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A PRELIMINARY HISTOLOGICAL STUDY ON OVARIUM DEVELOPMENT IN MIRROR CARP AND SCALED CARP (*Cyprinus carpio* L., 1758) INTRODUCED INTO GELINGÜLLÜ RESERVOIR, TURKEY

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Abstract:

Carp (*Cyprinus carpio*) is the most common species of the Cyprinidae family and is among the important species commercially caught in the Gelingüllü Reservoir, Yozgat, Turkey. In this study, the reproductive biology of mirror carp and scaled carp in Gelingüllü Reservoir was studied in terms of the gonadosomatic index (GSI), fecundity, and ovarian histology. Ovaries were classified into 5 maturity stages based on histological data, as follows: 1. Chromatin-nucleolus stage (oocyte diameter is 20-60 µm); 2. Peri-nucleolar stage (oocyte diameter is 60-200 µm); 3. Cortical alveolar stage (oocyte diameter is 200-600 µm); 4. Vitellogenic stage (oocyte diameter is 600-1000 µm); 5. Maturation stage (oocyte diameter is 1000-1400 µm). When ovulation was completed, post-ovulatory follicles and atretic follicles were noted in the ovaries. The spawning period in both populations extended from April to July, but peak-spawning activity occurred in April and May. Absolute fecundity reached 2.133 million eggs in mirror carp and 1.628 million eggs in scaled carp. Ovaries that contained both yolk stage oocytes and postovulatory follicles indicated that carp is a multiple spawner. Histological analysis in the present study showed that female mirror carp and scaled carp had mature eggs that contributed to sustaining the both populations in Gelingüllü Reservoir.

Keywords: *Cyprinus carpio*, Spawning season, Fecundity, Gonadal development, Gelingüllü Reservoir, Introduced fish

Introduction

As the human population continues to increase the world is faced by significant nutritional challenges. As such, the importance of inland fisheries in natural and artificial lakes in meeting human nutritional requirements and providing employment opportunities is increasing (Welcomme, 2001). Today, carp (*Cyprinus carpio*) is one of the valuable species for inland fisheries and aquaculture settings in many countries; therefore, it is widely introduced into natural lakes and reservoirs throughout the world (Kottelat and Freyhof, 2007). Governmental organizations are responsible for fish introductions into lakes and reservoirs in Turkey in order to support inland fisheries. Until recent time, two domestic forms of common carp -mirror carp and scaled carp- have been the most preferred fish for introduction programs in Turkey.

The carp (*C. carpio*) is the most common species of the Cyprinidae family and it is commonly found in warm, still or slowly flowing waters, especially in well vegetated lakes (Kottelat and Freyhof, 2007). Several studies on biology of carp have been previously undertaken in several region including Turkey, and they have mainly focused on life-history properties and yield assessment (e.g: Hulata et al., 1974; Karakoç and Sarıhan, 1987; Fernandez-Delgado, 1990; Vilizzi and Walker,1999; Karataş, 2000; Balık and Çubuk, 2001; Kırankaya and Ekmekçi, 2013; Vilizzi et al., 2015). Studies of fish reproduction often favour commercial or valuable fish species such as carp (Smith and Walker, 2004). Crivelli (1981) studied maturation and spawning of carp in southern France and Jankovic (1971) examined oogenesis of carp from Lake Skadar. Histological interpretation of ovarian development is one of the most favourable studies in fish populations, since histological examinations provide details within the maturation cycles (Sivakumaran et al., 2003). However, limited information is available on histological development of carp gonads, especially of carp introduced into artificial lakes and ponds. Gupta (1975) examined development of carp gonads in warm water aquaria under controlled conditions, but some other researchers (e.g. Dubost et al., 1997; Sivakumaran et al., 2003; Smith and Walker, 2004) histologically assessed the development of carp gonads in natural environments. Although histological studies may highlight vulnerable aspects of the reproductive biology of commercially valuable introduced fish such as carp, there is no study about histological development of gonad of carp in Turkey.

