Genetic Effects of Florida Largemouth Bass Introductions into Selected Texas Reservoirs

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Abstract: Florida largemouth bass (*Micropterus salmoides floridanus*) have been stocked extensively into Texas reservoirs containing the northern largemouth bass (*M. s. salmoides*) subspecies, and knowledge of the genetic make-up of these potentially intergraded populations is important to their continued management. Bass populations from 19 such reservoirs were analyzed by electrophoretic determination of individual fish genotypes. As measured by variation at 3 loci, intergradation ranged from 2% to 92%. Variations in percent intergradation among reservoirs were related to individual reservoir conditions and Florida largemouth bass stocking histories. Regression analysis suggested that repeated annual stocking of Florida largemouth bass had the greatest effect on the frequency of Florida largemouth bass genes. Water clarity and total number of Florida largemouth bass stocked were correlated with intergradation rate, but did not explain significant additional variation beyond that due to number of years stocked.

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The largemouth bass (*Micropterus salmoides*) is one of the most widely-distributed species of fish in the United States. It is intensively managed as both a sport and predator species. To enhance existing bass populations by increasing genetic diversity, Florida largemouth bass (*M. s. floridanus,* hereafter referred to as FLMB) have been stocked since 1972 by the Texas Parks and Wildlife Department into more than 170 public reservoirs containing endemic populations of northern largemouth bass (*M. s. salmoides,* hereafter referred to as NLMB). Many reservoirs have received a single FLMB stocking while others have received as many as 6 annual

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stockings. Size of fish stocked has included fry, 2- to 8-cm fingerlings, and adults. Stocking rates have varied from <0.5 to >40.0 fish/hectare for fingerlings and adults, as well as fry stockings of >400 fish/hectare.

This widespread stocking of FLMB into Texas waters with existing NLMB populations has resulted in the potential for interaction of 2 previously isolated gene pools. Genetic differences exist between the subspecies, and thus, individual fish can be identified using electrophoresis on the basis of different enzyme patterns at certain gene loci (Philipp et al. 1981). A genetic evaluation of these potentially intergraded Texas populations is necessary to evaluate the effect of FLMB introductions on NLMB populations and to direct future stocking practices. This evaluation can provide indications as to which reservoir types and stocking techniques are most suitable for FLMB introductions, both in terms of establishment of FLMB and maximizing intersubspecific hybridization, which results in increased heterosis within the bass population. Knowledge of the genetic evolution of mixed stocks of largemouth bass will be necessary for the development of genetically-sound stocking programs. In this study, we related variations in the percent of subspecific intergradation between reservoirs to the diversity of FLMB stocking techniques and reservoir types, in order to identify those parameters that might enhance the frequency of intergradation.

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Methods

Nineteen public Texas reservoirs that had received 1 or more stockings of FLMB were sampled. Although no genetic analyses were performed prior to FLMB introductions, each of these reservoirs contained previously established populations of NLMB when FLMB were stocked. Reservoirs included in this study were chosen to represent a wide range of FLMB stocking strategies and reservoir conditions.

Largemouth bass were collected from several sites in each reservoir from September through December 1983 by electrofishing, seining, and spot-rotenone treatment. Spring sampling was avoided to eliminate bias due to differences in spawning times of the 2 subspecies. The sampling goal at each reservoir was 30 young-of-the-year (Age 0) and 30 yearling (Age I) fish. Fish were placed on ice immediately after collection and either processed at the reservoir or transported to laboratory facilities. Total length (mm) and weight (g) were recorded for each fish. Scales for age determination were taken from the left side of the fish below the lateral line, near the tip of the pectoral fin. Livers were excised from the fish, placed in pre-numbered vials, frozen, and transported to the electrophoresis laboratory where they were stored at −40° C.

Liver samples were individually prepared for electrophoretic analysis by homogenizing in an equal volume of Tris-HC1 buffer (pH 7.0) and centrifuging for 15 minutes at 12,500 rpm and 4° C. Supernatants were refrozen at −40° C prior to subsequent evaluation. Horizontal starch gel electrophoresis was performed using the techniques of Selander et al. (1971), as modified by Harvey et al. (1980). Three diagnostic enzyme loci were evaluated: isocitrate dehydrogenase (IDH), glutamate-oxaloacetate transaminase (GOT-1) and tetrazolium oxidase (TO). With 1 exception, designation and description of allozymes (alleles) at the 3 diagnostic loci followed the description of Harvey (1983). While Harvey (1983) noted only 3 allozymes at the GOT-1 locus, Philipp et al. (1982) reported 4. A fourth allozyme, intermediate between GOT-1-100 and GOT-1-135, was also noted in the present survey and designated as GOT-1-115. Both the GOT-1-100 and GOT-1-115 allozymes were unique to NLMB.

