosswig, 1955; Hrbek and Meyer, 2003; Freyhof et al., 2017). They occur all over the southern part of the Western Palaearctic. Several tooth-carp species are restricted to one or a few catchments, and some are even restricted to single spring fields (e.g., *A. ginaonis* and *A. kavirensis*), making them highly vulnerable to habitat alterations in their often semi-arid or arid environments (see Freyhof et al., 2017). Its present-day species diversity and distribution have largely been shaped by vicariance events since the early Miocene, when the ancient Tethys Sea closed and uplift of the Anatolian and Iranian plateaus began (e.g., Hrbek and Meyer, 2003; Teimori et al., 2014).

As currently understood, there are four sympatric species, *A. mesopotamicus* and *A. stoliczkanus* in the Shadegan Wetland, Tigris River, southwestern Iran and also *A. hormuzensis* and *A. furcatus* in the Mehran River, Hormozgan basin (Masoudi et al., 2016; Esmaeili et al., 2018a, b; Teimor et al., 2018).

The Hormuz tooth-carp, *A. hormuzensis* is endemic to the Mehran River, Hormuzgan basin in south of Iran. It occurs mainly in two types of habitats, that is, brackish rivers of exorheic drainages and hot sulfur springs. In both habitat types, *A. hormuzensis* can be found sympatrically with *A. furcatus* and the endemic cichlid, *Iranocichla hormuzensis* Coad, 1982 (Teimori et al., 2018).

The scaleless tooth-carp, *A. furcatus* is found the Shur River, Kol River, Mehran River, Khurgu and Faryab hot sulphuric springs in the Hormuzgan drainage, Southern Iran (Teimori et al., 2014). The

[*]Corresponding author: Hamid Reza Esmaeili
E-mail address: hresmaeili@shirazu.ac.ir
DOI: https://doi.org/10.22034/ijab.v6i4.490

rivers and spring streams in Southern Iran are often seasonal and characterized by shallow water and white layers of salt around and within the river (Teimori et al., 2014). *Aphanius furcatus* individuals are often dominant on the riversides, where the water is shallow, warm and water flow is slow (Teimori et al., 2014).

Species of *Aphanius* are well-known for their remarkable capacity to adapt to adverse ecological conditions and evolve into new species when populations become isolated. This has made them particularly attractive as model species for biologists, and many researchers have studied their phenotypic variation, diversity, zoogeography, hybridization, sympatricity and phylogenetic relationships (see Masoudi et al., 2016; Teimori et al., 2018). The sympatricity increases the chance of natural hybridization between closely or distantly related species (Masoudi et al., 2016). The natural hybridization has been reported for two sympatric species of *A. mesopotamicus* and *A. stoliczkanus* but there is not such record for *A. hormuzensis* and *A. furcatus*. As this phenomenon preliminary requires the same period of reproductive activity of two sympatric species (e.g., spawning season), hence, in this study some aspects of reproductive biology of *A. hormuzensis* and *A. furcatus* including gonadosomatic index, modified gonadosomatic index and Dobriyal index which are indicators of spawning season which in turn causing or preventing natural hybridization, have been investigated.

Materials and Methods

Study area and sampling: The fish individuals were collected from the Mehran River (Hormozgan Basin) near Bastak city, Hormozgan Province, south of Iran (27°8'39.38"N., 54°15'42.38"E.). Fishes were sampled regularly by hand net (mesh size, 5 mm) from August 2014 to July 2015. The specimens were immediately preserved in buffered formalin solution of 10% until they could be examined.

Data analysis: Tooth-carp individuals were measured to the nearest 0.05 mm total length (TL) and standard length (SL) using a vernier caliper. The total body weight (TW) of all preserved fishes was measured using electronic balance to the nearest 0.001g. The sexes were determined based on the body color pattern (Figs. 1).

The parameters of length-weight relationships were estimated by linear regression of the log-transformed weight and length (Koutrakis and Tsikliras, 2003). Prior to regression analysis, log–log plots of length and weight values were performed for visual inspection of outliers (Froese, 2006) which is $W=aL^{b}$, where W is the total weight in gr, L the total length in mm, *a* a coefficient related to body form and *b* an exponent indicating allometric growth when unequal to 3. The Fulton's condition factor (k) was calculated monthly by $k=w/L3$ x 100 where W= whole body wet weight in grams and L= length in cm; the factor 100 is used to bring K close to unity (Murphy and williams, 1996).

