Introgression of Florida Largemouth Bass Introduced into Northern Largemouth Bass Populations in Oklahoma Reservoirs[1]

Eugene R. Gilliland, *Oklahoma Fishery Research Laboratory, 500 E. Constellation, Norman, OK 73072*

Julianne Whitaker, *Oklahoma Fishery Research Laboratory, 500 E. Constellation, Norman, OK 73072*

Abstract: Populations of largemouth bass (*Micropterus salmoides*) in Oklahoma reservoirs were sampled in 1986 and 1987 to determine the degree of introgression of Florida largemouth bass (*M. s. floridanus*), introduced over a 16-year period, into populations of native northern largemouth bass (*M. s. salmoides*). Florida subspecific alleles were present in 28 of 30 populations (93%) and were found in >50% of the fish from 8 (27%) reservoirs. Correlations with selected physical and biological parameters indicated that the percentage of bass with Florida alleles increased as the size of fish stocked increased and as cold weather and water level fluctuation decreased. Reservoirs in southern portions of the state with stable water levels that were stocked with fingerlings >100 mm in length at rates >25/ha for several consecutive years had the highest degrees of Florida allele introgression into existing bass populations.

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 43:182–190

Florida largemouth bass (FLMB) were introduced into 82 Oklahoma reservoirs between 1970 and 1986 to increase overall bass growth rates, provide larger trophy fish, and to hybridize with native northern largemouth bass (NLMB) to pass on those qualities to intergrade offspring (Bottroff and Lembeck 1978, Pelzman 1980, Mulford 1984). Although survival of introduced FLMB in Oklahoma varied among previous studies (Rieger and Summerfelt 1976, Nieman and Clady 1979, Wright and Wigtil 1980, Harper 1984, Mauck 1984), each reported that growth was equal or superior to that of NLMB and that the Florida sub-species had merit in Oklahoma fishery management programs.

The Oklahoma Department of Wildlife Conservation (ODWC) obtained FLMB

---

[1] Contribution No. 213 of the Oklahoma Fishery Research Laboratory, a cooperative unit of the Oklahoma Department of Wildlife Conservation and the University of Oklahoma Biological Survey.

broodstock in 1970, 1975, and 1979 from Florida through the U.S. Fish and Wildlife Service or from the Texas Parks and Wildlife Department (TPWD). Genetic contamination found in some FLMB stocks in Texas, other southeastern United States hatcheries (Harvey et al. 1980, Phillip et al. 1983), and at the ODWC Durant hatchery (Gilliland and Whitaker 1989) created doubt about the genetic integrity of the fingerlings supplied for past research and management stockings. Stock contamination and/or sub-specific misidentification, as opposed to environmentally or genetically based differences, may explain the varied results seen in FLMB research in Oklahoma and elsewhere.

Understanding the dynamics of FLMB introgression into NLMB populations is essential for the development of sound hatchery, stocking, and management policies. Electrophoretic capabilities were developed at the Oklahoma Fishery Research Laboratory (OFRL) in 1985 to aid in a survey of Oklahoma reservoirs to determine the impact FLMB and hybrid largemouth bass stockings had on native largemouth bass population genetic structure. The objective of this study was to relate variations in the frequency of largemouth bass expressing Florida sub-specific alleles to physical and biological parameters in Oklahoma reservoirs.

We thank ODWC fishery management, hatchery, and research personnel for field and laboratory assistance; and W. Harvey (TPWD), D. Phillip (Ill. Nat. Hist. Surv.), and K. Kulzer (formerly TPWD) for advice on electrophoretic technique. This research was funded through Oklahoma Federal Aid to Sport Fish Restoration Project F–39-R.

Methods

Samples of 20 to 80 age–0 largemouth bass were collected from each of 55 Oklahoma reservoirs that had received FLMB introductions between 1970 and 1986. Fish were collected by electrofishing in the fall and spring of 1986 and 1987. Age–1 and age–2 bass were collected to provide adequate sample sizes when sufficient age–0 fish were unavailable. Sampling was done in areas away from stocking sites to reduce possible bias from collection of recently stocked fish. All bass were placed on ice in the field and returned to the OFRL where otoliths were removed for age determination and samples of liver tissue were excised for electrophoretic analysis.

