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BLOOD PARAMETERS IN FISHES. V. ACTIVITY LEVEL AND TYPE OF RESPIRATION IN SOME MARINE ESTUARINE AND FRESHWATER FISHES OF VENEZUELA

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RESUMEN: Parámetros sanguíneos de 31 especies de peces marinos, dulceacuícolas y estuarinos de Venezuela, se correlacionaron con el habitáculo, el nivel de actividad y el tipo de respiración. Se encontró polimorfismo de la hemoglobina en *Astyanax bimaculatus*, *Plecostomus watwata* y *Thalassophryne maculosa*, pero estos polimorfismos no presentaron relación con el nivel de actividad, tipo de respiración o estabilidad del ambiente (termolábil o termoestable). No se encontró relación del número de hemoglobinas con actividad, tipo de respiración o habitáculo (marino, estuarino o dulceacuícola), pero la concentración de la hemoglobina, hematocrito y número de eritrocitos fueron mayores en especies muy activas. Niveles altos de actividad se correlacionaron con respiración branquial, mientras que los niveles bajos de actividad se correlacionaron con respiración aero-branquial. Los peces con hemoglobina de elevada afinidad por el oxígeno presentaron elevados valores de pH y efecto Root. Las especies dulceacuícolas presentan una afinidad mayor por el oxígeno que las especies marinas o estuarinas; el efecto Root fue mayor en las especies marinas.

ABSTRACT: Blood parameters for 31 species of marine, estuarine and freshwater fishes from Venezuela were correlated with habitat, activity level and type of respiration. Hemoglobin polymorphism was found in *Astyanax bimaculatus*, *Plecostomus watwata* and *Thalassophryne maculosa*, but no relationship was found between presence of polymorphism and activity level, type of respiration, or unstability of the environment (thermolabile or thermostabile). The number of hemoglobins was unrelated to activity level, type of respiration and habitat (marine, estuarine or freshwater), but hemoglobin concentration, hematocrit and number of erythrocytes were higher in highly active species than in species having low activity levels. High activity level was correlated with branchial respiration and low activity level with aero-branchial respiration. Fishes having hemoglobin with high oxygen affinity had higher values of pH and Root effect. Freshwater species showed a greater oxygen affinity than estuarine or marine species, and Root effect was higher in marine species.

INTRODUCTION

Fishes have developed a number of adaptive strategies to insure their survival in extreme variations in temperature, salinity, pressure, pH, oxygen and carbon dioxide concentrations. Among these adaptations are (a) the development of a larger proportion of red muscle and an increased gill surface to facilitate aerobic metabolism in water having low oxygen concentration; (b) the lowering of the activity level to reduce the oxygen requirement; (c) the development of aerial respiration and accompanying buffering systems in the blood (HEISLER, 1984); (d) seasonal movement to locations having relatively high oxygen concentration (MAGNUSON, *et al.*, 1985); and (e) the development of hemoglobin heterogeneity as a possible mechanism for adapting to unstable environments. During the last decade considerable attention has focused on the last strategy (see, for example,

TABLE I. SPECIES OF FISH IN THIS STUDY, WITH TYPE OF RESPIRATION (AB = AERO-BRANCHIAL, B = BRANCHIAL); ACTIVITY LEVEL (H = HIGH, I = INTERMEDIATE, L = LOW); AND HABITAT (M = MARINE, E = ESTUARINE, F = FRESHWATER). TAXONOMY FOLLOWS GREENWOOD ET AL. (1966).

Superorder Elopomorpha	
Order Anguilliformes	
Familiy Muraenidae	
Gymnothorax moringa (Cuvier)	B L M
Superoder Clupeomorpha	
Order Clupeiformes	
Family Clupeidae	
Lile piquitinga Schereiner & Miranda Ribeiro	B H M
Superorder Ostariophysi	
Order Cypriniformes	
Family Characidae	
Astyanax bimaculatus (Linnaeus)	B H F
Family Erythrinidae	
Hoplias malabaricus (Bloch)	B I F
Order Siluriformes	
Family Ariidae	
Cathorops spixii (Agassiz)	B I E
Arius herzbergii (Bloch)	B I E
Family Cetopsidae	
Hemicetopsis minutus Eigenmann	B I F
Family Callichthydae	
Hoplosternum littorale (Hancock)	AB L F
Family Loricariidae	
Lasiancistrus maracaiboensis Schultz	AB L F
Plecostomus watwata (Hancock)	AB L F
Superorder Paracanthopterygi	
Order Batrachoidiformes	
Family Batrachoididae	
Batrachoides manglae Cervigon	AB L M
Amphichthys cryptocentrus (Valenciennes)	AB L M
Thalassophryne maculosa Gunther	AB L Mo
Superorder Acanthopterygii	
Order Perciformes	
Family Centropomidae	
Centropomus undecimalis (Bloch)	B H E

