BLOOD RESPIRATORY PARAMETERS IN THREE SPECIES OF CULTIVATED FISH

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Abstract

The study was carried out on three species of fish which are commercially important and presently cultivated in Sri Lanka. These are Oreochromis mossambicus, Oreochromis niloticus and Chanos chanos. Blood parameters measured in the resting state for the three species were haematocrit, erythrocyte counts and haemoglobin concentrations. From these findings the following were calculated. The Hb content per single erythrocyte, the Hb concentration per single erythrocyte and volume of a single erythrocyte. It was found that all these values were higher in the Oreochromis species compared to Chanos. Inverse relationships between erythrocyte count and volume of a single erythrocyte and between cell count and Hb content per single erythrocyte were observed. O2 capacities and O2 dissociation curves were also determined. The effect of pH on O2 affinity was also studied. In vivo experiments were conducted to measure rates of O2 consumption in the resting state of fish.

Introduction

The diversity of blood components is thought to reflect the variety of ways in which fish adapt to their environment (Goel, 1984). The study of haematology of fish has contributed significantly to understanding the comparative physiology, phylogenetic relationships, mode of animal life, food selection and ecology (Murachi, 1959; Gunter et. al. 1961). The application of haematological techniques including measurements of haemoglobin concentration, haematocrit and erythrocyte counts have proved valuable for fishery biologists in assessing the health of fish (Blaxhall, 1972) and monitoring stress responses including those due to sublethal concentrations of pollutants as quoted by Kori-Siakpere (1985). The need for the establishment of normal haematological values for fish species; providing standards for physiological, pathological or toxicological investigation have been stressed (Mawdesly — Thomas, 1971). The aim of this study was to investigate the normal blood physiological properties of three species of fish Oreochromis mossambicus, Oreochromis niloticus and Chanos chanos which are commercially important and are presently being cultivated in Sri Lanka.

Materials and Methods

Cultures of the three species of fish were maintained in outdoor ponds. Two weeks prior to experiments the fish were brought into the laboratory and kept in aerated tap water at room temperature (28±1.5°C) in aquaria, fitted
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with under gravel water filter systems. All fish were considered healthy on the basis of their appearance and the absence of obvious signs of disease. No sexual selection was made.

The experiments were carried out on fish of weight 25-60g. and total length of 14-20cm. The fish were fed on pellets prepared in the laboratory using fish meal (30%), wheat flour (10%), rice bran (30%), poonac (30%) vitamins and minerals. To obtain a blood sample, a fish was made unconscious by a blow on its head. Total duration of the procedure was less than 60 sec. Blood was obtained by cutting off the tail peduncle and allowing it to drain into a deep watch glass into which heparin was added, so that its concentration in blood would be approximately 20iu/ml. About 0.5-ml of blood could be obtained from a single fish, so that the blood samples had to be pooled for some measurements (Hb concentration, O₂ dissociation curves, O₂ capacity). Blood haematocrit values were determined using a haematocrit centrifuge (Hawksley). About 40µl of blood was obtained into heparinized capillary tubes and centrifuged at 11000rpm for 5min. To measure the erythrocyte counts the blood was diluted by a factor of 200 with hayems fluid and counts were made using a haemocytometer (neubaur, improved, double). Haemoglobin concentrations were determined by the acid hematin method of Cohen and Smith (Oser, 1965). The standard acid hematin solution was prepared by the method of Wong (Oser, 1965). The absorbancy of the unknown acid hematin solution was read against the standard at 520nm using a spectrophotometer (Baush and Lomb, spectronic 20).

The haematological indices: the haemoglobin content per single erythrocyte (MCH), the haemoglobin concentration per single erythrocyte (MCHC) and the volume of a single erythrocyte (MCV) were calculated from Hb values, haematocrit values and erythrocyte counts. O₂ capacity was measured by the Haldane method, using the Haldane apparatus. O₂ dissociation curves and the effect of low pH on O₂ affinity was determined as follows. The blood cells were first hemolysed by addition of two volumes of distilled water to one volume of blood. Then a clear Hb solution was prepared by centrifuging this sample for 10min at 5000rpm and filtering first through glass wool and then through a millipore filter 0.5m pore size, 2mm diameter). O₂ dissociation curves were plotted by exposing the Hb solution to various partial pressures of O₂ ranging 0-155mmHg in a tonometer and measuring the degree of oxygenation by determining the absorbance using a spectrophotometer at 625nm, (Hoar and Hickman, 1967). To determine the effect of low pH on O₂ affinity of blood, the pH of the blood was varied by addition of the required phosphate buffer to the Hb solution. The normal blood pH was measured using a pHmeter(WTW,pH522). Experiments were carried out on whole animals to measure the O₂ consumption rates in resting state of the fish. A respirometer was used where water passed continuously at a constant rate through a chamber containing the fish. O₂ consumption was calculated
as the product of the flow rate through the chamber and out of the chamber. Metabolic rate was calculated by dividing the total $O_2$ consumption rate by the weight of the fish. $O_2$ content of water was found by the Winkler method (Hoar and Hickman, 1967).