We dissected the fishes and examined the ovary to determine stage of maturity. To examine the monthly changes in the gonads as a means for estimating the spawning season of theses tooth-carp, 3 indices were used: gonado-somatic index (GSI), modified gonado-somatic index (MGSI) and Dobriyal Index (DI), which were calculated by GSI = (weight of gonads / weight of fish)×100 (Nikolsky, 1963); MGSI = (weight of gonad / weight of fish - weight of gonad)×100 (Nikolsky, 1963); DI $=\sqrt[3]{GW}$ (Dobrial et al., 1999), where GW is the average gonad weight.

The gonads were examined and absolute fecundity was measured in terms of the number of oocytes [($F=\frac{n\times G}{gw}$), F = absolute fecundity, n = number of oocytes, G = gonad weight, gw= gonad sample weight] with a diameter greater than 0.2 mm (Leonards and Sinis, 1998), using 15 female gonads for each species. The relative fecundity (number of ova per unit of body weight) [($R=\frac{F}{TW}$) , R= relative fecundity, F= absolute fecundity, TW= total weight] was also estimated using the method suggested by Bagenal (1967). To determine the ovum diameter, the ovaries were preserved in 10% formalin solution. The diameters of 100 ova from each female fish were measured using a Zeiss light microscope which was

Figure 1. Male (left) and female (right) *Aphanius hormuzensis* collected from Mehran River, south of Iran.

Figure 2. Male (left) and female (right) *Aphanius furcatus* collected from Mehran River, south of Iran.

fitted with an ocular micrometer. The ovum diameters which were measured in ocular divisions were transformed into mm. The Chi square test was used to assess deviation from a 50:50 sex ratio (Robards et al., 1999). All the collected specimens were deposited in ZM-CBSU (Zoological Museum, Collection of Biology Department, Shiraz University).

Results

Descriptive information: We examined 427 specimens of *A. hormuzensis* (with ranging in total length from 253 to 521 mm, standard length 212 to 483 mm and total weight of 0.068-1.866 g) and 214 specimens of *A. furcatus* (with ranging in total length from 172 to 427 mm, standard length 130 to 381 mm and total weight of 0.061-0.701 g), from Mehran River, Hormozgan Province in southern Iran.

Sexual dimorphism: Males of *A. hormuzensis* have 2-3 dark and light blue alternating bars on the caudal fins, the last bar being yellow. The bars are crescent-shaped, with concave side posterior. Males outside the breeding season are less brightly coloured with silvery on the flanks with a grey or black-brown back and irregular flank bars. Females are brown-grey to silvery with 15-17 narrow dark brown flank bars (Fig. 1).

Males of *A. furcatus* (Fig. 2) usually have 7-11 vertical flank-bars, their dorsal, anal, caudal, pelvic and pectoral fins are white but with a dark pigmentation on the base of the 1^{st}-4^{th} dorsal rays. Females (Fig. 2) display 7-9 dark circular blotches on their flanks, starting behind the operculum and extending until the base of the caudal fin. Similar to the males, their dorsal, anal, caudal, pelvic and pectoral fins are white. Both males and females display a yellowish dorso-posterior part of the eyes.

Sex ratio: Of 427 fish specimens of *A. hormuzensis* caught, 183 were males and 244 were females, giving an overall sex ratio of 1.34F:1M (Chi square=17, $P<0.05$). In case of *A. furcatus*, out of 214 collected individuals, 118 were female and 96 were male with sex ratio of was 1.23F:1M (Chi square= 18, $P<0.05$).

Length-weight relationship: Descriptive statistics and estimated parameters of LWRs for the *A. hormuzensis* and *A. furcatus* species are given in Table 1. The value of parameter b ranged from 2.643 for male *A. furcatus* to 3.385 for female *A. hormuzensis*, which remains within the expected range of 2–4 reported by Tesch (1971) and almost to $2.5 < b < 3.5$ by Froese (2006).

Gonado-somatic and modified Gonado-somatic Indices: The results showed that female invest more in gonads than males (ANOVA, $P<0.001$). Significant differences were observed in female and male GSI and MGSI in different months (ANOVA, $P<0.05$). The spawning of the *A. hormuzensis* was determined by

Table 1. Length-weight relationship of *Aphanius hormuzensis* and *A. furcatus* from Mehran river, Bastak (in south of Iran).

