

Annual Fluctuation of Sex Steroid Hormones in Pre-spawning Female Kutum (*Rutilus frissi kutum*)

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Abstract: Kutum is one of the most popular and economical species in Caspian sea and the stocks of it decrease during last decades therefore with due attention to importance of Kutum, The aim of this study was investigation of fluctuation sex steroid hormones during spawning migration and Annual variation of them in Kutum. There were year-to-year differentiation in plasma levels of some sex steroid hormones (testosterone and progesterone) and sexual maturation and they were related to the year-to-year variation of sea and river surface temperature. Changes in the plasma levels of testosterone and progesterone during pre-spawning migration differed from year to year in association with the sea and river surface temperature, whereas the changes in plasma levels of 17 β -Estradiol was not apparently related to the sea and river surface temperature. Furthermore we observed changes in sex steroid levels hormone in pre-spawning female Kutum during their migration from Caspian Sea to river. Fluctuation of main sexual migratory hormones including 17 β -Estradiol; Testosterone and Progesterone were monthly measured in 320 female of kutum which caught 15th of every month at 11:00 Am at artificial reproduction station in Shihood from September to April 2003 and 2004 during spawning migration. The results reveal that the levels of sex steroid hormone are low in stage of maturation II (T=0.06 \pm 0.0018, P=0.03 \pm 0.009, E2=17.03 \pm 0.14 ng/ml) and suddenly the levels of 17 β -Estradiol and Testosterone significantly increased in stage of maturation III and IV respectively (E2 III&V=424.2 \pm 25.08, 349.2 \pm 13.28, T III&V=1.26 \pm 0.098, 1.10 \pm 0.08 ng/ml) and decreased in Stage of V. In spite of these two hormones the levels of progesterone significantly increased in stage of V (0.89 \pm 0.06 ng/ml). And it seems that it can be use as one of the best indicator for distinguishing stage of V from other stages.

Key words: Kutum % 17 β -Estradiol % Testosterone % Progesterone % Migration

INTRODUCTION

Caspian Sea is the largest lake in the world which located in northern Iran in Mazandaran and Gilan Province. Kutum, *Rutilus frissi kutum* is one of the main anadromous species in Caspian Sea, which lives in small schools in deep water, but spawns in shallow water in the rivers like Shihood and Sefidrood from March to April. Spawners start to migrate from Caspian Sea to the rivers at the end of stage of maturation III and will be found in stage of IV and V in rivers. The eggs are laid among weeds and over gravel and hatch in about 10 days. Shihood is the one of the main spawning rivers in the Caspian Sea and fish production of it, especially Kutum,

is accounted by more than 50 percent of the total caught in the Mazandaran province [1, 2]. Stocks of Kutum were found to have decreased about 50% during last decade, in spite of releasing about 7 to 10 million fingerlings into the Caspian Sea. Main reasons for occurring this phenomenon include: overfishing, attack of *Mnemiopsis leidye*, mechanization of farming, disperses of pollution and fertilizer in rivers [1, 3].

During sexual maturation, dramatic shifts in steroid biosynthetic activity occur in both sexes, having effects on both somatic [4-7] and gonadal tissues. In female fish, levels of maturation inducing steroids (usually progesterone) are elevated during final oocyte maturation and ovulation [8]. All fish have testosterone in their

blood; however the ratio of the hormone varies between females and males and females often show rising levels of Estradiol and testosterone during vitellogenesis [9]. In fish species, many studies demonstrated the crucial role of 17 β -Estradiol in the development of ovary and especially in vitellogenesis [10-13] its direct stimulating effect on gonadotropin synthesis [14-18] and an indirect (via the brain) inhibitory effect of it on gonadotropin release [19, 20]. Progesterone have complementary additional effects on brain and pituitary and often act according to a sequence fashion [21-27] and has been described mainly as a precursor steroid in fish and no clear role by itself has been reported. Moreover, a cooperative effect of Progesterone (or another progestin) and 17 β -Estradiol in fish has never been reported to our knowledge [28].

