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Seasonal and sex related haematological variations of the freshwater spiny eel, *Mastacembelus armatus* (Lacepede) reared in the cemented cisterns

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Abstract

The seasonal and sex related haematological variations in the freshwater spiny eel *Mastacembelus armatus* were studied during summer from April to June 2001 and winter from November 2001 to January 2002. In total 60 fish (30 males and 30 females) ranging from 30- 55.6 cm in total length and from 55-95.5 g in weight were collected from the culture stock maintained in the cemented cisterns. It was observed that the values of the total erythrocyte count (TEC), haemoglobin concentration (Hb), haematocrit values, packed cell volume (PCV) and neutrophil count were significantly higher ($P < 0.05$) in males than the females during summer (April to June- on-set of breeding season). Females were observed to have remarkably higher values ($P < 0.01$) of erythrocyte sedimentation rate (ESR), total leucocyte count (TLC) and thrombocyte count during summer. On the other hand, insignificant variations in the various haematological parameters were noticed during winter (November to January) between the two sexes of *M. armatus*.
Key words: Haematology, Breeding season, *M. armatus*

Introduction

Haematological study in fish plays an important role to ascertain their defense mechanism. It has assumed greater significance with the increasing emphasis on aquaculture and even greater awareness on fish diseases, the pollution of natural water resources as well as determination of patho-physiological condition of fish (Mishra *et al.* 1977). Haematological parameters such as the erythrocyte count, haemoglobin concentration, haematocrit and erythrocyte sedimentation rate vary in fish in relation to age, sex activity and the physico-chemical properties of water (Ezzat *et al.* 1974). The freshwater spiny eel, *Mastacembelus armatus*, locally known as Baim, belongs to the family Mastacembalidae is commonly occurs in ponds, lakes, streams and rivers of Bangladesh, India, Pakistan and Sri Lanka (Rahman 1989). It is a delicious and widely accepted fish in this sub-continent but unfortunately no initiation has been made to

culture it economically in Bangladesh. The fish may be a suitable candidate for intensive culture in ponds.

At present no published information is available on the haematology of *M. armatus.* However, secretory cells in the gills of the fish was describe by Kapoor (1957), its cephalic sensory canals by Maheshwari (1971) and a case of abnormality in the testes was reported by Maheshwari (1966) as well. The present study was undertaken to explore some important haematological parameters of freshwater spiny eel, *M. armatus* and the variations of those parameters in relation to sex of fish and season.

Materials and methods

Live specimens of freshwater spiny eel, *Mastacembelus armatus*, 30 to 55.6 cm total length and 55 to 95.5 g weight, were obtained from the culture stock maintained in cemented cisterns of the Bangladesh Agricultural University (BAU) Mymensingh, Bangladesh, during summer (April to June) and winter (December 2001 to February 2002). Five fish of either sex were used every month. For anaesthetizing the fish, mixture of clove oil: ethanol: water was used in the ratio of 2:18:9980 (0.02%) as reported by Keene *et al.* (1998). After anaesthetizing the fish, blood samples were collected from the caudal vein with a sterile disposable plastic syringe coated with 3.6% sodium citrate as an anticoagulant according to Smith *et al.* (1952). To avoid contamination with mucus and water, the area of insertion of needle was wiped with cotton soaked in 70% alcohol before and after the blood collection. Collected blood was gently pushed into a sterilized small glass vial containing anticoagulant (potassium salt of ethylenediamine tetra-acetic acid, EDTA) to give a final concentration of 5 mg EDTA per ml of blood.

Erythrocytes (RBC) and leucocytes (WBC) counts were determined by Neubauer's double haemocytometer using Hayem's and Shaw's diluting fluids, respectively. Erythrocyte sedimentation rate (ESR) and packed cell volume (PCV) were measured by Wintrobe's haematocrit tube. The collected blood was inserted into dry Wintrobe's tube by Pasteur pipette exactly up to 0 or 100 mark according to Barnhart (1969). Care was taken not to allow any bubble in the tube. The tube was placed in a special rack in vertical position for 1 hour. ESR was calculated by measuring the distance the erythrocyte had sedimented in the scale from the top of the tube and the result was expressed in mm/h. The tube was then spun in haematocrit centrifuge at 3000 rpm for 30 minutes (Siddiqui and Naseem 1979). The haemoglobin concentration of blood was evaluated by Sahli's haemocytometer. Erythrocyte indices or absolute values *viz.* mean cell haemoglobin conecntration (MCHC) and mean cell volume (MCV) were calculated mathematically from the erythrocyte count, haemoglobin concentration and haematocrit according to Daice and Lewis (1977).

