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## Condition Relative to Phenotype for Bass Populations in Southern Arkansas Lakes

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### Abstract

Southern Arkansas reservoir largemouth bass populations (*Micropterus salmoides*) are often supplemented with stocks of Florida bass (*M. floridanus*) in an attempt to boost the frequency of hybrid and trophy bass. Stocking rates of Florida bass among these lakes are highly variable. We determined bass phenotype composition among 12 lake populations based upon stocking protocols: exclusively Florida bass, primarily Florida bass, mixed stocking protocol and primarily largemouth bass. We also compared condition among phenotypes ( $n = 2,100$ ) to test for hybrid or phenotype vigor. Mean relative weight of bass for most lakes but SWEPCO Lake (mean  $W_r = 72$ ) were  $\geq 90$ . Phenotype frequencies were inconsistent with FB stocking histories. No lake population was comprised only with pure Florida bass despite four of the lakes being stocked solely with this bass species. Numbers of  $F_1$  hybrid bass were low for all lake samples. Relative weight among phenotypes was also inconsistent among lake samples, allowing no conclusions to be made regarding relative weight and hybrid vigor or phenotype. Further testing increasing both the number of lake samples and sample size within lakes may provide insight into these questions of stocking effectiveness of Florida bass and hybrid or phenotype vigor.

### Introduction

Largemouth bass (*Micropterus salmoides*; LMB) are the most sought after species of freshwater gamefish in the world. They are native to the Mississippi River drainage system in North America, but have been stocked in waters of every continent in the world other than Antarctica (Jackson 2002). Maintaining stocks of native LMB populations within the southern United States is a top priority for fisheries managers. One common method of management has

been the stocking of Florida bass (*Micropterus floridanus*; FB) in southern U.S. reservoirs outside its natural range. These two putative species were until recently classified as subspecies and have recently been proposed as separate species (Near and Koppleman 2009). 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).

Two management goals are often targeted with the stocking of FB in native LMB lakes. One goal is that of introducing a faster and larger growing species into southern reservoirs (Week 1984; Fries et al. 2002; Johnson and Fulton 2004); this approach has been severely criticized as being both incorrect and damaging to native LMB stocks (Philipp 1991). The second goal is to apply the principle of hybrid vigor for enhancing fish size (Zolczynski and Davies 1976; Gilliland and Whitaker 1989; Noble 2002), which has also been criticized as leading to outbreeding depression (Cooke et al. 2001; Philipp et al. 2002; Cooke and Philipp 2006). Results of bass hybrid vigor studies have been mixed. Trophy bass management programs have indicated that most trophy bass genetically tested have contained both LMB and FB alleles (Oklahoma, Horton and Gilliland 1993; Texas, Lutz-Carrillo et al. 2002); controlled pond studies have indicated strong latitudinal gradients impacting both growth rates and relative weight among phenotypes (Philipp et al. 2002); in northern Arkansas, results were inconclusive for comparing relative weights among phenotype groups for bass populations in two reservoirs (Johnson and Fulton 1999; Johnson and Fulton 2004). However, reliability of bass phenotype determination of in situ studies has historically been limited due to reliance on only two genetic allozyme markers (Philipp et al. 1983); recently, microsatellite analysis has enabled greater reliability in phenotype identification (Lutz-Carrillo et al. 2006).

## Condition Relative to Phenotype for Bass Populations in Southern Arkansas Lakes

Here we discuss genetic characteristics and condition of bass from 12 Arkansas lakes. Each of these lakes has been stocked to varying degrees with FB, ranging from a single stocking of FB, to mixed stocking, to exclusive stocking of FB (Table 1). To accomplish this goal, we first employed Bayesian statistics to assign phenotypes (FB,  $F_X$ -FB,  $F_1$ -hybrids,  $F_X$ -LMB and LMB) for individual bass.  $F_1$  fish represent first generation crosses between FB and LMB, whereas  $F_X$  fish represent later generation crosses. We compared phenotype distribution to stocking histories.

In addition to total length of bass, anglers and fisheries managers are interested in bass within a system having high condition indices. We therefore compared relative weights among these differing phenotypes for each lake studied as a measure of meeting bass management goals, particularly as they pertain to hybrid vigor or phenotype.

