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Gonad Histology of the Persian Tooth-carp *Aphanius persicus* (Jenkins, 1910) (Cyprinodontidae) in Southern Iran

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Abstract: The histological changes of gonads were studied in *Aphanius persicus* from the Barm-e-Shoor spring (south of Iran). The gonads were extracted and prepared histologically. The maturity stage of each specimen was examined according to size and weight of gonad, degree of occupation in the body cavity, the type and number of germinal cells (in testis) and follicles (in ovary). We have described 5 sexual stages for females and 4 for males. The total length and body and gonad weight of each specimen increased with increasing maturity stages. In females, the appearance of ripe oocytes was in stage IV and V from November to May. Male's maturity was seen in stage III and IV from October to April. Therefore, the spawning period of female *A. persicus* is from May to November and for males it is from April to October. *A. persicus* has a long period of spawning, so it is a batch spawner.

Key Words: *Aphanius persicus*, ovary, testis, histology, Iran, Middle East

Introduction

Biologists have long been familiar with the maturation, or so-called ripening, of the gonads of teleost fishes and the subsequent onset of the spawning period. The reproductive biology of several species of cyprinodontids has been studied from a variety of viewpoints by Delgado et al. (1988); Bisazza, (1993); Leonardos and Sinis, (1998); Keyvani and Soofiani, (2004); Esmaeili and Shiva, (2006). Cyprinodontid fishes are small (less than 15 cm) and represented in every continent, but more or less confined to tropical and warm temperature climates (Sterba, 1989). The genus *Aphanius* consists of about 10 species occurring in fresh or brackish waters along the north coast of Africa, Spain, Italy, Greece, and Turkey, and along the coast of the Arabian Peninsula (Parenti, 1981; Krupp, 1983). The genus is sexually dimorphic and probably not monophyletic (Berra, 2001).

Reproductive studies of fishes, such as assessment of size at maturity and duration of the spawning season and fecundity, require knowledge of the state of gonad development and a large number of macroscopic maturity scales in individual fish (Carrasson and Bau, 2003). Although macroscopic staging can enable detailed recording of the seasonal occurrence of different reproductive stages, histological analysis of the gonads provide a more precise determination. Cellular substructures can be recognized in the growing follicles and ovarian tissue and allow for unambiguous grading and interpretation of reproductive status. Description of the general pattern of histology and development of teleost ovaries are given by Wallace and Selman (1981), Tyler and Sumpter (1996), and Tomkiewicz et al. (2003). In this study we provide a description of gonad development of *Aphanius persicus* in Barm-e-Shoor spring (southern Iran). It is a native species that is found only in Fars province. The histology of the gonads is examined in relation to the process of maturation and reproductive effort.

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Materials and Methods

The study was conducted in the Barm-e-Shoor spring (29°27′N-52°42′E, Alt. 1465 m) in the lake Maharlu basin, near Barm-e-Shoor village, 30 km southeast of Shiraz, Fars province, southern Iran. The fresh specimens of *Aphanius persicus* were collected monthly from June 2003 to May 2004 using a dip net and were immediately preserved in 10% formalin. In the laboratory the specimens were weighed using an electron balance (0.001 gr), then morphometric characters of them, such as total length (TL) and standard length (SL), were measured according to Coad (1988) using a vernier caliper with an accuracy of 0.05 mm. For histological studies the specimens were dissected and ovary or testis was extracted. Weight, length, width, color, and shape of each gonad were recorded and the maturity stage of them was recognized macroscopically according to Nikolsky (1963). Then the gonads were fixed in 10% formalin and prepared using routine techniques for histology: Dehydration by alcohol, clearing with xylol, embedding in paraffin wax, sectioning under 7 m thickness, and staining with haematoxylin-eosin according to Bancroft and Stevens (1990). Finally, photographs were taken from the prepared slides.

