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BLOOD PARAMETERS IN FISHES II. OXYGEN AFFINITY, ROOT EFFECT, PH AND THE NUMBER OF HEMOGLOBINS IN SOME MARINE FISHES OF EASTERN VENEZUELA

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ABSTRACT: Oxygen affinity of the blood, Root effect, blood pH and the number of hemoglobins were studied in 12 species of marine fishes. A high correlation between oxygen affinity and blood pH, activity level and pH were found. There was no significant differences in the various parameters studied between the aquatic and aerial-aquatic breathing species.

RESUMEN: En doce especies de peces marinos, se estudiaron los parámetros sanguíneos: afinidad de la sangre por el oxígeno, el efecto Root, el pH sanguíneo y el número de hemoglobinas. Se encontró una alta correlación entre la afinidad y el pH y, entre éste y el nivel de actividad. No se encontraron diferencias significativas entre los parámetros estudiados entre las especies de respiración acuática y las especies de respiración aéreo-acuática.

INTRODUCTION

Fishes represent an invaluable group of organisms to study from a physiological point of view, how these organisms are able to live in environments so different as fish do. Their distribution is limited by marked fluctuations of environmental factors such as temperature, pressure, pH, salinity and oxygen availability.

In an earlier paper of this series (PÉREZ *et al.*, 1981), we reported the hemoglobin concentration, hematocrit, number of red blood cells, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration, in 12 species of marine fishes. In species with aerial aquatic respiration, large red blood cells, but fewer in number were found, and in 10 species with aquatic respiration high values of hemoglobin content were found. In the most active species, a high number of small red blood cells were found, characterized by a high concentration of hemoglobin. In this paper, we present the results of some additional blood parameters, such as oxygen affinity, Root effect, blood PH and the number of hemoglobins, in the same twelve species of marine fishes studied earlier (PÉREZ *et al.*, 1981).

MATERIAL AND METHODS

12 species of adult, unsexed marine fishes were used in this study. Two of these, *Amphychthys cryptocentrus* and *Batrachoides manglae* (Batrachoidídae) present, in addition to aquatic respiration, aerial respiration. The resf ot the species *Mycteroperca rubra, M. cidi* (Serranidae); *Lile piquitinga* (Clupeidae); *Ttrachinotus goodei* (Carangidae); *Haemulon bonariense; H. steindachneri* (Pomadasyidae); *Abudefduf saxatilis* (Pomacentridae); *Diplodus argenteus* (Sparidae); *Balistes vetula* (Balestidae) and *Gymnothorax moringa* (Muraenidae), have only aquatic respiration.

Fishes were collected in the Gulf of Cariaco and near Margarita Island, in eastern Venezuela; inmediately after capture, blood was collected after severing the caudal peduncle. Specimens of *G. moringa* were anaesthetized using 75 mg/l of ethyl - m - aminobenzoate; the rest of the species were bled without anaesthetic

The oxygen affinity and the Root effect of the blood were calculated employing an Aminco Hem-O-Scan oxygen dissociation analyzer, and following POWERS *et al.* (1979). The gases employed were 25%

oxygen in nitrogen, and 100% nitrogen. In order to calculate the Root effect, a gas mixture consisting of 25% oxygen and 5,6 CO_2 in nitrogen was used. The data are divided in four arbitrary categories following FARMER *et al.* (1979), based on approximated percentages: absent, less than 10% deoxygenation; low, 10-20% deoxygenation, moderate, 20-40% deoxygenation; and large, more than 40%, deoxygenration. The oxygen affinity was measured at two temperatures; 20 & 30°C. Blood pH was measured employing a Beckman microelectrode. In order to know the hemoglobin electrophoretic pattern of each species, electrophoresis in polyacrylamide, was used as given by DAVIS (1964). The samples were prepared according to FYHN & SULLIVAN (1974). The hemolysate was mixed with upper buffer which was 0.1 M in P. mercaptoethanol and a small amount of sodium hydrosulfate, to make a final concentration of hemoglobin of about 1 mg/ml. Carbon monoxide was bubbled into this mixture, and the sample was then applied to the gel. Gels were stained with a Coomassie Brilliant Blue G250 (Sigma) in a perchloric acid solution (MCFARLAND, 1977). The method to obtain hemolysate was described by PÉREZ & MACLEAN (1974).

