Histomorphometrical study of gonads in the endemic cyprinid fish, *Cyprinion tenuiradius* Heckel, 1847 (Cypriniformes: Cyprinidae)

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Abstract

To describe the morphological and histological changes during the annual reproductive cycle of the gonads of *Cyprinion tenuiradius*, a total of 341 specimens (102 female and 235 male specimens) were collected monthly from the Rudbal River (in the Qarah Aghaj sub-basin, the Persian Gulf basin), Firuzabad, Fars Province, southern Iran. The randomly sampling was adequate to collect males and females at the immature, mature, spawning-active, and non-active phases of reproduction. Five ovarian and five testicular maturation stages (I–V) were described using macroscopic and light and electron microscopic criteria. Also based on the histological examination, six types of ovarian follicles (A-F) and five types of testicular cells were designated. The largest ovarian follicles were found in the stage V of ovarian developmental stages in females with mean body weight and standard length of 22.27 g and 95.13 mm, respectively. The highest number of sperms was observed in the stage V of testicular developmental stages in males with mean body weight of 25.67 g and mean standard length of 97.83 mm. Based on the percentage of late ovary and testis maturation stages (IV, V) and high frequency of large oocytes and sperms it was concluded that the Qarah Aghaj botak spawns once a year during spring and the beginning of summer from April to July.

Keywords: Annual reproductive cycle, *Cyprinion tenuiradius*, Ovary, Testis, Iran.

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Introduction

The genus *Cyprinion* Heckel, 1843 in Iran encompass four native and one endemic species distributed in western (Tigris River basin), southern (Lake Maharlu, Persian Gulf, and Hormuz basins), eastern (Lut and Sistan basins), and southeastern (Hamun-e Jaz Murian, Makran, and Mashkid River basins) of the country (Esmaeili *et al*., 2010; Esmaeili and Gholamifard, 2012; Coad, 2015). The Qarah Aghaj botak, *Cyprinion tenuiradius* Heckel, 1847 (Fig. 1) is the only endemic species of the genus which distributed in the Lake Maharlu and the Persian Gulf basins in southern Iran (Esmaeili *et al*., 2010; Coad, 2015). In spite of the key role and importance of the endemic elements for the biodiversity of each country, the Qarah Aghaj botak, along the most endemic fishes of Iran, have mostly been studied from the systematics viewpoint (Esmaeili and Ebrahimi, 2006; Esmaeili and Piravar, 2006; Esmaeili and Timoorei, 2006; Esmaeili and Gholamifard, 2012) and different aspects of its biology are needed to be studied. According to Berg (1949) *C. tenuiradius* reaches 16.3 cm in size (Coad, 2015). Esmaeili *et al.* (2014) recorded a significant length-weight relationship for *C. tenuiradius* based on 341 specimens from the Persian Gulf basin, where the total length and total weight ranges were measured 3.24-19.5 cm and 0.39-110.1 g, respectively. The values of *a* and *b* parameters in the length-weight relationship for this species are 0.011 and 3.05, respectively with a r^2 value 0.987 (Esmaeili *et al*., 2014) which remain within the expected range of 2–4 reported by Tesch (1971), and $2.5 < b < 3.5$ by Froese (2006). Also, Esmaeili and Ebrahimi (2006) recorded a fork length range of 5.04-13.49 cm based on 40 specimens of this species. The *a* and *b* parameters in the length-weight relationship were 0.0139 and 3.063, respectively (Esmaeili and Ebrahimi, 2006). Based on Esmaeili and Gholamifard (2012) in an ultrastructural study of the egg membrane surface of *C. tenuiradius*, the eggs of this species are almost circular in shape, have a smooth surface and one type II micropyle consisting of the flat pit and a long canal in the animal polar region. Cellular substructures can be recognized in the growing follicles and ovarian tissue and allow for unambiguous grading and interpretation of reproductive status (Monsefi *et al*., 2009). In this study, based on a comprehensive work for study of the reproductive biology of this poorly known endemic fish with limited distribution, we investigate the histomorphology of the gonadal tissue of *C. tenuiradius* in relation to the process of maturation and reproductive efforts.

Figure 1: Dissection of a male specimen of *Cyprinion tenuiradius* at the fifth stage of maturation shows position of its internal organ. T: testis (right), L: liver, I: intestine. Note the tubercles on the snout and operculum.

Materials and methods

Study area, fish collection

Between April 2007 and March 2008 fieldwork was conducted monthly (in the third decade of each month) in the Rudbal River (28º41' N, 52º37' E., 1168 m a.s.l.), in the Qarah Aghaj sub-basin of the Persian Gulf basin, near Rudbal village, 15 km southeast of Firuzabad, Fars Province, southern Iran. In this study only specimens of *C. tenuiradius* were caught. At this sample site, *Capoeta mandica* (Bianco and Bănărescu, 1982 It is author of species and no need to be cited) and *Garra rufa* (Heckel, 1843) (Cyprinidae) are sympatric with *C. tenuiradius*. All specimens of *C. tenuiradius* (102 females and 235 males) used in the present study were caught between 10 a. m. to 1 p. m. by an electrofishing device conducted with pulse (DC) generator and were immediately preserved in 10% formalin solution at the field and transported to the laboratory. The width of river at our sampling during different months of a year was between 10 to 15 meters. The sampling depth was between about 0.5 to 1.5 meters. The river bed was covered in the shallow parts with rubble and in the deeper parts with dirt and mud. In most months of the sampling period (times without rainfall), the Rudbal River had a relatively quiet flow. During this study, air and water temperatures varied from 21 C° to 36 C° and from 16.6 C° to 27 C°, respectively.

