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## Hematological profile of male and female *Anabas testudineus*

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**Abstract:** The haematological profile of air breathing fish *Anabas testudineus* male and female was studied. The parameters studied were Haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), erythrocyte red blood cells (RBC) or erythrocytes, lymphocytes, monocytes, neutrophils and eosinophils. Most haematological parameters indicated sex variations. Higher Hb concentration, RBC counts and HCT percentages were found in male compared to their counterparts but higher MCH values, WBC counts and ESR levels were detected in female than in the male. In contrast, higher level of ESR was observed in female than male. Total counts of RBC observed in both male were significantly higher than in the female. MCV was found higher in male and female. However, no significant differences were observed in neutrophils, lymphocytes and eosinophils. Findings of this study showed sex variations in the haematological parameters between *Anabas testudineus*.

**Keywords :** Blood, air breathing, *Anabas testudineus*, haematology

### INTRODUCTION

The blood volume of fishes varies between 2-8% of their body volume.<sup>1</sup> One third to one half of the total blood volume in fish consists of blood cells, the rest is fluid plasma.<sup>2</sup> In spite of systematic diversity all fishes possess two main types of blood cells erythrocytes (red cells) and leucocytes (white cells), a property shared by the land living vertebrates, which are derived from early fish like ancestors. A bibliography on fish hematology was assembled by Hawkins and Mawdesley-Thomas (1972).<sup>3</sup> The major proteins of fish blood plasma are globulin to a large extent antibodies- immunoglobulin. A peripheral blood serum from a healthy fish shows a preponderance of erythrocytes. Other cells are lymphocytes, neutrophils, monocytes, eosinophils, basophils and thrombocytes or

platelets. Blood parameters of fishes may have species specific variations in cellular counts and biochemical values.

Air breathing climbing perch in India especially in Bihar is a commercially important high value fish. However, although a few literatures are available on the haematological parameters in various fishes of Bihar in which haematological parameters of climbing perch have never been reported. Banerjee (1966)<sup>4</sup> has reported on the haematological profiles of *Anabas testudineus*. This study, therefore, was designed to see the variation between male and female in hematological parameters.

### MATERIALS AND METHODS

Sexually matured *Anabas testudineus* were used as experimental animals purchased from the local market of Madhepura of Bihar.

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The experimental variables were the various blood parameters such as haemoglobin (Hb), erythrocyte sedimentation rate (ESR), red blood cells (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), differential neutrophils, lymphocytes, monocytes, eosinophils and basophils counts.

Samples of blood were drawn by severing the caudal peduncle. A sample of 2 ml blood was collected by using 3 ml plastic syringes treated with an anticoagulant: EDTA from (n=5) and held in sample test tube containing K3 EDTA solution. Blood parameters were assayed by using Mythic 18 Automated Haematology Analyzer (China). This instrument analyzes the blood parameters using three detector blocks and two types of reagents. To analyze haemoglobin by automated methods, the cyanmet hemoglobin method or oxyhaemoglobin method was applied.

## RESULTS AND DISCUSSION

Higher Haemoglobin concentrations were found in male ( $13.27 \pm 0.69$  g d/l) than female ( $9.8 \pm 0.12$  g d/l)

The observed higher Hb concentration found in male compared to females indicates sex differences. Males are more active and aggressive than the female, which is in agreement with Smith *et al.* (1952).<sup>5</sup> Hb concentration in different fishes living in similar water bodies may vary with each other considerably, but the variation in the same species is very little. The observed similar Hb concentration in the same sex between two fish is expected.<sup>6</sup> Levels of Hb found in both sexes koi was above 9 g d/l, which denotes the occurrence of no anaemic condition<sup>7</sup>. The concentration of Hb found in *C. batrachus* ranged 8.0 - 19.2 g d/l with an average of  $14.24 \pm 0.66$  g d/l.<sup>8</sup>

The female ( $42.67 \pm 0.33$  mm, 1st h.) had significantly higher ESR levels than in the than male ( $1.67 \pm 0.33$  mm, 1st h.).

The observed level of ESR in male was fewer than 10 mm, 1<sup>st</sup> h which indicates the normal physiological condition. Higher levels of ESR detected in female than male could be because of the production of large number of eggs compared to the sperms in male. Similar observation was found by (Mishra *et al.* 1977)<sup>9</sup> in *Amphipnous cuchia*. The observed higher ESR in females indicates the chances of occurrence of infections because at high ESR chronic inflammatory conditions, connective

Table 1: Blood parameters of *Anabas testudineus*

Sl. No	Parameters	Male	Female
1	Haemoglobin (g d/l)	$13.27 \pm 0.69$	$9.8 \pm 0.12$
2	ESR (mm, 1 <sup>st</sup> h)	$1.67 \pm 0.33$	$42.67 \pm 0.33$
3	RBC (m/ml)	$3.03 \pm 0.23$	$1.9 \pm 0.10$
4	HCT (%)	$50.67 \pm 3.76$	$30.33 \pm 0.88$
5	MCH	$44.00 \pm 2.65$ pg	$52.00 \pm 2.08$ pg
6	Total WBC ( $\times 10^4$ cu/mm)	$5.04 \pm 0.32$	$9.05 \pm 0.36$
7	Neutrophils (%)	$21.67 \pm 1.33$	$18.33 \pm 2.03$
8	Lymphocytes (%)	$75.33 \pm 2.60\%$	$71.33 \pm 1.86\%$
9	Monocyte (%)	$2.33 \pm 0.33\%$	$75.33 \pm 2.60\%$
10	Eosinophils (%)	$4.33 \pm 0.67\%$	$3.67 \pm 0.67\%$

tissue diseases, anaemia etc. may attack fishes. ESR is used as the indicator of the general health of the fish.<sup>10</sup> It has also been used for the early detection of infections in *Salvelinus fontinalis*.<sup>11</sup>

While comparing, total counts of RBC observed in male ( $3.03 \pm 0.23$  m/ml) were significantly higher than in the female ( $1.9 \pm 0.10$  m/ml).