Results

Female fishes

The female gonad is composed of 2 sac-shaped ovaries extending along the body cavity in a dorsal position above the intestine. In a single ovary there were oocytes in several stages of development. The maximum diameter of mature oocytes was 1.71 mm. Generally, the size of the oocytes increased from November to May, peaking in the middle of spring, and then decreased slowly from end of May to November. According to size and weight of the ovary, degree of occupying the body cavity, presence or absence of ripe oocytes, and also diameter of the oocytes in the ovary, we have described 5 maturation stages of *A. persicus* ovaries. Figure 1 shows the macroscopic appearance of ovaries in different stages.

Stage I: Oogonia were dominant cells that had not stored lipid droplets and enough yolk yet. These cells had basophilic cytoplasm with a large circular nucleus and peripheral nucleolus that were near or adjoined to nuclear envelope. The ratio of nucleus to cytoplasm was high. The stroma was composed of matrix and intercellular space that were settled into mesovarium cover. A very thin layer of connective tissue originating from the ovarian capsule surrounded each oogonia (Figure 2A and 2B).

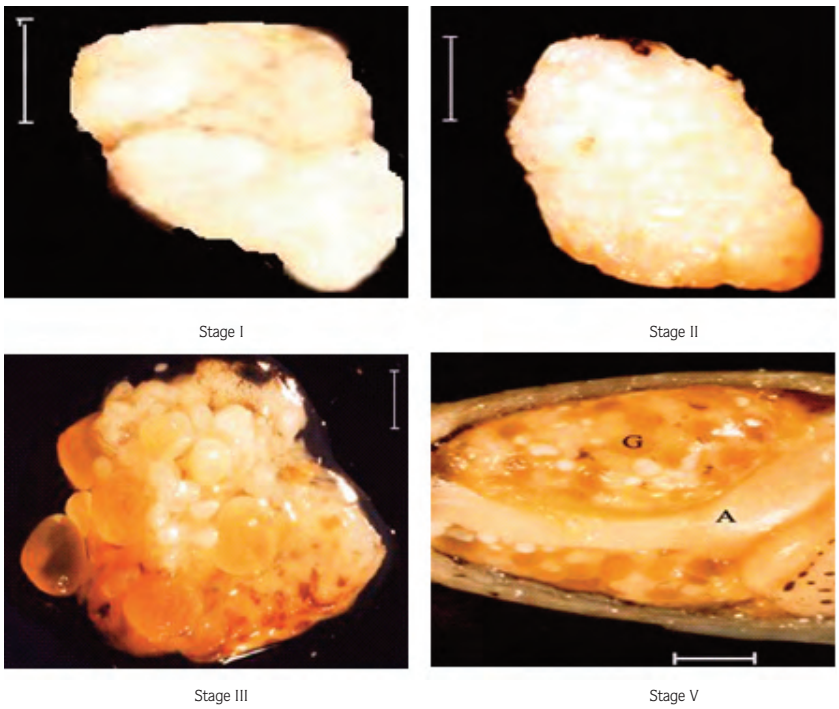

Figure 1. Morphology of ovary of *Aphanius persicus* in different stages. Gonad (G), alimentary canal (A), Scale bars of stages I-III = 1 mm and stage V = 2 mm.