Different study have been accomplished on fluctuation of sex steroid hormone levels during spawning migration and their relation with development of maturation in different species like *Acipenser oxyrinchus* by Sangalang *et al.* (1973) [29], *Acipenser transmontanus* by Lutes *et al.* (1987) (Plissero and Lemenn, 1991), *Acipenser baeri* by Plissero and Lemenn (1991) and Nagahama *et al.* (1993), *Poliodon spathula* Godovich *et al.* (1993) and *Morone saxatilis* by Mylonas *et al.* (1997) and Holland *et al.* (1998) [29].

Although several aspects of Kutum biology have been well examined in Iran at national levels and with support of Fisheries organization for increasing its stocks like stock assessment of the Caspian sea bony fishes [30], karyology (chromosomal study) of the Caspian Sea Kutum by white blood cells culture [2], toxicity and LC50 determination of Phenol, 1-Naphtol in Caspian kutum [31] and study on nutrient composition of Kutum [32] but there is a lack of knowledge on the reproductive endocrinology of it. Kutum are being caught from river in stage IV and V and transported to propagation center for breeding. After adaptation, they were injected with Pituitary extract. Kutum respond well to injecting hormone at stage IV in comparison to V and fishes in stage V do not need to inject of hormone and injecting may cause bleeding in ovary and decrease fecundity in them. According to injection of Pituitary gland, decrease of stress on brood stock and increase of fecundity and fertilization percentage, it is very important to find fast and easy way to determine the stage of maturation in them. Therefore the aim of this study is determining the levels of sex steroid hormones in different stage of maturation and finds the relation between fluctuation of

them and stage of maturation in Kutum specially in stage of IV and V from each other for the first time to improving the artificial propagation and releasing larva to Caspian Sea.

MATERIALS AND METHODS

Fish: In this study we used 320, 3, 4, 5 and 6 years old (n=80 in each age group) adult female fertile Kutum that have caught 15th of every month at 11:00 Am at artificial reproduction station in Shirood, main migratory river of Kutum in Caspian Sea in Stage of maturation IV and V and from Caspian sea and estuaries of its river in Stage of maturation II and III (n=20 samples for each mature group) from September to April in 2003 and 2004. The fishes were transported to the field in plastic tank (50-70 cm in diameter and 50 meter in depth) which supplied with oxygenated water containing 20 mg/l neutralized MS222 according to the method of Smith (1980). This method of transporting would overcome the effects of handling stress that may have been encountered during netting.

Sampling for Hormone Analysis: Blood Sampling was also conducted during September to April 2003 and 2004 monthly, after transporting captured fishes to the field (It take about half an hour). Sample of blood were taken from the caudal artery by heparinised syringes and were transported to laboratory for analysis in Ice. They were centrifuged at 2000g (10 min, at 4°C). The plasma was carefully decanted and kept at -20°C till further analysis. Radio-immunoassay (RIA) was used to measure 17 β -Estradiol, Testosterone and Progesterone. The concentrations of the steroid hormones were determined using appropriate FRANSA radioimmunoassay kits. (Cat Nos. Testosterone: CM-TESTO; Estradiol 17- β : SB-ESTR; Progesterone: CM-PROG supplied by FRANSA) and plasma was analyzed. All FRANSA RIA test kits made use of I125-labelled hormones, which are intended for use with human samples. All readings of radioactivity were taken using a Beckman Gamma 8500 Microprocessor Counter.

Biometry: Fishes were killed by a blow on the head immediately after blood sampling and length and weight of the fishes were measured. The abdominal cavity was opened and the ovary of the fishes were removed and weighted. The condition factor (K-factor) was calculated for each fish according to the formula: $K\text{-factor} = (\text{Weight (g)}/\text{Length (cm)}^3) \times 100$. Gonadosomatic Index (GSI) was

calculated [(Gonad weight/Somatic weight) × 100] just for samples that were caught from river, Absolute Fecundity was also measured.

