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RESEARCH ARTICLE

Macroscopical and Histological Analysis of Gonadal Development of *Squalius cephalus* (L., 1758) in Tödürge Lake, Turkey

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ABSTRACT

This study was carried out with the aim to analyze the gonadal development of *S. cephalus* in Tödürge Lake, Sivas, Turkey. Anatomical and histological structures of gonads, oogenesis and spermatogenesis processes along with the determination of sexual maturity age of female (n=43) and male (n=27) individuals and spawning periods of the species were determined. Seasonal variations of gonads were illustrated. Five different oogenesis phases (chromatin nucleolar, perinucleolar, cortical alveolar, vitellogenic and maturation) and three different spermatogenesis phases (immature, maturing and mature testis) were identified. Results indicated that chub has a group syncrone type ovary, has a short reproduction season and spawn at once or twice times per year. Males reached to sexual maturity at II and III ages, and females reached to sexual maturity at III age. In addition, spawning period started at the end of May and went on through the end of June. Both oocyte and spermatocyte development and maturation were similar with the species belong to the subfamily Leuciscinae.

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INTRODUCTION

Squalius cephalus (L., 1758) shows a wide outspread throughout the whole Europe, basins of Black Sea, Caspian Sea and Sea of Azov along with the Caucasian region. It is also found in the inland waters of Anatolia. It has a high population and has an economical importance in Tödürge Lake, Sivas, Turkey (Ünver and Tanyolaç, 1999; Caffrey *et al.,* 2008; Stefanova *et al.,* 2008; Ünver and Yıldırım, 2011). Tödürge Lake is one of the large karstic lakes in Turkey, and its distance is approximately 56 km to city of Sivas. It is limnologically an open lake. Its elevation is 1295 m, surface area is 350 hectare and average depth is 2 m. There are six fish species belonging to Cyprinidae family, one species of Balitoridae family and one species of Siluridae family in Tödürge Lake (Ünver and Erk'akan, 2005).

Studies on the reproduction characteristics and the gonadal development of fish are important for the identification of their reproduction period which includes developmental phases. Various assessing methods have shown show wide variation. Histological method is time consuming and an expensive method, it gives better results than the oocyte size measurement method. Reliability of phase allocation method depends on oocyte configuration and oocyte external appearance as compared to other methods (West, 1990; Ünver and Erk'akan, 2005). In Turkey, determination of the developmental phases in gonads is generally made by using morphological analysis or gonadal index methods because of their easy conducting characteristics. While there have been studies on Cyprinidae family in Turkey dealing with their reproduction characteristics, there are a small number of studies using histological analysis method (Ünal *et al.,* 1999; Ural and Özdemir, 2000; Şaşı and Balık, 2003; Ünver and Ünver Saraydın, 2004; Koç *et al.,* 2006). In the present study, the anatomical and histological structures of gonads, oogenesis and spermatogenesis processes along with the determination of the sexual maturity age and spawning periods have been assessed in *S. cephalus* population of Tödürge Lake.

MATERIALS AND METHODS

Fish samples (43 female, 27 male) were obtained via hunting in Tödürge Lake at 15 days intervals between April 2000 and November 2001. Fishing nets of various mesh sizes (15x15, 18x18, 20x20, 24x24, and 32x32 mm)

were used in order to catch the fish. Total and fork lengths (mm) were measured and body weights (g) were weighed. Scales were used in order to determine the age (Bagenal, 1974). The fish were dissected after being anaesthetized with MS-222 solution (0.1 g/l). Gonadal features (morphology, size, and color) were noted. Gonad maturity stages were assigned as young, quiescent, ripening, ripeness, reproduction and spent (Nikolsky, 1963). A portion of gonads were cut and fixed in Bouin's solution for 12 h. Following dehydration in the increasing concentrations of ethanol, clearing in xylene and embedding in paraffin, 7 µm thick transverse sections were cut by a rotary microtome (Reichert-RM 2045, Germany). Sections were stained by hematoxylen-eosin and studied under light microscope (Olympus BX51, Japan). Both oocyte and spermatocyte development and maturation are a continuous process, which have been sub-divided into different stages to simplify histological classification of ovaries and testicles.