RBC containing hemoglobin plays a vital role in respiration described by Wells *et al.* 1980.<sup>6</sup> The present data of RBC count was similar to the range found by Banerjee, 1966<sup>4</sup> in the same fish. Present study showed higher RBC counts in male than the female which denote sex variations. The observed higher RBC counts in male perhaps because of their hyper activeness compared to the female. RBC counts have proven to be a highly variable blood parameter among fishes and showed a remarkable difference in their sex. Svobodova *et al.* 2008<sup>12</sup> also found higher RBC in males of seven fish species.

HCT (%) observed in male ( $50.67 \pm 3.76\%$ ) were significantly higher than in the female ( $30.33 \pm 0.88\%$ ).

Males had significantly higher value of HCT than in the female that perhaps because of relatively higher RBC counts in male. The likely another reason could be of sex difference where male may remain stressed from hyperactivity in nature.<sup>7</sup> A lower haematocrit value in females indicates iron deficiency anaemia and relatively low RBC count etc.

The MCV value was found more in male ( $167.33 \pm 1.20$  fL) than female ( $160.00 \pm 4.16$  fl).

MCV value reflects the size of red blood cells by expressing the volume occupied by a single red blood cell. Normal value varies according to the species, sex, age etc.

MCH levels observed in female ( $52.00 \pm 2.08$  pg) were significantly higher than the levels measured in the male ( $44.00 \pm 2.65$  pg).

The higher MCH value in female than in the male indicates higher likelihood of occurrence of macrocytic anaemia in females than in male.<sup>13</sup>

The MCHC found in female ( $32.33 \pm 0.67$  g/dl) was significantly higher than in the male ( $26.33 \pm 0.67$  g/dl).

The observed higher level of MCHC in indigenous female than their male counterparts could be explained by sex differences. High levels of MCHC indicate more Hb in a unit of RBCs.<sup>12</sup> In case of fish MCHC there is no prescribed normal range. In teleosts this has been found species specific. The levels of MCHC have been detected 38.57, 29.93 and 27.16 g/dl in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus*, respectively.<sup>14</sup>

Total WBC counts detected in both female ( $9.05 \pm 0.36 \times 10^4$  cu/mm) were significantly higher than the levels measured in the male ( $5.04 \pm 0.32 \times 10^4$  cu/mm).

Observed WBC in female were significantly higher than the levels measured in the male, which indicate egg carriage, infection, adverse condition.<sup>15</sup> Levels of WBC in climbing perch are considerably higher than the level of man (Normal range: 4000-11000 cu/mm) as expected.<sup>16</sup> In fishes the significance of WBC and their biological function are not clearly understood. The number in some species of fish varies greatly with age, season and maturation.<sup>17</sup>

Percentage composition (%) of neutrophils found in both male and female male ( $21.67 \pm 1.33\%$ ) were similar ( $18.33 \pm 2.03\%$ ).

Neutrophils are the most important of the leucocytes as they show the greatest sensitivity to change in the environment. Their characterization and identification is, therefore, of significance for assessing the changes in the physiological state of fishes.

Sexually matured male and female ( $71.33 \pm 1.86\%$ ) had similar levels of lymphocytes count ( $75.33 \pm 2.60\%$ ). Similarly, no difference was detected in the lymphocyte (%) composition within male and female groups.

Lymphocytes were regarded as multi potential haemopoietic stem cells.<sup>18</sup> But now they are regarded as

highly differentiated cells with immunological stimuli in various ways and known as immune-competent cells. This similar immune level could be because of healthy fish farmed in farms and natural environment.

Similar level monocyte (%) was measured both in male ( $2.33 \pm 0.33\%$ ) and in the female ( $1.33 \pm 0.33\%$ ) and within groups.

Differential leucocyte counts in koi confirmed the presence of monocytes in teleost blood which is in agreement with Ellis (1977)<sup>19</sup>. Similar leucocytes count in both sexes of Thai and indigenous koi indicates that leucocytes count do not vary between sexes. The percentage of monocyte found in some fishes were 37% with an average of  $4.89 \pm 1.94\%$  in *Clarias batrachus*.<sup>8</sup>

Both ( $4.33 \pm 0.67\%$ ) male and female ( $3.67 \pm 0.67\%$ ) had similar composition of eosinophils.

According to Ellis (1977)<sup>19</sup> presence and absence of fish eosinophil are contradictory. While the previous workers opted for their absence, recent workers opted for their presence. During present investigation eosinophil was detected which deny the absence of eosinophil opted by earlier workers. The observed no difference in eosinophil counts between sexes shows similar physiological condition in terms of WBC differential counts. Eosinophil percentages detected in *C. batrachus* is 0-3% with an average of  $0.72 \pm 0.18 \%$ .<sup>8</sup>

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