In order to estimate the dependence of variables, the coefficient of correlation was calculated for the oxygen affinity, Root effect, and pH of the blood, the number of hemoglobins and the activity level and to know the variability between species, a variance analysis including the Ducan test was applied.

RESULTS AND DISCUSSION

Based on our observations, fishes used in this study were separeted into 8 levels of activity, beginning with the most active species: 8 = *L. piquitinga;* 7 = *T. goodei;* 6 = *H. bonariense* and *H. steindachneri;* 5 = *A. saxatilis;* 4 = *M. rubra* and *M. cidi; D. argenteus;* 2 = *B. vetula* and 1 = *G. moringa; B. manglae* and *A. cryptocentrus.*

The results obtained for the oxygen affinity of the blood (at 20 and 30°C) are expressed as P_{50} values. The partial pressures of oxygen at which hemoglobin is 50% saturated, are shown in Table I. The highest affinity (lower value of P_{50}) was found in *B. manglae* (at 20°C) and *A. saxatilis* (at 30°C) on the other hand, the lowest oxygen affinity (higher values of P_{50}) was found in the clupeoid *L. piquitinga.* The effect of temperature, which in generally lowers the affinity, was very small in both species of toad fish, and also in *A. saxatilis.* This effect was considerably higher both in *D. argenteus* and *G. moringa.* As RIGGS (1970) stated "The properties of fish hemoglobins reflect adaptations not only to the metabolic rate, but also to the prevailing external oxygen pressure:

TABLE I. BLOOD PARAMETERS IN TWELVE SPECIES OF MARINE FISHES: OXYGEN AFFINITY (P_{50}) AT 20 AND 30°C, ROOT EFFECT, pH AND NUMBER OF HEMOGLOBINS (Nº Hbs). THE MEAN ± SD. IS INDICATED. THE NUMBER OF SPECIMENS EXAMINED FOR EACH PARAMETER IS SHOWN IN PARENTHESIS.

Species	P_{50} (20°C)	P_{50} (30°C)	Root Effect	pH	Nº Hbs.
L. piquitinga	36.3 ± 1.2 (3)	51.3 ± 2.3 (3)	moderate (2)	6.78 ± 0.19 (11)	5 (49)
T. goodei	16.3 ± 0.4 (2)	23.8 ± 0.4 (2)	moderate (2)	7.03 ± 0.11 (6)	6 (20)
H. steindachneri	19.7 ± 0.8 (3)	27.7 ± 1.5 (3)	moderate (3)	7.24 ± 0.03 (4)	2 (35)
H. bonariense	18.7 ± 1.2 (3)	29.4 ± 2.1 (3)	moderate (3)	7.27 ± 0.02 (4)	2 (25)
A. saxatilis	12.9 ± 0.2 (3)	15.7 ± 0.6 (3)	moderate (2)	7.37 ± 0.06 (7)	1 (15)
M. rubra	21.7 ± 0.9 (2)	34.5 ± 0.7 (2)	large (2)	7.23 ± 0.10 (5)	2 (20)
M. cidi	22.7 ± 1.2 (2)	33.0 ± 0.7 (2)	large (2)	7.25 ± 0.07 (4)	2 (27)
D. argenteus	23.3 ± 0.3 (2)	42.2 ± 0.5 (2)	moderate (2)	7.30 ± 0.08 (7)	6 (15)
B. vetula	22.3 ± 1.1 (2)	32.1 ± 2.1 (2)	large (2)	7.40 ± 0.06 (4)	1 (21)
G. moringa	16.3 ± 1.8 (2)	34.5 ± 0.7 (2)	absent (2)	6.96 ± 0.16 (8)	2 (12)
B. manglae	12.0 ± 1.7 (6)	18.3 ± 1.5 (6)	moderate (4)	7.40 ± 0.09 (19)	4 (133)
A. cryptocentrus	15.9 ± 0.5 (3)	18.6 ± 0.6 (2)	moderate (3)	7.35 ± 0.07 (18)	7 (37)