Classification of Maturity Stages: Maturity stages were indexed for all groups of kutum following Kesteven scale (1960) with the following modifications. Color, shape and extension of the ovary into the body cavity as well as color and shape of ova, were considered to define stage of maturity in females. Degree of transparency of the ovary was also used as a criterion, since it is one of its characteristic features during early as well as fully matures phases.

Determination of Temperature of Surface Water of Caspian Sea and Shirood River: The Temperatures were determined by hydro biologic station of Ecological Institute of Caspian sea from 10 point of Caspian Sea and Shirood River every day during 2003 2nd 2004 with Thermometer.

Statistical Analysis: Statistical software (SPSS, version 11.5 & 13; Excel and Minitab 13.1) was used for all statistical analyses. Log-transformed to conform to the normality test and to homogeneity of variance (Levine's test). The 95% confidence intervals (95% CI) were calculated on the log-transformed data using the Univariate analysis. Tuckey test was performed on Log-transformed data followed by Dunett's test for post-hoc Differences between groups were analyzed by one-way ANOVA for normally distributed data.

RESULTS

The results of investigation of growth indices have been given in Table 1. Study of weight showed that there

are significant differences between Stages of maturation II, III and IV with V and also with stage of maturation II and III with IV and V ($p < 0.05$). Investigation of fork length and Cf reveal there are significant differences between Stage of maturation II and III with IV and V ($p < 0.05$).

Statistical analysis (Pearson Correlation) showed that there are no correlations between fluctuations of sex steroid hormones levels with growth indices (Pearson Coefficient < 0.2)

The results of considering fecundity indices have been given in Table 2. Study of Ovary weight, absolute fecundity and GSI show that there are significant differences between Stage of maturation II and III with IV and V ($p < 0.05$).

Statistical analysis (Pearson Correlation) showed that there are no correlations between fluctuations of sex steroid hormones with fecundity indices (Pearson Coefficient < 0.2) but there was strong correlation between absolute fecundity with weight and fork length (Pearson Coefficient = 0.95, $p < 0.05$). We also cannot determine the absolute fecundity in Captured fishes in stage II and III.

According to Table 3, the minimum levels of sex steroid hormones levels including Testosterone (0.06 ± 0.0018 ng/ml), Progesterone (0.03 ± 0.009 ng/ml) and 17 β -Estradiol (17.03 ± 0.14 ng/ml) are in Stage of maturation II and also statistical analysis shows that there is a significant differences between this stage with other stages ($p < 0.05$). Significant increase of 17 β -Estradiol and Testosterone were measured in stage of maturation III and IV and significant reduction of these hormones were also reported in stage of V ($p < 0.05$). In spite of 17 β -Estradiol and Testosterone we have significant increase of progesterone in stage of V (0.27 ± 0.009 ng/ml) ($p < 0.05$).

Significant increase of 17 β -Estradiol and Testosterone were measured in stage of maturation III and

Table 1: Mean ($\pm 1^\circ\text{C}$) Thermal variation ($^\circ\text{C}$) Of Caspian Sea and Shirood river surface water

Year	Spring	Summer	Autumn	Winter
2003	11	22	9	2
2004	16	30	12	7

Table 2: Mean (\pm SEM) of Kutum growth and fecundity indices in different stage of maturation in 2003 and 2004 (n= 80, 20 in each stage of maturation for every year)

