

REPRODUCTIVE BIOLOGY AND HISTOLOGICAL CHANGES IN THE GONADS OF BARB, *Capoeta trutta* (HECKEL, 1843) IN ATATÜRK DAM LAKE, TURKEY

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ABSTRACT

In this study, it was aimed at investigating the reproductive biology and histologically determining the developmental stages of oocytes of *Capoeta trutta* (Heckel, 1843) in Atatürk Dam Reservoir. The sex ratio was calculated as 1: 1.03 F/M (total n=422). It was determined that the mean length of the specimens considered sexually mature for both sexes was 200 mm. The gonado-somatic index (GSI) values peaked in May. The gonads obtained from males and females presented three and six development stages, respectively. The egg diameters were between 0.42 and 1.69 mm. The results indicated that the spawning activity observed in males and females, at 3 years of age or more, seems to be in May and June. The microscopic appearance of the gonads shows similar characteristics to other teleost species.

Key Words: *Capoeta trutta*, Gonadosomatic index, Fecundity, Gonad histology, Atatürk Dam Lake.

ÖZET

ATATÜRK BARAJ GÖLÜ'NDE YAŞAYAN KARABALIKLARIN *Capoeta trutta* (HECKEL, 1843) ÜREME BİYOLOJİSİ VE GONADLARINDAKİ HİSTOLOJİK DEĞİŞİMLER

Bu çalışmada, Atatürk Baraj Gölü'nde yaşayan *Capoeta trutta*'nın üreme biyolojisi ve gonad değişimlerinin histolojisi incelenmiştir. Populasyonun Dişi/Erkek oranı 1:1,03 olarak belirlenmiştir (n=422). Eşeyssel olgunluğa ulaşmış bireylerin ortalama boy uzunluğu dişi ve erkek bireyler için 199 ± 11.15 mm olarak tespit edilmiştir. GSI değerinin Mayıs ayında en yüksek düzeye ulaştığı belirlenmiştir. Gonadların olgunlaşma safhaları mikroskopik olarak erkek bireyler için üç, dişi bireyler için altı safha olarak değerlendirilmiştir. Yumurta çapları 0,42-1,69 arasında bulunmuştur. Çalışma sonuçlarına göre, 3 ve daha yaşlı dişi ve erkek bireyler için üreme zamanı Mayıs-Haziran

ayları olduğu tespit edilmiştir. Her iki eşey için gonadların mikroskopik özellikleri diğer kemikli balıklarla benzerlik göstermektedir.

Anahtar Kelimeler: *Capoeta trutta*, Gonadosomatik indeks, Fekundite, Gonad histolojisi, Atatürk Baraj Gölü.

INTRODUCTION

Atatürk Dam Lake was built on the Euphrates River in South-Eastern Anatolia. The dam was completed in 1990 for irrigation and hydroelectric purposes. It is one of the largest man-made dams in Turkey. The lake occupies an area of approximately 817 km², thus the lake is the biggest artificial reservoir in Turkey. For its large size and ecological properties, it has high importance of fisheries and fishing potential. There are 28 species and subspecies belonged to 8 families in Euphrates River and its man made lakes in Turkey, Syria and Iraq (Kuru, 1979).

Capoeta trutta belonging to family Cyprinidae has a wide distribution in both Euphrates, Tigris basins, natural and artificial lakes in Mesopotamia for thousands of years (Ünlü, 1991; Polat, 1987; Özdemir & Kabukcu 1983). There are no recent gonad development and histological studies on *C. trutta* reproduction. Whereas its growth and some reproductive properties are well documented (Ünlü, 1991; Yapalak *et al.* 1997; Kalkan, 2008; Özdemir & Kabukçu, 1983; Gül *et al.* 1996). Most researches on fish were dealing with the histological studies (Takashima,1995; Shimuzu *et al.*, 1987; Ünver and Ünver-Saraydin, 2004), spermatocyte maturation (Grier, 1975), oocyte growth and development (Takashima *et al.*, 1986, Wallace & Salman 1981, Begovac & Wallace, 1988) and specialized aspects of formation of primary oocyte (Lagler, 1977; Hughes, 1992), cortical alveoli formation and vitellogenesis and oocyte maturation (Fishelson *et al.* 1996).

