Caspian J. Environ. Sci. 2018, Vol. 16 No. 2 pp. 135~148 ©Copyright by University of Guilan, Printed in I.R. Iran



[Research]

## Reproductive biology of an endemic fish, *Alburnoides qanati* Coad and Bogustkaya, 2009 (Teleostei: Cyprinidae) from Southern Iran

#### Tahami M.S., Esmaeili H.R.\*, Monsefi M.

Department of Biology, College of Science, Shiraz University, Shiraz, Iran

\* Corresponding author's E-mail: hresmaeili22@gmail.com

(Received: Nov. 15. 2017 Accepted: April 11. 2018)

#### ABSTRACT

This study provides fundamental information on some key aspects of the reproductive traits of qanati tailor fish, *Alburnoides qanati*, an Iranian endemic, poorly studied cyprinid fish species. Sampling was performed on a monthly basis during one year (from March 2011 through February 2012) from a tributary of endorheic Kor River Basin, Southern Iran. The results of data analyzing showed that the sex ratio in the population of qanati tailor fish is 1:1 except for those in January and April. Based on the size, shape and weight of the gonads, degree of occupation of the body cavity, presence or absence of ripe oocytes, diameter of the oocytes in the ovary, and histological observations, five typical gonad maturation stages were described for females using macroscopic and microscopic criteria. Based on the percentage of the late gonad maturation stage (V) and high frequency of large oocytes it was concluded that *A. qanati* spawns during spring with its peak in April. These results were in accordance with those of three reproductive indices (gonado-somatic, modified gonado-somatic and dobriyal). Absolute fecundity was obtained between 732 and 2368. Study on its eggs by scanning electron microscopy (SEM) revealed that the fish have adhesive eggs, which could explain its low fecundity compared to other cyprinids.

Key words: Dobriyal index; fecundity; gonado-somatic index; Kor Basin; Qanati Tailor fish.

#### INTRODUCTION

Reproduction is one of the most important biological aspects of fish community and the survival of populations depends on its success (Suzuki & Agostinho 1997). Each fish species lives in special ecological conditions, therefore it has a unique reproductive strategy, with special anatomical, behavioral and physiological adaptations (Bone *et al.* 1995; Moyle & Cech 2004).

These strategies have been studied for many cyprinid fishes (e.g. Rinchard & Kestemont 1996; Platania & Altenbach 1998; Asadollah *et al.* 2011; Gholamifard *et al.* 2017). In order to obtain a comprehensive insight into the biology and population dynamics of a fish taxon and to apply monitoring, conservation and

management programs of that taxon, its reproductive potentials should be investigated. Different reproductive parameters and indices such as sex ratio, gonado-somatic index, modified gonado-somatic index (MGSI) and dobryial index (DI), number of produced eggs (fecundity), ova diameter, condition factor, stages of maturity, have been used to determine reproductive status and spawning season of different fish species including cyprinids (see Seifal et al. 2012). Generally, members of the cyprinid fishes of the genus Alburnoides are lithophilic and rheophilic, meaning that they inhabit in barbell and grayling zones and spawn on gravel and rubble (Breitenstein & Kirchhofer 2000; Copp et al. 2010). One of the widely-distributed and well-studied member of the genus is *Alburnoides bipunctatus* (Bloch 1782).

For a long time, *A. bipunctatus* (Bloch 1782) was the name applied to most populations of spirlins (riffle minnows or tailor fish) from France, north of the Alps, eastwards to the Black, Caspian and Aral Sea basins to the Middle East (Coad & Bogutskaya 2012), but ongoing researches revealed that a greater diversity is found in Europe and the Middle East, especially Iran. Recent studies have shown that at least seven species exist in different basins of Iran including the Caspian Sea, Lake Orumiyeh (Urmia), Tedzhen River, Kavir, Namak Lake, Tigris River, Persian Gulf drainage and Kor River basins (Bogutskaya & Coad 2009; Coad & Bogutskaya 2009, 2012).

Alburnoides qanati, qanat tailor fish (qanat spirlin) is a recently-described species by Coad & Bogutskaya (2009), which is known to be an endemic species of Kor River basin and the adjacent basin of Sirjan (Esmaeili 2012). Information on different biological aspects of the ganati tailor fish is scarce and limited to its morphological characteristics and its distribution (Coad Bogutskaya 2009; & Esmaeili et al. 2010, 2011, 2012).

The main aim of this study is to provide basic information on some key aspects of the reproductive characteristics (e.g. sex ratio, reproductive indices, maturity stages, fecundity and spawning season) of poorlystudied cyprinid fish species, *Alburnoides qanati*, from endorheic Kor River basin of Iran which will be useful in conservation management of this fish.

#### MATERIALS AND METHODS Sampling and preparation

To study reproductive biology of *A. qanati,* sampling was made on a monthly basis from March 2011 through March 2012 in Moshkan spring, Kor River basin, Fras Province (30° 36'16.9' N and 52° 56' 40.1' E) by electrofishing method.

The collected fishes were then anesthetized by MS-222, fixed in 10% formaldehyde and transported to the Ichthyology laboratory of Shiraz University.

