

## Research Article

# Plasma levels of sex steroids (testosterone, progesterone and 17 $\beta$ -estradiol) in Rohu Carp *Labeo rohita* broodstock from Khuzestan, Iran

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### Abstract

Sex steroids (testosterone, progesterone and 17 $\beta$ -estradiol) play important roles in controlling reproduction and sexual maturity in teleosts. The aim of the present study was to evaluate the seasonal changes of these steroids in Rohu *Labeo rohita*. Forty gravid females and males *L. rohita* reared in different seasons (spring, summer, autumn, winter) were caught by a net with 1 cm mesh from earthen ponds of Aquaculture Research Institute, in south of Iran. The highest levels of 17 $\beta$ -estradiol ( $122.8 \pm 17.73$  ng mL<sup>-1</sup>), testosterone ( $0.11 \pm 0.003$  ng mL<sup>-1</sup>) and progesterone ( $0.86 \pm 0.01$  ng mL<sup>-1</sup>) in female fish were recorded in autumn. In addition, the highest concentration of testosterone in male was determined as  $0.84 \pm 0.003$  ng mL<sup>-1</sup> in autumn. The mean value of dissolved oxygen in four seasons (spring, summer, autumn, and winter) were  $9.21 \pm 0.77$ ,  $8.36 \pm 0.51$ ,  $10.41 \pm 0.9$  and  $13.14 \pm 0.72$  mg L<sup>-1</sup>, with temperature  $25.47 \pm 1.07$ ,  $29.88 \pm 1.42$ ,  $20.23 \pm 0.69$  and  $14.63 \pm 0.47$  °C, respectively. Therefore, it can be inferred that progesterone was considered as the critical index for seasonal breeding selection. There was an appropriate condition in the mid winter for broodstock selection and breeding of fish with hormones manipulation.

**Keywords:** Sex steroids, Seasonal changes, *Labeo rohita*, Khuzestan, Iran

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## Introduction

Rohu, *Labeo rohita*, is one of the most valuable commercial and endemic fish in northern and central India especially in rivers of Pakistan, Bangladesh and Myanmar. Rohu is the principal species reared in carp polyculture systems along with the other two Indian major carps, *Catla catla* and *Cirrhinus mrigala*. These three Indian major carps are also the dominant species cultured in other countries such as Bangladesh, Pakistan, Myanmar, Lao People's Democratic Republic, Vietnam and Nepal. In all these countries, silver carp, grass carp and common carp are the most important species reared with the three Indian major carps in aquaculture (Wingfield *et al.*, 1990; McGlothlin and Ketterson, 2008).

In vertebrates, sex steroids play important roles in regulation of reproductive development and maturation as well as homeostatic mechanisms for instance, water and energy balance, which is well established that these hormones regulate the processes of sexual maturation, sexual behavior, oocyte maturation and ovulation in most teleosts. In fishes, estrogens (17 beta-estradiol) and androgens (testosterone and 11-ketotestosterone) were identified during gonadal development and progesterone during ovarian and testicular maturation (Wingfield *et al.*, 1990; McGlothlin and Ketterson, 2008; Pankhurst, 2008). In teleosts, 17  $\beta$ -estradiol produced by ovarian follicles that is necessary for the development of

female reproductive functions. During vitellogenesis process, vitellogenin (VTG) as the major yolk protein precursor synthesized in the liver by hepatic cells under the influence of circulating estrogens (Hiramatsu *et al.*, 2002). Oocyte maturation is initiated by binding of MISs (oocyte maturation inducing steroids) to a new G protein-coupled membrane progesterin receptor. This type of progesterin is the essential mediator of the maturational action of gonadotropin hormone (GtH-II) in fish, which induce meiotic resumption, while its production did not occur until an advanced stage of germinal vesicle migration and breakdown process, and also the adrenal glucocorticoids (e.g. corticosterone) which, are elevated during the reproductive cycle in some species, and act to mobilize energy for costly reproductive behaviors (Idler *et al.*, 1961; Kindler *et al.*, 1989).