Electrophoresis was conducted by using horizontal starch gel procedures outlined by Selander et. al. (1971) and Harvey (1983). Gels were stained and banding patterns interpreted for 2 diagnostic enzyme-coding loci following Phillip et al. (1982): AAT-B and IDH-B. Electrophoretic phenotypes were assigned as (1) NLMB if only northern sub-specific alleles were expressed, (2) FLMB if they carried only Florida sub-specific alleles, (3) F_1 intergrades (first generation hybrid NLMB x FLMB) if sub-specific heterozygosity was present at both the AAT and IDH loci, and (4) F_x intergrades (second or subsequent generation hybrids) if sub-specific heterozygosity was present at only 1 locus.

Thirty lakes which had samples of at least 30 electrophoretically scoreable fish of any age were used in further analyses. Percentages of each phenotype and the percentage of total bass with any Florida alleles (TBFA) were calculated for each age group in each reservoir (Table 1). No differences in the phenotypic percentages of fish age were detected (signed rank paired test, SAS Inst. Inc. 1987), so age classes within populations were combined. Percent TBFA provided an index of total FLMB introgression into a population and the percent F_1 phenotypes gave an estimate of the potential each population had to produce trophy (>3.6 kg) bass (80% of the trophy bass examined from Oklahoma reservoirs have been identified as F_1 individuals: Gilliland and Whitaker 1989).

Pearson's correlation coefficient was used to investigate linear relationships among 15 independent variables, consisting of 6 reservoir physical characteristics and 9 fishery parameters (Table 2). Stepwise multiple regression analysis (SAS

Table 1. Phenotypes of Largemouth bass collected during 1986 and 1987 from 30 Oklahoma reservoirs stocked with Florida Largemouth bass between 1970 and 1986, showing: scoreable sample size (*N*); percent (%) of northern (NLMB), Florida (FLMB), and intergrade Largemouth bass (F_1 INTLMB, F_X INTLMB, and total INTLMB), and total bass with Florida alleles (TBFA).

Reservoir	*N*	NLMB (%)	FLMB (%)	Intergrade (%)F_1	(%)F_X	(%)INT	TBFA (%)
Arbuckle	49	96	0	0	4	4	4
Atoka Bluestem	30	83	0	0	17	17	17
Broken Bow	45	93	0	0	7	7	7
Clear Creek	35	31	3	23	43	66	69
Crowder	49	94	0	2	4	6	6
Dripping Springs	49	41	0	20	39	59	59
Ellsworth	36	64	5	8	23	31	36
Eufaula	40	95	5	0	0	0	5
Fort Gibson	35	89	0	3	8	11	11
Fuqua	40	48	0	13	40	53	53
Grand	44	93	0	0	7	7	7
Healdton	35	31	0	20	49	69	69
Hudson	45	95	0	0	5	5	5
Hugo	35	94	0	0	6	6	6
Humphreys	40	38	3	20	40	60	63
Jap Beaver	56	84	7	2	7	9	16
Kerr	45	91	0	4	5	9	9
Lawtonka	31	68	0	10	22	32	32
Murray	30	33	0	17	50	67	67
Oologah	35	94	0	0	6	6	6
Pine Creek	52	92	0	0	8	8	8
Sardis	35	34	6	23	37	60	66
Sooner	43	51	2	21	26	47	49
Tenkiller	37	100	0	0	0	0	0
Texoma	77	84	0	7	9	16	16
Thunderbird	44	82	0	5	13	18	18
Vincent	39	87	0	3	10	13	13
Waurika	47	21	0	26	53	79	79
Webbers Falls	45	93	0	0	7	7	7
Wister	35	100	0	0	0	0	0

Table 2. Correlation/regression variables used in analysis of 30 Oklahoma largemouth bass populations sampled in 1986 and 1987.

