

# Comparison of Growth of Selectively Bred and Resident Largemouth Bass in Texas Small Impoundments

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**Abstract:** The Texas Parks and Wildlife Department (TPWD) began the ShareLunker program (currently sponsored by Toyota) in 1986 to promote public involvement in the management of trophy largemouth bass (*Micropterus salmoides*; LMB) fisheries in Texas. The program provides anglers an opportunity to donate trophy LMB ( $\geq 5.9$  kg) to a selective breeding and stocking program managed by TPWD, with the goal of increasing the production of trophy-sized fish in Texas reservoirs. Although the program was known to be successful at promoting trophy LMB fishing in Texas, it was not known whether selective breeding results in growth advantages and subsequent increases in the likelihood of producing trophy-sized LMB. We assessed the efficacy of the Toyota ShareLunker program by comparing length and weight of age-4 ShareLunker LMB stocked as fingerlings with age-4 naturally produced resident LMB in six small impoundments. ShareLunker offspring were significantly heavier than resident cohorts (1.19 kg versus 0.99 kg, respectively), but not significantly longer. Our results suggest that the stocking of selectively bred LMB may be an effective tool to enhance trophy LMB production in populations.

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**Key words:** genetics, age and growth, selective breeding

Journal of the Southeastern Association of Fish and Wildlife Agencies 3:57–63

Fisheries managers have stocked Florida largemouth bass (*Micropterus salmoides floridanus*; FLMB) throughout the southern United States since the 1950s (Maceina and Murphy 1992). The goal of these stockings is to produce bigger fish faster as FLMB commonly attain larger overall sizes in less time where growth factors are favorable (Forshage and Fries 1995, Lutz-Carrillo et al. 2006). Florida largemouth bass were first introduced into Texas waters in 1972 as a fast-growing sportfish with the potential to reach large sizes ( $\geq 4.5$  kg; Botttroff and Lembeck 1978, Wright and Wigtil 1980, Forshage and Fries 1995). Since then, TPWD has stocked over 243 million FLMB, primarily fingerlings, into 451 reservoirs and small impoundments (TPWD, unpublished data). Successful introduction of the FLMB was evident by 1980, when a FLMB intergrade became the new Texas state record, and all subsequent largemouth bass (*M. salmoides*) state records have had some FLMB genetic influence (Forshage and Fries 1995).

To further enhance and promote trophy largemouth bass (LMB) fisheries in Texas, TPWD began the ShareLunker program (cur-

rently sponsored by Toyota) in 1986. The purpose of this program is to promote public involvement in the management of trophy LMB ( $\geq 5.9$  kg) fisheries in Texas by providing anglers an opportunity to donate legally caught trophy fish to a selective breeding and stocking program managed by TPWD. In return, anglers receive a fiberglass replica of their fish and recognition at an annual banquet. Because all these large fish have been females, TPWD then selectively breeds them with 100% FLMB male brood stock ( $\geq 0.95$  kg in weight or age 2) and typically stocks a portion of their offspring into the system from which the trophy fish were caught. As of 1 May 2015, 563 fish had been donated to the program from 65 public and 22 private water bodies.

Although the ShareLunker program is very popular with anglers, the costs associated with collecting, maintaining, breeding, and stocking these ShareLunker progeny are 20 times greater than those associated with standard FLMB stocking efforts (TPWD unpublished data). It is unknown if the ShareLunker program results in biologically significant growth advantages and subsequent

**Table 1.** Selected characteristics of study reservoirs. Regulations on these reservoirs were minimum-length limits (min), protected-slot limits (slt), and catch-and-release regulations (C&R). Percent FLMB alleles at stocking refers to the percentage of FLMB alleles present in the resident LMB population prior to ShareLunker offspring stockings.

	Meridian	Mill Creek	Raven	Purtis Creek	Pinkston	Marine Creek
Year impounded	1933	1976	1956	1985	1976	1958
Surface area (ha)	20	96	97	141	181	101
Shoreline development index	2.6	3.4	2.3	3.4	4.9	2.6
Mean depth (m)	2.5	3.0	1.8	3.4	4.6	4.6
Record LMB (kg)	5.5	6.6	6.1	6.2	7.7	4.9
LMB regulation (mm)	457 min	356–533 slt	C&R	C&R	356–533 slt	457 min
Geographic coordinates (Lat-Long)	31.8888 –97.7003	32.5370 –95.8500	30.6104 –95.5341	32.3576 –95.9951	31.7102 –94.3629	32.8289 –97.3995
Last FLMB stocking	1990	1999	1998	1985	1976	1978
FLMB alleles at stocking (%)	32	60	77	71	70	25

**Table 2.** Number of ShareLunker LMB stocked in six Texas reservoirs during 2005 and 2006. Percent mean coded wire tag (CWT) retention represents values 48 h post stocking.