Now specimens are deposited and cataloged in the Zoological Museum-Collection of the Biology Department of Shiraz University (ZM-CBSU), Iran.

Macroscopic examination

In the laboratory, a few days after sampling, each specimen after washing with water was dried using a filter paper and weighed to the nearest 1 mg (body weight), using an electron

balance, then its morphometric characters including: total length (TL) and standard length (SL) were measured to the nearest 0.05 mm, using a vernier caliper. For histological studies the examined specimens were dissected and ovaries or testes of each specimen were extracted and examined macroscopically and microscopically, then each gonad was preserved in 10% formalin solution in a specific vial for future histological preparation. The macroscopic characters included: gonadal weight (GW, to the nearest 1 mg) and length (GL, to the nearest 0.05 mm).

Histological preparation and examination

For histological preparation and microscopic examination of gonads, several samples of gonads of 35 fish specimens (both sexes) at different stages of maturation were prepared using routine histological techniques consist of dehydration by ethanol, clearing with xylol, embedding in paraffin wax, sectioning under 7 µm thickness using the rotary microtome (Zeiss, Germany), and staining with haematoxylin and eosin according to Bancroft and Stevens (1990). Histomorphometrical examination on the diameter of follicle, nucleus and cytoplasm of each type of the ovarian follicles and different testicular cells at different stages of maturation was done using an ocular micrometer (Zeiss, Germany). Finally, photographs were taken from each prepared microscopic slide using a Nikon (Moticam model 350, Japan) digital camera.

Ultrastructurual study

Thirty five samples of the same ovaries and testes of *C. tenuiradius* at different stages of maturation (gonads of the previous histological study) were removed and fixed in the Karnovsky's fixative composed of 4% paraformaldehyde, 25% glutaraldehyde and 0.3 M cacodylate buffer with a pH of 7.3, postfixed in 1% osmium tetroxide, embedded in TAAB resin (including TAAB, DDSA, dodecenyl succinic anhydride, MNA, methyl nadic anhydride, and DMP, 2, 2-dimethoxypropane) (TAAB co., England), sectioned at a thickness of 1 µm using an Om U3 ultramicrotome (C. Reichert, Austria) and stained with the toluidine blue. Ultra-thin sections of 60–70 nm thickness were obtained and stained with uranyl acetate and counterstained with lead citrate, then were examined with a transmission electron microscope (Philips CM10, Germany) (Haris, 1991; Hunter, 1993).

Gonadosomatic index (GSI)

The spawning period of *C. tenuiradius* was also established from the analysis of Gonadosomatic index (GSI) which was calculated by: GSI = (gonad weight/total weight) × 100 (Nikolsky 1963).

Statistical analysis

Statistical analysis was performed using the SPSS software (version 11.5) with

the One-Way ANOVA, Scheffe's and Tukey's tests. $p<0.05$ was considered as a significant level.

Results

Female gonad morphology

The mean body weight of females of *C. tenuiradius* at different stages of maturation varied from 1.31 to 22.27 g that increased 17 folds in a few months. The mean body length of the females was grown from 38.07 to 95.13 mm or 2.5 folds at the same time. The mean ovarian weight differed between 0.003 g in the smallest size of gonad and 0.81 g in the biggest size of gonad or increased as 270 folds during several months. The mean ovarian length increased from 7.18 to 26.33 mm or 3.67 folds among the ovary developmental stages (Table 1).

Female gonad histology

Developmental stages of the ovary and follicle classes

Histological examination revealed six different types of ovarian follicles (A–F) in different ovarian stages (I-V) (Figs. 2-4; Table 2). Each ovarian follicle consisted of an oocyte with large and clear nucleus and several nucleoli and surrounding cytoplasm. This type of classification was based on the size and color of oocytes, relation of cytoplasm to nucleus, presence or absence of lipid droplets, follicular layers etc.

The different types of ovarian follicles (A-F) are described as below:

Type A: The type A ovarian follicle was the smallest type of follicles (mean diameter 15 µm), each with a highly basophilic cytoplasm, and a round, large and clear nucleus with numerous peripheral nucleoli near or adjoining the nuclear envelope. The ratio of nucleus to cytoplasm volume was high (the ratio value 1.5) (Fig. 2A; Table 2). The semithin section of this follicle type (Fig. 3A) confirmed the high density of its cytoplasm and attachment of the nucleoli to the nuclear membrane. The peritoneal layer of ovary was entered to its stroma and covered the follicles (Fig. 3A).

Type B: The type B ovarian follicles were 1.68 folds bigger than to the type A or mean 25.25 µm (Table 2). This follicle type was similar to the type A, but the ratio of nucleus to cytoplasm volume was lower (the ratio value 1.3) (Figs. 2B and 3A). The dense cytoplasm of this follicle type is illustrated in its ultrastructure aspect (Fig. 3G) that caused by many ribosomes.

Type C: Developing follicles (type C) exhibit a less basophilic cytoplasm that was characterized by small lipid droplets and a very thin follicular layer composed of squamous cells appeared gradually and surrounded the oocyte. Large peripheral nucleoli adjoining the nuclear envelope were distinguished (Fig. 2C). The follicular and peritoneal layers around the follicles of type C were illustrated in the semithin sections (Fig. 3B and C).