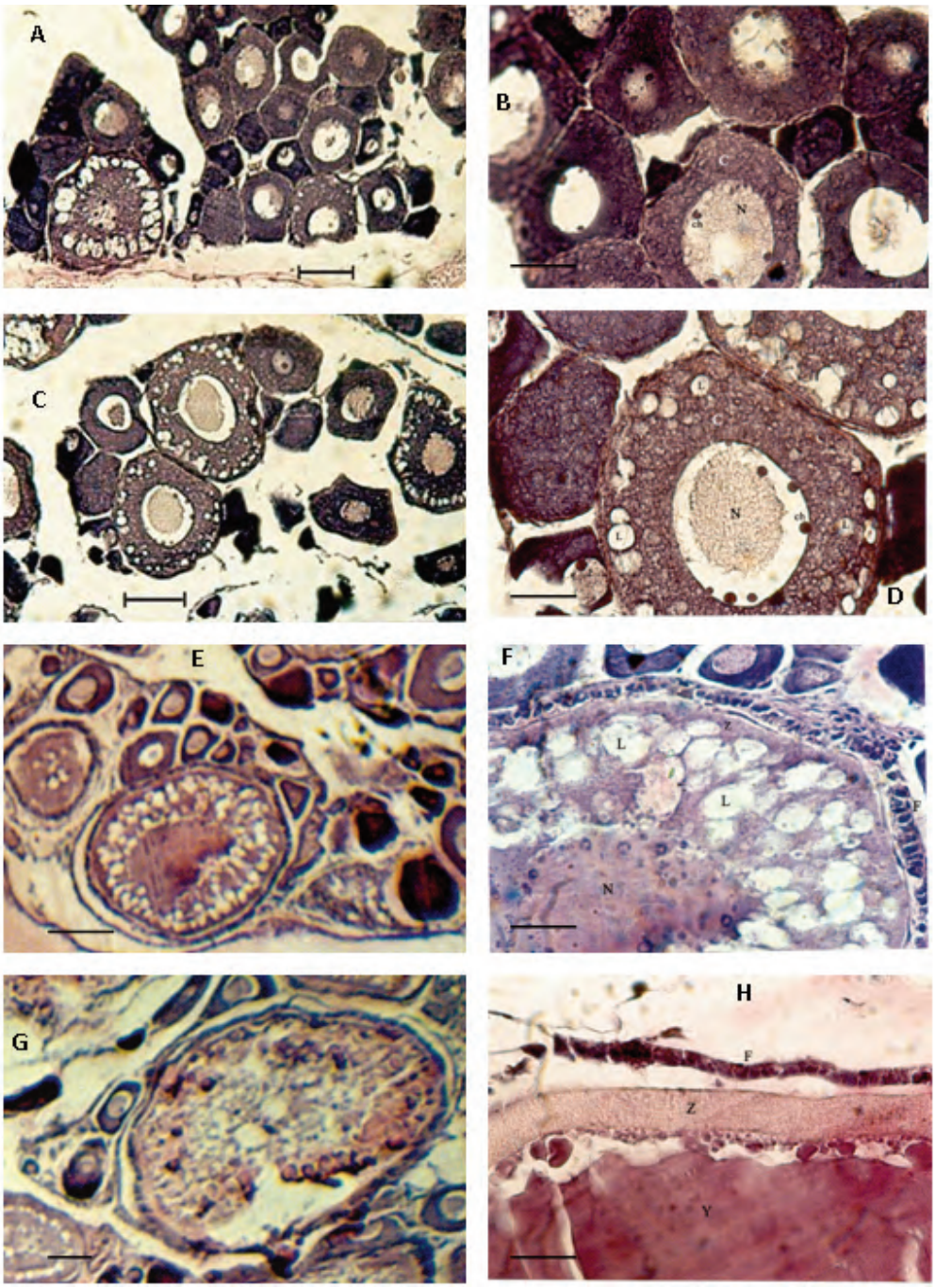

Figure 2. Photograph of ovary of *Aphanius persicus* in different stages. A. Oogonia with very low amount of yolk in stage I ovary. B. High magnification of A, cytoplasm of oocyte (C) , nucleus (N) and chromosomes (ch). C. In stage II, attention to larger size of oocyte and lipid droplet in cytoplasm. D. High magnification of C, lipid droplet (L). E. In stage III, follicles were large and the high amount of lipid droplet and surrounding layers of oocyte presented. F. High magnification of E. G. In stage IV, the small size follicles are at least, cytoplasm filled with yolk granules and lipid droplets. The surrounding layers of oocyte well defined. H. High magnification of part of very large follicle in stage V, the yolk mass (Y) and yolk granules, a thick layer of zona radiate (Z), and a single cuboidal layer of follicular cells (F) are seen. Hematoxylin and eosin staining, scale bars of A,C,E,G = 70 µm and in high magnification photographs = 20 µm.