	Meridian	Mill Creek	Raven	Purtis Creek	Pinkston	Marine Creek
Total stocked	1260	5949	5901	8742	11,174	6290
Stocking rate (fish ha <sup>-1</sup> )	63.0	61.9	60.8	61.9	61.6	62.3
Mean CWT retention (%)	99	95	99	96	92	95

increases in the likelihood of producing trophy-sized LMB. Our objective was to assess the efficacy of the ShareLunker program by comparing growth, in both length and weight, of age-4 ShareLunker LMB stocked as fingerlings with age-4 naturally produced resident LMB in six small reservoirs. We hypothesized that the selectively bred ShareLunker offspring would achieve significantly higher growth compared to their resident cohorts, indicating the selective breeding program is providing measurable benefits to anglers.

## Methods

### Study Sites

We conducted this study in six small Texas reservoirs. All reservoirs are located in the “Prairies and Lakes” and “Piney Woods” regions of east Texas, ranged from 20–181 ha in size, and were managed by a variety of harvest regulations (Table 1). Lake-record LMB varied from 4.9–7.7 kg across impoundments. Pinkston and Purtis Creek received a single stocking of FLMB in 1976 and 1985, respectively, while Raven was stocked with FLMB six times from 1979 to 1998. Marine Creek was stocked with FLMB in 1977 and 1978, while Mill Creek and Meridian each received three stockings of FLMB from 1976 to 1999 and from 1981 to 1990, respectively. At the time of stocking ShareLunker offspring, FLMB alleles present in the resident LMB populations ranged from 25%–77%, but was > 50% in 4 of the 6 impoundments (Table 1).

### ShareLunker Offspring Production and Stocking

ShareLunker offspring for the study were produced from four pairings during 2005 and seven pairings during 2006. After spawning, fry were grown to the advanced fingerling stage (approximately 150 mm in total length [TL]; range 130 to 300 mm TL) as described in Martinez and Owens (2009). We used advanced fingerlings because stocking success has been shown to increase with fish length (Wahl et al. 1995, Hoffman and Bettoli 2005, Mesing et al. 2008). Fish were then anaesthetized with a solution of tricaine methanesulfonate (0.08 g MS-222 and 0.4 g NaHCO<sub>3</sub> L<sup>-1</sup> water) and injected with a coded wire tag (CWT) in the left nape using a Mark IV tag injector (Buckmeier 2001). Injector function and tag retention was initially tested on a subsample of 100 ShareLunker LMB with a hand-held CWT wand detector upon set-up. ShareLunker offspring were then stocked into Meridian, Mill Creek, and Raven reservoirs in November 2005 and Purtis Creek, Pinkston, and Marine Creek reservoirs in November 2006 at a mean density of 62 fish ha<sup>-1</sup> (range; 61 to 63 fish ha<sup>-1</sup>; Table 2). Subsamples of 25 ShareLunker LMB were placed into each of three floating enclosures, at each lake, at the time of stocking. Tag retention was then observed again on these subsamples 48 h post stocking with a hand-held CWT wand detector. The TPWD Inland Fisheries Procedures Manual was followed for all ShareLunker offspring handling, hauling, acclimating, and stocking events.

## Fish Collections

Largemouth bass were collected via boat electrofishing (Smith-Root 5.0 or 7.5 GPP). All collections were made between 0.5 h after sunset and 0.5 h before sunrise, with effort concentrated on shoreline habitat and physical structure. All LMB were collected at each sampling site; however, we retained only confirmed ShareLunker LMB and resident individuals > 305-mm TL, which corresponded to the historical minimum length-at-age of age-4 resident LMB in these reservoirs (TPWD unpublished data). We selected age-4 LMB for analyses for two reasons. First, although it is generally believed that pure FLMB can attain larger sizes than either northern largemouth bass (NLMB) or intergrades between the strains, the age at which these differences might manifest themselves is less clear. Studies examining growth between the strains have found conflicting results for age—1–3 fish (Clugston 1964, Zolczynski and Davies 1976, Rudd 1985, Leitner et al. 2002). However, other studies have suggested that FLMB attained larger sizes by age 3 or age 4 (Inman et al. 1977, Bottroff and Lembeck 1978, Wright and Wigtil 1980, Maceina et al. 1988). Second, older fish are harder to capture, and power analyses suggested that sufficient sample sizes would be more likely to be attained for LMB that were age 4 or younger (TPWD unpublished data).

