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Original Article

The Reproductive Biology of Shirbot (*Barbus grypus* Heckel, 1843) in the Maroon River, Iran

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Abstract: Shirbot (*Barbus grypus*) is one of the species in south and southwest of Iran which is greatly favorable to residents of the region. Unfavorable ecological conditions in habitat of this species and overfishing have led to the reduction of the population of Shirbot. Therefore, to restore the natural stock of this species, identifying its reproductive cycle associated with its habitat is of a great importance. In this study, the reproductive status of Shirbot in the Maroon River in Khuzestan Province was studied in six sampling steps during four seasons. Also, morphological indicators, sex ratio, age of fish, gonadosomatic and hepatosomatic indices, histological changes in the testis and ovary of the fish were studied. The ratio of male fish to female was 2.35 to 1. The maximum value of gonadosomatic index (GSI) is among the specimens aged 3 to 5 years and in March and April. An increase of hepatosomatic index (HIS) during March may indicate the increased activity of liver during vitellogenesis and vitellogenin synthesis which is well verified by histological results of ovarian tissue. Based on our findings we recommend that the maximum reproductive activity of Shirbot in the Maroon River starts around the end of March and continues to middle of July.

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Introduction

Sustainable use of aquatic ecosystem resources depends on reproduction, and the availability of proper conditions for reproduction and growth of aquatic organisms. Thus, monitoring aquatic ecosystems is of a great importance. One of the main aspects of monitoring aquatic ecosystems is studying the reproductive cycle of varied aquatic organisms especially fishes, so that fishing without a thorough comprehension of the fish's breeding and evaluation of their resources may lead to complete disappearance of a species (Hosseinzadeh Sahafi et al., 2001; Khorashadizadeh et al., 2006). In other words, biology and ecology of different species of fish in aquatic ecosystems can play a significant role in developing a pattern for conserving and restoring indigenous and commercial fish stocks (Gharaei et al., 2011; Aliasghari and Parafkandeh Haghighi, 2013). Therefore, due to recent issues in breeding and population rehabilitation of many indigenous and commercial species, researchers in the field of fisheries have studied varied biological aspects of fish especially reproductive characteristics of fish (Ronnback et al., 2002; Orlando et al., 2003; Sinsneros et al., 2004; Guerriero et al., 2005; Guerriero, 2007). The studies on reproductive indices such as gonadosomatic index, morphology of gonads, histological changes of gonads in different seasons in Yellowfin tuna, *Thunnus albacares*, (Oryan et al., 2004), Javelin Grunt, *Pomadasys kaakan* (Valinasab et al., 2007), Klunzinger Mullet, *Liza klunzingeri* (Valinasab et al., 2004a, b), *Abudefduf sexfasciatus* (Hosseinzadeh Sahafi et al., 2002), Leaping mullet, *Liza saliens*, (Yousefian et al., 2003; Balik et al., 2011), Orange-spotted grouper, *Epinephelus coioides*, (Abbasi et al., 2005),

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Barbus esocinus (Eskandari et al., 2004), Kutum, *Rutilus frisii kutum*, (Najar Lashgari et al., 2007; Aminian at al., 2008), Silver sillago, *Sillago sihama*, (Hosseinzadeh sahafi et al., 2001) as a key method in determining the spawning season and studying the life cycle of the fish verifies this issue. Therefore, studying the changes in levels of sex steroid hormones, histology of gonads, and determining the biological indicators of the final maturation of wild fish along with constant monitoring of ecological and environmental conditions of aquatic ecosystems of Iran can help to have access to reproduction biotechnology and restoration of commercial and indigenous fish stock.

Shirbot (*Barbus grypus*) is one of the species of the genus *Barbus* and carps being usually found in the southern and west-southern river basins of Iran. This species can easily adapt to environmental conditions. However prefers slow flowing waters at 22°C. Although this species usually live in shallower waters than five meters depth, chooses very shallow waters with a gravel bed for spawning (Abdoli, 2000). This species is favorable among indigenous residents of south western provinces of Iran, especially Khuzestan Province.

