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Full Length Research Paper

Study on sexual maturity and levels of gonad steroid hormones in female kutum *Rutilus frisii kutum* (Kamenskii, 1901) during spawning season from river Sefid-Rood of the southern Caspian sea

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Sexual maturity and levels of two main steroid hormones in gonads, 17-β estradiol (E_2) and testosterone (T) relations which occur in female kutum *Rutilus frisii kutum* during spawning season from the river Sefid-Rood of the southern Caspian sea were studied using histological and radioimmunoassay. The study was carried out from February to May, 2008 using 105 fish specimen. The results of present study revealed that changes in plasma levels of gonadal steroids, (E_2) and (T) were closely correlated to ovarian development and increased in GSI ($p < 0.05$). Gonadosomatic index (GSI) was increased in March and reached the highest value (29.47 ± 4.2) in April then decreased sharply in early May. The highest peak of plasma level of (T) and (E_2) showed during spawning season, associated with the highest values of GSI. The results showed that levels of (E_2) and (T) in female kutum at the stage IV of sexual maturity was significantly highest compared to immature gonads (ovary in stages II and III) ($p < 0.01$). Plasma estradiol (E_2) and (T) levels increased in February, highest levels was observed in March and early April (105.6 ± 75.3 and 29.2 ± 96.6 ng/ml, respectively), and decreased in late April and in early May during the spawning season ($p < 0.05$).

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

INTRODUCTION

Caspian kutum, *Rutilus frisii kutum* (Kamenskii, 1901) populations generally recorded along near the coast, from the Trek river the north to the southern part more than 70% of fishermen catch in Iran coastal of the Caspian sea (Sharyati, 1993). This species is an endemic fish of Caspian sea and in natural environment; the fish spawn in groups in slow moving rivers at a temperature of 9-23°C (Sharyati, 1993). It has a group synchronous, single spawning behavior. Males normally mature between their third and fourth year, sometime earlier female mature during their fourth year. However, recently most spawners males and females maturating at age 3 and 4 years, respectively (Photo 1)

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, transported 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, re-

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Photo I. Kutum *Rutilus frisii Kutum* migrated to river sefid-Rood of the southern caspian sea during spawning season.

leasing the chromosomes into the cell. 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. Of these steroids, 17-β estradiol (E_2) stimulates in turn the hepatic synthesis and secretion of vitellogenin which is accumulated in the oocytes. Correlations between changes in plasma levels of gonad 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). Fish have evolved to reproduce under environmental conditions that are favorable to the survival of the young. Long before spawning, seasonal cues begin the process of maturation. In many fish, this can take up to a year. When the gametes have matured, an environmental stimulus may signal the arrival of optimal conditions for the fry, triggering ovulation and spawning. Examples of 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 aim of this work was to investigate the physiological role of gonadal steroids, the hormonal profiles of testosterone (T) and 17-β estradiol (E_2) and sexual maturity of 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 *R. frisii kutum* fish were collected from February to May in 2008, using a gill and seine net with a mesh size length 22 mm. 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 0.1 cm 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. After centrifugation, the plasma was stored at -45°C until steroid analysis. Plasma levels of 17-β estradiol (E_2) and testosterone (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 and 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).

Due to this being a field study, which may not be controlled as in the laboratory, a degree of stress may have been encountered. The significance of the variation is not as great as in controlled laboratory conditions (Cornish et al., 1993).

Statistical calculation

Data were statistically analyzed by analysis of variance (ANOVA) using the General Linear Models procedure coupled with Duncan's multiple range test in SPSS software (Ver. 11.0.) Significant F values were observed at $p \leq 0.05$ level. Correlation coefficients were calculated using the Pearson correlation procedure.

RESULTS

The cross section of different fish ovarian maturity stages were showed in Photo 1-3 (H&E, ×40). Figure 1 shows that relationship between standard length and body

Table 1. Maturity stages of the ovary of kutum.