## Biometry, data analysis and histological studies

Total length (TL) and standard length (SL) of the preserved specimens were measured to the nearest 0.05 mm using Vernier calipers. Preserved specimens were blotted dry, weighed (nearest 0.001 g; total mass, W) on an electronic balance and then the fish were dissected. The gonads were separated, weighed  $(\pm 0.001 \text{ g})$  and placed in 10 % buffered formalin for further studies. Sex was determined visually or by microscopic examination of the gonads. A chi-square test was used to assess deviation from 50:50 sex ratio (Robards et al. 1999). The ovaries were examined macroscopically and microscopically to determine maturity stages. The ovarian samples were dehydrated in ascending grades (70%, 90% and 100%) of alcohol, embedded in paraffin, sectioned (5–7  $\mu$ m) and stained with haematoxylin and eosin (H&E) (Bancroft & Stevens 1990; Esmaeili et al. 2009). To examine the monthly changes in the gonads for estimating spawning season of A. qanati, three indices were used including Gonadosomatic (GSI), modified gonadosomatic (MGI) and Dobriyal (DI) indices which were calculated for each fish and all values were in monthly average:

GSI = (gonad mass/fish mass) × 100 (Nikolsky 1963), MGI = (gonad mass/fish mass-gonad

mass) × 100 (Nikolsky 1963) DI =  $\sqrt[3]{GW}$  (Dobriyal *et al.* 1999), where W is whole fish mass in g, GW is gonad mass in g, L is fish length in mm.

Stages of maturation were classified as follows: I, immature (virgin); II, initial developing (recovery); III, developing (maturing); IV, ripe (follicular); V, spawning (running). The gonads were examined and absolute fecundity was measured in terms of the total number of oocytes presented in both ovaries using Bagenal's (1978) method. For this purpose, we carefully weighed the ovaries after removing excess water on filter paper and then prepared three subsamples from anterior, middle and posterior parts of ovaries. The number of eggs in subsamples was counted and then the total number was calculated (Esmaeili *et al.* 2009). The relative fecundity (number of ova per unit of body mass) was also estimated using Bagenal's (1978) method. Since fixation processes may lead to oocytes deformation, oocyte measurements (oocyte diameter) were made in two dimensions (Esmaeili 2001). We measured maximum width and length of 100 oocytes in each ovary preserved in 10% formalin solution using an ocular micrometer (Ziess model SV 6).

Ultra structure study of oocyte envelope was made using scanning electron microscope (SEM). So that, fully matured oocytes from the last maturity stage of gonads were dehydrated in 30%, 50%, 70%, 90% and 100% alcohol respectively, each for 10 minutes and were mounted on aluminum stubs. These mounts were then sputter-coated with gold, and were observed and photographed with a Cambridge 180 scanning electron microscope (SEM) at voltage of 20 KV at low probe current. Various images of the oocytes were recorded and studied (see Esmaeili & Johal 2005; Esmaeili & Gholamifard 2012). Data were analyzed statistically using SPSS (version 17) statistical software package. Differences were considered significant at an alpha level of 0.05. The collected specimens were deposited at ZM-CBSU, Zoological Museum of Shiraz University, Collection of Biology Department, Shiraz, Iran.

#### RESULTS

A total of 395 specimens of Alburnoides ganati (Fig. 1), were collected from Kor River basin, ranging from 24.8 to 118.7 mm in body length (TL) and from 0.14 to 24.3 g in body mass. Descriptive analyses of its length and weight for both sexes is presented in Table 1. As shown in the Table, females are larger in size and mass than males. Analyzing of the length frequency distribution based on sex reveals that the majority of the female specimens (accounting for 25.8%) were consisted of individuals between 60-70 mm and 70-80 mm in length classes, whereas male specimens in the length class of 60-70 mm (35.5%). We determined sex in 219 female and 168 male specimens. The overall sex ratio was significantly female biased and deviates from the hypothetical distribution of 1:1 (1.3:1, f/m; Chi square = 6.72, df = 1, p < 0.001) (Fig. 2).



Fig. 1. Alburnoides qanati, female, from Kor River basin, Iran.

| 1 able .          | <b>1.</b> Descriptive statistics of | lengul and weight of A | . quinti tot bout ses | tes.              |
|-------------------|-------------------------------------|------------------------|-----------------------|-------------------|
| parameters        | Sex                                 | Min                    | Max                   | Mean              |
| Total Length (mm) | Female                              | 24.76                  | 118.66                | $67.13 \pm 16.03$ |
|                   | Male                                | 24.88                  | 87.07                 | $59.52 \pm 14.28$ |
| Weight (g)        | Female                              | 0.140                  | 24.260                | $4.780 \pm 3.55$  |
|                   | Male                                | 0.110                  | 8.890                 | $3.046 \pm 2.08$  |

Table 1. Descriptive statistics of length and weight of A. qanati for both sexes

80 70 Frequency (%) 60 50 40 30 🛚 Female 🖾 Male 20 10 0 Sep. Jan. Feb. March Apr. May June July Aug. Oct. Nov. Dec. Month

Fig. 2. Monthly distribution of sex ratio of A. qanati, collected from Kor River basin, during 2011-2012.