Table 1: Mean and standard deviation of body and gonad weights (g), body and gonad lengths (mm) in different stages of ovarian development of *Cyprinion tenuiradius* from the Rudbal River.

Stage	Body weight	Gonad weight	Body length	Gonad length
I	1.31± 0.40	0.003± 0.003	38.07± 4.07	7.18± 1.14
II	4.01± 2.72	0.004± 0.005	54.39± 11.84	9.90± 2.19
III	14.14± 4.35	0.02± 0.01	81.83± 8.37	15.20± 4.79
IV	13.28± 4.11	0.56± 0.09	82.98± 11.15	24.66± 3.93
V	22.27± 15.73	0.81± 0.43	95.13± 23.46	26.33± 3.27

Table 2: Minimum and maximum size, mean and standard deviation of follicular diameter, nuclear diameter and cytoplasm diameter (µm) in different types of ovarian follicles of *Cyprinion tenuiradius* from the Rudbal River.

Type of follicles	Follicular diameter		Nuclear diameter		Cytoplasmic diameter	
	Min.-Max. levels	Mean and SD	Min.-Max. levels	Mean and SD	Min.-Max. levels	Mean and SD
I	12.00-17.00	15.00± 1.73	8.00-10.00	9.00± 0.57	3.00-8.00	6.00± 1.63
II	22.00-29.00	25.25± 2.05	12.00-17.00	14.25± 1.58	8.00-15.00	11.00± 2.67
III	31.00-44.00	36.25± 3.95	17.00-21.00	19.5± 1.41	13.00-24.00	16.75± 3.28
IV	48.00-62.00	53.83± 5.38	22.00-28.00	24.66± 1.96	23.00-35.00	29.16± 5.11
V	75.00-125.00	104.40±15.16	24.50-60.00	44.02± 8.65	52.00-65.00	59.77± 3.60
VI	92.00-142.00	108.33±19.69	30.00-63.00	44.66± 12.76	57.00-79.00	63.66± 8.16

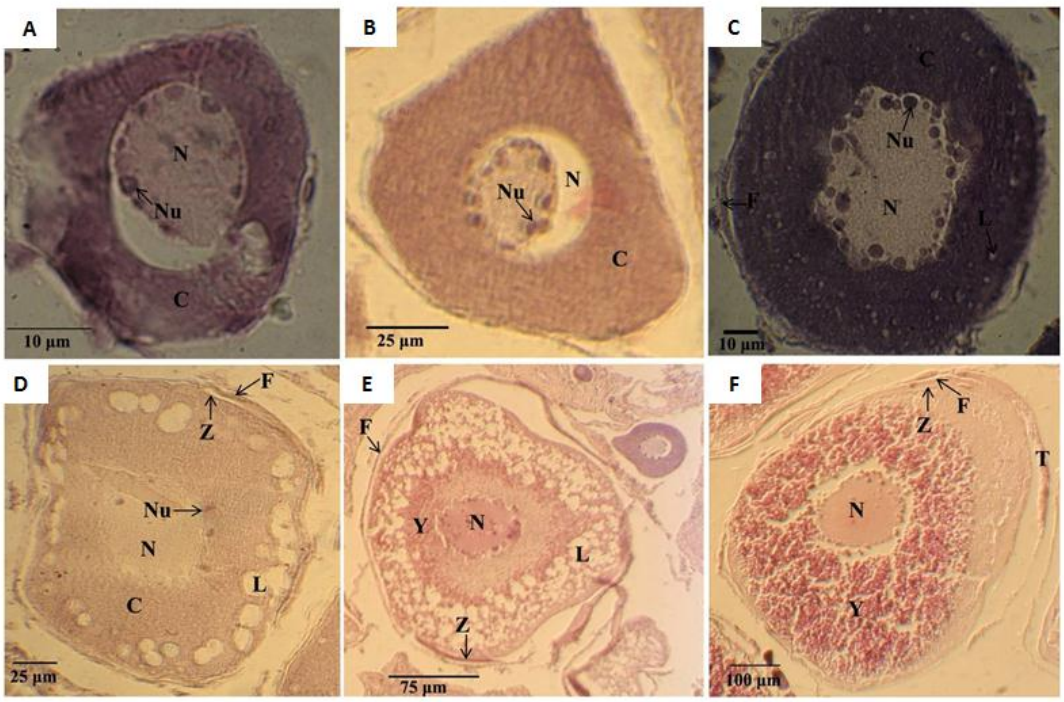

Figure 2: Different types of ovarian follicles of *Cyprinion tenuiradius* at different stages of maturation stained with hematoxylin and eosin (A-F). N: Nucleus, Nu: nucleolus, C: cytoplasm, L: Lipid droplet, F: follicular layer, Z: zona radiata, T: theca layer, and Y: yolk granules.