Physical Characteristics:
LAKE AGE-reservoir age at time of sampling (years)
AREA-reservoir surface area at conservation pool elevation (ha)
DEPTH-reservoir mean depth (m)
SECCHI-mean secchi disc transparency (m)
FLUCT-mean annual water level fluctuation (m)
TEMP-mean number of days with air temperature $\leq 0°C$

Fishery Characteristics:
FIRST-reservoir age at first FLMB stocking (years)
YEARS-number of years stocked with FLMB (years)
LAST-number of years since last FLMB stocking (years)
SIZE-mean size of FLMB stocked (mm)
RATE-mean annual FLMB stocking rates (*n*/ha)
PSD-Proportional stock density of LMB (%)
CFLMB-mean catch per electrofishing hour of LMB (*n*/hour)
FOODAVAL-forage abundance, as the sum of mean catch per electrofishing hour of gizzard shad and bluegill (*n*/hour)

Inst. Inc. 1987) was performed to determine which variables contributed most significantly to the percent TBFA and percent F_1 phenotypes. Significant correlation and regression variables were compared nonstatistically among reservoirs grouped by percent total bass with Florida alleles ($\leq$ 24%, 25%–49%, $\geq$50%). All residuals resulting from multiple regression procedures were found to be normal ($P \geq 0.10$). Probabilities of ≤ 0.05 were considered significant for all other statistical tests.

Results

None of the populations sampled contained exclusively FLMB; conversely, samples from 2 reservoirs (7%) were comprised entirely of NLMB (Table 1). Pure FLMB were found in 7 populations (23%), with percentages in the samples ranging from 2% to 7% (Table 1). Florida alleles were present in 28 populations (93%), with 11 populations (37%) having $\geq$25% of the bass with Florida alleles, and 8 populations (27%) with $\geq$50% of the bass with Florida alleles (Table 1).

Percent TBFA was found to be positively correlated with the size of FLMB fingerlings stocked ($r = 0.42, P = 0.019$) and negatively correlated with water level fluctuation ($r = -0.43, P = 0.019$) and the mean number of days with sub-freezing air temperatures ($r = -0.38, P = 0.037$). Percent F_1 Phenotypes was also positively correlated with the size of fish stocked ($r = 0.38$, $P = 0.038$) and negatively correlated with water level fluctuation ($r = -0.43, P = 0.018$). Percent F_1 Phenotypes, however, was not significantly correlated with the mean number of days with sub-freezing temperatures.

The regression models constructed predicted the percent TBFA would increase, on average, as the size of stocked fish increased and as the number of days below freezing, water level fluctuations, and the food available (based on catch rates of

Table 3. Stepwise multiple regression models explaining variation in percent total bass with Florida alleles (TBFA) and percent F_1 phenotypes constructed from electrophoretic data and 15 variables from 30 Oklahoma reservoir largemouth bass populations sampled in 1986 and 1987.

Model 1-Percent TBFA
%TBFA = 86.9365 − 5.2539 FLUCT − 0.6579 TEMP + 0.2007 SIZE − 9.5606 FOODAVAL
$R^2 = 0.48$; Model $F = 5.84$, $P = 0.0018$; All variables significant at $P < 0.05$

Model 2 − Percent F_1 phenotypes
%F_1 = 33.9160 − 0.1604 LAKE-AGE − 2.5823 FLUCT − 0.2422 TEMP
$R^2 = 0.3676$; Model $F = 5.04$, $P = 0.0069$; FLUCT, TEMP significant at $P < 0.05$.

gizzard shad and bluegill) decreased ($R^2 = 0.48$, $F = 5.84$, $P \leq 0.0018$; Table 3). Percent F_1 phenotypes increased as reservoir age, water level fluctuation, and number of days below freezing decreased ($R^2 = 0.3676$; $R^2 = 0.37$, $F = 5.04$, $P \leq 0.0069$; Table 3).

Mean values of the significant regression and correlation variables among reservoirs grouped by percent TBFA showed that populations with TBFA ≤24% were more likely to be in areas which had a higher mean number of days with subfreezing air temperatures (71 days) than were populations with TBFA ≥50% (59 days; Table 4). Similarly, reservoirs with high water level fluctuation were more likely to have populations with lower percent TBFA (2.7 m and 1.4 m for TBFA ≤24% and ≥50% groups, respectively; Table 4). Reservoirs which had been stocked for more years with FLMB were more likely to have higher percent TBFA (4.2 and 7.5 years for TBFA ≤24% and ≥50% groups, respectively; Table 4). Reservoirs in which larger mean size FLMB fingerlings were stocked were more likely to have higher percent TBFA (47 mm and 106 mm for TBFA ≤24% and ≥50% groups, respectively; Table 4). Differences in mean lake age and stocking rate were less striking. Significance of differences in mean values for temperature, fluctuation, years of stocking, and size of FLMB stocked among percent TBFA groups could not be determined due to small sample sizes and an inability to meet assumptions for statistical testing.

