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Relation between gonadal hormones and sexual maturity of female Kutum *Rutilus frisii kutum* during spawning season

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Abstract

Relation between sexual maturity and levels of two main steroid hormones in gonads, 17-□ estradiol (E_2) and testosterone (T) were studied by using histological and radioimmunoassay in female kutum *Rutilus frisii kutum* during spawning season of the southern Caspian Sea. The study was carried out from February to May 2008 using 105 migrated fish specimens catched from the River sefid-rood by various tools of catching including (Gillnet, Cast net, Seine net and Sheyl or Kulham). The results revealed that changes in plasma levels of gonadal steroids, (E_2) and (T) were closely correlated with ovarian development and increased in Gonadosomatic index (GSI) ($P<0.05$). GSI was increased in March and reached the highest value (29.47±4.2) in April. It was then decreased sharply in early May. The highest peak of plasma level of (T) and (E_2) was during spawning season and it was associated with the GSI. The results showed that levels of (E_2) and (T) in female kutum at the stage IV of sexual maturity was significantly higher than immature gonads (ovary in stages II and III) ($P<0.01$). Plasma (E_2) and (T) levels increased in February, the highest levels were observed in March and the early of April (105.6±75.3 and 29.2±96.6 ng/ml), respectively. It was decreased in the late of April and in the early of May during the spawning season ($P<0.05$).

Keywords: kutum, *Rutilus frisii kutum* (Kamenskii, 1901), sex steroid hormones, spawning season, Caspian sea

Introduction

Generally populations of Caspian Kutum, *Rutilus frisii kutum* (Kamenskii, 1901) recorded along near the coast, from the Trek River the north to the southern part of the Caspian Sea (Sharyati, 1993). This species is an endemic fish and more than 70% of fishermen catch it in Iranian coastal of the Caspian Sea. The catch ratio of catched Caspian Kutum of the southern coastal was about 17195 tons in 2007-08 (www.shilat.com). In natural environment, the fish spawning in groups, in slow moving rivers occur at the water temperature of 9-23^{0C} (Sharyati, 1993). The *Rutilus frisii kutum* is an order of Cypriniformes representative of cyprinid and Spawning strategy in this fish has a group synchronous and single spawning behavior. The principal spawning age groups are 2-5 years for males ad 3-6 years for females.

Several studies have been made in female teleosts to correlate the processes of ovarian follicular development and gametogenesis with seasonal fluctuations in plasma steroid levels Fostier et al., (1983); Kobayashi et al. (1989); Pankhurst and Conroy (1988); Rinchard et al. (1993); Rosenblum et al. (1987); Ramesh et al. (2009). Maturation of the egg is a long process that involves complex physiological and biochemical changes. Vitellogenesis is a process in which yolk proteins are produced in the liver, transport to the ovary and stored in the egg; resulting in tremendous egg enlargement. When conditions are appropriate for final maturation, nuclear development resumes, and the germinal vesicle migrates to one side. Finally, the walls of the germinal vesicle break down and maturity development completes. The association of changes in gonadal development with plasma levels of gonadal steroids has proven to be a valuable tool for understanding the endocrine control of reproduction in teleosts. Moreover, in teleosts, vitellogenesis and final oocyte maturation are regulated by gonadotropins via steroids secreted by the granolosa and theca cells of developing and mature oocytes. The occurrence of steroid production in different cells of the ovary may be related to different phases of oocyte development. Estradiol, (E_2) stimulates in

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Figure 1. Kutum *Rutilus frisii kutum* migrated to river Sefid- Rood of the southern Caspian Sea during spawning season.

turn the hepatic synthesis and secretion of vitellogenin which is accumulated in the oocytes. Correlations between changes of plasma levels of gonadal steroids and oocyte development have been well documented in a number of freshwater species including salmon forms (Whitehead et al., 1983; Truscott et al., 1986), cyprinids (Kobayashi et al., 1987), catfish *Heteropneustes fossilis* (Lamba et al., 1983), goldeye *Hiodon alosoides* (Pankhurst et al., 1986), walleye *Stizostedion vitrum* (Malison et al., 1994) and marine species including orange roughly *Hoplostethus atlanticus* (Pankhurst and Conroy, 1988; Putheti et al., 2008), Japanese whiting *Sillago japonica* (Matsuyama et al., 1990), Japanese sardine *Sardinops melanostictus* (Matsuyama et al., 1991) and common snook *Centropomus undecimalis* (Roberts et al., 1999). Environmental conditions and seasonal cues begin the process of maturation in many fish, this can take up to several years. When the gametes have matured, an environmental stimulus may signal the arrival of optimal conditions for the fry, triggering ovulation and spawning. Some environmental stimuli are changes in photoperiod, temperature, rainfall, and food availability. A variety of sensory receptors detect these cues, including the eye, pineal gland (an organ in the dorsal part of the forebrain that is sensitive to light), olfactory organs, taste buds, and thermo receptors. The objectives of this study are to investigate the relation between gonadal steroid levels, the hormonal profiles of (T) and (E_2) with sexual maturity stages in kutum *R. frisii kutum* during spawning season.