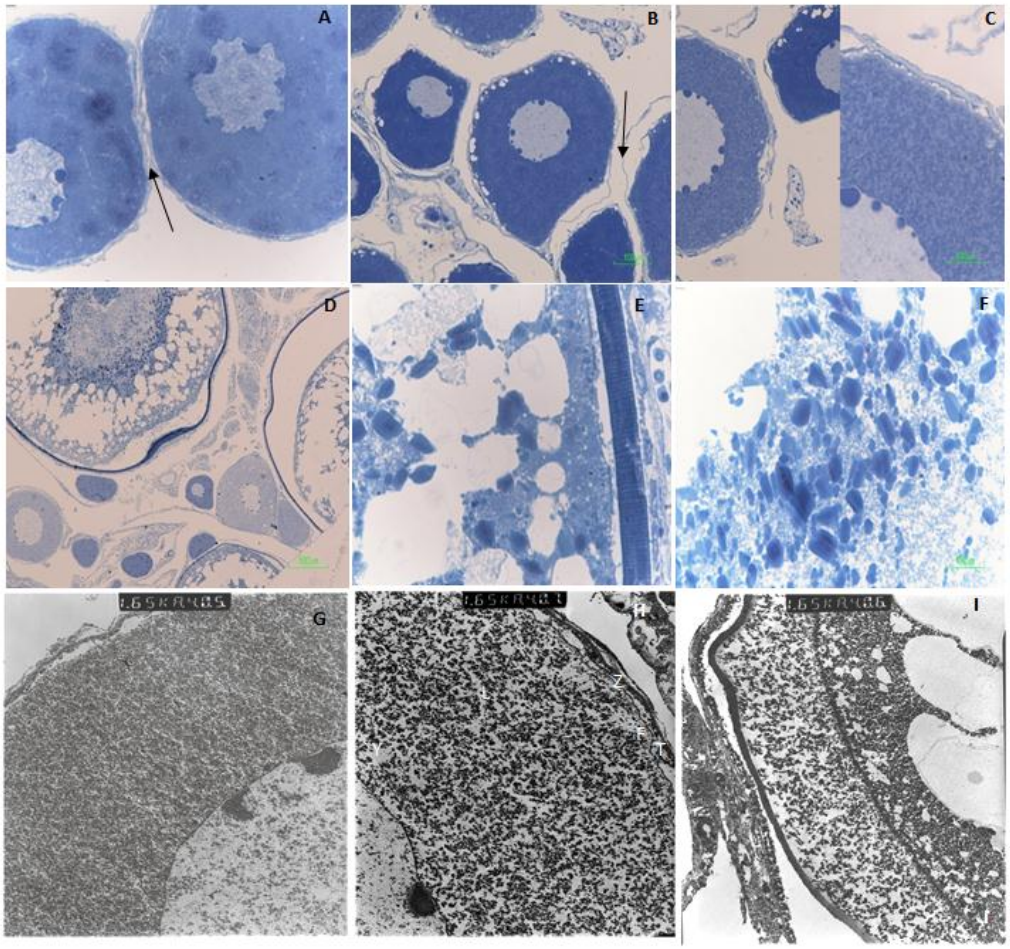

Figure 3: Semithin sections (A-F) and ultrathin sections (G-I) of ovaries of *Cyprinion tenuiradius* at different stages of maturation stained with toluidine blue and lead citrate and uranium acetate, respectively. Follicles of types A and B surrounded by peritoneal layer of ovary (arrow) as they show clear nuclei and several nucleoli (A). Follicles of type C consist of some lipid droplet (B and C). Note the peritoneal layer of ovary (arrow) and thin follicular layer around the follicles (B). Follicular layer were seen clearly in high magnification of type C follicle (C). (D) Follicles of type D, beside the types A and C, and its higher magnifications (E and F). High magnification of yolk granules showed in (F). Ultrastructure of type B follicle with dense cytoplasm, clear nucleus and peripheral nucleoli (G). The less density of cytoplasm in type C follicle (H), zona radiata and surrounding layers of type D follicle (I). Zona radiata: Z, follicular layer: F, theca layer: T, lipid droplet: L and yolk granule: Y.

Ultrathin section of this follicle type (Fig. 3H) showed its less dense cytoplasm than that of the type B follicle (Fig. 3G).

Type D: In the follicle of the type D many lipid droplets were observed as it considered clearer than the type C (Fig. 2D). This follicle type was 1.48 folds larger than the type C follicle (Table 2). A thin layer of zona radiata and cuboidal cells of the follicular layer were appeared around the oocyte (Figs. 2D and 3I).

Type E: This follicle type characterized by acidophilic cytoplasm fulfilled with many yolk granules and lipid droplets.

The zona radiata layer in this follicle type was thicker than that of the previous follicle type (Figs. 2E and 3D-F). Follicles of this type grew 1.94 folds compared to the type D or mean 104.40 µm (Table 2).

Type F: In this type of follicle the theca layer covered the cuboidal cells of the follicular layer. The zona radiata had the most thickness and the ratio of nucleus to cytoplasm volume showed the smallest value (the ratio value 0.70) (Fig. 2F). This follicle type increased 1.03 folds compared to the type E follicle (Table 2).

According to the macroscopic and microscopic features of gonads in females of *C. tenuiradius*, five developmental stages were described as follows:

Stage I (Immature ovaries): The stage I ovaries were very small, and transparent (Fig. 4-I). The thread-like ovaries were attached to the dorsal portion of the coelomic cavity by a mesentery. Their small and rounded oocytes with big central nucleus were not detectable macroscopically. The ovarian stroma was loose (Fig. 4-I). In this stage only follicles of types A and B were found

Stage II (developing ovaries–early stage): The stage II ovaries were still small and restricted to the posterior part of the body cavity, but the oocytes were observed with the naked eye (Fig. 4-II). Histological sections showed that in this stage the ovarian follicles consisted of types A, B, and C (Fig. 4-II).