To assess the states of maturation, three reproductive indices (GIS, MGIS and DI) were calculated. As it has been reported in many other fishes, regardless of the kind of examined reproductive index, females acquired more index value in gonads than did males (ANOVA, p < 0.001, df = 1) being about 3 times in females than in males. Monthly changes in the gonado-somatic index (GSI) of both sexes are presented in Fig. 3. One-Way ANOVA analysis showed significant differences of mean GSI between the two sexes (p < 0.05). Results showed the peak of GSI for females in April, with a small peak in July, while its minimum is in August. In males the peak of GSI was in April, whereas its minimum was in July. The results for MGSI were the same as GSI results for both sexes (Fig. 3). Monthly variation in the DI of both sexes was related to the GSI and MGSI results (Fig. 3). The oocyte diameter ranged from  $0.2 \pm 0.03$  to  $0.7 \pm 0.2$  mm. Mean oocyte diameter was highest in April which is the spawning season of A. ganati and lowest in July (Fig. 4). There was almost general increase in oocyte diameters from June to March. However, there was a decrease in the average oocyte diameter in February and March that might be the result of poor sampling in favor of mostly immature and juveniles. Estimation of

fecundity was performed for the last maturity stage of A. qanati by counting fully ripped oocytes (over 0.4 mm in diameter) from three subsections of the anterior, middle and posterior parts of gonads. Absolute fecundity ranged from 732 to 2386 with a mean value of  $1292 \pm 4$  oocytes per fish based on 20 females examined. Relative fecundity ranged 9-27 oocytes per gram body weight (Table 2). According to the size and weight of ovary, the volume of the abdominal cavity, presence or absence of ripe oocytes, and also oocytes diameter in the ovary, we have described 5 maturation stages of ovaries in A. qanati. Fig. 4 shows the macroscopic appearance of ovaries in different stages.

#### Stage I (immature, pre-vitellogenous period)

Gonads very thin and thread like covered with an epithelial tissue. Oocytes not visible to the naked eye having only one type of oocyte (Fig. 5a). In histological studies small round and transparent oocytes were observed. These oocytes called ooctye type I containing almost large central nucleus and numerous nucleoli developing within the margin of nucleus. The mean diameter of oocytes was 0.10 mm. The oocytes had basophilic cytoplasm and an acidophilic nucleus. The ratio of nucleus to cytoplasm volume was high (Fig. 6a).

Tahami et al.



**Fig. 3.** Monthly variation in GSI, MGSI and DI of female and male *A. qanati*, collected from Kor River Basin, during 2011-2012.

| Fecundity                 | Number | Mean    | Min    | Max     | S.D    |
|---------------------------|--------|---------|--------|---------|--------|
| Absolute Fecundity        | 20     | 1292.04 | 732.18 | 2566.88 | 150.09 |
| <b>Relative Fecundity</b> | 20     | 14.97   | 9.939  | 27.41   | 4.49   |



Fig. 4. Monthly variation in mean oocyte diameter of *A. qanati*, p < 0.001.



**Fig. 5.** Morphological profiles of the five female maturity stages. (a) Immature stage, (b) Initial development stage, (c) development stage, (d) Follicular stage, (e) Spawning stage.



**Fig. 6.** Five microscopic stages of female gonadal maturity in *A. qanati*. FCL, follicular layer; LD, lipid droplet; MC, Marginal cytoplasm; N, nucleus. NM; nuclear membrane; NU, Nucleolus; TL, thecal layer; ZR, zona radiata; Y, yolk.



**Fig. 7.** SEM microphotographs of egg capsule in *A. qanati* indicate (a) the whole shape, (b) the uneven surface, (c) the density of filaments.

### Stage II (initial development, vitellogenic period)

Ovaries increase in size. Small white oocytes hardly visible by naked eye (Fig. 5b). Two types of oocytes are distinguishable in histological sections of ovaries, which are oocytes type I and newly added oocytes type II. Developing oocytes type II exhibit a weak basophilic cytoplasm that is characterized by small lipid droplets at the margin. Yolk granules and a very thin follicular layer appear gradually. Interstitial tissue between oocytes is more than the previous stage. Mean diameter of oocytes was 13 mm (Fig. 6b).

#### Stage III (in development)

Ovaries size larger than before. High density of yellowish oocytes clearly visible by naked eyes. Three types of oocytes are observed in ovaries by histological studies (Fig. 5c). Oocyte type III are 5-6 times larger in size compared to oocytes type I. The ratio of cytoplasm to nucleus volume and also its ratio to the interstitial tissue volume is higher. Follicles are characterized by spherical acidophilic granules (lipid droplets) and also a thin zona radiata surrounded by cubic follicular cells and theca layer.

The size and number of yolk granules are also increased. Mean diameter of oocytes was 0.25 mm in this stage (Fig. 6c).

#### Stage IV (follicular, ripped)

Ovaries completely matured and filled almost the whole body cavity. Ovaries yellow in color with large, yellow and ripped oocytes. Membranous capsule around ovaries became thinner than before (Fig. 5d).

Four types of oocytes can be seen in ovaries. Oocyte type IV, which is called follicle from now on, has lower fraction of nucleus to cytoplasm. In this stage a coalescence of lipids and yolk granules and also the detachment of the follicular cell layer occurred.

