




Are haematological and biochemical properties of freshwater spotted snakehead *Channa punctata* (Bloch, 1793) season and sex dependent?


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Abstract

Freshwater murrel, *Channa punctata* commonly known as “spotted snakehead” is a popular fish because of its taste and price. Haematological and biochemical profile of fish helps in understanding the fish health and play an important role in fisheries management. The present work was carried out to determine season (summer, monsoon and winter) and sex dependent variations in the blood profile of *C. punctata*. Haematological [red blood cell counts (RBC), packed cell volume (PCV) and haemoglobin (Hb)] and serum biochemical parameters [blood glucose, total protein, cholesterol, triglyceride (Tg), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), serum calcium levels] of *C. punctata* were analysed which varied significantly with seasons ($p < 0.05$). The level of glucose, total protein, Tg, VLDL, RBC, Hb and PCV was low during monsoon season because of the growth and development of gonads compared to the summer and winter season. The level of cholesterol and LDL was high during monsoon. However, haematological and biochemical parameters were not sex dependent. The seasonal haematological and biochemical variations in the fish were mainly due to the physiological acclimatisation of the fish to variations in the environmental condition and feeding regime.

Keywords: *Channa punctata*; fish health; fish physiology; murrel; physiology; seasonal variation

1 | INTRODUCTION

Blood contains cells, proteins, sugars and fat particles from 1.3 to 7 percent of the total body weight of the fish (Acharya and Mohanty 2014). The studies of haematology and blood biochemistry of the fish are used to detect the physiological and pathological conditions in order to know the stress and health conditions which are considered important for fisheries management (Alyakrinskyaya and Dolgova 1984). The haematological and biochemical

profile of the blood of the fish can be affected by several factors including age, health condition, size, sex, reproduction, season, photoperiod, water temperature, food, stress, pollutions and diseases. The response of the fish to the different environmental conditions is dependent on the haematological and biochemical parameters of the fish. The knowledge of the blood parameters, therefore, helps in understanding the relationship of the blood characteristics of the fish to their habitats and environment.

In hatchery condition, haematocrit, red blood cells (RBC) and haemoglobin (Hb) concentrations are frequently used haematological parameters for the assessment of the condition of the fish (Bhaskar and Rao 1990). Ebeh *et al.* (2017) studied the effect of season and reproductive cycle on blood parameters of *Protopterus annectens*. Variations in the blood parameters of different fish populations were also revealed in different studies (e.g. Percin and Konyalioglu 2008; Zorriehzahra *et al.* 2010; Fazio *et al.* 2016).

The snakehead murrel, *Channa punctata* (Bloch, 1793) is an important freshwater fish, commonly known as “spotted snakehead”. It is a lean fish because of low lipid contents throughout the year (Ghosh 2006). This fish has great economic importance due to popularity as food and commercial value (Galib and Samad, 2009; Galib *et al.* 2010; Kashyap 2015). *Channa punctata* is distributed throughout the south Asian countries like Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar and Yunnan in China (Froese and Pauly 2019). The fish is commonly found in rivers and ponds (e.g. Chaki *et al.* 2014; Galib *et al.* 2016, 2018) but has also been reported from brackish water (Pethiyagoda 1991) and beels (wetlands) and ditches (Rahman 1989; Imteazzaman and Galib 2013). The survey of literature indicated the paucity of information on the blood parameters of *C. punctata*. Only few researchers carried out the work on the different aspects of the physiology and biochemistry of the *C. punctata*. Srivastav and Srivastav (1998) studied changes in serum calcium and phosphate levels during vitellogenesis and testicular maturation in *C. punctata*. Malathi *et al.* (2012) compared blood parameters between the two channa species, *C. punctata* and *C. striata*. The effect of heavy metals in effluents on biomolecules of different tissues of *C. punctata* were also reported (Javed and Usmani 2015; Kalsoom and Nasreen 2019). Several studies (e.g. Javed *et al.* 2016; Parveen *et al.* 2017; Talukdar *et al.* 2017) showed the effects of effluents and acid mine drainage discharged from tannery, thermal power plant and coal mines respectively on haematological parameters of *C. punctata*. Abdel-Hameid *et al.* (2017) worked to find the requirement of copper to maximize the growth of fingerlings of *C. punctata*. Therefore, keeping in mind the paucity of the information on the blood characteristics the present study was under taken to find out the seasonal and sex wise variability in blood parameters of *C. punctata*.

