Shifting Genetic Composition of Largemouth Bass Populations in Dendritic Arms of Two Large Arkansas Reservoirs Through Stocking of Florida Largemouth Bass

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Abstract: The Arkansas Game and Fish Commission has been annually stocking Florida largemouth bass (FLMB, *Micropterus salmoides floridanus*) at rates of 250 fish ha^{-1} into small dendritic sections of two large Arkansas reservoirs, Lake DeGray and Lake Ouachita, since 2006 and 2007, respectively, to alter local pre-existent northern largemouth bass (NLMB, *Micropterus s. salmoides*) populations. The management goal for these actions is to have 40% of the local largemouth bass (LMB) population possessing FLMB alleles within eight years of the initiation of the stocking program. Microsatellite analysis of ~1200 LMB were performed prior to and post stocking three and five years for Lake DeGray and three years for Lake Ouachita populations to measure progress towards this management goal. Stocked FLMB represented 2%–3% of sampled fish for both lakes. Following five years of stocking FLMB into Lake DeGray, 10% of the sample was identified F_1 LMB. Overall, the incorporation of FLMB alleles as measured by changing *q*-values into the native populations for both lakes has been slow (1.5–2.0/yr). Nonetheless, both reservoirs demonstrate ~ 25% of LMB containing FLMB alleles and appear to be moving towards the management goal of 40% established by the AGFC.

Key words: Florida largemouth bass, introgression, stocking success

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The goals of stocking gamefishes typically fall into one of three general categories: supplemental stocking (Boxrucker 1986, Buynak and Mitchell 1999, Mesing et al. 2008), controlling the abundance of another species (Meronek et al. 1996), or the introduction of a new species into a system (Pelzman 1980, Erdman 1984). However, Florida largemouth bass (FLMB; *Micropterus salmoides floridanus*) are commonly stocked into systems maintaining extant northern largemouth bass (NLMB; *M. s. salmoides*) populations to alter the genetic composition of the population due to the greater trophy fishery potential of the FLMB and resultant intergrades (Chew 1975, Gilliland and Whitaker 1990, Forshage and Fries 1995, Noble 2002, Lutz-Carrillo et al. 2006).

To this end, the Arkansas Game and Fish Commission (AGFC) currently stocks FLMB into 15 reservoirs. Typically, FLMB are stocked to impact an entire lake population, but AGFC has had mixed results for achieving this goal (Allen et al. 2009). Impacting the genetic composition of the entire largemouth bass (LMB) population in large reservoirs is both costly and difficult, requiring large numbers of stocked fish over a long time period to be successful (Forshage and Fries 1995). Stocking protocols that aim to increase the frequency of FLMB genetic influence in small areas within large reservoirs tend to have greater successes (Buckmeier et al. 2003). Two large reservoirs in Arkansas, lakes DeGray and Ouachita, represent daunting tasks for changing the genetic population structure of LMB. Therefore, approximately 100,000 FLMB fingerlings (50 mm) have been stocked annually by the AGFC in arms of Lake DeGray and Lake Ouachita since 2006 and 2007, respectively, to impact the genetic structure of the local, pre-existent NLMB populations. The goal of this action was to cause 40% of the localized LMB to possess FLMB alleles by the end of the eight-year stocking regimen. This 40% goal was established based upon previous stocking successes of FLMB for other Arkansas reservoirs. To measure progress toward this management goal, the objectives of this study were to 1) estimate the percent contribution of stocked LMB, and 2) measure incorporation of FLMB alleles into local LMB populations through time.

Study Areas and Stocking Protocol

Located in central Arkansas, Lake DeGray and Lake Ouachita are large tributary storage reservoirs, located at 34° N latitude. Lake DeGray was filled in 1972 and covers 5,423 ha; Lake Ouachita was filled in 1953 and covers 16,200 ha. Prior to this study, each reservoir possessed residential populations of NLMB with low levels of FLMB alleles identified (Philipp et al. 1983, Allen et al. 2009).

Because these were large systems, the AGFC opted to attempt to alter the genetics of local LMB populations rather than the entire reservoir. A single embayment approximately 400 ha in area was stocked at a rate of 250 fingerlings (approximately 50 mm total length [TL]) ha^{-1} annually in each reservoir. Fish were stocked by boat along the shoreline in the best available habitat, which consisted of gradually sloping banks with complex structure including the presence of emergent and submersed aquatic macrophytes and flooded terrestrial vegetation in less than 3 m of water.