Two layers of granulosa cells and one layer of zona radiata is visible. The nuclear envelope breaks down and the thickness of the zona radiata is higher than the previous stages. Mean diameter of follicles is 0.4 mm (Fig. 6d).

#### Stage V. (spawning, running)

Ovaries more massive and voluminous than the previous stage. Oocytes yellow in color and beads like. Adherence between follicles lessen (Fig. 5e). Oocytes are characterized by large mass of yolk and numerous large lipid droplets.

The zona radiata is completely thick and is separated from the follicular layers. Mean follicle diameter is 0.69 mm (Fig. 6e). Oocytes were studied under the electron scanning microscope to describe the structure of oocyte envelope.

The unfertilized eggs of qanati tailor fish at stage V are spherical in shape (Fig. 7) and egg envelops have a rough filamentous structure. This is considered as an adhesive type egg, which can stick on rocks and gravels of substratum after being released into the water, until its hatch. Generally, the annual average of water temperature, water velocity, dissolved oxygen (DO) and salinity were estimated to be 16.6° C, 2.17 cm sec<sup>-1</sup>, 7.41 ppm and 0.13 ppm, respectively.

#### DISCUSSION

This study provides the basic knowledge on the reproductive traits including sex ratio, spawning season, fecundity and gonad histology of a cyprinid endemic fish, *Alburnoides qanati* restricted to Kor and Sirjan endorheic basins of Iran (Eamaeili *et al.* 2012). To date, few studies have been carried out on reproductive biology of different *Alburnoides* species (Papadopol & Cristofor 1980; Yildirim *et al.* 1999; Türkmen *et al.* 2001; Polačik & Kováć 2006) to compare with our results in this study.

#### Sex ratio

According to our results, monthly sex ratios of A. qanati did not differ significantly from 1:1 except in January (Chi square = 5.452, p = 0.020, df=1) and April (Chi square = 5.063, p = 0.024), when the sex ratios were biased in favor of females (Fig. 2). Patimar et al. (2012) reported no significant differences from the ratio of 1:1 between females and males of Alburnoides bipunctatus (now A. eichwaldii) collected from a qanat system in south-east of the Caspian Sea basin (sex ratio 1:1.16 f:m,  $\gamma 2 = 0.24$ , p > 0.05). According to them a balanced sex ratio in A. eichwaldii could be attributable probably either to the consequence of the same survival rate and/or to the same longevity of the sexes and might reflex the stability of habitat condition in qanat system. This is in contrast to the general pattern of the sex ratio in cyprinid species in rivers. However, as an example, Raikova-Petrova et al. (2006) found the overall sex ratio of A. bipunctatus in the middle stream of the Iskar River, Bulgaria, to be 66.1F: 33.9M which is strongly female-biased. Yıldırım et al. (1999) also reported a significant deviation from parity in the ratio 1:1.26 (males to females) for A. bipunctatus (probably A. faciatus) in the Oltu River, Turkey. It seems that number of parameters e.g. age, size classes, season, fishing methods, migration and species can affect the sex ratio of fishes.

#### **Reproductive biology**

Macroscopic and microscopic studies of ovarian maturity of *A. qanati* revealed that this organ could be classified into 5 different maturity stages. These stages were found in

close agreement with the other species (see Seifali *et al.* 2012).

The spawning season of qanat tailor fish was determined on the basis of monthly variations in the mean gonado-somatic index, modified gonado-somatic index, Dobriyal index, the mean diameter oocytes and the proportion of the developmental stages of the ovaries. Overall, GSI, MGSI and DI indices exhibited positive relationships with gonad weight, therefore can show sexual maturity stages in both sexes. Dobrival Index (DI) is determinant factor of spawning season. DI does not consider weight of the fish in the calculation, which in turn is depended on feeding rate, food availability as well as environmental and physiological stresses. Therefore, it may be a more reliable index of gonad maturity and spawning season. The monthly average variation of GSI, MGSI and DI in A. qanati all showed a peak in April with a small peak in July, hence indicating the spawning season of this fish during this period. This was also confirmed by our histological analyses of gonads that showed the most matured oocytes (in case of level of maturity and number) in these two months. Parameters influencing these indices are temperature, light, good feeding, species-specificity and environmental conditions (Nikolsky 1963) which in turn yielding accumulation and storage of yolk in oocytes, increasing their sizes along with to increase in gonad mass and leading subsequent peaks in the three indices. Overall, means of GSI, MGSI and DI in spawning season were significantly higher in females compared to males. The reason is higher ovarian mass than testicular mass. A. qanati spawns during spring with its peak in April, which is almost consistent with other reports of genus Alburnoides on spawning season (Papadopol & Cristofor 1980; Yıldirim et al. 1999; Polačik & Kováć 2006). Considering various reported GSI patterns, it is evident that time and duration of the reproductive season is different among different regions; in April and May in Zarrin-Gol River, northern Iran (Patimar & Dowlati 2007), June-July in Kesselian stream, South Caspian Sea basin, northern Iran (Seifali et al. 2012), from April to August in a qanat in Caspian Sea basin (Patimar et al. 2012), between April and June in the Oltu River, Turkey (Yıldırım et al. 1999), between April and July in the Rudava River, western Slovakia (Polačik & Kováč 2006), and from April to July for A. *bipunctarus* in the basins of the rivers Upper San & Dunajec (Skóra 1972). Polačik & Kováč (2006) noted that, due to asynchronous spawning, A. bipunctatus shows diverse GSI patterns where they reported 3 peaks in GSI. However we found one main peak in April and another, shorter one in January for A. qanati (in the present study) confirming the effects of environmental conditions and fish species on spawning behavior of different Alburnoides species. Papadopol & Cristofor (1980) reported a large reproductive potential of spirlin in Romania, where it reproduces four to five times in a season. During the first episode of spawning, over a quarter of the eggs are released and the rest of the gametes are removed three to four times at intervals of approximately 15 days. Bless (1994, 1996) revealed that the potential spawning period in A. bipunctatus is very prolonged. At least some females spawn several times during one season. According to him, the spirlin therefore has to be considered as a seasonal multiple spawner. Nikolosky (1963) stated that gradual spawning in a long period is the characteristics of tropic and moderate water regions. Numbers of spawning shows reproductive strategies of any population that is dependent on environmental conditions and food availability (Nikolsky 1963; Baggerman 1990). This reproductive strategy is in favor of population survival.