The Maroon River is one of the habitats of this species. This river originates in the Dameh Mountain in Dehdasht region in Kohkilouyeh and Boyer Ahmad Province and after passing through Behbahan and Omidieh, joins the Jarahi River. The discharge of this river in highlands is over 119 m^3 per second and reaches 20 m^3 per second in estuary regions. Temperature of this river in different seasons of the year varies from 15 to 30°C. This river has a depth of 1 to 15 m and serves as a good habitat for different species of fish including Shirbot (*Barbus grypus*), Hemri (*B. luteus*), Berzem (B. barbulus), Common carp (*Cyprinus carpio*), Siahmahi (*Capoeta trutta*), Gel-cheragh (*Garra rufa*), Lotak (*Cyprinion tenuiradius*), Mesopotamian spiny eel (*Mastacembelus mastacembelus*), Shah kuli (*Chalcalburnus mossulensis*), Nazok (*Chondrostoma regium*), Arteshi catfish (*Glyptothorax silviae*), and Loach (*Nemacheilus Sp.*). Recently, many efforts have been done on the reproduction and breeding of indigenous species especially on the *Barbus* in the Khuzestan Province and other areas of Iran. However, further research could be helpful in overcoming problems and achieving its reproduction and breeding techniques. As previously mentioned, studying the life cycle of these fish in nature and with regard to the climatic conditions of each region may be a key to this problem. Therefore, this study aimed to investigate the life cycle of Shirbot in the Maroon River with regard to the climatic conditions of the Behbahan District and hydrologic conditions of the Maroon River.

Materials and Methods

The data collected from local fishermen in three stations were used for sampling from the Maroon River. These stations were located at the lake behind the Maroon Dam (30°42´N and 50°22´E), Kheirabad (30°21´N and 50°19´E), and Shohada regulatory dam (30°39´N and 50°18´E). Sampling was done at 6 stages and during summer, fall, winter and spring via a gill net. Samples were freshly transferred to the laboratory and the species intended for the purpose of this study was separated after identifying the fish. Then, using a digital scale, biometric board/scale, and calipers, the weight, morphometric characteristics of the fish including the total length, standard length, head length, snout length, and meristic characteristics such as number of whiskers, number of lateral line scales and scales up and down the lateral line were measured to assure the initial identification of the species. Their age was determined using the scales on different parts of their body. In this method, the age of fish was estimated by counting the annuli on the scale (Adeli, 2008).

Then, the specimens were autopsied and after sex identification, their gonad and liver was weighed using a digital scale to determine GSI and HSI. At this stage, fecundity of the fish which had reached sexual maturity was estimated based on the gravimetric method. For histological study of the gonads and determining different stages of sexual maturation, small pieces of the gonad from initial,

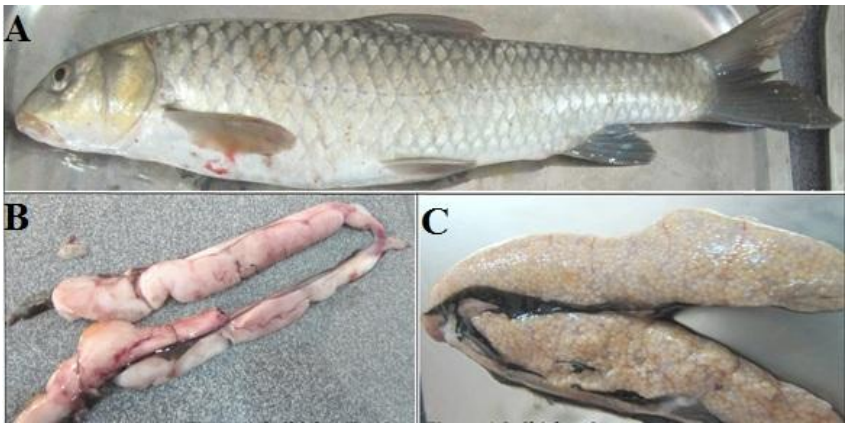

Figure 1. Shirbot, *Barbus grypus*, (A); Shirbot testis (B); and Shirbot ovary (C). Studying the gut content of these fish shows that they are omnivore, so that it is hard to accurately determine the food items these fish consume. However, these fish usually feed on plants.

middle, and final parts were sampled and fixed into Bouin's solution for 48 hours. After preparation, dehydration, and bleaching in alcohol and xylol solution, the tissues were embedded in paraffin and sectioned with a microtome. After preparing the slides, they were stained with hematoxylin and eosin stains.

Statistical analysis: Data were analyzed using one-way analysis of variance (ANOVA) by SPSS 15. Normality of the data was checked using the Kolmogorov-Smirnov test. The results were shown in mean ±S.E. The correlation between different variables was determined using the Pearson correlation method.

Results

Shirbot, *Barbus grypus*, is cylindrical in appearance. This fish is dark olive which looks lighter at the abdomen area (Fig. 1). Shirbot has 4 whiskers, protruding plump lips and the captured fish had the average length of 36.61 ± 7.95 cm and were 1-6. The sex ratio in the captured fish was 70.15 male to 29.85 female fish during the experiment. However, in some cases, sex identification was not possible.

There is a significant relationship (P<0.01) between the total length of the captured fish in different seasons with their sexual maturity and gonadosomatic index. The results indicate a significant relationship (P<0.01) between total weight and gonadosomatic index and between the age and sexual maturity. There was a significant relationship between changes in hepatosomatic index and that of gonadosomatic index in fish captured in different seasons (P<0.01) (Table 1).

