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K. A. Lamothe

Arkansas State University, karl.lamothe@smail.astate.edu

Ronald L. Johnson

Arkansas State University

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Microsatellite Analysis of Trophy Largemouth Bass from Arkansas Reservoirs

K.A. Lamothe* and R.L. Johnson

Department of Biological Sciences, Arkansas State University, State University, AR 72467

*Correspondence: karl.lamothe@smail.astate.edu

Running title: Microsatellite Analysis of Trophy Largemouth Bass from Arkansas Reservoirs

Abstract

The Arkansas Game and Fish Commission (AGFC) has introduced Florida largemouth bass (FLMB; *Micropterus salmoides floridanus*) to water bodies historically containing the northern largemouth bass (NLMB; *Micropterus salmoides salmoides*) subspecies since the late 1970s in an attempt to produce a trophy LMB fishery. Since 2006, the AGFC has been biannually sampling reservoirs stocked with FLMB to determine levels of admixture. Here, total sampling efforts between 2006 and 2011 have been combined, and LMB heavier than 2,268 g (5 lb) were analyzed in an effort to investigate distribution of bass by their genetic composition designated as trophy LMB by the AGFC. Of the 148 trophy LMB sampled, 123 possessed FLMB alleles (83.1%). Thirty-two of the heaviest 50 (64.0%) LMB sampled, including a potential state record that was nullified, were genetically confirmed to be FLMB. Distributions of trophy bass within reservoirs were preferentially represented by F_x-FLMB and FLMB.

Introduction

Largemouth bass (LMB; *Micropterus salmoides*) are the most targeted freshwater game fish sought out by anglers across the United States (USFWS 2006, Sutter et al. 2012). Despite compelling genetic evidence demonstrating species delimitation (Kassler et al. 2002, Near et al. 2003), the American Fisheries Society Committee on Names of Fishes continues to recognize 2 subspecies of largemouth bass: Florida largemouth bass (FLMB; *M. s. floridanus*) and northern largemouth bass (NLMB; *M. s. salmoides*) (Page et al. 2013). Therefore, for the purposes of this study, subspecies nomenclature will be maintained.

For the last 40 years, FLMB have been commonly stocked into southern United States reservoirs by state agencies that previously contained NLMB because of their reputation for greater growth potential than NLMB (Addison and Spencer 1971, Wright and Wigtil

1982, Horton and Gilliland 1993), a putative hybrid vigor between subspecies (Inman et al. 1978, Kleinsasser et al. 1990), and intense pressure from resident and nonresident anglers (Chen et al. 2003).

Previous studies focusing on comparing the performance of the 2 subspecies and their intergrades based on bass management parameters (e.g., mortality, growth, relative weight, catchability) have been inconsistent. Controlled pond studies were typically short in duration and showed varying levels of performance characteristics among NLMB, FLMB, and their F₁-intergrades (Isely et al. 1987, Kleinsasser et al. 1990, Horton and Gilliland 1993, Garrett 2002, Philipp et al. 2009). For example, outbreeding depression has been demonstrated among F₁ and later generational LMB intergrades (Cooke et al. 2001, Philipp et al. 2002, Cooke and Philipp 2006, Goldberg et al. 2005). In a study of a mixed bass population in a Texas reservoir, Maceina et al. (1988) determined that growth of female FLMB at Age 3 exceeded that of NLMB females, and therefore, conferred a selective advantage in terms of size-dependent fecundity. Allen et al. (2009) found no differences in relative weights among LMB subspecies and their intergrades in Arkansas reservoirs stocked with FLMB. Horton and Gilliland (1993) identified greater length and mass of FLMB related to NLMB in Oklahoma reservoirs.

An increase in frequency of LMB state records angled from states currently stocking FLMB, including Oklahoma (Horton and Gilliland 1993, Cofer 1993), Texas (Forshage and Fries 1995, Lutz-Carrillo et al. 2006, Tibbs 2008), and Louisiana (Hughes and Wood 1995), suggests positive management outcomes of stocking FLMB in the southern United States. In 2012, the AGFC had to disqualify what would have been the state record LMB (7.4 kg) due to a lack of angler licensure.

