Histological examination of ovarium development of shemaya *Chalcalburnus chalcoides* living in Lake Tödürge (Sivas/Turkey)

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A b s t r a c t . The process of oogenesis of *C. chalcoides* living in Lake Tödürge was histologically studied from monthly samples during April–November 2000. The process was divided into five stages (previtellogenic, cytoplasmic growth, vitellogenic growth, yolk oocyte and mature oocyte). Oocytes of the previtellogenic and mature phases were mainly seen in July and May, respectively. Oocytes and nuclei diameters were observed between 38–1470 μm and 29–270 μm until ovulation. *C. chalcoides* is thus considered to be synchronous. The ovulation period started in late May and ended in the beginning of July.

Key words: Kızılırmak Basin, karstic lake, Cyprinidae, oogenesis

Introduction

One of the most important studies of fishery biology is to determine the annual' breeding cycle of a fish species. There are a few methods, such as gonad indices, staging based on the external appearance of the ovary and whole oocytes, measurements of oocyte size and histology to assess the stage of gonad development of individual fish. Despite expensive and time-consuming procedures being used to determine gonad development, the most accurate technique is histological analysis (W e s t 1990). Shemaya *Chalcalburnus chalcoides* (Güldenstädt, 1772) is widely distributed in the river systems of the Black, Caspian and Aral seas (B o g u t s k a y a 1997). The species is benthopelagic and lives in fresh and brackish waters. The populations that live in lakes, migrate upstream for spawning from the beginning of May to the end of July (S l a s t e n e n k o 1955). Dense populations of this species are commonly found in the few streams (Sakarya, Kızılırmak, Yeşilırmak, Kura) that flow to the Black Sea and the tectonic lakes in the Marmara Region (Northwestern Anatolia) of Turkey (G e l d i a y & B a l ı k 1988). Lake Tödürge, which is in the Kızılırmak Basin, is a typical cyprinid lake. *C. chalcoides* inhabiting Lake Tödürge are of economical importance and the species is caught for consumption by individuals and cooperatives of local people (Ü n v e r 1998). There have been a number of investigations to determine some systematical and biological characteristics of *C. chalcoides* (B e r g 1949, S l a s t e n e n k o 1955, K u r u 1975, E r k'a k a n 1981, B a l ı k & S a r ı 1994, Ünver 1999). However, there are no investigations on the process of oogenesis of this species in Turkey. Because of this, in the present study we aimed to histologically determine the ovarium development of shemaya living in Lake Tödürge.

Study Area

Lake Tödürge is located north of highway E-23 (39°53' N, 37°36' E). It is approximately 56 kms from Sivas (Middle Anatolia) and is at an altitude of 1295 m (Fig. 1). Lake Tödürge

lies in an arid region surrounded by flat and wide grasslands and fields. The lake, which is in the Kızılırmak Basin, is of karstic origin and has a surface area of 350 hectares. The lake is roughly triangular in shape. Lake Tödürge has a mean depth of 2 m with a maximum depth around 28 m. The lake water is supplied by underground water, which percolates through karstic rocks. The outlet of the lake to the west discharges lake water into the Kızılırmak by means of a drainage canal around Yarhisar village. Lake Tödürge is one of the most important bird breeding areas of Turkey, with about 20 species of breeding birds. It is a typical cyprinid lake, as are most of the Turkish lakes. Eight species of fish (*Cyprinus carpio*, *Leuciscus cephalus*, *Chalcalburnus chalcoides*, *Chondrostoma nasus*, *Capoeta capoeta*, *Capoeta tinca*, *Orthrias angorae* and *Silurus glanis*) live in this lake. The most widespread species of macrophyte of the lake is *Phragmites australis* (Ü n v e r 1998).