Ovarian stage	Oocyte stages present in the ovary	Description of the most advanced Oocytes
Previtellogenic	Previtellogenic oocytes	Oocytes with vacuole-free cytoplasm.
Onset of endogenous vitellogenesis	Previtellogenic oocytes and oocytes in endogenous vitellogenesis.	Oocytes at primary yolk vesicle stage, glycoproteins appear and occupy 2 or 3 rings in the cytoplasm periphery (early endogenous vitellogenesis).
Complete of endogenous vitellogenesis	Previtellogenic oocytes and oocytes having complete endogenous Vitellogenesis.	Oocytes are full of glycoprotein inclusions. Follicular and cellular layers are differentiated (late endogenous vitellogenesis).
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.
Final maturation	Previtellogenic oocytes and oocytes in final Maturation.	Appearance of the micropyle and migration of the germinal vesicle to the micropyle.
Post-spawning	Previtellogenic oocytes and pre- and post-avoulatory follicles.	The follicle cells in the pre- and post-ovulatory follicles show hypertrophy, the yolk substance degenerates.

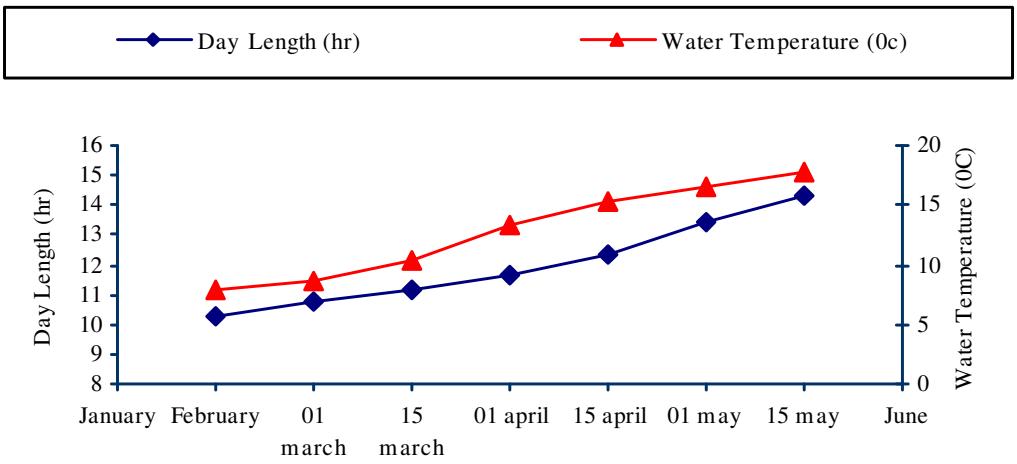

Figure 1. Show that relationship between standard length and body weight for all individuals is described by equation: $W = 0.0096 \times SL^{2.03735}$ ($r^2 = 0.96$, n= 105) and shows positive allometric growth for the kutum specimen

weight for all individuals and shows positive allometric growth of kutum, *R. frisii kutum*. Figures 2 and 3 shows the relation between water temperature (°C) with day length (h) and gonadosomatic index (GSI). Relationship between the values of monthly condition factor (CF) and gonadosomatic index (GSI) shows in Figures 4 and 5 represents monthly changes in the maturity stage (most advanced oocyte stage in the ovary) of kutum in Sefid-Rood river southern Caspian sea. In this study related Monthly condition factor (CF) and gonadosomatic index (GSI) of four group age kutum (Figures 6 and 7 represents monthly concentration of 17-β estradiol (E_2) and gonadosomatic index (GSI) in Sefid-Rood river southern Caspian sea. Monthly concentration of 17-β estradiol (E_2) and Testosterone (T) related in Figure 8. Plasma contained 17 β estradiol (E_2) and testosterone (T) concentration (ng/ml) value for the entire experiment period. Female plasma estradiol levels were low from February

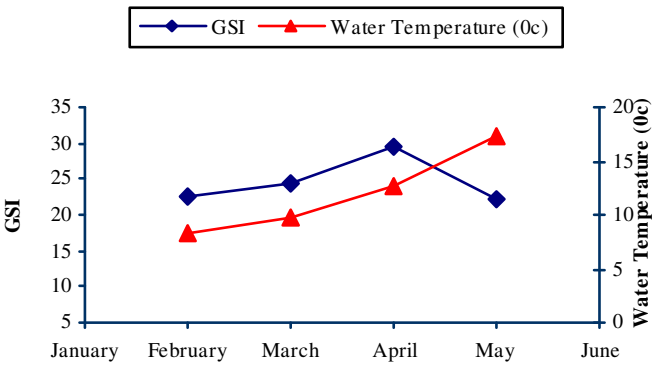

Figure 2. Relation between water temperature (°C) and day length (hr) in southern of Caspian Sea of River Sefid-rood.