In addition to potentially offering anglers an opportunity to catch a new state record bass, a primary goal of the AGFC Black Bass Management Plan (2002) is to increase the frequency of bass caught over 2,268 g (5 lb), which are designated as trophy LMB in

Arkansas (Hobbs et al. 2002). One approach to accomplish this goal has been for the AGFC to introduce FLMB to reservoirs in the southern half of Arkansas, which it has done since the late 1970s. Over the past decade, approximately 1,000,000 FLMB fingerlings have been stocked annually.

In order to assist in future management stocking decisions, the AGFC has sampled and genetically analyzed thousands of LMB over the previous 6 years from reservoirs stocked with FLMB. Historically, genetic distinctions between subspecies were determined by analyzing 2 allozyme loci which are fixed for different alleles between subspecies (Phillip et al. 1983). However, a 2-marker genetic system (sAAT-B and sIDH-B) often yielded incorrect identification (Maceina et al. 1988). Lutz-Carrillo et al. (2006) developed a protocol utilizing microsatellite markers to provide greater reliability in subspecies delimitation. To date, the AGFC in conjunction with Arkansas State University have microsatellite profiles using 7 microsatellite markers of almost 5,000 LMB from reservoirs sampled between 2006 and 2011.

We studied sample data from 13 Arkansas reservoirs that have been stocked with FLMB, and 1 reservoir that has not been stocked with FLMB. Each reservoir has a mixed population of FLMB, NLMB, and their intergrades (Allen et al. 2009). With these data, our goal was to determine if FLMB and their intergrades were preferentially represented among trophy LMB in comparison to the entire sample among stocking regimens. An increase in the frequency of FLMB relative to the overall population distribution could be indicative of greater survival and/or growth characteristics; a reduction in the frequency of FLMB could be indicative of thermal selection pressures from stocking FLMB outside their native range. Furthermore, changes in frequencies of F_1 and other intergrades could be indicative of hybrid vigor or outbreeding depression.

Methods

Stocking regimens

Stocking regimens put in place by the AGFC for the sampled reservoirs of the present study were classified into 3 categories: 1) creation of a new reservoir or performing a fish kill followed by stocking of FLMB (FLMB-initiated: lakes Atkins, Bois d'Arc, Columbia, Greenlee, and Monticello); 2) having an established NLMB population prior to regular FLMB introductions (NLMB-initiated: lakes Chicot, Erling,

Lower White Oak, and Millwood); or 3) NLMB stocking only or episodic instances of FLMB stocking on top of NLMB populations (Episodic: lakes Conway, DeGray, Greers Ferry, Ouachita, and Upper White Oak) [Table 1].

Reservoirs designated as FLMB-initiated were either newly created reservoirs initially stocked with FLMB (lakes Columbia and Monticello), or reservoirs that were drained and subjected to rotenone treatment (lakes Atkins, Bois d'Arc and Greenlee). Northern LMB-initiated reservoirs contained an established NLMB population prior to FLMB introductions. These reservoirs have been irregularly stocked with FLMB for at least 18 years (Table 1). For example, lakes Chicot and Erling were initially stocked with FLMB around 1985, then again around 1990, around 1995, 2001, and then continuously from 2005 to the present. Several intermittent stockings of NLMB also occurred during this period. Lower White Oak Lake has been stocked with FLMB annually from 1993 to 2011. Lake Millwood has had an intermediate stocking regimen relative to the other lakes of this category. Lake Chicot is a natural oxbow of the Mississippi River; the other 3 reservoirs were approximately 20 years old with pre-existent NLMB populations prior to FLMB stocking.

Of the lakes categorized as NLMB only or episodic stockings of FLMB, Greers Ferry has not been stocked with FLMB. Lake Conway received FLMB in 1993, 2001, and 2007, whereas Upper White Oak was stocked with FLMB in 1993 and 1994. Both lakes DeGray and Ouachita have had extensive stockings of FLMB in localized embayments since 2007 (Lamothe et al. 2012). However, the FLMB-stocking regimens at these lakes were not in place long enough (1 year prior to sampling) to produce LMB greater than 2,268 g or to be represented in sampling; therefore, LMB from these reservoirs were limited to control samples taken before FLMB-stocking regimens were implemented. Historically, Lake DeGray was stocked with 3,000 fingerlings and 60 adults in 1986, whereas Lake Ouachita had 1 introduction of 500,000 FLMB fingerlings in 1978.