Table 4. Mean values for reservoir variables found to be significant in correlation and regression analyses grouped by percent total bass with Florida alleles (TBFA).

Variable	≤24% TBFA	25%–49% TBFA	≥50% TBFA
FLUCT	2.7 m	1.0 m	1.4 m
TEMP	71 days	74 days	59 days
LAKE AGE	27 years	38 years	21 years
SIZE	47 mm	38 mm	106 mm
RATE	30/ha	21/ha	26/ha
YEARS	4.2 years	5.7 years	7.5 years

Discussion

Physical Parameters Regulating Florida Allele Persistence

The mean number of days with sub-freezing air temperatures at a given location, used as a measure of cold weather, was 1 of the most influential independent variables tested. This variable was highly negatively correlated with percent TBFA, was a significant component of both regression models (Table 3), and was substantially lower for reservoirs with high percent TBFA (Table 4). Florida largemouth bass are less tolerant of rapid water temperature drops such as those associated with strong cold fronts (Cichra et al. 1980, Guest 1982) and prolonged cold periods seen in much of Oklahoma (Johnson 1975, Rieger and Summerfelt 1976, Nieman and Clady 1979). Phillip et al. (1982) found strong evidence that climate (as estimated by latitude) was a key factor in the geographic distribution of northern, Florida, and intergrade largemouth bass, and researchers in Missouri, Ohio, and Michigan have concluded that the Florida sub-species was less fit than the native bass in northern environments (Graham 1973, Stevenson 1973, Latta 1977). Given that populations in Oklahoma with the highest percent TBFA and percent F_1 phenotypes were generally in the southern portion of the state (Fig. 1), the northern latitude at which FLMB survive and contribute to a fishery, may transect Oklahoma.

Water level fluctuation was highly negatively correlated with percent TBFA and percent F_1 phenotypes and was a significant variable of both regression models (Table 3). Populations with high percent TBFA had relatively stable water levels (Table 4). Water levels are known to be critical to successful bass reproduction (Rainwater and Houser 1975) and may have affected the sub-specific composition of the populations. Early spring water level instability would put FLMB at a disadvantage because of their tendency to spawn earlier than NLMB (Maceina et al. 1988). Delays in FLMB spawning would result in more hybridization because of

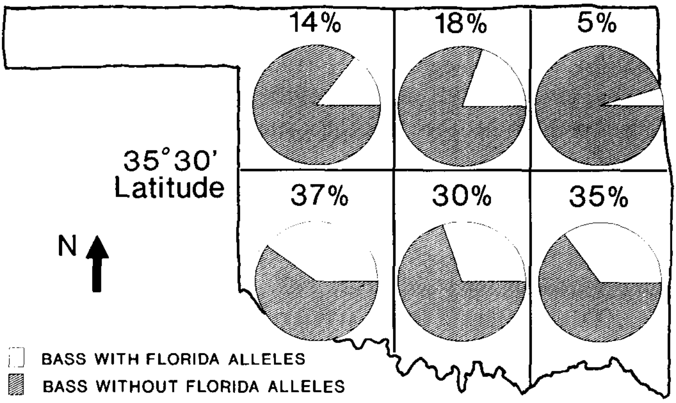

Figure 1. Mean percent total bass with Florida alleles (TBFA) in six geographic regions of Oklahoma calculated from phenotypic data from 30 reservoir populations sampled in 1986 and 1987.

greater overlap with the NLMB spawning period. Childers (1975) noted that maladaptive genes are easily introduced into a population, especially with strains that interbreed readily, but are removed very slowly by natural selection forces. In several populations, stocked FLMB apparently survived long enough to hybridize with the native NLMB reducing the rate at which the Florida alleles disappeared from the populations, but FLMB eventually disappeared because of reduced fitness for extreme conditions. Even reservoirs that were stocked recently showed very low percentages of pure FLMB, but often had moderate levels of intergradation.