Family Serranidae	
Diplectrum formosum (Linnaeus)	B I M
Diplectrum radiale (Quoy and Gaimard)	B I M
Mycteroperca cidi Cervigon	B I M
Mycteroperca rubra (Bloch)	B I M
Family Carangidae	
Trachinotus goodei Jordan and Evermann	B H M
Family Haemulidae	
Haemulon bonariense Cuvier	B I M
Haemulon steindachneri (Jordan and Gilbert)	B I M
Family Sparidae	
Diplodus argenteus (Poey)	B I M
Family Scienidae	
Umbrina coroides (Cuvier)	B I M
Micropogonias furnieri (Desmerest)	B I E
Stellifer sp.	B I M
Bairdiella ronchus (Cuvier)	B I E
Plagioscion squamosissimus (Heckel)	B I F
Family Cichlidae	
Petenia kraussii Steindachner	B H F
Family Pomacentridae	
Abudefduf saxatilis (Linnaeus)	B I M
Family Mugilidae	
Mugil curema Valenciennes	B H E
Order Tetraodontiformes	
Family Balistidae	
Balistes vetula (Linnaeus)	B I M

SHARP, 1973; FYHN, *et al.*, 1979; PÉREZ and RYLANDER, 1985; PÉREZ *et al.*, 1983; and PÉREZ, 1986).

In the present study, we report blood parameters for 31 species of fish representing 7 orders and 19 families (Table I). We tested for possible relationships between type of respiration, activity level, habitat, and the following blood parameters: hemoglobin concentration, hematocrit, erythrocyte number, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, oxygen affinity, Root effect, pH, and number of hemoglobins. We have interpreted our results in light of data from MAVARES and PÉREZ (1984), PÉREZ and RYLANDER (1985), PÉREZ *et al.* (1981, 1983, 1984, 1985) and MUNDARAY and PÉREZ(1988).

MATERIALS AND METHODS

Specimens were collected in eastern Venezuela as indicated in earlier papers (PÉREZ *et al.*, 1981, 1983, 1984, 1985; MAVARES and PÉREZ, 1984; MUNDARAY and PÉREZ, 1986). *Thalassophryne maculosa, Cathoreps spixii, Arius herzbergii, Mugil curema* and *Centropomus undecimalis* were collected from the Gulf of Cariaco, Sucre. Blood was withdrawn with a heparanized pipette from the severed caudal peduncle, transported on ice to the laboratory, and processed inmediately.

We followed the procedures described in PÉREZ *et al.* (1981, 1983) to measure hematocrit, hemoglobin

TABLE II BLOOD PARAMETERS IN 31 SPECIES OF FISH: CONCENTRATION OF HEMOGLOBIN (HB), HEMATOCRIT (HCT), NUMBER OF RED BLOOD CELLS (Nº RBC), MEAN CORPUSCULAR VOLUME (MCV) MEAN CORPUSCULAR HEMOGLOBIN (MCHBC), OXYGEN AFFINITY (P50) AT 20 AND 30ºC, ROOT EFFECT, PH AND THE NUMBER OF HEMOGLOBINS (Nº HBS). THE MEAN ± S.D. IS INDICATED. THE NUMBER OF SPECIMENS EXAMINED FOR EACH PARAMETER IS SHOWN IN PARENTHESIS.