Stage III (developing ovaries–late stage): Ovaries in the third developmental stage showed distinct growth in length and width, as small milky oocytes were easily visible to the naked eye (Fig. 4-III), but some yellow larger vitellogenic oocytes were found among them (Fig. 4-III). The majority of follicles of the ovaries of stage III were from types C and D, although some follicles of types A and B were still present (Fig. 4-III). In this stage, follicles were large and many yolk granules were accumulated in the growing follicles as the ovaries were observed yellow macroscopically (Figs. 4-III).

Stage IV (Mature ovaries): Ovaries of this developmental stage extended into the anterior portion of the body cavity and appeared more yellowish than those of the previous stages due to presence of a high proportion of vitellogenic oocytes (Fig. 4-IV). The weight and size of ovary increased but the ovaries have not attained their maximum weight and size. Due to increased volume of the ovaries in the abdominal cavity their shape was affected by the other internal organs. The connective tissue of the enlarged ovaries was slightly non-coherent, but larger ripe oocytes were still difficult to separate (Fig. 4-IV). Histological sections of this stage showed the majority of follicles of types E and F, but a few follicles of types C and D were also found (Fig. 4-IV).

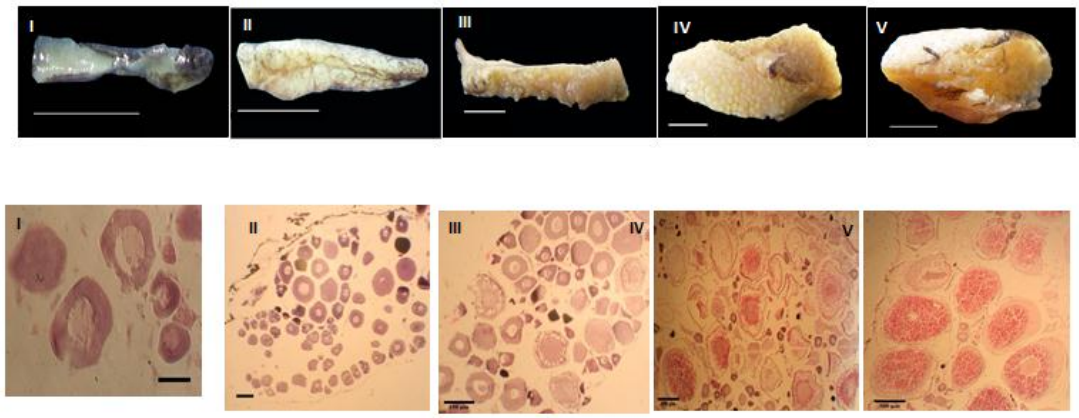

Figure 4: Macroscopic (upper row) and microscopic (below row) aspects of five ovarian developmental stages of *Cyprinion tenuiradius*. The upper row scale bars indicate 5 mm. Scale bars in histological sections of different ovarian stages stained with hematoxylin and eosin (below row) are 30, 100, 150, 300 and 300 µm, respectively.

Stage V (Ripe and running ovaries): Ovaries were occupied most of the body cavity and appeared dark yellowish (Fig. 4-V). Oocytes were released by slight pressure on the ovaries, as some of them were existed from the ovaries mass when ovaries were removed from the body cavity. In histological section (Fig. 4-V), the majority of follicles were from types E and F, but a few smaller follicles of type D also were found (Fig. 4-V).

Male gonad morphology

The mean body weight of males of *C. tenuiradius* at different stages of maturation varied from 1.69 to 25.67 g that increased 15 folds during a few months. The mean body length of males grew from 40.40 to 97.83 mm or 2.4 folds at the same time (Table 3). The mean body weight and length of males were less than the mean of these characters in the female fishes. The mean testicular weight and length grew from the lowest weight of 0.0001to the highest weight of 0.46 and from the shortest length of 7.78 to the tallest length of 31.60, respectively (Table 3). In the other hand testes were increased 4600 folds in weight and 4 folds in length at different stages of maturation during a few months.

Male gonad histology

The testes of *C. tenuiradius* were situated at the dorsal portion of the body cavity, the upper part of intestine and near to their vertebral column. Each testis was covered by capsular connective tissue that was entered to the gland and produced many lobules. The testicular lobules were consisted of tubular and convoluted seminiferous tubules.

Table 3: Mean and standard deviation of weight (g) and length (mm) of male body and gonad in different stages of testicular development of *Cyprinion tenuiradius* from the Rudbal River.

Stage	Body weight	Gonad weight	Body length	Gonad length
I	1.69± 1.07	0.0001± 0.00	40.40± 10.15	7.78± 2.47
II	6.75± 1.72	0.001± 0.001	65.67± 5.60	13.54± 1.10
III	9.33± 2.67	0.06± 0.03	75.88± 8.65	22.25± 3.87
IV	33.11± 25.04	0.36± 0.23	102.62± 32.24	31.39± 10.68
V	25.67± 20.78	0.46± 0.38	97.83± 25.34	31.60± 10.96

At different stages of testicular development, in the seminiferous tubules, different cluster of the germinal cells of spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid and spermatozoid in different densities were seen near together, therefore, each tubule showed the heterogeneous populations of the testicular cells (Fig. 5A).