Based on what is presented at Table 2, the male fish captured in different seasons are in a higher ratio compared with the female fish (2.35 to 1). The higher value of gonadosomatic index was found in fish aged 3-5.

The significant increase in hepatosomatic index (HIS) in March may indicate increased activity of liver during vitellogenesis and vitellogenis synthesis. The maximum value of gonadosomatic index (GSI) in the captured fish was found in March and April (Table 3).

Figure 2 illustrates the testis histological structure of Shirbot. In this figure, most spermatogonia have pale vesicular nuclei and granular cytoplasm. These cells are about 5-10 micrometers. Spermatogonia II are normally smaller than spermatogonia I and are usually visible as a cell mass in testis tissue.

Spermatocytes usually have a relatively dense nucleus and average cytoplasm content. Spermatocytes are about 4-6 micrometers. Primary spermatocytes are usually bigger than secondary spermatocytes. A significant proportion of the cells in the testis of the fish were primary and secondary spermatocytes.

Spermatid cells have a dense nucleus, which is surrounded by a ribbon-like cytoplasm and eosinophilic cytoplasm. These are the smallest cells that are found in generative epithelium (with an approximate size of 2-3 micrometers) and during spermatogenesis, their cytoplasmic connection

Table 1. The correlation between different biological factors in the captured Shirbot (*Barbus grypus*) during sampling

	Total length	Total weight	Age	Sex	HSI	GSI
Total weight	0.786**					
Sig.	0.00					
Age	0.513**	0.450**				
Sig.	0.00	0.00				
Sex	-0.311**	-0.362**	-0.319**			
Sig.	0.003	0.001	0.003			
HIS	-0.102	-0.164	-0.011	-0.030		
Sig.	0.345	0.129	0.992	0.782		
GSI	-0.477**	-0.437**	0.048	0.104	0.230*	
Sig.	0.00	0.00	0.656	0.336	0.032	
Maturity	-0.280	-0.169	-0.433	0.061	0.045	-0.063
Sig.	0.009	0.118	0.00	0.573	0.682	0.564

Table 2. Total length, hepatosomatic index, and gonadosomatic index of fish in male and female fish.

Age	Sex			Total length (cm)	Total weight (g)	Hepato-somatic-index	Gonado-somatic-index
	Male	Female	Unknown				
1	0	0	2	29.80±1.06	200.00±4.05	1.15±0.05	0.75±0.01
2	12	4	2	31.37±4.04	390.22±80.47	0.54±0.23	3.23±1.64
3	36	6	2	31.90±3.28	374.76±125.28	0.68±0.29	4.55±3.01
4	48	26	0	38.18±7.35	509.38±296.22	0.98±0.91	4.45±2.43
5	26	8	0	35.76±3.53	458.41±261.19	1.09±0.98	5.99±2.42
6	0	8	0	58.38±5.75	1800.05±476.1	0.68±0.14	1.64±0.95

Table 3. Total length, total weight, hepatosomatic and gonadosomatic index of fish according to the sampling time.

Sampling time	Frequency	Total length (cm)	Total weight (g)	Hepatic-somatic index	Gonodo-somatic index
September	26	38.04±9.87	518.93±359.05	0.68±0.15	2.84±0.47
November	24	37.07±5.84	376.18±76.80	0.82±0.25	3.61±1.07
February	42	39.92±9.65	664.46±589.14	0.69±0.68	4.19±1.93
March	28	31.98±2.35	315.34±61.38	1.83±1.32*	7.56±3.18*
April	18	32.88±4.23	521.05±366.46	1.06±0.50	7.58±2.65*
July	36	36.32±7.46	572.72±304.39	0.46±0.12	2.78±1.48

gradually breaks.

Spermatozoa cells have a dark round nucleus and very little cytoplasm which make cytoplasm identification in these cells very hard. Identifying the flagella/cilium-like tail was/is not possible in the tissue samples. These cells are about two micrometers and are found in the lumen of the testis. Sertoli cells have an elongated and triangular nucleus. Cytoplasm of these cells is not usually clear and it is hard to identify them. Cytoplasmic arms of a sertoli cell contain spermatogenic cells and form spermatocysts. Compared with germ cells, sertoli cells are less and usually found as single cells adjacent to testicular lobules walls. In some cases, sertoli hypertrophic cells are much like spermatogonia in appearance.