Species	Hb(g/100ml)	Hc (%)	Nºrbc(mm x10^6)	MCV(μ^3)	MCHb($\mu\mu_g$)
L. piguitinga	6.3 ± 1.0(54)	29.6 ± 1.9(58)	2.37 ± 0.55(46)	137.0 ± 47.2(24)	27.8 ± 9.8(18)
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)
M. curema	9.2 ± 1.3(38)	29.6 ± 3.9(56)	2.76 ± 0.51(55)	108.1 ± 10.8(54)	32.1 ± 5.0(35)
C. undecimalis	6.9 ± 1.0(34)	31.4 ± 2.9(34)	2.13 ± 0.37(34)	151.1 ± 28.4(34)	33.4 ± 10.8(34)
T. goodei	12.1 ± 1.8(19)	39.0 ± 2.7(20)	3.29 ± 0.36(20)	120.0 ± 19.0(20)	37.1 ± 6.6(19)
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)
H. steindachneri	7.8 ± 2.2(25)	36.1 ± 3.4(24)	2.19 ± 0.80(24)	183.2 ± 63.0(23)	38.7 ± 3.9(24)
H. bonariense	8.4 ± 1.6(20)	33.2 ± 4.1(20)	1.84 ± 0.53(20)	189.2 ± 39.0(20)	47.2 ± 8.4(20)
A. saxatilis	8.5 ± 2.0(25)	34.1 ± 4.7(25)	1.89 ± 0.53(25)	191.7 ± 58.8(25)	47.9 ± 18.5(25)
M. rubra	9.2 ± 1.9(18)	38.2 ± 4.4(19)	2.16 ± 0.53(25)	182.9 ± 29.3(19)	43.1 ± 9.5(18)
M. cidi	9.9 ± 1.7(27)	42.9 ± 7.3(27)	2.24 ± 0.62(27)	210.1 ± 81.8(27)	48.1 ± 17.1(27)
D. argenteus	8.1 ± 1.3(27)	36.7 ± 3.8(26)	2.10 ± 0.56(26)	185.4 ± 54.8(26)	40.6 ± 11.6(26)
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)
H. minutus	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)
U. coroides	7.3 ± 0.9(20)	30.7 ± 4.3(20)	2.58 ± 0.23(20)	120.4 ± 24.1(20)	28.3 ± 4.3(20)
Stellifer sp.	6.4 ± 1.7(20)	29.5 ± 2.9(20)	1.45 ± 0.11(20)	200.1 ± 24.3(20)	44.3 ± 12.9(20)
M. furnieri	5.0 ± 1.0(20)	26.9 ± 3.1(20)	1.56 ± 0.16(20)	172.9 ± 31.7(20)	32.5 ± 6.7(20)
B. ronchus	8.6 ± 1.9(20)	29.3 ± 2.8(20)	1.54 ± 0.19(20)	193.3 ± 27.9(20)	56.4 ± 14.7(20)
P. squamosissimus	7.3 ± 0.6(20)	39.0 ± 1.9(20)	2.99 ± 0.26(20)	131.4 ± 13.3(20)	24.7 ± 2.7(20)
D. formosum	10.8 ± 0.3(28)	44.0 ± 0.8(28)	1.52 ± 0.06(28)	289.4 ± 7.7(28)	70.7 ± 2.6(28)
D. radiale	11.7 ± 0.3(28)	43.7 ± 0.6(28)	2.48 ± 0.06(28)	176.1 ± 9.0(28)	47.0 ± 1.5(28)
C. spixii	7.0 ± 1.8(15)	28.9 ± 6.3(15)	1.68 ± 0.25(18)	174.9 ± 4.0(18)	40.1 ± 3.1(18)
A. herzbergii	6.8 ± 0.5(54)	32.0 ± 1.2(32)	1.74 ± 0.20(32)	185.9 ± 19.4(32)	38.8 ± 2.5(32)
B. vetula	8.7 ± 1.3(20)	42.5 ± 6.2(21)	2.91 ± 0.83(20)	163.8 ± 63.6(20)	32.1 ± 10.6(20)
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)
P. watwata	7.8 ± 1.8(23)	33.1 ± 6.0(23)	1.30 ± 0.27(23)	263.3 ± 62.2(23)	61.4 ± 12.7(23)
H. littorale	9.6 ± 1.9(38)	31.7 ± 5.9(29)	1.17 ± 0.27(24)	257.6 ± 40.8(24)	80.8 ± 15.1(23)
G. moringa	5.5 ± 1.4(18)	24.2 ± 4.7(18)	0.64 ± 0.11(20)	365.9 ± 61.7 (19)	82.1 ± 17.9(18)
B. manglae	4.6 ± 1.5(28)	26.8 ± 5.7(28)	0.57 ± 0.23(27)	537.4 ± 212.5(21)	91.7 ± 36.9(20)
A. cryptocentrus	3.6 ± 1.2(18)	29.9 ± 7.4(19)	0.43 ± 0.17(19)	742.6 ± 280.4(15)	97.1 ± 31.5(12)
T. maculosa	4.9 ± 1.1(13)	22.8 ± 4.3(13)	0.74 ± 0.22(12)	330.8 ± 134.6(12)	74.9 ± 31.7(12)