Indices	Stage of Maturation	Mean \pm SEM	Range of variation		
			Min	Max	
Growth indices	Weight (g)	II	870.30 \pm 8.09	275.00	1500.00
		III	890.83 \pm 9.08	310.00	1700.00
		IV	930.30 \pm 9.73	375.00	1800.00
		V	991.83 \pm 9.88	411.00	1950.00
		Fork length (cm)	II	33.45 \pm 0.50	27.00
III	35.22 \pm 0.60		29.00	48.00	
IV	40.84 \pm 0.70		30.00	51.00	
V	41.78 \pm 0.82		31.00	60.00	
Condition Factor or Cf (%)	II		1.20 \pm 0.02	0.10	1.65
	III	1.21 \pm 0.02	0.10	1.65	
	IV	1.26 \pm 0.02	0.10	1.67	
	V	1.27 \pm 0.02	0.80	1.67	

Table 3: Mean (\pm SEM) of Kutum fecundity indices in different stage of maturation in 2003 and 2004 (n= 80, 20 in each stage of maturation for every year)

Indices	Stage of Maturation	Mean \pm SEM	Range of variation		
			Min	Max	
Fecundity indices	Ovary weight (g)	II	100.56 \pm 8.09	25.00	150.00
		III	110.74 \pm 9.08	35.80	150.00
		IV	166.55 \pm 9.73	42.30	338.00
		V	180.37 \pm 10.78	54.00	338.00
		Absolute Fecundity	II	-	-
	III	-	-	-	
	IV	63768.94 \pm 1789.73	23046.00	99572.00	
	V	66493.44 \pm 2076.95	39975.00	107378.00	
GSI (%)	II	11.29 \pm 0.11	4.00	23.00	
	III	12.08 \pm 0.21	4.00	25.00	
	IV	18.06 \pm 0.47	6.00	29.00	
	V	18.29 \pm 0.39	12.00	29.00	

Table 4: Mean (\pm SEM) of Kutum Sex Steroid Hormones in different stage of maturation in 2003 and 2004 (n= 80, 20 in each stage of maturation for every year)

Sex Steroid Hormone	Stage of Maturation	Mean \pm SEM (ng/ml) (Min-Max)	
		2004	2003
Testosterone	II	0.06 \pm 0.0018 (0.01-0.1)	0.090 \pm 0.0019 0.050 \pm 0.0018
	III	1.26 \pm 0.098 (0.1-5.4)	1.55 \pm 0.095 0.97 \pm 0.010
	IV	1.10 \pm 0.08 (0.1-0.9)	1.309 \pm 0.1409 0.909 \pm 0.0940
	V	0.30 \pm 0.002 (0.1-5.2)	0.325 \pm 0.003 0.291 \pm 0.026
	Progesterone	II	0.03 \pm 0.009 (UD-0.09)
	III	0.15 \pm 0.009 (0.1-2.1)	0.19 \pm 0.009 0.11 \pm 0.008
	IV	0.24 \pm 0.007 (0.2-0.3)	0.250 \pm 0.009 0.239 \pm 0.008
	V	0.89 \pm 0.06 (0.5-2.1)	1.156 \pm 0.095 0.631 \pm 0.092
17 β -Estradiol	II	17.03 \pm 0.14 (10.0-50.0)	19.06 \pm 0.153 15.01 \pm 0.131
	III	424.2 \pm 25.08 (70-589)	405.1 \pm 23.76 440.31 \pm 26.91
	IV	349.2 \pm 13.28 (92.0-583.0)	361.50 \pm 23.015 366.91 \pm 13.319
	V	30.10 \pm 1.07 (20.0-49.0)	30.48 \pm 1.76 29.73 \pm 1.254