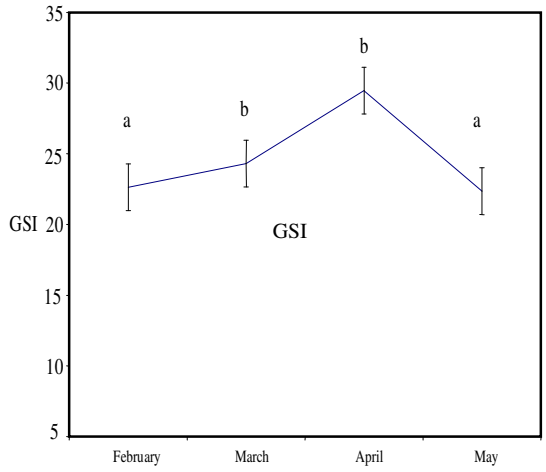

Figure 3. Changes in gonadosomatic index (GSI) and water temperature and spawning season relations with (GSI) of kutum in River Sefid-Rood southern Caspian Sea.

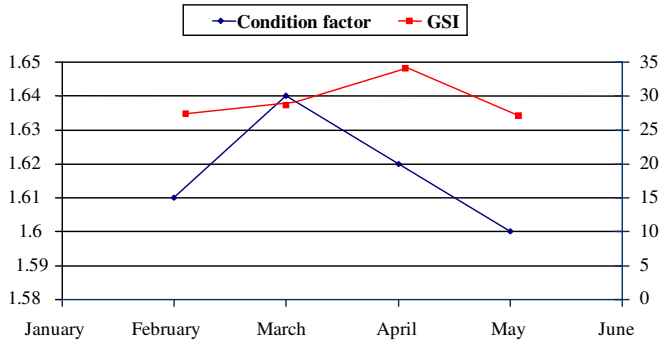

Figure 4. Monthly condition factor (CF) and gonadosomatic index (GSI) of kutum in River Sefid-Rood southern Caspian sea.

where after they increased significantly to March. In the case of female estradiol, the highest concentrations are

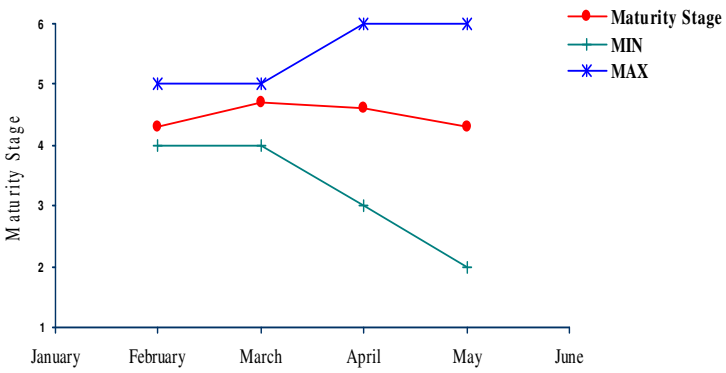

Figure 5. Monthly changes in the maturity stage (most advanced oocyte stage in the ovary) of kutum in river Sefid-Rood southern Caspian sea.

seen during March and April. Therefore in the female kutum, estradiol reaches a high concentration during March and April. It is during this period that female GSI levels reach a maximum. The histological pictures (Photos 2, 3 and 4 A and B respectively show the clear synchronicity of oocyte maturation.

DISCUSSION

Results clearly show that, the following processes occur in the ovaries of kutum females in the spawning seasonal migration from March to April 2008. In February 2008, the gonads of various individuals are at maturity stage IV. During February and early March, circulatory17 β estradiol ($E_{2)}$ and testosterone (T) levels were very low. Concentrations of these two steroids in plasma began to rise from March, and reached their highest values in the month of April, coinciding with the preponderance of vitellogenic follicles in the ovary. During this period, the females had an increased GSI. Although the GSI continued to increase further and reached high values in April (Figures 4 and 7), plasma (E_2), and (T) levels exhibited a sharp decline in the month of early May when oocyte maturation takes place.