The present study aimed to assess the impact of Atatürk Dam Lake on the gonad development, histological and reproductive biology of *C. trutta*, included the sex ratio, age and length at first sexual maturity, gonadosomatic index, fecundity and the histological evaluation of the gonads.

MATERIAL AND METHODS

A total of 214 male and 208 female of *C. trutta* were captured from September 2001 to August 2002 from Atatürk Dam Lake under the aid of fisherman who are fishing in the reservoir using gill nets with different mesh size (18x18, 25x25, 32x32, 40x40, and 55x55mm). After capturing, fresh fish were brought to the laboratory, each fish was recorded; data included the measurements for fork length (FL) to the nearest mm and total weight (W) to the nearest 0, 1 g. First males differ from females morphologically by the presence of breeding tubercles formed on the head during the spawning period. Scales were removed from each fish and cleaned in a 5% sodium hydroxide solution for age analyses according to Chugunova (1959).

The sex of immature samples was determined by the gonads under the aid of binocular microscope. The number of eggs was estimated by the gravimetric method using ovaries which had been preserved in 4% formalin solution (Laevastu, 1965) in

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order to enumerate the total number of eggs in the particular ovary (absolute fecundity). From each ovary, twenty ova were selected randomly and measured by means of a micrometer eye-piece, and then the mean egg diameter was computed for all of the samples. The monthly gonadosomatic index (GSI) was calculated as follows (Pantulu,1963); GSI: Gonad Weight(g)/Body Weight(g)x100. Fecundity - body weight (W), and fecundity - gonad weight (GW) relationships were calculated on the basis of the formula suggested by Pantulu (1963);

$$\text{Log F} = \log a + b \text{ Log W}, \text{ Log F} = \log a + b \text{ Log GW}.$$

The specimens were transported to the laboratory alive; the gonads were removed from each fish, fixed for 24 hours in Bouin's fixative in room temperature. Gonads samples were loaded the cassettes, placed into automatic processor (LeicaR), dehydrated in ascending grades of ethanol, cleared in two changes of xylene and impregnated in molten wax.

The specimens were removed from the processor and impregnated into paraffin with 58 °C melting point; the sections were obtained with rotary microtome. A single series of sequential histological sections 6 micrometer thick for ovary and 4 micrometer for testis was cut and stained with Hematoxylin and Eosin. Sections were examined under light microscope. Oocytes diameter were measured in oocyte cross sections on histological slides by microscope graticule (Luna, 1968).

Evaluation of the Stages of Female gonad

The process of oogenesis was classified according to size, appearance of nucleus, nucleolus and cytoplasm distribution in the ovary.

Female ovaries are located in the dorsal of the body cavity, showed thread-like surrounded by single layer germinal epithelium like a thick connective tissue which called tunica albugenea. The mature ovaries of females were large occupying 1-2/3 of body cavity and well supported with blood vessels. Ripe female had swollen green ovaries appeared flaccid.

RESULTS

As seen in Table 1, 49.29% of the samples were female and 50.71% male. The sex ratio was not significantly different from 1:1 ($P > 0.05$) in the samples observed. This ratio showed the male rate relatively higher than females in autumn 53.09 % and 46.91%, in winter the ratio was 54.12% in male and 45.88% in female respectively, while this value changed in summer to be in the ratio of female higher percentage to be 51.91% in spring and 50.40% in summer while the percentage of the male decreased to be 48.09% in spring and 49.60% in summer (Table 1).

Table 1. Seasonal variation in sex ratio for males and females of *C. trutta*.
Tablo 1. *C. trutta*'nın mevsimlere göre eşey oranları.