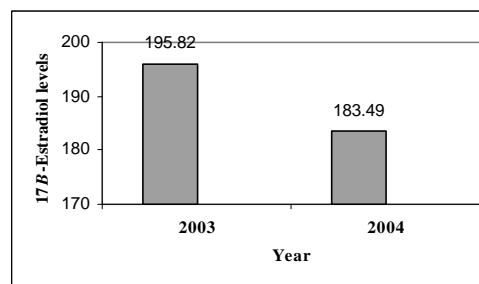
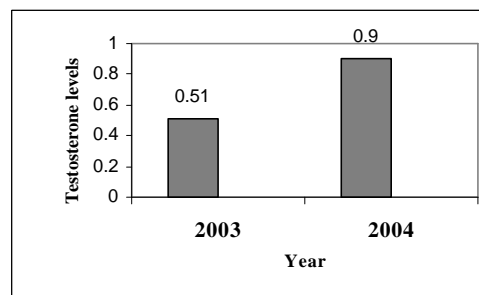
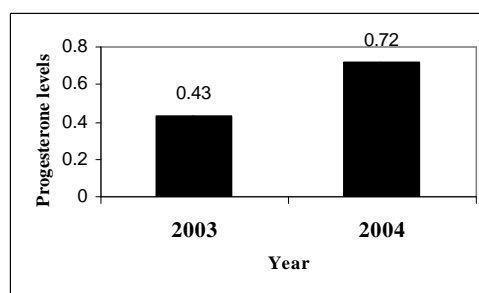


Fig. 1: Fluctuations of sex steroid hormones (ng/ml) during years of 2003 and 2004

IV and significant reduction of these hormones were also reported in stage of V. In spite of 17 β -Estradiol and Testosterone we have significant increase of progesterone in stage of V (30.10 \pm 1.07ng/ml).

Significant increase of testosterone and progesterone were also measured in stages of II, III, IV and V in year of 2003 in comparison to 2004 (P<0.05) but these changes was not clear in 17 β -Estradiol (Table 4 and Fig. 1).

DISCUSSION

Sexual maturation during homing migration is hormonally regulated by the hypothalamus-pituitary-gonadal axis (HPG axis) [33]. Steroid hormones secreted from the gonads have important roles in the control of gonadal maturation [34, 35].

Understanding of physiological and environmental mechanisms that govern sexual maturation of salmonids therefore requires elucidation of profiles of changes in plasma levels of steroid hormones and maturation of gonads during homing migration. Changes in the plasma levels of steroid hormones during gonadals maturation and spawning migration were reported, e.g., in pink salmon, *Oncorhynchus gorbuscha* [36]; sockeye salmon, *O. nerka* [37]; masu salmon, *O. masou* [38]; coho salmon, *O. kisutch* [34]; chum salmon, *O. keta* [39, 40] but there is no report on kutum and this is the first research on it.

Based on the comprehensive estimation of sexual maturation, the females at the hatchery in the 2003 were sexually more advanced than those in the 2004, although most of the females completed final oocyte maturation at the hatchery in all year examined. It seems that differences in sea and river surface temperature cause this phenomenon. The Mean of temperature in spawning season in 2004 was warmer than 2003 (Table 1). The mean levels of progesterone and testosterone in 2003 (0.70 ± 0.07 and 0.80 ± 0.09 ng/ml respectively) were significantly ($P < 0.05$) higher than 2004 but there are no significant differences between levels of 17 β -Estradiol in 2003 and 2004 (183.49 ± 22.42 and 195.82 ± 21.95 ng/ml respectively).

With due to the high levels of progesterone in year of 2003, we can understand that the captive kutum in 2003 were more sexually advanced than 2004. The present results showed that there are year-to-year differences in plasma levels of testosterone and progesterone on sexual maturation and they were influenced by the year-to-year variation of sea and river surface temperature. Onuma *et al.* (2004) [40] get same result on chum salmon.

Kutum started to migrate to river in stage III and we could find them in this stage just in Caspian Sea or in delta of river. Ascending migrant kutum cached after delta and toward upstream that they are in stage IV and V. Not only we do not have any kutum in stage V in down stream, but also there is no kutum in stage V in upstream and we have both of them in the midway of migration.

