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HAEMATOLOGICAL CHANGES IN *BARBODES CARNATICUS* (JERDON, 1849) IN RELATION TO SEASONAL VARIATION IN RIVER CAUVERY BASIN

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ABSTRACT : Assessment of haematological parameters provides an integrated measure of fish health status. We conducted this study to evaluate the seasonal variations on the haematological parameters of an important commercial freshwater fish, *Barbodes carnaticus* during three major tropical seasons; pre-monsoon, monsoon and post-monsoon. Fluctuations in haematological parameters such as total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of *B. carnaticus* were assessed. Haematological parameters such as TEC and haematocrit (Hct) recorded significantly higher values ($P<0.05$) during monsoon. Whereas, the MCH and MCHC values were significantly ($P<0.05$) higher in post-monsoon. The results indicate that TEC, TLC, Hb and Hct significantly correlated ($P=0.05$) with water temperature and Hb significantly correlated with rainfall. The values of TLC were found high (33207 cells/mm^3) during hot months of pre-monsoon, but no significant variations between seasons. The relation between seasonal variations in water temperature with haematological parameters is discussed. The baseline data generated in this study can be effectively used as a tool to monitor health status of *B. carnaticus* for sustainably managing the fish in tropical aquatic systems in a changing climate scenario.

Key words : Haematological parameters, health indicators, *Barbodes carnaticus*, climatic variations.

INTRODUCTION

Fish require an ambient aquatic environment for growth and development. However, the environment of fish is mainly affected by temperature, rainfall patterns and photoperiod, which indirectly affect the behaviour, physiology, breeding cycles of these species. It is well documented that the environmental conditions are directly or indirectly regulated by the season.

The most important body fluid, the blood, is very sensitive to environmental fluctuations (Adeyemo, 2008). Haematological parameters are considered as an important diagnostic tool to assess the health condition of fish. Some of the physiological factors of fish, such as sex, size, health status and reproduction can alter the haematological parameters (Farzin *et al*, 2014). These factors can also be influenced by external factors such as seasonal variations, water quality, diet, temperature, pollutions and diseases (Pradhan *et al*, 2012; Satheeshkumar *et al*, 2011; Adeyemo, 2003; Koteshwar Rao *et al*, 2015). Studies on haematological indices in fishes have assumed greater significance due to the awareness on impact of climatic variations and anthropological pollution of natural freshwater resources in the tropical region (Khan *et al*, 2012; Manveer & Rauthan, 2015). Hence, haematological studies have been used successfully as sensitive indices to monitor physiological and pathological changes in fishes which can be used as possible indicators in toxicological research and environmental monitoring for fishery management disease investigation (Mulcahy, 1975; Manveer & Rauthan, 2015). Recent reports on blood parameters of *Barilius bendelisis* (Sharma *et al*, 2016), *Mastacembelus armatus* (Manveer & Rauthan, 2015), *Channa marulius* (Latif *et al*, 2015), *Oreochromis niloticus* (Bezerra *et al*, 2014), *Esox lucius* (Farzin *et al*, 2014), *Tor putitora* (Gupta *et al*, 2013), *Alburnoides eichwaldii* (Kohanestani *et al*, 2013), the Indian major carps, *Catla catla, Labeo rohita* and *Cirrhinus mrigala* (Pradhan *et al*, 2012; Das *et al*, 2006), *Puntius sarana* (Das *et al*, 2012) and *Puntius filamentosus* (Vijayakumari & Murali, 2012) etc. indicate the impact of changing seasons on the haematological parameters. Scarcity of information on haematological parameters of minor carps made us to undertake the present study in order to assess the haematology of *Barbodes carnaticus,* a cyprinid fish, commonly known as Carnatic carp is an endemic species to the Western Ghats of India. Among the members of *Barbodes* genus, this species attains larger size (Talwar & Jingran 1991). This fish is a preferred food fish and forms minor fishery from the several reservoirs of the River (Ali & Raghavan, 2013). Despite the importance of minor carps in India very limited attempts has been made to study the haematological parameters. With this view, the present study was conducted to assess the variations in haematological parameters during different seasons of the year.