Interstitial cells (Leyding cells) have a round or oval nucleus, dense and vacuolated cytoplasm. Compared

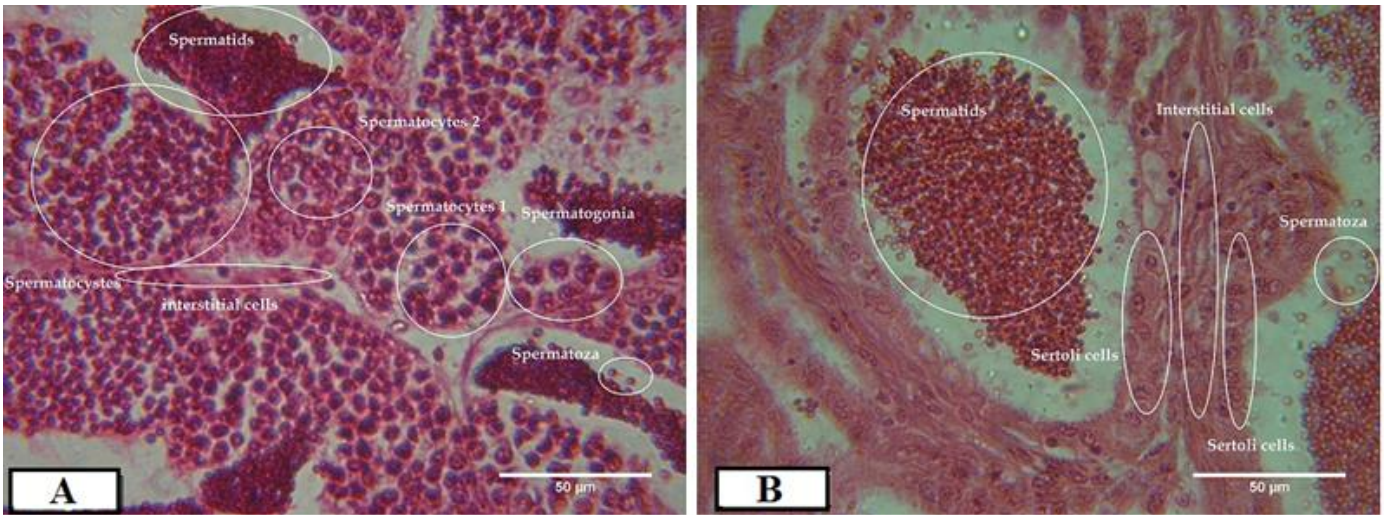

Figure 2. Histology of testes in Shirbot (*Barbus grypus*), different parts of testis including spermatogonia, spermatocytes I and II, spermatid, spermatocyst, spermatozoa, intestinal cells and sertoli cells are illustrated in Figure A and B.