On the basis of allozyme occurrences at the three diagnostic loci, individual fish were designated at 1) NLMB, if they exhibited unique NLMB allozymes at the IDH and GOT-1 loci, 2) F_1, if they were heterozygous for NLMB and FLMB allozymes at both the IDH and GOT-1 loci, 3) F_x, if they were heterozygous for at least 1 but not all of the 3 loci (indicating an intergrade other than an F_1 cross) or 4) FLMB, if they exhibited unique FLMB allozymes at IDH and GOT-1. The 2 allozymes present at the TO locus were of limited diagnostic value because both subspecies carry one of the allozymes, while the second allozyme is unique to FLMB, but only occurs with moderate frequency.

Scales collected from each fish were pressed into acetate slides and annuli counted. Errors in field determination of age frequently resulted in small sample size of Age I fish and numerous Age II fish. Consequently, Age I and Age II fish were combined into 1 group for all subsequent analyses. Fish older than Age II were excluded from all analyses.

For each reservoir sample, the percentage of NLMB, F_1, F_x, and FLMB was determined for Age 0 and Age I–II groups. To determine whether Age 0 and Age I–II groups could be combined for further analyses, it was necessary to test for differences in percent intergradation (combining F_1 and F_x individuals) between age groups in each reservoir, using Wilcoxon's signed-ranks text.

Simple linear correlation and stepwise multiple regression were used to relate reservoir conditions and FLMB stocking histories (Table 1) to percent intergradation in each reservoir. Values for many of the variables were obtained from hatchery stocking records and management reports. An attempt was made to obtain data for the year preceding first FLMB stocking in each reservoir; however, in many situations this was not possible and the closest year's data were used.

All statistical analyses performed in the study were accomplished with SAS (Stat. Anal. System, SAS Institute, Inc. 1982). Probabilities less than 0.05 ($P < 0.05$) were considered significant. Transformation of proportion values for para-

Table 1. Variables used in simple correlation and multiple regression analyses for largemouth bass populations from 19 Texas reservoirs, with abbreviations and corresponding units of measure. FLMB = Florida largemouth bass and NLMB = northern largemouth bass.

Variable	Abbreviation	Unit of measure
Intergradation	INT	Percent
Size of FLMB stocked	SZ	Centimeters
Mean annual FLMB stocking rate	RT	Number/hectare
Number of years stocked with FLMB	YR	Years
Time since last FLMB stocking	TM	Years
Age of reservoir when first stocked with FLMB	AG	Years
Standing crop of NLMB	SC	Number/hectare
Whether a power plant reservoir	PW	0 = yes; 1 = no
Secchi disk transparency	SD	Meters

metric analyses was not done since a plot of the distribution of percent values in this data set revealed normal distributions and 2 transformations (logarithmic and arcsine-square root) did not improve distributions. These transformations likewise had little effect on any of the independent variables, so these variables were also used in analyses without transformation.

For this study, we assumed that no significant FLMB genetic influence was present in Texas reservoirs prior to introductions by the Texas Parks and Wildlife Department. Also, because of FLMB broodstock contamination at state hatcheries (Harvey et al. 1980), some stockings may have included intergrade fish.

Results and Discussion

Genotype Data and Age Group Comparisons

FLMB allozymes were found in all 19 reservoir populations examined (Table 2). In 13 of the reservoirs, FLMB genotypes were noted, while in all reservoirs NLMB and intergrade genotypes were present. Percent intergradation in the reservoirs (age groups combined) ranged from 2% to 92%, with 10 reservoirs exhibiting 50% intergradation or higher.

Determination of differences in percent intergradation between age groups (Table 3) was conducted after excluding Long, Striker, Twin Buttes, and Winnsboro reservoirs because of small sample size ($N < 10$) of either age group. Analysis for the remaining 15 reservoirs indicated no significant difference in percent intergradation between Age 0 and Age I–II fish. Since reservoirs differed in their year of last FLMB stocking, differences in percent intergradation between age groups were plotted against number of years since last stocking, but no obvious trend was evident. This indicated results of the signed-ranks tests were not confounded by the influence of temporal differences in FLMB stocking. Thus, data from Age 0 and Age I–II groups were combined for all further analyses.

Table 2. Percent parental genotypes and percent intergradation for Age 0, I and II combined, for largemouth bass from 19 Texas reservoirs, 1983.