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. 17 β estradiol (E_2) is secreted by both the female gonads and inter-renal tissues. In general, (E_2) is responsible for stimulating vitellogenesis and is also secreted by female gonads during the pre-spawning period. Evaluation of the results between relationship with condition factor index (CF) and gonadosomatic index (GSI) with sexual maturity and Monthly concentration of 17-β estradiol (E_2) and Testosterone (T) in Figures 6 and 8 reflects the importance of

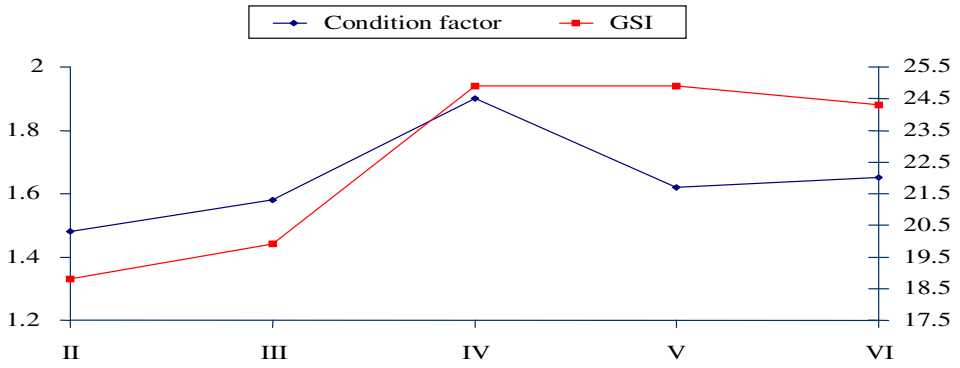

Figure 6. Relationship with Condition factor index (CF) and gonadosomatic index (GSI) with sexual maturity of kutum in river Sefid-Rood southern Caspian sea.

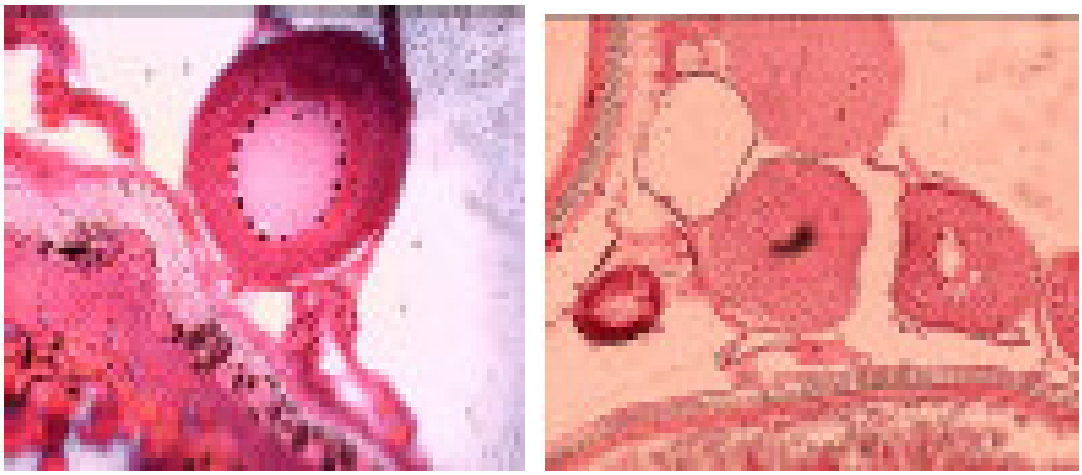

Photo 2. Histological picture, cross section of fish ovarian used in the study in 2008: A- maturity stage (Oogony) III (H & E, ×20). B- Maturity stage (Oogony) III in female kutum. (H & E, ×40).