The data were analysed by the "Student's t test," at the 5% level of significance.

Results

Table I

Blood respiratory properties and metabolic rates in three species of cultivated fish. All values refer to the mean ± standard error. $n = 6$)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>O. mossambicus</th>
<th>O. niloticus</th>
<th>C. chanos</th>
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</thead>
<tbody>
<tr>
<td>Haematocrit (%)</td>
<td>14.87 ± 1.35</td>
<td>13.313 ± 0.747</td>
<td>22.031 ± 1.658</td>
</tr>
<tr>
<td>Erythrocyte count ($\times 10^8$/mm$^3$ blood)</td>
<td>0.623 ± 0.488</td>
<td>0.648 ± 1.12</td>
<td>1.63 ± 0.404</td>
</tr>
<tr>
<td>Hb concentration (gHb/100ml blood)</td>
<td>5.49 ± 0.359</td>
<td>6.913 ± 0.226</td>
<td>4.054 ± 0.31</td>
</tr>
<tr>
<td>MCH ($\mu\mu$g)</td>
<td>88.15</td>
<td>113.18</td>
<td>24.83</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>36.92</td>
<td>47.12</td>
<td>18.405</td>
</tr>
<tr>
<td>MCV ($\mu$)</td>
<td>238.68</td>
<td>205.45</td>
<td>135.16</td>
</tr>
<tr>
<td>Normal blood pH</td>
<td>7.46</td>
<td>7.35</td>
<td>7.18</td>
</tr>
<tr>
<td>$O_2$ capacity (ml$O_2$/100ml blood)</td>
<td>7.26</td>
<td>8.93</td>
<td>4.1</td>
</tr>
<tr>
<td>$O_2$ consumption (ml$O_2$/h)</td>
<td>4.047 ± 0.206</td>
<td>4.75 ± 0.369</td>
<td>4.79 ± 0.783</td>
</tr>
<tr>
<td>Metabolic rate (ml$O_2$/h/g)</td>
<td>0.123 ± 0.0054</td>
<td>0.1685 ± 0.204</td>
<td>0.226 ± 0.022</td>
</tr>
</tbody>
</table>

Table I summarizes values for the blood respiratory parameters: haematocrit, erythrocyte count, haemoglobin concentration, mean cell haemoglobin, mean cell haemoglobin concentration, mean cell volume, $O_2$ capacity, pH and the metabolic rates.

Haematocrit values and erythrocyte counts were found to be significantly different between C. chanos and O. mossambicus and between C. chanos and O. niloticus. Both these values were higher in C. chanos. Haemoglobin concentrations were significantly different from each other in all three species,
while it was found to be lowest in *C. chanos*. All haematological indices i.e. haemoglobin content per single erythrocyte (MCH), haemoglobin concentration per single erythrocyte (MCHC), and volume of a single erythrocyte (MCV) were lower in *C. chanos* compared to the Oreochromis species. The inverse relationship between cell size and cell count for the three species is described by the regression equation \( Y = -87.97X + 278.16 \) \((r = -0.955)\). The relationship between haemoglobin content per single erythrocyte and cell count is described by the regression equation \( Y = -75.74X + 148.63 \) \((r = -0.955)\). The \(O_2\) capacity of blood of *O. niloticus* was found to be the highest while that of *C. chanos* was the lowest.

Metabolic rates for the three species in the resting state were determined using the rates of \(O_2\) consumption. The metabolic rate for *C. chanos* was significantly higher than in *O. mossambicus* and *O. niloticus*.