Environmental factors such as variations in temperature, salinity, light period, food availability as well as social parameters induce changes in the neuroendocrine equilibrium. Investigation on the interaction among steroids can be advantageous in elucidating many aspects of reproductive physiology. Effects of different light regimes were reported on the maturation progress of the spotted rabbit fish (*Siganus satur*) (Shirinabadi *et al.*, 2013). In particular, understanding the basic mechanism to design treatments for reproductive dysfunction helps broaden our knowledge about the diversity of

organism–environment interactions. Although, the structures and many of the functions of steroid hormones are conserved among vertebrates (Zohar and Mylonas, 2001; Oliveira *et al.*, 2002), interspecific differences in steroidal regulations of reproduction might reflect adaptations to different ecological pressures (Suresh *et al.*, 2008). On the other hand, the effects of external factors may be direct, e.g. the influence of an altered water temperature on enzymatic reactions, or more often, mediated chains of events of different duration.

In addition, the principle part of any effects of environmental factors on reproductive function may result from their interaction. Other similar factors, seasonal variation as well as social interactions can alter the expression of different GnRHs in the brain and pituitary (Jodo *et al.*, 2005; Au *et al.*, 2006). In fish species, the coordination between environmental stimuli and brain leads to stimulate the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from gonadotrophs, which can regulate the hormonal responses for successful reproduction (Butts *et al.*, 2012). These two factors are the most vital, so that, directly or through sense organs, act on the glands that produce hormones, which in turn stimulate appropriate physiological or behavioral responses that ultimately control the spawning time in fish (Mousavi and Yousefian, 2012). Androgens are known to play important roles in the reproductive

physiology of teleosts; however, not much is known about their roles in developing larval and juvenile fishes (Heppell, 2005).

Seasonal changes of sex steroids levels have been reported for carp species, including *Labeo rohita* (Scott *et al.*, 1983; Rinchard *et al.*, 1997; Lee and Yang, 2002; Sen *et al.*, 2002; Suresh *et al.*, 2008), *Catla catla* (Oliveira *et al.*, 2009; Hosseinzadeh Sehafii *et al.*, 2014), *Salvelinus fontinalis* (Fatima *et al.*, 2015; Fatima *et al.*, 2017) and *Alburnus chalcoides* (Pouresmaeilian *et al.*, 2017).

The aim of present study was to investigate the seasonal variations and profiles of sex steroids consist of progesterone,  $17\beta$ -estradiol and testosterone in males and females *Labeo rohita* as well as investigating the interaction of environmental factors and sex steroid levels in plasma on breeding in appropriate time in order to obtain the maximum efficiency for reproduction of *Labeo rohita* in specific climate of Khuzestan.

## Materials and methods

### *Preparation of fish*

The experiments were conducted between summer 2011 and spring 2012 in South of Iran Aquaculture Research Center (31° 40' N, 48° 79' E), Ahvaz, Khuzestan Province, Iran. *Labeo rohita* were transferred in 2010 from India to Iran. A total, 40 gravid *L. rohita* brooders (20 males and 20 females) were reared in ponds (1700 m<sup>2</sup> earthen ponds) and maintained in different

seasons (spring, summer, autumn, winter). Total body length (mm) and weight (g) were measured for collecting fish. Table 1 presents an average length and weight of the samples. Fish feeding was carried out with a special broiler diet, twice a day. In each season, 10

specimens of female and male fish were collected. Male and female fish maintained in ponds together. *L. rohita* like the other cyprinids become mature in three-year-old, but in this study the age of matured fish was 1.5 years. Male and female fish were caught by netting vetch with 1 cm mesh size.

