# **Biochemical Genetics of Largemouth Bass Populations in Alabama**

- **Kimberly G. Norgren,** Department of Fisheries and Allied Aquacultures Alabama Agricultural Experiment Station, Auburn University, AL 36849
- **Rex A. Dunham,** Department of Fisheries and Allied Aquacultures Alabama Agricultural Experiment Station, Auburn University, AL 36849
- **R. Oneal Smitherman,** Department of Fisheries and Allied Aquacultures Alabama Agricultural Experiment Station, Auburn University, AL 36849
- William C. Reeves, Fisheries Division, Alabama Department of Conservation and Natural Resources, Montgomery, AL 36104

Abstract: Thirty-six enzyme loci were surveyed for 22 largemouth bass (*Micropterus salmoides*) populations from 7 watersheds in Alabama. Twenty-one loci were polymorphic. Nei's normalized genetic identity (I) and Rogers' genetic similarity (S) showed little divergence among the populations (I = 0.990 and S = 0.9792). However, several drainages contained unique alleles in frequencies greater than 0.05 and several alleles were identified that were not found in a previous nationwide survey. Significant intrapopulational heterogeneity was indicated by hierarchical F-statistics and contingency chi-square analysis. Clinal distribution of MDH-B alleles significantly affected populational differentiation. Allelic variation at MDH, AAT and IDH loci accounted for 82% of the total population differences. Variability of largemouth bass populations differed among the physiographic regions of Alabama. Frequencies of alleles from the Florida subspecies, *M. s. floridanus*, were increased through supplemental stocking programs in Alabama.

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Genetic differences exist among largemouth bass populations in the United States (Philipp et al. 1983). Geographic distribution of several alleles shows a distinct latitudinal cline and suggests a thermal preference or tolerance by largemouth bass possessing certain alleles. Further studies into the local variability of largemouth bass populations are needed to allow more efficient regional management of this species.

Environmental heterogeneity is a major factor in maintaining and structuring genetic variation in natural populations (Wallis and Beardmore 1984). Alabama is geographically heterogeneous and contains a large number of watersheds. Geographical divisions separate the state into 6 distinct physiographic regions: Upper Coastal Plain, Lower Coastal Plain, Piedmont, Limestone Valley, River Drainage Basin and the Appalachian Plateau (Norgren 1986). Within these regions, aquatic systems are further partitioned into a multitude of isolated habitats. As populations become isolated and flow between gene pools ceases, genetic differentiation can accumulate through mutation, genetic drift and various forms of natural selection. The patchwork effect of freshwater habitats could have important consequences on the genetic structure and evolution of a species (Avise and Felley 1979).

The genetic structure of largemouth bass in Alabama may not only be affected by environmental diversity, but also by the stocking programs for Florida largemouth bass, M. s. floridanus, that have been initiated. More than 1 million Florida bass fingerlings have been stocked into the state's reservoirs and lakes during 1971 to 1983. A genetic survey of the native populations would provide information necessary to evaluate the impact of these stockings and to monitor any subsequent influence of management programs on the genetic structure of the populations as well as provide information for developing stocking strategies.

Objectives of this study were to: 1) describe the genetic structure of largemouth bass populations in Alabama; 2) establish a data base for future management programs and evaluations; 3) identify populations containing unique genetic variability; 4) ascertain the genetic relationships of populations within and between different watersheds; 5) determine the extent of intergradation of the northern and Florida subspecies, and 6) determine the influences of supplemental stocking programs.

### Methods

#### Collection and Electrophoresis of Samples

Samples of 20 to 50 largemouth bass were collected at 22 locations from seven watersheds for electrophoretic analyses (Fig. 1). All populations were native except the one in Lake Shelby. Lake Shelby dams were washed out in 1971; replacement stocking included 186 Florida bass fingerlings/ha. Collections were made by fishery biologists of the Alabama Department of Conservation and Natural Resources during the spring and summer of 1984. Largemouth bass were transported on ice or live in hauling tanks to field stations where eye, liver and muscle were dissected from each fish. Each sample was wrapped in foil and frozen until analysis.

