



Reproduction cycle and monthly alteration of serum testosterone, estradiol and cholesterol in *Capoeta trutta* (Heckel, 1843)

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Abstract This study was carried out between the March 2008 and February 2009 so as to search seasonal changes of serum testosterone and cholesterol of 180 male individuals and estradiol and cholesterol of 180 female individuals belonging to *Capoeta trutta* population in Karakaya Dam Lake. Reproduction period of the population was determined to be between May and June. The amount of testosterone which belongs to male individuals of *C. trutta* population was reported to be changeable between 20.20 ng/dl and 1423 ng/dl and the amount of cholesterol was found to be 112.62 mg/dl – 937.91 mg/dl. Amount of estradiol was determined to be between 20.10 pg/ml – 3855 pg/ml and the amount of cholesterol was between 83.67 mg/dl – 984.71 mg/dl for the female population.

Keywords *Capoeta trutta*, Testosterone, Estradiol, Cholesterol, Karakaya Dam Lake

Introduction

Gonadal steroid hormones can have profound influences on the central nervous system and behavior of vertebrates either through organizational effects during early development or through activational effects in adults [1]. The activational effects of gonadal hormones during reproduction are often associated with seasonal cycles of steroid hormone production and gametogenesis. Such seasonal changes can ultimately influence reproductive behaviors and are necessary for successful reproduction in all vertebrates. Cyclical changes in the reproductive hormones of teleost fishes are widely known to occur in association with reproductive cycles and have been investigated mainly to understand the mechanisms of reproductive behavior, gametogenesis, and gonadal steroidogenesis [2]. Seasonal changes in circulating levels of gonadal steroid hormones during the reproductive cycle was described for a variety of freshwater teleost species [3-12].

Cholesterol, a white, waxy, fat-like substance produced naturally in liver, triglyceride and other lipids are transported by the same way in body fluids by a series of lipoproteins [13]. According to Dindo and MacGregor III [14] gonadal steroid hormones stimulate the development of gonads, and lipids play an essential role in energy requirements during gonadal maturation. Seasonal changes in serum gonadal steroid hormones and serum lipids have been correlated with seasonal gonadal changes in fish [9]. There have been a lot of study on the level of cholesterol in fishes [9,13-25].

However, there have been not any study on the serum sex steroids and cholesterol in *C. trutta*. For this reason, we investigated the annual cycle of serum testosterone, estradiol and cholesterol in *C. trutta*.

Material and Methods

Karakaya Dam Lake (Figure 1) is the third largest dam lake on the River Euphrates (in respect to the surface area of lake) right after Keban Dam Lake and Karakaya Dam is situated 166 km downstream Keban Dam, in the locality of Seki Bağları, near the county of Çüngüş of Diyarbakır province. Apart from Euphrates as the main river, Sultansuyu, Tohma Brook, and other small brooks and streams join Karakaya Dam Lake [26].



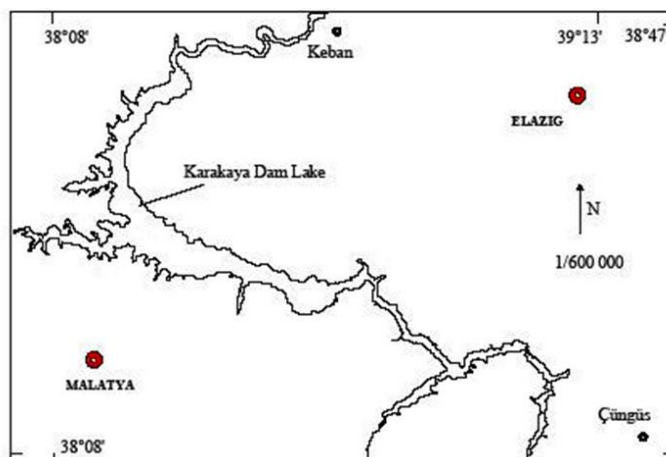


Figure 1: Karakaya Dam Lake, Malatya-Turkey [27]