2 | METHODOLOGY

2.1 Sampling

The specimens of *C. punctata* were collected using cast and drag nets with the help of a professional fisherman from the river Gomti at Lucknow (26°52' N 80°55' E) during monsoon, winter and summer seasons. A total of 30

specimens of the fish were used for the biochemical analysis after identification of the sex by observing their gonads. Ten specimens in each season (5 males and 5 females) were analysed. Total length (cm) and weight (g) of each specimen of the fish were measured using fish measuring board and weighing machine respectively. Blood was taken out from the heart of the fish with the help of syringe and immediately transferred into three different types of the vials: EDTA vial for haematological parameters, serum vial for calcium and biochemical parameters and fluoride vial for glucose. Blood samples of serum and fluoride vials were kept for half an hour at room temperature and centrifuged at 3000 rpm for 10 minutes to separate the serum and plasma for the analysis of biochemical parameters. All blood parameters were analysed at the Clinical Laboratory of the Department of Biochemistry, King George’s Medical University, Lucknow.

2.2 Estimation of haematological parameters

The haematological parameters such as RBC, Hb concentration, packed cell volume (PCV) were analysed using Haematology 5 Parts Auto-analyser. The values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using formulas as given by Dacie and Lewis (1984).

2.3 Estimation of biochemical parameters

The biochemical parameters such as glucose, total protein, triglyceride (Tg), cholesterol and high density lipoprotein (HDL) were estimated using their kits under Semi Biochemistry Auto analyser (Accurex AT112, Japan). Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated following Friedewald *et al.* (1972). Serum calcium was estimated with the help of the kit (Randox Laboratories Ltd. Crumlin, UK) based on colorimetric method using Electrolyte Auto Analyser (Caretium machine).

2.4 Data analysis

Means of different biochemical parameters were compared seasonally and sex wise using univariate analysis of variance (ANOVA) at 5% significant level ($p < 0.05$). All the calculations were carried out using SPSS (version 12.0).

3 | RESULTS

Total length (mean \pm SD) of fish was recorded as 19.7 \pm 1.56, 20.51 \pm 1.68 and 19.45 \pm 1.51 cm in the specimens of summer, monsoon and winter season respectively. The mean total weight (mean \pm SD) of fish in summer, monsoon and winter season was 78.12 \pm 18.80, 93.1 \pm 23.05 and 78.61 \pm 17.64 g respectively.

Biochemical parameters of blood plasma (glucose, total protein, cholesterol, Tg, LDL, VLDL, serum calcium) and

haematological parameters (RBC, Hb, PCV) were significantly different ($p < 0.05$) between the seasons but these parameters were not significantly different ($p > 0.05$) between sexes (Table 1). The levels of biochemical parameters such as glucose, total protein, cholesterol, Tg, LDL, VLDL and haematological parameters (RBC, Hb, PCV) recorded during monsoon were significantly different ($p < 0.05$) from winter and summer while no significant difference ($p > 0.05$) in any of these parameters was recorded during winter and summer (Figures 1 and 2). The level of glucose, total protein, Tg, VLDL, RBC, Hb, PCV were the lowest during monsoon compared to winter and summer while level of cholesterol and LDL were higher during monsoon. The serum Ca level of the fish varied significantly among seasons ($p < 0.05$) and maximum value was recorded during winter whereas minimum was recorded in monsoon (Figure 2).

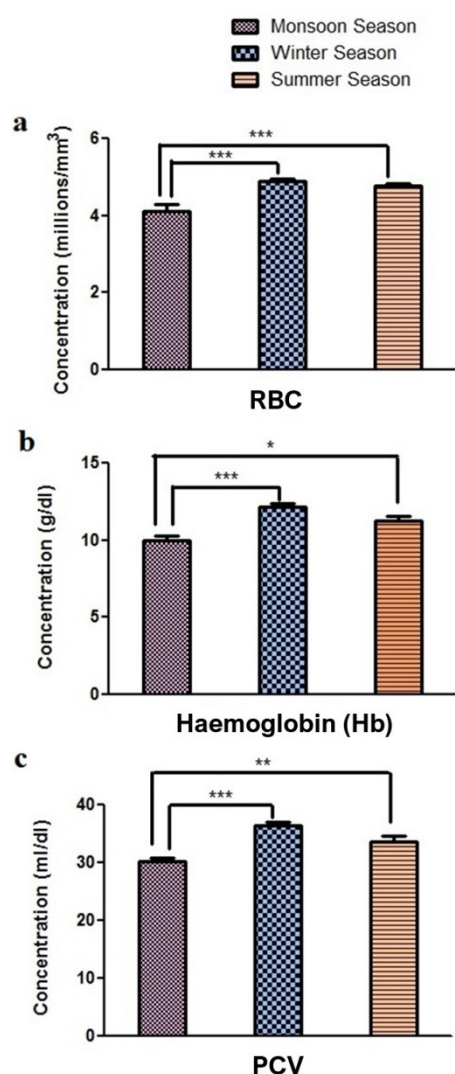


FIGURE 1 Mean \pm SEM of haematological parameters in different seasons: (a) RBC (b) Hb (c) PCV where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