the hemoglobin must not only be capable of combining with oxygen at the environmental PO_2, but also of unloading the oxygen at pressure appropiate for the tissue metabolism". In general, it is possible to generalize that very active fishes have hemoglobins with lower oxygen affinity (high values of P_{50}) than less active fishes (RIGGS, 1970). This seems to be true in this study, but only for some species such as *L. piquitinga, G. moringa, B. manglae, A. cryptocentrus,* but no in others. For example, we would expect higher values of P_{50} for *T. goodei, A. saxatilis* and lower P_{50} for *B. vetula;* but, it must be pointed out that we are dealing with species that belong to different phylogenetic lines and which may follow diverse adaptative strategies. As is shown in Table II, the correlation between oxygen affinity expressed as P_{50} and activity was not significant at any of the two temperatures. As one would expect, the correlation was very high between P_{50} at 20 and 30°C, and was high but negative with pH: low pH-high λ P_{50}.

TABLE II. CORRELATION COEFFICIENTS BETWEEN BLOOD PARAMETERS AND THE ACTIVITY LEVEL (ART.) SIMBOLS AS SHOWN IN TABLE I. 0.05 = 0.576; 0.01 = 0.708

	P_{50} (20°)	P_{50} (30°)	Root	PH	Nº Hbs.	Act.
P_{50} (20°C)	1.000					
P_{50} (30°C)	0.901**	1.000				
Root	0.251	0.007	1.000			
pH	—0.437	—0.641*	0.400	1.000		
Nº Hbs.	—0.102	—0.109	0.109	—0.031	1.000	
Act.	0.431	0.225	0.110	—0.584*	—0.042	1.000

The shape of the curves, that relate the degree of oxygenation of blood hemoglobin to oxygen pressure has long been held to be very important. Mammalian oxygen equilibrium curves are sigmoidal (n, the degree of interaction heme-heme = 2.2-2.9) and it is believed that this type of curve indicates a high unloading oxygen pressure in the tissues relative to the loading pressure in gills or lungs. The curves of many fish are nonsigmoidal (GRIGG, 1974) with much lower values of n. Any value of n above 1 is taken to indicate the presence of cooperativity between at least some of the oxgen binding sites (hemes). In the present study, we did find sigmoid curves only in one species *L. piquitinga* (n = 2.2), but in all cases n > 1: *A. saxatilis* = 1.8; *A. cryptocentrus* = 1.7; *T. goodei* = 1.6; *B. vetula* and *G. moringa* = 1.5; *H. bonariense* and *H. steindachneri* = 1.4; *D. argenteus* = 1.2; *M. ruba* and *M. cidi* = 1.2.

JOHANSEN & LENFANT (1972) compared the oxygen affinity of the blood of three species of water-breathing fish with selected air breathers and found that the affinity was lower in the last ones. However, POWERS *et al.* (1979) in a large quantity of Amazonian fishes belonging to 40 genera failed to find such difference. The obligate and facultative air-breathing fish presented blood oxygen dissociation curves that fall within the range occupied by the curves of the blood of the water breathers. As in our data, the general hypothesis that air breathers have lower oxygen affinity than water breathers as an adaptation to an aerial life was not true. As POWERS *et al.* (1979) suggested that perhaps the hypothesis in correct for amphibians as found by LENFANT & JOHANSEN (1969) but for a large variety of fish is less than compelling. JOHANSEN *et al.* (1978) indicated that in fishes the trend is not as clear, probably because of the diverse morphological adaptations for breathing air.

Temperature is a very important factor in those fishes from environments subject to either short term or seasonal temperature fluctuations. For example,

LENFANT & JOHANSEN (1968) compared the influence of temperature on the oxygen affinity of the blood of two species of fishes with aerial respiration, *Protopterus aethiopicus* and *Neoceratodus forsteri*. They found that the blood of *P. aethiopicus* that lives in a zone with stable temperature, is sensitive to changes in temperature, while in *N. forsteri*, a fish that inhabits areas with great variations in temperature, the blood is less sensitive. On the other hand PÉREZ *et al.* (1980) found that the blood of *Hoplosternum litterale*, a siluriform, with aerial respiration, presents blood even less sensitive than *N. forsteri* to changes in temperature and lives in a tropical environment. In the present research the tempererature is stable. However, the responses in oxygen affinity showed great variations: the toad fish, *A. saxatilis* in one extreme with little changes; while *D. argenteus* and *G. moringa* in the other extreme with great changes.