MATERIALS AND METHODS

A total of 120 *Barbodes carnaticus* ranging between 31.5cm to 39.5cm in length and 400g to 750g in weight were collected using cast nets between January 2015 and December 2015 from river Cauvery at Shivanasamudra (Fig. 1), Karnataka, India (latitude of 12.2706 N and longitude of 77.1648 E). Forty fishes were used for the haematological study in each season. The sampling was conducted every month and the data was pooled seasonally *viz.* pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January) based on the climatic conditions of South India. Fishes were handled with care to minimize the stress effects. The physico-chemical parameters like water temperature and dissolved oxygen were examined on a monthly basis by using standard digital field kit (HQ40d, 582580). The rainfall data were collected from Karnataka state natural disaster monitoring centre, Government of Karnataka. For collecting the blood samples, the surface of the fish at the caudal peduncle was cleaned with blotting paper to avoid contamination. The blood samples were drawn from caudal vein of fishes by using 2ml sterile disposable plastic syringe (23G) having a drop of an anticoagulant (10% EDTA) and expelled into the vials containing EDTA to avoid clotting. The vials containing blood samples were placed immediately in the ice box until to reach laboratory for analysis.

The total erythrocyte count (RBC) and leukocyte count (WBC) were done using an improved Neubauer-type haemocytometer (Pradhan *et al*, 2014a; Guijarro *et al*, 2003). The number of RBC's/WBC's was expressed as cells per cubic millimetre and was calculated as follows.

$$\text{Total number of RBC's /WBC's} = \frac{\text{Number of cells} \times \text{dilution factor} \times \text{depth factor}}{\text{Area counted}}$$

The haemoglobin (Hb) content was estimated by acid–Haematin method using Sahli's haemocytometer. The amount of haemoglobin was directly read in g/dL. The haematocrit (Hct) was determined by using micro-

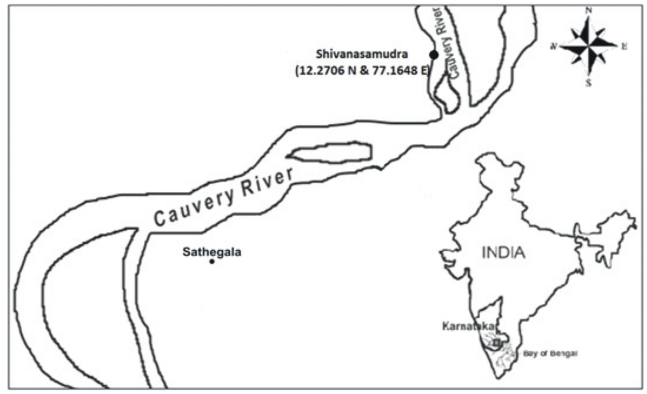

Fig. 1 : Map showing sampling location of *Barbodes carnaticus* from River Cauvery at Sivanasamudra, Karnataka, India.

Table 1 : Seasonal variations in the temperature and dissolved oxygen at the sampling station. All values are mean of 4 months (1 season) ±S.E (N=12) for each season.

Water parameters	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
Water temperature (^{0}C)	29.50±1.57^{a}	25.95±0.52^{b}	25.85±1.07^{b}
Dissolved oxygen (mg/l)	6.73±0.30^{b}	7.21±0.18^{ab}	8.11±0.45^{a}

Values with the different letters are significantly different (P<0.05).

haematocrit reader and the values were expressed in terms of % (Wintrobe, 1974). The erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the values of haemoglobin content, haematocrit and total erythrocyte count using the respective formula (Dacie & Lewis, 1975).

$$\text{MCV } (\mu^2\text{m}) = \text{Hct\%} / \text{RBC in million/mm}^3 \times 10$$

$$\text{MCH (pg)} = \text{Hb\%} / \text{RBC in million/mm}^3 \times 10$$

$$\text{MCHC (g/100ml)} = \text{Hb\%} / \text{Ht\%} \times 100$$

Statistical analysis

Statistical significance of the haematological data between seasons (mean ± SE) was determined using one-way analysis of variance (ANOVA). Duncan multiple range test was used to determine the differences between means (P<0.05). The correlation between haematological parameters by different seasons, temperature and rainfall was analyzed by Pearson coefficient (r) at P<0.05 by using the SPSS version 16.0.

RESULTS AND DISCUSSION

Monthly and seasonal average values of water temperature, dissolved oxygen and rainfall recorded during study period are summarized in Fig. 1 and Table 1, respectively. The river stretch is not polluted and the habitat is healthy (Panikkar *et al*, 2015). Both the water temperature and dissolved oxygen varied significantly ($P<0.05$) between different seasons. The seasonal average water temperature was found higher during pre-monsoon (29.5±1.57) followed by monsoon (25.95±0.52) and post-monsoon (25.85±1.07), respectively. The dissolved oxygen was observed to be higher (8.11±0.45) during post-monsoon followed by monsoon (7.21±0.18) and lower in pre-monsoon (6.73±0.30). The climate (raise in water temperature) and hydraulic variations (reduction in water discharge) causes changes in dissolved oxygen concentration (Kohanestani *et al*, 2013). The highest rainfall was received during monsoon season (88.81mm) and lowest in pre-monsoon (66.46mm). The water temperature, dissolved oxygen and rainfall were found to vary with seasons.