Stage II: The growing follicles exhibit a weak basophilic cytoplasm characterized by small lipid droplets. Very thin layer of zona radiata, yolk granules, and squamosal cells of follicular layer appeared gradually. This stage is called returning stage, because the ovaries after spawning will return to this stage to start the oogenesis (Figures 2C and 2D).

Stage III: The growing follicles were characterized by small spherical acidophilic yolk granules and many clear lipid droplets that had entirely filled the cytoplasm and also a thin zona radiata surrounded by cubic follicular cells. The size and number of yolk granules and also the thickness of zona radiata increased gradually (Figures 2E and 2F).

Stage IV: The ratio of nucleus to cytoplasm of oocytes decreased progressively. They also showed a coalescence of lipids and yolk granules. The thickness of zona radiata was higher compared to the previous stages (Figure 2G).

Stage V: The hydrated oocytes were irregular in shape and contained single yolk mass and large lipid droplets. The zona radiata was completely thick and separated from the follicular layers (Figure 2H).

Table 1 shows the information about the total length (L), body weight (W), gonad weight (GW), and ova diameter (OD) of each stage.

Table 1. Mean and SD of total length (L), body weight (W), gonad weight (GW), and ova diameter (OD) in different stages of female *A. persicus*.

Stage	Parameter	Min	Max	Mean & S.D.
1	L(mm)	23.90	32.60	27.171 ± 2.308
	W(g)	0.19	0.65	0.352 ± 0.123
	GW(g)	0.001	0.012	0.003 ± 0.002
	OD(mm)	0.03	0.24	0.097 ± 0.042
2	L(mm)	22.80	41.00	29.200 ± 4.980
	W(g)	0.18	1.56	0.516 ± 0.369
	GW(g)	0.001	0.035	0.010 ± 0.010
	OD(mm)	0.06	1.47	0.229 ± 0.166
3	L(mm)	22.20	41.20	29.050 ± 4.549
	W(g)	0.17	1.59	0.463 ± 0.296
	GW(g)	0.013	0.053	0.031 ± 0.010
	OD(mm)	0.06	1.65	0.570 ± 0.336
4	L(mm)	24.00	49.00	35.611 ± 6.376
	W(g)	0.26	2.57	1.045 ± 0.659
	GW(g)	0.036	0.191	0.095 ± 0.038
	OD(mm)	0.06	1.71	0.755 ± 0.372
5	L(mm)	42.90	59.50	50.688 ± 5.009
	W(g)	1.84	4.86	3.236 ± 0.947
	GW(g)	0.188	0.420	0.273 ± 0.073
	OD(mm)	0.18	1.71	0.884 ± 0.398

Male fishes

Male gonads are in different stages of maturity throughout a year. Testis weight range is between 0.001 and 0.028 g. Each testis is composed of many lobules. Different types of germinal cells (spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid, and spermatozoid) situated close to each other and consist of the population of the same cell in each lobule. Therefore you can see different populations of germinal cells closely in each lobule, and in each maturation stage some of these populations are dominant.

The number of spermatozoa clusters, which is a high maturity stage indicator, showed an increasing trend from October to April and then decreased gradually from April to October. According to the absence or presence of spermatozoa plus based on dominant cell lineage of sperm in the testicular lobules, histologically we distinguished 4 stages for testicular maturation of *Aphanius persicus*:

Stage I: Testis was lobuled by trabeculae of the loose connective tissue surrounding the testicular surface as a capsule. Lobules were relatively small and conspicuous. Spermatogonia and primary spermatocytes were the dominant cells of this stage. Spermatogonia had a light cytoplasm and a large nucleus. Primary spermatocytes were smaller than spermatogonia and had a dense nucleus covered with small and pale cytoplasm. A few spermatid and clusters of spermatozoa were seen in some lobules. Spermatids were the smallest cell in lobules exposed under spermiogenesis and converted to spermatozoa. Secondary spermatocyte was larger than spermatid but smaller than primary spermatocyte. Spermatozoa were seen as an aggregation mass consist of dark clusters of cell's nucleus around each lobule and pale tails and they were arranged close to each other regularly in the center of the lobules (Figure 3A).