Species	Basin	Sex	N	TL range (cm)	W range (g)	*a*	95% CI of *a*	*b*	95% CI of *b*	r^2
Aphanius hormuzensis	Hormuz	M	183	2.53-4.64	0.245-1.017	0.0095	0.0062–0.015	3.357	3.031–3.697	0.969
		F	244	2.12-5.21	0.068-1.866	0.0089	0.0079–0.0102	3.385	3.278–3.497	0.996
		A	427	2.53-5.21	0.068-1.866	0.0090	0.0081–0.0100	3.396	3.304–3.488	0.994
Aphanius furcatus	Hormuz	M	96	1.72-3.36	0.061-0.244	0.0126	0.0103-0.0158	2.643	2.353-2.921	0.964
		F	118	1.89-4.27	0.712-0.701	0.0098	0.0088-0.0109	3.035	2.882-3.154	0.987
		A	214	1.72-4.27	0.061-0.701	0.0010	0.0091-0.0111	2.981	2.845-3.091	0.972

M, male; F, female; A, sexes combined; N, sample size; TL, Total length; W, weight; *a*, intercept of log-log relationship; *b*, regression slope; r^2, coefficient of determination.

Table 2. Mean of gonadosomatic index (GSI) of female (F) and male (M) *Aphanius hormuzensis* and *A. furcatus* from the Mehran River, Bastak, south of Iran.

Species	Sex	No.	Mean±S.D.	Range	Sig.
A. hormuzensis	M	183	2.864±2.12	0.17-21.67	0.0001
	F	244	7.923±4.32	0.53-29.25	
	A	427	5.652±4.495	0.17-29.25	
A. furcatus	M	96	1.614±2.12	0.11-17.26	
	F	118	2.229±3.17	0.15-20.71	
	A	214	1.921±3.12	0.11-20.71	

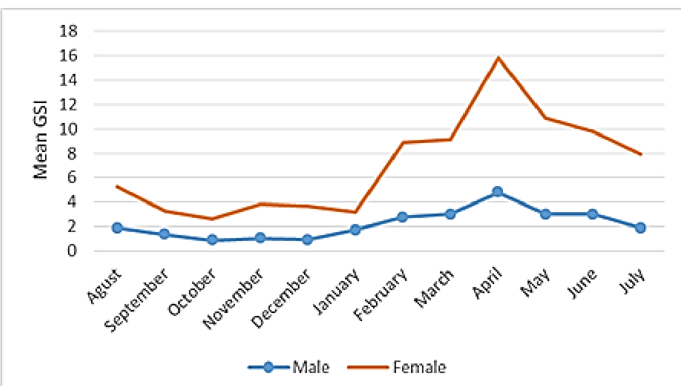

Figure 3. Variation of mean gonadosomatic index (GSI) in *Aphanius hormuzensis* in different months.

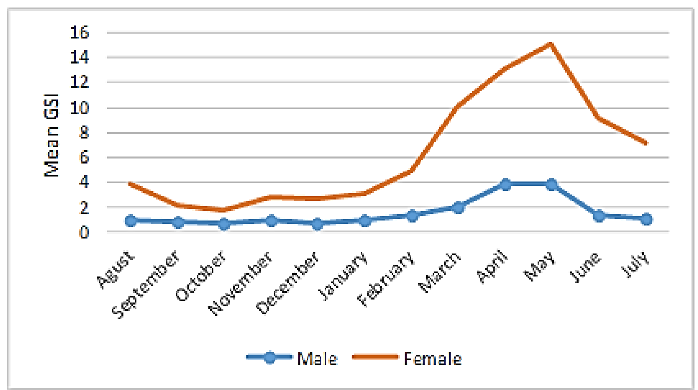

Figure 4. Variation of mean gonadosomatic index (GSI) in *Aphanius furcatus* in different months.

increased GSI during February to April showing two peaks in both sexes (Fig. 3). The first peak was observed in February with maximum mean value of 2.984±1.603 and the second one in April with maximum mean value of 4.789±5.106 for male individuals and 8.915±0.916 and 15.824±5.126 for female individuals, respectively. Spawning time of the *A. furcatus* was determined by increasing GSI value during February to May, showing two peaks in both sexes (Fig. 4). The first peak was observed in February with maximum mean value of 1.375±1.143 and the second one was seen in May with maximum mean value of 3.913±1.226 for male specimens and 3.501±1.712 and 11.198±3.326 for female specimens, respectively. The analysis of MGSI of for both *Aphanius* species (ANOVA, $P<0.05$) are also consistent with the results of the GSI index (Figs. 5, 6).

Dobriyal Index (DI): Another indicator of spawning season is the Dobriyal Index (DI). This index unlike the GSI index, does not consider the body weight, which is influenced by nutrition and environmental and physiological conditions. The results of the DI were also consistent with the results of GSI and MGSI analysis. In *A. hormuzensis*, the mean of DI for both sexes was maximum in April (0.338 for males and 0.558 for females) (Fig. 7). Respectively, for *A. furcatus* the mean of DI for males and females was high as 0.303 in April and 0.341 in May (Fig. 8).