RESULTS

Ovarium and Testis Morphologies: In post-larva and in young individuals, gonads could not be identified macroscopically as testis or as ovary since they were restiform-like and too small. Ovaries are in granulated appearance in mature individuals in which eggs could easily be seen beneath the ovarial membrane. It gets highly vascularized especially during the spawning period. Transverse sections of equal sized ovaries were oval shaped. On the other hand, in some samples, one of the ovaries could be smaller than the other one. While they were in bright green color during the out of spawning period, they were in dirty yellow or in gold yellow colors during the spawning period. Testicles had a smooth surface appearance and their transverse sections were in triangle shape. While testicles were in milky-white color during the spawning period, they were in creamy-pinkish color during the out of spawning period.

Oogenesis Process: Five consecutive oogenesis phases have been identified as a result of histological analysis. These consecutive phases were chromatin nucleolar phase, perinucleolar phase, cortical alveolar phase, vitellogenic phase and maturation phase respectively.

Chromatin nucleolar phase: Oogonia made triplet and ogdoad groups in the connective tissue. Primary oocytes had a dark and a large nucleus along with a very large nucleolus and a lightly stained fine cytoplasm. Each oocyte was surrounded by several flat shaped follicle cells. This phase oocytes were mainly seen in preparations taken at the end of June (Fig. 1).

Perinucleolar phase: Nuclear size increased parallel to the increase in oocyte size. In that phase, 9-20 nucleoli have been observed around each nucleus. Cytoplasm was stained uniformly and darker then nucleus. Fish in that phase was mostly caught in July and August (Fig. 2).

Cortical alveolar phase: The most common feature of cortical alveolar phase was the accumulation of spherical alveoles beneath the oocyte membrane. While the number of alveoli was fewer at the beginning, it increased to a few layers around the cytoplasm during the advancing developmental stages. The size of alveoli was also increased. Those alveolar structures were observed as empty when stained by hematoxylen-eosin. Chorion (vitellin membrane) was firstly seen in this phase of oogenesis. Chorionic thickness, the number and the diameter of blood vessels were also increased that was parallel to the oocyte development. Nuclear membranes of some oocytes were found to be partially degenerated at the end of the cortical alveolar phase. Fish in that phase was mostly caught in September and October (Fig. 3).

Vitellogenic phase: The most perennial phase of oogenesis is the vitellogenic phase. It is extensively seen throughout October. The very first samples collected following winter (April and May) could even demonstrate oocytes at the vitellogenic phase. This stage is characteristics with the vitellin granules around the nucleus. Granules were smaller and fewer at the beginning, whereas they became larger and numerous parallel with the oocyte development. As a result of this increase in number and volume of granules, alveoli were pushed towards the oocyte wall. Oocytes reached their maximum dimension and chorion became more evident (Fig. 4).

Maturation phase: While nucleus migrated towards the oocyte wall (animal pole), nucleus membrane was broken up and nucleoli scattered in the cytoplasm. Chorion made an invagination (animal invagination) towards the oocyte center. Vitellin granules spread around the cytoplasm and chorion reached to its maximum thickness. Oocytes in maturation phase were seen in the middle of May, and this has progressed to the end of June (Fig. 5).

Ovulation started at the end of May and continued through the end of June. Oocytes that reached to their maximum growth were delivered into the ovarial lumen. As a result of ovulation, granulosa and theca cells containing follicular wall, and atretic follicles were observed within the ovaries.

Spermatogenesis Process: The histological analyses of testis revealed three consecutive phases; immature, maturing and mature testis.

Immature testis: In the young individuals (1st age class), primary spermatogonia lined in the wall of seminiferous tubules. Their nuclei were rather large and stained dark. The number of primary spermatogonia has increased that was parallel to the testicular development. A lumen was formed in each tubule. Oval shaped Sertoli cells were evident in this phase and they had flat nuclei (Fig. 6).