Material and methods

Experimental fish

To investigate gonadal development during natural spawning season, each Thursday morning at 10:00, 105 female kutum *Rutilus frisii kutum*, were collected from February to May in 2008, fish specimens were catched by variious tools of catching (Gillnet, Cast net, Seine net and Sheyl or Kulham) with a mesh size length 22mm. The period of fish collection lasted for a full calendar year and water temperature was recorded whenever fish were collected. Scales were collected from the specimen in order to determine their age (Chungunova, 1959). Scales were measured to aging and total length and forke length measured the nearest 0.1cm and weighed (W) to the nearest 0.1 g. The ovaries were dissected out and weighed, the condition factor (CF) was determined using the following formula (Bagenal, 1978)

$$CF=W/L^{b}\times 100$$

Where W=total fish weight (g); L=fish standard length (cm) and b=slop of length-weight relationship.

Gonadosomatic index (GSI) was determined using the following formula (Roff, 1983).

GSI = gonad weight_100/body weight) for each fish analyzed throughout the sampling period was calculated and recorded.

Steroid assay and histological analysis

Fish were anaesthetized with clove oil (*Syzyglum aromaticum*) (75-115 ppm) and blood samples were taken from the caudal vessels by using heparinized disposable syringes. Sample was centrifuged for 10 min at 3000 rpm and the plasma was stored at -45 ^{0}C until steroid analysis. Plasma levels of (E_2) and (T) were measured by radioimmunoassay using the procedure described by (Rinchard et al., 1993).

Ovaries were fixed in Bouin’s solution, embedded in paraffin after dehydration-infiltration, sectioned at 5 µm and stained with Mayer’s hematoxylin and eosin for histological examination under binocular microscope. The developmental stage and the diameter of the 20 largest oocytes were recorded. Each gonad was classified according to the most advanced type of oocyte present (Table 1).

Table 1. Maturity stages of the ovary of kutum.

Ovarian stage	Oocyte stages present in the ovary	Description of the most advanced Oocytes
(I) Previtellogenic	Previtellogenic oocytes	Oocytes with vacuole-free cytoplasm
(II) Onset of endogenous vitellogenesis	Previtellogenic oocytes and oocytes in endo-genous vitellogenesis	Oocytes at primary yolk vesicle stage, glycoproteins appear and occupy 2 or 3 rings in the cytoplasm periphery (early endogenous vitellogenesis)
(III) Completion of endo-genous vitellogenesis	Previtellogenic oocytes and oocytes having complete endogenous vitellogenesis	Oocytes are full of glycoprotein inclusions. Follicular and cellular layers are differentiated (late endogenous vitellogenesis)
(IV) Exogenous vitellogenesis	Previtellogenic oocytes and oocytes at different stages of exogenous vitellogenesis	Oocytes accumulate yolk globules and yolk vesicles are in periphery of the cytoplasm
(V) Final maturation	Previtellogenic oocytes and oocytes in final maturation	Appearance of the micropyle and migration of the germinal vesicle to the micropyle
(VI) Post-spawning	Previtellogenic oocytes and pre- and post-avoulatory follicles	The follicle cells in the pre- and postovulatory follicles show hypertrophy, the yolk substance degenerates

Since this was a field study in river condition and during catching process, which may not be controlled as in the laboratory, a degree of stress may have been encountered in fish individuals but the significance of the variation is not as great as in controlled laboratory conditions (Cornish et al., 1993). Therefore in this study fish were anaesthetized with clove oil (*Syzyglum aromaticum*) (75-115 ppm)

