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## Temporal Changes of Largemouth Bass Alleles in a Northern Arkansas Reservoir Stocked with Florida Bass

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### Abstract

Southwest Electric Power Company (SWEPCO) Lake in northwest Arkansas is a thermal cooling pond for the Flint Creek Power Station. This reservoir has been regularly stocked with Florida bass (*Micropterus floridanus*) on top of a pre-existent largemouth bass (*M. salmoides*) population since its creation in 1976. Allozyme analysis of diagnostic loci in 1995-1996 revealed that 62% of the alleles were Florida bass alleles and that most fish were F<sub>x</sub> hybrids (77%). Microsatellite analysis of diagnostic loci in 2006 revealed that 78% of the alleles were Florida bass alleles and that most fish were F<sub>x</sub>-Florida hybrid bass (59%), with the remainder designated as Florida bass. The ongoing stocking of Florida bass and the possibility of a selective advantage for Florida bass alleles in a thermal pond may account for these temporal reductions in largemouth bass allele frequencies.

### Introduction

The Florida species of largemouth bass (*Micropterus floridanus*; FB) is commonly stocked in southern U.S. lakes where populations of largemouth bass (*M. salmoides*; LMB) are native. These two putative species were until recently classified as subspecies and have recently been proposed as separate species (Near and Koppleman 2007). Due to compelling genetic and meristic differences among these two species, in addition to strong biogeographic gradation, we will hereafter refer to these two fish as separate species (FB and LMB). State fisheries agencies often use the environmental variable of heating degree days to determine the northern limits of stocking FB (Gilliland 1992). Heating Degree Days (HDD) are defined as the sum over all days of the difference between 18.3 °C and the average daily temperature for those days below that temperature (Philipp et al. 1982). For example, a single day having a temperature of 12.3 °C would contribute six HDD to

the total. The value of 3400 heating degree days, which occurs in central Arkansas, is the critical value used by both the Oklahoma Department of Wildlife Conservation and Arkansas Game and Fish Commission (AGFC).

SWEPCO Lake, located in northwest Arkansas (36° 17' N), has HDD greater than that recommended for stocking FB (10 y mean = 3,450, SD = 450; NOAA, 1984-1994). However, this lake functions as a cooling reservoir for the coal-powered Flint Creek Power Station and the water temperature remains quite warm throughout the year (range 18-43 °C). Therefore, water temperature is largely independent of the effects of atmospheric temperature. SWEPCO Lake possessed a resident LMB population of unknown size residing within Flint Creek and has been stocked solely with FB since impoundment. A brief stocking history of FB fingerlings in this 215 ha reservoir is as follows: 1977, n = 40,000; 1980, 8,000; 1998, n = 49,000; 1999, n = 25,000; and 2004, n = 28,000. In 1995-1996, Johnson and Fulton (2004) used three diagnostic allozyme loci to measure FB allele frequencies of 141 bass in this reservoir. FB allele frequencies averaged 62% and phenotypic data identified the majority of the lake bass to be F<sub>x</sub>-hybrids (77%).

Recently, Lutz-Carrillo et al. (2006) published a suite of microsatellite primers capable of distinguishing between FB, LMB and their resulting hybrids. Microsatellite loci are highly polymorphic and are often used to finely resolve diversity within populations, sub-populations and individuals (Angers et al. 1995, Shaw et al. 1999, Banks et al. 2000). Our goal therefore was to compare recent to historic bass allele frequencies using these differing molecular markers. We expected the continued FB stockings since the initial allozyme study to reduce the frequency of LMB alleles.

### Methods

Fin clips were taken from bass (n = 150) collected by boat electrofishing in 2006 and preserved in ethanol

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by the AGFC for genetic analysis. Control samples from AGFC broodstock populations were also obtained, with LMB controls from the Joe Hogan and William Donham hatcheries in Lonoke (n = 33) and Corning, AR (n = 45), respectively, and FB controls from the Andrew Hulseley Hatchery in Hot Springs, AR (n = 103).