HSI index: An analysis of the values of the HSI index

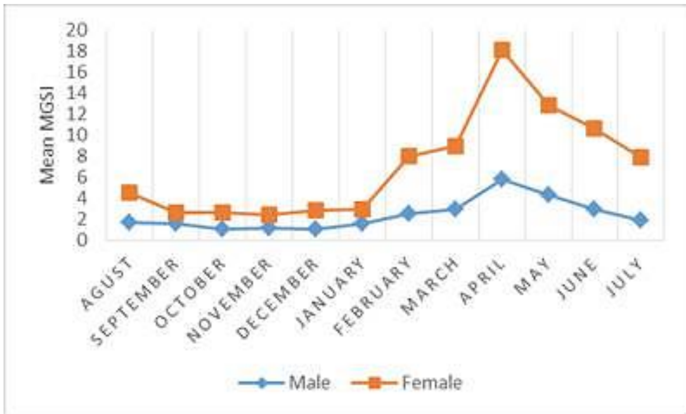

Figure 5. Variation of mean modified gonadosomatic index (MGSI) in *Aphanius hormuzensis* in different months.

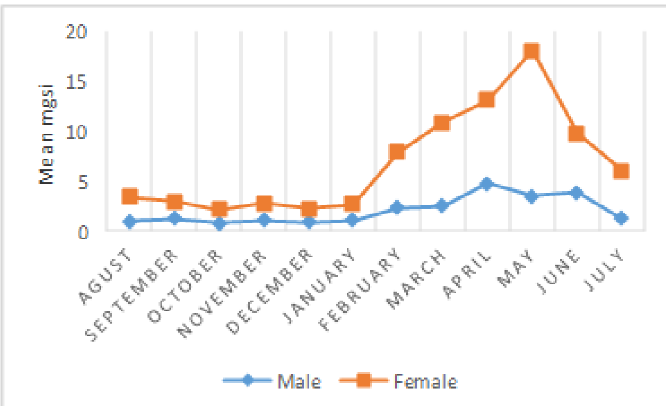

Figure 6. Variation of mean modified gonadosomatic index (MGSI) in *Aphanius furcatus* in different months. .

for male and female individuals in both *Aphanius* species are shown in the Figures 9-10. In general, the mean values of this index, especially in the female specimens, showed a decreasing trend during the spawning period.

Condition factor: We calculated the condition factor separately for each sex in different months, and found monthly differences in this factor. In *A. hormuzensis*, the condition factor of females was highest in February and March, and reached its lowest value in September. From September to December it showed fluctuations. We observed an increasing trend in the condition factor from December to March. In male specimens, the condition factor was lowest in October, and increased until March. It showed a virtually decreasing trend from July to December (Fig. 11). In *A. furcatus* the condition factor in both sexes, had a peak in March. In both males and females, the condition factor was the lowest in February, while

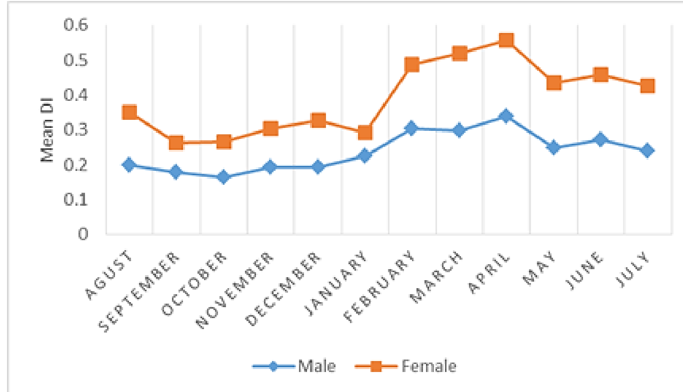

Figure 7. Variation of Dobriyal Index (D.I.) of *Aphanius hormuzensis* in different months.