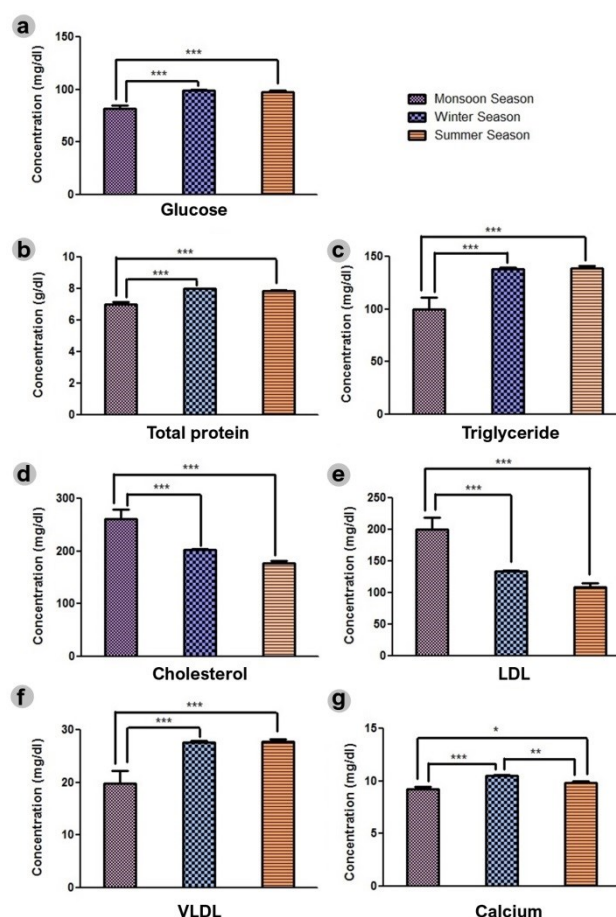


FIGURE 2 Mean \pm SEM of biochemical parameters in different seasons: (a) Glucose (b) Total Protein (c) Triglyceride (d) Cholesterol (e) LDL (f) VLDL (g) Ca where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4 | DISCUSSION

The significant seasonal variability of the haematological parameters (RBC, Hb and PCV) recorded in the present study may be due to the fluctuations in dissolved oxygen concentration of the water and metabolic rate of the fish. Low level of RBC, Hb and PCV recorded during the monsoon season which may be because of availability of high level of dissolved oxygen in water as rivers are rejuvenated with water due to rainfall and less number of RBC is required in the blood to carry out the oxygen (Valverde and García 2005). However, there was no significant difference in the amount of RBC, Hb and PCV during winter and summer seasons. Fallah *et al.* (2014) also reported the seasonal fluctuations in the levels of RBC, Hb and PCV and emphasised that the values of RBC, Hb and PCV may increase during winter because of the depletion of oxygen in the water in order to cater the demand of the fish. No seasonal variation in MCV, MCH and MCHC was recorded in *C. punctata*. Similarly, a number of studies (e.g. Fallah *et al.* 2014; Latif *et al.* 2015) also reported no seasonal variation in MCV, MCH and MCHC while Pradhan *et al.* (2014) reported similar result for MCV in *Labeo rohita*.

TABLE 1 Variation in blood parameters across seasons.

Parameters	SS	df	MS	F	p-value
Glucose					
Between groups	2046.8	2	1023.4	29.22	<0.001
Within groups	945.8	27	35.0		
Total	2992.7	29			
Total protein					
Between groups	6.6	2	3.3	41.63	<0.001
Within groups	2.1	27	0.1		
Total	8.7	29			
Cholesterol					
Between groups	31053.1	2	15526.5	19.23	<0.001
Within groups	21804.3	27	807.6		
Total	52857.4	29			
Tg					
Between groups	9182.9	2	4591.5	11.150	<0.001
Within groups	11118.1	27	411.8		
Total	20301	29			
HDL					
Between groups	1.854	2	0.9	0.087	0.917
Within groups	287.449	27	10.6		
Total	289.303	29			
LDL					
Between groups	56643.1	2	28321.6	19.54	<0.001
Within groups	39127.9	27	1449.2		
Total	95771.1	29			
VLDL					
Between groups	4688.3	2	2344.2	5.28	0.012
Within groups	11986.5	27	443.9		
Total	16674.9	29			
Serum Ca					
Between groups	9.4	2	4.7	23.65	<0.001
Within groups	5.4	27	0.2		
Total	14.8	29			
RBC					
Between groups	2.9	2	1.5	16.20	<0.001
Within groups	2.5	27	0.1		
Total	5.4	29			
Hb					
Between groups	22.1	2	11.0	14.58	<0.001
Within groups	20.5	27	0.8		
Total	42.6	29			
PCV					
Between groups	174.5	2	87.2	14.81	<0.001
Within groups	159.0	27	5.9		
Total	333.5	29			

TABLE 1 Continued.

