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Seasonal variations in the profile of sex steroids and ovarian development of catla (*Catla catla*, Hamilton) during the annual reproductive cycle

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Abstract

In South Asian carp culture, catla (*Catla catla*) is a major commercial species due to its higher growth, flesh quality and increased market demand. The present study investigated monthly variations in ovarian development and profiles of important steroids such as testosterone (T), 11-ketotestosterone (11-KT), estradiol-17β (E_2), cortisol (C) and 17α-hydroxyprogesterone (17α-HP) during annual reproductive cycle of catla (age = 18–29 months) under semi-arid climatic conditions. Ovarian development commenced after winter solstice (December) concomitant with gradual increase in levels of T and E_2. Profile of E_2 was showed positive relationship with gonadosomatic index (GSI). Peaks of both parameters were observed in June (GSI: 8.03 ± 0.46%; E_2: 0.41 ± 0.04ng/ml) showing major role of E_2 in maturation of oocytes. Mature females were observed during May until June. Spawning was not observed in captivity however, ova could be extracted on manual stripping during June. An inverse relationship has been observed between profiles of T and 17α-HP over the study period. Highest value of 17α-HP was noted in March (0.99 ± 0.04ng/ml) while levels of T dropped significantly at same sample point. Highest levels of T were observed during April (0.35 ± 0.03ng/ml), which gradually dropped afterwards. Highest levels of 11-KT were observed during June presumably indicating its role in growth of oocytes diameter thus increasing the GSI. Levels of C remained high over most of the study period except during maturation when concentration of T and E_2 was higher.

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Introduction

The major carp, catla (*Catla catla*), is one of the most economically important fish species in the South Asia particularly in Pakistan, India, Bangladesh, Myanmar, Laos, and Thailand (Costa-Pierce, 2005). Its higher growth rate, flesh quality and compatibility with other major carps in polyculture system and consumer preference have increased its popularity among fish farmers. Growth, survival and polycuture are the vastly investigated areas in this species (Sharma and Chakrabarti, 2008; Kadhar, 2014). However reproductive biology of catla has not been investigated in semi-arid region. Studies on fish reproduction assist the aquaculture industry in meeting the ever increasing demand for fish, by improving protocols for higher efficiency of egg production and enhanced viability of progeny.

Sex determination, gonadal differentiation and annual reproductive cyclehave been studied in several teleosts (Barcellos *et al.*, 2002; Cussac and Ortubay, 2002; Guerriero *et al.*, 2005; Chen *et al.*, 2006; Almeida *et al.*, 2008). Due to the importance of these parameters in applied aquaculture, these are commonly estimated for species of economic importance (Hunter *et al.*, 1992). Successful breeding, healthy fingerling production and optimization of harvest period are the major interests of carp farmers. Studying gonadal development in relation to environmental correlates (temperature and photoperiod) and endocrine control of reproductive cycle are critically important to control or manipulate reproduction in any species. Bhattacharyya *et al.* (2005) and Bhattacharyya and Maitra (2006) studied the gonadal development in relation with environmental correlates such as photoperiod and measured the seasonal variations in levels of T in catla but under sub-tropical climate conditions which are largely different from semi-arid climate in Pakistan. Variations in climate directly affect the hypothalamus-pituitary-gonadal axis therefore changing the time of occurrence of important stages such as recruitment for maturation and spawning season (Sivakumaran *et al.*, 2003). Lone *et al.* (2009, 2012) studied the histological development of gonads in catla in semi-arid climate conditions however, seasonal variations in important sex steroids during annual reproductive cycle of catla have not been studied in this region. As sex steroids play the most critical role in regulating the gonadal development therefore, the present study aimed at investigating seasonal variations in profiles of major sex steroids (T, 11-KT, E_2, C and 17-α HP) in commercially cultured female catla under semi-arid climate conditions of Pakistan. Findings of present study will be beneficial and applicable to commercial carp fish farm practices in the region.

Materials and methods

Procedures performed in this study were approved by the Government College University Animal Ethics Committee. Fish were obtained from a commercial fish farm (Himalaya Fish Hatchery, Sheikhupura) under natural water temperature and photoperiod over the study period (age: 18 months – 29 months; body weight: 892.08 ± 15.12g). Water quality parameters at farm were maintained at pH 6.8- 7.2, chlorine < 0.02mg/L, total ammonia < 2mg/L, nitrite < 1mg/L, nitrate < 80mg/L. Fish were fed with commercial diet at the rate of 2% of their body weight two times a day.