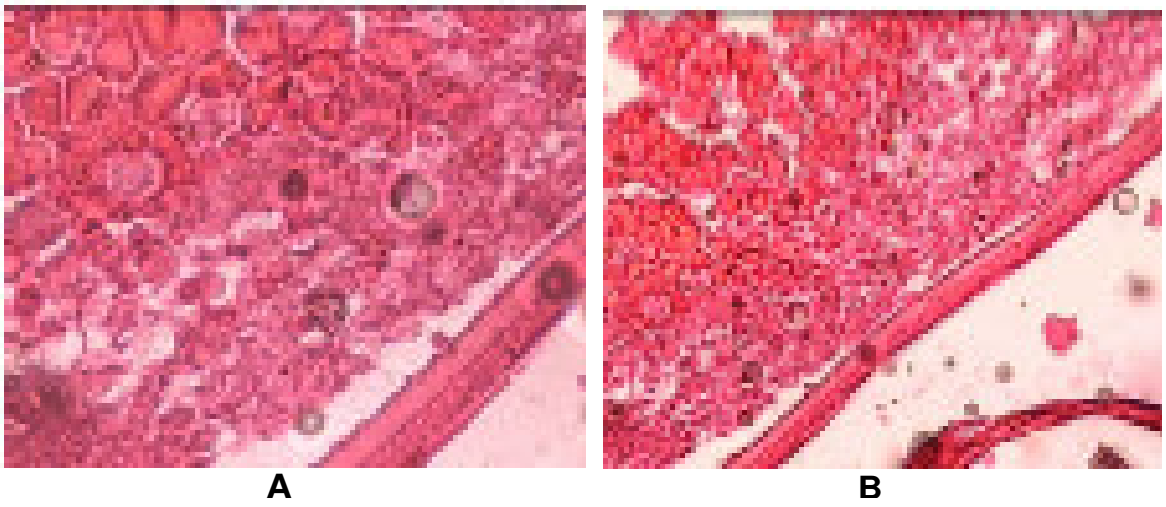

Photo 3. Histological picture, cross section of fish ovarian used in the study in 2008: A&B- maturity stage (Oogony) IV-V (H & E, ×40).

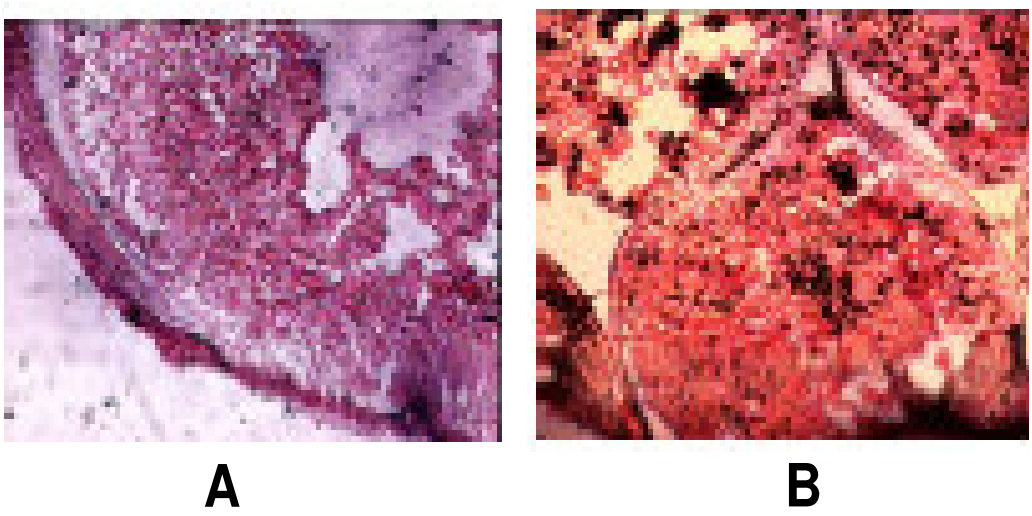

Photo 4:Histological picture, cross section of fish ovarian used in the study in 2008.A maturity stage (Oogony) V-VI in female kutum with total lenght 423 mm,1784 g weight and aging.5 (H & E, ×40).