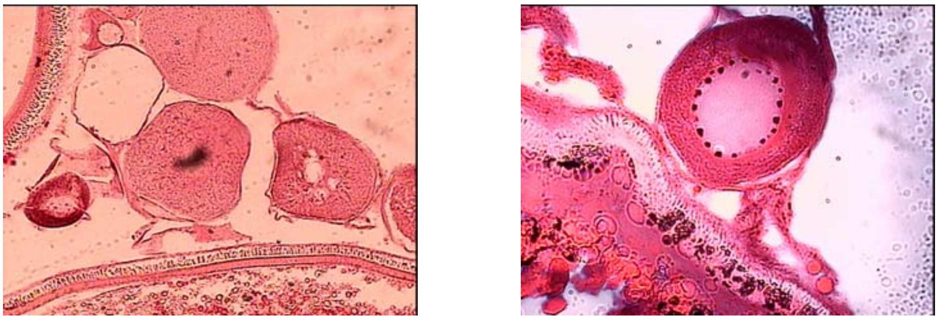
Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) in SPSS software (Ver. 11.0.)

Results

Results clearly show that, the following processes occurred in the ovaries of kutum females in the spawning seasonal migration from March to April 2008: in early February, the gonads of various individuals were at maturity stage IV. The cross section of different fish ovarian maturity stages are showed in figure 2-4 (H&E, X 40). The Table 1 Shows relationship between standard length and body weight for all individuals and shows positive allometric growth of kutum, *R. frisii kutum*. Figure 5 shows the relation between water temperature (^{0C}) with day length (hr) and gonadosomatic index (GSI). Relationship between values of monthly condition factor (CF) and gonadosomatic index (GSI) are presented in Figure 6. Monthly changes in the maturity stage (most advanced oocyte stage in the ovary) of kutum in Sefid- Rood river southern Caspian Sea was shown in Figure 7. In this study relation between Monthly condition factor (CF) and gonadosomatic index (GSI) with the maturity stage and monthly concentration levels of (E_2) with gonadosomatic index (GSI) of kutum in Sefid- Rood river southern Caspian Sea was shown in figure 8 and figure 9 respectively. Combination of monthly concentration of (E_2) and (T) related in (Figure 10). Circulatory plasma (E_2) and (T) concentration (ng/ml) values for the entire experimental period and female plasma estradiol levels were low from February but afterward they increased significantly to March coinciding with the preponderance of vitellogenic follicles in the ovary. In the case of female estradiol, the highest concentrations are seen during March and early April. Therefore in the female kutum, estradiol reaches a maximum in March (Figure 9, 10). Then plasma (E2) and (T) levels exhibit a sharp decline in early May when oocyte maturation takes place.

During this period, the females had an increased GSI and the GSI continued to increase further and reach high values in April (Figure 6, 8). The histological pictures show the clear synchronicity of oocyte maturation.



A B

Figure 2. Histological picture, cross section of fish ovarian used in the study in 2008: A- maturity stage (Oogony) II (H&E, X 20). B- Maturity stage (Oogony) III in female kutum. (H&E, X 40).