**Table 1: Mean length, weight of fish examined in the study.**

Seasons	Male		Female	
	Length $\pm$ SD (mm)	Weight $\pm$ SD (g)	Length $\pm$ SD (mm)	Weight $\pm$ SD (g)
Spring	517.25 $\pm$ 9.31 <sup>a</sup>	2815 $\pm$ 304.24 <sup>a</sup>	470 $\pm$ 8.16 <sup>a</sup>	1207.5 $\pm$ 69.58 <sup>a</sup>
Summer	528 $\pm$ 11.67 <sup>b</sup>	1981 $\pm$ 183 <sup>b</sup>	546 $\pm$ 16.73 <sup>b</sup>	2464 $\pm$ 387.01 <sup>b</sup>
Autumn	553 $\pm$ 7.58 <sup>c</sup>	2188 $\pm$ 79.73 <sup>c</sup>	564 $\pm$ 15.57 <sup>c</sup>	2718 $\pm$ 378.42 <sup>c</sup>
Winter	563.75 $\pm$ 7.5 <sup>d</sup>	2815 $\pm$ 304.24 <sup>a</sup>	556 $\pm$ 4.78 <sup>d</sup>	2511.25 $\pm$ 102.5 <sup>d</sup>

Note: a, b, c, d,  $p < 0.05$ , significantly different in length and weight of male and female.

#### Blood sampling

To determine the progesterone, 17 $\beta$ -estradiol and testosterone levels in males and females, blood samples were taken from live females and males from the lateral caudal vein with heparinized 22G needles in the morning within 10-20 min. Collected blood samples, in order to clot, was put at 37°C for 1 h, then centrifuged at 3000 $\times$ g for 20 min and the serum was used to estimate the progesterone, testosterone, and 17 $\beta$ -estradiol levels (Suresh *et al.*, 2008).

#### Measurement of steroid hormones

Concentrations of sex steroids (testosterone, progesterone and 17- $\beta$  estradiol) in plasma were measured by radioimmunoassay (Rinchard *et al.*, 1993). Quantitative immunoassay technique used to detect the level of 17  $\alpha$ - methyl testosterone, progesterone and 17  $\beta$ -estradiol in the plasma by measuring the precipitation formed by

the complex of the protein and the antiserum (Shimizu *et al.*, 1985; Takahashi *et al.*, 1985).

#### Water temperature and dissolved oxygen

During fish samplings, pond water temperature and dissolved oxygen were measured. The water temperature was measured by sampling from a depth of 2 meters with thermometer. In this study, Winkler method was used to measure dissolved oxygen (Eaton *et al.*, 2005).

#### Statistical analysis

The Normality of the data was evaluated by Shapiro-Wilk test. Differences between means were assessed using one-way ANOVA, followed by Duncan multiple comparison tests, and a significance level of 0.05 was used for all tests. All

data were expressed as mean $\pm$ SD using SPSS 17.

## Results

The results showed that the testosterone concentration in the male is higher ( $p<0.05$ ) than the female fish in all seasons. The highest level of 17  $\beta$ -estradiol, testosterone and progesterone in female *Labeo rohita* were recorded in autumn ( $p<0.05$ ). These steroid hormones were found to be the lowest in female *Labeo rohita* during summer. In the male fish, the highest and lowest levels of testosterone were recorded in

autumn and spring, respectively (Table 2).

