

## Study on Levels of 17-Beta Estradiol and Testosterone in Female Kutum *Rutilus frisii kutum* (Kamenskii, 1901) of Southern Caspian Sea

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**Abstract:** Caspian Kutum *Rutilus frisii kutum* is an endemic fish of Caspian Sea and one of the most economical species in natural environment. Levels of two main gonad steroid hormones, 17-beta Estradiol ( $E_2$ ) and Testosterone (T) in female kutum during spawning season from the River sefid- Rood of the southern Caspian Sea were studied by using radioimmunoassay (RIA). Throughout the experimental period from February to May 2008, 105 female fish specimens were used. Aspects of the reproductive physiology of kutum that were investigated included reproductive hormone profiles in serum supernatant. 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 ( $29.47\pm4.2$ ) in April then decreased sharply in early May. The highest peak of serum level of T and  $E_2$  during spawning season was 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) ( $P<0.01$ ). Serum  $E_2$  and T levels increased in February, highest levels was observed in March and early April ( $105.6\pm75.3$  and  $29.2\pm96.6$  ng/ml, respectively) and decreased in late April and in early May during the spawning season ( $P<0.05$ ). The results imply a close interaction between environmental cues and endocrine control of reproduction.

**Key words:** kutum • *Rutilus frisii kutum* • 17-Beta Estradiol • Testosterone • Caspian Sea

### INTRODUCTION

The Caspian Sea is the largest inland water bodies with no connection to others sea and oceans. Caspian kutum, *Rutilus frisii kutum* 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 [1]. The fish spawn in groups in slow moving rivers at a temperature of 9-23°C [1]. It has a group synchronous, single spawning behavior. Males normally mature between their third and fourth year, sometime earlier females mature during their fourth year. However, recently most spawners males and females maturing at age 3 and 4 years, respectively.

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 [2-6]. 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, releasing 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 granulosa and theca cells of developing and mature oocyte. The occurrence of steroid production in different cells of the ovary may be related to different phases of oocyte development. Of these steroids,  $E_2$  stimulates in turn the hepatic synthesis and secretion of vitellogenin which is accumulated in the oocyte. 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 [7, 8], cyprinids [9], catfish *Heteropneustes fossilis* [10], goldeye *Hiodon alosoides* [11], walleye *Stizostedion vitreum* [12] and marine species including orange roughy *Hoplostethus atlanticus* [4], Japanese whiting *Sillago japonica* [13] Japanese sardine *Sardinops melanostictus* [14] and common snook *Centropomus undecimalis* [15]. 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 T and  $E_2$  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 *Rutilus frisii kutum* fish were collected from February to May in 2008, using a gill and seine net 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 [16]. Scales were measured to aging and total length and fork length measured the nearest 0.1 cm and weighed (W) to the nearest 0.1 g. The ovaries were dissected out and weighed [17, 18]. Gonadosomatic index (GSI) was determined using the following formula [19].



Fig. 1: Kutum brood stock, *Rutilus frisii kutum* of the southern Caspian Sea

GSI= gonad weight/body weight $\times 100$ ) for each fish analyzed throughout the sampling period was calculated and recorded.

## Steroid Assay and Histological Microstructure Analysis:

Fish were anaesthetized with clove oil (*Syzygium 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 -80 °C until steroid analysis. Plasma levels of  $E_2$  and T were measured by radioimmunoassay (RIA) using the procedure described by Rinchard *et al.* [5]. Ovaries were fixed in Bouin's solution, embedded in paraffin after dehydration and infiltration, sectioned at 5  $\mu$ m and stained with Mayer's hematoxylin and eosin for histological examination under binocular microscope. The developmental stage and the diameter of the 20 largest oocyte were recorded. Each gonad was classified according to the most advanced type of oocyte [20].

**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 ( $P < 0.05$ ) level. Correlation coefficients were calculated using the Pearson correlation procedure.