The comparison of haematological values (mean±SE) of *B. carnaticus* in different seasons shown in Table 2 and Fig. 3 & 4. The correlation between haematological parameters in different seasons is given in Table 3&4 and their correlation with water temperature and rainfall are shown in Table 5. The haematological findings of *B. carnaticus* are complementing to the water parameters indicating that the seasonal changes in water parameters are directly affecting the fish physiology. Similar findings were reported by Lathif *et al* (2015) in *Channa marulius* indicating that changing water quality is directly affecting the fish health. In the present study, significant ($P<0.05$) seasonal differences were observed in TEC, Hct, MCH and MCHC. Whereas, the values of TLC, Hb and MCV did not show any remarkable significant differences ($P>0.05$) between seasons. Analysis of our results for TEC, Hb and Hct revealed that higher values of 2.56 $x10^6/mm^3$, 8.82 gm/dL and 35.62% respectively were recorded during monsoon season and lower values (2.08 $x10^6/mm^3$, 8.57 gm/dL and 30.33% respectively) in post-monsoon. The values of TLC were found high (33207 cells/mm^3) during warmer months of pre-monsoon, but no significant variations between seasons were observed. The higher values during the monsoon months may be due to the physiological changes taking place during the breeding seasons (Das *et al*, 2012), which are influencing the haematological parameters. *B. carnaticus* adults migrate upstream for spawning and breed in the flood waters along rivers during monsoon (Ranjit Daniels, 2002). Higher values of Hct, Hb, RBC and WBC in *Alburnoides eichwaldii* during warm seasons in an Iran stream were reported by Kohanestani *et al* (2013). They related the higher values to haematological response of fish to higher water temperature, dissolved oxygen variation for more gaseous exchange in warmer seasons. Similar findings for the seasonal variation were observed

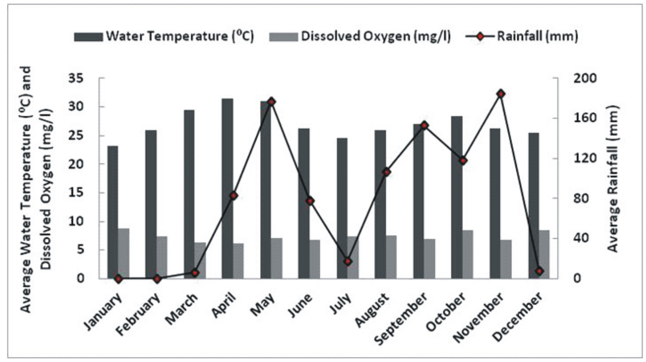

Fig. 2 : Monthly variations in the temperature, dissolved oxygen and rainfall at the sampling station.

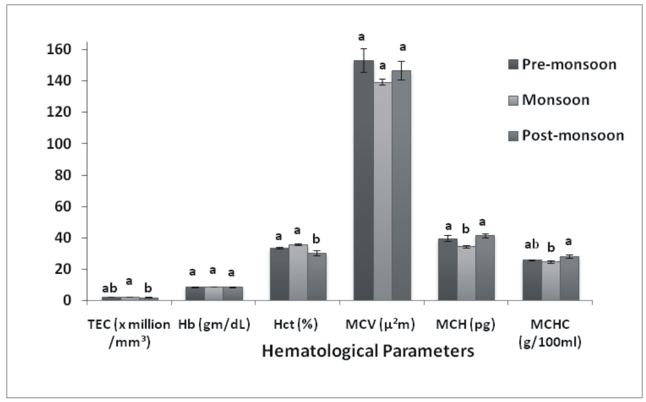

Fig. 3 : Graphical representation of seasonal variations in the haematological parameters of *Barbodes carnaticus.*

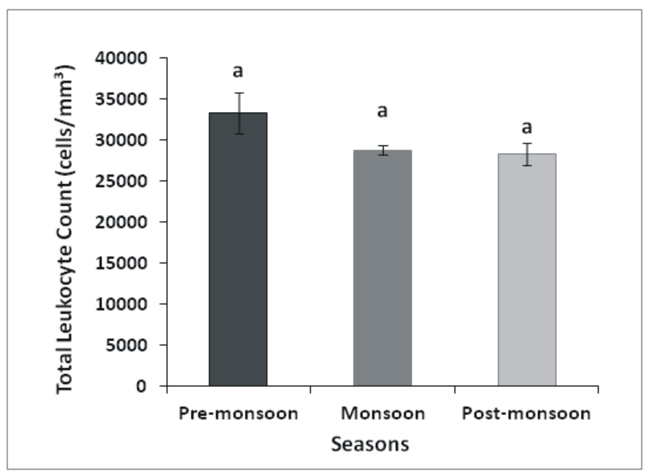

Fig. 4 : Seasonal variations in the total leukocyte count (cells/mm^3) of *Barbodes carnaticus.*