Sampling

Beginning in 2006, the AGFC has intensively sampled LMB for genetic analysis using a boom-mounted boat electrofishing unit. The primary goal of sampling LMB has been to target a broad representation of LMB sizes, particularly bass greater than stock size (200 mm; Guy et al. 2006), with a target of 10 fish per 25 mm size group per outing.

Microsatellite Analysis of Trophy Largemouth Bass from Arkansas Reservoirs**Table 1.** Physical characteristics and stocking protocol of study reservoirs by stocking regimen. Included are surface area (ha), year constructed/year renovated, the years AGFC stocked FLMB, the total number of stocking events, the total number of fingerlings/yearlings stocked, the total number of adults stocked, n = sample size, and the sampling years. Number of stocking events, the total number of fingerlings/yearlings stocked, and total number of adults stocked refer to data prior to most recent sampling.

Location	Surface area (ha)	Year Constructed/Renovated	Years Stocked with FLMB	No. of Stocking Events	Total No. of Fingerlings/Yearlings Stocked	Total No. of Adults Stocked	n	Sampling Years
FLMB-Initiated								
Atkins	304	1956/2002	2003-11	9	364,620	171	297	2009 2011
Bois d'Arc	263	1961/2002	2003-11	11	262,725	133	244	2009 2011
Columbia	1,194	1986	1986-2011	40	2,050,380 / 40,150	218	212	2008 2009
Greenlee	121	1961/2000	2001-11	18	101,640 / 42,267	151	300	2009 2011
Monticello	615	1992	1993-96, 2001-11	18	839,835	139	334	2008 2010
NLMB-Initiated								
Chicot	3,925	N/A	1986, 1994-96, 2000, 2004-11	14	594,758	23	285	2008 2010
Erling	2,833	1956	1984-85, 1991, 1993, 2006-11	10	1,113,405	0	209	2008 2010
Lower White Oak	665	1961	1993-2011	43	1,616,053 / 25,350	183	300	2009 2011
Millwood	11,938	1966	1984-86, 1991-94, 2001-11	46	3,885,742	223	334	2009 2011
NLMB and/or Episodic FLMB Stocking								
Conway	2,711	1948	1993-94, 2001, 2007	4	0 / 25,050	900	145	2009
DeGray	5,423	1972	1986	1	3,000	60	238	2006 2007
Greers Ferry	16,389	1963	-	0	0	0	118	2009
Ouachita	12,869	1953	2007-11	0	0	0	425	2006
Upper White Oak	417	1961	1993-94, 2010-11	5	121,732	21	146	2010

During collection, LMB were measured (total length (TL), mm), massed (g), and fin clips were taken and preserved in ethanol for molecular analysis. All sampling was performed between 2006 and 2011.

In addition to the above samples, we included the disqualified state record LMB caught from Lake Dunn. Lake Dunn, located in Village Creek State Park, has never been stocked with FLMB by the AGFC (K. Winningham, AGFC, *personal communication*).

Genetic Analysis

Genomic DNA extraction was performed using a modified version of the chloroform tris-acetate borate extraction method (Allen et al. 2009). With specifications outlined by Lutz-Carrillo et al. (2006), the polymerase chain reaction was carried for each individual using 7 fluorescent microsatellite primers (*Lma007*, *Lma12*, *Mdo3*, *Mdo6*, *Msa13*, *Msa021*, *Msa29*; Integrative DNA Technologies, Coralville, IA). One of the 7 loci (*Msa021*) was fixed between

subspecies; the other 6 loci had varying levels of allele frequencies in common between subspecies: *Lma007* (16.5% allelic overlap), *Lma12* (11.5%), *Mdo3* (39.3%), *Mdo6* (1.7%), *Msa13* (6.5%), and *Msa29* (1.1%).

Capillary electrophoresis was performed using a Beckman-Coulter CEQ8000 Genetic Analysis System (Beckman Coulter, Inc., Fullerton, CA). Fragment lengths were internally scored using a 400 bp standard and manually confirmed.

Largemouth bass were classified as 1 of 5 genetic groups (FLMB, F_x -FLMB, F_1 -intergrades, F_x -NLMB, NLMB) using the Bayesian clustering software *STRUCTURE* 2.3 (Hubisz et al. 2009). Control hatchery samples were provided to establish baseline subspecies parameters (NLMB: Joe Hogan and William Donham hatcheries in Lonoke ($n = 32$) and Corning, AR ($n = 42$), respectively; FLMB: Andrew Hulsey Hatchery in Hot Springs, AR ($n = 83$)). Florida LMB hatchery broodfish are genetically tested annually using allozyme analysis to maintain pure lines.

