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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. 0_2 capacities and 0_2 dissociation curves were also deter mined. The effect of pH on O_2 affinity was also studied. In vivo experiments were conducted to measure rates of O_2 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 enviornment (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 out door ponds. Two weeks prior to experiments the fish were brought into the laboratory and kept in aerated tap water at room temperature ($28 \pm 1.5c°$) in acquaria, fitted

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 lenght 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, O_2 dissociation curves, O_2 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. O_2 capacity was measured by the Haldane method, using the Haldane apparatus. O_2 dissociation curves and the effect of low pH on O_2 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). 0_2 dissociation curves were plotted by exposing the Hb solution to various partial pressures of 0_2 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 0_2 affinity of blood, the pH of the blood was varied by addition of the required phosphate buffer to the Hb solution. The normal blood pHwas measured using a pHmeter(WTW,pH522). Experiments were carried out on whole animals to measure the 0_2 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. 0_2 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 0_2 consumption rate by the weight of the fish. 0_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)

Parameters	*O. mossambicus*	*O. niloticus*	*C. chanos*
Haematocrit (%)	14.87±1.35	13.313±0.747	22.031±1.658
Erythrocyte count ($\times 10^6$/mm^3 blood)	0.623±0.488	0.648±1.12	1.63±0.404
Hb concentration (gHb/100ml blood)	5.49±0.359	6.913±0.226	4.054±0.31
MCH ($\mu\mu$g)	88.15	113.18	24.83
MCHC (%)	36.92	47.12	18.405
MCV (μ^3)	238.68	205.45	135.16
Normal blood pH	7.46	7.35	7.18
O_2 capacity (mlO_2/100ml blood)	7.26	8.93	4.1
O_2 consumption (mlO_2/h)	4.047±0.206	4.75±0.369	4.79±0.783
Metabolic rate (mlO_2/h/g)	0.123±0.0054	0.1685±0.204	0.226±0.022

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 ie 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 $Y = -75.74 \times + 148.63$ ($r = -0.955$). 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 dependance 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

Magnitude of Bohr effect in shift of P_{50} per unit change in pH

Species	*log P_{50}/pH*
O. mossambicus	−0.35
O. niloticus	−0.30
C. chanos	−0.33

The magnitude of the Bohr effect expressed as the change in P_{50} per unit change in pH (log P_{50}/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

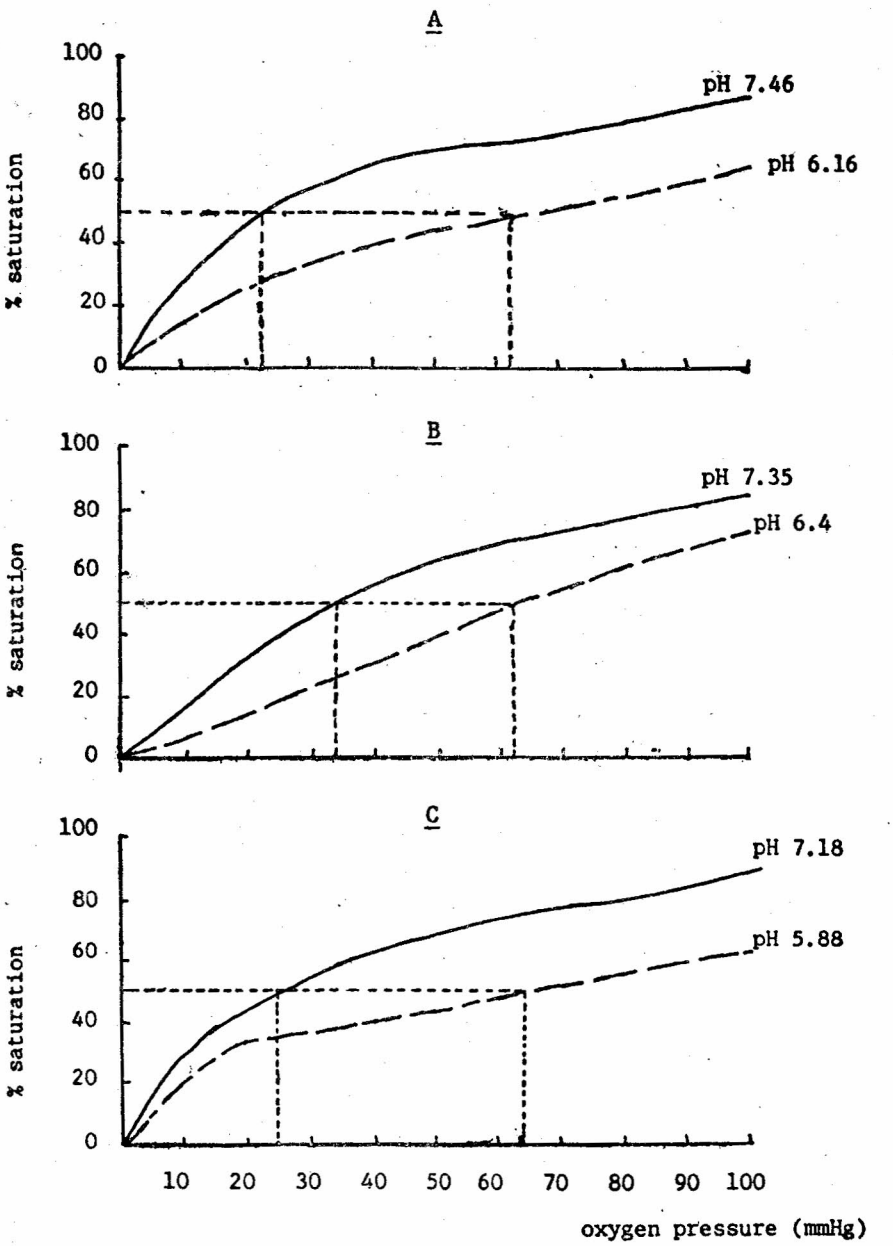

Fig. 1 Oxygen equilibrium curves at normal and low blood pH

(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 O_2 demand of a species (Houston, 1968). The values for O_2 capacity were also higher for the two Oreochromis species. Houston et.al. (1968) has reported that blood O_2 capacity is dependent upon the nature of dissociation relationships as well as upon the amount of O_2 carrier available This is in agreement with results obtained by us.

O_2 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 enviornment. Dissolved O_2 concentrations in waters which support a good growth of phytoplankton is usually high. In *O. niloticus* haemoglobin is half saturated at a higher O_2 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 O_2 with a low sensitivity to the presence of acid. Of the three species the metabolic rate was found to be highest in *C. chanos*.

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