Largemouth bass were sampled during March through May 2009 in Meridian, Mill Creek, and Raven reservoirs and March 2010 in Purtil Creek, Pinkston, and Marine Creek reservoirs. ShareLunker and resident LMB were always collected simultaneously when samples were conducted over a range of dates in a single impoundment, thus minimizing the effect of temporal bias on growth estimates between strains. We limited our analyses to female fish, as all trophy-sized LMB entered into the ShareLunker program have been female and female LMB are known to grow faster and attain larger sizes than males (Maceina et al. 1988). Individuals of both strains were sexed by the papilla and probe methods described by Benz and Jacobs (1986). When no CWT was detected, fish were assumed to be resident LMB; those confirmed to be male were released, while those suspected to be female were measured for TL (mm), weighed (g), and sacrificed. In the laboratory, sex was confirmed by visual examination of the gonads, otoliths were removed for aging, and a fin clip was collected for subsequent genetic analysis. Fish were aged using sectioned otoliths in accordance with Buckmeier and Howells (2003), and fish that were not age 4 were removed from analyses. Fin clips were only collected from fish with no CWT (i.e., assumed to be resident LMB) in 2009. We did not consider the possibility that LMB might contain foreign metal objects (i.e., fishing hooks) that could be detected with the CWT reader and therefore result in misidentification of resident and ShareLunker LMB; this issue was cor-

rected during 2010 by collecting fin clips from all LMB thought to be age-4 females.

## Genetic Analyses

To ensure correct assignment of ShareLunker offspring and resident fish, genetic analyses based on a 10-locus marker panel were used to assign each fish to the appropriate group. Fin clips were processed to isolate genomic DNA and polymerase chain reaction was performed on each sample using a tailed primer approach (Boutin-Ganache et al. 2001) in 10- $\mu$ L volumes (Eppendorf MasterCycler Pro S thermalcycler) as described in Lutz-Carrillo et al. (2008). All loci used (Lar7, TPW20, 50, 55, 60, 62, 70, 76, 96, 157) are described in DeWoody et al. (1998) and Lutz-Carrillo et al. (2008). Amplicons were resolved on a LI-COR 4300 DNA analyzer (LI-COR Lincoln, Nebraska).

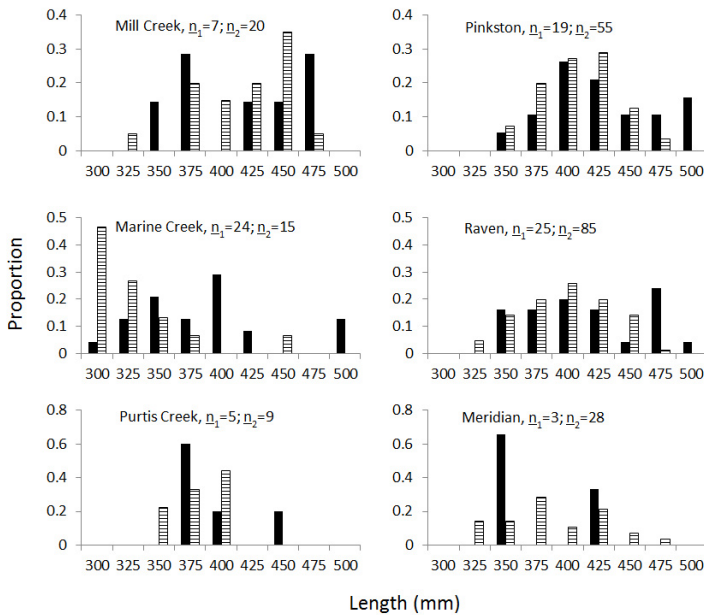
Using the likelihood approach implemented in CERVUS (version 3.0.3; Marshall et al. 1998, Kalinowski et al. 2007), each fish was classified as either ShareLunker or resident based on genotype compatibility with hatchery-crossed parental pairs. Simulations, assuming known sex parent-pairs and a typing error of 1%, were used to determine critical logarithm of odds (LOD) thresholds and quantify confidence for assignments. Putative ShareLunker fish (possible CWT detected) were designated as resident if the sample was not genotypically compatible with any crossed parental pair. Putative resident fish (no CWT detected) were designated as ShareLunker if the sample was compatible with a crossed parental pair and exceeded the simulated LOD 95% confidence threshold.