The Root effect (ROOT, 1931), the reduction in oxygen capacity of the blood in response to CO_2 or more properly to low pH, has been assumed to be a mechanism to facilitate O_2 into the swimbladder (FANGE, 1966). WILLMER (1934) found in some South American fishes that the blood of the air breathers was less sensitive to low pH than non-air-breathing fishes; thus supporting the idea of CARTER (1931) that the evolution of aerial respiration was preceeded by the evolution of blood less sensitive to the presence of CO_2 than in typical freshwater or marine fish. The results obtained by FISH (1956); JOHANSEN (1966); LENFANT *et al.* (1967); LENFANT & JOHANSEN (1968); LENFANT et al. (1966) showed a similar pattern: the greater the dependence of the lung fish species on air breathing, the smaller the Bohr effect (reduction of the affinity Hb-O_2 to low pH). In this study, no significant differences were found between the Root effect of fishes of aerial-aquatic and aquatic respiration. The results obtained by the R.V. Alpha Helix expedition to the Amazon River, FARMER *et al.* (1979) also revealed that Root effect in several species of air-breathing fishes not in agreement with Carter's hypothesis. Moreover, they found a positive correlation between the distribution of choroid retia mirabile in the eyes and the Root effect, but no relation was found with the presence of a swimbladder rete mirabile. Choroid rete is a vascular counter-current organ located behind the retina of the eye, and is responsible in part for the maintenance of a high partial pressure of oxygen due to the oxygen that is liberated from the hemoglobin by acidification of the blood. The choroid rete is found widely distributed among teleosts and is nearly always present in the superorder Acanthopterygii (WITTEMBERG & HAEDRICH, 1974), which includes most of the species of this study with the exception of *L. piquitinga, G. moringa, B. manglae* and *A. cryptocentrus*. Table II shows that Root effect is not significantly correlated with the rest of the parameters, being the highest correlation (not significant) with pH.

Blood pH was correlated significantly with oxygen affinity (at 30°C), low pH was associated with high values of P_{50}. A high correlation between blood pH and the activity level was found: highly active fishes as *L. piquitinga* and *T. goodei* presented lower values of pH. In contrast, the less active fishes as *B. manglae* and *A. cryptocentrus* showed high values of pH, the exception being *G. moringa*.

The number of hemoglobins (Table I & Fig. 1), varies between 1 in *B. vetula* and *A. saxatilis* and 7 in *A. cryptocentrus*. It has been suggested that multiple hemoglobin could be related to an adaptative strategy to cope up with thermal fluctuations in the environment (SULLIVAN, 1977). However the results of PÉREZ & RYLANDER 1985) do not support this explanation for the establishment of hemoglobin heterogeneity in fishes. On the other hand, correlation between the number of hemoglobins and the rest of the parameters was very low (Table II).

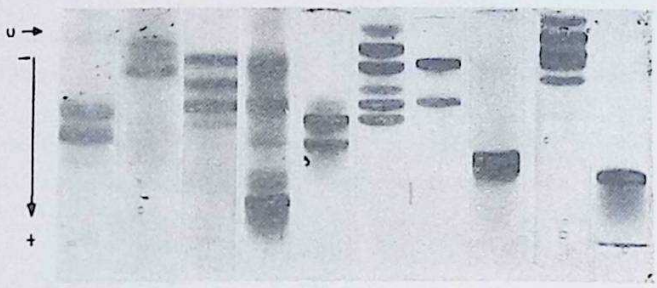

Fig. 1. Hemoglobin electrophoretic phenotypes of (from lefr to right): *G. moringa, L. piquitinga, B. manglae, A. cryptocentrus, M. cidi, T. goodei, H. steindachneri, A. saxatilis, D. argenteus* and *vetula*. O indicates the origen.

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REFERENCES

CARTER, G. S. & L. C. BEADLE. 1931. The fauna of the swamps of the Paraguayan Chaco in relation to its environment. II. Respiratory adaptations in the fishes. *J. Linn. Soc. Lond.*, 37: 327-366.

DAVIS, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci. 121:* 404-427.

FANGE, R. 1966. The physiology of the swimbladder. *Physiol. Rev.* 46: 299-322.