Maturing testis: In the II age class of individuals, secondary spermatogonia were formed as a result of the first meiotic division and they were lined above the primary spermatogonia. Secondary spermatogonia were smaller and darker then primary spermatogonia (Fig. 7).

Mature testis: It has been determined that testicles started to be mature from the end of the second age. In the mature phase, seminiferous tubule volumes were in the most developed stage. Spermatozoons have begun to be formed

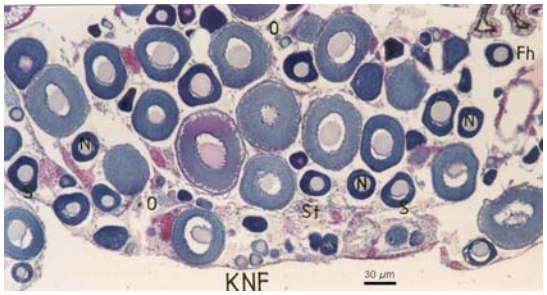

Fig. 1: Ovary in chromatin nucleolar phase [KNF: chromatin nucleolar phase, N: nucleus, S: cytoplasm, St: stroma, O: oogonium, Fh: follicular cells (H-E x 10)].

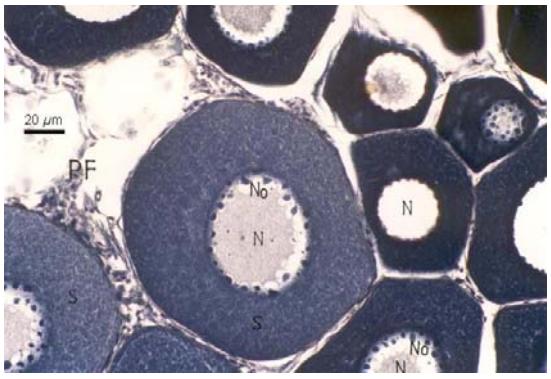

Fig. 2: Ovary in perinucleolus phase [PF: perinucleolar phase, N: nucleus, No: nucleolus, S: cytoplasm (WH x 40)].

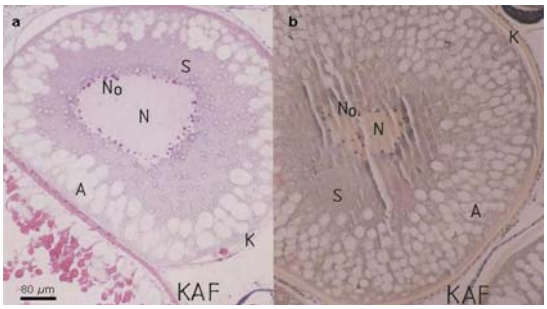

Fig. 3: Ovary in cortical alveolar phase [KAF: cortical alveolar phase, N: nucleus, No: nucleolus, S: cytoplasm, A: alveoli, K: chorion (a- H-E, b- WH x 20)].

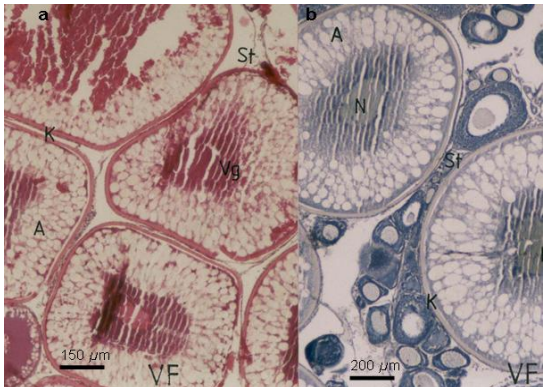

Fig. 4: Ovary in vitellogenic phase [VF: vitellogenic phase, N: nucleus, St: stroma, Vg: yolk globules, A: alveoli, K: chorion (a- H-E, b- WH x 10, WH x 100)].