Differential cell counts were made from the thin blood films prepared on glass slides immediately after collection of the blood samples, which were air dried, stained with Wright and Giemsa stain (Chinabut *et al.* 1991), washed in running tap water and dried followed by mounting in Canada balsam. A total of 100 leucocytes were counted under

microscope at × 45 for each sample and the percentage of different types of leucocytes were calculated.

Statistical analysis

The mean values of each parameter at each sampling were compared using analysis of variance (ANOVA) technique through complete randomized design (CRD).

Results

General values

General values of the haematological parameters are shown in Table 1. Haematocrit or packed cell volume (PCV) ranged from 26 to 33.5% with a mean of 29.62 ± 3.65%. The mean haemoglobin concentration (Hb) was 8.45 ± 1.85 g/100 ml of blood ranging from 6 to 10.0 g/100 ml of blood. Average erythrocyte sedimentation rate (ESR) was 2.57 ± 1.50 mm/h and ranged from 1.5 to 3.5 mm/h. The number of erythrocytes (TEC) ranged from 1.30 to $2.5\times10^6/mm^3$ and average was $1.97\times10^6/mm^3$. The leucocyte count (TLC) ranged from 30.60 to $60.0\times10^3/mm^3$ with a mean value of 49.40 ± $4.55\times10^3/mm^3$. The mean cell volume (MCV) ranged from 210 to 360 μm^3 with a mean value of 285.90 ± 4.80 μm^3 and mean cell haemoblobin concentration (MCHC) ranged from 25.0 to 44.50% with a mean of 30.0 ± 4.56%.

In differential counts of leucocyte population, large lymphocytes ranged from 6.0 to 8.50% with a mean of 7.55 ± 0.85%, small lymphocytes ranged from 28 to 31.50% with a mean of 29.45 ± 1.55%, thrombocytes ranged from 33 to 37% with a mean of 35.35 ± 1.65%, monocytes ranged from 2.0 to 4.0% with a mean of 3.65 ± 1.55% and neutrophils ranged from 23.0 to 26.5%, the mean being 24.05 ± 1.85%.

Haematological parameters in relation to sex of fish

Difference in the haematological parameters for males and females of *M. armatus* have been shown in Table 1. Haematocrit value in male was 30.75% and in female, 28.50%. The haemoblobin concentration was higher in male (9.0 g/100 ml of blood) than the female (7.90 g/100 ml of blood), the difference was significant ($p<0.05$). In erythrocyte count males had $2.15\times10^6/ mm^3$ cells and females, $1.80\times10^6/mm^3$, having an insignificant difference. Percent of the leucocytes were 7.50 and 7.60 for large lymphocytes, 29.5 and 29.4 for small lymphocytes, 34.50 and 36.20 for thrombocytes, 3.5 and 3.8 for monocytes and 25.0 and 23.0 for neutrophils in males and females respectively. These counts did not show any significant variation between male and female except for thrombocytes and neutrophils where the variation was significant ($p< 0.05$). Average erythrocyte sedimentation rate (ESR) was higher in female (3.15 mm/h) than in male (2.0 mm/h) showing a significant difference ($p< 0.05$). The values of mean cell volume were 285.60 and 286.20 μm^3 and mean cell haemoglobin concentration was

30.3% and 29.7% for males and females respectively, however, the difference was insignificant ($p > 0.05$).