Parameters	SS	df	MS	F	p-value
MCV					
Between groups	76.7	2	38.3	1.20	0.318
Within groups	866.6	27	32.1		
Total	943.3	29			
MCH					
Between groups	9.6	2	4.8	1.43	0.256
Within groups	90.7	27	3.4		
Total	100.4	29			
MCHC					
Between groups	88.5	2	44.3	2.13	0.139
Within groups	561.4	27	20.8		
Total	649.9	29			

Bold values indicate outcomes significant at $p \leq 0.05$.

The lowest level of glucose was observed during monsoon season which may be because of the demand of more energy for the growth and development of gonads of the fish as the monsoon is the breeding season of *C. punctata* and the gonadal activity of the fish is considered to be at its peak at this stage (Kashyap *et al.* 2016). A number of researchers such as Gutiérrez *et al.* (1988), Kocaman *et al.* (2005), Bani and Vayghan (2011) and Nasari *et al.* (2014) also reported the decreased level of glucose during breeding season because of the development of gonads in the various fish species. Bani and Vayghan (2011) emphasised that the gonadal development cause the decrease in glucose level to fulfil the high energy demand in fishes. Gutiérrez *et al.* (1988) reported that insulin level increases during pre-spawning period which resulted into the low level of glucose necessary for promoting uptake of vitellogenin in oocytes. There was no significant difference in glucose level during winter and summer seasons in the present study. Release of glucose from glycogen stored in liver may increase the serum glucose level to minimise stressful temperature conditions or low availability of food in these seasons. Lermen *et al.* (2004) reported decreased hepatic glycogen level at low and high temperature in their studies.

The lowest level of total protein was also recorded during monsoon in the present study because of the utilisation of protein in the development and building up of the gametes in the fish. Hutchinson and Manning (1996) and Svobodova *et al.* (1997) reported low level of total protein during reproduction. There was no significant difference in the level of total protein between winter and summer seasons because the fish maintain a constant serum protein level for the homeostatic condition at low and high temperature. Serum protein level was found to be high at stress condition like starvation.

The level of Tg was lowest during monsoon in the present study may be because of its use in gonadal development. The low level of Tg was also noticed at late gonadal maturation stage in other studies (e.g. Kocaman *et al.* 2005; Nasari *et al.* 2014). Cholesterol is essential for the production of gonadal and adrenal steroid hormones and the level of cholesterol was found to be high during monsoon season in the present study. Babin and Vernier (1989) pointed out that nutritional, physiological and developmental states highly influence the plasma cholesterol level in fish.

LDL level was also high during monsoon in the present study. Consumption of lipids rich diet might have caused its increased level. VLDL is a lipoprotein and was lowest during monsoon in the present study which carries mainly large quantity of triglyceride. Thus the decrease in the level of serum Tg may decrease the VLDL level in serum. Tg, cholesterol, LDL and VLDL levels were the same during winter and summer seasons. No significant difference was found in cholesterol level in low and high temperature in the study carried out by Rossi *et al.* (2017) which indicates that the fish maintain its homeostasis in these seasons.

Serum Ca level was high during winter while low in monsoon in the present study. Calcium plays important role in gonad maturation mainly in vitellogenin formation in female. Vitellogenin is a serum protein takes part in yolk formation. Deposition of vitellogenin bound Ca in yolk may lead the low level of serum Ca. High level of Ca was reported by Srivastav and Srivastav (1998) during winter also in fish.

In present study, no significant difference was found in levels of all studied blood parameters between male and female individuals in any season. Several studies (Percin and Konyalioglu 2008; Pradhan *et al.* 2014; Ebeh *et al.* 2017) also reported similar result. Same metabolic activity of male and female may be the reason of equal level of blood contents in the body.

5 | CONCLUSION

The haematological and serum biochemical parameters significantly vary with seasons in *C. punctata*. The level of glucose, total protein, Tg, VLDL, RBC, Hb and PCV decreases during monsoon season because of the growth and development of gonads. This study of haematology and blood biochemistry of the fish could be used to detect the physiological and pathological condition of the fish with a view to determining their stress and health conditions which is considered important for fisheries management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the datasets generated and analysed in this study are included in the manuscript and presented as tables and figures.

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CONTRIBUTION OF THE AUTHORS

M Singh designed the study, reviewed the literature, carried out the sample collection and data, laboratory work and prepared the first draft of manuscript; **AK** carried out the statistical analysis of collected data and helped in preparation of first draft of manuscript; **M Serajuddin** proposed and supervised the study, selected the species and revised the draft manuscript. All authors read and approved the final manuscript.



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