Lake age was a selected variable in the regression model for percent F_1 phenotypes (Table 3). Populations with high percent TBFA were found in reservoirs that were on average < 25 years old while those with lower percent TBFA were found in reservoirs with average ages > 25 years (Table 4). Florida largemouth bass influence was also greater in reservoirs that were stocked with FLMB when first impounded (Birch, Dripping Springs, Healdton, Konawa, Sardis, Sooner, and Waurika reservoirs; Table 2). Reduced competition with NLMB in younger reservoirs may have allowed greater survival of stocked fingerlings, avoiding the high natural mortality often associated with supplementally stocking bass into existing populations (Boxrucker 1986).

Biological Parameters Regulating Florida Allele Persistence

A sound stocking strategy involving annual stockings with high numbers of FLMB was more important than physical factors in the establishment of Florida genes in Texas reservoirs (Kulzer et al. 1985). The size of FLMB stocked, however, had no significant influence on their establishment in Texas, leading Kulzer et al. (1985) to conclude that stocking fry at high rates for several years would be a more cost effective means of establishing Florida genes. In Oklahoma reservoirs, stocking rate did not appear to be as important; however, the size of fingerlings stocked was a significant variable. Populations in the southwest region of the state had the highest mean percent TBFA (Fig. 1) and received, on average, more annual stockings, with larger fingerlings than did populations in regions with low mean percent TBFA such as the northeast (Fig. 1). Although some reservoirs in the northeast region received up to 6 stockings each from 1974 through 1985, they still showed low incidences of FLMB alleles, perhaps because stockings were for shorter periods of time and with small fingerlings.

The results of this and previous research may have been confounded by stocking F_1 and/or F_x phenotypes rather than pure FLMB. Conflicting results comparing growth and survival of FLMB, NLMB, and hybrid largemouth bass have been reported in Oklahoma (Nieman and Clady 1979, Wright and Wigtil 1980, Harper 1984, Mauck 1984). Mating among subsequent generations of offspring from contaminated or mis-identified fish introduced as FLMB could have provided the high numbers of F_x individuals now seen in these populations. It is probable that many trophy bass being caught by anglers are also F_1 individuals that were mistakenly stocked as FLMB.

Further evaluation of long-term changes in the bass genetic structure, reproduction, survival, recruitment, growth, and harvest in these reservoirs are needed. Identification of trophy bass should be continued to determine if the F_1 phenotype possesses the optimum complement of Florida alleles for environmental conditions in Oklahoma reservoirs. This information will be necessary to formulate genetically sound management and stocking plans.

Literature Cited

Bottroff, L. J. and M. E. Lembeck. 1978. Fishery trends in reservoirs of San Diego County, California following introductions of Florida largemouth bass, *Micropterus salmoides floridanus*. Calif. Fish and Game 64:4–23.

Boxrucker, J. 1986. Evaluation of supplemental stocking of largemouth bass as a management tool in small impoundments. N. Am. J. Fish. Manage. 6:391–396.

Cichra, C. E., W. H. Neil and R. L. Noble. 1980. Differential resistance of northern and Florida largemouth bass to cold shock. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 34:19–24.

Childers, W. F. 1975. Bass genetics as applied to culture and management. Pages 362–372 *in* R. H. Stroud and H. Clepper, eds. Black Bass Biology and Management. Sport Fish. Inst., Washington, D.C.

Gilliland, E. R. and J. Whitaker. 1989. Evaluation of genetic status of largemouth bass populations. Okla. Dep. Wildl. Conserv., Performance Rep., Fed. Aid Proj. F–39-R, Job 12. Oklahoma City. 27pp.

Graham, L. K. 1973. The introduction of Florida largemouth bass in Missouri. Mo. Dep. Conserv., Final Rep., Fed. Aid Proj. F–1-R–22. Jefferson City. 6pp.

Guest, W. C. 1982. Survival of adult Florida and northern largemouth bass subjected to cold temperature regimes. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 36:332–339.

Harper, J. L. 1984. Evaluation of supplemental stocking Florida and northern largemouth bass as a management procedure in small impoundments. Okla. Dep. Wildl. Conserv., Final Rep., Fed. Aid Proj. F–39-R, Job 5, Oklahoma City. 26pp.

