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Monthly variations in the profile of sex steroids and testicular development of Catla (*Catla catla*, Hamilton) during the Annual reproductive cycle: Basic information for future study

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Abstract

Catla (*Catla catla*) is the most important commercial carp in South Asia due to its higher growth, flesh quality and increased market demand. Present study investigated the seasonal variations in gonadosomatic index (GSI) and profiles of sex steroids such as testosterone (T), 11-ketotestosterone (11-KT), estradiol-17β (E_2), cortisol (C), progesterone (P) and 17α-hydroxyprogesterone (17α-HP) during annual reproductive cycle under semi-arid climate conditions. GSI increased during March concomitant with gradual increase in levels of T, P and 17α-HP. Highest values of these parameters were observed at different stages of gonadal development. T and 11-KT played key role in progress of testicular development and final maturation in June however, spawning in captivity was not observed. Role of P and 17α-HP was identified more as substrate of sex steroids. Higher levels of sex steroids suppressed the synthesis of cortisol presumably interfering with pituitary-inter-renal axis.

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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 and India. Its higher growth rate and compatibility with other major carps (*Labeo rohita*, *Cirrhinus mirigala*, *Cyprinus carpio*) in polyculture systems, consumer preference have increased its popularity among the fish farmers in India, Bangladesh, Myanmar, Thailand and Pakistan (Costa-Pierce, 2005). Growth, survival and polyculture are the vastly investigated areas in this species (Kadhar, 2014). However, various aspects of its reproductive biology have not been studied in semi-arid region.

Gonadal development is fundamental to fishery science as studied in several teleosts including catla in tropical region (Lone *et al.*, 2009; Bhattacharyya *et al.*, 2005; Bhattacharyya and Maitra, 2006). Successful breeding, healthy fingerling production and optimization of harvest period are the major interests of carp farmers. To achieve these goals, basic understanding of reproductive cycle of any species provides baseline information for further research. Unfortunately, traditional carp culture in south Asia lacks in advanced information about reproductive cycle of many species under different climate conditions. A fragmented work on reproductive cycle of catla has been done in sub-tropical region of India (Bhattacharyya *et al.*, 2005; Bhattacharyya and Maitra, 2006). They studied the gonadal development in relation with environmental correlates such as photoperiod and measured the seasonal variations in levels of T only. However, this study was performed under sub-tropical climate condition which is largely different from semi-arid climate in Pakistan which could alter the temporal pattern of gonadal development in both regions (Sivakumaran *et al.*, 2003). Lone *et al.* (2009) studied the histological development of gonads in catla during annual reproductive cycle in semi-arid climate, however seasonal variations in important sex steroids during this period have not been studied in this region. As sex steroids play the most critical role in regulating the gonadal development therefore present study aimed at investigating the seasonal variations in profiles of major sex steroids (T, 11-KT, E_2, C, 17-α HP and P) in commercially cultured male catla under semi-arid climate conditions of Pakistan. Findings of present study will be beneficial and applicable to commercial carp fish farm practices and future study on reproductive biology of this species in the region.

Materials and methods

Maintenance and Sampling

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 < 2 mg/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.

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 male 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 (30 µl L-1 AQUI-S). Total body weight (near to 0.1 g) and total body length (near to 0.1 cm) and gonadal weight (near to 0.1 g) were measured. GSI was measured by using following formula:

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

Analysis of Hormones

Testicular 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). Assays of sex steroids were validated before samples analyses [E_2 (Detection limit: 0.25 – 1 ng/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 – 2000 nmol/L, cross-reactivity: < 0.01 %, functional sensitivity: 0.030µg/dL; Precision 9.9% (intra-assay), 20% (inter-assay); P (Detection limit: 0.3 – 40 ng/ml; Precision: 7.1% (intra-assay), 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 (SPSS version 17) by one way ANOVA after analyzing by Levene's test of homogeneity. Tukey's post-hoc test was applied for comparison of means at confidence interval of 95%.