DNA analysis consisted of four distinct components: extraction, amplification, separation and analysis. DNA extraction followed a modified phenol-chloroform method (Saghai-Marooft et al. 1984). Genotypes were amplified using seven microsatellite loci: *Mdo003*, *Mdo006*, *Msa021*, *Lma007*, *Lma12*, *Msa13*, and *Msa29*, with PCR specifications outlined by Lutz-Carrillo et al. (2006). Amplification occurred in two multiplex reactions: one reaction using the primer sets *Mdo003*, *Mdo006*, *Msa13* and *Msa29*, whereas the second reaction contained the primer sets *Msa021*, *Lma007* and *Lma12*. Microsatellite primers were synthesized with distinct fluorescent tags (Integrative DNA Technologies, Coralville, IA), specific for capillary electrophoresis using a Beckman-Coulter CEQ8000 Genetic Analysis System. Fragment sizes were determined by the fluorescent scanner and manually confirmed.

Standard genetic diversity measures (alleles per locus, heterozygosity and polymorphism) were calculated for the bass population using GenAEx6.1<sup>TM</sup> (Peakall and Smouse 2005). Allele frequencies were calculated for each locus and alleles were determined to be exclusive to FB, LMB or shared between species using the hatchery samples as controls.

The program STRUCTURE (Pritchard et al. 2000) was first used with an admixture model with correlated allele frequencies and default settings to establish pure species lines and their hybrids (n = 321; 20,000 burn-in steps; 200,000 Markov Chain Monte Carlo steps). The result of this analysis was a statistical value for the individual admixture proportion (*q*) of each individual and for the population as a whole. Individual admixture proportions were used to classify individuals as either pure species or hybrid, following the 0.05 threshold used by Schwartz and Beheregaray (2008), in order to limit Type I errors. Individuals with  $q \geq 0.95$  were classified as pure LMB, whereas individuals with  $q \leq 0.05$  were classified as pure FB. All broodstock controls were within this threshold and distinguished as pure species. Individuals having intermediate *q*-values were classified as hybrid bass ( $F_X$ -LMB,  $F_1$ , and  $F_X$ -FB), as described below.

To further resolve bass phenotypes a second STRUCTURE analysis was then performed

implementing the same criteria as previously, but with "Population Information, K = 2" set to two generations back. This analysis was used to determine the probability that individuals were either pure species, or first ( $F_1$ ) or greater ( $F_X$ ) generation hybrids. Individuals of hatchery populations were included, with FB categorized as a "1" and LMB as a "2." First, the analysis generated a relative probability that each hatchery individual was categorized in the correct group (pure FB phenotype or pure LMB phenotype, respectively). Second, the analysis generated probabilities that hybrid bass sampled were correctly identified as  $F_1$  or  $F_X$  hybrid bass.

### Results and Discussion

Heterozygosity values were high for both allozyme (except sMDH-B) and microsatellite loci, which we expected due to their choice as loci as being both polymorphic and discriminative among species (Table 1). Heterozygosity values ranged from 0.360 to 0.633 (0 = 0.549) among the 7 microsatellite loci, as compared to 0.315 and 0.533, for the AAT-B and IDH-B loci, respectively (Johnson and Fulton 2004). The low heterozygosity for the sMDH-B locus (0.078) is somewhat misleading, as alleles for this locus are not fixed between species (Philipp et al. 1982). The LMB possesses both the sMDH-B\*1 and sMDH-B\*2 alleles, whereas the FB does not share the sMDH-B\*1 allele. Most alleles found in this population were the sMDH-B\*2 allele (Table 1). Microsatellite alleles per locus ranged from 4 to 8, with a mean of 5.9 (versus 2 for each allozyme locus). Heterozygosity and mean alleles per locus of the SWEPCO Lake sample population were also higher than that of the broodstock samples (range of heterozygosities of 0.000 to 0.786, Allen 2009), as hatchery populations tend to have low genetic diversity relative to wild populations (Miller and Kapuscinski 2003). A total of 41 alleles were identified within the SWEPCO Lake sample, which is also more than hatchery broodstock samples (range of 24-38, Allen 2009).