Methods

Adult LMB were collected from each stocking area within the reservoir in spring using a boom-mounted boat electrofishing unit. Fish were measured (TL, mm), weighed (g), and fin clips were taken and preserved in ethanol for genetic analysis. Largemouth bass collected were of age-appropriate size for the post-stocking samples based on pre-existing AGFC age and growth results from LMB of lakes DeGray and Ouachita (AGFC unpublished data). Pre-stocking collections of fin-clip samples were taken from Lake DeGray during 2006 and 2007 and from Lake Ouachita in 2007 during the initial lake FLMB stocking. Post-stocking sampling of Lake DeGray was conducted in 2009 and 2011, representing three and five years since the beginning of the stocking program, respectively. Post-stocking sampling of Lake Ouachita LMB occurred in 2010, following three years of FLMB stocking. Control samples of NLMB and FLMB were also obtained from AGFC broodstock populations to provide baseline genetic samples of both subspecies.

Genomic DNA was extracted from 20- to 25-mg tissue samples of each individual using a modified version of the chloroform tris-acetate borate extraction method (Allen et al. 2009). Genotypes were amplified using seven microsatellite loci: *Mdo*003, *Mdo*006, *Msa*021, *Lma*007, *Lma*12, *Msa*13, and *Msa*29 (Colbourne et al. 1996, DeWoody et al. 2000, Malloy et al. 2000), with PCR specifications outlined by Lutz-Carrillo et al. (2006). Microsatellite primers were synthesized with distinct fluorescent tags (Integrative DNA Technologies, Coralville, Iowa), specific for capillary electrophoresis using a Beckman-Coulter CEQ8000 Genetic Analysis System (Beckman-Coulter Inc., Fullerton, California). Fragment sizes were determined by the internal CEQ8000 analysis program using a 400-bp standard, and were manually confirmed.

Allele frequencies were calculated for each locus and alleles were determined to be exclusive to FLMB, NLMB, or shared between sub-species (mixed) using the hatchery samples as controls (Allen et al. 2009). Admixture proportions (q) were then calculated for each individual and population samples as described below.

The software program STRUCTURE 2.3 (Hubisz et al. 2009) was first used in an admixture model with correlated allele frequencies and default settings to establish pure sub-species lines and their intergrades (50,000 burn-in steps; 500,000 MC/MC steps). The number of clusters (k) has previously been analyzed for LMB within many Arkansas reservoirs, including populations in lakes DeGray and Ouachita, and was identified as 2, consistent with the two sub-species of study (Allen et al. 2009). The result of admixture analysis was a statistical value for the individual admixture proportion (q; 1 = NLMB; 0 = FLMB) of each individual. Individual admixture proportions were used to classify individuals as either pure sub-species or intergrade, 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 NLMB, whereas individuals with $q \leq 0.05$ were classified as pure FLMB. All broodstock controls were within this threshold and distinguished as pure sub-species. Individuals having intermediate q-values were classified as intergrade LMB (F_x-NLMB, F_1, and F_x-FLMB), as described below.

To further resolve LMB 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 sub-species, or 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 phenotype or pure NLMB phenotype, respectively). Second, the analysis generated probabilities that intergrade LMB sampled were correctly identified as F_1 or F_x intergrade LMB.

Percent contribution of stocked FLMB was determined by looking at the overall frequency of FLMB relative to post-stocking samples, and also among length groups (< 200 mm, 200–299 mm, 300–399 mm, and ≥400 mm).

Results

Pre-stocking Analysis of LMB Populations

Largemouth bass populations in both reservoirs were characterized by high q-values (Table 1). This was particularly true for the Lake DeGray sample, with a mean q-value of 0.990, consistent with a NLMB population, and phenotype proportions showed that almost all LMB (96.7%) were identified as NLMB (Table 2). In contrast, while the Lake Ouachita sample also had a high *q*-value (0.958), a greater proportion were characterized as F_x-NLMB (17.5%), with one third of F_x-NLMB having *q*-values >0.90. Neither lake sample had any FLMB or F_x-FLMB phenotypes identified.

Post-stocking Analysis of LMB Populations

Admixture proportions post-stocking showed high *q*-values, indicative of a predominance of NLMB alleles. Florida LMB *q*-values increased by 2.0% per year for Lake DeGray LMB, whereas rates of change were slightly lower for Lake Ouachita LMB at ~1.5% per annum.