Seasonal variation in haematological parameters

Results of the present study on seasonal variation of haematological parameters including leucocyte numbers have been shown in Table 2. Analysis of data showed that the haematocrit value, haemoglobin concentration and neutrophil values were significantly higher in males during summer. With the on-set of breeding season from April to June (summer), there was a decreasing tendency of haemoglobin concentration, haematocrit values and neutrophil count in female. The values of erythrocyte sedimentation rate, total leucocyte count and thrombocyte counts were higher during the summer in female. In winter (December to February) no significant variations in the haematological parameters in males and females of spiny eel, *M. armatus* was observed.

Discussion

It is very important to establish normal haematological values in fish with a view to aid in the diagnosis of disease and in connection with the effects of pollution on fish health (Mawdesley-Thomas 1971). The haematological findings observed in the present study were within the range found for other fish species. Haematocrit or packed cell volume (PCV) ranged from 26 to 33.5% which accorded with that of 25 to 50% in *Labeo rohita* (Siddiqui and Naseem 1979). McCarthy *et al.* (1973) found haematocrit values of 30 to 49% in rainbow trout *Salmo gairdneri*. Blaxhall and Daisley (1973) also found haematocrit value ranging from 20 to 43% in *S. trutta*. In the present investigation the haemoglobin concentration ranged from 6 to 10/100 ml of blood with a mean of 8.45/100 ml of blood, which is similar to 8.1/100 ml of blood as found in spiny eel, *Macrognathus aculeatum* (Prasad *et al.* 1977). Average erythrocyte sedimentation rate (ESR) of *M. armatus* was 2.57 mm/h in the present study which is similar to the result of Siddiqui and Naseem (1979) where the ESR value of *Labeo rohita* was 2.5 mm/h. Blaxhall and Daisley (1973) reported the range of ESR values of rainbow trout to be 1to 5 mm/h, McCarthy *et al.* (1973) reported 1 to 8 mm/h for rainbow trout and Sardar *et al.* (1999) reported 2 to 3.8 mm/h for *Clarias batrachus*. In this study ESR value ranged from 1.5 to 3.50 mm/h in *M. armatus* which was within the above ranges. However different plasma viscosity and specific gravity of the erythrocyte are responsible for different ESR values. Erythrocyte count of *M. armatus* ranged from 1.30 to 2.50×10^6 mm^3 with a mean of 1.97 $\times 10^6$ mm^3 which was similar to the findings of Prasad *et al.* (1977) who found the erythrocyte count of 1.78×10^6 mm^3 in *M. aculeatum*. In the present study the leucocyte counts ranged from 30.60 to 60.0×10^3 mm^3. Sardar *et al.* (1999) found 48.52 to 63.82×10^3 mm^3 in *C. batrachus*. Chinabut *et al.* (1991) found 64.75×10^3 mm^3 as an average leucocyte count of *C. punctatus*. Mahajan and Dheer (1979) reported the average leucocyte count of the same species to be 60.4×10^3 mm^3. These findings are similar to the results of the present study.

Table 1. Haematological parameters of male and female freshwater spiny eel, *Mastacembalus armatus* during summer

Sex	Length cm	PCV %	Hb g/100 ml blood	ESR mm/h	TEC $\times 10^6$ mm^3	TLC$\times 10^3$ mm^3	MCV (μm^3)	MCHC %	Differential count %				
									LL	SL	T	M	N
Male	34.50	30.75[a*]	9.0[a]	2.0[a]	2.15[a]	48.7[a]	285.6[a]	30.3[a]	7.50[a]	29.50[a]	34.50[a]	3.5[a]	25.0[a]
Female	28.25	28.50[b]	7.9[b]	3.15[b]	1.80[b]	50.0[b]	286.2[a]	29.7[a]	7.60[a]	29.40[a]	36.20[a]	3.8[a]	23.0[a]
Mean ± SD	31.37 ±3.12	29.62 ±1.12	8.45 ±0.54	2.57 ±0.57	1.97 ±0.15	49.4 ±0.65	285.90 ±0.3	30.0 ±0.3	7.55 ±0.05	29.45 ±0.05	35.35 ±0.85	3.65 ±0.15	24.0 ±1.0

* Numbers in the same column having the same superscripts are not significantly different ($p>0.05$) and different superscripts are significantly different ($p<0.05$).
ESR = Erythrocyte sedimentation rate; Hb = Haemoglobin concentration; LL = Large lymphocyte; M = Monocyte; MCHC = Mean cell haemoglobin concentration; MCV = Mean cell volume; N = Neutrophil; PCV = Packed cell volume; SL = Small lymphocyte; T = Thrombocyte; TEC = Total erythrocyte count; and TLC = Total leucocyte count.