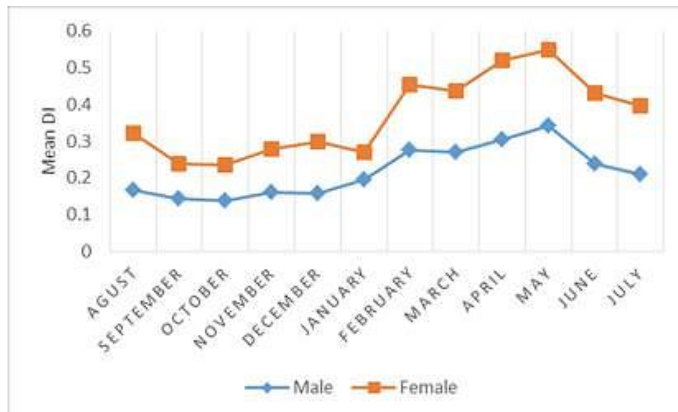

Figure 8. Variation of Dobriyal Index (D.I.) of *Aphanius furcatus* in different months

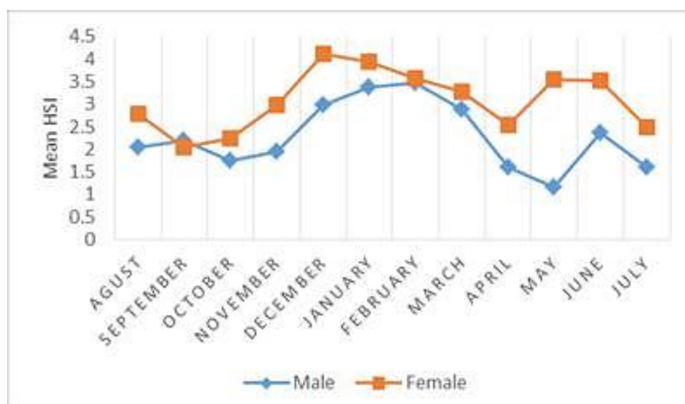

Figure 9. Variation of hepatosomatic Index (HSI) of *Aphanius hormuzensis* in different months.

increased up until March (Fig. 12).

Fecundity: The estimated absolute fecundity of *A. hormuzensis* ranged from 78 (TL = 32.2 mm) to 730 (TL = 51.1 mm), with a mean value of 219.78±66.50 oocytes per fish based on 15 females. The relative fecundity ranged from 14 to 173 oocytes/cm body length (Mean±S.D: 79.95±38.03 oocytes/cm) and 68.45 to 518.54 oocytes/g body mass

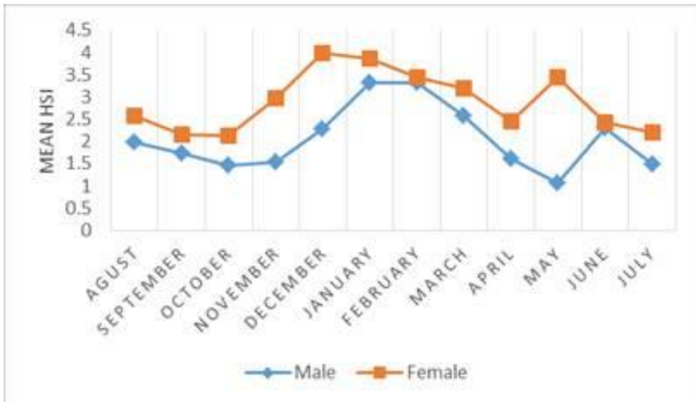

Figure 10. Variation of hepatosomatic Index (HSI) of *Aphanius furcatus* in different months.

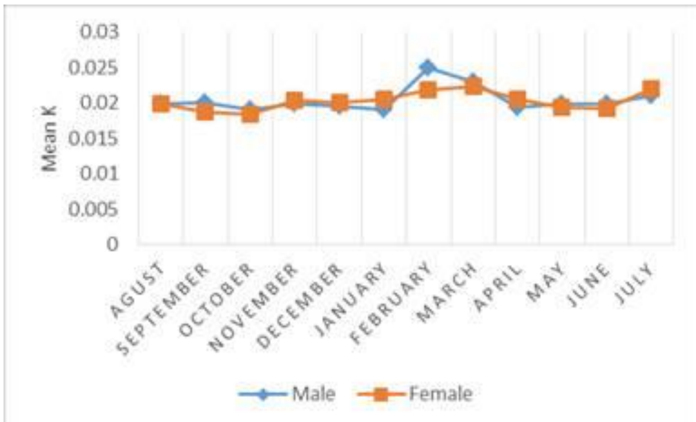

Figure 11. Variation of mean condition factor (K) of *Aphanius hormuzensis* in different months.

(Mean±S.D: 237.67±96.87 oocytes/g).

