Comparisons of Hemoglobins and Hematocrits of Blue, Brown Bullhead, Channel, White, Crossbred, and Hybrid Catfishes[1]

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Abstract: Hemoglobin patterns were identified by electrophoresis for channel catfish, *Ictalurus punctatus;* blue catfish, *I. furcatus;* white catfish, *I. catus;* brown bullhead, *I. nebulosus;* and (female x male) blue x channel, channel x blue, white x blue and channel x white hybrids. Hemoglobin patterns of each species were different with channel catfish having 11 bands; blue catfish, 8; white catfish, 5; and brown bullheads having 3 different patterns of 6, 10, and 11 bands. All hybrids except channel x white (10 bands) had 11 bands. The banding pattern exhibited by the hybrids was a combination of the parental pattern except for 1 or 2 hemoglobins unique to the hybrids. Total hemoglobin and hematocrit were higher for channel catfish and brown bullheads than blue catfish and white catfish. Maternal effects were evident for total hemoglobin in crossbred channel catfish and channel-blue hybrids. White x blue hybrids exhibited overdominance for total hemoglobin and hematocrit. The molecular weight of channel catfish hemoglobin was estimated to be 67,000 when compared to known compounds.

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Multiple hemoglobins are a common characteristic of fish blood. Electrophoretic separation showed distinct hemoglobin patterns varying from 2 bands in the skipjack tuna (Sharp 1969) to 18 bands in the toadfish (Fyhn and

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Sullivan 1974). Polymorphism within a given species occurs frequently and creates further diversity in fish hemoglobin patterns. Common polymorphs and rare variants produce up to 27 idiomorphic patterns in the eelpout (Hjarth 1975). Further complexity of hemoglobin patterns occurs in populations of hybridized species. Hybrid hemoglobins can display additive qualities of the 2 parent hemoglobins or distinctly separate patterns of their own.

The study of fish hemoglobin patterns provides an excellent means of identifying distinct populations or sub-populations of fish (Sick 1961). Analysis of hemoglobin patterns may also be a valuable tool in the taxonomy of different species and their hybrids. Skow (1971) examined the hemoglobins of black bullheads (*Ictalurus melas*), blue catfish (*I. furcatus*), channel catfish (*I. punctatus*), the hybrid channel ♀ x blue ♂ ; flathead catfish (*Pylodictus olivaris*), and white catfish (*I. catus*). His analysis indicated a close relationship between channel catfish and flathead catfish, and a close relationship between black bullheads and white catfish.

Hemoglobin and the hematocrit are related to the oxygen binding ability of blood and changes in both values are related to reactions to stress. Generally, values for these 2 parameters decrease when a fish is exposed to stress (Redding and Schreck 1983, Peters et al. 1984). Dwyer et al. (1983) found that hemoglobin percent and hematocrit of brook trout increase with temperature. Theoretically, this was a result of lower oxygen levels in the warmer water and higher metabolism in the brook trout. Limsuwan et al. (1983) determined that hemoglobin percent and hematocrit increased in channel catfish anesthesized with etomidate. Wedemeyer and Yasutake (1977) stated that the possible significance of low blood values was anemia, gill damage, or nutritional disease and that of high blood values was dehydration or stress polycythemia.

Qualitative or quantitative characteristics of blood parameters could be related to physiological performance and culture traits. Manwell et al. (1963) suggested that the greater capacity of hemoglobin to transport oxygen increased the tolerance of centrarchid hybrids to low dissolved oxygen. Skow (1971) stated 1 hemoglobin band of blue catfish appeared related to anoxia.

The objective of this experiment was to compare the electrophoretic separation of hemoglobin, total hemoglobin, and hematocrit of 4 strains of channel catfish and their crossbreeds and of 4 species of catfish: blue, brown bullhead (*I. nebulosus*), channel, white, and their hybrids. Data was for determination of taxonomic relationships among fish, inheritance of these blood parameters, and correlations to culture traits.

Methods

Experimental Fish

All fish used in this study except the brown bullheads were fingerlings from the 1976 spawn at the Alabama Agricultural Experiment Station, Auburn,

Alabama. Four strains of geographically distinct channel catfish, (Auburn, Marion, Kansas, and Rio Grande strains), were used (Dunham and Smitherman 1984). Blue catfish and white catfish also came from Auburn University populations (Dunham and Smitherman 1984). Intraspecific crossbreeds and interspecific hybrids were produced by mating adults from these populations. The population of brown bullheads was collected from West Point Reservoir, Chambers County, Alabama. The geographic distribution of the bullhead population would cover the upper Chattahoochee River and farm ponds innundated by West Point Reservoir.