Spermatogonia were the largest type of testicular cells (mean 4.82 µm) inside the germinal epithelium (Table 4). They showed a large and euchromatic nucleus that was surrounded by the thin and dense cytoplasm. Some of the spermatogonia showed a heterochromatic nucleus and the less condense cytoplasm (Fig. 5). The primary spermatocytes were smaller than spermatogonia (mean 2.97 µm), with more heterochromatic nucleus and dense cytoplasm (Fig. 5 and Table 4). The secondary spermatocytes and spermatids showed the smaller size (mean 1.62 and 1.30 µm, respectively) and darker color than the above mentioned testicular cells (Table 4). Finally, spermatozoids (sperms) were arranged as regular clusters. Their tails were attached together and their heads similar to small beads were situated near them (Fig. 5C). They were the smallest cells of the seminiferous tubules (mean 0.71 µm).

Based on the macroscopic and microscopic features of the examined testes of *C. tenuiradius*, testicular development was divided to five stages as follows:

Stage I (Virgin testes): Testes are very small, thin and filiform. There are small seminiferous tubules in the loose and thin stroma, they encompass only spermatogonia (Fig. 6I).

Stage II (Immature testes): Testes become larger and thicker than in the previous stage and testicular lobulations were visible to the naked eye. Spermatogonia and the primary spermatocytes were the dominant cells of the seminiferous tubules (Fig. 6-II).

Stage III (Maturing testes): Testes are large and their shape is affected by the abdominal viscera. Histological examination showed the less population of the spermatogonia and primary spermatocytes and the prominent clusters of spermatids and sperms. There were many compact seminiferous tubules between the thin stroma (Fig. 6-III).

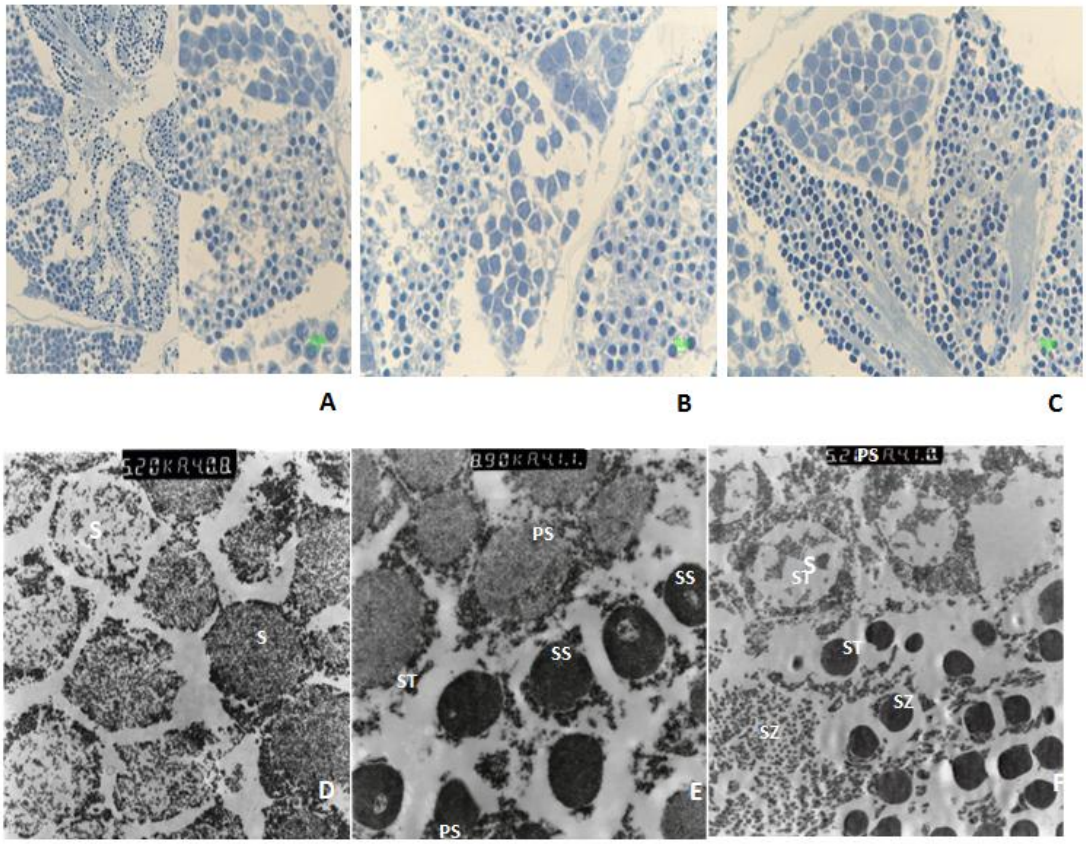

Figure 5: Semithin sections (A-C) and ultrathin sections (D-F) of testes of *Cyprinion tenuiradius* at different stages of maturation stained with toluidine blue and lead citrate and uranium acetate, respectively. (A) Seminiferous tubules of testis at stage III with two different magnifications (left and right). (B) and (C) show clusters of other germinal cells in the seminiferous tubule in stage III. Two types of spermatogonia were seen in photomicrograph (D). Euchromatic and heterochromatic nuclei in these two types were noted. More dense and smaller secondary spermatocyte compared to spermatogonium in photomicrograph (E). The spermatids and spermatozoids cluster were seen in (F). S: Spermatogonium, PS: Primary spermatocyte, SS: Secondary spermatocyte, ST: Spermatid and SZ: spermatozoids.