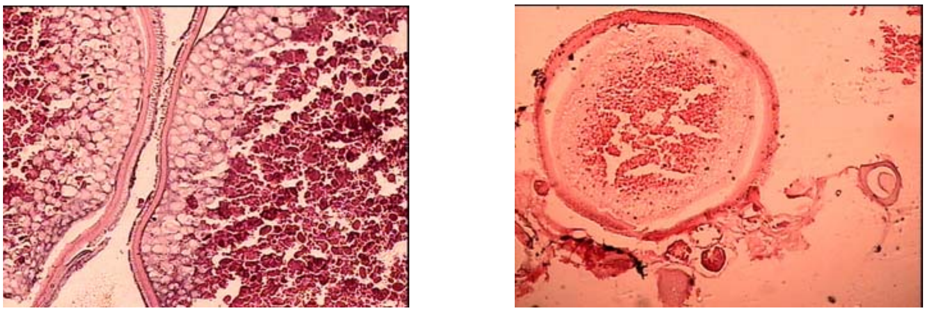


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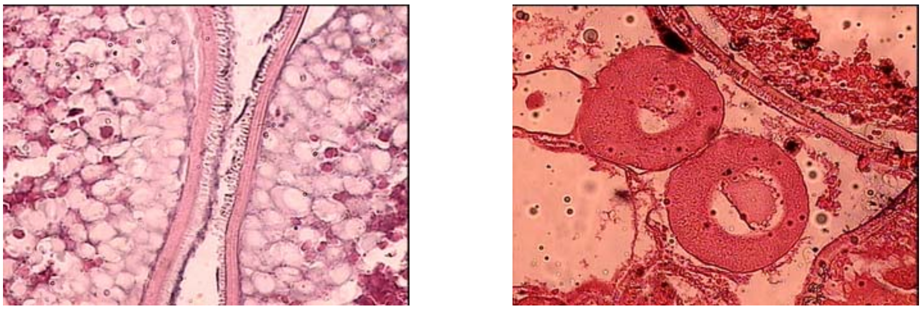
Figure 3. Histological picture, cross section of fish ovarian used in the study in 2008: A&B- maturity stage (Oogony) IV-V (H&E, X 40).



A B

Figure 4. Histological picture, cross section of fish ovarian used in the study in 2008: A maturity stage (Oogony) IV; B- maturity stage(Oogony) III in female kutum with total length 423mm, 1784gr weight and aging 5.(H&E,X 40).

Table 1. Relationship between standard length and body weight for all individuals which is described by equation: W= 0.0096×SL $^{2.03735}$ (r^2= 0.96, n= 105), there is a positive allometric growth for the kutum specimen. MOSLS, mean observed standard length and standard deviation; MOWS, mean observed weight and standard deviation. A significantly different was MOSL and age (P= .027).

Age	MOSLS (mm)	MOTWS (gr)	Number
3	304 ± 6.12	549 ± 63	25
4	337 ± 10.24	896 ± 94	50
5	378 ± 13.58	1219 ± 85	18
6	441 ± 19.85	1593 ± 114	12

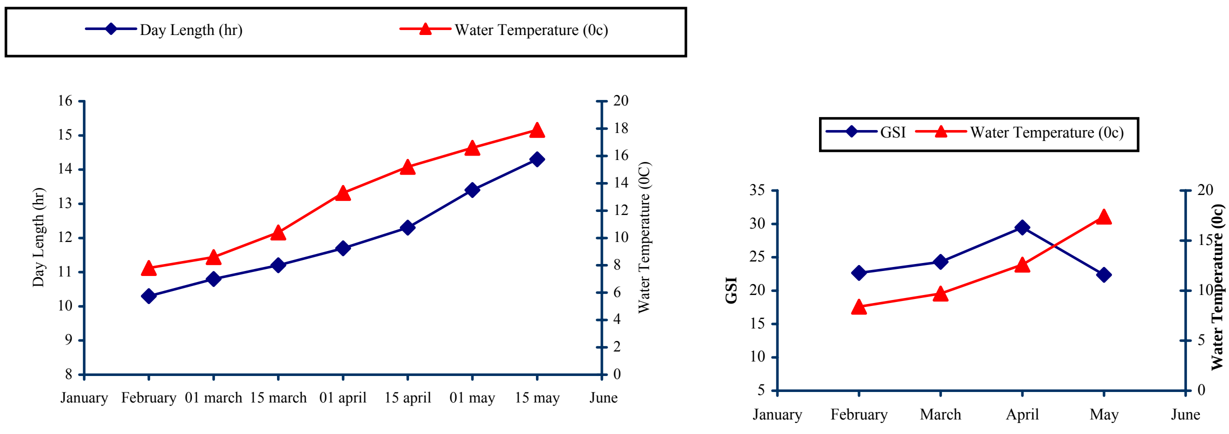


Figure 5. Relation between water temperature (0C) with gonadosomatic index (GSI) and day length (hr) in southern of Caspian Sea of River Sefid-Rood.

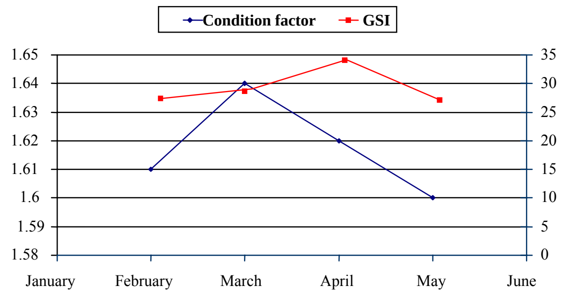


Figure 6. Monthly condition factor (CF) and gonadosomatic index (GSI) of kutum in River Sefid- Rood southern Caspian Sea.