in the RBC, Hb and Hct of pirarucu, *Arapaima gigas* by Bezerra *et al* (2014). Joshi *et al* (1989) also reported that increase in TEC, TLC, Hb and Ht to be closely related with breeding activity and gonadal maturation in *Channa gachua*. Lathif et al. (2015) observed significantly higher PCV, WBCs, RBCs and MCV of *Channa marulius* during April month of summer. In support to the present study, the low value of WBC (TLC) in *Alburnoides bipunctatus, Chalcalburnus mossulensis* and *Cyprinion macrostomus*; *Parachanna obscura* and

Table 2 : Seasonal variations in the haematological parameters of *Barbodes carnaticus.* All values are mean ± S.E, n = 40 fishes in each season.

Haematological parameters	Seasons			P -value
	Pre-monsoon	Monsoon	Post-monsoon	
TEC ($x10^6/mm^3$)	2.21±0.13[ab]	2.56±0.03[a]	2.08±0.14[b]	0.042
TLC ($Cells/mm^3$)	33207±2477	28733±562	28248±1312	0.117
Hb (gm/dL)	8.71±0.23	8.82±0.13	8.57±0.38	0.815
Hct (%)	33.48±0.62[a]	35.62±0.43[a]	30.33±1.44[b]	0.010
MCV (μ^2m)	153.04±7.54	139.44±1.84	147.00±5.99	0.285
MCH (pg)	39.78±1.75[a]	34.58±0.80[b]	41.54±1.35[a]	0.014
MCHC (g/100ml)	26.01±0.26[ab]	24.81±0.71[b]	28.36±1.18[a]	0.036

Values with the different letters are significantly different (P<0.05).
TEC=Total erythrocyte count, TLC=Total leukocyte count, Hb=Haemoglobin, Hct= Haematocrit, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration.

Table 3 : Correlation analysis of haematological parameters by different seasons in *B. carnaticus*.

Season	Variable	TEC	TLC	Hb	Hct	MCV	MCH	MCHC
Pre-monsoon	TEC	1	0.898**	0.881**	0.816**	-0.888**	-0.806**	0.418
	TLC	0.898**	1	0.926**	0.733**	-0.912**	-0.827**	0.580*
	Hb	0.881**	0.926**	1	0.817**	-0.771**	-0.643*	0.624*
	Hct	0.816**	0.733**	0.817**	1	-0.593*	-0.517	0.516
	MCV	-0.888**	-0.912**	-0.771**	-0.593*	1	0.967**	-0.343
	MCH	-0.806**	-0.827**	-0.643*	-0.517	0.967**	1	-0.254
	MCHC	0.418	0.580*	0.624*	0.516	-0.343	-0.254	1
Monsoon	TEC	1	0.129	0.425	0.266	-0.005	-0.139	-0.022
	TLC	0.129	1	0.217	-0.080	0.106	0.243	0.215
	Hb	0.425	0.217	1	-0.412	-0.384	0.755*	0.843**
	Hct	0.266	-0.080	-0.412	1	0.291	-0.728*	-0.572
	MCV	-0.005	0.106	-0.384	0.291	1	-0.082	-0.546
	MCH	-0.139	0.243	0.755*	-0.728*	-0.082	1	0.817*
	MCHC	-0.022	0.215	0.843**	-0.572	-0.546	0.817*	1
Post-monsoon	TEC	1	0.773*	0.904**	0.812**	-0.573	-0.764*	-0.081
	TLC	0.773*	1	0.672*	0.907**	-0.016	-0.624	-0.464
	Hb	0.904**	0.672*	1	0.622	-0.699*	-0.475	0.259
	Hct	0.812**	0.907**	0.622	1	-0.028	-0.764*	-0.587
	MCV	-0.573	-0.016	-0.699*	-0.028	1	0.306	-0.687*
	MCH	-0.764*	-0.624	-0.475	-0.764*	0.306	1	0.468
	MCHC	-0.081	-0.464	0.259	-0.587	-0.687*	0.468	1