Enzymes examined were aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), adenylate kinase (AK), aldolase (ALD), calcium binding protein (CBP), creatine kinase (CK), esterase (EST), glycerol-3-phosphate dehydrogenase



**Figure 1.** Relative proportion of Florida bass alleles in largemouth bass populations in Alabama, as indicated by the percentages of IDH-B<sup>3</sup>, AAT-B<sup>3</sup> and B<sup>4</sup> alleles, locations sampled: (1) Mobile Delta (2) Coffeeville, (3) Demopolis, (4) Aliceville, (5) Gainesville, (6) Tuscaloosa, (7) Lewis Smith, (8) Purdy, (9) Jordan, (10) Weiss, (11) Martin, (12) Harris, (13) Point A, (14) Pea, (15) Eufaula, (16) West Point, (17) Little Bear, (18) Cedar Creek, (19) Pickwick, (20) Guntersville, (21) Big Creek, and (22) Shelby.

 $(\alpha$ -GP), glyceraldehyde-3 phosphate dehydrogenase (GAP), glucosephosphate isomerase (GPI), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), peptidase (PEP), 6-phosphogluconate dehydrogenase (6-GPDH), phosphoglucomutase (PGM), sorbitol dehydrogenase (SDH), and superoxide dismutase (SOD).

Techniques for horizontal starch gel electrophoresis followed those of Steiner and Joslyn (1979), Philipp et al. (1983), and Hallerman et al. (1986*a,b*). Locus and allele nomenclature were that of Philipp et al. (1982). For comparison with the nationwide study of largemouth bass completed by Philipp et al. (1983), similar enzymatic procedures were employed with the following exceptions: an EDTAborate-tris gel (EBT) was substituted for the tris-citrate gel (TC), for evaluation of adenylate kinase (AK), as it provided better resolution, and glucosephosphate isomerase (GPI) was evaluated on both TC and EBT gels.

Polymorphic expression varies with the gel substrate selected for GPI-A. A few individuals homozygous on one substrate are polymorphic on another substrate (Hallerman et al. 1986b). To maintain consistency with the nationwide study, GPI-A alleles observed on EBT gels were assigned the nomenclature of GPI-A<sup>2</sup> and GPI-A<sup>3</sup> (Philipp et al. 1982). Although the A<sup>2</sup> allele was common to both gel systems, fast variant alleles specific to TC gels were designated A<sup>4</sup>.

Additional genetic information was obtained by including 2 enzyme systems, esterase and peptidase (Hallerman et al. 1986b), not examined in the nationwide study. The substrate for the esterase stain was  $\alpha$ -napthyl acetate (Shaw and Prasad

1970). The dipeptide leucyl-tyrosine was used in the peptidase stain (Steiner and Joslyn 1979). Two undescribed liver loci,  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogense, were examined on histidine citrate gels.

#### Analysis of Data

To measure the overall genetic variability of each population, the percentage of loci polymorphic (P) and the mean observed and expected heterozygosity (H) (Nei 1978) were calculated from the genotype data. Allele frequencies were then analyzed with chi-square tests to determine departures from Hardy-Weinberg expectations. Population differentiation was further analyzed with hierarchical F-statistics (Wright 1978). Population heterogeneity was tested for significance using contingency chi-square (Workman and Niswander 1970). Indices for genetic identity (I) (Nei 1978) and genetic similarity (S) (Rogers 1972) were calculated for each pair-wise comparison.

To test for temporal shifts in allele frequencies, four Alabama populations represented in the nationwide survey (Philipp et al. 1983) were resampled. A modified Student's *t*-test (Hallerman et al. 1986b) was used to test for differences between the 2 samples.

