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BLOOD PARAMETERS IN FISHES. III. HEMOGLOBIN CONCENTRATION, HEMATOCRIT AND THE NUMBER OF RED BLOOD CELLS IN SOME FRESHWATER FISHES OF EASTERN VENEZUELA.

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RESUMEN: En 7 especies de peces de agua dulce, con diferentes niveles de actividad, algunas con respiración aérea, además de la respiración acuática, se estudió: el hematocrito, la concentración de la hemoglobina, el número de eritrocitos. Con estos parámetros se calcularon las relaciones: hemoglobina corpuscular media, concentración de la hemoglobina corpuscular media y el volumen corpuscular medio. El nivel de actividad se correlacionó positivamente con el número de eritrocitos y con el hematocrito y negativamente con el volumen de los eritrocitos. Los peces con respiración acuática exclusiva presentaron un hematocrito y un número de eritrocitos mayor que los peces que poseen además, respiración aérea; estos últimos, a su vez, poseen un volumen y un contenido en peso de hemoglobina por glóbulo mayor que los primeros.

ABSTRACT: In 7 species of freshwater fishes, with different levels of activity, some with aerial respiration, in addition to aquatic breathing, hematocrit, hemoglobin concentration and erythrocyte number were measured. These parameters were used to calculate mean corpuscular hemoglobin (by weight and percentage) and the mean corpuscular volume. The activity level was correlated positively with the number of erythrocytes and the hematocrit and negatively with the mean corpuscular volume of the red blood cells. Fishes with aquatic respiration exhibit a higher number of red blood cells and hematocrit than fishes with aerial respiration; these showed a higher volume of erythrocytes and hemoglobin content (in weight) in each red blood cell than fishes with aquatic breathing only.

INTRODUCTION

The activity of fishes varies widely from sedentary bottom forms to highly active pelagic species. These differences in activity must be related to differences in metabolic requirements. Several kinds of adaptations characterize the more active species: changes in the body shape to reduce resistance (BAINBRIDGE, 1963); larger gill surface areas (GRAY, 1954; HUGHES, 1966); increased proportion of red muscle, which is better for aerobic metabolism than white muscle (BODDEKE, *et al.* 1959); and some adjustements in various hematological parameters to increase the amount of oxygen in the blood (HALL & GRAY, 1929; PUTNAM & FREEL, 1978; PEREZ, *et al.* 1981, 1983). On the other hand, fishes live in environments that vary greatly in several physico-chemical properties, such as the concentration of oxygen. Thus, their blood must transport oxygen under different conditions and have diverse respiratory properties.

In the present study, we have selected freshwater species that present different levels of activity and that inhabit waters with considerable variation in oxygen concentrations.

This study is one of a series (PEREZ, *et al.* 1981, 1983) dealing with the types of hematological adjustments that have been developed by marine and freshwater fishes of South America.

MATERIALS AND METHODS

Twenty specimens of each species were analyzed; their names, systematic position and the respiration type are given in Table I. All specimens were adult. They were collected in eastern Venezuela, from the Manzanares River and Campoma Lake in Sucre state, and from temporary ponds in Monagas state, south of Maturin. Immediately after capture blood was collected from the specimens by severing, without anesthetic, the caudal peduncle.

Methods for determining hematocrit (Hem), hemoglobin concentration (Hb), red blood cell count (N° rbc), mean cell volume (MCV), mean cell hemoglobin (MCHb), and mean cell hemoglobin concentration (MCHbC) have been previously described (PEREZ *et al.* 1980).

A coefficient of correlation was calculated to determine the dependence of variables for these parameters, and an analysis of variance, including a Du can test, was applied to determine the variability b ween species with aquatic vs aerial-aquatic types respiration.

RESULTS AND DISCUSSION

Fishes used in the present study were placed five categories, depending on level of activity, beg ning with the most active species: 5 ^ *A. bimaculat* 4 ^ *P. kraussii;* 3 ^ *H. minutus.* 2 ^ *H. malabaricus* ^ *H. littorale, P. watwata* and *L. maracaiboensis.* Le of activity was determined from field and laborato observations.