Table 2. Haematological parameters of male and female freshwater spiny eel, *Mastacembalus armatus* during winter

Sex	Length cm	PCV %	Hb g/100 ml blood	ESR mm/h	TEC $\times 10^6$ mm^3	TLC$\times 10^3$ mm^3	MCV (μm^3)	MCHC %	Differential count %				
									LL	SL	T	M	N
Male	28.20	28.4[a*]	8.3[a]	2.10[a]	1.9[a]	48.7[a]	285.6[a]	30.4[a]	7.40[a]	29.70[a]	34.80[a]	3.7[a]	24.40[a]
Female	30.0	28.0[a]	8.0[a]	2.50[a]	1.8[a]	49.0[a]	286.2[a]	29.7[a]	7.50[a]	29.80[a]	35.00[a]	3.8[a]	23.90[a]
Mean ± SD	29.1 ±0.90	28.2 ±0.2	8.15 ±0.15	2.30 ±0.2	1.85 ±0.05	48.8 ±0.14	285.90 ±0.3	30.05 ±0.25	7.45 ±0.05	29.75 ±0.05	34.90 ±0.01	3.75 ±0.05	24.15 ±0.25

* Values of the haematological parameters are not significantly different ($p>0.05$).
ESR = Erythrocyte sedimentation rate; Hb = Haemoglobin concentration; LL = Large lymphocyte; M = Monocyte; MCHC = Mean cell haemoglobin concentration; MCV = Mean cell volume; N = Neutrophil; PCV = Packed cell volume; SL = Small lymphocyte; T = Thrombocyte; TEC = Total erythrocyte count; and TLC = Total leucocyte count.

Among the leucocytes the number of thrombocytes were less and that of small lymphocytes were high in the present study in comparison to that of Sardar *et al.* (1999) for *C. batrachus* and Mahajan and Dheer (1979) for *C. punctatus*. The confusing structure of small lymphocytes and rounded thrombocytes, as well as the reduction in number of thrombocytes during smear preparation due to their fragility might contribute to this findings.

Haematocrit value, haemoglobin concentration and erythrocyte count were significantly higher in male than in female as observed during the present study. Similar observations were reported by Kapila *et al.* (2000) in golden mahseer *Tor putitora*, Sardar *et al.* (1999) in *C. batrachus* and Siddiqui and Naseem (1979) in *Labeo rohita*. Ezzat *et al.* (1974) found higher erythrocyte count in males than females of *Tilapia zillii*. Average ESR value and total leucocyte count (TLC) was higher in female than the male during summer (on-set of breeding season). In the present study, these results were similar to the above results. This appeared to be related to activity of the sex, males were being more active, due to the gonadal activity and endocrine factor as pointed out by them.

Little information is available with regard to changes in the blood value associated with season and breeding. Decreasing tendency of blood value in the present study during the months of April to June (on-set of breeding season) and increasing tendency in December to February coincides with the findings of Sardar *et al.* (1999) in *C. batrachus* and Mahajan and Dheer (1979) in *C. punctatus*. Among the leucocytic population, only neutrophils and thrombocytes showed changes in different seasons, which accorded with the results of Sardar *et al.* (1999) in *C. batrachus* and Mahajan and Dheer (1979) in *C. punctatus*.

It may be concluded from the above observations that though *M. armatus* is a freshwater spiny eel, its haematological parameters were found to be similar, at least within or nearer to the range, to those of *C. batrachus*, *L. rohita*, *Tilapia*, *S. trutta*, *Macrognathus aculeatum*, *S. gairdneri*, *C. punctatus* and *T. putitora*. It will imply that the physiological susceptibility and the pathobiology of the spiny eel *M. armatus* in response to any physico-chemical or biological factors might be similar to that of the above fishes at least.

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