

Original Research Article

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## Seasonal Changes in the Testosterone Level in Different Age Groups of Amur Common Carp

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### ABSTRACT

The role of testosterone in reproductive performance of Amur common carp has been studied during different seasons using the RP-HPLC method from gonad and plasma of two different age groups of both sexes. Gonadal and plasma T levels in both the age groups showed similar seasonal trend with highest level in spring season, which decreased in summer and continued to decrease to the lowest level in autumn followed by slight increase in winter season. Statistically significant differences ( $p < 0.05$ ) were observed in 1+ and 2+ year's age groups in relation to age, seasons and interaction (age & seasons) for gonadal and plasma T. In both the age groups, levels of gonadal and plasma T were higher in male as compared to female. Pearson's correlations ( $p < 0.01$ ) showed significant positive correlation between GSI with gonadal and plasma T. The present study showed that seasonal changes in hormones were found to have profound effect on the scale of success of reproduction of Amur common carp, *Cyprinus carpio haematopterus*, during spring and summer seasons and 2+ year's age group showed better reproductive potential.

#### Keywords

Amur common carp, testosterone, seasonal, reproduction

#### Article Info

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### Introduction

Role of steroidal hormone testosterone on reproduction of the fish are well recognised and explored. This hormone plays a cascading effect through direct or feedback mechanisms on the reproductive functions in the fishes. Fish testes synthesize the androgenic hormones, testosterone (T) which play role in spermatogenic process, gamete maturation,

development of secondary sexual characters and induction of reproductive behaviour are well documented (Matty, 1985). Steroidal hormones and their metabolites released in water act as sex pheromone in attracting opposite sex and affecting sexual behaviour (Fostier, 1983). It controls the development and function of both male and female reproductive systems (Segner *et al.*, 2017). Seasonal change in sex steroid over the course

of their annual reproductive cycle was observed in catfish, *Clarias batrachus* (Singh & Singh, 1987) and in both sexes of *Capoeta capoeta umbla* (Erdoğan *et al.*, 2002), male and female tench (*Tinca tinca*) (Pinillos *et al.*, 2003) and relative to gonadal development in plainfin midshipman fish, *Porichthys notatus* (Sisneros *et al.*, 2004). The seasonal profile of plasma T seems to be correlated with the major changes in spermatogenesis from the renewal of stem cells to spawning of spermatozoa in freshwater spotted snakehead *Channa punctatus* (Basak *et al.*, 2016). The role of T in seasonal reproductive performance of Amur common carp has not been documented and an attempt has been made to elucidate their character in reproductive physiology.

## Materials and Methods

### Maintenance of Experimental Specimens

Amur common carp (*Cyprinus carpio haematopterus*) specimens of 1+ and 2+ year's age groups obtained from Instructional Fish Farm (IFF) of the College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar (Uttarakhand) were held in earthen ponds and later sampled for analysis. Fish were fed once daily @ 3% body weight with supplementary pellet floating feed containing 25% protein.

### Experimental Site and Climatic Conditions

The experimental site is situated at IFF inside the Experimental farm facility of College of Fisheries, G.B. Pant University of Agriculture & Technology, Pantnagar (Uttarakhand) located at the latitude of 29.01°N, longitude 79.3°E, 344 metres above mean sea level (MSL) at Tarai region, the Shivalik range of the Himalayas. The region has a sub-tropical type of climatic with very hot, humid and dry summer, monsoon and very cold winter.

### Sample Collection, Schedule and Anaesthesia

Samples for 1+ and 2+ year's age group were collected only from pond A and B during summer, autumn, winter and spring seasons. Clove oil @ 30 mg/l (Velisek *et al.*, 2005) was used to anesthetize the fish prior to regular handling or experimental procedure of the specimens. After collection of specimens from experimental ponds, the fish were anesthetized using clove oil for collection of blood for hormonal and biochemical assay. Blood samples were collected within 5-10 mins (as far as possible). The blood drawn was dispensed into a lithium heparin coated plasma tubes for hormonal estimation and another into normal serum tubes for biochemical analysis.

The heparinized blood was centrifuged at 10,000 rpm (11180 x g) for 12 mins at 4°C and the supernatant was collected in 2 ml microcentrifuge tubes and analyzed immediately or stored at below -20°C sealed with parafilm till analysis. Similarly, the whole blood sample for the biochemical study was allowed to clot for 15 - 30 mins at normal room temperature and centrifuged at 4000 rpm (1788 x g) for 10 mins at 4°C and the supernatant was collected in 2 ml microcentrifuge tubes and analyzed immediately or stored at below -20°C sealed with parafilm till analysis.