Fish samples were collected from Karakaya Dam Lake by gill nets. Total of 360 *C. trutta* (180 female and 180 male) were captured between March 2008 – February 2009. Blood samples were collected from the caudal vein of each fish in the research field monthly. Immediately after collection, the blood samples were brought to the laboratory by cold chain application and were centrifuged at 3500 rpm for 10 minute and serums were separated. After, serum samples were stored at -25 °C until analysis. Body weight (g), gonad weight (g) and sex were determined for each fish. In order to find out spawning period of the fish, the gonadosomatic index (GSI) values were calculated by formula $GSI = [\text{gonad weight} / (\text{body weight} - \text{gonad weight})] \times 100$ according to Avşar [28]. Estradiol (in females) and testosterone (in males) were determined using an automatic system (Bio DPC-Immulate® 2000; electrochemiluminescence) with Immulate® 2000 total testosterone and estradiol test kits. Serum cholesterol levels were measured previously described [29]. Microsoft Office Excel 2007 and SPSS 12.0 package programs were used to get the statistical analysis (t-test and one-way ANOVA-Duncan).

Results

In order to determine the reproduction period of *C. trutta* population, the average gonadosomatic index (GSI) values of each sex and every individual were calculated on a monthly basis, and these values are shown in Table 1 and Figure 2. It has been found that the gonadosomatic index values of males ranged from 0.10 to 11.06 %, while gonadosomatic index values of females ranged from 0.07 to 15.03 %. It has been observed that GSI values of *C. trutta* population reached the peak level at the beginning and then they scattered their eggs and sperms in May and June (Table 1 and Fig. 2). Also, secondary sex feature has been seen on the tip of the nose in the male fishes during the reproduction period.

Table 1: Monthly alteration of gonadosomatic index (GSI) in *C. trutta* population

Months	Gonadosomatic index (Male)					Gonadosomatic index (Female)				
	N	Min.	Max.	Mean	S. Error	N	Min.	Max.	Mean	S. Error
March	15	0.10	1.30	0.56	0.098	15	0.25	3.30	1.16	0.218
April	15	0.22	3.78	1.41	0.337	15	0.28	2.40	1.09	0.183
May	15	3.36	11.06	6.66	0.601	15	0.35	15.03	5.19	1.151
June	15	0.14	7.31	1.98	0.572	15	0.21	9.04	1.86	0.626
July	15	0.16	3.14	0.59	0.186	15	0.53	5.62	1.53	0.414
August	15	0.19	0.58	0.36	0.027	15	0.26	0.91	0.53	0.053
September	15	0.24	0.98	0.48	0.051	15	0.23	1.47	0.67	0.087
October	15	0.13	1.13	0.54	0.072	15	0.07	1.27	0.74	0.117
November	15	0.28	0.94	0.52	0.055	15	0.30	1.69	0.87	0.125
December	15	0.19	1.17	0.55	0.065	15	0.21	2.25	1.01	0.156
January	15	0.27	1.14	0.56	0.064	15	0.15	3.27	0.96	0.260
February	15	0.34	1.74	0.81	0.104	15	0.07	2.36	0.91	0.213