*Correlation is significant at P=0.05 **Correlation is significant at P=0.01

Esox lucius during autumn which could be due to low ambient temperature and low metabolic rate (Orun *et al*, 2003; Adebayo *et al*, 2007; Farzin *et al*, 2014). Sharma *et al* (2016) observed peak value of WBC in summer and least in winter season that might be due to increase in water temperature, which positively increases the number of circulating WBCs in the blood. A similar rise in the white blood cell count was found in *Cirrhinus mrigala* and *Oncorhynchus mykiss* at a higher temperature (Pradhan *et al*, 2014b; Houston *et al*, 1996). The values of secondary blood indices (erythrocyte indices) such as MCH and MCHC have shown significantly (P<0.05) higher values during post-monsoon (41.54 pg and 28.36 g/100ml, respectively). The low values of MCH (34.58 pg) and MCHC (g/100ml) recorded in monsoon season. But there were no significant changes

Table 4 : Correlation analysis of Hematological parameters of *B. carnaticus* for the entire study period.

Variable	TEC	TLC	Hb	Hct	MCV	MCH	MCHC
TEC	1	0.444*	0.739**	0.808**	-0.676**	-0.847**	-0.387*
TLC	0.444*	1	0.578**	0.482**	-0.228	-0.326	-0.172
Hb	0.739**	0.578**	1	0.538**	-0.609**	-0.416*	0.110
Hct	0.808**	0.482**	0.538**	1	-0.245	-.0774**	-0.722**
MCV	-0.676**	-0.228	-0.609**	-0.245	1	0.653**	-0.196
MCH	-0.847**	-0.326	-0.416*	-0.774**	0.653**	1	0.589**
MCHC	-0.387*	-0.172	0.110	-0.722**	-0.196	0.589**	1

*Correlation is significant at P=0.05 **Correlation is significant at P=0.01

Table 5 : Correlation of the hematological parameters with temperature and rainfall.

Parameters	Correlation with temperature	Correlation with rainfall
TEC	0.391*	0.575
TLC	0.953**	0.381
Hb	0.569**	0.578*
Hct	0.483**	0.427
MCV	-0.118	-0.525
MCH	-0.247	-0.472
MCHC	-0.176	-0.082

*Correlation is significant at P = 0.05
**Correlation is significant at P = 0.01

in MCV between different seasons. Our results are in conjunction with the findings of Gupta *et al* (2013) in *Tor putitora* and Sharma *et al* (2016) in *Barilius bendelisis* where maximum values of MCH and MCHC were recorded in the rainy season. However, Pradhan *et al* (2012) recorded higher MCV values in summer and lower in winter for *Catla catla*, though there were no significant differences in MCH and MCHC between seasons.

The correlation coefficient analysis of haematological parameters during different seasons is given in Table 3. The highest positive correlation obtained for MCH-MCV (r=0.967, p=0.01) and Hb-TLC (r=0.926, p=0.01) during pre-monsoon. In the monsoon season MCHC-Hb (r=0.843, p=0.01) and MCHC-MCH (r=0.817, p=0.05) have shown higher positive correlation. During post-monsoon correlation between Hct-TLC (r=0.907, p=0.01) and HB-TEC (r=0.904, p=0.01) were higher. For the overall study period the highest positive correlation occurs between Hct-TEC (r=0.808, p=0.01) and Hb-TEC (r=0.739, p=0.01).

Haematological parameters such as TEC, TLC, Hb and Hct shows significant correlation (P=0.05) with water temperature and Hb had significant correlation with rainfall (Table 5). Our results complement the findings of Collazos *et al* (1998) and Latif *et al* (2015), which reports that blood parameters levels were affected by variation in water temperature in *Tinca tinca* and *Channa marulius*, respectively. Das *et al* (2012) established a positive correlation between TEC, Hb, Hct and temperature in *Puntius sarana.*

As reported by Sharma *et al* (2016), haematological parameters in fishes are considerably influenced by wide range of environmental factors, nutritional state, seasons, spawning and stress. Fish being ectothermic, can adapt to fluctuations in ambient temperature and an elevated water temperature can increase the metabolic activity. Fluctuations in water temperature reduce the oxygen concentration in water. This causes the fish to display an increase in erythrocyte number and haemoglobin concentration because of hypoxia/lesser-dissolved oxygen condition (Witeska *et al*, 2013; Pradhan *et al*, 2012).

CONCLUSION

The investigation indicates that haematological parameters in *B. carnaticus* are impacted by seasonal variations, especially the temperature and rainfall. Hence, seasons play a vital role as reference ranges when fish haematology is concerned. The results of this study would provide a baseline for haematological parameters that can be used as a scientific tool for monitoring the health status of *B. carnaticus* in a climate change scenario. This study can be helpful for sustainably managing the fish in tropical aquatic systems.

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