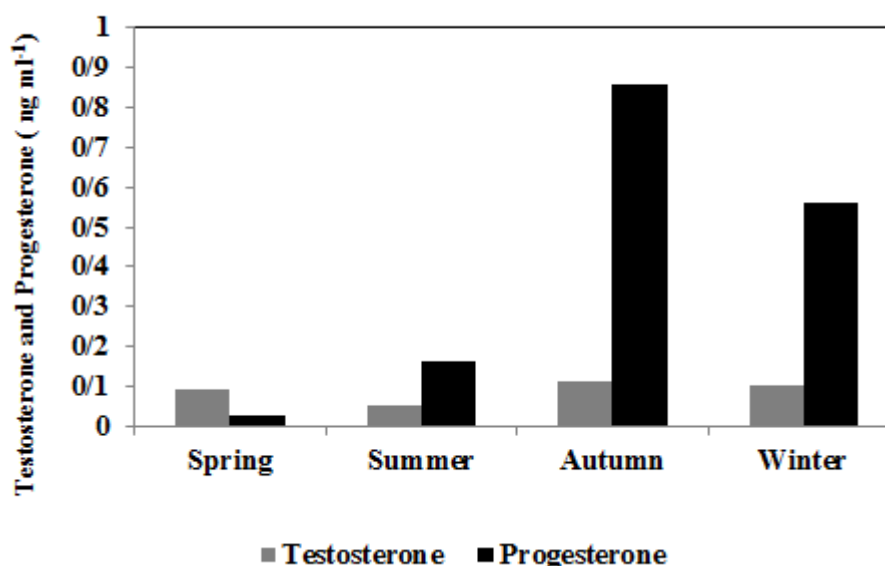
Seasonal change of DO (dissolved oxygen) and temperature are presented in figure 1. DO level was higher in winter than in summer ( $p<0.05$ ). The average temperature was higher in summer than winter ( $p<0.05$ ).

Seasonally, there were also significant differences in plasma levels of testosterone and progesterone in females *Labeo rohita* ( $p<0.05$ ) (Fig. 2). 17 $\beta$ -estradiol in summer was lower compared to spring, autumn and winter ( $p<0.05$ ) (Fig. 3).

**Table 2: Serum steroid hormones (ng mL<sup>-1</sup>) in *Labeo rohita* in different seasons, samples from Khuzestan, Iran.**

Seasons	Male (ngml <sup>-1</sup> )		Female (ngml <sup>-1</sup> )	
	testosterone	progesterone	testosterone	17 $\beta$ - estradiol
Spring	0.19 $\pm$ 0.005 <sup>a</sup>	0.027 $\pm$ 0.04 <sup>a</sup>	0.092 $\pm$ 0.004 <sup>a</sup>	107.75 $\pm$ 12.82 <sup>a</sup>
Summer	0.55 $\pm$ 0.007 <sup>b</sup>	0.16 $\pm$ 0.02 <sup>b</sup>	0.05 $\pm$ 0.002 <sup>b</sup>	80.2 $\pm$ 13.66 <sup>b</sup>
Autumn	0.84 $\pm$ 0.003 <sup>b</sup>	0.86 $\pm$ 0.01 <sup>c</sup>	0.11 $\pm$ 0.003 <sup>c</sup>	122.8 $\pm$ 17.73 <sup>c</sup>
Winter	0.32 $\pm$ 0.006 <sup>c</sup>	0.56 $\pm$ 0.05 <sup>d</sup>	0.1 $\pm$ 0.006 <sup>c</sup>	104.25 $\pm$ 17.72 <sup>a</sup>

Note: a, b, c, d,  $p<0.05$ , significantly different in hormones of male and female.



**Figure 1: Seasonal changes of testosterone and progesterone (ng mL<sup>-1</sup>) in female of *Labeo rohita* from Khuzestan, Iran.**