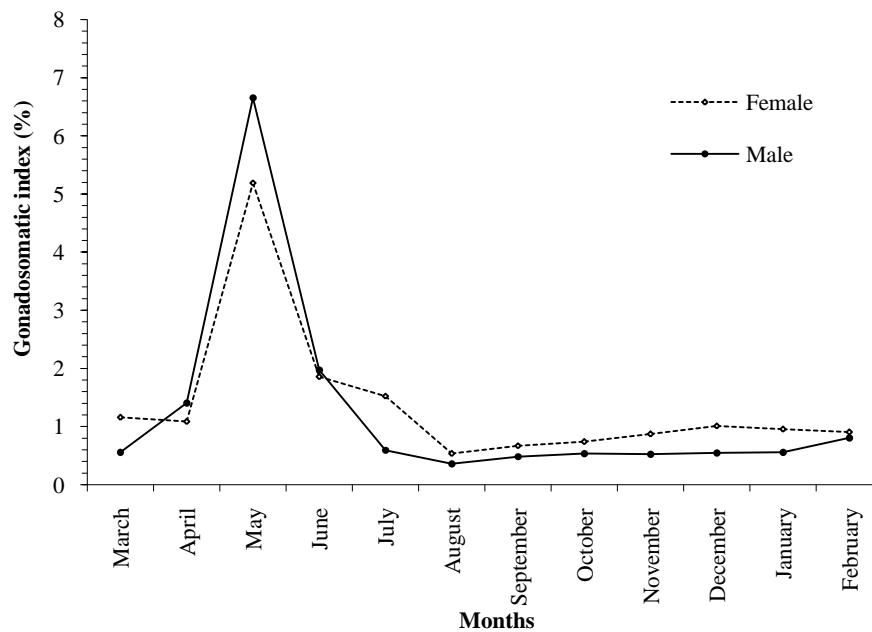


Figure 2: Monthly alteration of gonadosomatic index (GSI) in *C. trutta* population according to mean values

The average values of testosterone and estradiol were higher in May (testosterone: 754.67 ± 94.679 ng/dl; estradiol: 1516.60 ± 301.230 pg/ml) than in other months (Table 2 and Figure 3). After the reproduction period, the gonadal steroid hormone levels significantly decreased ($P < 0.05$). The highest value of testosterone was determined 1423 ng/dl in May, while the lowest value was 20.20 ng/dl in August and September. The highest value of estradiol was determined 3855 pg/ml in May, while the lowest value was 20.00 ng/dl in February (Table 2).

Also, there has been seen highly correlation ($r = 0.87$) between monthly average values of testosterone and estradiol (Figure3).

Table 2: Monthly alteration of testosterone (ng/dl) and estradiol (pg/ml) in *C. trutta* population

Months	Testosterone (ng/dl)					Estradiol (pg/ml)				
	N	Min.	Max.	Mean*	S. Error	N	Min.	Max.	Mean*	S. Error
March	15	51.90	304.00	131.55 ^{ab}	15.822	15	59.20	947.00	186.72 ^a	57.567
April	15	44.20	1212.00	332.43 ^c	77.801	15	39.80	2000.00	655.84 ^b	163.889
May	15	308.00	1423.00	754.67 ^d	94.679	15	175.00	3855.00	1516.60 ^d	301.230
June	15	52.40	439.00	174.97 ^b	36.798	15	162.00	1937.00	1010.87 ^c	144.451
July	15	22.00	90.80	38.17 ^a	5.665	15	144.00	962.00	420.53 ^{ab}	66.836
August	15	20.20	35.80	21.97 ^a	1.009	15	63.90	705.00	215.94 ^a	44.125
September	15	20.20	31.30	25.20 ^a	0.945	15	48.00	461.00	134.23 ^a	27.148
October	15	20.40	115.00	38.55 ^a	7.699	15	89.80	614.00	230.42 ^a	39.877
November	15	26.10	172.00	82.33 ^{ab}	12.455	15	43.40	506.00	112.51 ^a	30.004
December	15	20.80	257.00	79.57 ^{ab}	20.135	15	20.50	334.00	108.39 ^a	25.389
January	15	20.60	142.00	63.46 ^{ab}	11.338	15	41.60	833.00	274.17 ^a	57.425
February	15	20.80	468.00	110.61 ^{ab}	31.448	15	20.00	608.00	133.31 ^a	51.364

* There is no statistical difference between the months indicated by the same letter ($p > 0.05$).