Blood Collection

All fish were held in 400-liter fiberglass troughs at 20° C for 3 days prior to bleeding. Approximately 0.25 ml of blood from each fish was extracted by caudal vein puncture. Collected blood was expelled into 3 heparinized microhematocrit tubes. Two microhematocrit tubes were centrifuged for 10 minutes, and 1 microhematocrit tube was left uncentrifuged.

Total Hemoglobin

Total hemoglobin concentration was determined by the cyano-methemoglobin method (Larsen 1964). A 0.02 ml sample of blood was removed from the uncentrifuged microhematocrit tube and mixed with 5.0 ml cyanomethemoglobin reagent (Hycel). This mixture was then centrifuged to remove any suspended debris and read at 540 nm on a Bekman D. U. Spectrophotometer. Test readings were compared to a standard curve prepared with a commercially produced hemoglobin standard (Hycel No. 117). Due to consistently lower readings given by catfish blood, the test readings were adjusted by a predetermined factor (Larsen 1964).

Hematocrit

The 2 centrifuged microhematocrit tubes were used to determine hematocrit with a Spirocrit (Clay-Adams) reading device. The 2 values for each sample were averaged.

Hemoglobin Patterns

Five microliters of packed red blood cells (RBC) were taken from 1 of the centrifuged microhematocrit tubes. Due to rapid clotting factors in catfish blood, the packed RBC were not washed in saline but were used directly from the tube. The RBC were lysed with 60 μl of a commercial lysate (Helena Labs). Samples were loaded onto cellulose acetate plates (Titan III-H, Helena Labs) and electrophoresed for 45 minutes at 340 volts using a commercial Tris- EDTA-Borate buffer, ph 8.4 (Helena Labs). Plates were stained with Ponceau S stain, cleared, and read at 525 nm using a Fleur-vis auto-scanner (Helena Labs).

Molecular Weight of Channel Catfish Hemoglobin

The molecular weight of channel catfish hemoglobin was determined using a Sephadex G100–120 column, 20 mm in diameter and packed to a height of 250 mm. Supre Heme buffer (Helena Labs) pH 8.4, was used to eleute the column. Void volume of the column was 32 ml with a flow rate of 5 ml/min. A 1.0 ml sample of hemoglobin was placed on the column and fractions were collected. Molecular weight was determined by measuring the volume of eluate (Ve) versus the void volume (Vo). The Ve/Vo ratio was compared to similar ratios of compounds with known molecular weights (Cytochrome C, trypsin inhibitor, human hemoglobin, hexokinase, and PHA).

Results and Discussion

Molecular Weight of Channel Catfish Hemoglobin

The Ve/Vo ratio of channel catfish hemoglobin was 1.79. When plotted along with Ve/Vo ratios of various known compounds, the molecular weight of channel catfish hemoglobin was approximately 67,000.

This molecular weight is slightly different from the value of 55,000 reported for channel catfish by Skow (1971). He also reported values at 57,000 for flathead catfish and white catfish and 70,000 for black bullheads and blue catfish.

Total Hemoglobin and Hematocrit

Generally, total hemoglobin and hematocrit decrease when fish are subjected to stress (Redding and Schreck 1983, Peters et al. 1984). A genetic group that tolerates stress may rapidly decrease its total hemoglobin to counteract the stress, or if the stress is tolerated, the total hemoglobin may be less affected or increase as is the case with the channel catfish anesthesized with etomidate (Limsuwan et al. 1983). A normally high level of hemoglobin level may facilitate oxygen transport or a normally low level might indicate efficiency of that hemoglobin type.

Brown bullheads and channel catfish had higher ($P < 0.01$) total hemoglobin than blue or white catfish (Table 1). Total hemoglobin of crossbred channel catfish was similar to their female parent. Channel-blue hybrids were intermediate to their parents but maternally influenced, their means nearer to their female parent. Hemoglobin values for the hybrids where white catfish was one of the parents were overdominant, falling outside ($P < 0.01$) both the low and high sides of the parental range.

Rank of species was similar to that of hemoglobin for hematocrit (Table 1). However, there was no maternal effect on hematocrit. Hematocrits of the white x blue were overdominant in the positive direction as was the case for total hemoglobin. Hematocrit of the channel x white was similar to white catfish.

Table 1. Hematocrit and total hemoglobin values for *Ictalurus* spp.

