

BLOOD PARAMETERS IN FISHES. IV OXYGEN AFFINITY, ROOT EFFECT, pH AND THE NUMBER OF HEMOGLOBINS IN SOME FRESHWATER FISHES OF EASTERN VENEZUELA

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RESUMEN: En siete especies de peces dulceacuícolas de diferentes niveles de actividad, algunas con respiración aérea, además de la acuática, se estudiaron los parámetros sanguíneos pH, afinidad por el oxígeno y efecto Root y número de hemoglobinas separadas por electroforesis. Se encontró una mayor afinidad de la sangre por el oxígeno en los peces más activos que también fueron más afectados en esta propiedad por la temperatura. La afinidad estuvo relacionada, además, con el pH, efecto Root y número de hemoglobinas. Los valores de pH fueron mayores en las especies más activas que son las que presentan respiración branquial exclusiva.

ABSTRACT: Oxygen affinity, Root effect, pH and the number of hemoglobins separated by electrophoresis were studied in the blood of seven freshwater fish species, with different levels of activity, some species with aerial respiration, in addition to aquatic breathing. A greater oxygen affinity was found in fishes, which were highly active that were also more affected by temperature in this property. The affinity was related with pH, Root effect and the number of hemoglobins. pH values were higher in the more active fishes, which are also the ones that showed only branchial respiration.

INTRODUCTION

In recent years fish hematology has become an important tool to the research ichthyologist, in fisheries and fish culture studies. It offers not only a reliable indication of the physiological state of the organism, but also gives some clues as how fishes are able to cope with different environments.

In an earlier paper of this series (PEREZ *et al.* 1984), we reported the hematocrit, number of red blood cells, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, in 7 species of freshwater fishes with different levels of activity, some species with aerial respiration in addition to aquatic breathing. In the most active species, a high hematocrit, and a high number of red blood cells of smaller size were found. Similar results were

detected in fishes with aquatic respiration when they were compared with fishes with aerial respiration. In this paper we present the results of some additional blood parameters, such as oxygen affinity, Root effect, blood pH and number of hemoglobins, in the same 7 species of freshwater fishes studied earlier (PEREZ *et al.* 1984).

MATERIAL AND METHODS

Unsexed adults, of 7 freshwater species were used in this study, three of these, *Hoplosternum littorale* (Callychthyidae), *Plecostomus watwata* and *Laciancistrus maracaiboensis* (Loricariidae) possess in addition to aquatic respiration, some kind of aerial respiration. The rest of the species, *Petenia kraussii*, (Cichlidae), *Hoplias malabaricus*, (Erythrinidae) *Hemicetopsis minutus* (Cetopsidae) and *Astyanax bimaculatus* (Characidae), use only aquatic respiration.

Fishes were collected in eastern Venezuela as indicated by PEREZ *et al.* (1984). Immediately after capture, blood was collected after severing the caudal peduncle.

The oxygen affinity and Root effect of the blood were calculated employing an Aminco Hem-O-Scan oxygen dissociation analyzer, following POWERS *et al.* (1979). The gases employed were 25% oxygen (balanced nitrogen), and 100% nitrogen. In order to calculate the Root effect, a gas mixture consisting of 25% oxygen and 5.6% CO₂ in balanced nitrogen was used, the data are divided in 4 arbitrary categories following FARMER *et al.* (1970), based on approximated percentages: *absent*, less than 10% deoxygenation; *low*, 10-20% deoxygenation; *moderate*, 20-40% deoxygenation; and *large*, more than 40% deoxygenation. The oxygen affinity was measured at 2 temperatures; 20 and 30°C. Blood pH was measured employing a Beckman microelectrode.

In order to determine the hemoglobin electrophoretic pattern of each species, electrophoresis in polycryamide was used, as described by DAVIS (1964). The samples were prepared according to FYHN & SULLIVAN (1974). Gels were stained with a Coomassie Brilliant Blue G250 (Sigma) in a perchloric acid solution (McFARLAND, 1977). The method used to obtain hemolysate was described by PEREZ & MACLEAN (1974).

In order to estimate the dependence of variables, the Pearson coefficient of correlation was calculated for the oxygen affinity, Root effect, blood pH, the number of hemoglobins and the activity level. A variance analysis (SOKAL & ROHLF, 1969), was used to determine whether significant variation existed between the aquatic and aerial aquatic and the aerial aquatic groups.