Seasons	Male		Female	
	N	%	N	%
Autumn	43	53.09	38	46.91
Winter	46	54.12	39	45.88
Spring	63	48.09	68	51.91
Summer	62	49.60	63	50.40
Total	214	50.71	208	49.29

Age groups of 422 *C. trutta* specimens from Atatürk Dam Lake ranged from II to IX. The majority of the specimens were in age groups IV and V. The mean fork length from 144.2 to 297.4 mm and mean weight from 34.3 to 859.7 g (Table 2).

Table 2. The mean length and weight, of the different age groups of *C. trutta*.
Tablo 2. *C. trutta*'nın yaşlara göre ortalama boy ve ağırlığı.

Age	N	Fork Length (mm)	Body Weight (g)
II	15	144.2	34.3
III	37	199.0	94.4
IV	117	227.7	140.9
V	121	251.0	189.7
VI	66	290.4	309.1
VII	33	328.9	485.9
VIII	22	383.3	702.9
IX	11	397.4	859.7

The mean gonad weight and GSI values peak reach the maximum in May. This GSI values 8,653 in females 5.534 in males (Figure 1), as the same case the egg diameter at maximum suggesting that eggs were laid on May - June (Figure 2). In July, all the ovaries were empty and only a few remaining eggs were observed.

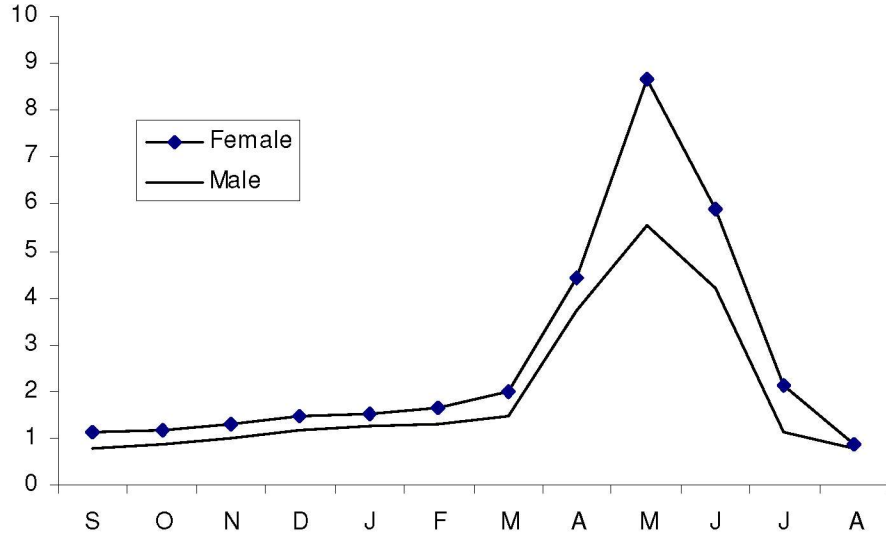


Figure 1. Monthly variation of gonadosomatic index (GSI) for females and males of *C. trutta*.
Şekil 1. *C. trutta*'nın erkek ve dişilerinde gonadosomatik index değerinin aylık değişimi.

Fecundity was estimated in 208 females captured just prior to spawning. The mean number of eggs ranged from 6871 to 33252. A positive correlation was found between fish weight and gonad weight versus fecundity and the relationships are given below;

$$\log F = 4.09718 + 0.31298 \log GW \quad (r = 0.986)$$

$$\log F = 3.31034 + 0.43901 \log W \quad (r = 0.973)$$

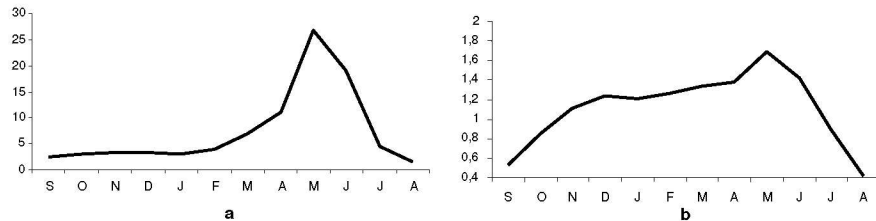


Figure 2. Monthly variation of Ovary weight (a) and Egg diameter (b) of *C. trutta*.