### Methods

#### Study sites

Boat electrofishing for bass was performed by the Arkansas Game and Fish Commission (AGFC) in 2007-2008 within 12 Arkansas lakes (Figure 1; 11 reservoirs and 1 oxbow lake;  $n = 2100$ ). Lake samples were grouped into four categories based on bass stocking practices: lakes exclusively stocked with FB and no LMB (Lakes Bois d'Arc, Greenlee, Monticello, and SWEPCO); lakes stocked primarily with FB (Lakes Atkins, Columbia, Lower White Oak, and Millwood) lakes stocked irregularly with both FB and LMB (mixed stocking protocol; Lakes Chicot and Erling); and lakes primarily stocked with LMB (Lakes DeGray and Ouachita). SWEPCO (215 ha) and Monticello (615 ha) lakes have been stocked solely with FB since their construction in 1977 and 1993, respectively. Lakes Bois d'Arc (263 ha) and Greenlee (121 ha) were renovated in 2002 and 2000, respectively, and subsequently stocked with FB. Lakes Atkins, Columbia, Lower White Oak, and Millwood had pre-existent stocks of LMB prior to AGFC stocking of FB. Lake Atkins (304 ha) has had 250,000 FB fingerlings stocked since 2003, Lake Columbia (1,194 ha) has had over 1.7 million stocked FB fingerlings since 1986, Lake Millwood (11,938 ha) over 3 million FB fingerlings since 1984, and Lower White Oak Lake (702 ha) over 1 million FB fingerlings since 1993. Both of the lakes having a mixed stocking protocol of FB and LMB, lakes Chicot and Erling, had pre-existing LMB populations prior to initial stocking

of FB. Lake Chicot (3,925 ha) is an oxbow lake of the Mississippi River, whereas the source waters for Lake Erling (2,833 ha) and the bass of that lake is the Bodcau River. Since the AGFC began stocking Lake Chicot, most fish stocked have been LMB (60%), whereas, Lake Erling has had primarily FB (78%) stocked. Lakes DeGray (5,423 ha) and Ouachita (12,869 ha) have had one to two stockings of FB two to three decades ago.

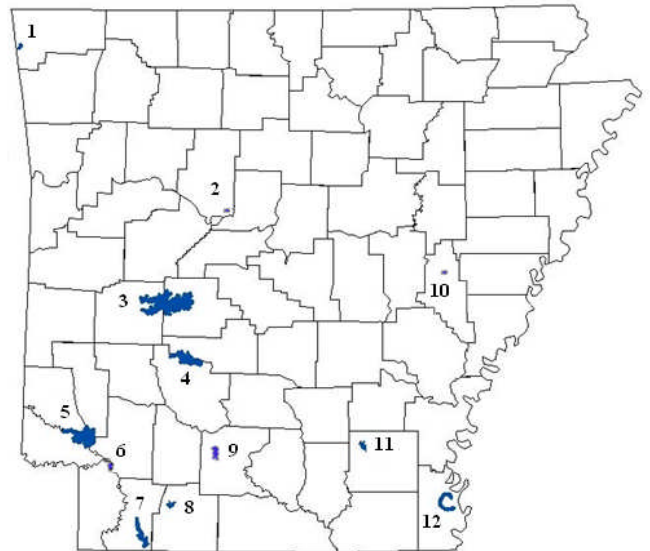


Figure 1. Arkansas lakes sampled for FB and LMB phenotypes: 1. SWEPCO Lake; 2. Lake Atkins; 3. Lake Ouchita; 4. Lake DeGray; 5. Lake Millwood; 6. Lake Bois d'Arc; 7. Lake Erling; 8. Lake Columbia; 9. Lower White Oak Lake; 10. Lake Greenlee; 11. Lake Monticello; and 12. Lake Chicot.

### Genetic analysis

Fin clips were taken from each bass for genetic analysis. DNA was isolated and analyses were performed using the methods of Allen and Johnson (2009; this issue). Allele frequencies were calculated for each of the seven microsatellite loci, and alleles were determined to be exclusive to FB, LMB or shared between species using hatchery samples (LMB, Joe Hogan and William Donham hatcheries in Lonoke ( $n = 33$ ) and Corning, AR ( $n = 45$ ), respectively; FB, Andrew Hulsey Hatchery in Hot Springs, AR ( $n = 103$ )) as controls. The program STRUCTURE (Pritchard et al. 2000) was used to provide a statistical value for the individual admixture proportion ( $q$ ) of each individual. 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

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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 in Allen and Johnson (2009).