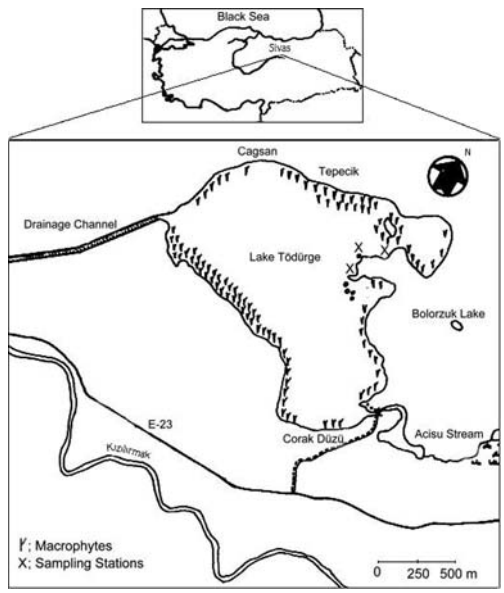

Fig. 1. Lake Tödürge and the fish sampling areas (1/25000).

Material and Methods

Specimens of *C. chalcoides* were collected in Lake Tödürge between April and November 2000. The fishes were caught by using 15–32 mm mesh, gill-nets monthly. The body cavity of each specimen was opened, then the ovaria were taken out. For light-microscopical examination, samples from the middle part of each ovarium were fixed routinely in Bouin's fixative and embedded in paraffin. Serial sections of 7 μm were prepared and stained with hematoxylin-eosin, Heidenhain's iron hematoxylin and Weigert's iron hematoxylin. A total of 84 specimens, which belong to same age and length groups, were used to determine gonad development. Ten fish were examined in each month except for May and June (12 specimens). For analysis of the ovarian cycle, the observed oocytes were divided into five stages according to sizes and development stages of oocytes: previtellogenic, cytoplasmic growth, vitellogenic growth, yolk oocyte and mature oocyte. The diameter of oocyte and nucleus, the thickness of vitelline membrane were measured under a binocular stereo microscope using an ocular micrometer.

Results

The oocytes in previtellogenic phase were seen during all months except for May, the maximum frequency was established in July (Fig. 2). In this phase, oocyte diameter was 38–61 μm and that of the nucleus was 29–49 μm (Fig. 3). The oogonia were seem in stroma in groups of 6–10 cells in this stage. The oocytes had a large nucleus and a thin layer of cytoplasma, staining dark and homogenous. Each oocyte was surrounded by 2–3 follicular cells (Fig. 4).

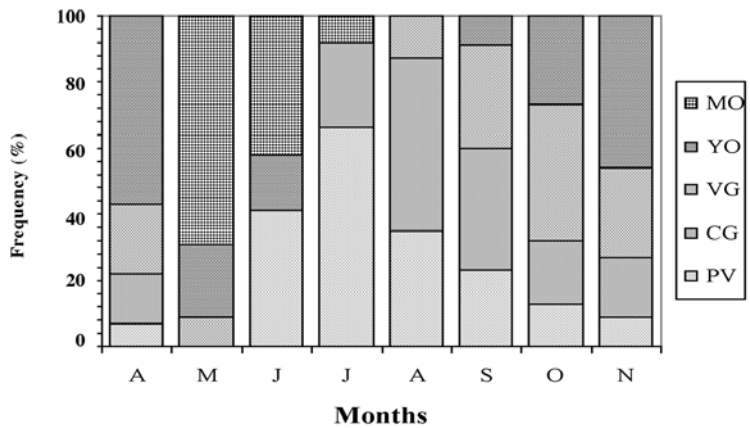

Fig. 2. Monthly changes in frequency (%) of oogenesis stages in each histological section (PV, Previtellogenic; CG, Cytoplasmic growth; VG, Vitellogenic growth; YO, Yolk oocyte; MO, Mature oocyte).

In the cytoplasmic growth phase, the nuclei of oocytes contained 7–20 nucleoli, which were arranged in the periphery of the nuclei. The ooplasma stained dark and uniformly. The oocytes were surrounded by a single layer of squamous follicle cells (Fig. 5). Together with oocyte growth, the size of nucleus also increased. The diameter of oocyte and vesicle varied between 123–273 μm and 58–135 μm, respectively (Fig. 3). The percentage of frequency of the oocytes in this phase was at its maximum in August and at its minimum in April (Fig. 2).