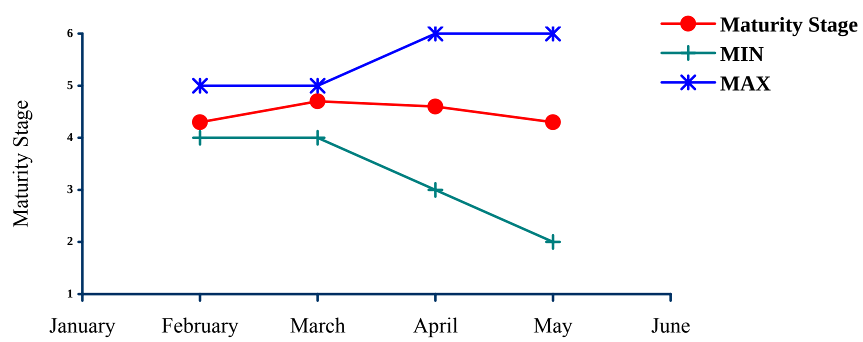


Figure 7. Monthly changes in the maturity stage (most advanced oocyte stage in the ovary) of 105 individuals kutum in River Sefid-Rood southern Caspian Sea.

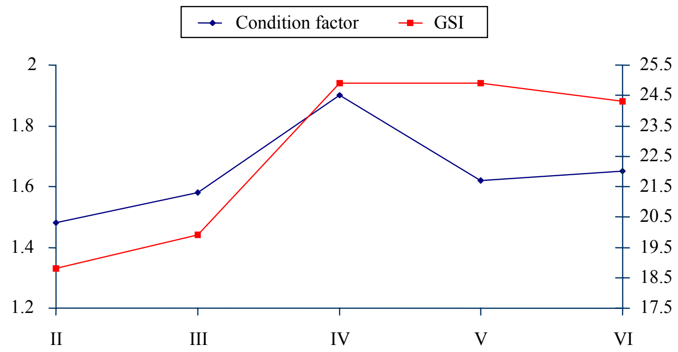


Figure 8. Relationship with Condition factor index (CF) and gonadosomatic index (GSI) with sexual maturity of 105 individuals kutum in River Sefid-Rood southern Caspian Sea. (n=105).

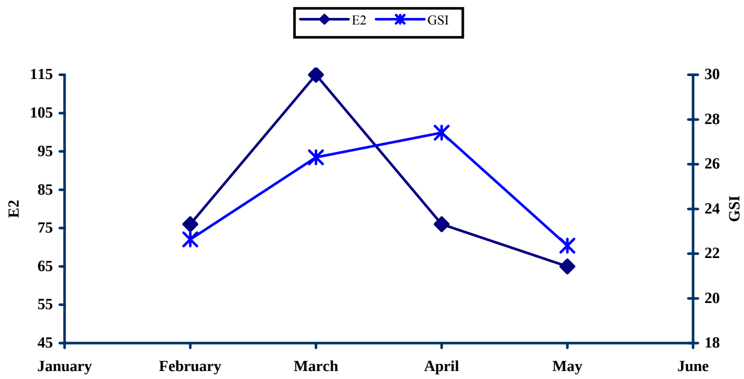


Figure 9. Monthly concentration of (E_2) and gonadosomatic index (GSI) of 105 individuals kutum in River Sefid-Rood southern Caspian Sea. (n=105).

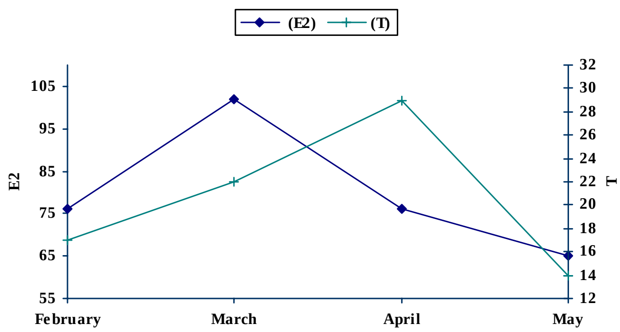


Figure 10. Monthly concentration of (E_2) and (T) of 105 individuals kutum in River Sefid-Rood southern Caspian Sea. (n=105).