Monthly diagram of oocytes diameter also confirms spawning behavior of fish (Nikolsky 1963). According to this diagram, maximum mean diameter of oocytes was in April. However, Polačik & Kováć (2006) reported maximum mean diameter of oocytes for *A*. *bipunctatus* from Slovakia in April and May.

The observed size range of oocytes (0.2 to 0.7 mm) was different from that of 0.50-1.70 mm

reported by Patimar *et al.* (2012). Others reported various size ranges as following; 0.20 to 1.20 mm for *Alburnoides* cf. *eichwaldii* from southeastern Caspian Sea basin, (Polačik & Kováč 2006), 0.50 to 1.40 mm for *Alburnoides* sp. in the Rudava River in western Slovakia (Soric & Ilic 1985), and that of 0.02-1.67 mm presented by Seifali *et al.* (2012) for *Alburnoides* sp. from Talar River, Southern Caspian Sea basin. The diameter of oocytes is dependent on the quality and quantity of food, light intensity, temperature and fish age (Fleming & Gross 1990; Morita & Takashima 1998).

Absolute fecundity of A. qanati ranged between 732 and 2368 and relative fecundity ranged between 9 and 27 per body gram. There are other reports presenting almost the same results for sprilin in different countries (Papadopol & Cristofor 1980; Polačik & Kováć 2006). The low number of fecundity is correlated with the higher rate of females to males to insure the survival of generations. Higher sex ratio toward females has been reported for members of this genus (Raikova-Petrova et al. 2006). It seems that in studies of fish fecundity, it is essential to define whether fecundity is determinant or indeterminate (Hesp et al. 2004). In determinant fecundity, the numbers of each egg batches which are supposed to be spawned in spawning season, are determined before releasing. In other words, there is no indeterminate oocyte in the ovaries of fish with determinant fecundity (Hunter et al. 1985; Murua et al. 1998). According to Polačik & Kováć (2006) report on from spirlin fecundity Slovakia, non vitellogenous oocytes (immature) constantly develop to vitellogenous oocytes in spawning season and there is no significant gap between oocytes of an ovary. In the present study we also did not observe a significant gap between oocytes with different stages of development during spawning season. In addition, the majority of oocytes were of vitellogenoushydrated ooctyes which were the ripest type in the last maturity stage, while the abundance of undeveloped oocytes was extremely low. Consequently, A. qanati may have determinant

fecundity with an asynchronous ovary. However, egg abundance in fecundity is dependent on individuals, time and number of spawning as well as fish size. Increasing in length and weight of fish and abdominal cavity leads to increase in fecundity. Age, quality and quantity of available food are other affective parameters on fish fecundity (Nikolsky 1963; Bagenal 1969; Vondracek *et al.* 1988; Kingdom & Allison 2011).

Study on the oocyte envelope of A. qanati by scanning electron microscopy revealed presence of high density filamentous on the surface of chorion membrane. Demersal eggs in teleosts are divided in two categories: non adhesive and adhesive (Mito 1979). Environmental status contributes in the final morphology of eggs (Huysentruyt & Adriaens 2005). Observations showed that A. qanati has demersal adhesive eggs. These eggs will stick to gravel on the substratum which is the characteristic of A. qanati's habitat. According to experiments carried out by Bless (1994, 1996), the eggs of A. bipunctatus are positioned in portions at equal depth in the interstices of the substrate. Only a few eggs could be observed adhering to the surface of the sediment at the spawning place. According to Rizzo et al. (2002) adhesive eggs are larger and less in number, which represents the reproductive strategy of sedentary species and parental caring behavior. This may be one of the strategies for A. qanati survival regarding the high velocity of its habitat.

#### CONCLUSION

Based on the GSI, MGSI and DI patterns and also distribution of oocytes diameter, *Alburnoides qanati* spawns once a year in spring. Macroscopic and histological studies showed 5 stages of maturity and asynchronous ovaries in females. According to SEM analyses, eggs in *A*. *qanati* are of adhesive-demersal type.