Species (strain)	*N* sampled	Hematocrit (%) (1 standard deviation)	Total hemoglobin (gm %) (1 standard deviation)
Channel catfish (Auburn)	208	37.10 (5.20)	9.32 (2.24)
(Marion)	8	39.12	8.33
(Kansas)	8	31.43	9.66
(Rio Grande)	8	33.62	7.60
(A x RG)	8	38.37	9.78
(M x K)	8	30.12	8.70
Blue catfish	24	27.00 (2.10)	7.08 (0.66)
Blue x Channel	16	33.65 (4.05)	7.83 (0.72)
Channel x Blue	16	32.60 (2.30)	8.47 (1.46)
White catfish	24	27.10 (3.60)	8.02 (1.20)
White x Blue	16	30.10 (2.40)	9.66 (1.39)
Channel x White	16	27.30 (3.80)	6.51 (0.97)
Brown bullhead	49	35.62 (7.13)	10.34 (2.24)

The hematocrit and total hemoglobin are highly correlated in groups of rainbow trout grown in different environmental conditions (Peters et al. 1984). No correlation ($r = 0.06$) existed among strains and crosses of channel catfish but high correlations ($r = 0.90$, $r = 0.68$) existed among species and among species and hybrids of catfish, respectively. Correlations among species should be higher than that among strains since genetic differences are greater among species than among strains. Similar results were obtained for the correlation between body conformation and dressing percentage among species and strains of catfish (Dunham et al. 1983*a*).

The quantity or quality of hemoglobin may be related to physiological performance or culture traits of catfish. The crossbred channel catfish in this experiment have heterotic growth rate (Dunham and Smitherman 1983) and disease resistance (Dunham and Smitherman 1984). Channel catfish grow faster and have greater disease resistance than other species of catfish, and bullheads and white catfish tolerate lower dissolved oxygen than blue or channel catfish (Dunham and Smitherman 1984). The channel x blue hybrid tolerates lower dissolved oxygen than channel catfish (Dunham et al. 1983*b*).

There was no consistent trend among channel catfish crossbreeds that could be related to culture traits. If high hematocrits aid in combating stress caused by pathogens, the species rank of channel, blue and white catfish corroborate this hypothesis but that of the brown bullhead contradicts such a hypothesis. The 2 species with the greatest tolerance of low oxygen, brown bullhead and white catfish, had the highest and lowest hematocrits. The total hemoglobin and hematocrit do not appear strongly related to culture traits of catfish.

Hemoglobin Patterns

Hemoglobin patterns of each species were different (Fig. 1). White catfish had 5 bands, blue catfish had 8 bands, and channel catfish 11 bands. The

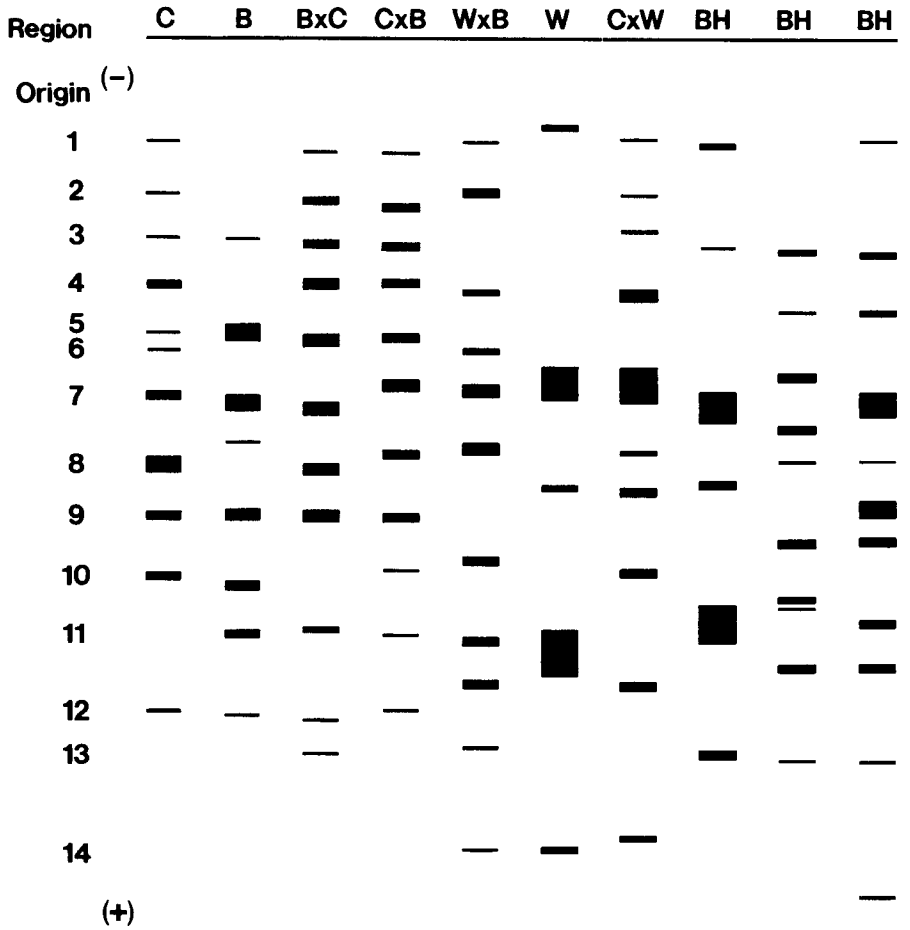

Figure 1. Electrophoretic separation of catfish hemoglobins. C = channel catfish, B = blue catfish, W = white catfish, BH = brown bullhead.

population of brown bullheads had 3 different patterns, 6 bands, 10 bands, and 11 bands (Fig. 1).