## RESULTS

Relationship between standard length and body weight for all individuals and shows positive allometric growth of kutum, *R. frisii kutum* in table 1. Results show the relation between water temperatures ( $^{\circ}$ C) with day length (h). Figs (3-5) show Monthly changes in 17-beta Estradiol and Testosterone concentration in Kutum from southern of Caspian Sea respectively. Estradiol fluctuation during gonad development stages signed in (4-6). Figure (7) Monthly changes in maturity stage

Table 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.

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

MOSLS, mean observed standard length and standard deviation; MOWS, mean observed weight and standard deviation. A significantly different was MOSL and age ( $P = 0.027$ ).

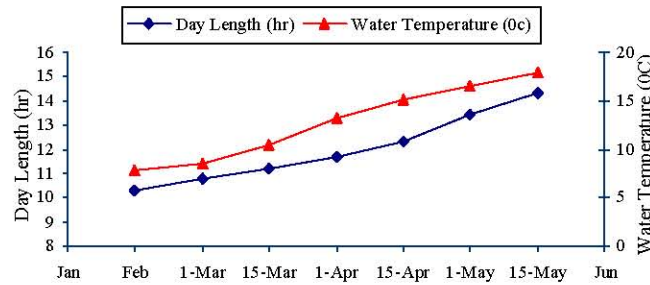


Fig. 2: Relation between water temperature ( $^{\circ}\text{C}$ ) and day length ( $^{\text{hr}}$ ) in southern of Caspian Sea.

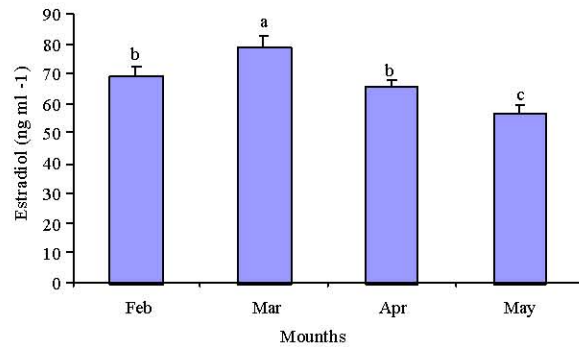


Fig. 3: Monthly changes in Estradiol concentration in Kutum from southern of Caspian Sea.

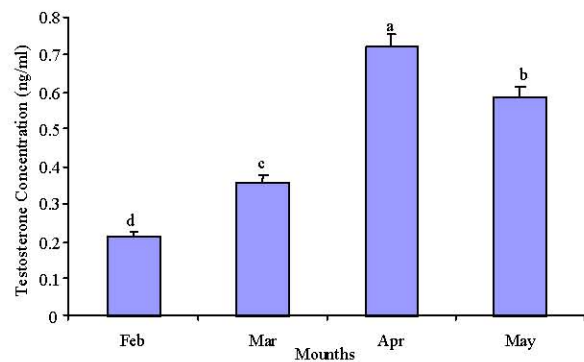


Fig. 5: Monthly changes in Testosterone concentration in Kutum from southern of Caspian Sea.

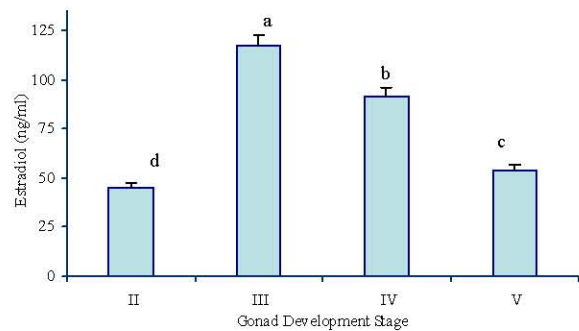


Fig. 4: Estradiol fluctuation during gonad development stages in Kutum from southern of Caspian Sea