The hemoglobin content of the blood var between 9.6 g/100 ml for *H. littorale,* which posses instestinal and branchial respiration, and 7.5 g for *maracaiboensis,* which also exhibits aerial (stomach and aquatic breathing. Both of these species show the lowest level of activity Furthermore, *A. bimacu*

TABLE I. FISH SPECIES WITH THEIR RESPECTIVE FAMILIES AND TYPE OF RESPIRATION (AQUATIC = A AND AERIAL AA).

Species	Family	Respiration
Astyanax bimaculatus	Characidae	A
Petenia kraussii	Cichlidae	A
Hemicetopsis minutus	Cetopsidae	A
Hoplias malabaricus	Erithrinidae	A
Hoplosternum littorale	Callichthyidae	AA
Plecostomus watwata	Loriicaridae	AA
Lasiancistrus maracaiboensis	Loriicaridae	AA

TABLE II. BLOOD PARAMETERS IN SEVEN SPECIES OF FISHES: CONCENTRATION OF HEMOGLOBIN (Hb) IN g/100 HEMATOCRIT (HEM) IN% NUMBER OF RED BLOOD CELLS (N° Rbc) Xmm^{3}X10^6 MEAN CORPUSCULAR VOLUME (MC IN μ, MEAN CORPUSCULAR HEMOGLOBIN (MCHb) IN $\mu\mu$g, AND THE MEAN CORPUSCULAR HEMOGLOBIN CONCE TRATION (MCHbC) IN %. THE MEAN ± S.D. AND THE NUMBER OF SPECIMENS EXAMINED FOR EACH PARAMETER SHOWN IN PARENTHESIS.

	Hb	Hem	N° R.b.c.	MCV	MCHb	MCHbC
A. bimaculatus	8.6 ±1.1(20)	37.9 ±4.5(20)	1.70 ±0.16(20)	229.3 ±29.1(20)	50.4 ± 8.8(20)	22.8 ±2.7(15)
P. kraussii	8.7 ±1.3(36)	34.9 ±4.0(30)	2.43 ±0.64(30)	151.1 ±34.6(30)	38.4 ±10.0(30)	25.6 ±4.0(30)
H. malabaricus	9.1 ±1.5(20)	35.3 ±6.4(20)	1.76 ±0.63(20)	217.3 ±65.6(19)	55.3 ±17.2(19)	25.9 ±2.5(21)
H. minutos	8.8 ±0.9(26)	37.1 ±4.4(22)	1.50 ±0.39(14)	242.1 ±38.9(13)	59.9 ±12.2(13)	23.6 ±2.8(12)
P. watwata	7.8 ±1.8(23)	33.1 ±6.0(23)	1.30 ±27(23)	263.3 ±62.2(23)	61.4 ±12.7(23)	23.9 ±4.5(23)
L. maracaiboensis	7.5 ±1.6(24)	31.0 ±1.6(22)	1.01 ±0.36(23)	319.8 ±96.4(22)	78.3 ±17.3(23)	25.3 ±4.2(22)
H. littorale	9.6 ±1.9(38)	31.7 ±5.9(29)	1.17 ±0.27(24)	275.6 ±40.8(24)	80.8 ±15.1(23)	29.5 ±6.3(28)

, which had the highest activity level, possesses less moglobin content than *H. littorale*, *H. malabaricus*, *kraussii* and *H. minutus* (Table II). As in the first per of this series (PEREZ *et al.* 1981) the relationship tween high activity level-high hemoglobin concen-ıtion, that has been found in several species of fish [ALL & GRAY, 1929; ENGEL & DAVIS, 1964; LARS-)N *et al.* 1976; PUTNAM & FREEL, 1978; WELLS *et al.*)80), was not shown here to be significantly correla-d (Table III) when all species are compared. The :moglobin concentration was not significantly co-elated with the other parameters (Table III), not 'en with the hematocrit, as found in several other)ecies (HAES & GOODNIGHT, 1962; HATTINGH,)72; HASTINGH & DU TOIT, 1973 a,b,c; MISHORA *et* . *1977*; PEREZ *et al.* 1980; and PIETERSE *et al.* 1981). nalysis of variance indicated no significant diffe-:nces between hemoglobin concentration in fishes ith air breathing capacity ($\bar{X}$ = 8.3) and those that ad only branchial respiration ($\bar{X}$ = 8.8). Considera-le differences were found by DUBALE (1959), who :udied the amount of iron present in the blood (as a ıeasure of hemoglobin) in 5 species with obligatory ranchial respiration (15.1 to 27.5 mg of iron in 100 ıl of blood) and 5 species of air breathers (29.4 to 0.0 mg). Dubale's study included both freshwater nd marine teleosts. On the other hand, PEREZ *et al.* 1981) found significant differences by comparing the oncentration of hemoglobin between 9 marine ıranchial respiring species and two species of toad ish, which are aerial-water breathers.