Species	MCHbC(%)	P_{50}(20ºC)	P_{50}(30ºC)	Root	pH	Nº Hbs
L. piguitinga	21.7 ± 2.8(52)	36.3 ± 1.2(3)	51.3 ± 2.3 (3)	++(2)	6.78 ± 0.19(11)	5(49)
A. bimaculatus	22.8 ± 2.7(15)	6.0 ± 1.0(3)	14.5 ± 0.6 (2)	++(4)	7.52 ± 0.10(8)	7-8-9(5-52-35)
M. curema	29.7 ± 4.9(36)	19.3 ± 0.4(2)	24.0 ± 0.0 (2)	+(2)	6.99 ± 0.07(7)	3(28)
C. undecimalis	22.1 ± 6.0(34)	19.5 ± 0.7(2)	35.4 ± 0.8 (2)	+(2)	7.05 ± 0.05(5)	3(28)
T. goodei	30.9 ± 4.1(19)	16.3 ± 0.4(2)	23.8 ± 0.4 (2)	++(2)	7.03 ± 0.11(6)	6(20)
P. kraussii	25.6 ± 4.0(30)	8.1 ± 0.1(3)	11.1 ± 1.6 (2)	(-) (2)	7.41 ± 0.10(13)	7(36)
H. steindachneri	21.9 ± 5.3(24)	19.7 ± 0.8(3)	27.7 ± 1.5 (3)	++(3)	7.24 ± 0.03(4)	2(35)
H. bonariense	25.3 ± 3.2(20)	18.7 ± 1.2(3)	29.4 ± 2.1 (3)	++(3)	7.27 ± 0.02(4)	2(25)
A. saxatilis	24.9 ± 4.1(25)	12.9 ± 0.2(3)	15.7 ± 0.6(3)	++(2)	7.37 ± 0.06(7)	1(15)
M. rubra	23.8 ± 3.5(18)	21.7 ± 0.9(2)	34.5 ± 0.7 (2)	+++(2)	7.23 ± 0.10(5)	2(20)
M. cidi	23.8 ± 6.0(27)	22.7 ± 1.2(2)	33.0 ± 0.7 (2)	+++(2)	7.25 ± 0.07(4)	2(27)
D. argenteus	22.1 ± 2.2(27)	23.3 ± 0.3(2)	42.2 ± 0.5 (2)	++(2)	7.30 ± 0.08(7)	6(15)
H. malabaricus	25.9 ± 2.5(20)	5.6 ± 0.6(2)	10.3 ± 0.4 (2)	++(2)	7.35 ± 0.09(9)	3(12)
H. minutus	23.6 ± 2.8(26)	10.0 ± 0.4(3)	13.7 ± 0.2 (2)	(-) (2)	7.43 ± 0.09(5)	3(25)
U. coroides	23.9 ± 2.9(20)	19.3 ± 5.0(3)	37.3 ± 3.1 (3)	+++(3)	7.31 ± 0.04(20)	5(20)
Stellifer sp.	22.0 ± 6.6(20)	18.8 ± 1.8(3)	24.2 ± 3.3 (3)	++(3)	7.22 ± 0.20(20)	4(20)
M. furnieri	18.8 ± 3.9(20)	17.7 ± 1.6(3)	30.0 ± 8.0 (3)	++(3)	7.33 ± 0.65(20)	3(20)
B. ronchus	29.4 ± 7.4(20)	19.2 ± 6.4(3)	23.5 ± 9.9 (3)	++(3)	7.31 ± 0.09(20)	5(20)
P. squamosissimus	18.9 ± 1.7(20)	22.7 ± 1.5(3)	44.5 ± 6.2 (3)	++(3)	6.80 ± 0.20(20)	6(20)
D. formosum	24.4 ± 0.6(28)	40.5 ± 1.6(5)	47.0 ± 1.1 (5)	++(5)	7.15 ± 0.6(28)	7(39)
D. radiale	26.7 ± 0.6(28)	26.8 ± 2.2(5)	32.3 ± 1.5 (5)	++(5)	6.98 ± 0.09(28)	3(27)
C. spixii	25.0 ± 2.2(18)	7.3 ± 0.4(2)	10.7 ± 0.2 (2)	++(2)	7.26 ± 0.04(6)	5(15)
A. herzbergii	20.9 ± 0.9(32)	10.1 ± 0.6(2)	17.2 ± 0.8 (3)	++(2)	7.21 ± 0.04(7)	1(29)
B. vetula	20.1 ± 3.0(20)	22.3 ± 1.1(2)	32.1 ± 2.1 (2)	+++(2)	7.40 ± 0.06(4)	1(21)
L. maracaiboensis	25.3 ± 4.2(22)	14.2 ± 0.6(4)	16.5 ± 0.4 (4)	(-) (3)	7.32 ± 0.08(7)	3(19)
P. watwata	23.9 ± 4.5(23)	12.0 ± 0.0(2)	18.8 ± 0.4 (2)	(-) (2)	7.18 ± 0.04(7)	2-4(55-24)
H. littorale	29.5 ± 6.3(28)	13.7 ± 0.6(3)	16.6 ± 0.0 (3)	(+) (3)	7.34 ± 0.09(7)	2(34)
G. moringa	22.5 ± 3.4(18)	16.3 ± 1.8(2)	34.5 ± 0.7 (2)	(-) (2)	6.96 ± 0.16(8)	2(12)
B. manglae	17.4 ± 5.3(28)	12.0 ± 1.7(6)	18.3 ± 1.5 (6)	++(4)	7.40 ± 0.09(19)	4(133)
A. cryptocentrus	12.8 ± 4.1(16)	15.9 ± 0.5(3)	18.6 ± 0.6 (2)	++(3)	7.35 ± 0.07(18)	7(37)
T. maculosa	22.3 ± 4.7(12)	11.0 ± 1.3(23)	19.5 ± 1.3(24)	++(24)	7.44 ± 0.13(16)	7-8(133-78)