Reservoir	*N* fish	% NLMB[a]	% FLMB[b]	% intergradation
Benbrook	48	8.3	0.0	91.7
Brownwood	62	29.0	3.3	67.7
Buchanan	48	77.1	0.0	22.9
Conroe	63	61.9	1.6	36.5
Graham	59	76.3	0.0	23.7
Halbert	60	55.0	1.7	43.3
Houston County	58	5.2	12.0	82.8
Long	40	20.0	5.0	75.0
Medina	58	5.2	6.9	87.9
Mexia	60	48.3	1.7	50.0
Murvaul	56	37.5	1.8	60.7
Nasworthy	60	80.0	0.0	20.0
Quitman	56	73.2	1.8	25.0
Striker	39	43.6	7.7	48.7
Texoma	59	86.4	0.0	13.6
Twin Buttes	51	23.5	11.8	64.7
Tyler East	61	44.3	4.9	50.8
Winnsboro	57	45.6	1.8	52.6
Wright Patman	59	98.3	0.0	1.7

[a]Northern largemouth bass (*Micropterus salmoides salmoides*).
[b]Florida largemouth bass (*M. s. floridanus*).

Table 3. Percent subspecific intergradation for Age 0 and Age I–II largemouth bass from 19 Texas reservoirs, 1983.

	Age 0		Age I–II	
Reservoir	*N* fish	% intergradation	*N* fish	% intergradation
Benbrook	31	90.3	17	94.1
Brownwood	31	70.9	31	64.5
Buchanan	35	28.6	13	7.7
Conroe	26	38.5	37	35.1
Graham	30	20.0	29	27.6
Halbert	30	60.0	30	26.7
Houston County	30	73.3	28	92.9
Long	32	75.0	8	75.0
Medina	38	86.8	20	90.0
Mexia	31	67.7	29	31.0
Murvaul	34	52.9	22	72.7
Nasworthy	31	19.4	29	20.7
Quitman	45	26.7	11	18.2
Striker	30	50.0	9	44.4
Texoma	28	14.3	31	12.9
Twin Buttes	2	100.0	49	63.3
Tyler East	37	51.4	24	50.0
Winnsboro	48	54.2	9	44.4
Wright Patman	29	3.4	30	0.0

Factors Related to Genetic Variation

Reservoirs used in this study were characterized by a wide range of physical and biological conditions (Table 4). Impoundments ranged from 6 to 50 years old at time of first FLMB stocking and 3 served as power plant cooling lakes. Mean Secchi disk transparency varied from 0.3 to 2.0 m. Standing crop of NLMB, as estimated from cove rotenone samples, ranged from 9.3 to 170.0 kg/hectare. Stocking rates varied from 0.04 to 33.60 FLMB/hectare; lowest rates were used when large fish were stocked. Reservoirs had been stocked annually for 1 to 4 years, and time from last FLMB stocking to genetic sampling was at least 3 years.

Simple correlations between percent intergradation and each of the 8 independent variables listed in Table 1 showed significant (positive) correlations with 2 variables, YR and SD (Table 5). The variables YR and SD were also correlated with each other ($r = 0.45$, $P = 0.05$).

Because RT was calculated as an average annual stocking rate for reservoirs receiving multiple year stockings, a separate variable, YR × RT, was generated to reflect the total number of FLMB stocked in each reservoir. Simple correlation analysis between YR × RT and percent intergradation revealed a significant correlation ($r = 0.47$, $P = 0.04$). This significance was intermediate between that of YR with percent intergradation ($r = 0.61$, $P = 0.01$), and that of RT with percent intergradation ($r = 0.31$, $P = 0.19$). This suggests repetitive stocking is more important in establishing FLMB than the total number stocked. Due to annual variations

Table 4. Values for physical and biological variables[a] used to describe 19 Texas reservoirs in simple correlation and multiple regression analyses.

	Variables								
Reservoir	INT	SZ	RT	YR	TM	AG	SC	PW	SD
Benbrook	91.7	3.18	15.00	2	7	23	63.6	1	0.85
Brownwood	67.7	2.54	15.80	4	5	42	63.9	1	1.43
Buchanan	22.9	3.81	5.30	1	5	45	34.4	1	0.71
Conroe	36.5	5.10	10.50	1	4	6	83.4	1	1.29
Graham	23.7	3.81	6.90	1	4	21	27.6	0	0.38
Halbert	43.3	3.18	17.80	2	8	49	76.1	1	0.38
Houston County	82.8	2.54	20.60	3	6	13	168.8	1	1.58
Long	75.0	5.10	5.70	2	3	14	50.2	1	0.90
Medina	87.9	2.54	5.70	3	5	63	40.5	1	2.11
Mexia	50.0	2.54	27.50	3	6	13	15.8	1	0.30
Murvaul	60.7	34.29	0.04	2	3	13	170.0	1	0.82
Nasworthy	20.0	3.81	2.00	1	3	50	9.3	0	0.37
Quitman	25.0	34.29	0.16	1	3	18	114.9	1	1.03
Striker	48.7	3.81	33.60	1	7	19	44.1	0	1.19
Texoma	13.6	2.54	0.80	2	6	31	48.2	1	1.30
Twin Buttes	64.7	2.54	8.50	4	5	13	62.8	1	1.33
Tyler East	50.8	34.29	0.40	1	4	11	143.7	1	0.81
Winnsboro	52.6	3.81	20.20	1	9	12	55.1	1	1.01
Wright Patman	1.7	3.81	6.10	1	5	22	58.3	1	0.65

[a] Abbreviations are defined in Table 1.