An admixture model with correlated allele frequencies and default settings were first used to establish pure subspecies lines and their intergrades ($n = 3,744$; 20,000 burn-in steps; 200,000 Markov Chain Monte Carlo steps). The result of this analysis was a statistical value for the admixture proportion (q) of each individual. Admixture proportions were used to classify individuals as either subspecies or intergrades, 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 NLMB, whereas individuals with $q \leq 0.05$ were classified as FLMB. All broodstock controls were within this threshold and distinguished as subspecies. Individuals having intermediate q -values were classified as intergrade bass (F_x -NLMB, F_1 , and F_x -FLMB), as described below.

To further resolve bass phenotypes a second *STRUCTURE* analysis was then performed implementing the same criteria as previously stated, but with "Population Information, $K = 2$ " set to 2 generations back. This analysis was used to determine the probability that individuals were either pure subspecies, first (F_1), or greater (F_x) generation intergrades. Individuals of hatchery populations were included, with FLMB categorized as a "1" and NLMB as a "2." First, the analysis generated a relative probability that each hatchery individual was categorized in the correct group (pure FLMB or pure NLMB, respectively). Second, the analysis generated probabilities that intergrade bass sampled were

correctly identified as F_1 or F_x -intergrade bass. All individuals designated as F_1 were then manually verified as being appropriately heterozygous for all 7 loci. It was not the intent of this study to delineate later generation intergrades, hence the use of F_x .

Statistical Analysis

In addition to looking at representation of trophy bass among genetic groups, we investigated whether the distribution of trophy bass was different from the overall sample for each stocking regimen. To achieve this, a Chi-square goodness of fit test was performed. The data set was divided into 3 stocking categories: NLMB-initiated, FLMB-initiated, and NLMB and/or episodically stocked with FLMB reservoirs. The individual collected from Lake Dunn was not included in the stocking regimen analysis because no additional sampling was performed at this location.

If there were no differences among frequencies of trophy bass by genetic group relative to the general population, then the genetic distributions of trophy bass should equal that of the overall sampled population. Expected frequencies were derived from the overall distribution of sampled groups for each stocking regimen.

Results

Of the 148 trophy LMB collected in Arkansas, 56 were characterized as FLMB (37.8%; Table 2), including the disputed state record bass angled illegally from Lake Dunn. A total of 124 LMB sampled contained FLMB alleles (FLMB, F_x -FLMB, F_1 , and F_x -NLMB; 83.8%). Of the 50 heaviest sampled LMB, there were 32 FLMB, 5 F_x -FLMB, 5 F_1 -intergrades, 4 F_x -NLMB, and 4 NLMB. Most trophy LMB collected ($n = 114$) were sampled from FLMB-initiated reservoirs, particularly from Lake Monticello ($n = 66$) and Lake Atkins ($n = 28$; Table 2).

Reservoirs designated as NLMB-initiated were dominated by NLMB and F_x -NLMB. In contrast, FLMB-initiated reservoirs were composed primarily of FLMB and their intergrades. The observed and expected frequencies of trophy FLMB, F_x -FLMB, F_1 -intergrades, and F_x -NLMB in NLMB-initiated reservoirs were combined for the Chi-square analysis due to low expected frequencies of these genetic groups (Roscoe and Byars 1971). A Chi-square analysis could not be performed for reservoirs stocked with only NLMB or episodically with FLMB due to low observed frequencies of trophy LMB.

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Table 2. Total number of trophy LMB and overall sampled LMB from each FLMB-initiated reservoir. Included are counts by genetic group for bass greater than 2,268 g, total counts of sampled reservoirs, and *STRUCTURE* mean *q*-values for each reservoir.