TABLE III: CORRELATION COEFFICIENTS BETWEEN BLOOD PARAMETERS, ACTIVITY LEVEL (ACT.), ENVIRONMENT (ENV.) AND TYPE OF RESPIRATION (RESP.). SYMBOLS AS SHOWN IN TABLE II BETWEEN BLOOD PARAMETERS, ACTIVITY LEVEL (ACT.), ENVIRONMENT (ENV.) AND TYPE OF RESPIRATION (RESP.) SYMBOLS AS SHOWN IN TABLE II A = 0.05* 0.355 0.01** = 0.456

	Hb	Hcto	Nº.rbc	MCV	MCHb	MCHbc
Hb	1.000**					
Hcto	0.746**	1.000**				
No.rbc	0.714	0.604	1.000**			
MCV	-0.552	-0.311	-0.941**	1.000		
MCHb	-0.261**	-0.264	-0.861**	0.913	1.000	
MCHbc	0.791	0.191	0.479	-0.511	-0.124	1.000
P_{50}(20º C)	0.124	0.238	0.249	-0.205	-0.260*	-0.051
P_{50}(30º C)	0.057	0.222	0.311	-0.290	-0.407	-0.144
Root	0.055	0.265	0.242	-0.149*	-0.249*	-0.152
pH	-0.150	-0.055	-0.330	0.391	0.390	-0.158
Nº. Hbs	-0.166	-0.122	-0.131	0.102	0.064	-0.091
env.	-0.199*	-0.129*	-0.035**	0.005	-0.085**	-0.181
resp.	0.444**	0.395*	0.720**	-0.712	-0.693**	0.263
act.	0.457	0.389	0.726	-0.714	-0.677	0.295

	P_{50}(20º C)	P_{50}(30º C)	Root	pH	Nº. Hbs	env.	resp.	act.
Hb								
Hcto								
No.rbc								
MCV								
MCHb								
MCHbc								
P_{50}(20º C)	1.000**							
P_{50}(30º C)	0.909*	1.000**						
Root	0.451**	0.466**	1.000					
pH	-0.618	-0.647	0.044					
Nº. Hbs	-0.037**	0.001**	0.054**	-0.079	1.000			
env.	0.580	0.553	0.551	-0.171	-0.109	1.000		
resp.	0.201	0.353	0.207	-0.305	-0.137	0.222	-1.000**	
act.	0.071	0.226	0.120	-0.228	-0.010	0.096	-0.900	1.000

TABLE IV: VARIANCE ANALYSIS RESULTS FOR THE 14 VARIABLES AND 5 GROUPS OF FISHES ACORDING TO ENVIRONMENT AND RESPIRATION: MB MARINE BRANCHIAL; MAB = MARINE - AEREOBRANCHIAL; ES = STUARINE BRANCHIAL; FB = FRESHWATER - BRANCHIAL AND FAB = FRESHWATER - AEROBRANCHIAL + INDICATES SIGNIFICANT DIFFERENCES.