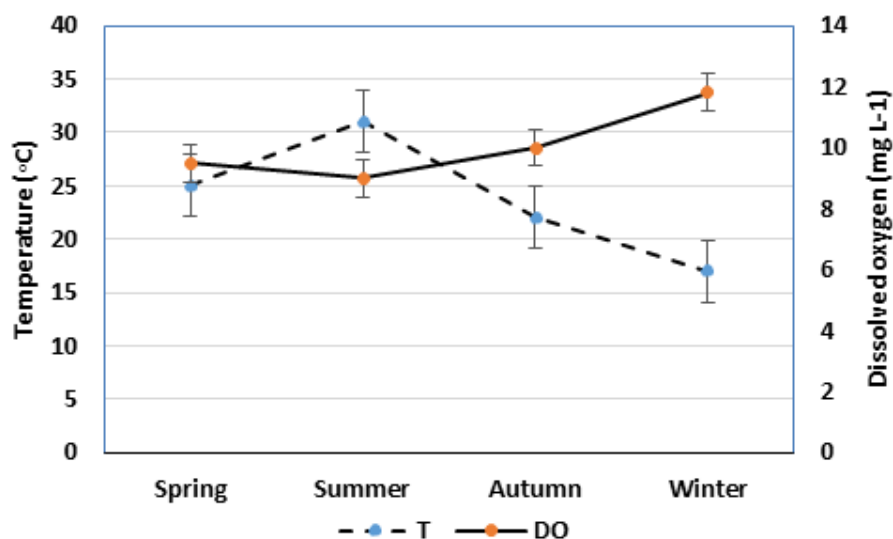


Figure 2: Seasonal changes of dissolved oxygen (DO) (mg L<sup>-1</sup>) and temperature (°C) in earthen ponds.

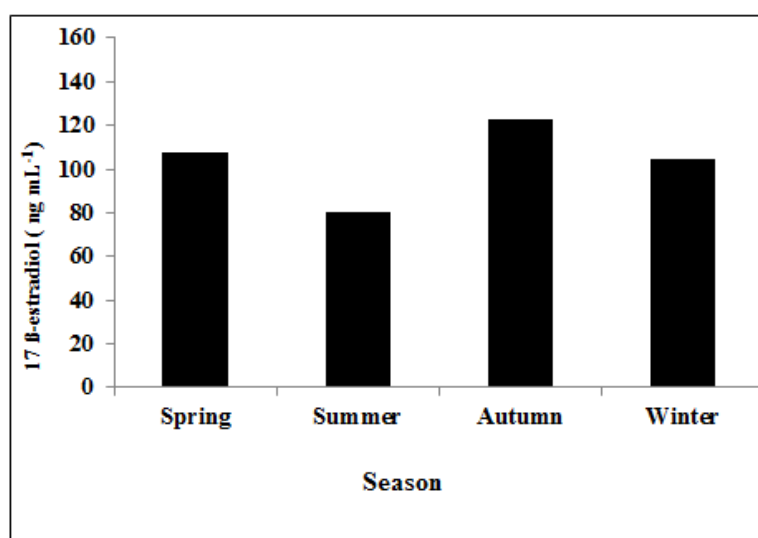


Figure 3: Seasonal changes of 17  $\beta$ -estradiol (ng mL<sup>-1</sup>), in female of *Labeo rohita* from Khuzestan, Iran.

## Discussion

In the present study, based on the measuring hormonal profiles in *Labeo rohita* the appropriate time for breeding of this species was determined in the southern of Iran.

17  $\beta$ -estradiol level in Indian major carp showed a decreasing trend during the spawning period (Suresh *et al.*, 2008). Sen *et al.* (2002) reported a

similar result in this species. In roach (*Rutilus rutilus*), 17  $\beta$ -estradiol level decreased during the spawning period (Rinchard *et al.*, 1997). Probably, the decrease in the steroid levels is because of a shift in the steroidogenic pathway prior to the germinal vesicle breakdown (Zohar and Mylonas, 2001; Oliveira *et al.*, 2002, Fatima *et al.*, 2017), which reduces not only the negative feedback

on pituitary but also gonadotropin surge, which is needed for maturation (Scott *et al.*, 1983; Lee and Yang, 2002, Pal *et al.*, 2018). In the present study, the highest mean values of  $17\beta$ -estradiol ( $122.8 \pm 17.73$  ng mL<sup>-1</sup>), testosterone ( $0.11 \pm 0.003$  ng mL<sup>-1</sup>) and progesterone ( $0.86 \pm 0.01$  ng mL<sup>-1</sup>) in female *Labeo rohita* were recorded in autumn. Surge of estradiol during the pre-spawning period of the reproductive cycle in females of several species, including *Oncorhynchus mykiss* (Scott *et al.*, 1980), *Oncorhynchus rhodurus* (Kagawa *et al.*, 1983), *Catostomus commersoni* (Scott *et al.*, 1984), *Acanthopagrus schlegeli* (Chang and Yueh, 1990), *Paralichthys dentatus* (Merson *et al.*, 2000), *Gadus morhua* (Dahle *et al.*, 2003), and *Solea senegalensis* (Oliveira *et al.*, 2009) have been reported. Hosseinzadeh Sehafii *et al.* (2014) indicated the mean values of  $17\beta$ -estradiol in spring, summer, autumn and winter were 85.50, 101.50, 128.50 and 88.75 ng mL<sup>-1</sup>, respectively. There was a significant difference in level of  $17\beta$ -estradiol between spring and winter with summer.