#### ACKNOWLEDGMENTS

We wish to thank R. Khaefi, B. Parsi, G. Sayadzadeh and S. Mirghiasi for their valuable helps with the fish collections, Bioethics

committee of Biology Department and Shiraz University for the financial support.

#### REFERENCES

- Asadollah1, S, Soofiani, NM, Keivany, Y & Shadkhast, M 2011, Reproduction of *Capoeta damascina* (Valenciennes, 1842), a cyprinid fish, in Zayandeh-Roud River, *Iranian Journal of Applied Ichthyology*, 27: 1061–1066.
- Baggerman, B 1990, Sticklebacks In: AD, Munro, AP Scott, TJ Lam (eds.) Reproductive Seasonality in Teleosts: Environmental Influences, CRC Press Inc. Boca Raton, FL, USA, Chap. 261 p.
- Bagenal, TB 1969, The relation between food supply and fecundity in brown trout, *Salmo trutta*. *Journal of Fish Biology*, 1: 167–182.
- Bagenal, TB & Braum, E 1978, Eggs and early life history In: TB Bagenal (ed.) *Methods for assessment of fish production in freshwaters*. Blackwell, Oxford, pp. 165–210.
- Bancroft, JD & Stevens, A 1990, Theory and practice of histological techniques. Churchill Living stone, New York, 726p.
- Bless, R 1994, Contributions to the spawning behaviour of the spirlin- *Alburnoides bipunctatus* (Bloch) under laboratory conditions. *Fischokologie Koln*, 7: 1-4.
- Bless, R 1996, Reproduction and habitat preference of the threatened spirlin (*Alburnoides bipunctatus* Bloch) and soufie (*Leuciscus soufia* Risso) under laboratory conditions (Teleostei: Cyprinidae). *Conservation of Endangered Freshwater Fish in Europe*, 249-258.
- Bogutskaya, NG & Coad, BW 2009, A review of vertebral and fin-ray counts in the genus *Alburnoides* (Teleostei: Cyprinidae) with a description of six new species. *Zoosystematica Rossica*, 18: 126-173.
- Bone, Q, Marshal, NB & Blaxter, JHS 1995, Biology of Fishes. London, Blackie Academic and Professional, An important of Chapman and Hall, 332 p.
- Breitenstein, ME & Kirchhofer, A 2000, Growth, age structure and species association of the cyprinid *Alburnoides bipunctatus* in the

River Aare, Switzerland. *Folia Zoolica*, 49: 59-68.

- Coad, BW & Bogutskaya, NG 2009, Alburnoides qanati, a new species of cyprinid fish from southern Iran (Actinopterygii, Cyprinidae). ZooKeys, 13: 67-77.
- Coad, BW 2014, Freshwater Fishes of Iran, (www.briancoad.com).
- Copp, GH, Kováč, V & Siryová, S 2010, Microhabitat use by stream-dwelling spirlin Alburnoides bipunctatus and accompanying species: implications for conservation. Folia Zoolica, 59: 240–256.
- Dixit, RK & Agarwal, N 1974, Studies on the developmental rhythm in the oocyte of *Puntius sophore, Acta anatomica* 90:133-144.
- Esmaeili, HR 2001, Some aspects of biology of an exotic fish *Hypophthalmicthys molitrix* (Val., 1844) from Gobindsagar reservoir Himachal paradesh, India. PhD Dissertation, Department of Zoology, Panjab University, Chandigarh, 287p.
- Esmaeili, HR & Johal, MS 2005, Ultrastructural features of the egg envelope of silver carp, *Hypophthalmichthys molitrix* (Osteichthyes, Cyprinidae). *Environmental Biology of Fishes*, 72: 373-377.
- Esmaeili, HR, Gangali, Z & Monsefi, M 2009, Reproductive Biology of the endemic Iranian cichlid, *Iranocichla hormuzensis* Coad, 1982 from Mehran River, southern Iran. *Environmental Biology of Fishes*, 84: 141-145.
- Esmaeili, HR, Coad, BW, Gholamifard, A, Nazari, N & Teimori, A 2010, Annotated Checklist of the Freshwater Fishes of Iran. Zoosystematica *Rossica*, 19: 361- 386.
- Esmaeili, HR, Nazari, N, Saifali, M & Gholamhosseini, A 2011, Morphometric and meristic comparisons of populations of Qanat Tailor Fish, *Alburnoides qanati* Coad & Bogutskaya, 2009 (Actinoptyrygii: Cyprinidae) in Kor River basin of Iran. *Iranian Journal of Animal Biosystematics*, 7: 1-11.
- Esmaeili, HR, Tahami, MS, Parsi, B, Sayyadzadeh, G & Hojati, A 2012, First record of *Alburnoides qanati*

(Actinopterygii: Cyprinidae) from Sirjan River *Basin. Journal of Animal Environment*, 4: 73-76. (In Persian).