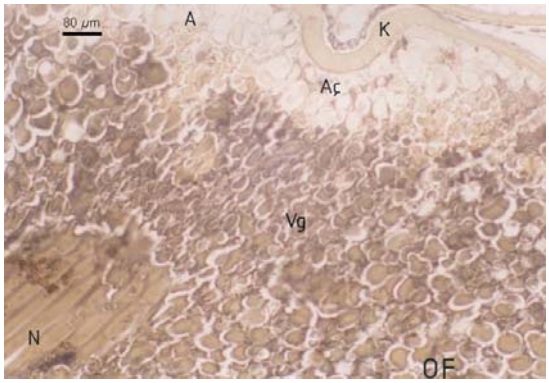

Fig. 5: Ovary in mature phase [OF: mature phase, N: nucleus, Vg: yolk globules, A: alveoli, K: chorion, Aç: animal pole (WH x 20)].

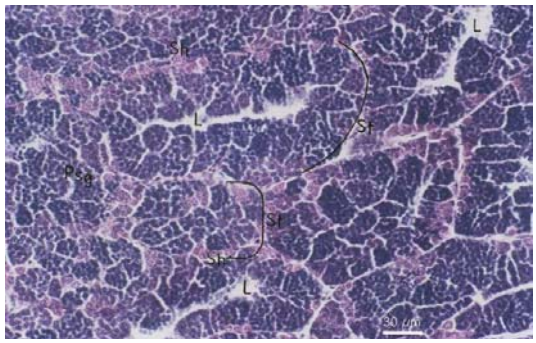

Fig. 6: Immature testis [L: lumen, St: seminiferous tubules, Sh: Sertoli cells, Psg: primary spermatogonium (H-E x 20)].

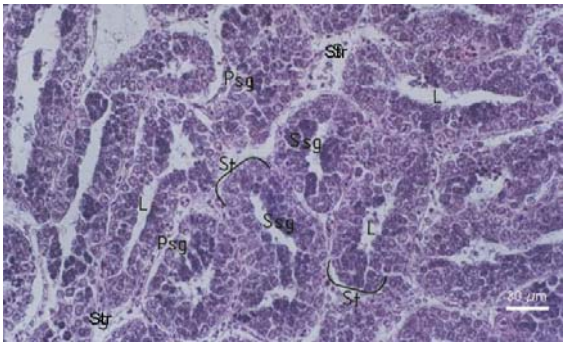

Fig. 7: Maturing testis [L: lumen, St: seminiferous tubules, Str: stroma, Psg: primary spermatogonium, Ssg: secondary spermatogonium (H-E x 40)].

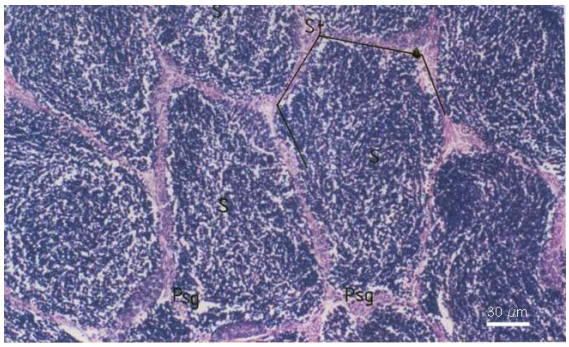

Fig. 8: Mature testis [St: seminiferous tubules, Psg: primary spermatogonium, S: spermatid Ssg: secondary spermatogonium (H-E x 40)].

in November. Mature spermatozoons filled the whole luminal space. In this stage, sperm heads were stained darker thus were clear. A small number of primary spermatogonia were evident around the seminiferous tubules (Fig. 8).