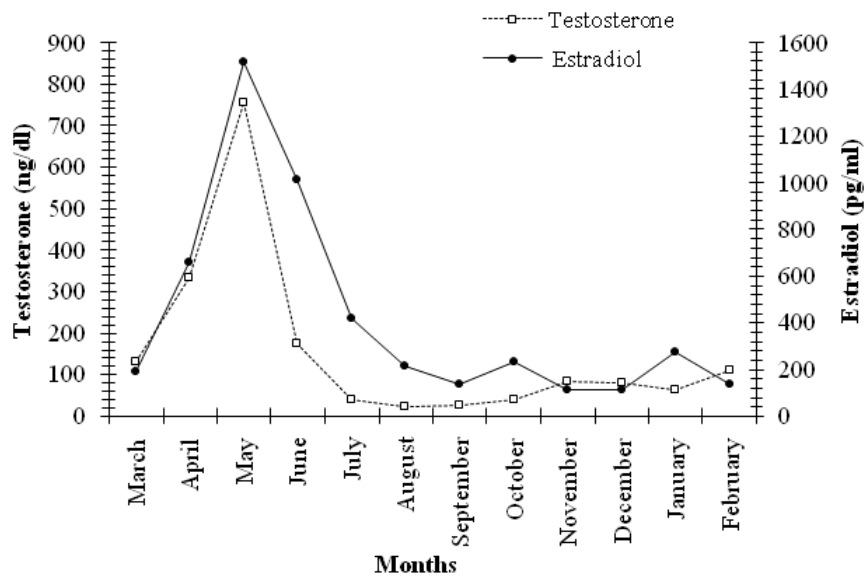


Figure 3: Monthly alteration of testosterone and estradiol in *C. trutta* population according to mean values. In both sexes the highest values of cholesterol were determined in April (937.91 mg/dl for male; 984.71 mg/dl for female). While the lowest value of cholesterol was determined as 112.62 mg/dl in November for males, the lowest value of cholesterol was 83.67 mg/dl (March) for females (Table 3). It has been observed that cholesterol concentration reached the maximum level in the prespawning month (April). Also, it has been found that cholesterol concentrations fluctuate throughout the year (Figure 4).

Table 3: Monthly alteration of cholesterol (mg/dl) in *C. trutta* population

Months	Cholesterol (mg/dl)									
	Male					Female				
	N	Min.	Max.	Mean*	S. Error	N	Min.	Max.	Mean*	S. Error
March	15	216.80	492.69	344.39 ^a	22.124	15	83.67	674.65	372.74 ^{ab}	40.232
April	15	383.15	937.91	741.80 ^f	44.213	15	142.59	984.71	570.55 ^{cd}	50.740
May	15	404.06	840.56	578.74 ^{de}	29.857	15	377.22	565.37	438.54 ^b	15.215
June	15	280.50	821.22	472.49 ^{bcd}	42.794	15	222.46	756.94	477.94 ^{bc}	40.962
July	15	348.28	848.59	600.02 ^e	38.902	15	232.92	630.41	443.78 ^b	26.295
August	15	299.84	647.28	397.71 ^{ab}	29.552	15	190.36	637.01	396.12 ^{ab}	32.219
September	15	199.69	718.95	409.31 ^{abc}	42.408	15	136.52	644.07	308.52 ^a	33.916
October	15	268.51	665.13	485.57 ^{bcd}	33.373	15	251.00	718.28	464.31 ^{bc}	38.771
November	15	112.62	672.31	522.34 ^{cde}	37.146	15	172.00	652.31	432.43 ^b	36.671
December	15	224.11	800.00	482.89 ^{bcd}	37.576	15	263.37	654.10	450.16 ^b	33.681
January	15	276.68	754.93	502.56 ^{bcd}	32.235	15	316.43	821.60	587.15 ^d	40.213
February	15	176.91	715.13	520.73 ^{cde}	39.602	15	229.02	646.80	437.82 ^b	36.968

* There is no statistical difference between the months indicated by the same letter ($p > 0,05$).