## Statistical Analyses

Differences in length and weight between age-4 ShareLunker and resident LMB within each reservoir were assessed with an ANOVA using fish as the replicates within each reservoir. Differences in TL and weight between age-4 ShareLunker and resident LMB among reservoirs were likewise assessed with an ANOVA, using reservoirs as replicates and fish as subsamples. The treatment effect (i.e., parentage of the offspring) and the year effect were considered fixed effects for all analyses. In the among reservoirs analysis, reservoir was considered a random effect, as was the interaction between treatment and reservoir. All models used the variances components covariance structure. Because the weight data were right-skewed, we used the  $\log_{10}$ -transformation of weight as our response variable; length data were not transformed. All analyses were conducted using Proc Mixed (SAS Institute 2008). Statistical significance was set at  $P=0.05$ .

**Results**

We stocked a total of 39,316 tagged ShareLunker LMB into six reservoirs (Table 2). Combined mortality for all tagging, handling, hauling and acclimating procedures was 0.7% and 0.2% in 2005 and 2006, respectively. Coded wire tag retention rates were consistently high for all stocked ShareLunker cohorts (Table 2). Five ShareLunker LMB were originally misidentified as resident LMB due to the lack of a detectable CWT. Additionally, two fish originally identified as ShareLunker LMB, due to the false detection of a CWT, were actually resident LMB based on genotype.



**Figure 1.** Length frequencies of age-4 female LMB collected during March through May 2009 and March 2010. Black columns denote ShareLunker LMB and shaded columns denote resident LMB. Sample sizes are given for ShareLunker LMB ( $n_1$ ) and resident LMB ( $n_2$ ).

The largest fish collected in each reservoir were usually ShareLunker LMB but there was considerable overlap between the strains among smaller fish (Figure 1). Catches of age-4 females varied greatly among the six reservoirs sampled, ranging from a total of 14 fish in Purtis Creek reservoir, to 110 fish in Raven reservoir (Table 3). Within reservoirs, both length and weights of age-4 ShareLunker LMB were significantly greater than resident LMB in three of the six reservoirs examined. Among reservoirs, age-4 ShareLunker LMB were significantly heavier than resident LMB, but mean lengths were similar (Table 3).

**Discussion**

Our study results suggest that selective breeding of trophy-sized LMB entered into our ShareLunker program produced offspring with a measureable growth advantage, particularly in weight, over naturally produced resident cohorts of the same age. Increased growth rates and body weights have been observed in other fishes subjected to selective breeding (Donaldson and Olson 1957, Bolivar and Newkirk 2002, Maluwa and Gjerde 2007, Eknath and Hulata 2009, Gjedrem 2010, Thodesen et al. 2013); thus, finding that the offspring of selectively bred FLMB were heavier than resident LMB was not completely unexpected. However, offspring in previous studies were typically evaluated under controlled conditions and over multiple generations of selection. In our study, offspring were evaluated in uncontrolled environments, against age- and sex-matched cohorts, and after minimal selective breeding. These results suggest that rather than simply focusing on fish with FLMB genomic contribution, managers interested in trophy LMB should consider selective breeding of fish that have demonstrated that they can obtain trophy sizes, regardless of genetic composition.

Higher growth of ShareLunker offspring was not evident in ev-

**Table 3.** Total numbers ( $n$ ) and sizes of female LMB collected by electrofishing at age-4, by reservoir. “ShareLunker” refers to individuals stocked as ShareLunker LMB offspring, whereas “resident” refers to same-age naturally produced resident LMB offspring. Standard deviations are in parentheses. The  $F$ -test and  $P$ -value reflects the comparison of ShareLunker offspring to resident offspring within and across reservoirs. In the “Across Reservoirs”  $F$ -test, fish are considered subsamples within reservoirs.

	Meridian	Mill Creek	Raven	Purtis Creek	Pinkston	Marine Creek	Across reservoirs
ShareLunker $n$	3	7	25	5	19	24	83
Resident $n$	28	20	85	9	55	15	212
Mean weight (kg)							
ShareLunker	0.87 (0.29)	1.45 (0.60)	1.20 (0.41)	1.02 (0.32)	1.46 (0.44)	0.96 (0.57)	1.19 (0.51)
Resident	0.82 (0.29)	1.24 (0.34)	1.01 (0.29)	0.88 (0.18)	1.10 (0.27)	0.54 (0.26)	0.99 (0.33)
$F$ -test	0.17	0.59	5.97	1.02	14.18	12.80	12.13
$P$	0.688	0.449	0.016	0.333	<0.001	0.001	0.018
Mean TL (mm)							
ShareLunker	389 (32)	435 (51)	428 (46)	413 (37)	443 (42)	398 (55)	421 (50)
Resident	400 (41)	430 (36)	409 (36)	393 (22)	419 (30)	337 (39)	407 (40)
$F$ -test	0.19	0.10	4.60	1.72	7.03	14.36	6.49
$P$	0.644	0.758	0.034	0.214	0.010	<0.001	0.051