The carp is among the important species commercially caught in the Gelingüllü Reservoir, Yozgat, Turkey. The Gelingüllü Reservoir is located in Central Anatolia (35°032′02″ E, 39°36′30″N, 1050 m a.s.l) and is the result of the dam that was completed on the Delice River, tributary of the Kızılırmak River at the end of 1993. During some summer periods, there has been a significant decrease in the water level of the reservoir, which was built in order to provide an agricultural water supply in an arid region. After completing the dam, the State Hydraulic Works (DSI) introduced mirror carp into the reservoir in 1994 and replenished the stock in subsequent years in order to advance commercial fishery in the region. The reproductive success of mirror carp in the harsh continental climate of the region was considered doubtful, as the species is a warm water culture form; therefore, scaled carp were introduced into the reservoir beginning in September 2000 as a candidate species for fishery enhancement.

The present study aimed to describe the reproductive pattern of mirror and scaled carp populations introduced into Gelingüllü Reservoir. We examine trends in gonadosomatic index, fecundity and gonad histology to better understand their reproductive biology. We use these to identify the potential of both populations to establish self-sustaining populations in Gelingüllü Reservoir.

Materials and Methods

Sampling was performed between March and November 2006. In total, 64 mirror carp and 51 scaled carp were caught using gill nets with mesh ranging in size from 20 × 20 mm to 100 × 100 mm. Total length (TL) and fork length (FL) were measured to the nearest 0.5 cm and total body weight (W) was recorded to the nearest 0.01 g. Fish samples were dissected for sex determination, and ovaries were removed and weighed to the nearest 0.01g**.** For histological analysis samples obtained from the middle part of each ovarium were fixed in 10% formalin.

Monthly changes in water temperature were monitored. While the water temperature varied between 8 and 10 °C in the first 5 m below the

surface in the spring, it can increase to 20 °C during summer.

The spawning season of mirror and scaled carp was investigated by using monthly variation in the mean GSI and ovarian maturity stages. Temporal changes in gonad development were determined based on the gonadosomatic index (GSI) and the following equation: GSI = (GW/W) × 100, where GW is gonad weight and W is total body weight. ANOVA was used to determine the significance of difference in the GSI by month.

The absolute fecundity (AF) was estimated as AF = W_G × D, where W_G is gonad weight and D is number of the oocytes per gram of ovarian tissue (Begenal and Braum, 1971).

For histological examination the ovary parts that were fixed in 10% formalin were washed in running water and stored in 70% alcohol until sectioning. In all, 115 female gonads were histologically examined. A transverse section from the central part of each gonad was dehydrated in alcohol, embedded in paraffin, and subsequently sectioned transversely to 5-7 µm using a microtome. The sections were fixed on glass slides with distilled water and stained using hematoxylin and eosin (H&E) (Culling et al., 1985; Roberts, 2001). Ovary developmental staging was based on the terminology proposed by West, 1990 and Wallace and Selman, 1981.

Results and Discussion

In total, 64 mirror carp and 51 scaled carp specimens were collected, with FL ranging from 29.7 cm to 71.5 cm (SD = 13.23) and 26.3 cm to 57 cm (SD= 8.17), respectively. Body weight of the specimens varied from 897 g to 12,500 g (SD = 2497) in mirror carp and 555 g to 4220 g (SD = 900) in scaled carp.

GSI differed significantly both in mirror and scaled carp according to the month they were collected (ANOVA: F = 2.46, $p<0.05$ for mirror carp; F = 4.61, $p<0.05$ for scaled carp). Monthly variation in mean GSI is shown in Figure 1. Mean GSI in mirror and scaled carp was 10 and 11.9, respectively, in March, peaked at 13.38 in mirror carp and 12.92 in scaled carp in April, and then gradually decreased to 0.37 and 0.42, respectively, in mirror carp and scaled carp in August. These data show that mirror carp had a prolonged breeding period - observed from April to August - whereas scaled carp spawned between April and July.

Absolute fecundity (AF) reached 2,133,990 in mirror carp and 1,628,526 in scaled carp. Mean AF ranged from 308,713 to 830,297 in mirror carp and 116,540 to 604,031 in scaled carp (Figure 2). AF in mirror carp was significantly higher than in scaled carp (ANOVA: F =2.84, $P < 0.05$). No mature eggs were observed in the gonads of scaled carp in July or mirror carp in August.