Of the 41 microsatellite alleles identified, 20 were designated as diagnostic largemouth bass alleles, 16 as Florida bass alleles, 2 were shared between species, and 3 were unique to the SWEPCO Lake sample. The 3 alleles unique to this sample may be remnants of alleles from LMB native to Flint Creek or from FB of previous brood stocks. For example, SWEPCO Lake has a long-standing history of FB stocking dating back to 1977, before broodstock hatchery programs were

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Table 1. Allele frequency and direct-count heterozygosity ( $H_{DC}$ ) using allozyme (1995-1996; n = 141) and microsatellite (2007; n = 150) data for a bass population in SWEPCO Lake, AR.

	Allozyme Loci				Microsatellite Loci					
	AAT-B	IDH-B	MDH-B	<i>Mdo003</i>	<i>Mdo006</i>	<i>Ms021</i>	<i>Lma007</i>	<i>Lma012</i>	<i>Msa13</i>	<i>Msa29</i>
FB	0.64	0.59	0.00	0.29	0.83	0.86	0.74	0.80	0.79	0.82
LMB	0.36	0.41	0.17	0.20	0.17	0.14	0.25	0.19	0.21	0.18
Shared	0.00	0.00	0.83	0.51	0.00	0.00	0.01	0.00	0.00	0.00
$H_{DC}$	0.315	0.533	0.078	0.620	0.607	0.607	0.480	0.360	0.533	0.633

established in Arkansas. As a result, the early stocking efforts were through direct-stock transfer from either Florida lakes where FB are native, or from established FB populations in Texas (C. Dennis, AGFC, pers. comm.). Private alleles, however, only accounted for 2% of the alleles in the sample, and, due to lack of ability to verify their ancestry, were discarded from further analysis. Conversely, the shared alleles accounted for 8% of the total alleles in the sample, mostly from one allele in the *Mdo003* locus, where it represented 51% of the alleles of that locus (Table 1).

As the *Mdo003* locus has a high percentage of shared alleles, similar to the allozyme locus sMDH-B, calculation of FB allele frequencies was performed using the other 6 microsatellite loci. The FB alleles accounted for 78% of the total, whereas 20% were from the LMB alleles. These FB allele frequencies are higher than found for allozymes by Johnson and Fulton (2004). The two exclusive diagnostic allozyme loci used by Johnson and Fulton (2004), AAT-B and IDH-B, showed lower FB allele frequencies, 0.64 and 0.59 respectively, than this study (Table 1). Supplemental stockings of FB in 1998, 1999 and 2004 may explain the increase in FB allele frequencies. Selection may also be involved in this temporal reduction in LMB allele frequencies. Thermal selection has been demonstrated for FB (or their alleles) as compared to LMB, in the laboratory using enzyme kinetics (Hines et al. 1983), artificial pond settings established in different latitudes (Fields et al. 1987; Philipp et al. 2002) and in altered lakes serving as cooling ponds (Childers 1979). Even though microsatellite alleles are considered non-selective, phenotype selection on individuals carrying certain allozyme alleles would similarly impact microsatellite allele frequencies.