### **Results and Discussion**

#### Isozyme Expression

Fifteen of the 36 enzyme loci surveyed for 22 largemouth bass populations in Alabama were monomorphic. These loci included AAT-A, AAT-M, ADH-A, AK-A, CBP-A, CK-A, CK-B, EST-A,  $\alpha$ -GP-A, GAP-A, IDH-A, LDH-A, LDH-B, LDH-C, and SDH-A. Polymorphic loci included AAT-B, AK-B, ALD-A, ALD-B, CK-C, EST-B, EST-C, EST-E,  $\alpha$ -GP-B, GAP-B, GPI-A, GPI-B, IDH-B, MDH-A, MDH-B, PEP-A, PEP-B, PEP-C, 6-PG-A, PGM-A, and SOD-A loci. Allele frequencies for these loci have been described (Norgren 1986). Four of the polymorphic loci (ALD-B, CK-C, GPI-B, and SOD-A) possessed variant alleles in frequencies less than 0.05.

The following summary provides only the descriptions of enzymes not previously analyzed or in which the isozyme expression differed from earlier accounts (Philipp et al. 1982, Hallerman et al. 1986b). Rates of migration for the allele of these new isozymes are found in Norgren (1986).

*Esterase*—Hallerman et al. (1986b) described this complex system as expressing 5 loci. Four loci (A, B, D, and E) were expressed in the liver and 3 loci (B, C, and E) were expressed in the eye. Although similar results were found in the present study, the EST-A locus was found to be the fastest migrating locus in the eye, and not the EST-B.

An allelic variant slower than the common  $EST-B^1$  was identified in this survey and designated  $B^0$ . Eleven populations were fixed for  $EST-B^1$  allele. Variant  $B^0$ alleles were found in a few individuals in West Point Lake, Lake Eufaula, and Demopolis Lake with higher frequencies of 0.09 and 0.125 observed in the Mobile Delta and in Gainesville Lake, respectively. EST-B<sup>2</sup> was unique to the Alabama River drainage and neighboring Lake Shelby, with frequencies ranging from 0.025 to 0.125.

All populations except 3 were fixed for  $EST-C^2$ . The variant  $EST-C^1$  alleles occurred randomly in different watersheds with 1 individual containing the allele in Aliceville Lake, and 2 individuals each in Guntersville Lake and Point A Lake.

EST-D was not scored as it occurred in the lower portion of the liver gels and resolution was obscured by EST-C and EST-E expression. One unique EST-E variant was found in Guntersville Lake with  $EST-E^1$  being the common allele.

*Isocitrate Dehydrogenase*—Greater levels of variability were observed at the liver locus, IDH-B, than previously identified (Philipp et al. 1982, Hallerman et al. 1986*b*). Hallerman et al. (1986*b*) identified an allele, B<sup>0</sup>, which migrated more slowly than the B<sup>1</sup> and B<sup>3</sup> alleles originally reported by Philipp et al. (1982). IDH-B<sup>0</sup> was observed in 2 heterozygotes in Point A Lake. IDH-B<sup>1</sup> was the most common allele in Alabama. It was fixed in 5 populations and had frequencies greater than 0.800 in 15 other populations. Values of 0.550 to 0.750 were found in Harris Lake, West Point Lake, Little Bear Creek Lake, and Cedar Creek Lake. Low frequencies of IDH-B<sup>1</sup> were found in 2 populations, Lake Eufaula, and Lake Shelby (0.375 and 0.200, respectively).

In all except 1 population the alternate allele to B<sup>1</sup> was B<sup>3</sup> or B<sup>0</sup>. However, in Weiss Lake unusual isozyme expression was observed. Two new alleles designated IDH-B<sup>2</sup> and IDH-B<sup>4</sup> were expressed at frequencies of 0.191 and 0.103, respectively. Both homozygotes and heterozygotes were detected for B<sup>2</sup> and heterozygotes only for B<sup>4</sup>. Genotypes at the AAT-B and  $\alpha$ -GP-B loci confirmed that the fish with the B<sup>2</sup> and B<sup>4</sup> alleles were largemouth bass sampled and not spotted bass, *M. punctulatus*, which are also in Weiss Lake.