Table. 4 ._ Varience gnalysis results for the 14 variables and 5 groups of fishes acording to environment and respiration: MB = marine branchial ; MAB = marine - aereobranchial ; ES = stuarine branchial ; FB = freshwater-branchial and FAB = freshwater - aereobranchial + indicates significant differences.

	Hb					Hcto					Nª rbc					MCV					MCHb				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1. MB		+	+	+	+		+	+		+		+			+		+	+		+		+	+		+
2. MAB	+		+			+		+	+	+	+		+	+	+	+		+	+	+	+		+	+	+
3. ES	+	+		+	+	+	+		+		+	+			+	+	+		+	+	+	+		+	+
4. FB	+		+				+	+		+		+			+		+	+		+		+	+		+
5. FAB	+		+			+	+		+		+	+	+	+		+	+	+	+		+	+	+	+	

	MCHbC					P_{50} 20					P_{50} 30					Root				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1. MB		+			+		+	+	+	+		+	+	+	+			+	+	+
2. MAB	+		+	+	+	+					+		+					+	+	+
3. ES		+			+	+			+		+	+			+	+	+			+
4. FB		+			+	+		+			+					+	+			+
5. FAB	+	+	+	+		+					+		+			+	+	+	+	

	pH					Nª Hbs					Resp.					Act.					Env.				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1. MB		+	+		+		+		+			+			+		+		+	+			+	+	+
2. MAB	+		+	+	+	+		+	+	+	+		+	+		+		+	+				+	+	+
3. ES	+	+					+		+			+			+		+			+	+	+		+	+
4. FB		+			+	+	+	+		+		+			+	+	+			+	+	+	+		
5. FAB	+	+		+			+		+		+		+	+		+		+	+		+	+	+		

concentration, erythrocyte number, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, oxygen affinity, Root effect, pH and the number of hemoglobins. We classified activity level as high, medium or low, and type of respiration as branchial or aerobranchial (Table I), based on field and laboratory observations (MAVARES and PÉREZ 1984; MUNDARAY and PÉREZ, 1988; PÉREZ *et al.* 1981, 1983, 1984, 1985; and the present study). We classified Root effect according to the following arbitrary categories (FARMER *et al.*, 1979): (a) absent (-), less than 10% deoxygenation; (b) low (+), 10-20% deoxygenation; (c) moderate (++), 20-40% deoxigenation; and (d) large (+++), more than 40%.

The data on the blood parameters, type of respiration, habitat, and activity level were incorporated into a matrix of 14 columns (variables) and 31 rows (species). Another matrix based on this matrix contained 31 columns (species) and 14 rows (variables). To identify the order of blood parameters and species, we employed a cluster analysis and a principal component analysis using the BMDP programs (DIXON *et al.*, 1981) and SPSS (NIE *et al.* 1975). To compare the order of the parameters (one at a time), the matrix of 31 columns x 14 rows was modified successively in a matrix of 31 x 13, thereby producing 14 dendrograms that could be compared to the first, wich included the 14 blood parameters. The Pearson coefficient of correlation was calculated to determine the dependence of variables, and an analysis of variance (SOKAL and ROHLF, 1981) was used to determine if significant variation existed between species groups on the basis of habitat and type of respiration. An estimate of similarity and dissimilarity was calculated using Sorensen's equation (SORENSEN, 1948).

RESULTS

Table II shows the results for the 11 blood parameters in the 31 species under consideration. Hemoglobin polymorphism was found in *A. bimaculatus*, *P. watwata*, and *T. maculosa*.. Table II indicates the number of specimens per species that exhibited a particular number of hemoglobins. For example, in the case of *T. maculosa* 133 specimens had 7 hemoglobins and 78 specimens had 8 hemoglobins. No relationship was found between presence of polymorphism and activity level or type of respiration, nor with instability of the envirónment, as suggested by SULLIVAN (1977).