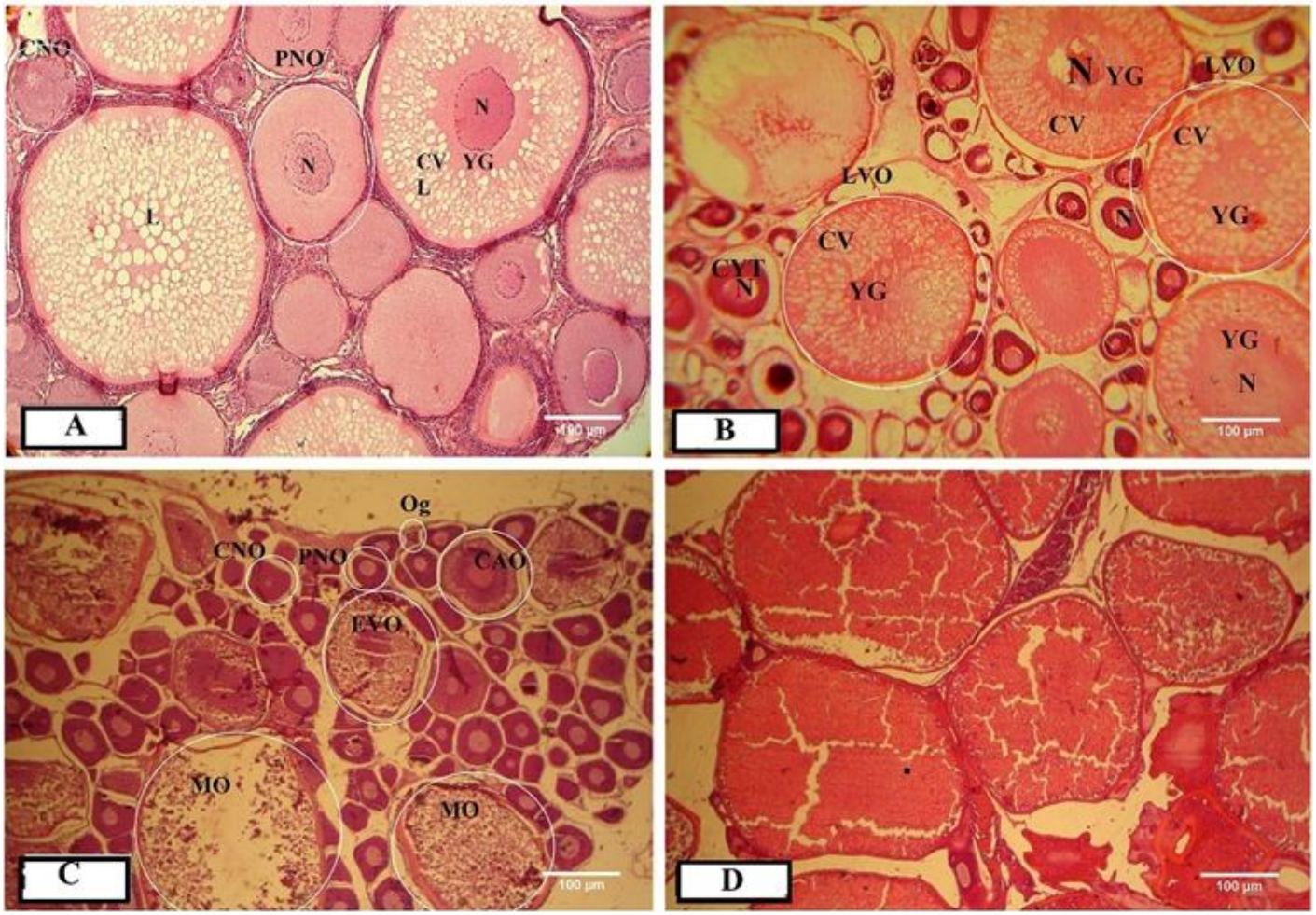

Figure 3. Histology of Shirbot (*Barbus grypus*) ovary, different parts of ovary tissue are presented in Figures A to D including oogonia (Og), oocytes at chromatin nucleus stage (CNO), oocytes at pronucleus stage (PNO), oocytes at cortical alveolar stage (CAO), oocytes at early (EVO) and late stages of vitellogenesis (LVO), final maturation of oocytes (MO), yolk granules (YG), cortical vesicles (CV), and nucleus (N) in oocytes.

with germ cells, these cells are usually single or as small cell communities in interstitial space and testis lobules. However, in appearance, they are more like spermatocytes, with interstitial cells just in the space between lobules.

Spermatocysts are functional units of testis and are

composed of spermatogenic cellular communities including spermatogonia, spermatocytes and spermatids which are surrounded by sertoli cells' cytoplasm. In spermatocycts, cells look like a cell mass and the cells are connected to each other by cellular connections and remain like this up to the final maturation and the release of spermatozoa (Fig. 2).

Figure 3 illustrates the histological structure of Shirbot ovary. In this Figure, the oogonia are the smallest oocyte cells. The main characteristics of these cells are a big nucleus, which is sometimes indistinguishable, and a little cytoplasm. At chromatin nucleus stage, oocytes look a bit bigger than oogonia. At this stage, the oogonia are surrounded by pre-follicular cells (which are probably granulose cells) and this is when follicular layers begin to develop. As oocytes growth, size and volume of cytoplasm increases, which looks more dense and granular compared with the oogonia. Now, oocytes have a relatively big nucleus containing a big and single nucleolus. The size of the nucleolus (germinative vesicle) increases along with oocytes growth, and several nucleoli appear which are usually around the nucleus. Therefore, at this stage the oocytes are called pronuclear stage. Irregular dark spots can be seen in cytoplasm, although pronuclear oocytes may have clear or amphophilic vacuoles in their cytoplasm. These cells are abundantly found in ovary tissue of mature fish (Fig. 3).

Oocytes are usually bigger at cortical alveolar stage compared with pronucleus oocytes. At this stage, cortical alveolar appear in ooplasm. There is no doubt that alveolar cortical is different from yolk and has no role in feeding the fetus/embryo. Chorionic layers appear at this stage and pre-follicle cells are easily visible. At the early stage of vitellogenesis, oocytes are bigger than those in cortical alveolar stage. At this stage, the most striking features of oocytes are their spherical shape and eosinophilic characteristics, as well as the presence of granules or yolk globules in their cytoplasm. In some cases, accumulation of yolk in the central region of the cell, especially in slides stained with hematoxylin and eosin may be mistakenly seen as nucleus. At the late stages of vitellogenesis, yolk granules accumulate in oocytes and it increases the cell size. The increase of yolk in oocyte cells marginalizes the corticals alveolar content. Gradually, the nucleus moves to the cell margin.