Although most LMB were categorized as NLMB phenotypes in post-stocking samples in both reservoirs (Table 2), all phenotypes (NLMB, F_x-NLMB, F_1, F_x-FLMB and FLMB) were identified in post-stocking samples. Although there was a reduction in the proportion of FLMB in the 2011 Lake DeGray sample, F_1 LMB increased to 10% of all phenotypes. The proportion of F_1 LMB was unchanged in the 2010 Lake Ouachita sample relative to the pre-stocking sample. Percent contribution of FLMB (and therefore stocked FLMB) ranged from 2%–3% of the total sample among lake populations (Table 3). There were no observable trends in frequency of FLMB among length groups. Most F_1 LMB were primarily age-1 LMB (<200 mm) in the 2009 Lake DeGray sample, but by the 2011 sample, F_1 individuals were more common throughout the length groups studied. Within the 2010 Lake Ouachita LMB sample, F_1 individuals were mostly <200 mm TL, and therefore recent progeny of stocked FLMB and resident NLMB.

Discussion

Despite concerted and continuous stocking of FLMB within tributary embayments of large reservoirs, incorporation of FLMB and their alleles as demonstrated by *q*-values into the existing LMB population was consistently low in all samples. However, the large increase in the proportion of intergrades into the existing DeGray LMB population was encouraging (22% increase after five years). Stocking densities used for the present study were much greater than typical stocking densities used in other studies (e.g., 10–41 fish ha^{-1}, Buckmeier and Betsill 2002; 15–60 fish ha^{-1}, Mesing et al. 2008; except see Boxrucker 1986, 450 fish ha^{-1}). Because age-0 LMB relate to shoreline structure (Irwin et al. 1997), determination of stocking densities must take into account the topography of individual lakes. Both lakes DeGray and Ouachita have high shoreline to surface area ratios in the study areas, and thus optimal stocking densities (on a per hectare basis) may have been greater in these systems compared to a less dendritic lake.

Contribution levels of stocked LMB vary widely among studies,

Table 1. Admixture proportions (*q*-values) for each sample as determined by *STRUCTURE* where *q* = 1.00 for NLMB and 0.00 for FLMB. DeGray (2006–2007) and Ouachita (2007) represent pre-stock samples.

	FLMB	NLMB
Population	**1 – *q***	***q***
Hatcheries		
Hulsey	0.998	0.002
Joe Hogan	0.004	0.996
William Donham	0.011	0.989
Lakes		
DeGray (2006–2007)	0.010	0.990
DeGray (2009)	0.061	0.939
DeGray (2011)	0.115	0.885
Ouachita (2007)	0.042	0.958
Ouachita (2010)	0.085	0.915

Table 2. Proportions of bass phenotypes for lakes DeGray and Ouachita as determined by STRUCTURE. DeGray (2006–2007) and Ouachita (2007) represent pre-stock samples.

Lake	*n*	NLMB	F_x-NLMB	F_1	F_x-FLMB	FLMB
DeGray (2006–2007)	90	0.967	0.033	0.000	0.000	0.000
DeGray (2009)	376	0.886	0.053	0.027	0.005	0.029
DeGray (2011)	450	0.736	0.118	0.102	0.029	0.016
Ouachita (2007)	200	0.820	0.175	0.005	0.000	0.000
Ouachita (2010)	471	0.788	0.155	0.004	0.017	0.036

Table 3. Proportion of bass phenotypes following the stocking of FLMB by length group as determined by STRUCTURE.

Lake	*n*	NLMB	F_x-NLMB	F_1	F_x-FLMB	FLMB
DeGray (2009)	371	0.895	0.040	0.030	0.005	0.030
< 200 mm	27	0.852	0.000	0.111	0.000	0.037
200–299 mm	135	0.941	0.044	0.000	0.007	0.007
300–399 mm	176	0.869	0.040	0.045	0.006	0.040
≥ 400 mm	33	0.879	0.061	0.000	0.000	0.061
DeGray (2011)	450	0.736	0.118	0.102	0.029	0.016
< 200 mm	33	0.788	0.061	0.121	0.030	0.000
200–299 mm	141	0.730	0.142	0.085	0.021	0.021
300–399 mm	174	0.707	0.132	0.115	0.034	0.011
≥ 400 mm	102	0.775	0.078	0.098	0.029	0.020
DeGray Totals	821	0.807	0.085	0.067	0.019	0.022
< 200 mm	60	0.822	0.033	0.108	0.017	0.020
200–299 mm	276	0.834	0.093	0.043	0.015	0.015
300–399 mm	350	0.790	0.086	0.080	0.019	0.025
≥ 400 mm	135	0.804	0.076	0.067	0.022	0.031
Ouachita (2010)	471	0.788	0.155	0.004	0.017	0.036
< 200 mm	15	0.667	0.200	0.067	0.000	0.067
200–299 mm	161	0.739	0.186	0.006	0.031	0.037
300–399 mm	295	0.820	0.136	0.000	0.010	0.034
≥ 400 mm	0	N/A	N/A	N/A	N/A	N/A