Results

An increase in value of GSI was observed in March however significantly increase was noted during May (Fig. 1). Highest value of GSI was observed in June (0.48 ± 0.07%) when 100% male population was observed to be running ($F_{11, 56}$ = 8.40, P < 0.5). It dropped later in July (one month post-summer solstice) and remained low during rest of the study period. Levels of T gradually increased in January (one month post-winter solstice) and showed the highest concentration in July (0.67 ± 0.10ng/ml) ($F_{11, 56}$ = 5.05, P < 0.5) (Fig. 2a). Levels of T dropped in August and remained low till end of the study. Highest levels of 11-KT was observed in May (7.04 ± 0.01ng/ml) ($F_{11, 56}$ = 3.96, P < 0.5) (Fig. 2b). Concentration of 11-KT dropped in June and increased again during September (6.38 ± 0.02ng/ml). Levels of E_2 remained within the range of 0.5 ± 0.01–0.13 ± 0.02ng/ml over the study period ($F_{11, 56}$ = 13.10, P < 0.5) (Fig. 2c). A significant rise in levels of C was observed during July (620.00 ± 30.50 ng/ml) (Fig 2d). Over the study period, concentration of C remained within the range of 329.01 ± 40.43–639.15 ± 59.35 ng/ml ($F_{11, 56}$ = 4.30, P < 0.5). Observed range of P ($F_{11, 56}$ = 5.30, P < 0.5) and 17α-HP ($F_{11, 56}$ = 7.30, P < 0.5) over the study period were 0.47 ± 0.02–0.47 ± 0.03ng/ml (Fig. 2e) and 0.20 ± 0.01 – 0.66 ± 0.03 ng/ml, respectively (Fig. 2f).

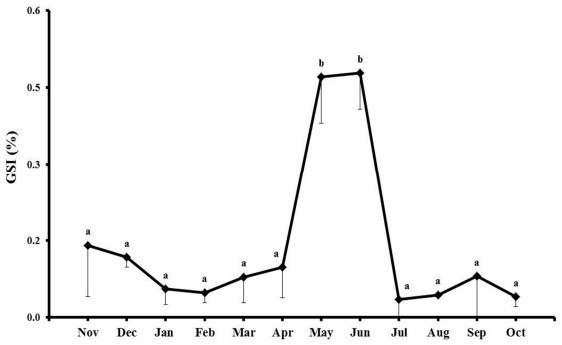
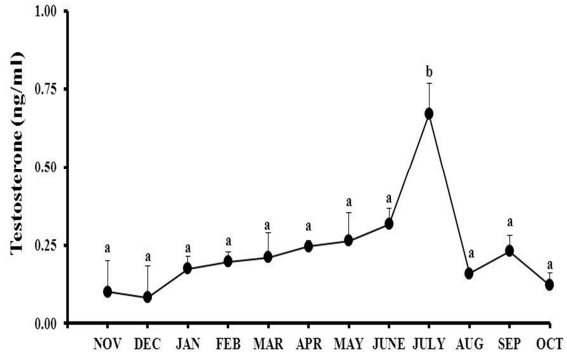
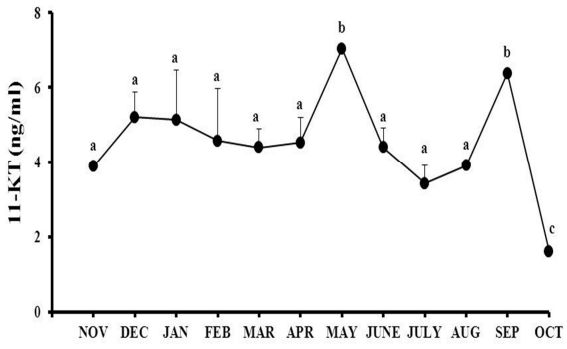


Fig. 1. Monthly variations (mean ± SE) in gonadosomatic index (GSI) during the annual reproductive cycle of male *Catla catla* (November, 2007 – October, 2008; age = 18 – 29 months). Significantly different subsets (P<0.05) given by Tukey's HSD are indicated by letters.



(a)