However, the persistence of LMB alleles within this reservoir, despite receiving no stocks of LMB is intriguing. A likely source of these alleles is the original population of LMB within Flint Creek, prior to the reservoir's construction (Johnson and Fulton 2004). These fish may have been established prior to the first stocking event of FB. The recent stockings of FB were in response to poor recruitment in the preceding years (R. Moore, AGFC, pers. comm.). The success of stocking fish on top of established populations has been questioned due to heavy predation and competition, possibly reducing genetic impacts of stocking (e.g., Forshage and Fries 1995, Buckmeier et al. 2003, Hoffman and Bettoli 2005). Nonetheless, the high incidence of pure FB as defined by STRUCTURE provides evidence of a high success rate of these continued stocking efforts. A second possibility for the high incidence of LMB alleles could be the result of the early stocking efforts of FB, which were not genetically verified as pure, as genetic methods for distinguishing between the species of bass had not yet been developed. LMB alleles may have been unintentionally introduced as a result of contaminated brood stock. Brood stock contamination in hatcheries of several states including Arkansas has been problematic, requiring extensive genetic testing to reestablish pure lines (D. Brader, Manager, Hulsey State Fish Hatchery, pers. comm., Harvey et al. 1980, Philipp et al. 1982, Gilliland and Whittaker 1989). Another source of LMB alleles could be from direct-stock transfer by local fisherman and bass clubs, a common practice by fishermen attempting to enhance the fisheries of "their" lake (C. Dennis, AGFC, pers. comm.).

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The software STRUCTURE identified an average admixture proportion that was consistent with the stocking history of this lake ( $q = 0.120$ ). This value indicates that LMB alleles, while common in the overall frequency of the sample, have not diminished the identity of the population from its traditional FB ancestors. Phenotypic analysis using STRUCTURE supports these findings, identifying 74.6% of the sample population as being pure FB, and the remaining fish being  $F_X$ -FB hybrids (Table 2). The  $F_X$ -FB hybrid individuals were at least second generation hybrids, and all were predominantly influenced by FB alleles (low  $q$  values). These findings differed from those of Fulton (1998), who found predominantly  $F_X$ -hybrid phenotypes (77%), as well as identifying pure LMB and  $F_1$ -phenotypes (Table 2). As discussed above, the recent supplemental stockings of FB could contribute to our increase in pure FB phenotypes.

The increase in the number of loci used in the present study as compared to Johnson and Fulton (2004) should provide greater resolution into bass phenotype. For example, during the previous study there were a small percentage of individuals diagnosed

Table 2. Phenotype frequencies of bass using allozyme (1995-1996;  $n = 141$ ; Fulton 1998) and microsatellite (2007;  $n = 150$ ) data for a bass population in SWEPCO Lake, AR.

Phenotype	% Frequency	
	Allozymes	Microsatellites
FB	11.0	41.0
$F_X$ -FB	N/A	59.0
$F_1$	9.0	0.0
$F_X$ -LMB	N/A	0.0
$F_X$	77.0	N/A
LMB	3.0	0.0

as pure LMB (3%). With the lack of reproductive selection among species (Maceina et al. 1988; Gilliland and Whittaker 1989; Philipp and Whitt 1991), there were numerous generations for hybridization events to take place; the presence of pure LMB in that study was probably due to a Type II error. For example, the mating of two individuals heterozygous for each of the 3 allozyme loci previously studied could result in the diagnosis of a 'pure' LMB offspring 1.6% of the time

by probability ( $1/4^3$ ); other allelic combinations where a mating individual was homozygous for one locus would further increase the probabilities of a Type II error. With an increase in the number of loci studied to 7 as in this study, the probability of a heterozygous mating resulting in the diagnosis of a 'pure' LMB offspring drops to  $< 0.01\%$ . Nonetheless, most individuals diagnosed in the present study using STRUCTURE were identified as pure FB. A membership coefficient of 0.95 or greater was used for the discriminating of species, common to studies of this type (Schrey et al. 2006, Schwartz and Beheregaray 2008).

### Conclusions

Here, we present a study of a lake that has historically been stocked with only FB and been previously evaluated with allozyme markers. Although we used differing molecular tools, loci used were diagnostic among species and useful for distinguishing bass phenotypes. Over the decade between studies we found both an increase in FB allele and phenotype frequencies, which may be associated with the continued stocking of FB or the selection of those alleles and phenotypes in an altered setting. Our microsatellite phenotypic data is consistent with the lake's stocking history of pure FB. Florida bass were detected in high frequency, despite the presence of LMB alleles. This data suggests that microsatellite analysis is an effective tool for evaluating stocking success in fisheries management.

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