For *A. furcatus* the estimated absolute fecundity ranged from 53 (TL = 26.9 mm) to 102 (TL = 32.04 mm), with a mean value of 93.73±45.37 oocytes per fish based on 15 females. The relative fecundity ranged from 12 to 85 oocytes/cm body length (Mean±S.D: 55.35±22.02 oocytes/cm) and from 22.41 to 123.65 oocytes/g body mass (Mean±S.D: 64.98±23.37 oocytes/g).

Discussion

This study provided the details on some aspects of reproductive biology of *A. hormuzensis* and *A. furcatus*, two endemic tooth-carps of Iran. Both species exhibit a clear external sexual dimorphism as found in the other aphaniid species and also cyprinodontid and poecilid fishes (Esmaeili et al., 2014; Teimori et al., 2014, 2018). Sexual dimorphism includes color pattern differences as seen in aphaniid

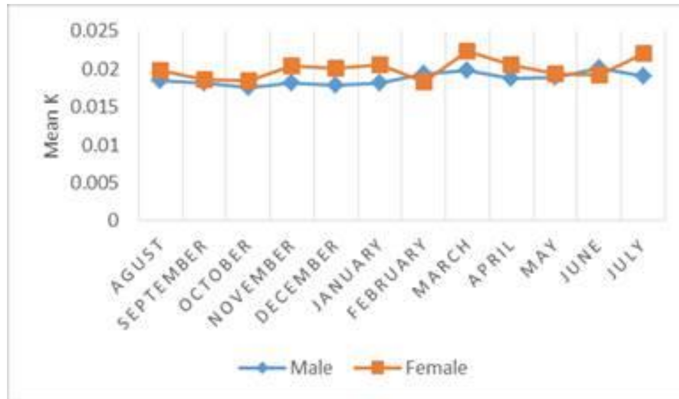

Figure 12. Variation of mean condition factor (K) of *Aphanius furcatus* in different months.

fishes or size differences and presences of gonopodium in poecilid fishes. It has been suggested/ shown that sexual selection promotes the evolution of costly secondary sexual traits in males of many animal species (Darwin, 1871; Sedlácek, et al., 2014). The evolutionary analysis suggests that both sexes of the common ancestor of killifish were relatively plainly coloured (Sedlácek et al., 2014). During evolution of the group, changes in male and female colouration were correlated across most killifish lineages (Sedlácek et al., 2014). This interspecific pattern suggests that intersexual genetic correlation is an important factor in the evolution of killifish colouration (Sedlácek et al., 2014). In the presence of genetic correlation, females bear fully developed or rudimentary colour ornamentation and such female ornaments were more intensively developed in species with more variegated males as predicted by macroevolutionary scenario (Sedlácek et al., 2014).

The results of the present study showed that both tooth-carps spawn in April and May. The mean values of reproductive indices (GSI, MGSI and DI) and high frequency of large oocytes confirmed the spawning season. Spawning season for other *Aphanius* species have already been reported. Reproduction of *A. dispar* (now *A. kruppi*) occurs throughout the day and throughout the year in Oman with 69% of females having ripe eggs (up to 41) in April-June. Peak spawning in southern Iraq in *A. dispar* (now *A. stoliczkanus*) is April to June when only a small proportion of eggs in the single ovary are fully developed eggs (Al-Daham et al., 1977). Shafi and

Shalli (1986) record up to 73 mature eggs in southern Iraq specimens with a diameter of 220 microns. Breeding season was May-July. In Saudi Arabia mature and developing oocytes are observed in fish during the whole year. The egg cycle may be more than one year with spawning in March and April (El-Hawawi and Al-Imam, 1984). On the Mediterranean coast of Egypt, *A. dispar* spawning occurs from March to September with a peak in July and August. Maximum egg diameter is 2.2 mm and size at maturity for females is 30 mm total length. Iranian specimens of *A. stoliczkanus* with large eggs (2.0 mm) on 16 March but young fish (8.9 mm standard length) were caught on 26 November suggesting reproduction is almost year around (see Coad, 2017). Bibak et al. (2012) examined reproduction *A. dispar* (now *A. stoliczkanus*) in the Dalaki River, Bushehr and found females to predominate (390 fish versus 140), males had a maximum length of 49 mm and females 39 mm, and gonadosomatic indices indicated a spawning period of April to June, with peaks in each of these months of 6.449 and 6.632.