Table 5. Simple correlations and associated levels of significance of physical and biological variables used to describe 19 Texas reservoirs, with percent intergradation.

Variable[a]	Simple Correlation (r)	Probability (P)
YR	0.61	0.01
SD	0.49	0.03
RT	0.31	0.19
PW	0.30	0.21
SC	0.28	0.25
TM	0.18	0.45
SZ	−0.07	0.78
AG	−0.04	0.86
YR×RT	0.47	0.04

[a]Abbreviations are defined in Table 1.

in factors affecting survival, repeated annual stockings of FLMB should increase chances of establishing FLMB genes in subsequent generations. Therefore, it would be preferable to stock a given number of FLMB in smaller units over several years than to stock them all at one time.

When the 8 independent variables were subjected to stepwise multiple regression analysis with percent intergradation as the dependent variable, only YR yielded a significant regression coefficient. However, the overall model was significant ($R^2 = 0.53$, $P = 0.02$) with the addition of 3 more variables as follows:

$$\text{INT} = -0.019 + 0.007\text{RT} + 0.108\text{YR} + 0.130\text{SD} + 0.001\text{SC}$$

Significance of YR makes biological sense. As discussed above, chances of genetic effects resulting from FLMB influence are increased by the implementation of multiple stockings. In conjunction with this, RT appeared in the model, although it did not produce a significant regression coefficient. The inclusion of both YR and RT in the model reveals an important management concept. Since hatcheries often have a limited number of FLMB for stocking, fishery managers must consider the benefit of stocking a few reservoirs at an increased rate and concentrating stocking efforts at these same few reservoirs for several years, versus stocking several different reservoirs at lesser rates.

Inclusion of SD in the model was somewhat surprising because of the intercorrelation of this variable with YR ($r = 0.45$, $P = 0.05$). However, inclusion of SD in the model may suggest clearer reservoirs produce more highly intergraded bass populations. Work done by 1 of the authors in private ponds in Texas has indicated clearer water is more conducive to FLMB survival.

An ecological factor often associated with hybridization frequency is a disparity in numbers of 1 species (or subspecies) versus the other (Hubbs 1955) and this may explain the inclusion of SC as a positive factor in the model. Individuals of the rare species (in this case, the FLMB subspecies) are not as likely to encounter proper mates and therefore tend to hybridize. Of course, if the difference in numbers is too great, gene flow from the rare type would be negligible.

The fact that YR was the only variable in the multiple regression model with a significant regression coefficient limits inferences relative to other variables in the model. Intercorrelation between YR and other variables also affects their probability of significance in the model, and limits conclusions about the direct effect of YR on INT. Consequently, inferences about any variables, including YR, should be interpreted with caution and be considered primarily as bases for further hypotheses.

The present electrophoretic evaluation of largemouth bass from 19 Texas reservoirs has demonstrated conclusively that stocking FLMB into existing NLMB populations can affect the genetics of NLMB. Results suggest, within the ranges of conditions evaluated, a sound stocking strategy is more important than physical parameters of reservoirs in the establishment of FLMB genes. However, the influence of other variables was important and contributed to the overall effectiveness of the model. All variables chosen for this analysis could, and probably do, affect survival and/or reproductive contribution of introduced FLMB.

Numerous factors not included in the present analysis could also greatly affect the success of FLMB introductions into Texas reservoirs. Data on many potentially important variables were not available for this study, thereby precluding their use in our analysis. Also, because values for Secchi disk transparency and standing crop of NLMB were not always available for the exact year or years each reservoir was stocked with FLMB, data from up to 3 years prior to or following stocking were used. Despite these limitations, such data were useful in providing direction for future research.

Several general management recommendations can be suggested from the results of the present study. In addition to the importance of stocking the same reservoirs for several years to ensure FLMB survival, the failure of SZ to yield a significant regression coefficient suggests stocking small fingerlings or fry may be successful in the establishment of FLMB. State hatcheries could reduce expenses by producing smaller fish for stocking programs. Also, although AG did not yield a significant regression coefficient, the negative correlation of this variable with percent intergradation conforms to the currently-held management belief that FLMB establishment is more successful in newer reservoirs.

In addition to their use in choosing stocking strategies, the baseline electrophoretic data generated in this study will also prove valuable for future evaluation of genetic changes within these reservoir bass populations, which must be monitored if future genetic impacts are to be used in management.

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