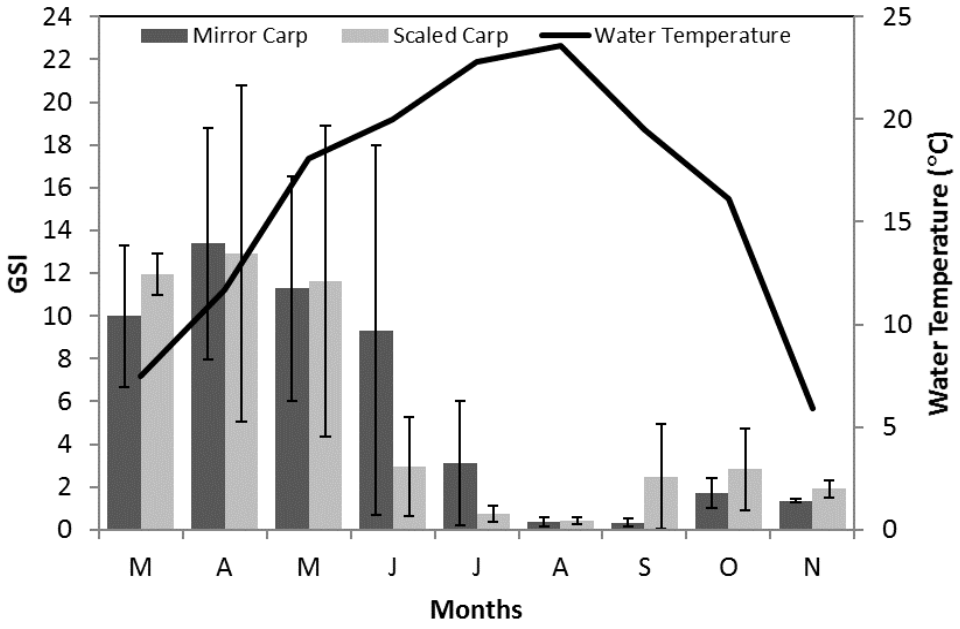

Figure 1. Monthly variation in gonadosomatic index of mirror carp and scaled carp

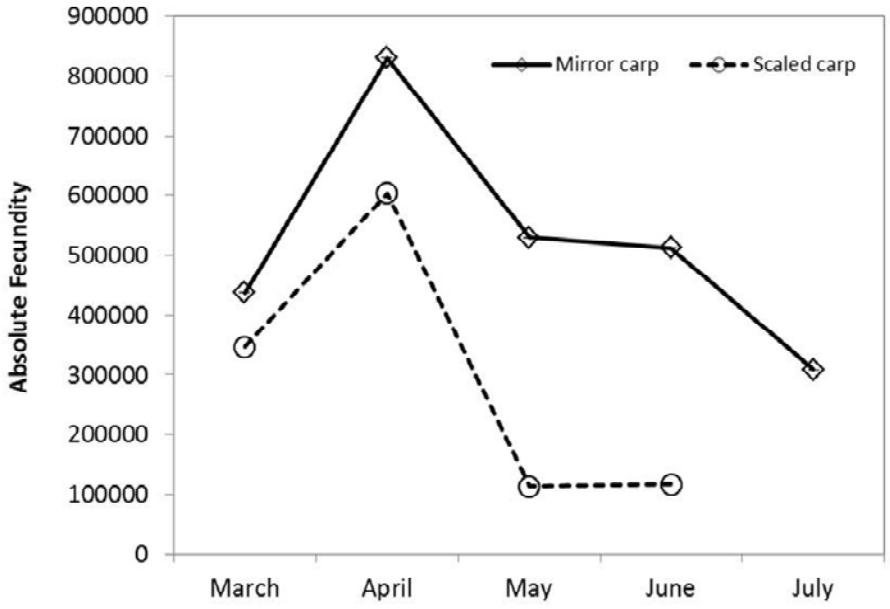

Figure 2. Monthly variation in absolute fecundity of mirror carp and scaled carp

For examination of the ovarian cycle, the observed oocytes of female mirror carp and scaled carp were classified into 5 developmental stages, and the characteristic features of each stage are given below. This categorization was based on previously published criteria: Oocyte size, appearance of nucleus in the cell and nucleolus number, type of cytoplasmic objects and their location in the cytoplasm (Wallace and Selman, 1981; West, 1990).