Stage IV (Mature testes): Testes appeared as large and thick glands with dark creamy color. There any sperm were existed when the testis was pressured with the forceps. Spermatids and spermatozoids were the most prominent cells of the seminiferous tubules. A few spermatogonia were also found (Fig. 6-IV).

Stage V (Ripe and running testes): Testes were dark yellowish and occupied most of the body cavity (Fig. 6-V). Testes of this developmental stage were similar to the fourth stage, but many sperms were released by a slight pressure. The lumen of the seminiferous tubules was fulfilled with sperm populations that caused their dark and dense appearance in the histological section (Fig. 6-V).

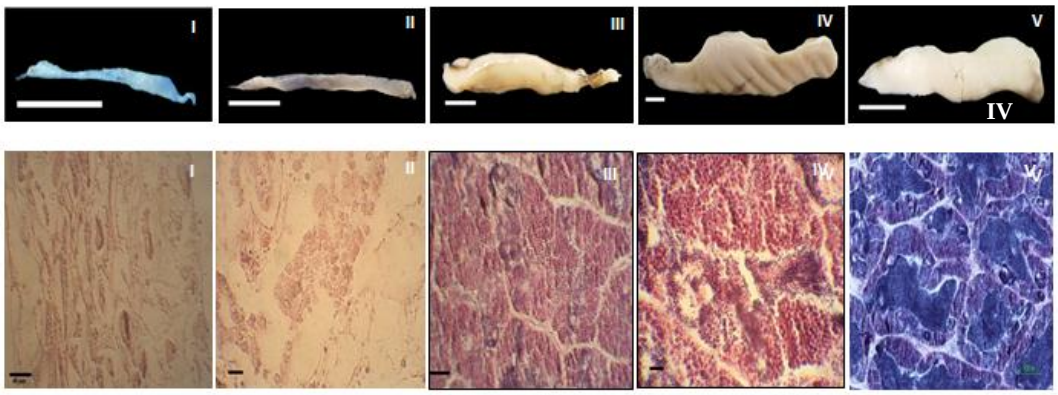

Figure 6: Macroscopic (upper row) and microscopic (below row) aspects of five testicular developmental stages of *Cyprinion tenuiradius*. The upper row scale bars indicate 5 mm. Scale bars in histological sections of different testicular stages stained with hematoxylin and eosin (below row) are 40, 10, 40, 10 and 100 µm, respectively.

Table 4: Mean and standard deviation of cells diameters in testes of male *Cyprinion tenuiradius* from the Rudbal River in micrometer.

Cell type	Diameter
Spermatozoid	0.71± 0.00
Spermatid	1.30± 0.17
Secondary spermatocyte	1.62± 0.18
Primary spermatocyte	2.97± 0.29
Spermatogonium	4.82± 0.86

Gonadosomatic index (GSI)

The gonadosomatic index was calculated from 100 females and 232 males of *C. tenuiradius* (Table 5). Maximum mean of the monthly GSI values were 8.97 and 2.55 for females and males, respectively (Table 5 and Fig. 7). The mean of GSI values for the whole study period were 0.781± 1.081 for males and 1.798± 2.643 for females. Females of *C. tenuiradius* showed the monthly GSI values significantly higher than those of males (Table 5 and Fig. 7). Also, monthly evolution of the GSI followed the same tendency for both males and females and showed the maximum values during spring (April–June) and beginning of summer (July, in males) with a peak at May (Fig. 7).

Table 5: Minimum and maximum, mean and standard deviation of the GSI for male and female specimens of *Cyprinion tenuiradius* from the Rudbal River, within a year (April 2007 to March 2008).

Month	Sex	Number	Minimum- Maximum levels	Mean & SD
1	Male	12	0.09-4.20	2.37± 1.47
	Female	5	0.24-9.34	2.27± 3.96
2	Male	25	0.007-4.95	2.55± 1.83
	Female	1	8.97-8.97	8.97
3	Male	26	0.003-5.70	2.42± 1.61
	Female	8	0.066-12.94	5.17± 4.64
4	Male	21	0.004-3.75	1.46± 1.32
	Female	8	0.058-3.59	1.09± 1.48
5	Male	23	0.001-2.19	0.160± 0.447
	Female	7	0.168-0.648	0.412± 0.184
6	Male	24	0.001-0.072	0.025± 0.019
	Female	7	0.198-0.541	0.347± 0.103
7	Male	18	0.001-0.098	0.028± 0.032
	Female	10	0.244-0.616	0.425± 0.127
8	Male	16	0.002-0.094	0.020± 0.031
	Female	9	0.026-0.681	0.524± 0.205
9	Male	14	0.002-0.037	0.011± 0.011
	Female	11	0.328-1.091	0.586± 0.254
10	Male	15	0.005-0.089	0.032± 0.034
	Female	15	0.202-1.03	0.633± 0.228
11	Male	22	0.003-0.178	0.054± 0.056
	Female	8	0.277-0.750	0.508± 0.160
12	Male	16	0.005-1.53	0.251± 0.402
	Female	11	0.243-1.65	0.652± 0.388