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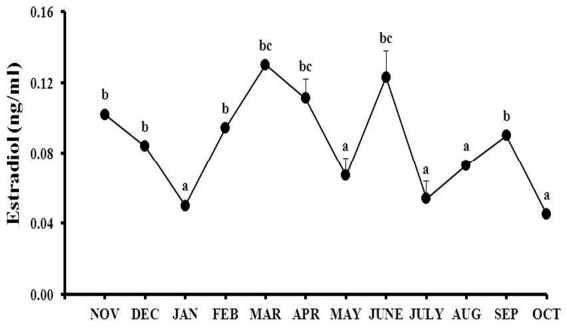
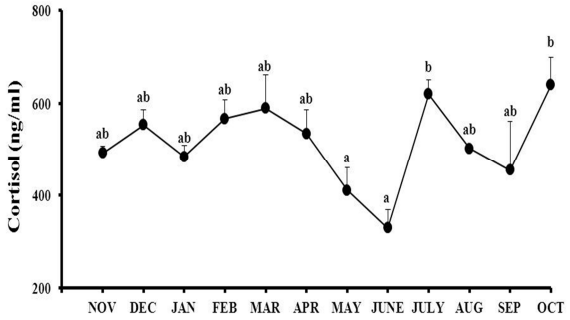
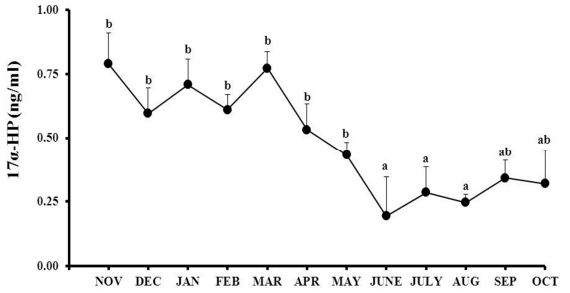


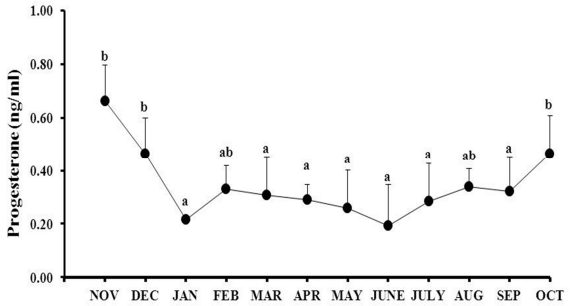
(c)



(d)



(e)



(f)

Fig. 2. Monthly variations (mean ± SE) in profile of testosterone (a), estradiol-17β (b), 11-ketotestosterone (c), cortisol (d), 17α-HP (e) and progesterone (f) during the annual reproductive cycle of male Catla catla. Significantly different subsets ($P<0.05$) given by Tukey's HSD are indicated by letters.

Discussion

In present study, gradual increase in GSI during February – March concomitant with rise in T, 11-KT might be result of spermatogonial proliferation following the winter solstice in December (Lone *et al.*, 2009). Higher levels of P and 17α-HP at this stage can be correlated to their role as substrate of T and 11-KT thus initiating spermatogenesis (Miura *et al.*, 2007) in this species. Relatively higher concentration of E_2 at this stage could be associated with its role in spermatogonial proliferation as reported previously (Song and Gutzeit, 2003).

Further testicular development i.e. initiation of meiosis and formation of spermatids might be regulated by high concentration of T during May and June as observed in other teleosts including catla (Bhattacharyya and Maitra, 2006). Peak in GSI was observed in June preceded by the peak of 11-KT while concomitant with peak in T. This finding is in contrast with that Bhattacharyya and Maitra (2006), who reported the highest value of GSI in July in same species. At this stage, milt could be extracted on manual stripping showing the final stages of development (milt hydration). These developmental changes were presumably found as a result of action of T and 11-KT and release of other growth factors mediated by these both androgens from sertoli cells (Kobayashi *et al.*, 1991).

In catla, cortisol profile showed a negative correlation with those of T and 11-KT. Cortisol secretion started decreasing from April and reached at its minimum value in June which is the period of the active spermeiogenesis. During this period concentrations of sex steroids were very high opposite to that of C consistent with the studies on rainbow trout (*Onchorhynchus mykiss*) (Pottinger *et al.*, 1996) and stellate sturgeon (*Acipenser stellatus*) (Semenkova *et al.*, 2002). According to Mommsen *et al.* (1999) elevated levels of gonadal steroids modify the pituitary-inter-renal axis thus suppressing the levels of cortisol. In present study, this inference was supported by an immediate rise in cortisol level after June, concomitant with the end of reproductive activity and low levels of progestins (P and 17α-HP) and androgens.

Spawning was not observed in catla under captivity due to lack of natural environmental cus as observed in previous study on catla (Bhattacharyya and Maitra, 2006; Lone *et al.*, 2009). Decreased values of GSI observed after July (August-October) showed the regression of testes associated with decline in levels of T and 11-KT at this stage (Tveiten *et al.*, 1998). This decrease might reflect a shift in steroidogenesis pathways from synthesis of C19 androgens (T and 11-KT) to production of C21 progestins (P and 17α-HP) (Kokokiris *et al.*, 2000) as levels of P and 17α-HP showed slight increase during this period of regressed testicular activity.