The concentrations of sex steroid hormones ($T = 0.06 \pm 0.001$, $P = 0.03 \pm 0.009$, $E2 = 17.03 \pm 0.14$) are low in Kutum in stage II in comparison to other stages. In stage II or on the other hand before pre vitelogenic stage or

cytoplasm growth of ova, which they are small and animal and vegetal pole are not distinguishable, the concentration of steroid sex hormones are low in fishes [41, 42]. Production of sex steroid hormones and growth of sexual glands are related with production rate and concentrations of gonadotropin hormones. Therefore the concentrations of sex steroid hormones are lower in immature fishes and during the first stage of maturations (I, II) [41, 42].

Furthermore other researches got similar results in other species that we touch on some of them. Majazi Amiri *et al.* (1996) [43] reported low concentrations of 17 β -Estradiol (0.6 ng/ml) and testosterone in immature and stage I an II in Bester (Hybrid of *Huso huso* \times *Acipenser nudiiventris*). Frantzen *et al.* (1997) [44] reported low concentration of Testosterone (2-4ng/ml) and 17 β -Estradiol (2-5 ng/ml) in Arctic Char (*Salvelinus alpinus*) brood stock in previtelogenic stage. Nazari (2001) [45] reported low levels of sex steroid hormones ($T = 0.25$, $E2 = 0.55$, $P = 0.32$ ng/ml) in *Acipenser persicus*. We could not find any similar research on Kutum and this is the first report of sex steroid hormones in these fishes.

The levels of sex steroid hormones in Kutum ($T = 1.26 \pm 0.098$, $P = 0.15 \pm 0.098$, $E2 = 424.20 \pm 25.08$ ng/ml) increased significantly in stage III in comparison to stage II ($p < 0.05$). Amiri Majazi *et al.* (1996) in Bester reported significant increase of 17 β -Estradiol (2-4 ng/ml) and Testosterone (20-80 ng/ml) in vitelogenic stage. Frantzen *et al.* (1997) [44] were reported high concentration of Testosterone (11ng/ml) and 17 β -Estradiol (71ng/ml) in Arctic Char (*Salvelinus alpinus*) brood stock in stage III. Barannikova *et al.* (1997) [46] reported levels of Testosterone, 17 β -Estradiol and Progesterone in *Acipenser gueldenstaedtii* 16.7, 1.2 and 3, 7 ng/ml in stages of III respectively. Nazari (2001) [45] also reported significant increase of Testosterone (8.55 ng/ml), 17 β -Estradiol (4.35 ng/ml) and Progesterone (0.52 ng/ml) in *Acipenser persicus* at stage III.

The increases in the levels of testosterone in early stage of upstream migration coincide well with that reported with in Chum Salmon [40]. Sexual maturation is hormonally regulated by the HPG axis [33, 38] and environmental condition has influence on this axis and can modulate neuroendocrine function of HPG axis in the homing fish which were prevented to arrive to their natal river. The levels of mRNAs encoding precursors of gonadotropin-releasing hormone (sGnRH-I and-II) and gonadotropin (GTH) subunits elevated during migration of fish. While homing fish were prevented to reach their natal river, sGnRH probably stimulated synthesis and

release of GTHs and then the released GTHs further facilitated secretion of testosterone. Several reports indicated that HPG axis promotes homing behavior [47-49]. Implantation of testosterone induced upstream migratory behavior in masu salmon [42] and shortened homing duration of sockeye salmon [47, 49]. Furthermore androgens had a positive feedback effect on GnRH neurons in the brain [38, 50, 51]. Therefore, the higher levels of testosterone seen in the warm years may have positive feedback effects on GnRH neurons that have critical roles in control of upstream migration [40].