RESULTS AND DISCUSSION

Based on our observation, fish species used in this study were separated into 5

levels of activity, beginning with the most active species:

5 = *A. bimaculatus*; 4 = *P. kraussii*; 3 = *H. minutus*; 2 = *H. malabaricus*; and 1 = *H. littorale*, *L. maracaiboensis* and *P. watawata*.

Results for oxygen affinity of the blood (at and 30°C) are expressed as P₅₀ values. (the partial pressure of oxygen necessary to saturate 50% of the blood), these are shown in Table 1. The lowest values of P₅₀, indicating high affinity (less pressure of oxygen to reach 50% saturation), were observed at both temperatures in *H. malabaricus*, followed by *A. bimaculatus*, *P. kraussii* and *H. minutus*, the 4 species which possess only aquatic respiration. Duncan test showed significant differences between the two breathing groups. A different situation was found in marine fishes (PEREZ *et al.*, 1983), where *Batrachoides manglae* and *Amphychtys cryptocentrus*, with aerial-aquatic respiration showed higher oxygen affinity than the other 10 species with only aquatic respiration.

It is also important to mention that the oxygen affinity in freshwater fishes is in general, much higher, than in marine fishes (PEREZ, *et al.* 1983).

On the other hand, the hypothesis that highly active fishes possesses lower oxygen affinity (RIGGS, 1970), is not supported by the results of this study (Table II). In fact there is a significant negative correlation between values of P₅₀ and activity, highly active fishes presents lower values of P₅₀ (high affinity).

The effect of temperature on oxygen affinity (at 20 and 30°C) was more important in *A. bimaculatus* and in general in the group of fishes with only aquatic breathing. The same pattern was observed in the group of marine fishes (PEREZ *et al.* 1983), where the effect of the temperature in oxygen affinity was also much more important than in the freshwater species. This is the logical

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TABLE 1. BLOOD PARAMETERS IN SEVEN SPECIES OF FISHES: OXIGEN AFFINITY (P_{50}) AT 20 AND 30°C, ROOT EFFECT, pH AND NUMBER OF HEMOGLOBINS (N° Hbs) THE MEAN \pm SD AND THE NUMBER SPECIMENS EXAMINED FOR EACH PARAMETER IS SHOWN IN PARENTHESIS.

SPECIES	$P_{05}(20^{\circ}\text{C})$	$P_{50}(20^{\circ}\text{C})$	ROOT EFFECT	Ph	N° Hbs
<i>A. bimaculatus</i>	6.0 \pm 1.0(3)	14.5 \pm 0.6(2)	MODERATE (4)	7.52 \pm 0.10 (8)	7-8-9(5-52-35)
<i>P. kraussi</i>	8.1 \pm 0.1(3)	11.1 \pm 1.6(2)	ABSENT. (2)	7.41 \pm 0.10(13)	7(36)
<i>H. malabaricus</i>	5.6 \pm 0.6(2)	10.3 \pm 0.4(2)	MODERATE (2)	7.35 \pm 0.09 (9)	3(12)
<i>H. minutus</i>	10.0 \pm 0.4(3)	13.7 \pm 0.2(2)	ABSENT. (2)	7.43 \pm 0.09 (5)	3(25)
<i>P. watwata</i>	12.0 \pm 0.0(2)	18.8 \pm 0.4(2)	ABSENT. (2)	7.18 \pm 0.04 (7)	2-4(55-24)
<i>L. maracaiboensis</i>	14.2 \pm 0.6(4)	16.5 \pm 0.4(4)	ABSENT. (3)	7.32 \pm 0.08 (7)	3(19)
<i>H. littorale</i>	13.7 \pm 0.6(3)	16.0 \pm 0.0(3)	LOW (3)	7.34 \pm 0.09 (7)	2(34)

response if we consider that temperature is more variable in rivers, lakes and ponds than in the ocean.

As one can expect, there was a significant correlation of oxygen affinities at both temperatures, with pH, higher values of pH were associated with higher, affinity (Table II), Values of P_{50} at 20°C were also significantly correlate with Root effect, number of hemoglobins and activity level. High affinity was correlated whit high Rood effect, high number of hemoglobins and high activity level.

The Root effect, measured at 30°C was generally not very important and was only correlated with P_{50} at 20° C. This is similar to our observations in marine fishes (PEREZ *et al.* 1983).