- Esmaeili, HR & Gholamifard, A 2012, Ultrastructure of the chorion and the micropyle of an endemic cyprinid fish, *Cyprinion tenuiradius* Heckel, 1849 (Teleostei: Cyprinidae) from southern Iran. *Iranian Journal of Fisheries Sciences*, 11: 657-665.
- Fleming, IA & Gross, MR 1990, Latitudinal clines: a trade-off between egg number and size in pacific salmon. *Ecological Society of America*, 71: 1-11.
- Gholamifard, A, Monsefi, M & Esmaeili, HR
  2017, Histomorphometrical study of gonads in the endemic cyprinid fish, *Cyprinion tenuiradius* Heckel, 1847
  (Cypriniformes: Cyprinidae). *Iranian Journal of Fisheries Sciences*, 16: 477-493.
- Hesp, SA, Potter, IC & Schubert, SRM 2004, Factors influencing the timing and the frequency of spawning and fecundity of the goldlined seabream (*Rhabdosargus sarba*) (Sparidae) in the lower reaches of an estuary. *Fishery Bulletin.*, 102: 648-660.
- Hunter, JR, Lo, NCH & Leong, R 1985, Batch fecundity in multiple spawning fishes. (ed. Lasker, R) In: An egg production method for estimating spawning biomass of pelagic fish: application to the northern anchovy (*Engraulis mordax*). US Dep. Commer, NOAA Tech. Rep. NMFS, 36: 67-78.
- Huysentruyt, F & Adriaens, D 2005, Adhesive structures in the eggs of *Corydoras aeneus* (Gill, 1858; Callichthyidae). *Journal of Fish Biology*, 66: 871-876.
- Kingdom, T & Allison, M 2011, The Fecundity, Gonadosomatic and Hepatosomatic Indices of *Pellonula leonensis* in the Lower Nun River, Niger Delta, Nigeria. *Current Research Journal of Biological Sciences*, 3: 175– 179.
- Koç, ND 2010, A study on ultrastructure of zona radiata during oocyte development of zebrafish (*Danio rerio*). *Journal of Fisheries Sciences*, 4: 144-151.

- Mito, S 1979, Fish egg. Gekkan Kaiyo-Kagaku, 11: 126-130. (In Japanese).
- Morita, K & Takashima, Y 1998, Effect of female size on fecundity and egg size in whitespotted charr: comparison between sea-run and resident forms. *Journal of Fish Biology*, 53: 1140-1142.
- Moyle, PB & Cech, JJ 2004, Fishes. An introduction to ichthyology. 5<sup>th</sup> Ed., Prentice Hall, New York, NY, 744 p.
- Murua, H, Motos, L & Lucio, P 1998, Reproductive modality and batch fecundity of the European hake (*Merluccius merluccius* L.) in the Bay of Biscay. Calif. Coop. California Cooperative Oceanic Fisheries Investigations Report, 39: 196-203.
- Nikolsky, GV 1963, The ecology of fishes. Academic Press, London, 352 p.
- Papadopol, M & Cristofor, S 1980, Recherches sur L'ologie de deux populations de spirlin, *Aburnoides bipunctatus*, des eaux de la Romanie. Grigore. *Antipa.*, 22: 483-493. (In French)
- Patimar, R & Dowlati, F 2007, Investigation on age, growth and reproduction of riffle minnow Alburnoides bipunctatus (Bloch, 1782) in Zarrin-Gol River, East Alborz Mountains. J. Fish. 1: 55-62.
- Patimar, R, Zare, M & Hesam, M 2012, On the life history of spirlin *Alburnoides bipunctatus* (Bloch, 1782) in the qanat of Uzineh, northern Iran. *Turkish Journal of Zoology*, 36: 383-393.
- Polačik, M & Kováč, V 2006, Fecundity and annual course of maturation in spirlin, *Alburnoides bipunctatus*. *Folia Zoolica*, 55: 399-410.
- Raikova-Petrova, GN, Petrov, IK, Marinova, D & Hamwi, N 2006, Structure of riffle minnow's population (*Alburnoides bipunctatus* Bloch, 1782) in the middle stream of the Iskar River, Bulgaria vzv. Acta Zoolica Bulgarica, 58: 395-400.
- Rinchard, J & Kestemont, P 1996, Comparative study of reproductive biology in singleand multiple-spawner cyprinid fish. I. Morphological and histological features.

*Journal of Fish Biology*, 49: 833-894. doi10.1111/j.1095-8649.1996.tb00087.x.

- Rizzo, E, Sato, Y, Barreto, BP & Godinho, HP 2002, Adhesiveness and surface patterns of eggs in neotropical freshwater teleosts, *Journal of Fish Biology*, 61: 615–632.
- Robards, MD, Piatt, JF & Rose, GA 1999, Maturation, fecundity and intertidal spawning of pacific and lance in the northern Gulf of Alaska. *Journal of Fish Biology*, 54: 1050–1068.
- Seifali, M, Arshad, A, Esmaeili, HR, Kiabi, BH, Yazdani Moghaddam, F & Fardad, N 2012, Fecundity and maturation of South Caspian spirlin, *Alburnoides* sp. (Actinopterygii: Cypriniade) from Iran. *Iranian Journal of Science and Technology*, A2: 181-187.
- Skóra, S 1972, The cyprinid Alburnus bipunctatus Bloch from the basins of the rivers Upper San and Dunajec. Acta Hydrobiologica, 14: 173-204.
- Soric, VM & Ilic, KR 1985, Systematic and ecological characteristics of *Alburnoides bipunctatus* (Bloch) in some waters of Yogoslavia. *Icthyologyia*, 17: 47-58.
- Platania, SP & Altenbach, CS 1998, Reproductive strategies and egg types of seven Rio Grande basin cyprinids. ASIH 3: 559-569. DOI: 10.2307/1447786.