Harvey, W. D. 1983. An electrophoretic evaluation of bi-specific populations of largemouth bass in small Texas impoundments. Ph.D. Diss., Texas A&M Univ., College Station. 98pp.

———, I. F. Greenbaum and R. L. Noble. 1980. Electrophoretic evaluation of five hatchery stocks of largemouth in Texas. Annu. Proc. Texas Chap. Am. Fish. Soc. 3:49–63.

Johnson, D. L. 1975. A comparison of Florida and northern largemouth bass in Missouri. Ph.D. Diss., Univ. Mo., Columbia. 111pp.

Kulzer, K. E., R. L. Noble and A. A. Forshage. 1985. Genetic effects of Florida largemouth bass introductions into selected Texas reservoirs. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 39:56–64.

Latta, W. C. 1977. Survival and growth of Florida largemouth bass in ponds and reservoirs in Michigan. Mich. Dep. Nat. Resour., Final Rep., Fed. Aid Proj. F–35—R–3, Study No. 27, Lansing. 14pp.

Meceina, M. J., B. R. Murphy and J. J. Isely. 1988. Factors regulating Florida largemouth bass stocking success and hybridization with northern largemouth bass in Aquilla Lake, Texas. Trans. Am. Fish. Soc. 117:271–231.

Mauck, P. E. 1984. Evaluation of Florida and northern largemouth bass when stocked into new small impoundments. Okla. Dep. Wildl. Conserv., Final Rep., Fed. Aid Proj. F–39-R, Job 6, Oklahoma City. 20pp.

Mulford, C. J. 1984. Florida largemouth bass stocking evaluation. Texas Parks and Wildl. Dep., Final Rep., Fed. Aid Proj. F–31-R, Obj. XXVII, Austin. 17pp.

Nieman, D. A. and M. D. Clady. 1979. Florida and northern largemouth bass growth and survival in a heated reservoir. Proc. Okla. Acad. Sci. 59:47–50.

Pelzman, R. J. 1980. Impact of Florida largemouth bass, *Micropterus salmoides floridanus,* introductions in selected northern California waters with a discussion of the use of meristics for detecting introgression and for classifying fish of intergrade populations. Calif. Fish and Game 66:133–162.

Phillip, D. P., W. F. Childers and G. S. Whitt. 1982. Electrophoretic analysis of largemouth bass, *Micropterus salmoides.* Pages 1–156 *in* D. P. Phillip, W. F. Childers, and G. S. Whitt, eds. Biochemical genetics of largemouth bass, *Micropterus salmoides.* Electric Power Inst., Palo Alto, Calif.

———. 1983. A biochemical genetic evaluation of northern and Florida subspecies of largemouth bass. Trans. Am. Fish. Soc. 112:1–20.

Rainwater, W. C. and A. Houser. 1975. Relation of physical and biological variables to black bass crops. Pages 306–309 *in* R. H. Stroud and H. Clepper, eds. Black Bass Biology and Management. Sport Fish. Inst., Washington, D.C.

Rieger, P. W. and R. C. Summerfelt. 1976. An evaluation of the introduction of Florida largemouth bass into an Oklahoma reservoir receiving a heated effluent. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 30:48–57.

SAS Institute, Inc. 1987. SAS/STAT Guide: For Personal Computers; Version 6 Edition. SAS Inst., Inc., Cary N.C. 378pp.

Selander, R. K., M. H. Smith, S. Y. Yang, W. F. Johnson and J. B. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus.* I. Variation in the old field mouse (*Peromyscus polionotus*). Studies in Genetics VI, Univ. Texas Publ. 7103:49–90.

Stevenson, F. 1973. A preliminary evaluation of a Florida strain of largemouth bass, *Micropterus salmoides floridanus,* in Ohio ponds. Ohio Dep. Nat. Resour., Final Rep., Fed. Aid Proj. F–29-R–13, Columbus. 8pp.

Wright, G. L. and G. W. Wigtil. 1980. Comparisons of growth, survival, and catchability of Florida, northern, and hybrid bass in a new Oklahoma reservoir. Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 34:31–38.