### Observations on Water Quality Parameters

Water quality parameters of the two experimental ponds which included water temperature, total dissolved solids (TDS), pH, dissolved oxygen (DO) and free carbon dioxide (CO<sub>2</sub>) were recorded during all the four sampling seasons. Temperature, TDS (accuracy ±2%) and pH (accuracy 0.01 pH) were measured using a digital meter whereas DO and CO<sub>2</sub> were analyzed using titrimetric method (APHA, 1992).

## Hormonal Estimation

Estimation of the steroidal hormones – Testosterone (T) in blood plasma were carried out using reversed phased high-performance liquid chromatography (RP-HPLC) of Dionex Ultimate 3000, operated by Chromeleon software (version 6.8).

Chromatographic condition including validation, quantitation, linearity of the assay, accuracy, stability, repeatability and precision were carried according to Soranganba and Singh, (2018).

Each aliquot was pre-treated with SPE (Solid Phase Extraction) LiChrolut RP-18 (40-63  $\mu\text{m}$ ) 100 mg 1 ml standard PP-tubes [119855] as per Budzinski *et al.*, (2006) and Chen-Hao Zhai *et al.*, (2009) with certain modifications.

## Statistical Analysis

Data were statistically analyzed by analysis of variance (ANOVA – one way). Differences were considered for significance at  $p < 0.05$ . Data were expressed in mean $\pm$ SEM.

## Results and Discussion

Observations on T in plasma and gonadal tissue of 1+ and 2+ year's age groups of Amur common carp in different seasons are shown in Tables 1 and 2 respectively. Gonadal and plasma T levels in both the age groups showed similar seasonal trend with highest level in spring season, which decreased in summer and continued to decrease to the lowest level in autumn followed by slight increase in winter season (Figures 1 & 2).

Statistically significant differences ( $p < 0.05$ ) were observed in 1+ and 2+ year's age groups in relation to age, seasons and interaction (age & seasons) for gonadal and plasma T. In both the age groups, levels of gonadal and plasma

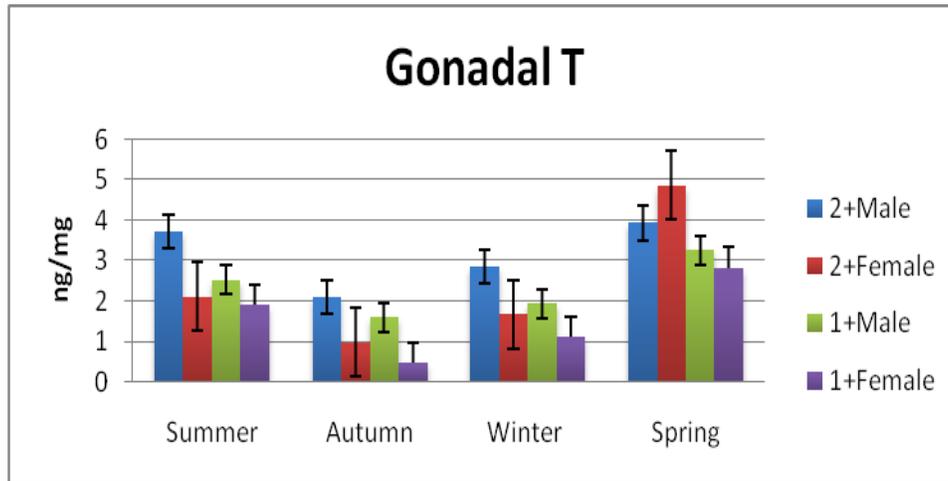
T were higher in male as compared to female. Pearson's correlations ( $p < 0.01$ ) showed significant positive correlation between GSI with gonadal and plasma T.

Gonadal and plasma testosterone (T) levels in both the age groups showed significant differences showing two major peaks – spring and summer seasons, with male having the higher androgen levels. Changes in T level corresponded with the spawning season and higher level in 2+ year's age group seems to be correlated with higher GSI and might be an indication of more active involvement and higher production demand for gonadal maturation in this groups. Roy *et al.*, (2001) observed a significant variation in catfish, *Clarias batrachus* testosterone level correlated with its annual reproductive cycle except post spawning phase. High level of seasonal changes in plasma T level was reported in other species including *Gobio gobio* (Rinhard *et al.*, 1993), *Clarias microcephalus* (Tan-Fermin *et al.*, 1997) and *H. nemurus* (Adebiyi *et al.*, 2013).