Sampling

At each monthly sample point, a total of ten fish were randomly collected and transferred to University Aquaculture facility. Fish were kept in tanks for one week to be released from stress of capture. A total of five female fish were sampled every month however this number varies due to lack of sexual dimorphism. At sampling, fish were killed by killed by anesthetic overdose (30µl L-1 AQUI-S). Total body weight (near to 0.1g) and total body length (near to 0.1 cm) and gonadal weight (near to 0.1g) were measured. GSI was measured by using following formula:

GSI= [Gonadal Weight (g) / Total body weight (g)] × 100

Ovarian development refers to changes in values of GSI over the study period. Blood samples were collected from caudal vein and centrifuged at 3000 rpm for 15 minutes. Serum samples were stored at -80°C until assayed to determine the levels of sex steroids by ELISA (BIORAD).

Analysis of Hormones

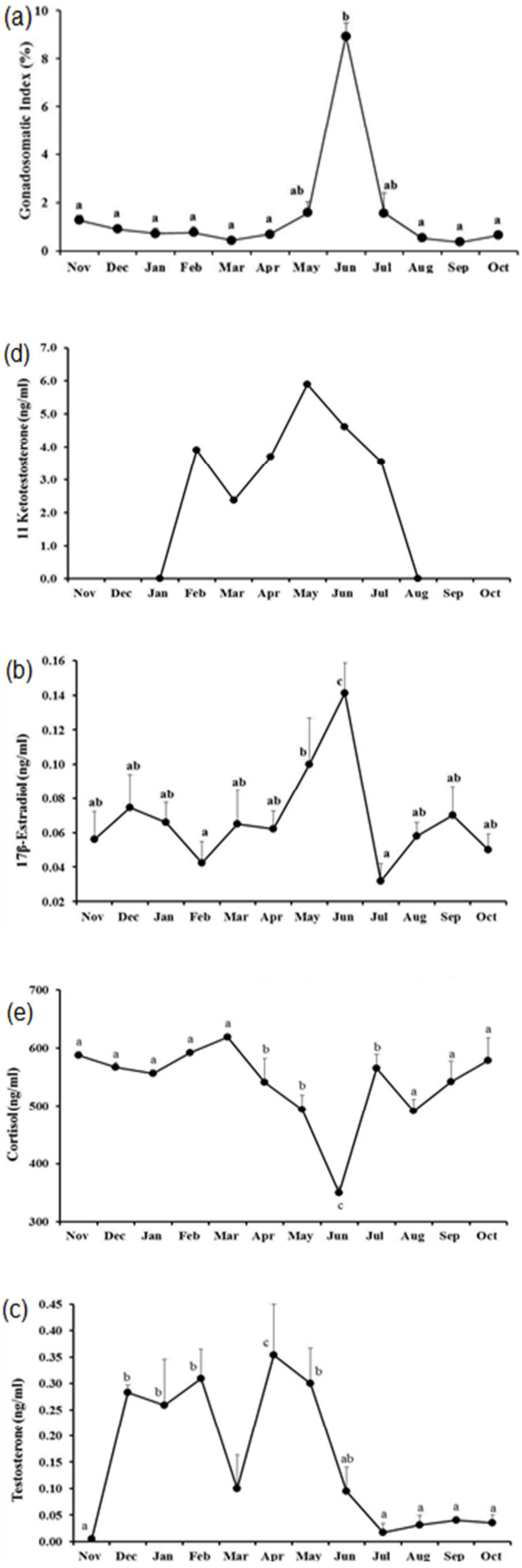
Assays of sex steroids were validated before samples analyses [E_2 (Detection limit: 0.25–1ng/ml; Precision: 2.4% (intra-assay), 7.3% (inter-assay); Specificity (cross-reactivity): estrone, 3.4%; estriol, 0.84%); T (Detection limit: 0 – 20ng/ml; Precision: 2.5% (intra-assay), 6.8% (inter-assay); Specificity (cross-reactivity): Dehydroepiandrosterone, 3.2%; 11-KT, 1.2%); 11-KT (Detection limit: 0.00078 – 0.1ng/ml; Specificity (cross-reactivity): 11-KT, < 0.01%); C (Detection limit: 10 – 2000nmol/L, cross-reactivity: < 0.01%, functional sensitivity: 0.030µg/dL; Precision 9.9% (intra-assay), 20% (inter-assay); P (Detection limit: 0.3 – 40ng/ml; Precision: 7.1% (intr-aassay), 12.6% (inter-assay), Specificity (cross-reactivity): Testosterone, 0.1%; E_2, < 0.01%); 17α-HP (Detection limit: 0 – 1ng/ml; Precision: <10% (intra-assay), <12% (inter-assay), Specificity (cross-reactivity): Progesterone, 1.93%; 17-Hydroxypregnenolone, 1.69%)].

Statistical Analysis

Data were analyzed by one way ANOVA after analyzing by Levene's test of homogeneity using SPSS version 18. Tukey's post-hoc test was applied for comparison of means at confidence interval of 95%.

