

Reproductive phase wise fluctuations in protein, carbohydrate and cholesterol in the blood serum of female spotted snakehead, *Channa punctata* (Bloch, 1793)

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Abstract

Spotted snakehead, *Channa punctata* found throughout water bodies of Central India. Snakehead, *C. punctata* is predaceous fish and breed once in a year in the months of June to October. This annual breeder fish has five phases in its breeding cycle viz., resting phase (mid. December- February), preparatory phase (March- April), prespawning phase (May), spawning (June- October) and postspawning phase (November- mid. December). During spawning oocytes accumulates yolk which is heterogeneous material comprising all nutrient component. For vitellogenesis; protein, carbohydrate and fats derive from different tissues particularly from liver and supplied to maturing oocytes. Present work by biochemical estimation of protein, carbohydrate and cholesterol of blood serum in different phases of reproductive cycle tried to understand the trends of fluctuations of protein, carbohydrate and cholesterol in serum along different phases of reproductive cycle.

Keywords: *Channa punctata*, protein, carbohydrate and cholesterol.

1. Introduction

Spotted snakehead, *Channa punctata* (Bloch, 1793) are ambush predators, living in still waters although some inhabit the larger rivers and are commercially high prized fish (Chakravarti, 2006). Fry Snakehead mostly feeds on zooplankton and phytoplankton following yolk sac absorption (Munshi and Hughes, 1992). As juveniles, they feed on insect larvae, small crustaceans, and fry of other fishes (Munshi and Hughes, 1992). Predaceous adult consumed crustaceans, frogs, smaller reptiles, and sometimes young birds and small mammals, with many species preferring other fishes (Welcomme, 1985).

Depending upon the frequency of spawning in a year, teleosts are termed as annual breeder or multiple breeder. Annual breeder teleosts have different phases in single annual reproductive cycles.

These different phases or stages of the reproductive cycle are characterized by different parameters like the presence of morphology of ovary, oocyte stages, gonadosomatic index (GSI) etc. On the basis of GSI, different phases of reproductive cycles are identified in *Heteropeustes fossilis* (Sonparote *et al.*, 2012), *Channa striatus* (Sonparote *et al.*, 2015), *Labeo rohita* (Sonarghare *et al.*, 2015) and *C. punctata* (Kulsange and Masram, 2017). *Channa punctata* is annual breeder and many tissue including ovary undergo drastic morphological changes during different phases of reproductive cycle (Kulsange and Masram, 2016) and its reproductive cycles has five phases viz., resting phase (mid. December- February), preparatory phase (March- April), prespawning phase (May), spawning (June- October) and postspawning phase (November- mid. December) (Kulsange and Masram, 2017). The cyclical changes occurred during different phases of reproductive or breeding cycle affect different organs. Prominent among them are ovary, kidney, liver, and blood. It altered histology and profile of different biomolecules including proteins, carbohydrates, and cholesterol.

2. Materials and Methods

Sexually mature female *Channa punctata* were collected from the Futala Lake, Shukravari Lake and Gokulpeth fish market, Nagpur (M.S.). All fishes were collected and transported alive to the laboratory and maintained in fish aquarium of size 3×2×1.5 feet rectangular tanks, and acclimatize them for at least 8 days. The tank will supply with fresh water maintained at 28°C ±2. Fish were fed with a diet of goat liver once in the day under normal photoperiodic condition. Blood was collected from the caudal vein in non-heparinized syringe. Blood samples were centrifuged at 3000g for 20 min. Serum thus obtained were stored at - 40°C. Serum samples were analysed for total protein, total carbohydrate and total cholesterol following Lowry method (Lowry *et al.*, 1951),

Anthrone reagent method (Dubois *et al.*, 1956) and ferric chloride method (Zlatkis *et al.*, 1953) using Elico SL 177 spectrophotometer.

In all the estimations, at least six samples were tested from different fishes (n=6).

3. Results

3.1 Protein

Maximum protein content 27.86 ± 0.71 mg/ gm of wet tissue in blood serum is reported in resting phase (Table 1, Graph 1) and it lowered very significantly ($p < 0.01$) to 19.69 ± 0.32 mg/ gm of wet tissue in preparatory phase (Table 1, Graph 1). In prepsawning phase, protein content is 17.11 ± 0.40 mg/gm of wet tissue (Table 1, Graph 1). From preparatory phase no significant change occurred in protein content in blood serum of prepsawning phase ($^+p > 0.05$), but when compare the protein content of prepsawning phase with resting phase, extreme fall in protein content observed ($p < 0.0001$). Protein content (14.81 ± 0.28 mg/ gm of wet tissue) in blood serum observed further lowered significantly when compare with resting phase ($p < 0.001$) and significantly when compare with prepsawning phase ($^+p < 0.05$). Protein content increases very significantly in postspawning phase from prepsawning phase ($^+p < 0.01$) and it is 23.04 ± 0.45 mg/ gm of wet tissue in postspawning (Table 1, Graph 1) but still lowered significantly than the protein content of resting phase blood serum ($p < 0.05$). Thus there is fluctuation in protein content in blood serum in different phases of reproductive cycle in *C. punctata*. Protein content lowered from resting phase to spawning phase and later rise in protein content observed in postspawning phase.