Table III shows the correlation coefficients for blood parameters, activity level (6 = high, 4 = intermediate, 2 = low), habitat (6 = marine, 4 = estuarine, 2 = freshwater) and type of respiration (4 = branchial, 2 = aero-branchial). The number of hemoglobins

separated by electrophoresis — the most independent of the parameters — was not correlated with habitat, type of respiration, activity level, or any other blood parameter.

We found correlations between hemoglobin concentration, hematocrit, and erythrocyte number and volume. Activity level and type of respiration were also correlated, branchial respiration being found only in highly active fishes (correlation between activity level and type of respiration = -0.900; Table III). Also, hemoglobin concentration, hematocrit and number of small erythrocytes were higher in highly active species (which have branchial respiration) than in species having low activity and aero-branchial respiration.

Table IV shows the results of an analysis of variance for each variable in the 5 groups, based on habitat and type of respiration. Percentages of similarity were calculated from significant differences between means (Fig. 1). Table IV and Fig.1 clearly show the importance of respiration type in determining most of the blood parameters. A large number of differences (13) were found between groups MB-FAB and EsB-MAB (similarity of 7.1%), followed by MB-MAB. The fewest differences (7) were between MB-FB and EsB-FB, with a similarity of 50.0%, followed by MAB-FAB and MB-EsB (similarity of 35.7%).

We also prepared a dendrogram (Fig. 2) using all 14 parameters, and calculated the relative weight of each variable by sequentially removing them one at a time from the data. The results of this process were similar when respiration type and activity level were not considered. Cluster analysis also indicated that the order in which the species are grouped is determined primarily by the type of respiration and secondarily by the environmental variables. In Fig. 2 it can be seen that the 6 species on the right posses aero-branchial respiration, while all other species, on the left, posses only branchial respiration. Marine and freshwater species showed a tendency to form two separate groups, with the estuarine species distributed among them.

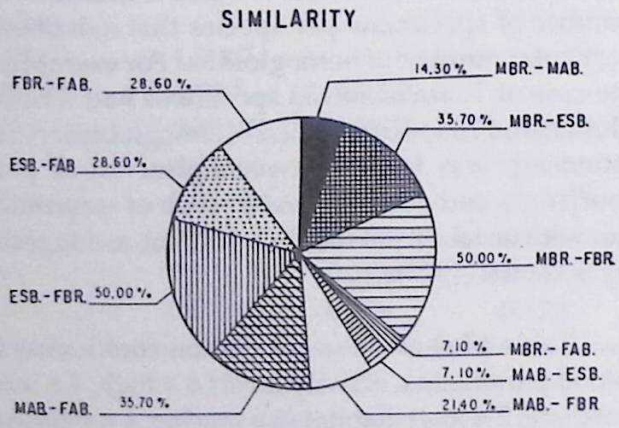

Fig.1 Similarity – percentages between 5 groups of fishes according to habitat and type of respiration

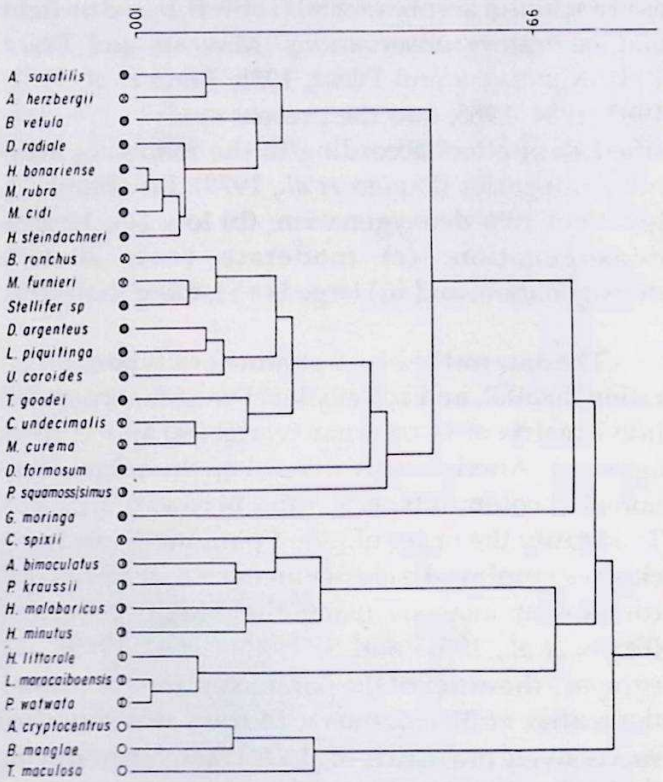

Fig. 2 Phenogram showing the distribution of species based on the parameters

DISCUSSION

In this study we have identified correlations between blood parameters, habitat, respiratory level, and type of respiration. We have no satisfactory explanation for the correlation between branchial respiration and high activity level or between aero-branchial respiration and low activity level, as there seem to be no obvious causal relationships between these variables.