According to Zare et al. (2015) size composition analysis of oocyte diameter showed that females contained mature oocytes (>1.1 mm) throughout year. Absolute fecundity of *A. ginaonis* ranges from 36 to 832 oocytes per individual (mean: 341±209 oocytes), varying considerably at given length and mass (Zare et al., 2015). Relative fecundity to total length of *A. ginaonis* fluctuates from 12 to 169 oocytes/mm (mean: 78±41 oocytes/mm) while relative fecundity to total mass varies from 61-526 oocytes/g (mean: 213±106 oocytes/g) (Zare et al., 2015). *Aphanius ginaonis* is an asynchronous, iteroparous spawner producing more than one oocyte clutch in a single reproductive season (Zare et al., 2015). The absolute and relative fecundity in *A. farsicus* have been reported to be 115.7 and 90.01 respectively and it has been related to fish size (total length and total weight) and also to gonad weight (Esmaeili and Shiva, 2006). The relative fecundity of another aphaniid species *A. fasciatus* is reported to be136.2 for the Mesolongi Lagoon, 104.4 and 94.7 for Alykes, both in Greece (Leonardos and Sinis, 1998). The mean value of absolute fecundity in *A. hormuzensis* (219.78±66.50) is greater than *A. ginaonis* (Zare et al., 2015) and lower than *A. farsicus* (Esmaeili and Shiva, 2006). The mean absolute fecundity of *A. furcatus* (93.73±45.37) is lower than *A. ginaonis, A. hormuzensis* and *A. farsicus*. Fecundity is affected by several factors, such as the size and age of the female (Thorpe et al., 1984), life history strategy (Morita and Takashima, 1998), food supply and temperature (Fleming and Gross, 1990).

Reduction of number of produced eggs (fecundity) in *A. furcatus* might be another case of regressive evolution in this fish. In *A. furcatus*, several phenotypic characters show reductions; most remarkable are the complete absence of scales and the lower degree of ossification (the reduced thickness of the neural and haemal spines of PU3, in the short length of the spines of the preceding caudal vertebrae and in the presence of thin and short ribs) (see Teimori et al., 2014).

The fecundity of the studied tooth-carp increases with fish size (total length and body weight). According to Jonsson and Jonsson (1999), fecundity increases with body size because the amount of energy available for egg production and the body cavity accommodating the eggs increases with fish size.

Conclusion

Aphanius hormuzensis and *A. furcatus* from southern Iran demonstrate some reproductive strategies including sexual dimorphism, female biased sex ratio and a lengthy spawning period in response to its habitat. The small body size is another significant factor in the life history of these tooth-carps, which allows fish to colonise and exploit micro-environments. However, the distribution of these species is limited to the waters in southern Iran. This limited distribution makes the species highly vulnerable and could result in significant loss or even extinction, if habitats are disturbed or destroyed. Due to its restriction to the southern part of Iran, the population of these small and beautiful fishes should be conserved because it is an important part of the natural heritage of this country. Due to overlapping of spawning season in these two sympatric species, it seems that other pre- and post-zygotic factors are responsible for absence of natural hybrids in these studies species in the Mehran River.

Acknowledgments
The research work was funded by Shiraz University and Alzahra University and it was approved by the Ethics Committee of Biology Department (SU-909830).

References
Al-Daham N.K. (1977). Fishes of Iraq and the Persian Gulf. Volume 1. Squaliformes to Atheriniformes. Publication 9, Centre for Arab Gulf Studies, University of Basrah, Iraq. 546 p. (In Arabic)

Bagenal T.B. (1967). A short review of fish fecundity. In: S.D. Gerking (Ed.). The Biological Basis of Freshwater Fish Production, Oxford. pp: 89-111.

Bibak M., Rakhshani M., Hosseini S.A., Koohani M., Moein M. (2012). Reproduction of *Aphanius dispar dispar* (Rüppell, 1829) in Dalaki River, Bushehr, south of Iran. World Journal of Fish and Marine Sciences, 4: 594-596.

Coad B.W. (2017). Freshwater Fishes of Iran. (www.briancoad.com). Retrieve 1 December 2017.

Darwin C.R. (1871). The Descent of Man, and Selection in Relation to Sex. John Murray, London.

Dobriyal A.K., Rautela K.K., Rautela A.S. (1999). Invention of a new index for the determination of sexual maturity in fishes. Uttar Pradesh Journal of Zoology, 19: 207-209.

El-Hawawi A.S.N., Al-Imam M.S.E. (1984). Oöcyte differentiation and yolk formation in the Arabian barred fish *Aphanius dispar*. Journal of the College of Science, King Saud University, 15: 443-452.

Esmaeili H.R., Shiva A.H. (2006). Reproductive biology of Persian tooth-carp, Aphanius persicus (Jenkis, 1910) (Actinopterygii: Cyprinodontidae) in South of Iran. Zoology in the Middle East, 37: 39-46.