Location	FLMB	F _x -FLMB	F ₁	F _x -NLMB	NLMB	Total	Mean <i>q</i> -value
Atkins > 2,268 g	22 (78.6%)	1 (3.6%)	5 (17.9%)	0 (0.0%)	0 (0.0%)	28	0.111
Atkins Total	50 (16.8%)	18 (6.1%)	73 (24.6%)	46 (15.5%)	110 (37.0%)	297	0.634
Bois d'Arc > 2,268 g	1 (50.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2	0.327
Bois d'Arc Total	13 (5.3%)	3 (1.2%)	21 (8.6%)	39 (16.0%)	168 (68.9%)	244	0.855
Columbia > 2,268 g	0 (0.0%)	3 (37.5%)	0 (0.0%)	5 (62.5%)	0 (0.0%)	8	0.583
Columbia Total	1 (0.5%)	20 (9.4%)	26 (12.3%)	144 (67.9%)	21 (9.9%)	212	0.637
Greenlee > 2,268 g	2 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (80.0%)	10	0.798
Greenlee Total	253 (84.3%)	13 (4.3%)	13 (4.3%)	1 (0.3%)	20 (6.7%)	300	0.103
Monticello > 2,268 g	30 (45.5%)	26 (39.4%)	10 (15.2%)	0 (0.0%)	0 (0.0%)	66	0.209
Monticello Total	111 (33.2%)	156 (46.7%)	46 (13.8%)	18 (5.4%)	3 (0.9%)	334	0.245
Total > 2,268 g	55 (48.2%)	31 (27.2%)	15 (13.2%)	5 (4.4%)	8 (7.0%)	114	0.406
Category totals	428 (30.9%)	210 (15.1%)	179 (12.9%)	248 (17.9%)	322 (23.2%)	1387	0.495

Table 3. Total number of trophy LMB and overall sampled LMB from each NLMB-initiated reservoir. Included are counts by genetic group for bass greater than 2,268 g, total counts of sampled reservoirs, and *STRUCTURE* mean *q*-values for each reservoir.

Location	FLMB	F _x -FLMB	F ₁	F _x -NLMB	NLMB	Total	Mean <i>q</i> -value
Chicot > 2,268	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	4 (66.7%)	6	0.873
Chicot Total	0 (0.0%)	17 (6.0%)	18 (6.3%)	96 (33.7%)	154 (54.0%)	285	0.850
Erling > 2,268	0 (0.0%)	0 (0.0%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	3	0.739
Erling Total	2 (1.0%)	2 (1.0%)	3 (1.4%)	67 (32.1%)	135 (64.6%)	209	0.902
Lower White Oak > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (80.0%)	1 (20.0%)	5	0.786
Lower White Oak Total	5 (1.7%)	6 (2.0%)	9 (3.0%)	135 (45.0%)	145 (48.3%)	300	0.856
Millwood > 2,268	0 (0.0%)	1 (8.3%)	4 (33.3%)	3 (25.0%)	4 (33.3%)	12	0.682
Millwood Total	3 (0.9%)	4 (1.2%)	8 (2.4%)	114 (34.1%)	205 (61.4%)	334	0.889
Total > 2,268 g	0 (0.0%)	2 (7.7%)	5 (19.2%)	9 (34.6%)	10 (38.5%)	26	0.770
Category totals	10 (0.9%)	29 (2.6%)	38 (3.4%)	412 (36.5%)	639 (56.6%)	1128	0.874

Table 4. Total number of trophy LMB and overall sampled LMB from reservoirs not stocked with FLMB or those with episodic FLMB stocking. Included are counts by genetic group and proportions for bass greater than 2,268 g, total counts of sampled reservoirs, and *STRUCTURE* mean *q*-values for each reservoir.

Location	FLMB	F _x -FLMB	F ₁	F _x -NLMB	NLMB	Total	Mean <i>q</i> -value
DeGray > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0	N/A
DeGray totals	0 (0.0%)	0 (0.0%)	2 (0.8%)	40 (16.8%)	196 (82.4%)	238	0.925
Ouachita > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0	N/A
Ouachita totals	0 (0.0%)	1 (0.2%)	1 (0.2%)	110 (25.9%)	313 (73.6%)	425	0.942
Conway > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	1	0.991
Conway totals	0 (0.0%)	1 (0.7%)	1 (0.7%)	28 (19.3%)	115 (79.3%)	145	0.958
Greers Ferry > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	1	0.997
Greers Ferry totals	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (9.3%)	107 (90.7%)	118	0.979
Upper White Oak > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)	4 (80.0%)	5	0.977
Upper White Oak totals	0 (0.0%)	0 (0.0%)	0 (0.0%)	36 (32.7%)	110 (75.3%)	146	0.946
Totals > 2,268	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	6 (85.7%)	7	0.982
Category totals	0 (0.0%)	2 (0.2%)	4 (0.4%)	225 (21.0%)	841 (78.5%)	1072	0.950

The observed frequencies of trophy LMB by genetic group sampled from FLMB-initiated reservoirs differed significantly from the overall expected frequency of LMB in FLMB-initiated reservoirs ($\chi^2 = 46.68$, $df = 4$, $p < 0.001$; Table 2). Most notably increased over expected were FLMB and F_x-FLMB, whereas there were large declines in F_x-NLMB and NLMB.