The number of hemoglobins (Table 1 & Fig. 1) varied between 2 in *H. littorale* and

P. watwata To 9 in *A. bimaculatus*. The species *P. watwata* and *A. bimaculatus* presented *polymorphism*. The first species have individuals with 2 and with 4 hemoglobins (as shown in Fig. 1), the second species presented specimens with 7,8 and 9 hemoglobins, showing polymorphisms in 2 different zones Fig. 1, shows a sample with 7 hemoglobins. In general, fishes with aerial-aquatic respiration (or less active) showed fewer homoglobins than fishes with only aquatic respiration. The relationship between number of hemoglobins and the rest of the parameters (Table 2) was significant with P_{50} at 20°C (higher number correlated with high affinity), with pH (high number correiated with high pH values) and with activity (the most active species presented more hemoglobins) In marine fishes (PEREZ *et al.* 1983) the correlation between the number of hemoglobins and the other parameters was very low.

TABLE II. CORRELATION COEFFICIENTS BETWEEN BLOOD PARAMETERS AND THE ACTIVITY LEVEL (ACT.) SYMBOLS AS SHOWN IN TABLE I

γ 0.05 = 0.532; 0.01 = 0.708

	20°C	30°C	ROOT	pH	N°,Hbs	Act.
20°	1,000					
30°	0,746**	1,000				
ROOT	-0,638*	-0,371	1,000			
pH	-0,577*	-0,556*	0,428	1,000		
N° Hbs	-0,632*	-0,361	0,284	0,708**	1,000	
Act.	-0,747*	-0,517	0,309	0,853**	0,917**	1,000

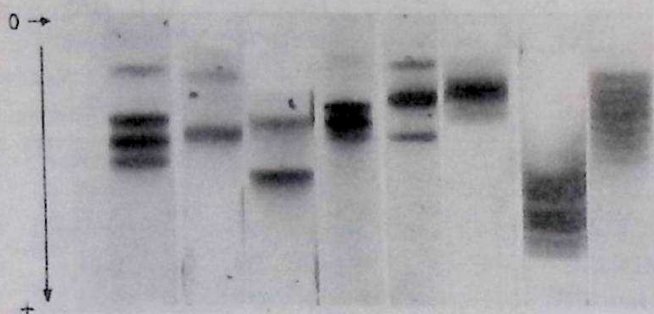


Fig. 1. Hemoglobin Electrophoretic Phenotypes (from left to right). *P. watwata*, *P. watwata*, *H. littorale*, *L. maracaiboensis*, *H. minutus*, *H. malabaricus*, *A. bimaculatus* and *P. kraussii*.

One explanation for the occurrence of multiple hemoglobins and hemoglobin polymorphism in fishes is that hemoglobin heterogeneity is an adaptative mechanism for unstable environments. It has been suggested that this hypothesis is supported by studies that showed more hemoglobins in fish from thermolabile than from thermostable environments (SULLIVAN, 1977). However PEREZ & RYLANDER (1985) argued that fishes with few hemoglobins, could just be as capable of surviving in heterogeneous environments, if their hemoglobins possess diverse capabilities, or even in case these capabilities are limited, fishes could compensate by periodically migrating to an environment for which the hemoglobins were suited, or even if these species could not compensate very well during favorable periods, in the long run these species could have greater chance of survival and reproduction if their limited hemoglobins functioned more efficiently during critical period such as the breeding season.

pH values range from 7.18 in *P. watwata* to 7.52 in *A. bimaculatus* (Table 1). Comparing blood pH of the 2 groups, in relation to breathing system, we found higher values ($X = 7.42$) in the branchial group in comparison to the aereo-branchial group ($X = 7.28$), the differences being significant. Although some values overlap, the general tendency was the opposite in marine fishes (PEREZ, *et al* 1983), in which the two aereo-

aquatic species showed the lowest levels of activity).

The significant correlation between pH and activity is clear in Table 2, most active fishes presented higher pH values. This result is clearly not logical, not in agreement with the results obtained from marine fishes (PEREZ *et al* 1983). The possible explanation could be that it is the type of breathing (higher CO_2 content in the air) that determines the lower values of pH the aerial branchial group and perhaps not their activity level. For the freshwater fish species aerial respiration is much more important than in the case of the marine species (PEREZ *et al*. 1983) with their cutaneous type of breathing.

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