It was indicated that peak level of testosterone was observed during the spring, simultaneous with spawning, while it was decreased throughout the rest of the year. Testosterone levels showed a little seasonal variation in juveniles (0–1 and 2–3 age groups), but a seasonal increase was found from autumn to spring in older fish (4–5 and 6<sup>+</sup> age groups) (Heppell, 2005). The hormonal profile indicated that gravid

fish is ready to ovulate. Other researchers have been reported that the level of testosterone in female *Catla catla* in spring, summer, autumn and winter were 0.1, 0.04, 0.1 and 0.13 ng mL<sup>-1</sup> respectively, than the higher level belonged to the winter season. Also, the level of testosterone in male *Catla catla* in spring, summer, autumn and winter were 0.05, 0.04, 0.04 and 0.16 ng mL<sup>-1</sup>, that the higher level belonged to the winter season and there was a significant difference in the level of testosterone among the winter with spring, summer and autumn (Oliveira *et al.*, 2009). Also, the level of this hormone in *Cyprinus carpio* was 0.05, 0.72, 0.71 and 0.56 ng mL<sup>-1</sup> in spring, summer, autumn and winter, so that higher level belongs to summer, and there was a significant difference in the level of testosterone among spring to summer, autumn and winter seasons (Fatima *et al.*, 2017; Pouresmaeilian *et al.*, 2017; Pal *et al.*, 2018).

The higher level of mentioned hormones in gravid species is attributed to a shift in the activity of key enzyme involved in steroidogenesis as follicles are prepared for synthesis of progestogens (Nagahama, 1987). Also the same studies demonstrated that testosterone level in goldfish *Carassius auratus* was high when gametes are fully mature and ready to ovulate (Kobayashi *et al.*, 1987).

In the present study, the highest and lowest levels of progesterone in female *Labeo rohita* was recorded in autumn and spring. Hosseinzadeh Sehafii *et al.*

(2014) indicated that progesterone in female *Catla catla* were higher than winter as like as the other seasons. Levels of progesterone in spring, summer, autumn and winter were 0.38, 0.51, 0.46 and 1.32 ng mL<sup>-1</sup>. In another study, progesterone in *Cyprinus carpio* was higher than the summer and there was a significant difference between summer and spring seasons. Levels of progesterone in spring, summer, autumn and winter seasons were 0.42, 0.71, 0.62 and 0.46 ng mL<sup>-1</sup>, respectively (Kobayashi *et al.*, 1987).

We found that a measured dissolved oxygen concentration in winter was higher and significantly differed in comparison with summer. So, there was an inverse relationship between dissolved oxygen and temperature. Similarly, in vitro study on African cichlid, *Pseudocrenilabrus multicolor victoriae*, demonstrated that testosterone and estradiol levels were higher during pre-ovulation than to ovulation or post-ovulation periods (Friesen *et al.*, 2012). This would be related to the GnRH levels that control hypothalamus-pituitary-gonad axis. Ovulation and spawning induction in fishes by administration of GnRH $\alpha$  and catecholaminergic pharmaceutical compounds as well as the effects of pimozide and chlorpromazine on ovarian development and levels of gonadal steroid hormones were investigated in the controlled environment conditions (Ahmadnezhad *et al.*, 2013; Koohilai *et al.*, 2016). As much as progesterone is a precursor for

testosterone and estradiol, so it was introduced for seasonal breeding selection in *L. rohita*. There was a proper condition in mid-winter for broodstock selection and breeding programs (under hormonal treatment).

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