**Peptidase**—Resolution of the PEP-A liver locus, not previously scored, was improved by staining center gel slices immediately following electrophoresis. PEP-A was observed to have 3 alleles with PEP-A<sup>2</sup>, the most common, fixed in 18 populations. A slower-migrating allele, PEP-A<sup>1</sup>, was unique to Lake Eufaula and found in 2 individuals, 1 heterozygous and 1 homozygous. PEP-A<sup>3</sup> occurred only in low frequencies (0.015–0.025) in the most southern populations sampled (Mobile Delta, Pea River, and Lake Eufaula).

All except four populations were fixed for the PEP-B<sup>1</sup> allele. Populations containing the PEP-B<sup>2</sup> variant were randomly distributed in all watersheds sampled. The PEP-C<sup>1</sup> allele (Hallerman et al. 1986*b*) was the predominant allele at PEP-C. A number of populations, however, had PEP-C<sup>2</sup> at frequencies ranging from .025 to .350. A single individual in Lake Purdy was heterozygous for the fast variant allele, PEP-C<sup>3</sup>, not previously identified.

Superoxide Dismutase—A unique allele, SOD-A<sup>3</sup>, not identified by Philipp et al. (1982) or Hallerman et al. (1986b), was found in Demopolis Lake in a single heterozygous individual. Most populations were either fixed for SOD-A<sup>2</sup> or had the

allele at frequencies greater than 0.900. Five populations (West Point Lake, Lake Eufaula, Lake Shelby, Weiss Lake, and Martin Lake) contained high levels of SOD-A<sup>1</sup>. An unusually high concentration of the A<sup>1</sup> allele was observed in Martin Lake. Fifteen heterozygous individuals and 1 homozygous individual were observed in a sample of 20 fish.

 $\alpha$ -Glycerophosphate Dehydrogenase—In addition to the  $\alpha$ -GP-A muscle locus described by Philipp et al. (1982), a liver locus,  $\alpha$ -GP-B, was also observed. Sixteen populations were fixed for the  $\alpha$ -GP-B<sup>2</sup> allele. The  $\alpha$ -GP-B<sup>1</sup> was unique to the Alabama River drainage and occurred in six populations at frequencies ranging from 0.025 to 0.150.

Aldolase—Philipp et al. (1982) described aldolase as possessing 3 loci— ALD-A (muscle), ALD-B (liver), and ALD-C (brain and heart)—of which only muscle and brain were evaluated. Liver and muscle loci were evaluated in the present study.

Most populations were fixed for the ALD-A<sup>1</sup> allele. A new allele, ALD-A<sup>2</sup>, was found in frequencies less than 0.05 in the Alabama River drainage.

The most common of the 3 ALD-B alleles was  $ALD-B^2$ , fixed in 19 populations.  $ALD-B^1$  and  $ALD-B^3$  were unique to the Tennessee River drainage and occurred at low frequencies. Single heterozygotes were found in Guntersville Lake and Cedar Creek Lake for  $ALD-B^1$ ; 3 individuals in Cedar Creek Lake possessed  $ALD-B^3$ .

*Glucosephosphate Isomerase*—GPI activity was originally described by Philipp et al. (1982) using EBT gels. Hallerman et al. (1986*b*), however, observed that some individuals monomorphic for GPI-A (liver locus) on 1 gel, TC or EBT, were polymorphic on the other. The altered electrophoretic conditions provided by the different gels permitted expression of the allele GPI-A<sup>4</sup> that would otherwise remain undetected. One individual from Jordan Lake and 2 from Little Bear Creek Lake were observed to be  $A^2A^3$  heterozygotes. The genotype  $A^2A^4$  was found for 2 individuals in West Point Lake. Fourteen populations were fixed for the GPI-B<sup>2</sup> allele with 5 populations containing low frequencies of GPI-B<sup>3</sup>, and 2 with GPI-B<sup>4</sup> alleles present.

Adenylate Kinase—Expression at 2 loci in muscle and eye was summarized by Philipp et al. (1982). One new allele at the AK-B locus, designated AK-B<sup>3</sup>, was found at a frequency of 0.05 in Lake Demopolis. Seven of the more northern populations (Lake Purdy, Weiss Lake, Little Bear Creek Lake, Cedar Creek Lake, Pickwick Lake, and Guntersville Lake) exhibited the AK-B<sup>1</sup> allele in low frequencies of 0.025 to 0.075, with the remaining populations fixed for the AK-B<sup>2</sup> allele.