Results

GSI in females remained low after winter solstice in December till March. A slight increase was observed in May while the highest value was noted in June (9.00 ± 0.56%) ($F_{11, 56}$ = 4.40, P < 0.5) (Fig. 1a). It dropped later in July and remained low during rest of the study period. A rise in levels of E_2 was observed in March which reached its peak in June (0.14 ± 0.02 ng/ml) and remained low afterwards ($F_{11, 56}$ = 6.00, P < 0.5) (Fig. 1b). Levels of T remained high during December till February but significantly dropped in March ($F_{11, 56}$ = 4.95, P < 0.5) (Fig. 1c). Levels of T increased afterwards and remained high till May. Concentration of 11-KT remained within the range of 5.90 ± 0.56 – 2.38 ± 0.67ng/ml over the study period ($F_{11, 56}$= 0.69, P > 0.5) (Fig. 1d). Levels of C remained high throughout the study period ($F_{11, 56}$ = 21.42, P > 0.5) except in June when a significant drop was observed (Fig. 1e). Highest level of 17α-HP was observed in March (0.99 ± 0.03) ($F_{11, 56}$= 4.76, P < 0.5) which significantly dropped during April and May. Levels of 17α-HP increased afterwards and remained high till end of study (Fig. 1f).



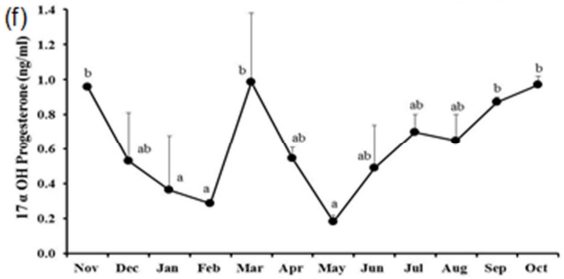


Fig. 1 Monthly variations (mean ± SE) in profile of gonadosomatic index (a), 17β-estradiol (b), testosterone (c), 11-ketotestosterone (d), cortisol (e) and17α- OH Progesterone (f) during the annual reproductive cycle of female catla. Significantly different subsets (P<0.05) given by Tukey's HSD are indicated by letters. Monthly values of 11-KT below detection limit were not shown.

Discussion

In present study, initial gradual increase in GSI was result of oocyte growth (as observed macroscopically) correlated with high levels of E_2 during this period. Stimulation of vitellogenin (precursor of egg yolk protein) expression in the liver which in turn promotes oocyte grows this primarily regulated by E_2 in teleosts (Kobayashi *et al.*, 2009; Kazeto *et al.*, 2011). Higher levels of T during this period could be related to its role as substrate of E_2 and 17α, 20β dihydroxypregnenone (DHP) (Borg, 1994). 11-KT has been also identified as the major androgen in females, produced in ovarian follicles (Lokman *et al.*, 2002; Matsubara *et al.*, 2003). During April-May, quantitatively higher levels of 11-KT than T might be related to its role in oil droplet accumulation in the oocytes which in turn enhances the oocyte growth (Endo *et al.*, 2008).

Oocytes might have attained their maximum growth and final maturation (hydration of oocytes) during end of May until June when highest values were observed in both E_2 and GSI. A gradual increase in concentration of 17α-HP was observed before this period. This hormone is substrate for DHP which induces uptake of water by oocytes, initiating their final maturation to be released from ovary in cypriniformes (Nagahama and Yamashita, 2008). Although natural spawning could not be observed in tank conditions however, ova could be manually extracted.

This indicates that spawning in catla occurs during July under natural environmental conditions as observed in previous studies in tropical India (Bhattacharyya *et al.*, 2005; Bhattacharyya and Maitra 2006). GSI declined in July and remained low afterwards showing the regression of ovaries associated with decline in levels of T and E_2 at this stage. This decrease might reflect a shift in steroidogenesis pathways from androgens and E_2 to 17α-HP production (Kokokiris *et al.* 2000) as levels of 17α-HP remained high in late phase of this study.

In catla, cortisol profile showed a negative relationship with those of sex steroids. Cortisol secretion decreased after March and minimum level was observed in June, the period of vigorous oocyte growth. Elevated levels of gonadal steroids were found to modify the pituitary-interrenal axis thus suppressing the levels of cortisol (Pottinger *et al.* 1996; Semenkova *et al.* 2002). In present study, this inference was supported by an immediate rise in cortisol level after June, concomitant with the end of reproductive activity, low levels of 17α-HP and sex steroids. Overall, this study provided basic information on endocrine control of ovarian development in catla under semi-arid conditions. As reproductive cycle in this species is finely controlled by environmental correlates therefore present study is scientifically significant for advanced future studies on the control of reproduction of this species in semi-arid regions.

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