a. Chromatin nucleolus stage (Figure 3a)

This stage is characterized by the youngest and smallest oocytes. The large nucleus is surrounded by the cytoplasm and the oocytes appear basophilic after staining. Oocyte diameter is 20-60 µm.

b. Peri-nucleolus stage (Figure 3b)

In this stage oocyte cytoplasm stains deeply with hematoxylin and appears darker than the nucleus. Many nucleoli varying in size are observed within the nucleus periphery. Oocyte diameter is 60-200 µm.

c. Cortical alveolus stage (Figure 3c)

At the beginning of this stage the cortical alveoli begin to appear near the nucleus in the cytoplasm. As these spherical structures are stained with hematoxylin-eosin they seem to be empty. The cytoplasm becomes paler and homogenous. Oocytes continue to grow while cortical alveoli increase in size and number. Chorion (zona pellucida) first appears during this stage of oocyte development. Oocyte diameter is 200-600 µm.

d. Vitellogenic stage (Figure 3d)

This is the longest stage of oogenesis and begins with the appearance of yolk globules around the nucleus. Cortical alveoli are displaced to the peripheral cytoplasm due to the increase in size and number of yolk globules. Oocytes almost reach maximum size during this stage. Chorion becomes rather distinct and oocyte diameter is 600-1000 µm.

e. Mature oocyte stage (Figure 3e)

This is the final stage of oogenesis. The nucleus moves towards the animal pole where the micropyle is situated. The nucleus membrane breaks up and nucleoli are scattered in the cytoplasm. The yolk globules completely fuse together and cover the entire the cytoplasm. Oocyte diameter is 1000-1400 µm.