In the vitellogenic growth phase, the appearance of cortical vesicles, which are spherically on the periphery of the cytoplasma, was observed. The ooplasma seemed

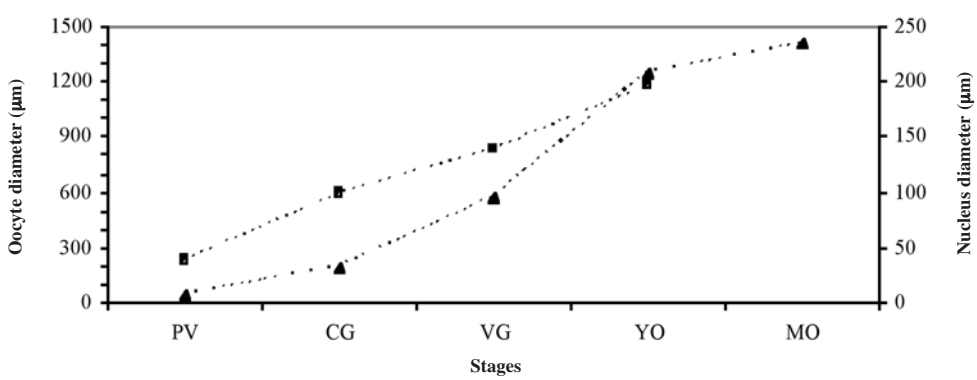

Fig. 3. Changes in mean diameter of oocyte (▲) and nucleus (●) during oogenesis process (PV, Previtellogenic; CG, Cytoplasmic growth; VG, Vitellogenic growth; YO, Yolk oocyte; MO, Mature oocyte).