Şekil 2. *C. trutta*'da ovaryum ağırlığı ve yumurta sayısının aylık değişimi.

The percentage of mature *C. trutta* at age II was 12.5 %, while 95% of the fish were reaches the maturity at age III and 5% not mature. On the other hand, 28.6% of male *C. trutta* reaches the maturity at age II, while 71.4% of fish were immature, but this percentage increase to 100% at age III (Table 3). The sexual maturity of males and females was reached at age III, when they reached a fork length of about 199 ± 11.15 mm.

Table 3. The age of maturity of females and males of *C. trutta*.

Tablo 3. *C. trutta*'da erkek ve dişilerin olgunlaşma yaşı.

Months	Ovary Weight (g)	GSI (Female)	GSI (Male)	Egg Diameter (mm)
September	2.43	1.110	0.76	0.53
October	2.97	1.159	0.886	0.86
November	3.16	1.312	1.012	1.11
December	3.21	1.480	1.158	1.23
January	3.08	1.524	1.246	1.21
February	3.86	1.641	1.313	1.26
March	6.72	1.978	1.474	1.33
April	11.03	4.411	3.713	1.38
May	26.75	8.653	5.534	1.69
June	18.93	5.896	4.213	1.42
July	4.46	2.114	1.112	0.89
August	1.58	0.886	0.796	0.42

Perinucleotic and Cortical alveolar stage I- II

The ovary in this stage contains oocytes in perinucleotic and previtellogenic stage. Perinucleotic form contains oocyte in a 12-130 micrometer and the nucleus was about 50% of the oocyte diameter. Nucleolus divides to two or more nucleoli and resides in the periphery of nucleoplasm and intense basophilic cytoplasm. In previtellogenic stage, the nucleus occupies about 2/3 of the cell and contains numerous nucleoli arranged under the nuclear membrane. In addition, balbiani bodies appear in the ooplasm. Oocyte is surrounded with single layer of follicle cells (Fig. 3-a).

Vitellogenic stage III

Oocyte in this stage measured 320 ± 28 micrometer and 730 ± 41 micrometer containing yolk granules and large vacuoles. The nucleus was about 13-15 % of the total oocyte diameter. The major event in this stage is the appearance zona radiata in the periphery of the oocyte. The nuclear membrane takes irregular structure and the nucleoli number increased. At this stage, accumulation of hepatically derived yolk protein called vitellogenin that responsible for majority of oocyte growth. The follicle consisted of two layers, an outer and inner layer glandular granulose (Fig. 3-b).

Maturation stage IV

At this stage, oocyte reaches its maximal size between 800 ± 28 and 1600 ± 61.2 micrometer. The ovary was packed with vitellogenic and the massive accumulation of yolk protein occupies the central portion of the oocyte. The nucleus disappeared due to the condense yolk accumulation (Fig. 3-c, d).

Ovulation and Post ovulation stage V - VI

After the germinal vesicle break down the oocyte ovulated into the ovarian lumen and becomes mature eggs. Past ovulation process a few small oocyte were present and characterized by presence of atresia of remaining vitellogenic ova after spawning (Fig. 3-e, f).