FARMER, M., FYHN, H. J., FYHN, U. E. H. & R. W. NOBLE. 1979. Ocurrence of Root effects hemoglobins in Amazonian fishes. *Comp. Biochem Physiol.* 62A: 115-124.

FISH, G. R. 1956. Some aspects of the respiration of six species of fish from Uganda. *J. Exp. Biol.* 33: 185-195.

FYHN, U. E. H. & SULLIVAN. 1974. Hemoglobin Polymorphism fishes. I. Complex phenotypic patterns in the toad fish. *Opsanus tau. Biochem. Genetics.* 11: 373-385.

GRIGG, G. C. 1974. Respiratory function of blood in fishes. *In Chemical Zoology.* (Eds. Florkin, M & Sceer, B.T.). Vol. VII: 331-368.

JOHANSEN, K. 1966. Air breathing in the teleost *Symbranchus marmoratus Comp. Biochem. Physiol.* 18: 383-395.

JOHANSEN, K. & C. LENFANT. 1972. A comparative aproach to the adaptability of 0_2-Hb Affinity of Hemoglobin and Red Cell Acid Base Status. *In* P. Astrup and M. Rorth (eds.), *Oxygen affinity of hemoglobin and red cell acid base status.* Academic Press, Munksgaard, Copenhagen-Dinamarca.

————. MANGUM, C. P. & G. L. LYKKEBBE. 1978. Respiratory properties of the blood of Amazonian fishes. *Can J. Zool.* 56: 898-906.

LENFANT, C. & K. JOHANSEN. 1968. Respiration in the African Lungfish. *Protopterus aethiopicus. J. Exp. Biol.* 49: 437-452.

LENFANT, C. JOHANSEN, K. & G. C. GRIGG. 1966. Resriratory properties of blood and pattern of gas exchange in the lungfish, *Neoceratodus forsteri* (Krefft). *Resp. Physiol.* 2: 1-21.

————. 1967. Respiratory adaptations in selected amphibians. *Ibid.*, 2: 247-260.

MCFARLAND, F. 1977. The staining of starch gels with Coomassie Brilliant Blue G-250 perchloric solution. *Experientia* 33: 1688.

PÉREZ, J. E. & N. MACLEAN, 1974. Ontogenetic changes in hemoglobins in roach, *Rutilus rutilus* (L). and rudd, *Scrardinius erythrophthalmus* (L). *J. Fish Biol.* 6: 479-482.

PÉREZ, J. E., CORTES, H., GONZÁLEZ, D. & G. OJEDA. 1981. Blood parameters in fishes I. Hemoglobin concentration, hematocrit and the number of red blood cells in some marine fishes of eastern Venezuela. *Bol. Inst. Oceanogr. Univ. Oriente* 20(182): 33-38, 3 tals.

PÉREZ, J. E. & M. K. RYLANDER. 1985. Hemoglobin heterogeneity in Venezuelan fishes. *Comp Biochem. Physiol.* (in press).

POWERS, D. A., FYHN, H. J. FYHN, U. E. H., MARTÍN, J. P., GARLICK, R. L. & S. C. WOOD. 1979. A comparative study of the oxygen equilibria of blood from 40 genera of Amazonian fishes. *Comp. Biochem. Physiol.* 62A: 67-85.

RIGGS, A. 1970. Properties of fish hemoglobins. In: *Fish Physiology.* Vol. 4. (Ed. Hoar, W. S. & Randall, D. J.) 209-252. Academic Press, New York.

————. 1979. (Ed). The Alpha Helix Expedition to the Amazon for the study of fish bloods and hemoglobins. *Com. Biochem. Physiol.* 62A: 1-272.

ROOT, R. W. 1931. The respiratory function of the blood of marine fishes. *Biol. Bull. 61,* 427-456.

SULLIVAN, B. 1977. Hemoglobin variation and its significance in fish. US-URSS. Workshop on *Physiology and Biochemistry of aquatic animals. Georgetown. S. C.* (mimeographed).

WILLMER, E. N. 1934. Some observations on the respiration of certain tropical freshwater fishes. *J. Exp. Biol* 11: 283-306.

WITTENBERG, J. B. & R. L. HAEDRICH. 1974. The choroid rete mirabile of the fish eye. II Distribution and relation to the pseudobranch and to the swimbladder rate mirabile. *Biol. Bull.* 146: 137-156.

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