Fig. 4

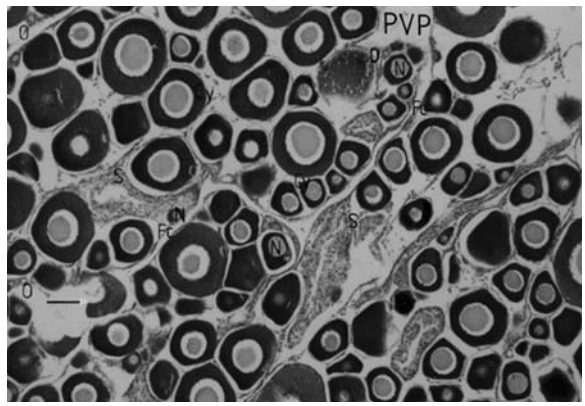

Fig. 5

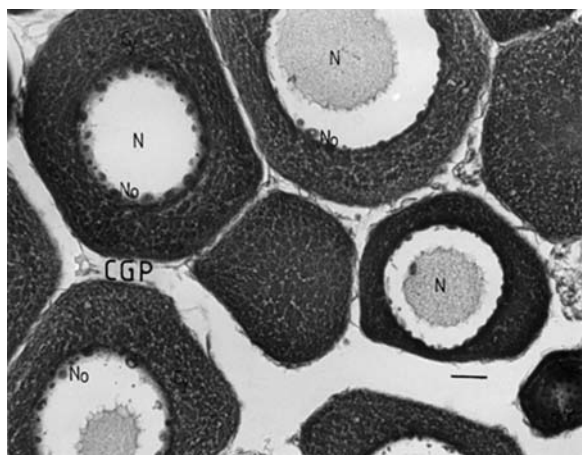

Fig. 6

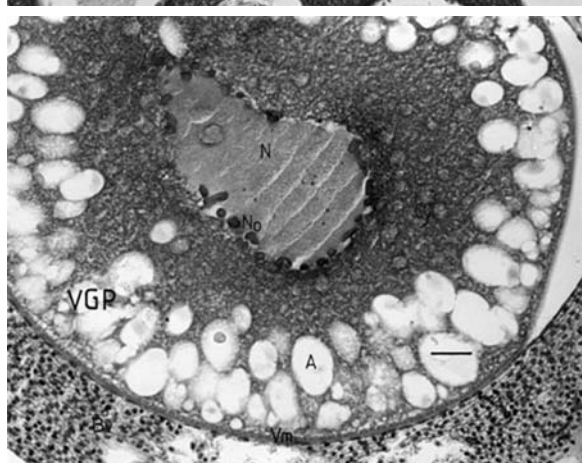

Fig. 4. Ovary of *C. chalcoides* with oocytes at the previtellogenic stage (Hematoxylin-eosin x 10- PVP; Previtellogenic phase, O; oogonium, S; stroma, N; nucleus, Fc; follicle cell, Cy; cytoplasma-Scale: 50 μm).
Fig. 5. Ovary of *C. chalcoides* with oocytes at the cytoplasmic growth stage (Weigert's iron hematoxylin x 40- CGP; cytoplasmic growth phase, N; nucleus, No; nucleolus, Cy; cytoplasma-Scale: 50 μm).
Fig. 6. Ovary of *C. chalcoides* with oocytes at the vitellogenic growth stage (Weigert's iron hematoxylin x 40- VGP; vitellogenic growth phase, N; nucleus, No; nucleolus, Cy; cytoplasma, A; alveol, Vm; vitelline membrane, Bv; blood-vessel-Scale: 50 μm).

Fig. 7a

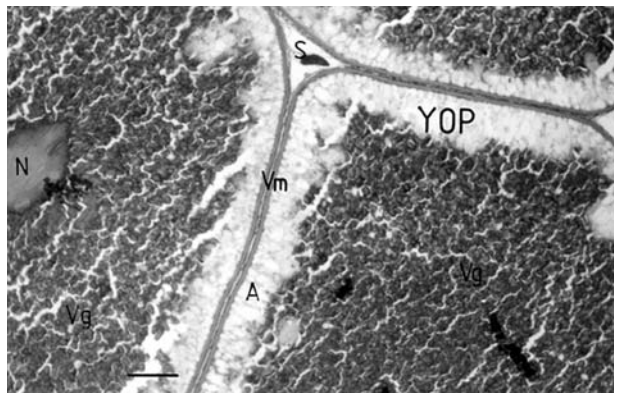

Fig. 7b

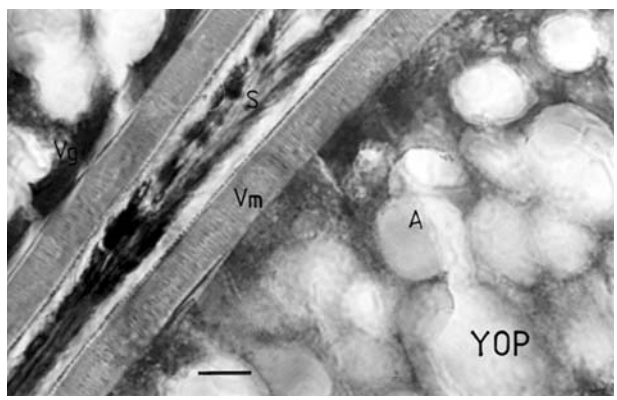

Fig. 8

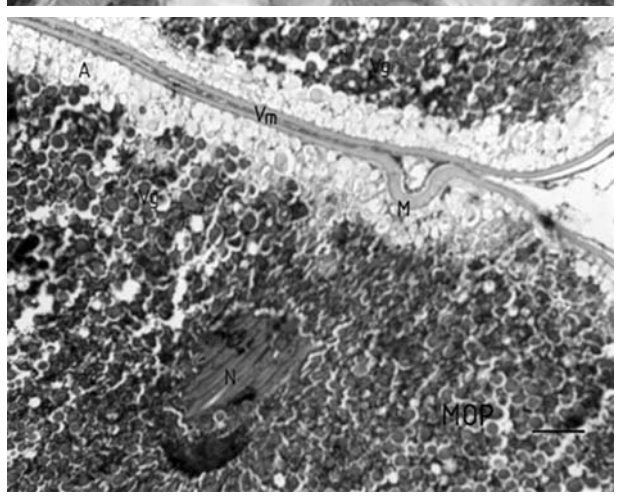

Fig. 7a. Ovary of *C. chalcoides* with oocytes at the yolk oocyte stage (Weigert's iron hematoxylin x 40- YOP; yolk oocyte phase, S; stroma, Vm; vitelline membrane, A; alveol, Vg; vitelline granule, N; nucleus-Scale: 100 μm).
Fig. 7b. Ovary of *C. chalcoides* with oocytes at the yolk oocyte stage (Weigert's iron hematoxylin x 100- YOP; yolk oocyte phase, S; stroma, Vm; vitelline membrane, A; alveol, Vg; vitelline granule-Scale: 10 μm).
Fig. 8. Ovary of *C. chalcoides* with oocytes at the mature oocyte stage (Weigert's iron hematoxylin x 20- MOP; mature oocyte phase, M; micropyle, Vm; vitelline membrane, A; alveol, Vg; vitelline granule, N; nucleus-Scale: 50 μm).