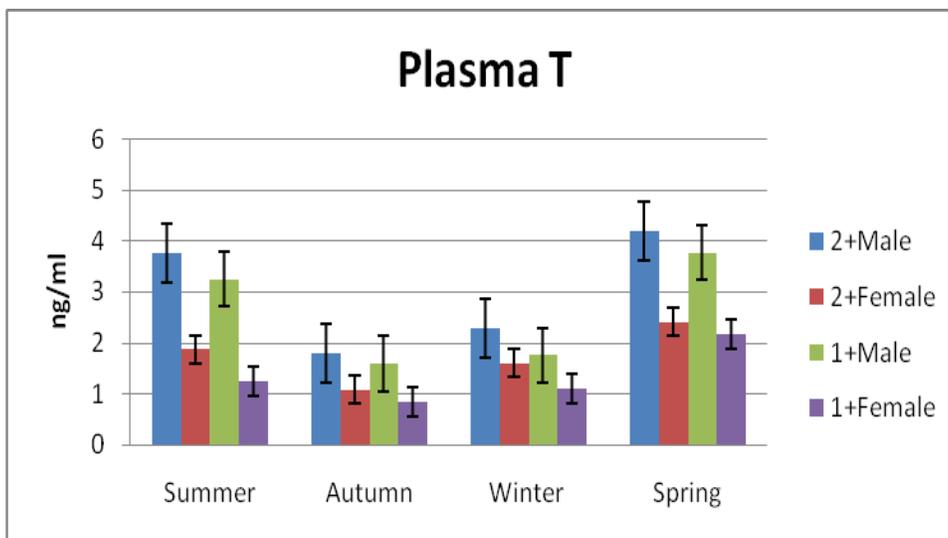
Significant positive correlation between gonadal and plasma T levels with GSI might be due to biosynthesis of this hormone in gonadal tissue and circulation via blood which occurred proportionately during spawning season required for gonadal maturation and reproduction of the species. A surge in testosterone concentrations during gametogenesis, which lasted until the end of the spawning period and coincided with the increased GSI values, have been reported in *Dentex dentex* (Fostier *et al.*, 2000; Assem *et al.*, 2016) and sturgeons (Barannikova *et al.*, 2004).

Low T level in female of both the age groups could be correlated with its involvement as a substrate for biosynthesis of E2 through aromatization (Barannikova *et al.*, 2002; Pellegrini *et al.*, 2005).

**Fig.1** Gonadal Testosterone level (ng/mg) of 1+ and 2+ male and female of Amur Common carp during different seasons



**Fig.2** Plasma Testosterone level (ng/mg) of 1+ and 2+ male and female of Amur Common carp during different seasons



**Table.1** Gonadal T (ng/mg) Levels of 1+ and 2+ Year's old Amur Common Carp in Different Seasons

Age groups	Summer	Autumn	Winter	Spring
Male 2+	3.70±0.02	2.09±0.02	2.85±0.02	3.92±0.02
Female 2+	2.10±0.04	0.96±0.02	1.66±0.03	4.85±0.06
Male 1+	2.52±0.06	1.58±0.03	1.93±0.04	3.25±0.08
Female 1+	1.89±0.04	0.46±0.02	1.09±0.03	2.81±0.07
[Data are given as mean±SEM (n=5)]				

**Table.2** Plasma T (ng/ml) Levels of 1+ and 2+ Year's old Amur Common Carp in Different Seasons

Age groups	Summer	Autumn	Winter	Spring
Male 2+	3.76±0.02	1.79±0.02	2.43±0.03	4.20±0.06
Female 2+	1.87±0.03	1.07±0.02	1.54±0.03	2.40±0.03
Male 1+	3.25±0.01	1.59±0.02	1.92±0.02	3.77±0.06
Female 1+	1.24±0.02	0.85±0.02	1.12±0.04	2.17±0.02
[Data are given as mean±SEM (n=5)]				

Based on the steroidal hormonal levels, the spring season was found to be most potent period for reproduction and seed production of Amur common carp, in the Tarai region of Uttarakhand. The seasonal changes in the levels of steroidal hormones with highest level in spring season followed by next higher level in summer seasons seem to be correlated with higher level of gonadal development and possibility of spawning under favourable environmental conditions. This study might be helpful in formulating for future research programmes towards achieving off season breeding and seed production of Amur common carp. Based on the hormonal observed during the study adult of 2+ years group may be considered more preferred age group for use in the breeding programme than 1+ year's age group. Seasonal changes in hormones were found to have profound effect on the scale of success of reproduction of Amur common carp, *Cyprinus carpio haematopterus*, during spring and summer seasons and 2+ year's age group showed better reproductive potential.

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