At the final maturation, oocytes reach their maximum volume due to absorbing the yolk and water intake/dehydration. The nucleus migrates toward the micropyle and the cell margin and germinal vesicle breakdown (GVBD) occurs. The loss of nucleus does not help identifying the features of this stage of oocytes that much. Anyway, in most big oocytes, the nucleus is indistinguishable. Due to the fragile nature of cells at this stage, it is hard to make histological slides. Therefore, in most cases it was hard to see cells at the final maturation (Fig. 3).

Discussion

Shirbot is one of the main edible species in the Maroon River which is of a great importance to local people, so that due to local fishermen's great interest to this species and over fishing, and also river pollution, and loss of habitats caused by drought in recent years, the population of this fish has decreased dramatically and these fish are facing the risk of extinction. Therefore, obtaining information on the reproductive physiology and biology of this fish may help restoring fish stock, reproducing, and cultivating them in cultural ponds. The findings of this study suggest that female Shirbots reach sexual maturity at the age 3-4 years old, while males reach sexual maturity at the age 2-3 years old. Furthermore, these fish start spawning as the weather warms since the beginning of April up to late July. Changes in gonadosomatic index during this period verify these results. In this study, the male fish had a higher ratio compared with the female ones among the captured fish. The sex ratio in the Shirbot that captured approximately was 2 male to 1 female fish during the experiment. Sex ratio of *B. barbulus* and *B. esocinus* was 1:1 to 1:4 (female to male), respectively (Mortezavizadeh et al., 2010;

Eskandary et al., 2001).

There was a significant relationship between total length and age of the fish captured in different seasons and their sexual maturity. A significant correlation between the gonadosomatic index with length and weight of fish was observed. Patterns in the monthly GSI fluctuations that shows similar pattern to the reproductive cycles are common among other barbus fish (Eskandary et al., 2001; Mortezavizadeh et al., 2010; Ghafari and Jamili, 2010). The significant relationship between the numerical changes in hepatosomatic index with those of gonadosomatic index in the fish captured in different seasons suggests a relationship between the liver activity in different stages of sexual maturation and the growth of gonads. This relationship is evident in female fish during vitellogenesis.

According to histological studies, most fish captured in September and even November are at the second, and some are at the first, stage of sexual maturity. At this stage, testis and ovary look like a dark red semi-transparent ribbon. The ovary is only recognizable under the microscope. These fish are usually at their second sexual cycle. At this stage of sampling, a few of the fish are experiencing their first sexual maturity; and in some cases it is hard to distinguish their gender. For instance, in some mature fish captured in November, it was hard to identify their gender by the gonad appearance due to absence of sexual differentiation. In these fish, primary germ cells were developing. The main characteristics of these cells are their relative big size, lower ratio of nucleus to cytoplasm, and the presence of one or two nucleolus in them. Stromal tissue is a dense and filament connective tissue with many collagen fibers which are seen red in hematoxylin and eosin staining. Moreover, in this group of fish and another group of immature fish in which sex identification was difficult, only primary oogonia and oocytes were detectable in female fish, and primary spermatocytes and spermatogonia. In other words, in a few cases, besides oogonia, pronuclear oocytes were detectable in the ovary of juvenile fish. In the ovary of fish which were at the first phase of maturity, more than 90% of oocytes were at pre-vitellogenesis, and often at pronuclear and cortical alveolar stage. However, various oogonia were found among larger ovarian follicles in the sexual gland of mature fish. At this stage, only spermatogonia were detectable in the testis of male fish.

Most of the fish captured in November or even some of the fish captured in February were at the second stage of sexual maturity; at this stage, oocytes were visible as polygons and dispersed granular particles in cytoplasm. Most primary oocytes are morphologically spherical, oval, or polygon. These cells have a fairly large nucleus which occupies a great part of ovule/ovum and cytoplasm surrounds it as a thick layer. At this point, oocytes are normally at the first or second phase of vitellogenesis. By cytoplasm development, the diameter of the oocyte increases too. Follicular layers surrounding developing oocytes are easily detectable. Now, not only the spermatogonia, but also the primary and secondary spermatocytes, and in some cases the spermatozoa are observable in the testis of male fish. At this stage, size of the ovary and the testis has increased and they are full of capillaries. The oocytes are easily detectable and sex identification is easy.

In most fish captured in March and a few of those captured in February, the ovary was at the final stage of vitellogenesis; at this stage, the cells were round and cytoplasm was full of yolk granules. Lipid particles looked like intercellular hollow cavities. Primary and secondary spermatocytes and spermatids were seen in the testis of male fish. Seconday spermatocytes and spermatids were abundantly found in the testis tissue. The testes were white, but the ovaries were still red. The increased rate of secondary oocytes was evident compared with the previous stage. Due to vitellogenesis, the volume of oocytes is increasing. The numerical value of hepatosomatic index (HIS) reaches its peak. This pattern is similar to that reported in *B. capito* by Shajiei et al. (2002).