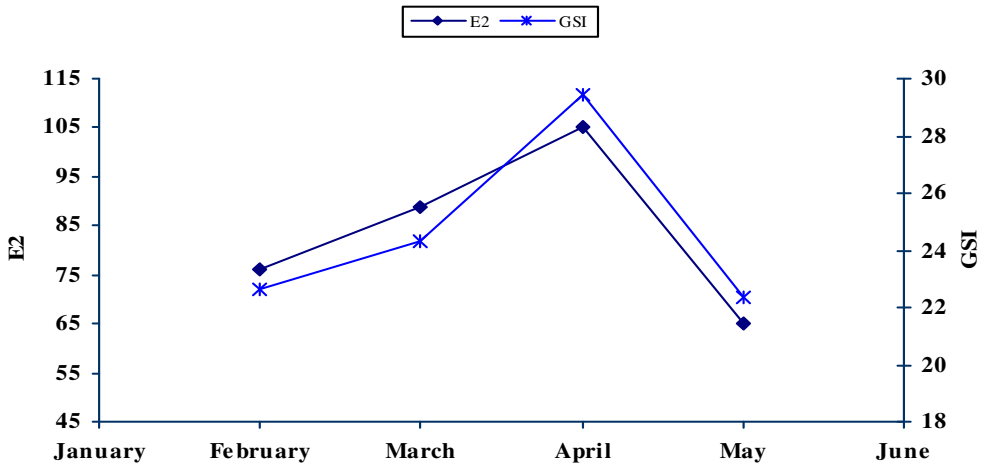

Figure 7. Monthly concentration of 17-β estradiol (E_2) and gonadosomatic index (GSI) of kutum in river Sefid-Rood southern Caspian sea.

this hormone. The latter observation suggests that most females were in the immediate post spawning period prior to gonadal recrudescence at this time. 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. 17 β estradiol (E_2) 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 other hormones) is responsible for controlling the female sexual cycle. Also 17 β estradiol (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 17 β estradiol (E2) levels once spawning commences and that it remains high throughout the period of oocyte growth. Sen et al. (2002) reported that concentration of plasma testosterone (T) in Indian major carp *Labeo rohita* is expected to be high when it is no longer needed for aromatization, while, actually (T) levels during post-vitellogenic stage exhibited a quick decline in this fish, coinciding with the fall of plasma17 β estradiol (E_2) concentration. A sudden drop in the level of plasma (E_2) in *Labeo. rohita* from vitellogenic to post-vitellogenic stage may be explained in terms of switching off the aromatize (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

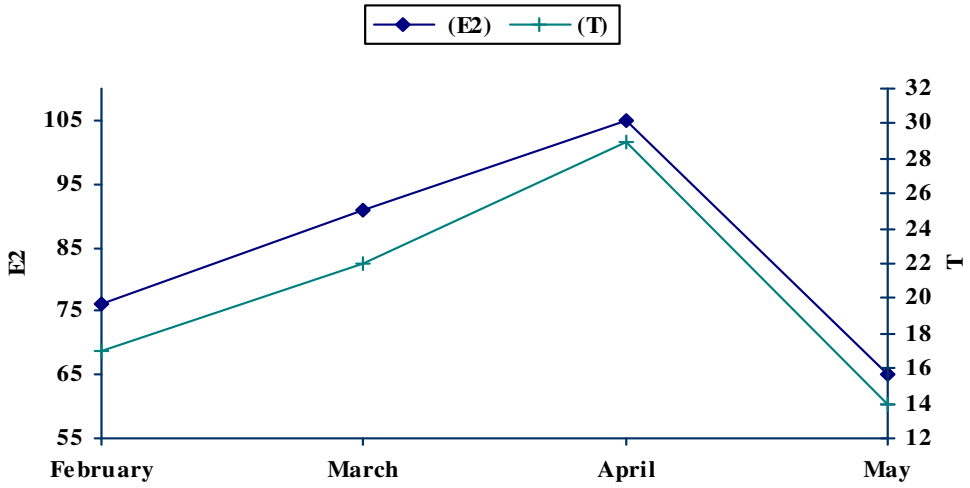

Figure 8. Monthly concentration of 17-β estradiol (E_2) and testosterone (T) of kutum in river Sefid-Rood southern Caspian sea.

the occurrence of hypothalamus-mediated GtH surge, which is required for the development of oocyte maturational competence (OMC). In this context, 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 17 β estradiol (E_2) and calcium levels in female teleosts. Increases in plasma 17 β estradiol (E_2) in female Tilapia *Oreochromis mossambicus* paralleled increases in both GSI and calcium levels Cornish, (1993), thereby confirming a role for estradiol in vitellogenesis. In present results for kutum *R. frisii kutum*, showed that correspond with those for most teleosts fish and vertebrates, testosterone has been reported in the blood of a number of female teleosts. The slight increase of testosterone (T) levels during oocyte development can be related to its role as precursor of 17 β estradiol (E_2) synthesis, as a precursor of (E_2) production, (T) is available in the ovary for aromatization. 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 work shows that there is an increase in the level of testosterone (T) in the plasma which could be associated with the increase in the River sefid- Rood water temperature which occurs at the same time (March–April, Figure 9). There is also an increase in day length during this period, which has been shown to be an environmental cue to a pre-ovulatory surge in hormonal secretion in cyprinids (Aida, 1988).

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