3.2 Carbohydrate

A total carbohydrate in resting phase blood serum is 12.98 ± 0.32 mg/gm of wet tissue (Table 1, Graph 2). No significant change observed in carbohydrate content in preparatory phase ($p > 0.05$) and it is reported to be 12.10 ± 0.43 mg/ gm of wet tissue (Table 1, Graph 2). In prepsawning phase, carbohydrate content remains almost stable to 12.62 ± 0.12 mg/gm of wet tissue (Table 1, Graph 2) which is not significantly different from the carbohydrate contents of resting phase blood serum ($p > 0.05$) and preparatory phase blood serum ($^+p > 0.05$). Similarly, in spawning phase blood serum, carbohydrate content is 12.08 ± 0.17 mg/ gm of wet tissue (Table 1, Graph 2) which do not vary significantly from carbohydrate content of resting phase blood serum ($p > 0.05$) or prepsawning phase blood serum ($^+p > 0.05$). Carbohydrate content of postspawning blood serum is 11.85 ± 0.50 mg/ gm of wet tissue (Table 1, Graph 2). No variation in carbohydrate content of blood serum noted when compare with carbohydrate

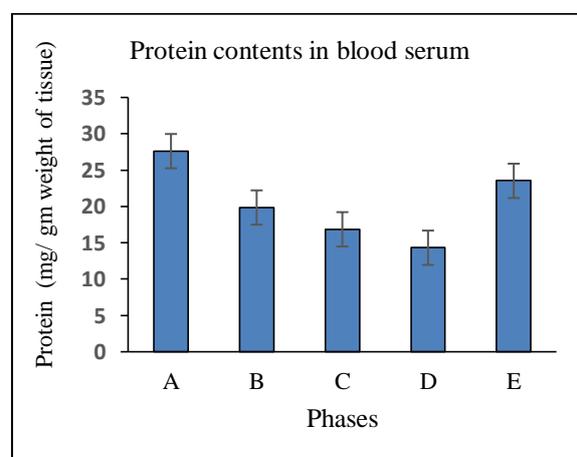
content of resting phase blood serum ($p > 0.05$) and spawning phase blood serum ($^+p > 0.05$). With respect to carbohydrate content, no variation observed in different phases of reproductive cycle of *C. punctata*.

3.3 Cholesterol

Cholesterol content of resting phase blood serum is 3.79 ± 0.23 mg/gm of wet tissue (Table 1, Graph 3) which lowered very significantly ($p < 0.01$) to 3.52 ± 0.28 mg/ gm of wet tissue (Table 1, Graph 3) in preparatory phase. In prepsawning phase, cholesterol content (1.89 ± 0.11 mg/gm of wet tissue) (Table 1, Graph 3) of blood serum significantly decreases from resting phase ($p < 0.05$) and preparatory phase ($^+p < 0.05$). Further the cholesterol content remains stable at 1.67 ± 0.86 mg/gm of wet tissue (Table 1, Graph 3) in spawning phase blood serum ($^+p > 0.05$) but if compared the value of cholesterol content of spawning blood serum with resting phase blood serum significant decline observed ($p < 0.05$). From spawning phase, significant rise ($^+p < 0.05$) in cholesterol of blood serum is noted in postspawning at 3.37 ± 0.75 mg/gm of wet tissue (Table 1, Graph 3) and almost attained the level of cholesterol content of resting phase blood serum ($p > 0.05$). In case of cholesterol thus from resting phase to spawning phase there is gradual decline. Cholesterol content of blood serum rises in postspawning phase equalized with cholesterol content of resting phase blood serum.

3.4 Correlation

Correlation matrix indicate strong negative correlation between protein and carbohydrates throughout reproductive cycle ($r = -0.97$). Protein show moderate correlation with cholesterol ($r = 0.65$). Carbohydrate have moderate negative correlation with cholesterol all along the reproductive cycle in blood serum of *C. punctata* ($r = -0.52$) (Table 2).



Graph 1: Variation in serum protein, A- Resting phase, B- preparatory phase, C- prepsawning phase, D- spawning phase, E- postspawning phase.

Table 1. Variation in total proteins, carbohydrates and cholesterol contents (mg/gm weight of tissues) in blood serum.