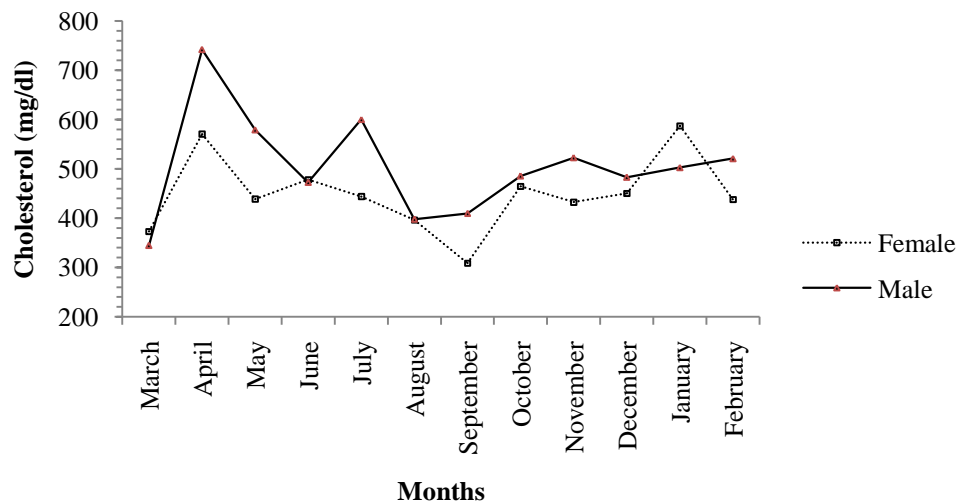


Figure 4: Monthly alteration of cholesterol in *C. trutta* population according to mean values

Discussion and Conclusion

The gonadosomatic index values for both sexes reached the maximum level in May. And GSI values were higher in May and June than in other months. Similarly, it has been previously reported that the GSI values of the *Capoeta trutta* population living in the Karakaya Dam Lake are higher in May and June than in the other months [30].

Throughout the prespawning period, testosterone and estradiol levels were very low. Our data are consistent with those of other researchers [9,14,31-33]. Testosterone and estradiol levels of both sexes were highest in May. After the spawning period steroid hormone levels decreased significantly ($P < 0.05$). The rapid increase in serum gonadal steroids in both sexes of *C. trutta* is highly correlated with increases in the gonadosomatic index. Seasonal patterns of correlation between serum steroids and gonadal maturation have been reported previously [9,14,34,35].

Cholesterol concentrations for both sexes are fluctuate throughout the year. However, the highest values of cholesterol were determined in April in *C. trutta*. Dindo and MacGregor [14] reported that serum total lipid concentrations in females were often significantly higher than in males but the seasonal fluctuations were similar. In addition, they also reported that cholesterol concentrations for both sexes were similar throughout the year in the striped mullet. The same cholesterol result was reported in *Salvenilus fontinalis* [36], *Capoeta capoeta umbla* [9]. Associated with an increase in serum cholesterol during April is the period of gonadal enlargement and elevated serum estradiol and testosterone in *C. trutta*. According to Erdoğan et al. [9], cholesterol is also the precursor of steroid hormones [36]. Cholesterol decreased with the beginning of reproduction activity in May. It is estimated that deposited energy was used for reproduction activity. Low level of cholesterol at the time of spawning have been confirmed by Dindo and MacGregor [14], Cerda et al. [33], Erdoğan et al. [9] and Kocaman et al. [13]. Many researchers have reported that fish deposit the source of foods in their tissue and liver. Then these deposited foods are used in reproduction and other activities [9].

In conclusion, it was seen that monthly alterations in gonadosomatic index, serum gonadal steroids and serum cholesterol levels in both sexes were associated with the reproduction cycle.