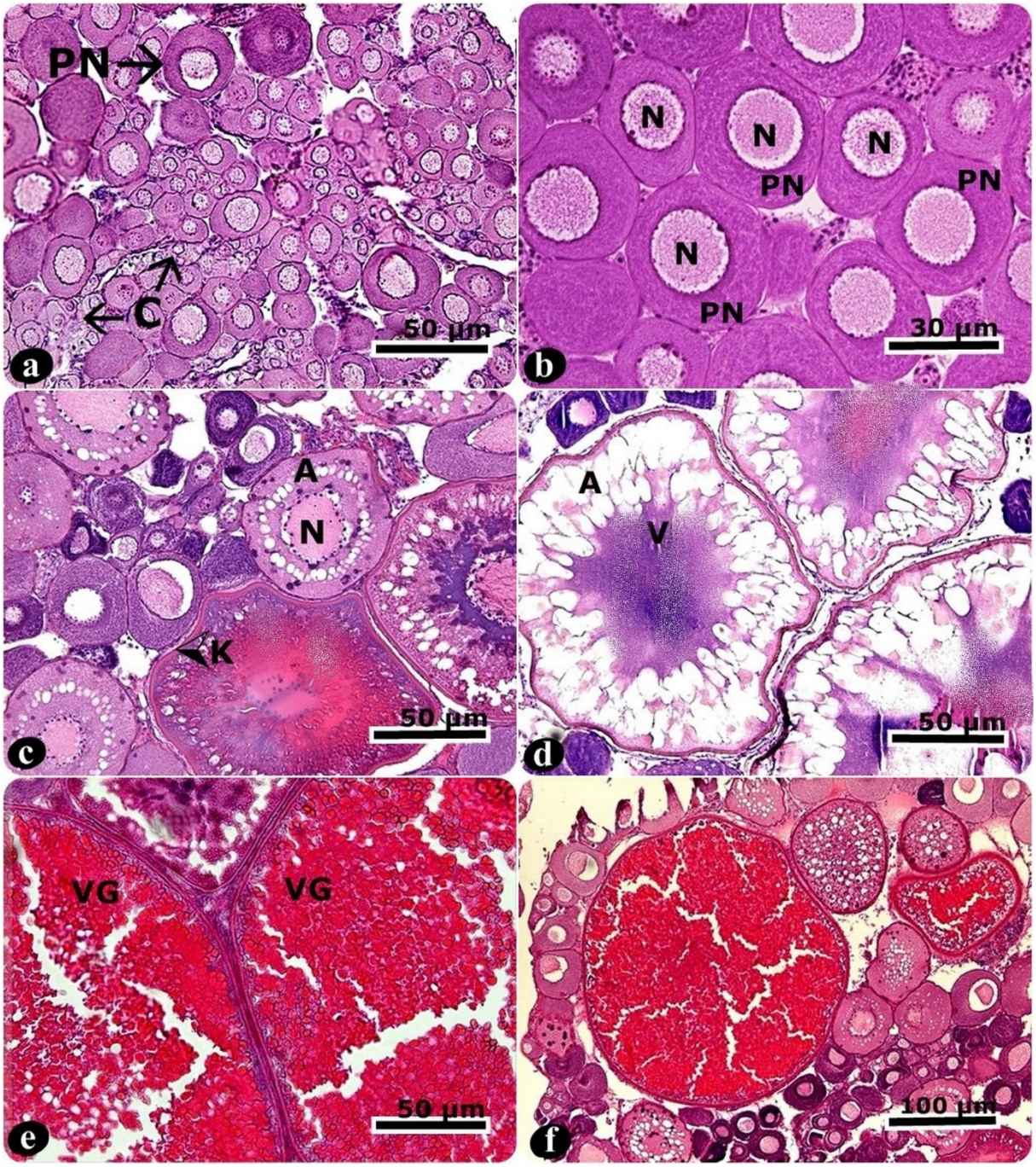

Figure 3. Histological observations of the oocytes of Cyprinus *carpio*: **(a)** chromatin stage (C) (arrow; hematoxylin-eosin), **(b)** perinucleolus stage (PN) (arrow; hematoxylin-eosin), **(c)** cortical alveolus stage (A) (K-chorion; hematoxylin-eosin), **(d)** vitellogenic stage (V) (hematoxylin-eosin), **(e)** mature oocyte stage (Vg) (hematoxylin-eosin), **(f)** different oocyte developmental phases (hematoxylin-eosin).

The reproductive characteristics of fish vary according to species, and the physical, chemical, biological, and hydrographic properties of the water systems in which they live (Dubost et al., 1997; Karataş, 2000; Balık et al., 2006). Establishment of a self-sustained population in a new environment depends on the reproductive success of introduced fish species (Minos et al., 2010). Definition of the stages of gonadal development used to evaluate such reproductive characteristics as breeding period and fecundity facilitates the collection of data on the reproductive success of a fish species.

In the present study, breeding season, GSI and fecundity were evaluated in order to determine the reproductive success of mirror carp and scaled carp populations in a reservoir environment located in a region with a harsh continental climate. It was previously reported that female mirror and scaled carp in Gelingüllü Reservoir begin to reach sexual maturity at age 3 years (Kırankaya and Ekmekçi, 2013); sexually mature (age ≥3 years) individuals were examined in the present study.

According to histological analysis and monthly changes in GSI, the mirror carp breeding period was April-August, versus April-July in scaled carp. Mean GSI in female mirror carp was 10.00 in March, whereas the maximum was 13.38 in April, after which time it gradually decreased (Figure 1). This changes in mean GSI indicated that spawning had begun. Similarly, GSI in female scaled carp was 11.93 in March and reached the maximum of 12.92 in April; the minimum mean GSI was 0.42 in August. According to these data, spawning in female mirror carp began during May when the water temperature was 18°C and continued during June and July, whereas scaled carp spawned between April and July. The wide range of GSI in both the mirror carp and scaled carp populations in the present study between April and June indicates that the samples included females with asynchronous ovaries that had both spawned and not spawned. Gupta (1975) found that carp specimens have laid all their eggs fractionally under controlled warm water aquaria conditions.