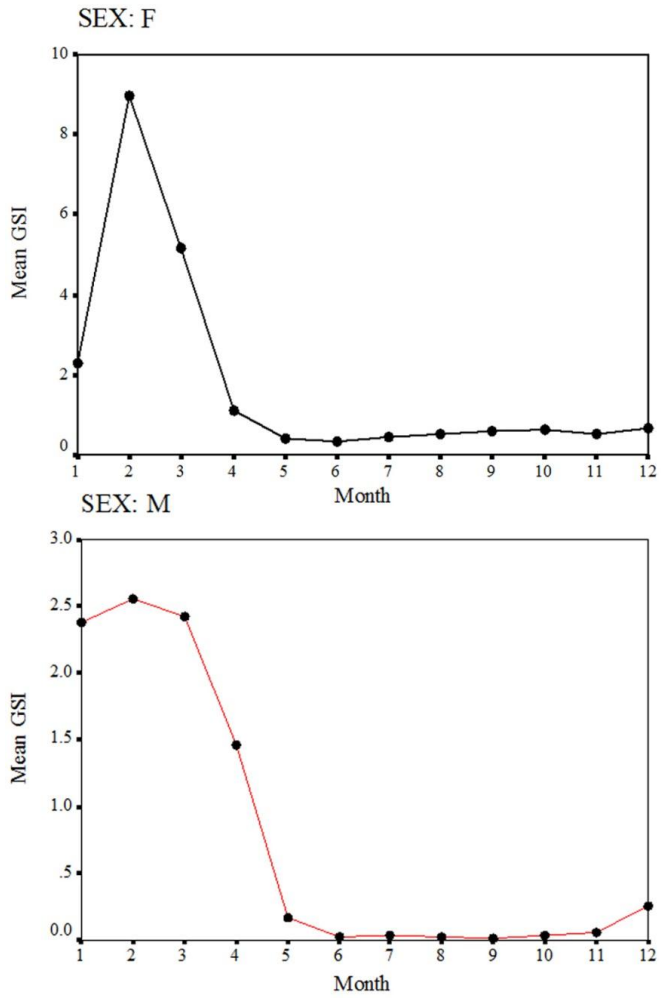

Figure 7: Graphs show monthly variation in mean of the GSI for female (above) and male (below) specimens of *Cyprinion tenuiradius* from the Rudbal River.

Discussion

This study represents the first attempt to investigate the histomorphology of gonad development in *C. tenuiradius*. The percentage of late gonad maturation stages (IV and V) and high frequency of large oocytes (follicles of types E and F) indicated the spawning period of this species in the Rudbal River is in spring and beginning of the summer (April to July). Macroscopic and microscopic development of the testes followed a trend similar to those of the ovaries. Testes volume increased along with the maturity stages, but it was always smaller than the ovaries. Also, macroscopic examination of the belly in the larger captured fishes of the present study in the field during spring and beginning the summer showed and confirmed the above conclusion as with slight pressure on their belly the ripe germinal cells were released. A similar period of spawning has also been reported in *C. macrostomum* Heckel, 1843 from Iraq, "which most fish are

mature by April, spawning occurs principally in May and June, with some in early July, but by July most fish are spent" (Coad, 2015). According to Coad (2015) "a spawning season of May and June has been recorded for *C. macrostomum* in Iraq on gravel beds in shallow water with fast current, also the maturity is attained there at 2-3 years, 15 cm length and 50 g weight". Based on our results (Tables 1 and 3) mean of the standard length and body weight for females and males of *C. tenuiradius* at the last stage of maturation (V) are 95.13 mm, 22.27 g and 97.83 mm, 25.67 g, respectively.

The minute, but developing eggs have been recorded for a specimen of *C. macrostomum* from Iran with 71.3 mm standard length caught on 31 January and specimens caught on 5 July had eggs with diameter of 1.4 mm (Coad, 2015). In *C. watsoni* from Pakistan at Islamabad spawning takes place from mid to late March to mid-April and near Islamabad in April and May (Coad, 2015). According to Uçkun and Gökçe (2015) mean oocyte diameter ranged between 0.13 and 1.34 mm for *C. macrostomus* (*sic*) and between 0.14 and 0.86 mm for *C. kais* from the Euphrates River in Turkey. Based on the gonadosomatic index (GSI), the spawning periods of *C. macrostomus* (*sic*) and *C. kais* were determined to be between June and August (Uçkun and Gökçe, 2015). Histological analysis of the gonads of *C. tenuiradius* have shown one maturation per year, which is consistent with annual fluctuations in water temperature, while there is no report that fishes of this genus spawn twice a year. A rapid increase in the weight of gonads (Tables 1 and 3) takes place when the temperature rises and increasing amount of food is available (Wootton, 1979). The reproductive index (GSI) for females was always higher than that of for males. Although the number of gametes produced does not necessarily need to be a function of the size of the gonad, the difference in male and female indices suggests that the energy invested in gamete production by males is probably less than that invested by females (Esmaeili *et al.*, 2009; Mousavi-Sabet *et al.*, 2012). According to our findings, it is clear that the gonad mass gives a better correlation with reproductive capacity than the body mass. Hence, this could be used for determination of spawning season, sexual maturity and frequency of spawning. This requires only the data related to sexual organs, it is easy for interpretation and calculation and provides a narrow range of index if the gonad mass is very low or very high. Results of this research can be helpful in determination of the exact taxonomic status of this species, maintain the genetic resources and its use in the farming of this endemic species.

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Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

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