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References

- [1]. Nelson, R.J. (2000). *An Introduction to Behavioral Endocrinology*, Second Ed. Sinauer Associates, Inc., Sunderland, USA.
- [2]. Sisneros, J.A., Forlano, P.M., Knapp, R., & Bass, A.H. (2004). Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. *General and Comparative Endocrinology*, 136:101-116.
- [3]. Kobayashi, M., Aida, K., & Hanyu, I. (1986). Annual changes in plasma levels of gonadotropin and steroid hormones in goldfish. *Bulletin of the Japanese Society of Scientific Fisheries*, 52:1153-1158.
- [4]. Liley, N.R., Berton, B., Fostier, A., & Tan, E.S.P. (1986). Endocrine changes associated with spawning behavior and social stimuli in a wild population of rainbow trout (*Salmo gairdneri*). *General and Comparative Endocrinology*, 62:145-156.
- [5]. Scott, A.P., & Sumpter, J.P. (1989). Seasonal variations in testicular germ cell stages and in plasma concentrations of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old, *General and Comparative Endocrinology*, 73:46-58.
- [6]. Trudeau, V.I., Peter, R.E. & Sloley, B.D. (1991). Testosterone and estradiol potentiate the serum gonadotropin response to gonadotropin-releasing hormone in goldfish. *Biology of Reproduction*, 44:951-960.
- [7]. Gazola, R., Borella, M.I., Donaldson, E.M., Val-Sella M.V., Sukumasavin, N., Fava-De-Moraes, F., & Bernardino, G. (1996). Plasma steroid and corticosteroid levels of female pacu *Piaractus mesopotamicus* Teleostei-Characidae. *Brazilian Journal of Medical and Biological Research*, 29:659-664.
- [8]. Gazola, R., & Borella, M.I. (1997). Plasma testosterone and 11-ketotestosterone levels of male pacu *Piaractus mesopotamicus* (Cypriniformes, Characidae). *Brazilian Journal of Medical and Biological Research*, 30:1485-1487.
- [9]. Erdoğan, O., Haliloğlu, H.İ., & Çiğtaş, A. (2002). Annual cycle of serum gonadal steroids and serum lipids in *Capoeta capoeta umbla*, Gündenstein, 1772 (Pisces: Cyprinidae). *Turkish Journal of Veterinary and Animal Science*, 26:1093-1096.
- [10]. Guerriero, G., Ferro, R., & Ciarcia, G. (2005). Correlations between Plasma Levels of Sex Steroids and Spermatogenesis during the Sexual Cycle of the Chub, *Leuciscus cephalus* L. (Pisces: Cyprinidae). *Zoological Studies*, 44(2): 228-233.
- [11]. Guerriero, G. (2007). Seasonal steroids variations and maturity stages in the female chub, *Leuciscus cephalus* L. (Pisces, Cyprinidae). *Italian Journal of Zoology*, 74(4):317-324.
- [12]. Argungu, L.A., A. Christianus, A., Amin, M.S.N., Daud, S.K., & Siraj, S.S. (2015). Annual dynamics of the plasma sex steroid hormones of the Malaysian walking catfish *Clarias batrachus* (Linnaeus 1758). *Journal of Fisheries and Aquatic Science*, 10:24-34.
- [13]. Kocaman, E.M., Yanık, T., Erdoğan, O. & Çiğtaş, A.K. (2005). Alterations in cholesterol, glucose and triglyceride levels in reproduction of rainbow trout (*Oncorhynchus mykiss*). *Journal of Animal and Veterinary Advances*, 4(9):801-804.
- [14]. Dindo, J.J., & MacGregor III, R. (1981). Annual cycle of serum gonadal steroids and serum lipids in striped mullet. *Transactions of the American Fisheries Society*, 110:403-409.
- [15]. Deb, S., Mukherjee, D., & Bhattacharya, S. (1983). Interrelationship between plasma and ovarian cholesterol in a teleost fish. *Cellular and Molecular Life Sciences*, 39(4):27-428.
- [16]. Farrell, A.P., & Munt, B. (1983). Cholesterol levels in the blood of Atlantic salmonids. *Comparative Biochemistry and Physiology Part A: Physiology*, 75(2): 239-242.
- [17]. Dutta, H.M., & Haghghi, A.Z. (1986). Methylmercuric chloride and serum cholesterol level in the bluegill (*Lepomis macrochirus*). *Bulletin of Environmental Contamination and Toxicology*, 36:181-185.
- [18]. Babin, P.J., & Vernier, J.M. (1989). Plasma lipoproteins in fish. *Journal of Lipid research*, 30:467-489.
- [19]. Ghazaly, K.S. (1991). Physiological alterations *Clarias lazera* induced by two different pollutants. *Water, Air & Soil Pollution*, 60(1-2):181-187.