However, the testes of many fish captured in March, and April was completely white and contained some

seminal fluid. Increased spermatids and the appearance of spermatozoa are the most notable histological changes of the testes. At this stage, vitellogenesis is done and a few of oocytes have reached their final maturation. Oocytes are generally round and big. At this point, the ovary is at the fourth stage of sexual maturation. In the ovary of mature fish, oocytes are at the final phase of vitellogenesis and mature oocytes are easily detectable. Follicules reach their biggest size, especially after water intake. After ovulation of the oocytes, the fish start to spawn. This is evident in some of the fish captured in April and most of those captured in July. Since due to the fragile nature of oocytes, preparing histological samples was very difficult, some of the samples were useless. At this stage, sex cells in sexual glands had reached their final maturity and the oocytes and sperm liquid were easily secreted with a slight pressure. Gonadosomatic index (GSI) was in its highest peak.

In a few of fishes captured in September, the ovaries and testes have shrunk and are full of capillaries. In the ovary of these fish, only the remains of follicular layers, including theca and granulose cells are visible. These histological changes in the ovarian and testis can be typical in many species of freshwater fish (Eagderi et al., 2006; Eagderi et al., 2013).

Therefore, since estimating the sexual maturation time and the fish's spawning season is done by several authors with regard to histological changes of gonads in breeding and pre-breeding fish in various aquatic ecosystems and is proposed as a reliable method (Eagderi et al., 2006; Eagderi et al., 2013). Our results showed that the spawning season of Shirbot (*B. grypus*) in Maroon River normally starts early in March and continues till the middle of July. Spawning season of *B. xanthopterus* in April (Eskandary, 1999), *B. esocinus* in May (Eskandary et al., 2001), *B. grypus* and *B. sharpeyi* (Nikpey, 1996), *B. barbulus* (Mortezavizadeh et al., 2010) and *B. pectoralis* (Ghafari and Jamili, 2010) between March to April in Karoon River were reported.

Therefore, capturing the broodstock for the purpose of artificial breeding of these fish by experts and applying restrictive laws of fishing during this period could help restoring the stock of these important fish.

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Reference

Abbasi F., Oryan Sh., Matinfar A. (2005). Histology and morphological study of gonad in *Epinephelus coioides* in the Persian Gulf. Pajouhesh-va-Sazandegi, 66: 68-74.

Abdoli A. (2000). The inland water fishes of Iran. Iranian Museum of Nature and Wildlife. 378 p.

Aliasghari M.., Parafkandeh Haghighi F. (2013). A comparative study on reproduction of kilka species (Clupeidae) in southeastern parts of the Caspian Sea. Iranian Journal of Fisheries Sciences, 12(3): 522-532.

Aminian Fatideh B., Hosseinzadeh Sahafi H., Shaabani A., Yaghmaei F., Shafiei Sabet S. (2008). Determination of stages of sex maturity of *Rutilus frisii kutum* Kameskii, 1901. In the Caspian Sea using biological indexes. Journal of Science, 8(2): 107-120.

Balik I., Emre Y., Sümer Ç., Tamer F.Y., Oskay D.A., Tekşam I. (2011). Population structure, growth and reproduction of Leaping grey mullet (*Liza saliens*, Risso, 1810) in Beymelek Lagoon, Turkey. Iranian Journal of Fisheries Sciences, 10(2): 218-229.

Eagderi S., Mojazi Amiri B., Adriaens D. (2013). Description of the ovarian follicle maturation of the migratory adult female Bulatmai barbel (*Luciobarbus capito*, Güldenstädt 1772) in captivity. Iranian Journal of Fisheries Sciences, 12(3): 550-560.

Eagderi S., Mojazi Amiri B., Mirvaghefi A.R. (2006). A histological study of testis structure and reproductive cycle in male Bulatmai barbell (*Barbus capito*), migratory to Sefidrood and Polrood Rivers. Iranian Journal of Natural Resources, 59(1): 139-149.

Eskandari G., Dehghan S., Nikpay M. (2004). The reproduction biology of *Barbus esocinus* in Dez Dam Reservior. Iranian Journal of Fisheries Sciences, 13(1): 1-24.

Eskandary G.R. (1999). The biology of *Barbus xanthopterus* in Karkhe River and hour-Al- Azim area (Khuzestan Province). The Scientific Research Results of the Iranian fisheries Org., Tehran-Iran, 91 p.

Eskandary G.R., Dheghan S., Nikpey M., Miahi Y. (2001). The biology of *Barbus esocinus* in Dez dam lake (Khuzestan Province). The Scientific Research Results of the Iranian fisheries Org., Tehran-Iran, 75 pp.