Histological analysis of ovaries in mirror carp and scaled carp yielded findings that mirrored the monthly changes observed in GSI. In both fish populations mature oocytes were first observed from the beginning of April until July. When ovulation was complete, post-ovulatory follicles (consisting of granulosa and theca cells) and atretic follicles were noted in the ovaries. Vazirzadeh et al. (2014) showed that wild carp populations from the south-eastern Caspian Sea were asynchronous multiple spawner. Sivakumaran et al. (2003) also reported that wild carp was both single and multiple spawners in South Victorian water of Australia. Smith and Walker (2004) also stated that spawning was asynchronous in common carp population from River Murray and each female may have spawned up to three discrete batches of eggs.

Ovarium structure in animals with cyclical reproduction characteristics varies according to the different phases of the cycle; there are 3 types of ovary—synchronous, group synchronous, and asynchronous (Wallace and Selman, 1981; Nagahama, 1983). According to histological analysis of ovarium tissues taken from mirror carp and scaled carp during the breeding period, it was determined that mirror carp and scaled carp have asynchronous ovaries and that different oocyte developmental phases are seen together (Figure 3f). It is known that fish species with asynchronous ovaries have a prolonged breeding season (Dubost et al., 1997; Smith and Walker, 2004). Generally, ovaries in multiple-spawners contain both post-ovulatory follicles and vitellogenic oocytes simultaneously; in this type of ovary, post-ovulatory follicles gradually disappear, but vitellogenic oocytes continue developing (Iqbal et al., 2007). In the present study, vitellogenic oocytes and mature oocytes were observed with post-ovulatory follicles simultaneously, indicating that carp is a multiple spawner, spawning more than once in a single spawning season. Yoneda et al., 1998 reported that there were post-ovulatory follicles and vitellogenic with matured oocytes in the ovaries of *Lophiomus setigerus* between May and November, which is evidence of multiple spawning.

Histological analysis in the current study showed that female mirror carp and scaled carp had mature eggs that contributed to sustaining the population. It was previously reported that there were 0+ age mirror carp fry in Gelingüllü Reservoir, whereas there were no scaled carp fry during the same period, and that there might be problems for the scaled carp population in terms of the spawning area and larval development in the reservoir (Kırankaya and Ekmekçi, 2013). Carp is known as a phytophilic fish species (Nikolsky, 1963) that depends on submerged macrophytes in the littoral zone for egg laying. Thus, there is a strong relationship between the structure of the littoral zone and reproductive success of phytophilic fish. Reservoirs are unstable land-water ecotones exposed to irregular changes in water level, and macrophyte colonization in such water is weak due to a narrow littoral zone and a high degree of steepness (Duncan and Kubecka, 1995). A narrow littoral zone and irregular water level in the littoral zone negatively affect carp spawning, egg development, and larval growth, as they lay their eggs on littoral macrophytes (Fernando and Holcik, 1991; Duncan and Ku-

becka; 1995). During the study period, it was determined that there was a tendency to decrease in water level in Gelingüllü Reservoir (Kırankaya and Ekmekçi, 2013). In this case, the narrow littoral zone, which has rare submerged macrophyte colony, might be more declined and therefore breeding area for carp might be limited. It is also possible that different populations with phytophylic features compete for use of this limited reproduction area. In an earlier study 0+ age mirror carp were observed with rate of 1.5%, but no 0+ age scaled carp were noted (Kırankaya and Ekmekçi, 2013), which suggests that mirror carp may have better adaptation for reproduction to the limited environments than scaled carp, and that scaled carp has lower reproduction performance in this environment. On the other hand, F_t values indicate that scaled carp had lower reproductivity than mirror carp.

Conclusion

In conclusion, data of the current study shows that there were no completely atrophied gonads and that ovaries contained both vitellogenic and mature eggs during the breeding season. These findings suggest that there were no developmental problems that prevented reproduction of mirror carp and scaled carp, but as reservoirs have typical limnological features, the area in which these fish can lay eggs is limited; therefore, it might be that this is a factor that determines the reproductive success of carp in this environment.

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