**Condition comparisons**

Condition, expressed as relative weight ( $W_r$ ), was calculated for stock size individuals using the parameters of Henson ( $W_s = -5.528 + 3.273 \log_{10}$  Total Length; 1991). Due to the high variability in phenotype distributions, sample sizes, and in relative weights both among lakes and seasonally, we did not attempt to make state-wide comparisons among phenotype relative weights. For example, lake bass populations other than Lake Erling were sampled pre-spawn in late March and early April. Lake DeGray and Ouachita bass populations had additional samples collected during late October and November, and Lake Erling bass were sampled solely during November. To standardize relative weight data within these three lakes having multi-season collections, each fish sampled was measured as + or - the sample mean for that lake sample. For example, if the lake mean for relative weight was 100, then a bass having a relative weight of 110 would have a score of 10. This removed sampling variability associated with season (Johnson and Fulton 1999).

Each lake was dominated by two to three of the five phenotypes, primarily the pure species (LMB or FB), and secondarily the  $F_X$  towards that species. Data was normally distributed for all samples, so ANOVAs were performed comparing differences in relative weight of individuals among the numerically dominant phenotypes ( $n > 6$ ). ANOVAs demonstrating significance were followed with an *a posteriori* Tukey's multiple comparison test to test for treatment and interaction effects. All significance levels were set at  $\alpha = 0.05$ . Additionally, individual lakes are subject to a wide range of variables that impact the relative weight and growth of that lake. Rather than attempt to combine data among all lakes, we therefore kept our analyses to within lakes, and looked for trends relative to stocking patterns.

**Results and Discussion****Stocking Regimens and Bass Phenotypes**

Phenotype analysis showed a high range of incorporation of FB alleles and therefore phenotypes among the sample populations. Our data are

inconsistent in regards to phenotype trends relative to FB stocking history (Table 1). None of the lakes stocked solely with FB had 100% FB phenotypes. Most individuals of SWEPCO and Greenlee lakes had FB phenotypes (75 and 78%, respectively). Most of the remainder of the SWEPCO Lake bass were categorized as  $F_X$ -FB, whereas bass phenotypes in Lake Greenlee were distributed among each grouping other than  $F_X$ -LMB. As stated previously, Lake Greenlee was completely renovated and drained with a complete fish kill, with new stockings of FB beginning in 2000. However, Lake Greenlee is a small reservoir (121 ha), and was flooded shortly thereafter, with waters of Piney Creek entering the reservoir. This possibly contaminated the bass population with native LMB. Evidence of this Piney Creek source of LMB may be the presence of mature adults (8% of sample; 325 – 554 mm total length) that had LMB phenotypes. Lake Monticello had lower numbers of pure FB (35%), and was numerically dominated by  $F_X$ -FB (48%). Lake Monticello has several small streams feeding it; it is possible that native LMB from these streams have reduced the effectiveness of FB stocking in this reservoir. Lake Bois d'Arc was also renovated in 2002 similar to Lake Greenlee above, with only FB stocked since that time. However, the success of the fish kill in removing pre-existing LMB prior to stocking is suspect. Most individuals collected from Bois d'Arc were LMB (76%).

Lakes Atkins, Columbia, Millwood and Lower White Oak Lake have each had extensive FB fingerling stockings over prolonged periods, yet few sampled individuals of any of these lakes were pure FB; most individuals were distinguished as  $F_X$ -LMB or pure LMB (Table 1). Lake Atkins had a higher proportion of both FB (17%) and  $F_1$  hybrids (27%) than the other three reservoirs within this stocking regimen. The Columbia Lake sample was unique among this group, with few pure LMB identified.

Both of the lakes having a mixed stocking protocol of FB and LMB, lakes Chicot and Erling, had a numerical dominance of LMB and  $F_X$ -LMB phenotypes (Table 1). The greater proportion of LMB stocked into Lake Chicot relative to Lake Erling is reflected by phenotype proportions (57 versus 42% LMB, respectively).

Both lakes DeGray and Ouachita have had limited stocking events of FB, yet very high and continuous stocking of LMB. Phenotypes observed in both reservoirs are consistent with a LMB stocking protocol (Table 1). Most fish in both reservoirs were LMB (Lake DeGray 70%; Lake Ouachita 74%), with most of

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Table 1. Comparison of phenotype frequencies and relative weights (SE) of common phenotypes in Arkansas lake samples based upon Florida bass stocking protocols. Phenotypes sorted by pure species, F<sub>1</sub>, and F<sub>X</sub> hybrids. Lake Greenlee FX-FB individuals were less than stock size so relative weight was not calculated for this group.