*Glyceraldehyde-3-Phosphate-Dehydrogenase*—Philipp et al. (1982) identified 3 loci. GAP-A locus (predominantly muscle) and the GAP-B locus (predominantly liver) were observed in the present study. GAP-B, not previously evaluated, was extremely polymorphic. Two alleles with slight cathodal migration similar to that in muscle were observed near the origin and were designated GAP-B<sup>1</sup> and GAP-B<sup>2</sup>. Heterozygotes of B<sup>1</sup>B<sup>2</sup> were the most frequent genotype in all populations.

### Description of Populations Within Each River System

In addition to several rare alleles found in populations along the Alabama River system (Tombigbee, Black Warrior, Cahaba, Coosa, and Tallapoosa rivers), several alleles—ALD-A<sup>2</sup>, EST-B<sup>3</sup>, IDH-B<sup>2</sup>, IDH-B<sup>4</sup>, SOD-A<sup>3</sup>, and  $\alpha$ -GP-B<sup>2</sup>—were unique to this drainage. Certain populations had greater tendency for exhibiting unique or rare alleles. Along the Alabama River, populations from Lake Purdy, Gainesville Lake, Demopolis Lake, and Weiss Lake were observed with high frequencies of both rare (occurring in less than 5% of all bass populations) and unique alleles. Weiss Lake (upper Coosa River) had the highest level of rare and unique alleles (GPI-A<sup>3</sup>, IDH-B<sup>2</sup>, and IDH-B<sup>4</sup>) observed in Alabama. Although the frequencies ranged from 0.029 (GPI-A<sup>3</sup>) to 0.191 (IDH-B<sup>4</sup>) for the unique alleles, downstream dispersal of fish with these alleles appeared effectively restricted or subjected to severe selection pressures. Overall genetic variability (P = 13.9, H = 0.041) in the Alabama River system appeared intermediate to that of other drainages within the state and had a low level of Florida bass alleles.

Low levels of genetic variability were observed in the Conecuh River-Perdido River-Escambia River system. Several rare alleles, 1 unique, were present. The Pea River, the largest tributary of the Choctawhatchee River system, had moderate levels of genetic variability; several rare alleles were observed.

Populations sampled from the Chattahoochee River system exhibited the highest levels of genetic variability (P = 26.4, H = 0.077) observed in Alabama. The heterozygosity in the Chattahoochee drainage is probably due to an interaction between the 2 subspecies, *M. s. floridanus* and *M. s. salmoides*, and the exchange of genetically accumulated differences, since this drainage is the farthest east in Alabama and in the intergrade zone.

The Tennessee River drainage had 3 unique alleles, ALD-B<sup>1</sup>, ALD-B<sup>3</sup>, and EST-E<sup>2</sup>. Populations sampled from the Tennessee River expressed high frequencies of MDH-B<sup>1</sup> and AAT-B<sup>2</sup> alleles, genetically separating them from other populations.

Samples from Big Creek Lake on the Escatawpa River system exhibited the lowest levels of genetic variability found in Alabama. No rare or unique alleles were observed. This drainage empties into the Gulf of Mexico and is the farthest west of those sampled.

### Genetic Variability in Alabama Populations of Largemouth Bass

The average percentage of loci polymorphic was 17% (range 5.6 - 30.6%) with a mean heterozygosity of 0.043 (range 0.015 - 0.086). The average percentage of polymorphic loci was slightly higher than those identified by Philipp et al. (1983) for populations of bass occupying the intergrade zone (P = 15.5%). Observations of populations from 4 lakes common to both studies (Lake Eufaula, West Point Lake, Big Creek Lake, and Guntersville Lake) showed a 1% to 5% increase in polymorphism over the previously reported levels. This increase was largely due to the 9 additional loci examined in the present study, 5 of which were highly polymorphic.