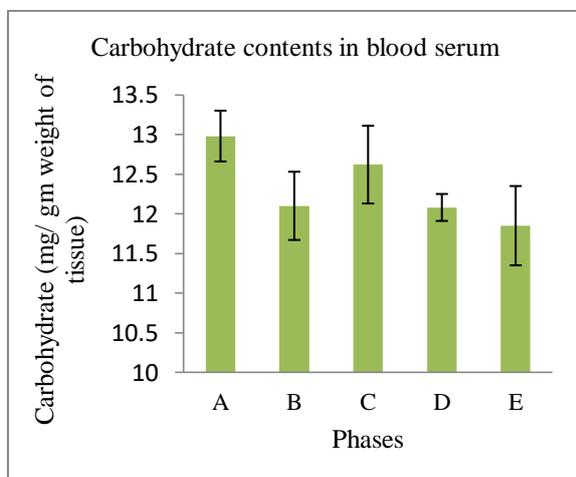
Phases	Biochemical contents (mg/ gm of wet tissue) Mean ± SE		
	Proteins	Carbohydrates	Cholesterol
Resting	27.86 ± 0.71	12.98 ± 0.32	3.79 ± 0.23
Pre-paratory	19.69 ± 0.32 p < 0.001	12.10 ± 0.43 p > 0.05ns	3.52 ± 0.28 p < 0.001
Pre-spawning	17.11 ± 0.40 p < 0.0001 +p > 0.05ns	12.62 ± 0.49 p > 0.05ns +p > 0.05ns	1.89 ± 0.11 p < 0.05 +p < 0.05
Spawning	14.81 ± 0.28 p < 0.001 +p < 0.05	12.08 ± 0.17 p > 0.05ns +p > 0.05ns	1.26 ± 0.20 p < 0.05 +p > 0.05ns
Post-spawning	23.04 ± 0.45 p < 0.05 +p < 0.001	11.85 ± 0.50 p > 0.05ns +p > 0.05ns	3.37 ± 0.75 p > 0.057ns +p < 0.05

p- When value is compared with value of resting phase.
+p when compared with preceding phase. ns – non significant difference, significant at p < 0.05, very significant at p < 0.01, extremely significant at p < 0.001, extremely significant at p < 0.0001, Data of all phases passed the Normality test with p > 0.05.

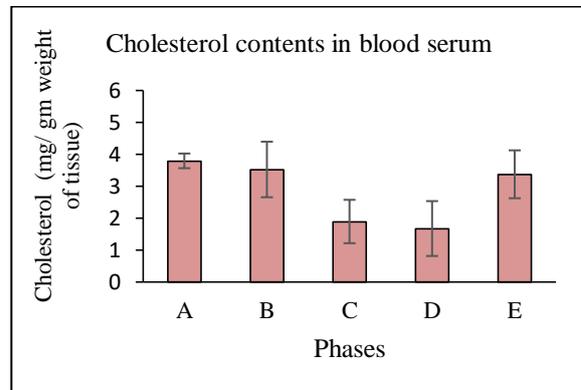
Table 2. Correlation between total protein, carbohydrates and cholesterol contents in blood serum during reproductive phases of *C. punctata*.

	A	B	C
A	1.00		
B	-0.97	1.00	
C	0.65	-0.52	1.00

A-Protein, B- Carbohydrate and C- Cholesterol



Graph 2. Variation in carbohydrate contents in blood serum during reproductive phases, A- Resting phase, B- preparatory phase, C- prespawning phase, D- spawning phase, E- postspawning phase.



Graph 3. Variation in cholesterol contents in blood serum during reproductive phases of *C. punctata*, A- Resting phase, B- preparatory phase, C- prespawning phase, D- spawning phase, E- postspawning phase.

4. Discussion

Present study recorded gradual decrease in protein, carbohydrate and cholesterol in blood from resting phase to spawning phase of reproductive cycle and subsequent rise of this component in postspawning phase.

Relationship of fluctuation of serum protein with plasma volume was revealed in Caspian kutum, *Rutilus frisil kutum* (Imanpoor *et al.*, 2011). Decline in plasma volume and serum protein further would in turn influenced by prolonged starvation or stress (Azarin *et al.*, 2012) to which females of teleosts fish are subjected during the spawning. With respect to other tissue like ovary, testis, liver and muscles lowest protein content was reported in blood serum during preparatory phase in *Notopterus notopterus* (Khaparde *et al.*, 2016)

Similarly, carbohydrate depletion in serum during spawning is due increased depletion of liver glycogen (Ojolick *et al.*, 1995).

There is contradiction in report of serum cholesterol during different phases of reproductive cycle. Increase in cholesterol level along vitellogenesis progression arriving peak during the during prespawning and thereafter lowered cholesterol level in postspawning phase is reported in *Cyprinus carpio* (Abbassi *et al.*, 2011) while in Tench, *Tinca tinca*, lowest blood cholesterol was reported during the spawning phase with high GSI (Svoboda *et al.*, 2001). Similar to *T. tinca*, in *C. punctata* also lowest serum cholesterol reported which further rise during the postspawning phase.

5. Conclusions

In *C. punctata*, from resting phase to spawning phase of reproductive cycle, protein and cholesterol lowered and then there is rise in protein and cholesterol content of serum in postspawning phase. No alteration observed in carbohydrate content in serum throughout the reproductive cycle.

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