The 4 intraspecific crosses of channel catfish had patterns identical to the parent species. The channel-blue hybrids showed 11 bands and most bands were parental type. The white x blue hybrid had 11 bands and the channel x white hybrid, 10 bands. Each of these 2 hybrids expressed several unique bands. The percent composition of hemoglobin bands for each species and the hybrid crosses are shown in Table 2.

Skow (1971) reported 6, 9, 7, 18, 8, and 6 bands for black bullheads, blue catfish, channel catfish, channel x blue hybrid, flathead catfish, and white catfish, respectively, using starch gel electrophoresis. All but 1 or 2 of the

Table 2. Band composition (% of total hemoglobin) of catfish hemoglobins.

	Species and hybrid crosses[a]									
Band	C	B	W	B x C	C x B	W x B	C x W	BH	BH	BH
1	1.83		5.98	0.37	0.24	4.57	4.40	8.73		4.80
2	3.34			2.68	2.36	4.16	4.86			
3	8.81	10.03		6.03	6.61		3.92	2.80	7.60	8.00
4	14.82			10.52	10.74	10.27	8.70		3.20	7.35
5	7.52	17.37		14.36	14.76					
6	6.66					5.20			12.50	
7	20.18	18.40	36.53	16.44	16.53	13.89	22.32	35.93	17.93	25.92
8	12.99	6.80	3.49	15.81	16.41	17.10	10.35	6.31	3.94	3.19
9	9.99	24.69		13.50	14.40		12.36			15.73
10	10.52	8.47			10.51	17.20	14.36		25.89	11.89
11		7.62	49.75	10.70	6.02	6.85			10.00[b]	8.70
12	3.34	6.64		6.65	1.42	8.51	13.15	40.77	14.08	8.32
13				2.94		8.40		5.46	1.23	5.71
14			4.25			3.85	5.58			0.39

[a] W, white catfish; B, blue catfish; C, channel catfish; BH, brown bullhead.
[b] Two bands in this region, 1st band = 10.00, 2nd band = 3.63.

bands of the channel x blue hybrid were parental types as was found in this study. Hemoglobin patterns and concentrations of bands on cellulose-acetate gels (Skow 1971) were similar to those reported in this study. The dissimilar patterns on the 2 gel types indicates the complexity of the genotypes for hemoglobin in catfish. The hemoglobin of individual species is more unique based on 2 gel types than 1 gel type.

The data from the starch gels indicates catfish and flathead catfish are closely related, and bullheads and white catfish are closely related. Data from the acetate gels indicates a close relationship between the bullheads and white catfish, and between blue catfish and channel catfish. These results agree with other taxonomic classifications utilizing karyotype analysis (LeGrande 1981). Hemoglobin patterns, karyotype analysis (LeGrande et al. 1984), isozymes (Dunham and Smitherman 1984), and morphology (Dunham et al. 1983*a*) combine to provide several genetic markers for use in hybridization programs.

Brown bullheads were the only species polymorphic for hemoglobin pattern. Skow (1971) found no polymorphism for hemoglobin patterns in black bullheads, 2 strains of blue catfish, channel catfish, flathead catfish, or white catfish.

The quantity of hemoglobin and the number of hemoglobin bands is greater for channel catfish compared to blue and white catfish. Greater range of body conformation (Dunham et al. 1983*a*) and greater length variation (Brooks et al. 1982) are also characteristic of channel catfish compared to blue and white catfish. This large amount of genetic variation may explain the greater adaptability of channel catfish as illustrated by its greater geographic range and suitability for aquaculture.

The electrophoretic separation of the hemoglobins may be more related

to the culture traits of the species than the total hemoglobin and hematocrit. The banding pattern may be split into 14 regions. Regions 1 and 7 to 13 are most strongly expressed by brown bullheads and white catfish, regions 2 to 4 by channel catfish, and regions 2 to 6 by blue catfish, channel catfish and their hybrids. Region 14 is exclusive to brown bullheads, white catfish, and hybrids of white catfish. These distinct regions need to be tested for their oxygen binding ability to determine their relationship to the resistance to stress of these fish.

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