Category/ Lake		Lake	LMB	F <sub>X</sub> -LMB	F <sub>1</sub>	F <sub>X</sub> -FB	FB
<b>Exclusive FB stocking</b>							
Bois d'Arc	n	108	81	8	7	0	12
	W <sub>r</sub>	107	107	106	102	---	104
	SE	(0.7)	(0.8)	(2.4)	(1.8)	---	(3.0)
Greenlee	n	147	12	0	9	12	114
	W <sub>r</sub>	120	114	---	127	---	121
	SE	(1.2)	(2.7)	---	(2.8)	---	(1.4)
Monticello	n	180	0	6	25	86	63
	W <sub>r</sub>	102	---	---	98	98	107
	SE	(1.0)	---	---	(1.8)	(1.2)	(1.5)
SWEPCO	n	150	0	0	6	32	112
	W <sub>r</sub>	72	---	---	---	73	72
	SE	(0.8)	---	---	---	(1.2)	(0.8)
<b>Primary FB stocking</b>							
Atkins	n	134	58	13	36	4	23
	W <sub>r</sub>	99	95	99	100	---	109
	SE	(0.8)	(1.0)	(2.0)	(1.2)	---	(1.9)
Columbia	n	60	3	47	8	1	1
	W <sub>r</sub>	96	---	94	94	---	---
	SE	(1.4)	---	(4.5)	(1.4)	---	---
Millwood	n	190	95	87	6	2	0
	W <sub>r</sub>	97	97	98	---	---	---
	SE	(1.1)	(1.6)	(1.7)	---	---	---
White Oak	n	147	50	84	9	1	3
	W <sub>r</sub>	100	102	99	96	---	---
	SE	(0.6)	(0.9)	(0.9)	(2.6)	---	---
<b>Mixed stocking protocol</b>							
Chicot	n	150	85	49	14	2	0
	W <sub>r</sub>	99	99	101	100	---	---
	SE	(0.9)	(0.9)	(1.5)	(2.5)	---	---
Erling	n	60	25	32	2	1	0
	W <sub>r</sub>	100	97	103	---	---	---
	SE	(1.4)	(1.7)	(1.3)	---	---	---
<b>Primary LMB stocking</b>							
DeGray	n	349	245	98	3	3	0
	W <sub>r</sub>	93	93	89	---	---	---
	SE	(0.7)	(0.7)	(1.1)	---	---	---
Ouachita	n	425	313	110	1	1	0
	W <sub>r</sub>	90	90	91	---	---	---
	SE	(0.4)	(0.4)	(0.8)	---	---	---
<b>Total n</b>		<b>2100</b>	<b>967</b>	<b>534</b>	<b>126</b>	<b>145</b>	<b>328</b>

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the remaining bass distinguished as FX-LMB (28% and 26%, respectively).

### Bass Phenotypes and Relative Weight

Relative weights of sampled fish were high for most lake samples other than SWEPCO Lake (Table 1). Mean  $W_r$  of most other populations were at that recommended by Anderson (1980) and Gutreuter and Childress (1990) for quality bass populations, where relative weight ranges of 95-105 are indicative of optimal condition and deviations from this range potentially indicate stresses related to growth, predator-prey imbalances, population density, recruitment problems or physiological abnormalities (Gutreuter and Childress 1990).

There was no consistent trend between bass phenotype and  $W_r$ . Six of the 12 lake bass populations sampled showed significant differences in  $W_r$  among phenotypes (Table 2). Four of these six significant sample groups demonstrated bass having higher proportions of FB alleles as having higher  $W_r$  than bass having predominantly LMB alleles; the other two samples were opposite this trend. Likewise, hybrid vigor was not evident with these data. Few  $F_1$  hybrids are found in these lakes studied, the phenotype thought to have the fastest growth and greatest condition (Kleinsasser et al. 1990; Noble 2002). 'Pure' species had greater relative weights than hybrids in four of the six samples. Therefore, differences in relative weights