paler. The nucleus contained between 26 and 44 nucleoli. The number and size of yolk vesicles increased. The vitelline membrane began to develop at this phase. The thickness of the vitelline membrane was measured to be 2.9–4.1 μm. The nuclear membrane had an irregular structure. The blood-vessels in the stroma increased in size (Fig. 6). Oocyte diameter was 382–779 μm and that of the germinal vesicle was 105–205 μm at this stage (Fig. 3). Vitellogenic oocytes were mostly present from September to mid October (Fig. 2).

In the beginning of the yolk oocyte phase, spherical yolk proteins (yolk spheres, granules) appeared around the oocyte nucleus. Concomitant with oocyte growth, the granules increased in both size and number (Fig. 7a). The vitelline membrane became completely visible (Fig. 7b). In this phase, the diameters of the oocyte and nucleus were measured to be 1199–1381 μm and 258–270 μm, respectively (Fig. 3). The vitelline membrane had a thickness of 8.8–11.0 μm. During oogenesis, the oocytes in this stage first appeared in September. The frequency of oocytes at this phase of development attained maximum level in April (Fig. 2).

Mature oocyte phase is the final stage of the process of oogenesis. In the beginning of this stage, the micropyle developed, the membrane of nucleus dissolved, then the nucleus migrated toward the animal pole of the oocyte. Because the entire oocyte was covered with yolk proteins at the end of this phase, the cortical alveoli were arranged into several peripheral rows in form (Fig. 8). Oocyte diameter reached 1310–1470 μm, which is the maximum size of oocytes during oogenesis (Fig. 3). The vitelline membrane had a thickness of 14.7–18.3 μm. The oocytes in this phase were seen in May, June and July. The frequency of the oocytes at this phase reached the highest level in May (Fig. 2).

Following the final stage of oogenesis, ripe oocytes were released into the ovarian lumen. After ovulation, concomitant with ovarium shrink, the fold and curls were seen on the ovarium wall. The spent ovaria were composed of atretic follicles, immature oocytes and mature eggs left unspawned and were often bloodshot in appearance. Ovulation appears to have started in late May and ended in the beginning of July (Fig. 2). Mean oocyte diameter increased significantly between September and April, and the highest oocyte diameters were attained in May (Fig. 9).

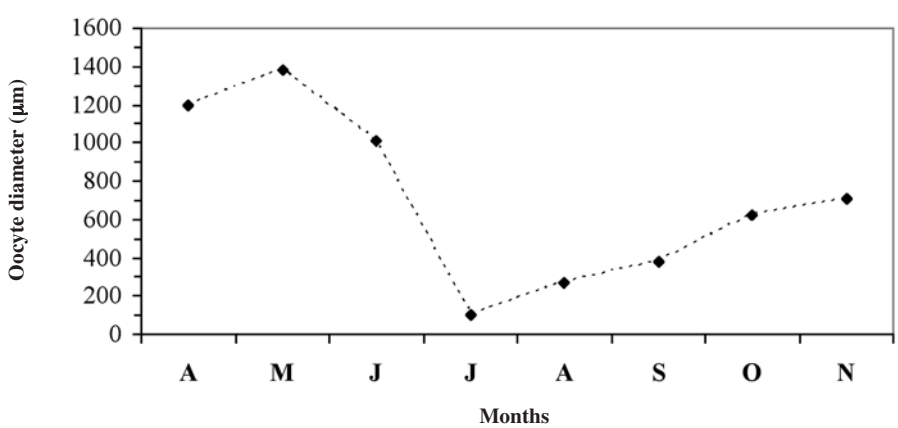

Fig. 9. Monthly changes in mean diameter (μm) of oocytes.