Hematocrit varies between a mean of 31.0% in *L. maracaiboensis* to 37.9% in *A. bimaculatus* (Table I). This parameter only shows significant correlation vith the activity level (Table III) as found by PUTNAM & FREEL (1978); WELLS *et al.* (1080); PIETERSE *et al.* (1981). No correlation was found with the number of red blood cells, as in the first paper of this series (PEREZ *et al.* 1980), nor with the size (MCV), as PIETERSE *et al.* (1981) later demonstrated. However, significant differences were found between the mean values of hematocrits of branchial respiring fishes ($\bar{X}$ = 36.3%) and the aerial-water breathing fishes ($\bar{X}$ = 31.9%).

The number of red blood cells shows a great variation, the range extending between *P. kraussii*, a highly active fish that possesses a high number ($\hat{X}$ 2.4 million/mm³) and *L. maracaiboensis*, a species with low activity level, that possesses a low number ($\hat{X}$ 1.01 million/mm³). The relationship between high activity level and high N° rbc, found in several species of fish (ENGEL & DAVIS, 1964; Atkinson & JUDD, 1978; PEREZ *et al.* 1981), was also found in this study. However, *A. bimaculatus*, the most active species, shows a relatively low number of erytrocytes. Comparing the two groups of fish with respect to the type of respiration, wefound significant differences. The aquatic group showed a higher number of rbc ($\hat{X}$ 1.85 million/mm³) than the aerial-aquatic group ($\hat{X}$ 1.16 million/mm³). In the first paper of the series (PEREZ *et al.* 1981), we also found that fishes that exhibit some kind of aerial respiration possess lower N°rbc than fishes with only aquatic breathing capacity.

The mean corpuscular volume of the erytrocytes (MCV) was correlated in a significant way with the number of red blood cells (large N°rbc: small MCV). On the other hand MCV was correlated with MCHb, large MCV: high MCHb. The situation found in marine fishes of eastern Venezuela (PEREZ *et al.* 1981) was the opposite. The MCV was also correlated with

TABLE III. CORRELATION COEFFICIENT BETWEEN BLOOD PARAMETERS AND THE ACTIVITY LEVEL (Act). SYMBOLS AS SHOWN IN TABLE II. 0.05* = 0.754 0.01** = 0.874.

	Hb	Hem	N°rbc	MCV	MCHb	MCHbC	Act.
Hb	1.000						
Hem	0.279	1.000					
N°Rbc	0.295	0.564	1.000				
MCV	0.403	0.594	-0.981**	1.000			
MCHb	0.076	-0.715	-0.930**	0.920**	1.000		
MCHbC	0.553	-0.742	-0.182	0.145	0.482	1.000	
Act.	0.664	0.874*	0.759*	-0.734	-0.817*	-0.406	1.000

the activity level, highly active fishes possessing small cells. The MCV showed significant differences between aquatic and aerial-aquatic breathing fishes, the first group possessing smaller cells ($\bar{X} = 209.9\,\mu^3$) than the second ($\bar{X} = 286.2\,\mu^3$).

The MCHb showed a significant correlation with the activity level: highly active fishes had a low MCHb. There was also a significant difference between the aerial ($\bar{X} = 73.5\,\mu\mu g$) and the aquatic groups ($X = 51.0\,\mu\mu g$).

MCHbC did not show correlation with the other blood parameters, nor with the activity level. This parameter did not differ between the two respiring groups (aerial $\bar{X} = 26.2\%$ and aquatic 24.5%).

Therefore, in general, highly active freshwa fishes have a high hematocrit, and a large number of blood cells that are smaller in size, with a lower c tent of hemoglobin, as compared to fishes with activity level. We can also conclude that fishes w some kind of aerial respiration possess a lower he tocrit and a lower number of bigger red blood c with higher MCHb than fishes that exhibit aqu respiration only.

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