Overall, present study provides detailed data on seasonal variations in testicular development of catla and profile of important hormones involved in its regulation. This study is the first of its kind in semi-arid region of South Asia and provided the basal line reference values for reproductive parameters and will become the base for many advanced studies on the control of reproduction of this fish in semi-arid regions.

References

Bhattacharyya S, Dey R, Maitra SK. 2005. Photoperiodic regulation of annual testicular events in the Indian major carp *Catla catla*. Acta Zoologica (Stockholm) **86**, 71–79.

Bhattacharyya S, Maitra SK. 2006. Environmental correlate of the testicular events in a major carp *Catla catla* in an annual reproductive cycle. Biological Rhythm Research **37**, 87-110. http://dx.doi.org/10.1080/09291010500124605

Costa-Pierce B. 2005. Urban Aquaculture. CABI Publishers., Willingford 234 p.

Hunter JR, Macewicz BJ, Lo NCH, Kimbrell CA. 1992. Fecundity, spawning and maturity of female Dover sole *Microstomus pacific*, with an evaluation of assumption and precision. Fisheries Bulletin **90**, 101-128.

Kadhar A, Kumar A, Ali J, John A. 2014. Studies on the Survival and Growth of Fry of *Catla catla* (Hamilton, 1922) Using Live Feed. Journal of Marine Biology **2014,** 1-7.
http://dx.doi.org/10. 1155/2014/842381

Kobayashi M, Aida K, Stacey NE. 1991. Induction of testis development by implantation of 11-ketotestosterone in female goldfish. Zoological Science **8,** 389-393.

Kokokiris L, Mourot B, Le Menn F, Kentouri M, Fostie A. 2000. Endocrine changes during the annual reproductive cycle of the red porgy, *Pagrus pagrus* (Teleostei, *Sparidae*). Fish Physiology and Biochemistry **23,** 1-11.
http://dx.doi.org/10.1023/A:1007882807782

Lone KP, Fatima S, Sahar S. 2009. Gross and histological variations in testes of a major carp, *Catla catla* (Hamilton, 1822), during its first maturation cycle in pond culture system. Pakistan Journal of Zoology **41**, 483-494.

Miura C, Higashino T, Miura T. 2007. A progestin and an estrogen regulate early stages of oogenesis in fish. Biology of Reproduction **77**, 822-828.
http://dx. doi.org/10.1095/biolreprod.107.061408

Mommsen TP, Vijayan MM, Moon TW. 1999. Cortisol in teleosts: Dynamics, mechanism of action and metabolic regulation. Reviews of Fish Biology and Fisheries **9,** 211-268.

Pottinger TG, Carrick TR, Hughes SE, Balm PHM. 1996. Testosterone, 11-ketotestosterone and estradiol-17β modify baseline and stress induced internal and corticotrophic activity in trout. General and Comparative Endocrinology **104**, 284-295. http://dx.doi.org/10.1006/gcen.1996.0173

Semenkova T, Barannikova I, Kime DE, Mc Allister BG, Bayunova L, Dyubin V, Kolmakov N. 2002. Sex steroid profile of female and male stellate sturgeon (*Acipenser stellatus*) during final maturation induced by hormonal treatment. Journal of Applied Ichthyology **18**, 375-381.
DOI: 10.1046/ j.1439-0426.2002.00368.x

Sivakumaran KP, Brown P, Stoessel, Giles A. 2003. Maturation and reproductive biology of female wild carp, *Cyprinus carpio* in Victoria, Australia. Environmental Biology of Fish **68**, 321-332. http://dx.doi.org/10.1023/A:1027381304091

Song M, Gutzeit HO. 2003. Effect of 17α-ethynylestradiol on germ cell proliferation in organ and primary culture of medaka (*Oryzias latipes*) testis. Dev Growth Differ 45: 327–337.
http://dx.doi.org/10.1046/ j.1440-169X.2003.00701.x

Tveiten H, Mayer I, Johnsen HK, Jobling M. 1998. Sex steroids, growth and condition of Arctic charr broodstock during an annual cycle. J Fish Biol **53,** 714-727.