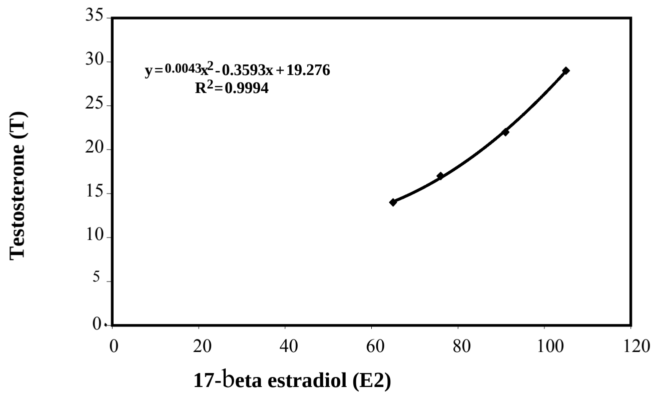


Figure 11. Relationship between levels of (T) and (E2) concentration in plasma which occurs at the same time in kutum of the River Sefid- Rood southern Caspian Sea. (n=105).

Discussion

Khalko and Talikina 1993 described that in the ovaries of bream females *Abramis brama* in the Rybinsk reservoir from autumn to spring during winter months, trophoplasmic growth of eggs proceeds with a corresponding enlargement of yolk globules. Yolk deposition comes to an end, and oocytes become functionally mature in late March to early April. Generally, (E_2) is responsible for stimulating vitellogenesis and also released by female gonads during the pre-spawning period. According to the results Condition factor index (CF) and gonadosomatic index (GSI) related with sexual maturity stages and Monthly concentration of sexual steroids hormones demonstrated in (figure 8 and 10) reflects the importance of this hormone. Over the period from February to April a gradual increase in plasma levels was observed a bimodal increase from both the gonads and the inter-renal tissues. Estradiol is known to be secreted by the cells of the ovarian follicles that promote the development and maintenance of the female sexual characteristics. In humans this hormone (together with some other hormones) is responsible for controlling the female sexual cycle. Also (E_2) has been reported to stimulate vitellogenesis in teleosts changed the plasma levels of sex steroid hormones during gonadal maturation (Silversand et al., 1993); (Smith and Haley, 1988). These authors reported an increase in plasma (E2) levels once spawning commences and remains high throughout the period of oocyte growth. Sen et al. 2002 reported that concentration of plasma testosterone (T) in Indian major carp *Labeo labeo rohita* is expected to be high when it is no longer needed for aromatization, while (T) levels during postvitellogenic stage exhibited a quick decline in this fish, coinciding with the fall of plasma (E_2) concentration. A sudden drop in the plasma (E_2) level in *Labeo rohita* from vitellogenic to postvitellogenic stage may be explained in terms of switching off the aromatise (CYP19) activity as the oocytes progressed to maturation. Almost a similar profile of E_2 has been reported during the transition from vitellogenic to maturational stage in rainbow trout (Fostier et al., 1983). This drop in circulatory (E_2) levels probably reduces the intensity of sex steroid feedback, allowing the occurrence of hypothalamus-mediated gonadotropnes surge, which is required for the development of oocyte maturational competence. Rinchard et al. 1993 mentioned that in other teleosts such as gudgeon, *Gobio gobio*, there was no decrease of E_2 level during oocyte maturation; meanwhile this study has shown decreased E_2 in some specimens of kutum. (Rosenblum et al. 1987) observed a good correlation between circulating (E_2) and calcium levels in female teleosts. Increases in plasma (E_2) in female Tilapia *Oreochromis mossambicus* paralleled increases in both GSI and calcium levels (Cornish, 1993), thereby confirming a role for estradiol in vitelogenesis in present study for Kutum *R. frisii kutum*, is in agreement that correspond with those for most teleosts fish and vertebrates. The slight increase of testosterone (T) levels during oocyte development may be related to its role as precursor of (E_2) synthesis. At high concentration, (T) might also be involved in hepatic vitellogenin synthesis (Rinchard et al., 1993). The sudden peak was measured when most fish were in final maturation (stage V), an effect of the release of testosterone (T) into the plasma when this was no longer needed for aromatization. This acute rise in testosterone indicates that oocytes are fully mature and ready to ovulate (Kobayashi et al., 1989). Although the same relationship was established between oocyte stages and testosterone levels in fish in river Sefid- Rood during spawning season. The present study shows that an increase in the level of (T) in the plasma could be associated with correlation between levels of (T) and (E2) concentration in Kutum of the River Sefid- Rood, southern Caspian Sea (Figure 1). There is also an increase in day length during this period, which has been shown to be an environmental cue to a preovulatory surge in hormonal secretion in cyprinids (Aida, 1988).

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