SOME BLOOD PARAMETERS OF THE YELLOWFISH (*BARBUS HOLUBI*) AND THE BARBEL (*CLARIAS GARIEPINUS*)

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ABSTRACT

Haemoglobin concentrations, haematocrit values, red blood cell counts, red blood cell diameter, white blood cell counts and plasma haemoglobin concentrations were performed on the yellowfish (*Barbus holubi*) and the barbel (*Clarias gariepinus*). Wide variations were observed in haematocrit values and haemoglobin concentrations and statistically significant correlations existed between mean corpuscular volume and average corpuscular haemoglobin and between the red blood cell count and mean corpuscular volume in the case of the yellowfish and between the red blood cell count and haemoglobin concentration in the case of the barbel. It was found that the determination of one variable is not sufficient for the routine assessment of other haematological parameters in these fish due to poor correlations observed. This can only be done in the case of the variables mentioned.

INTRODUCTION

Haematology will in future no doubt play an important role in the diagnosis of disease in fish and also in the assessment of the effects of pollution on fish life. Unless basic work is done to establish normal haematological ranges, this will be difficult (McCarthy *et al.*, 1973).

As part of an extensive programme concerning the physiology of local fish, various blood parameters of the yellowfish (*Barbus holubi*) and barbel (*Clarias gariepinus*), were investigated. No detailed observations concerning the haemoglobin concentrations, haematocrit and erythrocyte counts of these fish are available, and it was decided to investigate the relationships (if any) existing between these variables. It is known that in some fish, e.g. carp, definite relationships exist, but in the brook-trout no such correlations are evident (Houston & DeWilde 1972). If present, such correlations are useful in the routine analysis of physiological response to environmental stress and pathological status. (Schumacher 1958, Anthony 1961 and DeWilde & Houston 1967). The present report shows that in these fish some correlations are present but care should be taken in using one parameter as a general index of haematological status.

MATERIALS AND METHODS

Adult, healthy fish of both species were obtained from the Provincial Fisheries during October 1972. Prior to sampling of blood, individual animals were anaesthetized in aqueous tricane methanesulphonate (MS 222, 75 mg/L). Blood samples were collected by cardiac puncture using 5 ml syringes with minimal suction and immediately analysed. In the case of the yellowfish the syringes were heparinized with heparin (5 000 units/ml) and in the case of the barbel, EDTA was used. Both males and females were used and none of the fish were in a spawning or pre-spawning condition.

Haemoglobin content of the blood was determined as cyanmethaemoglobin according to the method of Kleihauer & Betke (1957). After the determination of the haematocrit value (P.C.V.) by centrifugation (Korzheuv 1964) the plasma was analysed for haemoglobin in

TABLE 1

HAEMATOLOGICAL PARAMETERS IN *Barbus holubi* AND *Clarias gariepinus*: POPULATION MEANS ±S.D. AND CORRELATION COEFFICIENTS (N—10)

Body weight g	*Heart as % body weight. Heart weight g*	*Hb g %*	*Haematocrit %*	*R.B.C. × 10⁶ cells/mm³*	*R.B.C. length and breadth µm*	*M.C.V. µ³ m*	*A.C.H. ng*	*M.C.H. %*	*Fish*
274,93±13,63	0,110±0,01 0,296±0,04	9,47±0,77	42,31±5,64	1,094±0,333	18,47±0,98× 11,24±1,06	413,12±126,49	93,80±29,3	22,80±62,78	*Barbus holubi*
1222,2±363,6	0,18±0,03 2,13±0,77	8,23±2,04	28,36±8,17	2,00±0,61	10,97±0,76× 9,64±0,76	147,5±42,2	45,8±13,0	31,09±12,31	*Clarias gariepinus*
correlation coefficients	R.B.C. vs. P.C.V., r=0,402; Hb. vs. P.C.V., r=0,684; R.B.C. vs. Hb., r=0,341; P.C.V. vs. M.C.V., r= 0,125; M.C.V. vs. A.C.H., r=0,935; R.B.C. vs. M.C.V.; r= −0,814; R.B.C. vs. Area of red cell; r= −0,710; Body weight vs. Heart weight, r=0,049.								*Barbus holubi*
correlation coefficients	R.B.C. vs. P.C.V., r=0,732; Hb. vs. P.C.V., r=0,515; R.B.C. vs. Hb., r=0,859; P.C.V. vs. M.C.V., r= 0,146; M.C.V. vs. A.C.H., r=0,698; R.B.C. vs. M.C.V., r= −0,559; Hb. vs. M.C.V., r= −0,629; Body weight vs. Heart weight, r=0,790								*Clarias gariepinus*

solution, or plasma haemoglobin, according to the method of Crosby & Furth (1956). Red blood cell and white blood cell counts were performed in Bright-Line Haemocytometers (American optical corporation) using Toisson's Fluid as diluting medium. Blood was diluted 1 to 200 for erythrocyte determinations and 1 to 20 for white blood cell determinations. The dimensions of the cells were obtained from dry films using a stage micrometer. For histological studies on the white blood cells, blood was spun down in a microhaematocrit tube and a thin smear was made of the cells contained in the buffy layer. When dry, these cells were stained according to the method of Giemsa (1904). Mean corpuscular volume (M.C.V.), average corpuscular haemoglobin (A.C.H.) and mean corpuscular haemoglobin concentration per cent (M.C.H.) was calculated according to Korzhuev (1964).