- [20]. Lund, E.D., Sullivan, C.V. & Place, A.R. (2000). Annual cycle of plasma lipids in captive reared striped bass: effects of environmental conditions and reproductive cycle. *Fish Physiology and Biochemistry*, 22:263-275.
- [21]. Chatzifotis, S., Muje, P., Pavlidis, M., Ågren, J., Paalavuo, M. & Mölsä, H. (2004). Evolution of tissue composition and serum metabolites during gonadal development in the common dentex (*Dentex dentex*). *Aquaculture*, 236:57-573.
- [22]. Çelik, E.Ş., & Çakıcı, H. (2005). Determining of some biochemical blood parameters of scorpion fish (*Scorpaena porcus* Linneaus, 1758) in dardanelles. *Nineteen May University Journal of Agriculture Faculty*, 20(2):15-23 (in Turkish with English Abstract)
- [23]. Asadi, F., Masoudifard, M., Vajhi, A., Lee, K., Pourkabir, M. & Khazraeinia, P. (2006). Serum biochemical parameters of *Acipenser persicus*. *Fish Physiology and Biochemistry*, 32(1):43-47.
- [24]. Afaq, S., Rana, K.S., & Lone, M.A. (2010). toxicological effects of leather dyes on serum cholesterol of fresh water teleost, *Cirrhinus mrigala* (ham.). *International Journal of Pharma and Bio Sciences*, 1(2):1-4.
- [25]. Abassi, Z., Shaikh, S.A., & Abbassi, J. (2011). Serum cholesterol level during vitellogenesis of teleost fish, *Cyprinus carpio*. *Pakistan J. Zool.*, 43(4):739-745.
- [26]. Anul, N. (1995). Karakaya Dam Lake Limnological Report. Turkish Republic The Ministry of Public Works and Settlement, General Directorate of State Hydraulic Works, The 9. Regional Directorate, Elazig, Turkey, (in Turkish).
- [27]. Pala, G., Tellioglu, A., Eroglu, M., & Şen, D. (2010). The digestive system content of *Mastacembelus mastacembelus* (Banks & Solander, 1794) inhabiting in Karakaya Dam Lake (Malatya-Turkey). *Turk J Fish Aquat Sci.*, 10:229-233.
- [28]. Avşar, D. (2005). *Fisheries Biology and Population Dynamics*, Adana, Nobel Press, 289 pp. (in Turkish).
- [29]. Wedemeyer, G.A., & Yasutake, W.T. (1977). Clinical Methods for the Assessment of the Effects of Environmental Stress on Fish Health. *U.S. Fish and Wildlife Service Technical Paper*, 89:1-18.
- [30]. Düşükcan, M., & Çalta, M. (2012). The comparison of reproduction periods of *Capoeta trutta* (Heckel, 1843) Populations from Keban, Karakaya and Atatürk dam lakes. *Firat University Journal of Science*, 24(2):57-61.
- [31]. Galas, J., & Bieniarz, K. (1989). Seasonal changes of sex steroids in mature female and male carp (*Cyprinus carpio* L.). *Pol. Arch. Hydrobiol.*, 36(3):407-416.
- [32]. Barry, T.P., Santos, A.J.G., Furukawa, K., Aida, K., & Hanyu, I. (1990). Steroid profiles during spawning in male common carp. *Gen. Comp. Endocrinol.*, 80:223-231.
- [33]. Cerda, J., Zanuy, S., Carrillo, M., Ramos, J., & Serrano, R. (1995). Short and long term dietary effects on female sea bass (*Dicentrarchus labrax*): seasonal change in plasma profiles of lipids and sex steroids in relation to reproduction. *Comp. Biochem. Physiol.* 111:83-91.
- [34]. Matty, A.J. (1985). *Fish Endocrinology*, Timber Press, Portland, Oregon, USA, 267pp.
- [35]. Pinillos, M.L., Delgado, M.J., & Scott, A.P. (2003). Seasonal changes in plasma gonadal steroid concentrations and gonadal morphology of male and female tench (*Tinca tinca*, L.), *Aquaculture Research*, 34:181-1189.
- [36]. Farrell, A.P., & Munt, B. (1983). Cholesterol levels in the blood of Atlantic salmonids. *Comp. Biochem. Physiol.*, 75A (2):239-242.