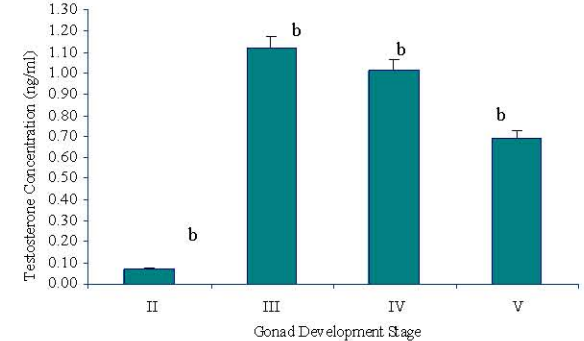


Fig. 6: Monthly changes in Testosterone concentration in Kutum from southern of Caspian Sea.

(most advanced oocyte stage in the ovary) of kutum during study period. Fig. 8 show Monthly changes in gonadosomatic index (GSI) in kutum during sampling period. Fig. 9 represents monthly changes in the maturity

stage (most advanced oocyte stage in the ovary) of kutum in sefid- Rood river southern Caspian Sea. Plasma contained estradiol  $E_2$  and Testosterone T concentration (ng/ml) value for the entire experiment period.



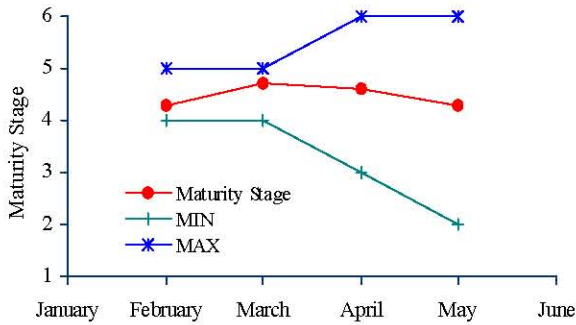


Fig. 7: Monthly changes in maturity stage (most advanced oocyte stage in the ovary) of kutum in southern Caspian Sea.

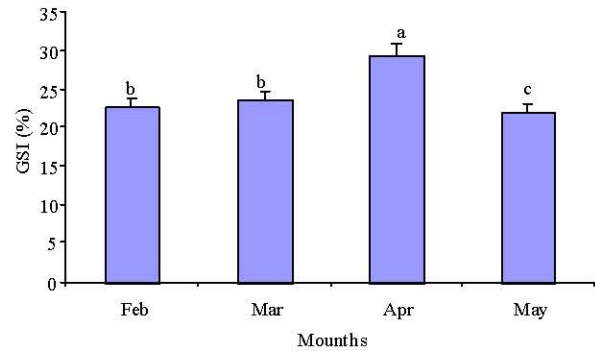
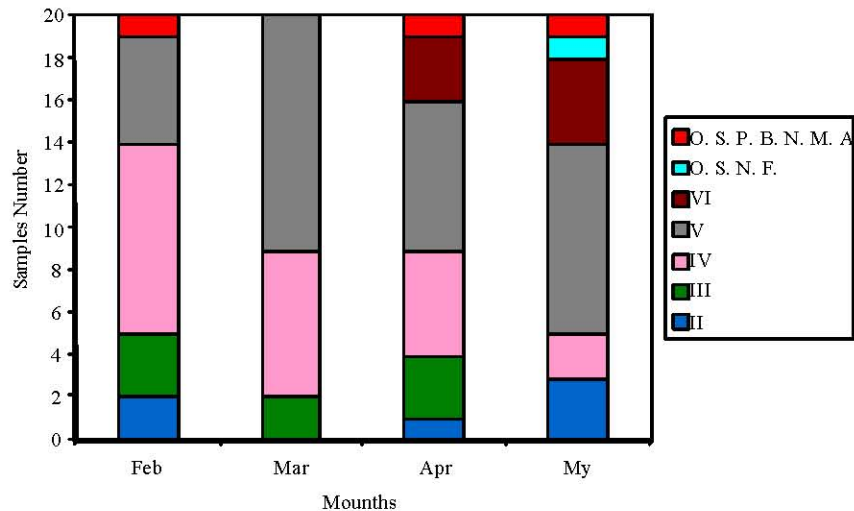


Fig. 8: Monthly changes in gonadosomatic index (GSI) of kutum in southern Caspian Sea.