between phenotypes may be only reflective of patterns within an individual lake rather than hybrid vigor or differential length-weight ratios of one phenotype relative to another. Preliminary length at age data is similarly inconclusive regarding hybrid vigor or phenotype advantage (Allen 2009). Other studies comparing bass phenotype to condition have similarly had confounding results. For example, Fulton (1998) identified no significant differences among phenotypes in regards to relative weight for bass in two northern Arkansas lakes, whereas other studies have shown FB and  $F_1$  hybrids to have greater relative weights than LMB in Oklahoma (Wright and Wigtil 1980) and Texas (Inman et al. 1977); conversely, another study showed stock sized FB to have lower relative weights than LMB in Texas (Maceina and Murphy 1988). Previous research has found that hybrid vigor is lessened with further backcrossing (Philipp et al. 2002). That stated, previous studies have also relied on two allozyme loci for determination of first or later generation crosses (Philipp et al. 1983; Johnson and Fulton 1999). With an increase in the number of loci studied, it is possible that some fish previously designated as  $F_1$  hybrids in allozyme studies were in reality FX bass. We are continuing to increase both lake sample sizes and number of lake populations studied, which may provide further insight into this question of hybrid or phenotype vigor among bass species.

Table 2. ANOVA comparing  $W_r$  among bass phenotypes by lake.

Category/ Lake	df	F	p	Significances
<b>Exclusive FB stocking</b>				
Bois d'Arc	3,107	1.53	0.211	None
Greenlee	3,146	3.81	0.026	$F_1 > LMB$ ; $p < 0.01$
Monticello	2,167	11.77	$< 0.001$	$FB > F_1 = F_X-LMB$ ; $p < 0.01$
SWEPCO	1,143	0.43	0.513	None
<b>Primary FB stocking</b>				
Atkins	3,104	16.92	$< 0.0001$	$FB > F_X-FB = LMB = F_1$ ; $p < 0.01$
Columbia	1,54	0.13	0.720	None
Millwood	1,139	0.33	0.567	None
White Oak	2,142	3.58	0.031	$LMB > F_1$ ; $p < 0.05$
<b>Mixed stocking protocol</b>				
Chicot	2,142	1.40	0.250	None
Erling	1, 54	6.90	0.011	$F_X-LMB > LMB$ ; $p = 0.011$
<b>Primary LMB stocking</b>				
DeGray	1,342	5.99	0.015	$LMB > F_X-LMB$ ; $p = 0.015$
Ouachita	1,422	0.79	0.375	None

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### Conclusions

Although none of the lakes stocked solely with FB were 100% pure FB, three of the four lakes were numerically dominated by FB and F<sub>x</sub>-FB hybrids. The renovation and stocking efforts for a pure FB stock of Lake Bois d'Arc largely failed. The stocking of FB on top of existing LMB stocks has resulted in limited changes in phenotypes in southern Arkansas bass populations, with the possible exception of Lake Atkins. Among the four lakes stocked primarily with FB a small proportion were identified as F<sub>1</sub> bass (11%). If hybrid vigor is a management goal of the AGFC, then a more effective approach for increasing numbers of F<sub>1</sub> progeny must be developed. For example, the AGFC is presently heavily stocking isolated pockets of large reservoirs (Lakes DeGray and Ouachita), in efforts to increase the numbers of F<sub>1</sub> progeny on a local level.

There has been limited study regarding the long-term stocking success of FB in large lakes and reservoirs for several reasons. Included among these reasons are the difficulties in accounting for dynamic changes in habitat, a limited knowledge of resident populations, unknown variables relating to dispersal of stocked fish, and attaining an adequate sample size relative to the population (e.g., Fieldhouse 1971, Dunham et al. 1992, Buckmeier and Betsill 2002). Forshage and Fries (1995) recommend that introductions of FB occur for newly constructed reservoirs, as established populations are difficult to alter. Our phenotype analysis supports these recommendations, as the greatest proportions of FB and hybrids occurred in lakes initially stocked with FB following construction or renovated lakes with a fish kill (other than Lake Bois d'Arc). Smaller lakes (e.g., Lakes Atkins and Monticello) have also historically had better records of FB incorporation (Forshage and Fries 1995). The low success rate of FB stocking in most lakes previously housing LMB populations may also be attributed to predation of stocked fingerling FB by larger fish of an established bass population or other predators (Modde et al. 1996, Buckmeier et al. 2005, Hoffman and Betolli 2005).

Lastly, to date, we have not identified hybrid vigor in these Arkansas bass populations, nor is there a clear demonstration of phenotype advantage in regards to relative weight. Further work should be performed on differing performance of bass phenotypes in regards to relative weight and differing growth patterns in Arkansas lakes, which perhaps represent the northern limits of successful FB stocking.

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