\(O_2\) dissociation curves and their dependence on pH for the three species are shown in Fig. I. The affinity for \(O_2\) expressed as the partial pressure at which, half the haemoglobin molecules are oxygenated (\(P_{50}\) value) reveals that *O. niloticus* has a low \(O_2\) affinity (\(P_{50}\) value of 32 mmHg at pH 7.35) compared to *O. mossambicus* (\(P_{50}\) value of 22 mmHg at pH 7.46) and *C. chanos* (\(P_{50}\) value of 24 mmHg at pH 7.18). All three species showed a shift in the dissociation curve to the right when the pH was lowered.

**Table II**

<table>
<thead>
<tr>
<th>Species</th>
<th>(\log P_{50}/\text{pH})</th>
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<tbody>
<tr>
<td><em>O. mossambicus</em></td>
<td>...</td>
</tr>
<tr>
<td><em>O. niloticus</em></td>
<td>...</td>
</tr>
<tr>
<td><em>C. chanos</em></td>
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</tr>
</tbody>
</table>

The magnitude of the Bohr effect expressed as the change in \(P_{50}\) per unit change in pH (\(\log P_{50}/\text{pH}\)) reveals that the pH effect on the dissociation curve for *O. niloticus* is less than that for *O. mossambicus* and *C. chanos*.

**Discussion**

Results in the present study are similar to those reported for other fresh water teleosts, (Smith et.al. 1979; Goel et.al. 1984). Kori—Siakpere (1985) has reported that within the teleosts the three haematological parameters are found to vary from species to species, probably as physiological adaptation to their different modes of life, and ecological habitats. This may limit their
Fig. 1 Oxygen equilibrium curves at normal and low blood pH

(A) Oreochromis mossambicus
(B) Oreochromis niloticus
(C) Chanos chanos
distribution throughout the world. Intraspecies variation in haematological values attributed to many factors can be put into two main groups, namely physical factors and factors due to stress. In the second group stress can occur during capture, handling and sampling procedure while physical factors include length, weight, nutritional state, season, spawning sex and genetic variations.

In this study haematocrits and erythrocyte counts were higher while haemoglobin concentrations were lower in *C. chanos* compared to the two *Oreochromis* species. Hb content per single erythrocyte, Hb concentration in a single erythrocyte and volume of a single erythrocyte were all higher in the two Oreochromis species compared to Chanos. The negative correlation between RBC count and mean cell volume (high number: small size) has been found for other species of fish, *Tilapia zilli* (Farghaly et al., 1972) and *Salmo gairdneri* (Dewilde and Houston, 1968). An inverse relationship was also observed between haemoglobin content per single erythrocyte and cell count. This may be an adaptation for mutual compensation. That is when the cell size and Hb content in a cell increase it may compensate to a certain extent the loss caused due to decreased RBC number and haematocrit. Goel et al. (1984) reports that active fish with a higher number of erythrocytes in their blood are found to have low MCV and MCHC values while in sluggish fish vice versa a low RBC count is correlated with higher MCV and MCH values.

Apparently there is also a general correlation between the habits of fishes and Hb concentration of their blood (Hall and Gray, 1929) and this gives some indication of the O₂ demand of a species (Houston, 1968). The values for O₂ capacity were also higher for the two Oreochromis species. Houston et al. (1968) has reported that blood O₂ capacity is dependent upon the nature of dissociation relationships as well as upon the amount of O₂ carrier available. This is in agreement with results obtained by us.

O₂ dissociation at normal blood pH values are similar in *O. mossambicus* and *C. chanos* whereas the curve is shifted to the right in *O. niloticus*. *O. niloticus* is a phytoplankton feeder in its natural environment. Dissolved O₂ concentrations in waters which support a good growth of phytoplankton is usually high. In *O. niloticus* haemoglobin is half saturated at a higher O₂ partial pressure than in *O. mossambicus* and *C. chanos*. Therefore it could be said that *O. niloticus* is more likely to suffer from anoxia and respiratory distress than *O. mossambicus* and *C. chanos*.

All three species showed a shift in the dissociation curves to the right when the blood pH was lowered. The largest Bohr effect was found in *O. mossambicus* while the smallest was found in *O. niloticus*. Blood of *O. niloticus* combines a low affinity for O₂ with a low sensitivity to the presence of acid. Of the three species the metabolic rate was found to be highest in *C. chanos*. 

References