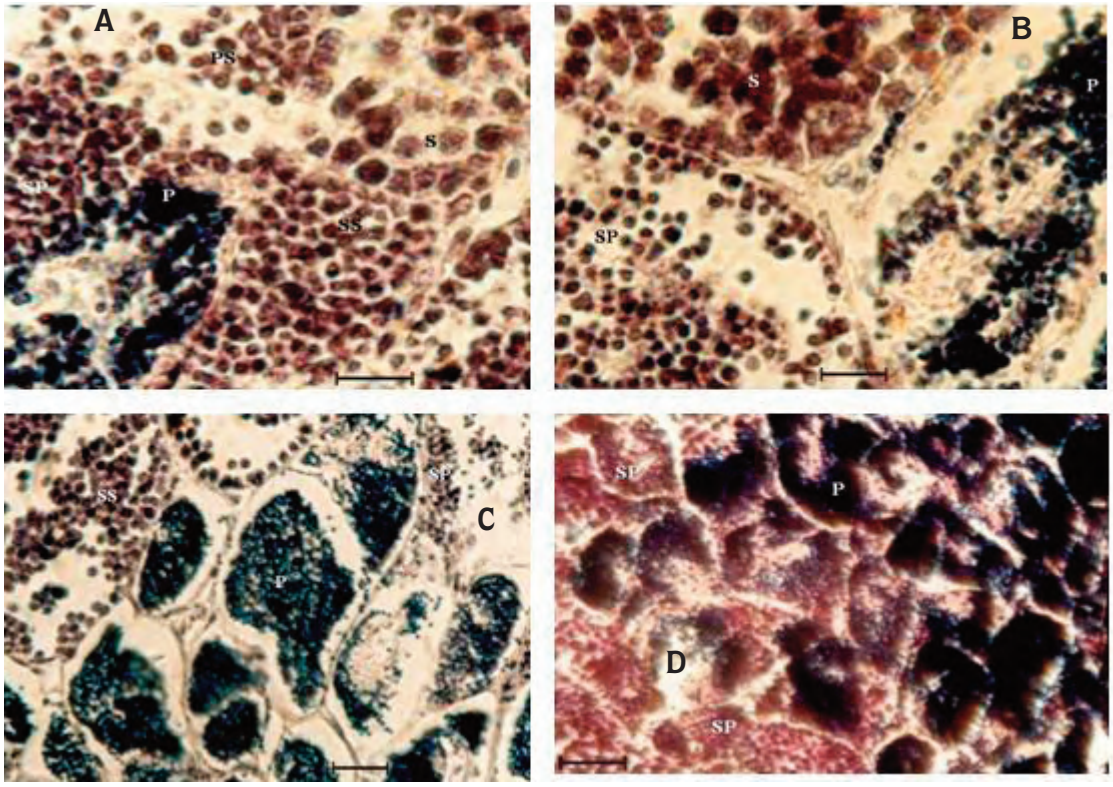

Figure 3. Photograph of testis of *Aphanius persicus* in different stages. A. Stage I, the dominant cells are spermatogonia (S) and primary spermatocytes (PS) but the other cells such as secondary spermatocyte (SS) and spermatid (SP) are seen. In some lobule the cluster of sperms (P) existed. B. In stage II, population of small germinal cells (eg. secondary spermatocyte and spermatid) is abundant. Connective tissue between lobules is seen. C. In stage III, the cluster of sperms fulfilled the most lobules. D. Only cluster of sperms and some spermatid are seen dominantly. Hematoxylin and eosin staining, scale bars in A – C = 10 µm and scale bar in D = 20 µm.