Ghafari S.M., Jamili Sh. (2010). Certain Aspects of the Reproductive Biology of Berzem (*Barbus pectoralis*) in Karoon River. Journal of Fisheries and Aquatic Science, 5(1):33-41.

Gharaei A., Rahdari, A.A., Ghaffari M. (2011). Artificial reproduction of *Schizothoraz zarudnyi* using synthetic hormones. Journal of Marine Science and Technology Research, 10(1): 1-12.

Guerriero G. (2007). Seasonal stroids variations and maturity stages in the female chub, *Leuciscus cephalus* L. (Pisces, Cyprinidae). Italian Journal of Zoology, 74(4): 317-324.

Guerriero G., Ferro R., Ciarcia G. (2005). Correlations between plasma levels of sex steroids and spermatogenesis during the sexual cycle of the Chub, *Leuciscus cephalus* L. (Pisces: Cyprinidae). Zoological Studies, 44(2): 228-233.

Hosseinzadeh Sahafi H., Shariefpour E., Nazemi S.H. (2002). The reproduction biology of *Abudefduf sexfasciatus* in the Persian Gulf (Larak Island). Iranian Scientific Fisheries Journal, 11(2): 17-30.

Hosseinzadeh Sahafi H., Soltani M., Dadvar F. (2001). Some aspects of reproductive biology of Sillago sihama in Persian Gulf. Iranian Scientific Fisheries Journal, 10(1): 37-54.

Khorashadizadeh M.A., Abtahi B., Kazemi R., Fazli H. (2006). Anatomical and histological study of ovary development stages of bigeye kilka (*Clupeonella grimmi*) in Babolsar area, Iran. Iranian Scientific Fisheries Journal, 15(3): 61-74.

Mortezavizadeh S.A., Hashemi S.A., Eskandary G.R. (2010). Some aspects of the reproductive biology of *Barbus barbulus* Heckel, 1847, from Karoon River, Iran. Iraqi Journal of Aquaculture, 7(2): 123-136.

Najar Lashgari S., Khara H., Nezami Blouchi S.A. Saeidi A.A. (2007). A survey of the characteristics the sperm of producers of the With Fish (*Rutilus frisii kutum*) migrating to the Khoshkrood River. Journal of Biology Science, 1(1): 75-84.

Nikpey M. (1996). The biology of Barbus sharpeyi and *Barbus grypus* in Khuzestan Province. The Scientific Research Results of the Iranian fisheries Org., First program, Tehran-Iran, 83 pp.

Orlando F.E., Binczik G.A., Thomas P., Guillette Jr. (2003). Reproductive seasonality of the male Florida gar, *Lepisosteus platyrhincus*. General and Comparative Endocrinology, 131(3): 365-371.

Oryan Sh., Hosseinzadeh Sahafi H., Abdaly S. (2004). Histological study on ovary of Yellowfin Tuna (*Thunnus albacares*) in the Gulf of Oman. Iranian Journal of Fisheries Sciences, 12(4): 85-96.

Ronnback P., Bryceson I., Kautsky N. (2002). Coastal aquaculture development in eastern Africa and the west Indian Ocean: Prspects and problems for food security and local economies. Ambio, 31: 537-542.

Shajiei H., Vosughi G.H., Oryan S., Ramin M. (2002). Biological characteristic of growth and reproduction in *Barbus capito* in south coasts of the Caspian Sea, Gilan Province. Journal of Marine Science and Technology, 1(4): 85-98.

Sinsneros J.A., Forlano P.M., Knapp R., Bass A.H., (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. General and Comparative Endocrinology, 136(2): 101-116.

Valinasab T., Keyvan A., Sedghi Marouf N., Kamali I. (2007). Reproduction characteristics of Javelin Grunt, *Pomadasys kaakan*, in Hormuzgan waters of the Persian Gulf. Journal of Marine Sciences and Thechnology, 5(3-3): 87-99.

Valinasab T., Seyfabadi S.J., Javadzadeh N., Safikhani H. (2004a). Fecundity of Klunzinger Mullet, *Liza klunzingeri*, in coastal waters of Hendijan (Persian Gulf). Journal of Marine Science and Technology Research, 3(1): 75-82.

Valinasab T., Seyfabadi S.J., Javadzadeh N., Safikhani H. (2004b). Reproduction of Klunzinger Mullet, *Liza klunzingeri*, in coastal waters of Khuzestan

Province (Persian Gulf). Iranian Journal of Fisheries Sciences, 6(2): 129-142.

Yousefian M., Oryam Sh., Asaeian H., Farokhi F. (2003). A study on oogenesis of *Liza saliens*. Iranian Journal of Fisheries Sciences, 12(1): 131-152.