Observed frequencies of trophy LMB by genetic group sampled from NLMB-initiated reservoirs were not statistically different from the overall (expected) frequencies of LMB in NLMB-initiated reservoirs ($\chi^2 = 2.76$, $df = 1$, $p = 0.097$), yet the sample size for this group was small ($n = 26$; Table 3).

Only 7 trophy LMB were sampled from reservoirs episodically stocked with FLMB. Six of the 7 trophy bass were genetically confirmed NLMB, whereas 1 was an F_x-NLMB (Table 4).

Florida LMB-initiated reservoir bass populations had the lowest mean *q*-value (0.495; Table 2), whereas NLMB-initiated populations' *q*-values ranged from 0.850-0.902 and reservoir populations stocked only with NLMB or episodically stocked with FLMB

ranged from 0.925-0.979.

Consistent with the analyses above, mean *q*-values for trophy bass were lower than for overall lake samples for both FLMB and NLMB-initiated reservoirs other than lakes Greenlee and Chicot, respectively (Tables 2-3). Mean *q*-values for trophy bass were similar for episodically stocked reservoirs to overall samples.

Discussion

Florida LMB was the most represented genetic group in our analysis of trophy LMB sampled from Arkansas reservoirs. Further, these trophy bass were primarily sampled from FLMB-initiated reservoirs. The frequencies of trophy bass in FLMB-initiated reservoirs were related to the levels of FLMB alleles, declining in frequency from FLMB to F_x-FLMB, to F₁ intergrades, etc. Thus, neither hybrid vigor nor outbreeding depression were evident based upon trophy bass frequencies. Despite similar sample sizes among reservoir types, the numbers of trophy bass from FLMB-initiated lakes were 4-fold greater than

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from NLMB-initiated lakes, where were another 4-fold greater than trophy bass from NLMB reservoirs. As the sample size of trophy LMB in Arkansas has increased over several years of sampling (2006-2011), a strong trend is emerging that these trophy fish tend to contain a high number of FLMB alleles.

This trend is consistent among southern states currently stocking FLMB. For example, in Oklahoma Horton and Gilliland (1993) analyzed a sample ($n = 251$) of angled trophy LMB from cooperating Oklahoma taxidermists using allozyme analysis and determined that 93% of these Oklahoma LMB contained FLMB alleles. In Louisiana, Hughes and Wood (1995) reported that 26 of the top 30 heaviest LMB were caught from Caney Creek Reservoir in north central Louisiana, a reservoir designated as a FLMB stocking site. In Texas, the current state record and 35 of the top 50 heaviest LMB were caught from Lake Fork, a reservoir stocked with FLMB since the early 1980's (Chen et al. 2003, Myers and Allen 2005). The mass of the current Texas state record LMB (18.18 kg) is 26% greater than the record prior to FLMB introductions (Tibbs 2008). Myers and Allen (2005) determined that Texas lakes stocked with FLMB had a 7-fold increase in their likelihood of producing a trophy LMB versus lakes not stocked with FLMB; in Arkansas there was a 16-fold increase in frequency.

The 5 FLMB-initiated reservoirs of the present study were either stocked with FLMB as new reservoirs (lakes Columbia and Monticello, constructed in 1986 and 1992, respectively) or drained and treated with rotenone in order to eliminate the resident population between 2000 and 2002 (lakes Atkins, Bois d'Arc, and Greenlee). The anomalous dataset for this stocking regimen are the Lake Greenlee trophy LMB which proved to be NLMB; however, age data demonstrated that these bass were holdovers from an incomplete fish kill prior to stocking FLMB (Allen et al. 2009). Reservoir ages for the NLMB-initiated and episodic FLMB-stocked reservoirs in Arkansas were much greater, ranging from 34 to 61 years (Lake Chicot is an oxbow).