Stage II: The number of spermatozoa clusters was more than the previous stage. There were a large number of spermatogonia, some primary spermatocytes and few spermatids. Therefore, the size of the cells was still large in each lobule. Spermatocytes were recognized by their smaller nucleus and darkly staining chromatin material (Figure 3B).

Stage III: The number of large cells decreased but the population of small cells (spermatids and sperms) increased progressively. Therefore, the clusters of spermatozoa were more than those of the other cells (Figure 3C).

Stage IV: The gonads had well-defined lobules with a large number of spermatids and spermatozoa on their inner margin. Therefore, the predominant cells were spermatozoa with dark blue stain related to their nucleus. Very small amount of spermatogonia were seen in the subcapsular lobules (Figure 3D).

Table 2 shows the data about the total length (TL), body weight (W), and gonad weight (GW) of different stages.

Discussion

Gonad development was characterized by the presence of more advanced oocyte or spermatozoid in its higher stage of maturation. Many studies have been performed on histological and morphological changes of ovary in fishes (Biswas, 1993). Indices like color, size of the egg, and degree of occupation of body cavity in teleost fishes were used to gain classification keys. Maturity stages of ovary in fishes were divided into different steps based on interspecies similarities. These divisions varied between 5 and 7 stages (Bhatti and Al-Daham, 1978; Suluehanamma et al., 1981; Nee Lakamtan et al., 1989; Salem et al., 1999). The results of histological studies on ovaries of *Aphanius persicus* have shown 5 sexual stages which resemble many species such as *Thunnus albacares*, *Cyprinus carpio*, *Sillago sihama*, and *Lutjanus fulviflamma*. Study on testes of *A. persicus* have shown 4 sexual stages corresponding to *Cyprinus carpio* and *Aidablennius sphynx*. Ripe specimens were present in higher stages. In males, the appearance of high amount of spermatozoid was in stage III and IV. The number of these full filled spermatozoa clusters was very high in stage IV specimens.

Table 2. Mean and SD of total length (L), body weight (W), and gonad weight (GW) in different stages of male *A. persicus*.

Stage	Parameter	Min	Max	Mean & SD
1	L(mm)	22.30	25.40	23.933±1.169
	W(g)	0.140	0.340	0.233±0.065
	GW(g)	0.001	0.001	0.001±0.000
2	L(mm)	22.60	30.20	25.537±2.577
	W(g)	0.190	0.510	0.282±0.119
	GW(g)	0.002	0.008	0.004±0.002
3	L(mm)	25.50	38.50	31.312±4.238
	W(g)	0.290	1.110	0.606±0.275
	GW(g)	0.003	0.013	0.009±0.004
4	L(mm)	36.00	41.90	38.950±4.171
	W(g)	1.060	1.570	1.315±0.360
	GW(g)	0.021	0.028	0.025±0.005

Decreasing pattern in number of these clusters, which was seen from April to October, indicated the spawning period of male *A. persicus*. In females, the appearance of ripe oocytes was in stage IV and V. Histological examination showed that all stages of sexual maturity were represented in the ovaries from May to November; therefore, the spawning in female *A. persicus* is asynchronous, occurring over an extended period from May to November. Esmaeili and Shiva (2006) studied the GSI of this fish and our studies regarding the histological changes in gonads confirmed their analysis. Also, decreasing pattern in the size of oocytes of this fish confirmed that the Persian tooth carp is a batch spawner. Batch spawning is undoubtedly an advantage for this fish, which lives in unstable and changeable environments, such as a temporary lagoon or a very small pool. Batch spawning allows relatively large eggs to be laid, which have a greater chance of survival (Wooton, 1990; Leonardos and Sinis, 1998). Because temperature and some other environmental factors change at spawning time, investigations on these factors and their effects on spawning can be useful for identification of biology and reproduction of this fish.

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