- Suzuki, HI & Agostinho, AA 1997, Reprodução de peixes do reservatório de Segredo. pp. 163-181. (ed. Agostinho, AA, Gomes, LC) In: *Reservatório de Segredo*, Bases ecológicaspara o manejo, Maringá, Eduem, 387p. (In French)
- Türkmen, M, Erdoğan, O, İbrahim Halıloğlu, H & Yildirim, A 2001, Age, Growth and Reproduction of *Acanthalburnus microlepis*, Filippi 1863 from the YaÛan Region of the Aras River, Turkey. *Turkish Journal of zoology*, 25: 127-133.
- Vondracek, B, Wurtsbaugh, WA & Cech, JJR 1988, Growth and reproduction of the mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: consequences for life history. *Environmental Biology of Fishes*, 21: 45–57.
- Yıldırım, A, Erdoğan, O, Turkmen, M & Demir, BC 1999, The investigation of some reproduction characteristics of the *Alburnoides bipunctatus faciatis* (Nordman, 1840) living in Oltu stream, Coruh basin. *Turkish Journal of Veterinary and Animal Sciences*, 23: 679-686. (In Turkish).
- Zhukov, P 1965, Ryby Belorussii. (The fishes of Belorussia), Minsk, Nauka Tekhnika, 415 p. (In Russian).

# زیست شناسی تولیدمثل ماهی بومزاد خیاطه قناتی Alburnoides qanati Coad and زیست شناسی تولیدمثل ماهی بومزاد خیاطه قناتی Bogustkaya, 2009

تهامی م.س.، اسماعیلی ه.ر.\*، منصفی م.

گروه زیست شناسی، دانشکده علوم، دانشگاه شیراز، شیراز، ایران

(تاریخ دریافت: ۹۶/۰۸/۲۴ تاریخ پذیرش: ۹۷/۰۱/۲۲)

#### چکیدہ

مطالعه حاضر به بررسی برخی جنبههای زیستشناسی تغذیه در ماهی بومزاد خیاطه قناتی Alburnoides qanati در آبهای داخلی ایران می بردازد. نمونه برداری به صورت ماهیانه در طول یک سال (از فروردین ۸۹ تا اسفند ۸۹) از یکی از سرشاخههای حوضه آبریز رودخانه کر در جنوب ایران انجام شد. نتایج حاصل از آنالیز دادهها نشان داد که نسبت جنسی در این جمعیت از ماهی خیاطه قناتی به جز ماههای دی و فروردین ۱۱۱ است. بر پایه بررسیهای شکل ظاهری، اندازه و وزن گنادها، میزان اشغال ماهی خطوه شکل ظاهری، اندازه و وزن گنادها، میزان اشغال حفره شکمی، وجود یا عدم وجود تخمکهای رسیده، قطر تخمکهای تخمدانها، و مشاهدات بافتشناسی، پنچ مرحله بلوغ گنادی برای مادههای این گونه ماهی با استفاده از خصوصیات ماکروسکوپی و میکروسکوپی توصیف شد. بر اساس درصد گنادهای گنادی برای مادههای این گونه ماهی با استفاده از خصوصیات ماکروسکوپی و میکروسکوپی توصیف شد. بر اساس درصد گنادهای میزان اشغال معره مرحله آخر (V) و فراوانی بالای تخمکهای درشت، نتیجه گرفته شد که ماهی تفصی قدر طول فصل بهار تخمریزی میکند و اوج آن در ماه فروردین است. این نتایج حاصل از محاسبات سه شاخص گنادی بردی، قدر در ماهی با استفاده از خصوصیات ماکروسکوپی و میکروسکوپی توصیف شد. بر اساس درصد گنادهای میکندی برای مادههای این گونه ماهی با استفاده از خصوصیات ماکروسکوپی و میکروسکوپی توصیف شد. بر اساس درصد گنادهای میکندی برای ماده های این گونه ماهی بالای تخمکهای درشت، نتیجه گرفته شد که ماهی تافی گنادی بدنی، گنادی بدنی تغییریافته، میکند و اوج آن در ماه فروردین است. این نتایج حاصل از محاسبات سه شاخص گنادی بدنی، گنادی بدنی تغییریافته، و دوبریال مطابق بودند. هماوری مطلق بین ۷۳۲ و ۲۳۶۸ به دست آمد. مطالعه تخم این گونه ماهی توسط میکروسکپ الکترونی و دوبریال مطابق بودند. هماوری ماهی خله قناتی دارای تخم های چسبنده است. این یافته ماهی نتی و میکروسکوپی توسکی می زونه ماهی توسط میکروسکپ الکترونی و دوبریال مطابق بودند. هماوری میلام قناتی دارای تخمهای چسبنده است. این گونه ماهی خوسکی این گونه ماهی خوسکی ایک و راین کرورماهیان توجیه میکند.

\*مؤلف مسئول