Consistent with our findings, newly constructed or renovated reservoirs are often associated with rapid growth and production of large bass (Horton and Gilliland 1993, Crawford et al. 2002, Myers and Allen 2005). Further, Myers and Allen (2005) identified reservoir age to be a greater predictor for a Texas lake presenting a trophy bass than was bass genetic composition. Nonetheless, within FLMB- and NLMB-initiated reservoirs, the frequency of trophy FLMB and F_x -FLMB, and trophy bass mean q -values,

demonstrated a strong genetic difference from the overall population.

In contrast to hypotheses and studies suggesting outbreeding depression of introduced FLMB (Philipp et al. 2002, Cooke et al. 2001, Goldberg et al. 2005), genetic factors have been proposed as a causal factor for the increased number of FLMB trophy bass in southern states (Addison and Spencer 1971, Horton and Gilliland 1993). Maceina et al. (1988) did identify an increase in weight of Age 3 FLMB relative to NLMB in a Texas reservoir, indicating a genetic basis for differing growth potential. Horton and Gilliland (1993) found that mature female FLMB had significantly greater mean growth rates than other genetic groups (F_1 and NLMB) in Oklahoma reservoirs. Further investigations comparing growth patterns among genetic groups of mature bass in reservoir systems are needed.

In contrast to differing growth patterns among subspecies, it has been proposed that an increase in the frequency of trophy FLMB relative to NLMB is due to differences in subspecies susceptibility to angling (Garrett 2002, Lutz-Carrillo and Dumont 2012). The basis of this hypothesis is that NLMB are more aggressive than FLMB, and therefore may be removed from populations at a younger age. Garrett (2002) demonstrated a reduced vulnerability to angling and therefore potential harvest for NLMB versus FLMB in a multi-generational pond study. Earlier pond studies were inconclusive in demonstrating differences in angling susceptibility (Zolczynski and Davies 1976, Inman et al. 1978, Wright and Wigtil 1980, Kleinsasser et al. 1990). In a study of 5 Texas reservoir populations with varying levels of subspecies introgression, Lutz-Carrillo and Dumont (2012) compared creel surveys and electrofishing results relative to *STRUCTURE* q -value distributions. Three of the 5 reservoirs showed significant differences in angling versus electrofishing results among genetic categories, but only for FLMB and bass having greater than 80% FLMB alleles, supporting an angling-biased removal of NLMB. Crawford et al. (2002), in studying bass populations in Florida lakes, indicated that longevity is a critical factor in trophy LMB production. If this angling-selection hypothesis is indeed correct, this could explain in part the over-representation of FLMB as trophy bass in Arkansas reservoirs. Supportive evidence of angler-selection (and/or other variables related to survival) is an increase in older bass ($> \text{Age } 6$) having a greater number of FLMB alleles in Arkansas reservoirs (*unpublished data*).

Although Lake Dunn has never been stocked with

FLMB, the disputed state record LMB angled and reported to the AGFC was determined to be a FLMB. Unfortunately, volitional illegal transport of fish by anglers is a recurring problem across the United States (Rahel 2004, 2010) and may explain how a FLMB was caught from Lake Dunn in 2012. Lake Austell, located less than 2 km from Lake Dunn in Village Creek State Park, was historically stocked with FLMB by the AGFC, and therefore may have been the source of this trophy FLMB (K. Winningham, AGFC, *personal communication*). A study is currently in progress aimed towards determining the level of FLMB introductions by anglers in Lake Dunn.

Conservation biologists have disputed the stocking of fish outside their native range for many years (Allendorf 1991, Philipp 1991, Courtenay Jr. 1995, Leary et al. 1995, Philipp et al. 2002, Cucherousset and Olden 2011). The introduction of nonnative fish has led to increased similarities of freshwater communities across the United States and homogenization is a concern for regional, national, and global biological diversity (Rahel 2002). Furthermore, stocking of nonnative fish can introduce potentially maladaptive gene complexes leading to the loss of adaptation at the local level (Fields et al. 1987, Koppelman et al. 1988, Leary et al. 1995, Philipp et al. 2002). Despite these ecological and conservation concerns, state fisheries agencies continue to stock FLMB throughout much of the southern United States in order to enhance LMB fisheries and give anglers the opportunity to land a trophy bass (Chen et al. 2003).

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