with extremes of limited contribution (Buckmeier and Betsill 2002, Hoffman and Bettoli 2005, Diana and Wahl 2009), similar to our findings, to upwards of 60%–70% in other reservoirs (Boxrucker 1986, Hoxmeier and Wahl 2002). In a review of literature by Heitman et al. (2006), median contributions of stocked LMB by age-1 fish was 10% in southeast and southcentral reservoirs of the United States, which was much greater than that observed in our study. Buckmeier et al. (2003) found that percent contribution of FLMB stocked in a large Texas reservoir was density-dependent, with 10,000 fingerlings stocked per 2 km site to be most cost-effective (5.4% contribution after five months); however, a 10-fold increase in stocking rates tripled the contribution of FLMB.

Straightforward comparisons of success rates among studies of stocking densities relative to success rates are difficult. Reasons provided for variation of stocking success are wide-ranging, including immediate stocking mortality from a lack of tempering or stocking during temperature extremes (Wahl et al. 1995, Buckmeier and Betsill 2002), a lack of feeding by newly stocked fish (Heidinger and Brooks 2002, Porak et al. 2002), competition with native stock (Buynak and Mitchell 1999, Hoxmeier and Wahl 2002), predation and/or cannibalism (Buckmeier et al. 2005, Hoffman and Betolli 2005), overwinter mortality due to insufficient growth of stocked LMB to achieve piscivory (Timmons et al. 1980, DeAngelis and Coutant 1982, Olson 1996), and fitness of stocked fish (Neal and Noble 2002). Previous studies of Arkansas reservoir LMB populations have identified no consistent trends regarding differences in survival, condition, or growth parameters among NLMB, FLMB, or intergrade phenotypes (Johnson and Fulton 1999, Allen et al. 2009, Allen and Johnson 2009). Competition with wild fish and mortality of stocked FLMB were not directly studied in the present study, but need to be considered during future stocking efforts. Later generational intergrades became common following the fifth year of stocking FLMB in Lake DeGray, indicative of successful reproduction of stocked FLMB. Another explanation for low levels of FLMB is the emigration of fish once stocked; however, several studies have demonstrated that dispersal of LMB following stocking is low, typically with distance limits of about 1 km (Noble et al. 1994, Buckmeier and Betsill 2002, Hoffman and Bettoli 2005).

Lakes DeGray and Ouachita represent recent stocking efforts of FLMB. Fifteen Arkansas reservoirs of varying size have been stocked with FLMB for decades to impact entire lake populations having extant NLMB populations with varying degrees of success (Allen et al. 2009). Proportions of FLMB in those Arkansas lakes stocked intensively and repeatedly ranged from 2% to 35%. Even lakes stocked solely with FLMB after rotenone fish kills or after reservoir construction did not show 100% FLMB phenotypes. The presence of NLMB and their intergrades in these reservoirs were attributed to incomplete fish kills, introduction of NLMB from feeder streams, or flooding of adjacent streams (Allen et al. 2009, Allen and Johnson 2009).

Johnson and Fulton (1999) and Dumont and Lutz-Carrillo (2011) found no differences in distributions of phenotypes among age groups for Arkansas and Texas reservoirs, respectively, indicating a lack of long-term survival advantage for one phenotype over another. In contrast, Maceina et al. (1988) and Brown and Murphy (1994) hypothesized a greater long-term survival of introgressed phenotypes over NLMB in a Texas reservoir. Additionally, trophy LMB management programs of southern states have indicated that most trophy LMB genetically tested have contained high proportions of FLMB alleles, thus survival was apparently not a limiting factor among stocked FLMB or resultant intergrades (Horton and Gilliland 1993, Lutz-Carrillo et al. 2006, R. L. Johnson, Arkansas State University, unpublished data).

In conclusion, although stocking success of FLMB into these two lake populations has been low to date, particularly for Lake Ouachita, the slow but steady increase of FLMB alleles into the LMB population observed in this study is encouraging. Samples to date represent years three and five of the eight-year stocking strategy for both reservoirs. Frequencies of LMB containing FLMB alleles are 21%–26%, and appear to be moving towards the management goal established by the AGFC. As a result of these findings, the AGFC has decided to continue the present stocking plans and regimens of both reservoirs through the remainder of the study. Although both lake samples represent an extensive number of fish sampled, ongoing sampling of both lake LMB populations will provide greater insight into the population dynamics of varying phenotypes. The outcomes of this study will enable us to effectively determine the potential of locally impacting genetic structure in other large Arkansas reservoirs.

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