O.S.P.B.N.M.A: oocyte stage present but not most advanced and O.S.N.F: oocyte stage not found.

Fig. 9: Monthly changes in the maturity stages most advanced oocyte stage in the ovary) of kutum in southern Caspian Sea.

Female plasma estradiol levels were low from February where after they increased significantly to March. In the case of female estradiol, the highest concentrations are 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 show the clear synchronicity of oocyte maturation.

## DISCUSSION

Results clearly showed 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 different maturity stages but dominantly in IV. During February and early March, T levels were very low. Concentration of

this steroid in plasma began to rise from March and reached their highest value in the month of April, coinciding with the preponderance of vitellogenic follicles in the ovary. During this period, the females had an increased GSI (21). Although the GSI continued to increase further and reached high values in April (Fig. 8). Plasma  $E_2$  and T levels exhibited a sharp decline in the month of early May when oocyte maturation takes place.

Khalko and Talikina [22] 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 oocyte become functionally mature in late March to early April. 17-beta Estradiol 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 [23]. Evaluation of the results between Relationship with sexual maturity and Monthly concentration of E<sub>2</sub> and T in Fig. (4-6) reflects the importance of 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. 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 other hormones) is responsible for controlling the female sexual cycle. Also E<sub>2</sub> has been reported to stimulate vitellogenesis in teleosts changed the plasma levels of sex steroid hormones during gonadal maturation [24, 25]. These authors reported an increase in plasma Estradiol E<sub>2</sub> levels once spawning commences and that it remains high throughout the period of oocyte growth. Sen *et al.* [26] reported that concentration of plasma T in Indian major carp *Labeo labeo rohita* is expected to be high when it is no longer needed for aromatization, while, actually T levels during postvitellogenic stage exhibited a quick decline in this fish, coinciding with the fall of plasma Estradiol E<sub>2</sub> concentration. A sudden drop in the plasma E<sub>2</sub> level in *Labeo. rohita* from vitellogenic to postvitellogenic stage may be explained in terms of switching off the aromatize (CYP19) activity as the oocyte progressed to maturation. Almost a similar profile of E<sub>2</sub> has been reported during the transition from vitellogenic to maturational stage in rainbow trout [2]. This drop in circulatory E<sub>2</sub> levels probably reduces the intensity of sex steroid feedback, allowing the occurrence of hypothalamus-mediated GtH surge, which is required for the development of oocyte maturational competence (OMC). In this context, it was mentioned that in other teleosts such as gudgeon, *Gobio gobio*, there was no decrease of E<sub>2</sub> level during oocyte maturation; meanwhile this study has shown decreased E<sub>2</sub> in some specimens of kutum. Rosenblum *et al.* [6] observed a good correlation between circulating E<sub>2</sub> and calcium levels in female teleosts. Increases in plasma E<sub>2</sub> in female *Tilapia Oreochromis mossambicus* paralleled increases in both GSI and calcium levels [27], 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 T levels during

oocyte development can be related to its role as precursor of E<sub>2</sub> synthesis, as a precursor of E<sub>2</sub> production, T is available in the ovary for aromatization. At high concentration, T might also be involved in hepatic vitellogenin synthesis [5]: the sudden peak was measured when most fish were in final maturation (stage V), an effect of the release of T into the plasma when this was no longer needed for aromatization. This acute rise in Testosterone indicates those oocytes are fully mature and ready to ovulate [3]. 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 T in the plasma could be associated with the increase in the River sefid- Rood water temperature which occurs at the same time (March-April), (Figs. 2-5). 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 [28]. It was concluded that a close interaction were found between environmental cues and endocrine control of reproduction. The endocrine control cannot continue without the appropriate environmental cues required to stimulate reproduction.

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