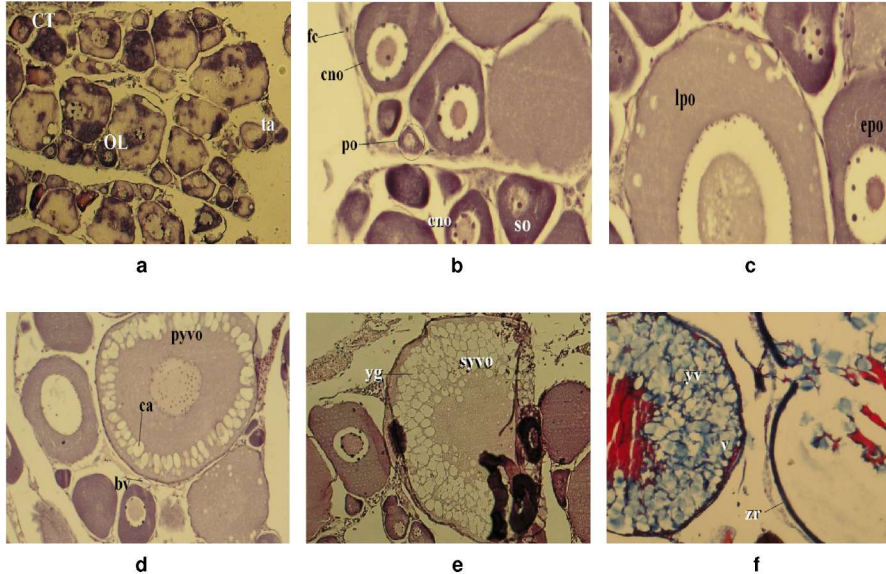


Figure 3. Illustrated the stages of *C. trutta* gonad development. **a)** Ovigerous lamella (OL), connective tissue (CT) **b)** primary oogenia (po), secondary oogenia (so), chromatin nuclear oocyte (cno), follicle cells (fc) **c)** late peri-nuclear oocyte (lpo), early peri-nuclear oocyte (epo) **d)** cortical alveoli (ca), primary yolk vesicle oocyte (pyvo) **e)** secondary yolk vesicle oocyte (syvo), yolk granules (yg) **f)** zona radiata (zr), advanced atresia yolk globules (yv), vacuoles (V). H&E X 100.

Şekil 3. *C. trutta*'da gonad gelişim safhaları. **a)** Ovigerous lamella (OL), connective tissue (CT) **b)** primary oogenia (po), secondary oogenia (so), chromatin nuclear oocyte (cno), follicle cells (fc) **c)** late peri-nuclear oocyte (lpo), early peri-nuclear oocyte (epo) **d)** cortical alveoli (ca), primary yolk vesicle oocyte (pyvo) **e)** secondary yolk vesicle oocyte (syvo), yolk granules (yg) **f)** zona radiata (zr), advanced atresia yolk globules (yv), vacuoles (V). H&E X 100.

DISCUSSION

Results obtained from our study showed that the female/male percent was 49.29/50.71 or 1:1.03 ratio. This is with the result reported by Nikolsky (1963), indicated the different sexual separation of the same species was to be found 1:1. Özdemir and Kabukçu (1988) reported that the sex percent of *C. trutta* living in Keban Dam Lake was 52% for female and 48% for male, Ünlü (1991) reported that the percentage of female/male of *C. trutta* living in Tigris river was 58.74 and 41.26. Polat (1987) reported 64.69% for female and 36.11% for male from Keban Dam Lake, Kalkan (2008) found that female/male ratio was 0.98:1 while Gül *et al.* (1996) found that the percentage of *C. trutta* living in Euphrates River 53.3 female and 46.7 male.

Age composition of the *C. trutta* in present study was determined II-IX. The age composition was determined by several papers; Yapalak *et al.* (1997) I-VI, Ünlü (1991) I-X, Polat (1987) I-VIII, Gül *et al.* (1996) I-X and Kalkan (2008) 0-VII. The differences among the age distribution values may be related to the kind of nets or mesh-size of the nets or may be explained as an adaptive response to the different ecological conditions of the study areas.