Statistical analyses of the results were made by calculating means, standard deviations and regression coefficients according to the methods of Freund (1952).

RESULTS

Table 1 shows the results obtained for both species. Wide variations were observed in the haematocrit values and a statistically significant correlation was found between haemoglobin concentration and haematocrit value for the yellowfish ($P<0,05$). Other statistically significant correlations observed in this species were between M.C.V. and A.C.H. ($P<0,001$), between R.B.C. and M.C.V. ($P<0,01$), and between R.B.C. and the area of the red cell ($P<0,025$). The area used in this case was calculated from the average length and breadth of the red cells of individual fish and is therefore not very precise. In the case of the barbel, significant correlations were found between R.B.C. and P.C.V. ($P<0,025$); between R.B.C. and Hb ($P<0,025$); between M.C.V. and A.C.H. ($P<0,025$); between Hb and M.C.V. ($P<0,05$) and between body weight and heart weight ($P<0,05$).

Plasma haemoglobin concentration varied widely and it was found that for a period of two to three weeks after capture (yellowfish) or up to one week (barbel) the plasma contained variable amounts of haemoglobin. Values of up to 700 mg % were recorded and subsequent analyses were performed only on fish freshly caught or specimens which had been kept for at least two weeks in tanks at the laboratory after capture. (The same applied to the other variables investigated). Subsequent analyses showed that *Clarias gariepinus* contained a mean of 7,33 mg % haemoglobin in the plasma. A wide variation in this parameter was especially characteristic of the yellowfish.

With a blood dilution of 1 to 20 and using the diluting fluid mentioned, no white blood cells could be counted in either of the two species. A buffy coat was occasionally present in the haematocrit tube and blood cells obtained from this area (stained with Giemsa) showed the presence of large and small lymphocytes (8–13 μ), monocytes (9–15 μ), occasional macrophages (13 μ) and thrombocytes ($4 \times 10\mu$) in the case of the yellowfish. In the case of the barbel, basophils (7 μ), eosinophils (6,3 μ), large and small lymphocytes (7–9 μ), monocytes (11,9 μ) and thrombocytes ($2,1 \times 14,0\mu$) were observed. No differential counts were performed.

DISCUSSION

Bouch & Ball (1966) have reported on the influence of capture methods on blood characteristics in fish and showed that the haemoglobin concentrations, erythrocyte sizes and plasma protein concentrations varied according to the method of capture. Delayed shock was also of importance and influenced all the above parameters. Care was therefore taken in this study to use only freshly caught fish or animals acclimated for at least two weeks. The variation observed in the plasma haemoglobin concentration within the first few weeks or days after capture is probably due to a shock reaction and may indicate that the red blood cells are more susceptible to haemolysis immediately after capture than is normally the case. The same has been observed in the case of the mudfish (Hattingh, 1973).

Mott (1957) has summarized previous results from various fish species and it appears that red blood cell counts vary from 0,1 to 12 million/mm^3 blood, while the mean and S.D. for red cell diameters is 12,2 $\pm$ 7,3 $\times$ 25,0 $\pm$ 14,0μ in different fish. Houston & DeWilde (1972) found small variations in the haemoglobin concentration (7,1 $\pm$ 0,2 g %), red blood cell count (1,54 $\pm$ 0,02 $\times$ 10^6 cells/mm^3) and haematocrit (27,8 $\pm$ 0,6%) in the common carp. The same applied to the brook-trout. The values obtained in the present investigation fall within the range reported in the literature but much wider variations were evident. The low value of heart weight (as % body weight) corresponds to that given by von Skramlik (1935) for inactive fish. Both the yellowfish and barbel are mediumly active. Smith *et al.* (1952) recorded some degree of inverse correlation between corpuscle size and red cell count in a variety of freshwater teleosts. No significant correlation was obtained with *Clarias gariepinus*, but *Barbus holubi* showed a correlation of $-0{,}710$. Young (1949) noticed considerable fluctuations in the haematocrit of *Girella nigricans*, both between individuals and in single individuals examined at intervals over a period of months. I obtained similar results and this variation could not be correlated with sex, body weight or length. The possibility of a seasonal variation should, however, be kept in mind (see also Satchell 1971).

The only really good correlations I observed were between M.C.V. and A.C.H. and between R.B.C. and M.C.V. in the case of the yellowfish and between R.B.C. and Hb. in the case of the barbel. Why the red blood cell count does not correlate well with the haematocrit in the case of the yellowfish is not entirely clear. This could be due to inherent inaccuracies in the counting technique (Korzhuev 1964) or due to variations in the size of individual erythrocytes. The values given in Table 1 for erythrocyte size are mean values obtained from the individual means of 10 fish. The variation observed in the blood of a single fish for erythrocyte size was larger in the yellowfish than in the barbel. This individual variation is not shown in Table 1 and a more detailed study of this is necessary.

In spite of the fact that some degree of correlation exists between the haematological parameters in both species examined, these are in themselves not close enough to warrant the use of one parameter for routine assessment of the other variables. This procedure can only be used for the variables mentioned in the previous paragraph.

Puchkov (1964) maintains that fish blood contains on the average more white blood cells than mammalian blood. This was not found to be the case in these two species using the diluting fluid mentioned. Moreover, these species do not have white cell counts in the region of

100,00/mm³ (Puchkov 1964) nor do they show the normal neutrophil picture, thought to be present in fish flood. This raises some important questions concerning the natural resistance of these animals and this aspect is at present being investigated.

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