Populations from Coffeeville Lake, Jordan Lake, and Big Creek Lake contained the lowest levels of heterozygosity (0.018, 0.015, and 0.029, respectively) in Alabama, while Lake Eufaula (0.086) and Bear Creek (0.085) exhibited the highest levels of heterozygosity.

Chi-square analyses of the allele frequencies showed that 5 polymorphic loci (EST-B, AAT-B, IDH-B, MDH-B, and PEP-C) significantly deviated from Hardy-Weinberg expectations ( $P \le 0.05$ ) in several populations. The fixation indices ( $F_{ij}$ ) for these collections were all positive, reflecting heterozygote deficiencies. Deviations from Hardy-Weinberg expectations and increased homozygosity suggest that population substructuring may have occurred at these locations (Avise and Felley 1979).

#### **Relatedness Among Populations**

Analysis of the polymorphic loci with hierarchical F-statistics (Wright 1978) and contingency chi-square showed significant populational heterogeneity for 5 loci. These analyses revealed that MDH-B was an important source of genetic variation within and among populations. It contributed 33% of the total variance between populations. This influence on the genetic differences among populations can best be understood in terms of the clinal distribution of MDH-B<sup>1</sup> and MDH-B<sup>2</sup> discussed by Hines et al. (1983).

The distribution of the B<sup>1</sup> allele in Alabama followed a distinct latitudinal cline (Fig. 2), probably due to differences in thermal efficiency identified for the MDH-B alleles (Hines et al. 1983). Predominantly found in northern populations of Ala-



Figure 2. Distribution of the MDH-B alleles among largemouth bass populations in Alabama.

bama, the MDH-B<sup>1</sup> allele frequency diminished in southern progression until samples at the most southerly locations (Big Creek, Lake Shelby, and the Mobile Delta) expressed only the MDH-B<sup>2</sup> allele.

IDH-B and AAT-B loci also contributed to the significant populational heterogeneity. Twenty-seven percent of the total intrapopulational variance was due to the IDH-B variance, and 22% to that of AAT-B. By combining data from MDH-B, IDH-B, and AAT-B loci, 82% of the populational differences can be identified.

Nei's normalized genetic identity and Rogers' coefficient of similarity indicated little genetic divergence among largemouth bass populations. Mean genetic identity was 0.990 (range 0.944 - 1.00) with a mean genetic similarity of 0.964 (range 0.908-0.991).

Due to the additional loci examined, these observed mean values are higher than those found for intergrade populations of largemouth bass (I = 0.972 and S = 0.940) by Philipp et al. (1983). Calculation of these indices using only the loci common to both studies gave identical values for I and S. The genetic similarity values obtained for largemouth bass in Alabama indicate the observed variation to be at the populational level, with little divergence present.

The genetic identities generally clustered populations within a river together or with neighboring river populations. Lake Shelby had the most distinct population, as expected from the restocking of Florida bass.

Hierarchical F statistics (Wright 1978) and contingency chi-square indicated that greater differentiation existed among local populations than among rivers and watersheds (Norgren 1986) despite some unique alleles being found only in certain rivers. This may have been a result of the clinal distribution of MDH alleles, the distribution of AAT and IDH, and the random distribution of rare and unique alleles.

### **Physiographic Influences**

Genetic diversity among the different physiographic regions of Alabama ranged from H = 0.021 to H = 0.054. Mean genetic variability levels for the River Drainage Basin were the lowest observed among the physiographic regions. Although highest mean heterozgosity was expressed in the Lower Coastal Plains (primarily due to the Lake Eufaula population), overall genetic diversity was greatest among the Limestone Valley populations, which also exhibited the greatest degree of rare and unique alleles. The greater diversity of the Limestone Valley is further illustrated in the Coosa River which has three distinct physiographic regions, the Limestone Valley, the Piedmont Plateau, and the Lower Coastal Plain. High levels of genetic variability were observed in the Limestone Valley of the Coosa River, but variability diminished as the river descended into the other 2 regions (Weiss Lake: H = 0.064; Lake Mitchell: H = 0.029 (Philipp et al. 1983); Jordan Lake: H = 0.015).