The levels of sex steroid hormones in kutum were also significantly high in stage IV ($T=1.10\pm 0.08$, $P=0.24\pm 0.007$, $E_2=349.20\pm 13.28$ ng/ml) in comparison to stage II but the levels of 17 β -Estradiol and Testosterone decreased a little and not significantly in comparison to stage IV. Majazi *et al.* (1996) [43] in Bester and Nazari (2001) [45] reported in *Acipenser persicus* reported significant decrease of 17 β -Estradiol in stage IV in comparison to III and after vitellogenic stage but insignificant increase and decrease of Testosterone respectively. Rosenblum *et al.* (1987) found increase of 17 β -Estradiol before spawning and decrease of it in early stages of spawning in Catfishes (Nazari, 2001) [45]. Barannikova *et al.* (1997) [46] reported levels of testosterone in *Acipenser gueldenstaedtii* 14.9 ± 0.68 and 9.9 ± 0.5 in stages III and IV respectively.

The levels of 17 β -Estradiol decreased during upstream migration. The decreases in the levels of 17 β -Estradiol coincide well with those reported in sockeye salmon [37], pink salmon [36] and chum salmon [39] and chum salmon [40]. According to Previous researches, these changes indicate that the steroidogenic shift from the production of 17 β -Estradiol to 17 α -20 β -dihydroxy-pregnene-3-one [40, 52] occurs just prior to spawning, from the midway of migration to the hatchery during upstream.

The functional development of the GnRH system in immature teleost is under stimulatory control of gonadal steroids, especially testosterone and 17 β -Estradiol. This has been demonstrated for the masu salmon (*Oncorhynchus masou*), rainbow trout (*Oncorhynchus mykiss*), platyfish (*Xiphophorus maculatus*) and African catfish (*Clarias gariepinus*) [53].

In fish species, many earlier studies have demonstrated the crucial role of 17 β -Estradiol in the development of ovary and especially in vitellogenesis [8, 10-13].

An increase in plasma 17 β -Estradiol levels occurs during vitellogenesis and 17 β -Estradiol acts on the liver

to stimulate biosynthesis and release of vitellogenin (Vg) [29, 54-58]. Moreover, it has been also reported an effect on brain-pituitary complex where 17 β -Estradiol is responsible of a direct stimulating effect on gonadotropin synthesis [14, 16, 18, 20] and an indirect (via the brain) inhibitory effect on gonadotropin release [15, 19]. Females often show rising levels of 17 β -Estradiol and testosterone during vitellogenesis and sharp increases of 17 α -20 β -dihydroxy-pregnene-3-one during ovulation [9].

The levels of 17 β -Estradiol and testosterone decreased significantly in stage V in comparison to III and IV ($p<0.05$) but the level of progesterone has increased significantly in comparison to other stages in stage V ($T=0.30\pm 0.002$, $P=0.89\pm 0.06$, $E_2=30.10\pm 1.07$).

In amphibian, Progesterone induces oocyte maturation [59] but 17 α -20 β -dihydroxy-pregnene-3-one has such a role in rainbow trout and in other fish species [52, 60-62]. Progesterone has been described mainly as a precursor steroid in fish and no clear role by itself has been reported. Moreover, a cooperative effect of Progesterone (or another progestin) and 17 β -Estradiol in fish has never been reported to so far. In female fish, levels of maturation-inducing steroids (usually progesterone) are elevated during final oocyte maturation and ovulation [8]. The results of this research on kutum confirm this subject.

According to the results of this project, there were year-to-year differentiation in plasma levels of some sex steroid hormones (testosterone and progesterone) and sexual maturation and they were related to the year-to-year variation of sea and river surface temperature. Changes in the plasma levels of testosterone and progesterone during pre-spawning migration differed from year to year in association with the sea and river surface temperature, whereas the changes in plasma levels of 17 β -Estradiol was not apparently related to the sea and river surface temperature. Furthermore we observed changes in sex steroid levels hormone in pre-spawning female Kutum during their migration from Caspian Sea to river. Also, progesterone can be used as an indicator for distinguishing stage of maturation II, III and IV from V. There is no other similar research on kutum that we can use progesterone as one of our indicator to determine the exact alternations range of progesterone.

ACKNOWLEDGMENTS

Our special thanks are presented to Mr. H. Mousavi and their colleagues for their helping.

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