Discussion

Reproductive studies of fishes require knowledge of the stage of gonad development in individual fish. There are a few methods such as measurement of oocyte size, staging based on the appearance of whole oocytes, staging based on the external appearance of the ovary, gonad indices and histology to determine of the stage of gonad development. However, despite the fact that histology is time-consuming and expensive, it is the most accurate method used to assess gonad development (West 1990, Hibiya 1982). In the majority of teleost fishes, the process of oogenesis may be divided to five, six or eight stages (Fishelson et al. 1996, Nagahama 1983, Ünal et al. 1999, West 1990, Poortenaar et al. 2001). According to Fishelson et al. (1996) and West (1990), the nucleus was large in the previtellogenic (chromatin nuclear) phase, with 2–4 nucleoli situated in the centre of the nucleoplasma, at the cytoplasmic growth (perinucleolar) phase, in the periphery of the vesicle there were 8–12 nucleoli. Yolk vesicles were seen on the cytoplasma and the germinal vesicle possessed 35–45 nucleoli at the vitellogenic growth (cortical alveolar) phase, the yolk proteins appeared around the nucleus and the nucleus possessed 65–75 nucleoli at the yolk oocyte (vitellogenic) phase. The nucleus membrane dissolved and the peripheral migration of the nucleus started in the mature oocyte (ripe) phase, and then the matured oocyte was released into the ovary lumen. In the present study, all stages were identified in a similar manner. Because of the size of oocyte, the nucleus diameter and the number of nucleolus may vary between species, and also they are closely related with the ecological factors that affect populations (Fishelson et al. 1996). The diameters of the oocyte and the nucleus and the number of nucleolus were found to be different. There is a close relationship between the amount of vitellogenin and oocyte size. Concomitant with increases in the accumulation of vitellogenin in the oocytes, the size of oocytes also begins to increase. Therefore, the nutritional situation of the population, especially during the period of vitellogenesis, is the main biological factor to effect the process of vitellogenesis (Hibiya 1982, West 1990). The vitelline membrane appears commonly at the yolk vesicle stage and sometimes at the late perinucleolus or at the end of the yolk vesicle stage (West 1990, Ünal et al. 1999). This situation may vary from species to species. In the present study, the vitelline membrane began to develop at the vitellogenic growth stage (Fig. 6). According to Hibiya (1982), after movement of the nucleus to an animal pole, the first meiotic division occurs and the first polar body is released. Both the movement of nucleus and the breakdown of the membrane of the nucleus is a commonly used indicator of final maturation (West 1990). In the present study the final maturation occurred in May (Fig. 8), and the atretic oocytes commonly appeared in the post-spawning period in oocytes of any stage. Similarly, Hibiya (1982) stated that the ovary just after spawning is composed of many postovulatory follicles, immature oocytes and mature eggs left unspawned. Environmental stress is especially the main cause of follicular atresia (West 1990). In the present study, the atretic follicules were mostly seen at end of the ovulation (in July) and were occasionally seen in the maturation stage (in May). As mentioned in the results section, ovulation started in late May (Figs 2, 9). This was the time of the beginning of the reproduction period. The spawning period of *C. chalcoides* continued approximately a month and ended in early July. In the beginning of the spawning period, the adults of *C. chalcoides* migrated to Acısu Stream, which flows into the lake (Fig. 1). The adults returned into the lake after spawning. Where the populations of *C. chalcoides* live in lakes, they migrate upstream for spawning from the

beginning of May to the end of July (Slastenenko 1955, Geldiay & Balık 1988). However, during the spawning period, adult fish were captured by local people. Because of this, fishing must be prohibited during the reproductive period to protect the sustainability of the population of *C. chalcoides*. However, young oocytes appeared at all stages of oogenesis, they were seen especially at the chromatin nucleolar and perinucleolar stages. Occasionally, four but mainly two or three stages of oocyte development were observed in the ovaria of the studied samples at any stage (Fig. 2). According to West (1990), group synchronous ovaries are those in which at least two size groups of oocytes are present at the same time. Therefore, the ovarium type of *C. chalcoides* is group synchronous and this species spawns once in a breeding season. The oocyte diameter varied between 38–470 μm. There is a significant relationship between the size of ripe eggs, fish age and size. Egg size increases concomitant with the age and size of the females (Lagler 1956, West 1990). Because, there are few investigations on the reproduction properties of *C. chalcoides*, we expect that the results of the present study, which aims to determine histologically of ovarium development of shemaya living in Lake Tödürge, will contribute considerably important knowledge to the research on the process of oogenesis of *C. chalcoides*.

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