Esmaeili H.R., Teimori A., Gholami Z., Reichenbacher B. (2014). Two new species of the tooth-carp *Aphanius* (Teleostei: Cyprinodontidae) and the evolutionary history of the Iranian inland and inland-related *Aphanius* species. Zootaxa, 3786: 246-268.

Esmaeili H.R., Asrar T., Gholamifard A. (2018a). Cyprinodontid fishes of the world: an updated list of taxonomy, distribution and conservation status (Teleostei: Cyprinodontoidea). Iranian Journal of Ichthyology, 5: 1-29.

Esmaeili H.R., Sayyadzadeh G., Eagderi S., Abbasi A. (2018b). Checklist of freshwater fishes of Iran. FishTaxa, 3: 1-95.

Fleming I.A., Gross M.R. (1990). Latitudinal clines: a trade-off between egg number and size in Pacific salmon. Ecology, 71: 1-11.

Freyhof J., Weissenbacher A., Geiger M. (2017). *Aphanius kruppi,* a new killifish from Oman with comments on the A. dispar species group (Cyprinodontiformes: Aphaniidae). Zootaxa, 4338: 557-573.

Froese R. (2006). Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology, 22: 241-253.

Hrbek T., Meyer A. (2003). Closing of the Tethys Sea and the phylogeny of Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). Journal of Evolutionary Biology, 16: 17-36.

Jonsson N., Jonsson B. (1999). Trade-off between egg mass and egg number in brown trout. Journal of Fish Biology, 55: 767-783.

Kosswig C. (1955). Zoogeography of the Near East. Systematic Biology, 4: 49-73.

Leonardos I., Sinis A. (1998). Reproductive strategy of *Aphanius fasciatus* Nardo, 1827 (Pisces: Cyprinodontidae) in the Mesolongi and Etolikon lagoons (W. Greece). Fisheries Research, 35: 171-181.

Masoudi M., Esmaeili H.R., Teimori A., Gholami Z., Gholamhosseini A., Sayyadzadeh G., Keivany Y., Reichenbacher B. (2016). Sympatry and possible hybridization among species of the killifish genus *Aphanius* Nardo, 1827 (Teleostei: Cyprinodontidae) in Southwestern Iran. Limnologica-Ecology and Management of Inland Waters, 59: 10-20.

Morita K., Takashima Y. (1998). Effect of female size on fecundity and egg size in white-spotted charr: Comparison between sea-run and resident forms. Journal of Fish Biology, 53: 1140-1142.

Murphy B.R., Willis D.W. (1996). Fisheries techniques. American Fisheries Society, Bethesda.

Nikolsky G.V. (1963). The Ecology of Fishes, Academic Press, New York. 352 p.

Robards M.D., Piatt J.F., Rose G.A. (1999). Maturation, fecundity and intertidal spawning of pacific and lance in the northern Gulf of Alaska. Journal of Fish Biology, 54: 1050-1068

Sedlácek O., Baciaková B., Kratochvílet L. (2014). Evolution of body colouration in killifishes (Cyprinodontiformes: Aplocheilidae, Nothobranchiidae, Rivulidae): Is male ornamentation constrained by intersexual genetic correlation? Zoologischer Anzeiger - A Journal of Comparative Zoology, 253: 207-215.

Shafi M., Shalli M.R. (1986). On the taxonomy, distribution and ecology of genus *Aphanius* Nardo (Cypriniformes (*sic*): Cyprinodontidae) of Iraq. Bangladesh Journal of Zoology, 14: 129-133.

Teimori A., Esmaeili H.R., Erpenbeck D., Reichenbacher B. (2014). A new and unique species of the genus *Aphanius* Nardo, 1827 (Teleostei: Cyprinodontidae) from Southern Iran: A case of regressive evolution. Zoologischer Anzeiger-A Journal of Comparative Zoology, 253: 327-337.

Teimori A., Esmaeili H.R., Hamidan N., Reichenbacher B. (2018). Systematics and historical biogeography of the *Aphanius dispar* species group (Teleostei: Aphaniidae) and description of a new species from Southern Iran. Journal of Zoological Systematics and Evolutionary Research. (doi.org/10.1111/jzs.12228)

Thorpe J.E., Miles M.S., Keay D.S. (1984). Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture, 43: 289-305.

Zare P., Naderi M., Asghari S. (2015). Reproductive biology of the Geno hot spring tooth-carp (*Aphanius ginaonis* Holly, 1929) in southern Iran. Environmental Biology of Fishes, 98: 1365-1371.