According to the average GSI, the highest values were observed for female 8.653 and for male 5.534 in the samples caught in May. The lowest values were observed for female 0.886 in August and for male 0.76 in September. There are decreasing in mean GSI values of June samples because almost all individuals were spawned. The mean GSI values were found a gradual increased from October until the new spawning season. The highest GSI value was determined by Kalkan (2008) form Karakaya Dam Lake, for females to be 7.91 in May, the lowest value 0.29 in September. These values were determined by Ünlü (1991) from Tigris River, 12.03 in May, 1.45 in August for female and 6.00 in May, 1.05 in August for male.

The highest mean egg diameter was found in May (1.69 mm) and lowest value was found in August (0.42 mm). The highest and the lowest diameter of eggs were determined by Ünlü (1991) to be 0.55-1.38 mm, Polat (1987) was found to be 0.43-1.03 mm, Gül *et al.* (1996) reported the higher and lower diameter of oocyte was 0.46-1.04 mm and Kalkan (2008) found to be 0.37-1.04 mm.

In this study, the mean egg number varied between 6871 to 33252. This values was determined by Ünlü (1991) 4713-18240 and Yapalak *et al.* (1997) 4136-24152. It has been reported that fecundity can change in relation to fish age, length and weight and especially temperature which is the most important ecological factor effecting the egg number. As we know, the older individuals are more productive than the younger.

According to the monthly changes in the values of the GSI, egg diameter and egg number of the specimens in Atatürk Dam Lake, the spawning period was determined to be May and June. This period has been determined to be by Kalkan (2008) between March and July, Gül *et al.* (1996) May-July, Özdemir & Kabukçu (1983) May-September, Polat (1987) April-June and Unlu (1991) May-June. Because the ecological and climatical conditions are different, the starting and finishing time of the reproduction period may include different months. The sexual maturity of male and female III age groups when they reached a fork length about 199 ± 11.15 mm respectively. According to Kalkan (2008) the sexual maturity age in *C. trutta* population of Karakaya Dam Lake is II-III. The maturity age of the specimens was found to be II-III in both sexes by Ünlü (1991) from Tigris River.

To evaluate the reproductive study of *C. trutta* living in Ataturk Dam Lake including in the measurement of oocyte size, staging at the external appearance of the ovary. Histological examination and gonad indices to determine the stages of gonad development. However, the histological examination is expensive and time consuming, but it is the most accurate method employed to study the gonad development (Ünal *et al.*, 1999; West, 1990). Generally, the process of teleost oogenesis may be divided to 5 – 8 stages (West, 1990; Fishelson *et al.* 1996; Ünver and Ünver-Saraydin, 2004).

The histological studies of gonad development of *C. trutta* are basically the same in all teleost species. The process of oogenesis has been divided into six stages. The size of oocytes at the early stages increase due mainly to accumulation of non yolk cytoplasm and the major growth of oocyte at the later stages is mainly due to accumulation of the hepatic derived materials, as protein, vitellogenin yolk materials (Hibiya, 1982; West, 1990). The oocyte reaches maximum size 1.76 mm in *C. trutta* living in Atatürk Dam Lake, this is directly found that the nutritional state of the fish affects the vitellogenic and maturation of oocyte because there is a close relationship between the vitellogenesis and oocyte size (Wallace & Salman 1978). The ecological factors, as temperature, photoperiod and nutrition are important environmental factors for regulations of reproductive pattern in most teleost (De Vlaming 1972), and the high temperature suppressed gonadal activity in Cyprinid fish (Okuzawa *et al.* 1989).

The nucleus membrane fused and the migration of the nucleus started in the mature oocyte, (ripe phase), 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 rational management of a fishery necessitates some understanding of the dynamics of the population, this study covers essential basic criteria which are well understood for other Cyprinidae species. Therefore this paper provides the first systematic observations on population structure of *C. trutta* living in Atatürk Dam Lake, age and length for the species and the first on reproductive status.

In conclusion, the economical importance of *C. trutta* in fishing in Euphrates, Tigris and man made reservoirs must be coordinated by government to prevent the realer fisheries. The prohibition of fishing during April- August period can be advised, and the prohibition include using small nets to capture under 22 cm must fix low to forbid capturing.

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