Impact of the Florida Bass Stockings

Among the public water systems examined in the present study, the Escatawpa River system was the first (e.g., 1971) to receive the Florida bass. Although the majority of the stockings occurred during 1978 to 1982, incidental stockings from washed-out dams and through spillway overflows could have introduced Florida bass genes into Alabama watersheds earlier and in greater numbers than stocking records indicate. Figure 1 indicates the extent of the Florida bass presence in Alabama as measured by the percentage of IDH-B<sup>3</sup>, AAT-B<sup>3</sup> and B<sup>4</sup> alleles.

Populations not supplementally stocked with Florida bass, excluding the Chattahoochee River which being nearest to the center of intergrade zone had the highest level of Florida bass alleles, exhibited 0.0% to 11.0% Florida bass alleles. Those stocked with Florida bass, exhibited 0.0% to 47.0% Florida bass alleles. In populations receiving the introductions, expression of Florida bass alleles was irregular and complex. For example, Jordan Lake expressed only 1% of the Florida alleles but received a higher stocking rate (2.05/ha) than Weiss Lake (0.23/ha) or Martin Lake (0.45/ha), 21% and 16% Florida alleles, respectively. Although Point A Reservoir received 22 Florida bass/ha in 1980, percentage of Florida alleles was identical to that observed in the Pea River in which no stockings were reported.

The association of Florida bass stocking density (*N*/ha) and incidence of Florida bass alleles (percent contribution) was evaluated using a simple correlation. An analysis pooling all the populations sampled indicated a correlation of r = 0.16. However, when correlations were evaluated for the three largest drainages the association between stocking density and presence of Florida bass alleles was higher. For example, in the 2 main branches of the Alabama River system (the Tombigbee/Black Warrior and the Cahaba/Coosa/Tallapoosa Rivers), r = 0.50, but was not statistically significant. A correlation of r = 0.99 ( $P \le 0.01$ ), however, was observed in the Tennessee river drainage and indicated a definite impact by the Florida bass stocking in this drainage.

The influences of *M. s. floridanus* on heterozygosity in largemouth bass of Alabama was evaluated by grouping populations according to their content of Florida bass alleles, and calculating the mean genetic variability measures for each group. As would be expected of intergrade populations, the greatest degree of heterozygosity was observed among populations containing 30% to 50% of the Florida alleles. Populations containing primarily northern bass alleles (0-2.5%) or primarily Florida bass alleles (70% to 80%) showed lower levels of heterozygosity, similar to those found by Philipp et al. (1983).

#### Allele Frequency Changes

Big Creek Lake, Guntersville Lake, Lake Eufaula, and West Point Lake were sampled in this study and by Philipp et al. (1983). Only 2 significant changes in allele frequency occurred between the two surveys.

A shift (P < 0.05) in the allele frequency of CK-C<sup>1</sup> from the survey of Philipp et al. (1982) was observed in Big Creek Lake. Although short-term evolutionary

responses to industrial and thermal conditions have occurred, the randomly distributed CK-C variants showed no correlations to environmental variables (Philipp et al. 1982).

In West Point Lake, a temporal shift at the SOD-A locus (P < 0.05) was observed. The SOD-A<sup>2</sup> alleles were in excess of the frequency reported (Philipp et al. 1982) for West Point, indicating a shift toward the northern bass genome. Changes could have been caused by selection, drift, sampling error or possibly subpopulations existed at different sampling sites.

In general, populations of largemouth bass in Alabama were genetically similar, although some populations did possess unique or rare alleles. Weiss Lake was one of the most distinctive populations. Allelic variation at MDH, AAT and IDH loci accounted for 82% of the total population differences. The variability for these 3 loci may be natural and/or influenced by stocking of Florida largemouth bass. Frequencies of alleles from the Florida subspecies were increased by the supplemental stocking programs in Alabama. Management decisions will need to be made on the relative value of preserving the more unique populations and alleles, and value of increasing the frequency of Florida bass alleles. Such questions may not be adequately answered without data on performance traits of various populations.

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