DISCUSSION

Double gonads in fish might develop longitudinally and medio-dorsally to the coelom. However, gonads might be seen asymmetrically because of their fusion or a cease in the development of one gonad (West, 1990). In some samples in the present study, gonads at different sizes were observed especially in females. Testicles were lobular type whereas ovaries were saccular type in samples evaluated in the present study. Ovaries and testicles in female and in male samples that reached to sexual maturation were different from each other. Those gonadal characteristics varied depending upon the seasonal reproductive alterations (Nikolsky, 1963). Testicles started to develop from the end of II age and all males became mature at the III age. On the other hand, ovaries became mature after the 3rd years of age and entered the oogenesis process from now on. Those findings indicate that *S. cephalus* population in Tödürge Lake that males are sexually mature at the age of II-III whereas it occurs at the age of III in females. Males in cyprinid species could reach to sexual maturity a year earlier than females because of physical parameters of the environment and their biological characteristics (Hliwa *et al.,* 2009). Spermatogenesis occurred at three distinct stages (Figs 6-8). Fishelson *et al.* (1996) analyzed the testicular development at 8 distinct stages, however, they also noted three main developmental phase; immature, maturing and mature testis. Ünal *et al.* (1999) divided the spermatogenesis process into three consecutive phases in *Chalcalburnus tarichi*. Sperm production in testis should be started well before the onset of the fertilization. This permits the occurrence of the fertilization (Fishelson *et al.,* 1996). It has been found out that sperms in males were formed at the end of November. It has also been suggested that sperms were formed in testis of *Chalcalburnus tarichi* population five months before the fertilization (Ünal *et al.,* 1999). The spermatogenesis did not present the remarkable variations over the development process as the oogenesis, with gradual decreasing from spermatogonia to spermatozoa (Gomes *et al.,* 2004). When the ovaries were analyzed, 2-3 different sized oocytes at different stages of development were found. Thus, it could be suggested that *S. cephalus* has a group syncrone type ovary, has a short reproduction period and leaves eggs at once or twice. It is not clear, however, whether the chub is a total or multiple spawner (Fredrich *et al.,* 2003). Mann (1976) observed half empty gonads after a spawning period. Hellawell (1971) described signs of resorption of oocytes and drew the conclusion that chub spawn once per year. Libosvarsky (1979) described unimodal oocyte size distributions before and during spawning. Libosvarsky and Sterba (1981) observed empty follicles, after a spawning period and they concluded that chub spawns twice per year at minimum. In general, the ovarian development process in teleost can be divided into two phases (Wallace and Selman, 1981); the previtellogenic phase, when growth is comparatively slow, with few cytoplasmic changes, and the vitellogenic phase, characterized by faster growth and the deposition of large amounts of yolk in the ooplasma. Oogenesis process in teleosts mainly occurs at five different stages; chromatin nucleolar, perinucleolar, chortical alveolar, vitellogenic and maturation phases. Several researchers add a sixth, ovulation, phase and define six consecutive oogenesis phases (West, 1990). Gomes *et al.,* (2004) and Pinon *et al.,* (2009) were identified six gonadal stages. The seven stages of oogenesis were described by Çek *et al.,* (2001) and Lucano-Ramirez *et al.,* (2001). In the present study, oogenesis was evaluated in five phases (Fig. 1-5). To do this, criteria used by several investigators such as oocyte size, appearance of nucleus and nucleolus, type of cytoplasmic bodies and their location in the cytoplasm were accepted (West, 1990; Fishelson *et al.,* 1996; Gomes *et al.,* 2004; Pinon *et al.,* 2009). Chorion was first observed at the cortical alveolar phase. Although chorion was observed either at the end of the vitellogenic phase or at the perinucleolar phase, it was mostly seen at the vitellogenic phase in several species. However, this varies among different species (West, 1990). In the present study, mature oocytes were observed at the end of May. The number of emptied ovaries was observed from the end of May. In addition, all ovaries were seen empty when fish caught after June. Cortical alveolar phase ovaries were seen beginning from June. These data demonstrate that the population start the spawning period at the end of May and continue until the end of June. This study demonstrates the importance of histological analysis of gonads in order to confirm the results of the macroscopic analyses routinely carried out in studies on reproduction biology of cyprinid fish. Data determined in the present study were that while oogenesis had five consecutive phases, spermatogenesis occurred at three different stages. It can be described that the chub has a group syncrone type ovary, has a short reproduction season and spawn at once or twice times per year. Both oocyte and spermatocyte development and maturation were similar with the species belong to the subfamily Leuciscinae.

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