Activity level and type of respiration were also correlated with several blood parameters: hemoglobin concentration, hematocrit and number of erythrocyes were higher in highly active (branchial respiration) species than in species having low activity levels (aero-branchial respiration). Such a relationship was not demonstrated by PÉREZ *et al.* (1981, 1983), but the relationship may have been missed due to the small size of the sample. When in the present study we added 5 marine, 6 estuarine and one freshwater species to their sample, we obtained the significant correlations shown in Table III. The same relationship has been reported by HALL and GRAY (1929), ENGEL and DAVIS (1964), LARSSON *et al.* (1976), PUTNAM and FREEL (1978) and WELLS *et al.* (1980).

The increased number of erythrocytes in highly active species, such as *L. piquitinga*, is not doubt a function of the greater surface/volume ratio of small cells that would make gas exchange more efficient. Apparently the number of cells in wich the hemoglobin is distributed is more important for respiratory efficiency than the amount of pigment contained in the erythrocytes. Hematocrit would also be expected to differ according to oxygen demands.

As in another study of tropical fishes (PÉREZ and RYLANDER, 1985), we did not find that hemoglobin heterogeneity was adaptive for unstable environments, as was previously suggested by SULLIVAN (1977). SULLIVAN based his conclusion on published data that showed more hemoglobins in fish from thermolabile than from thermostable environments (in temperate waters). PÉREZ and RYLANDER (1985) pointed out that "although it is reasonable to expect more hemoglobins in species that are subjected to numerous environmental variations, it is conceivable that in some instances species with fewer hemoglobins could be just as capable of surviving in a heterogeneous environment." For example, fishes with fewer hemoglobins may have hemoglobins which have multiple capabilities that adapt them to diverse environmental stresses; or their few hemoglobins may be the very ones that enable them to survive periods critical for their reproductive success, such as the breeding season. Thus, hemoglobin heterogeneity could be an adaptive strategy under certain circumstances but eclipsed by more effective strategies under others.

The oxygen affinity of the blood was correlated with Root effect and pH. Fishes with high affinity (lower values of p50) had higher values of pH and Root effect. Thus, the hypothesis that highly active fishes possess lower oxygen affinity (RIGGS, 1970) is not supported (Tables II y III). The high oxygen affinity was also correlated with environmental variables: freshwater species had higher oxygen affinity than estuarine or marine species. On the other hand, Root effect was higher in marine species. We have no explanation for these correlations in terms of their possible adaptative significance.

Other factors not considered in the present study may be important in interpreting hematological characteristics of fishes, *e.g.*, the age and size of the specimen (SIAKPERE, 1985) and health of the fish (BLAXHALL, 1972). Also, temperature, mass and oxygen consumption have been shown to be related in ways that are probably characteristics of the species (ECCLES, 1985), and a comprehensive study of multivariate relationships would consider morphological, as well as hematological, characters to predict the habitat in wich a species is found (see FELLEY, 1984). Sample size must also be carefully considered (RAILO *et al.*, 1985).

Finally, it is of interest to consider these data from a taxonomic point of view. At the left of Fig. 2, between *A. saxatilis* and *G. moringa*, there are 20 species, 17 of them in the Superorder Acanthopterygii (15 of which are in the Order Perciformes, including the estuarine species *M. curema* and the freshwater species *P. squamossisimus*). There are 11 species on the right: *A. spixii* (estuarine) and *A. bimaculatus*, *H. malabaricus*, *H. minutus*, *H. littorale*, *L. maracaiboensis*, *P. watwata*, and *P. krausii* (all freshwater). All belong

to the Superorder Ostariophysi (with the exception of *P. krausii*), 5 being in the Order Siluriformes and 2, in the Order Cypriniformes. The remaining 3 species are marine aero-branchial forms and belong to the Superorder Paracanthopterygii and the Order Batrachoidiformes.

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