ery reservoir, which may have likely been caused by limited sample sizes from some locales. Collecting a large enough sample size of age-4 female LMB proved more difficult than expected in some of our study lakes. The literature shows survival of stocked LMB can be quite variable, ranging from as low as 0% (Barron 1966, McCammon and von Geldern 1979) to as high as 57% (Sammons and Maceina 2005). Sammons and Maceina (2005) results were from predator-free ponds and survival estimates were from stocking until the following spring. In contrast, Buckmeier and Betsill (2002) stocked fingerling LMB into a predator-rich reservoir and found that only 2%–3% of the stocked fish survived 5 months post-stocking. In addition, mean annual mortality of adult LMB is typically reported to be 30%–50% (Allen et al. 2002, Bulak and Crane 2002, Driscoll et al. 2007, Daugherty and Smith 2012). Therefore, it is not surprising that we were unable to collect as many age-4 fish as we desired. Although small sample sizes may have precluded us from resolving significant differences in some study populations, the magnitude and consistency of differences in weight between ShareLunker and resident LMB were sufficient to allow us to have confidence in our results.

Growth in LMB, particularly in weight, is likely a highly polygenic trait similar to stature-related phenotypes in other animals with many loci, each of very small effect, contributing to the phenotype (Flint and Mackay 2009, Yang et al. 2010). The genetic variation contributing to growth is also expected to be primarily additive and thus amenable to directional selection (Kruuk et al. 2001, Pakkasmaa et al. 2003, Visscher et al. 2008). This study examined size at age 4 of two different groups of LMB while controlling for spatial and temporal factors. Finding that differences in weight between ShareLunker and resident LMB existed within each population suggests that the phenotypic expression of growth in weight was heritable and rather robust across a variety of environments, although scope of growth and weight differences between groups were still reservoir-dependent. Because it has been demonstrated that size is a heritable trait, we expect these benefits could be passed on to future offspring (Visscher et al. 2008).

Although ShareLunker offspring were never previously stocked into our study lakes, resident LMB populations were genetically introgressed and influenced by previous stockings of FLMB (Forshage and Fries 1995, Buckmeier et al. 2003, Tibbs 2008). Introgression levels prior to the initiation of the study ranged from 25% to 77% FLMB alleles. Given the introgressed resident LMB populations, one might expect the magnitude of differences between the cohorts to be minimal. Still, our results showed significantly higher weights in selectively bred ShareLunker LMB over resident LMB at age-4. In fact, the greatest differences in both length and weight between these groups were observed in Marine Creek (25% FLMB

alleles in the resident population) and Pinkston (70% FLMB alleles in the resident population), which represented almost the entire range in observed allele frequencies across the study lakes. If the greater size of the ShareLunker LMB was solely due to FLMB influence then one would expect size differences between ShareLunker and resident fish to be minimized in high FLMB-influenced populations and maximized in low FLMB-influenced populations. We did not observe that pattern among our small group of reservoirs. Whereas introgression levels of LMB across reservoirs were variable in this study, future research may wish to control the levels of introgression. One approach would be to compare LMB stocked under the standard TPWD stocking program (which are routinely 100% Florida influenced) with ShareLunker offspring.

ShareLunker offspring were stocked as advanced fingerlings to gain a survival advantage, and hence were likely longer and heavier than the naturally produced resident cohorts at the time of stocking. However, it is unlikely that the size differential between the cohorts at the time of stocking confounded our results at age 4. Diana and Wahl (2009) found no long-term differences after two years of growth when stocked LMB were larger than wild fish at the time of stocking. Similarly, Howells (2003) found that both slow- and fast-growing (as designated at age-0) FLMB were of comparable size by ages 3–5. These studies both suggest that in the absence of genetic differences, we could expect both cohorts to be of comparable size by the time we sampled at age 4. Future studies should consider determining whether ShareLunker offspring stocked at the same size as naturally produced resident offspring also show a size advantage at age 4.

It is not known whether the higher growth of ShareLunker offspring demonstrated in our study at age 4 can be expected to continue among older age groups resulting in the achievement of ultimately larger maximum sizes when compared to resident cohorts. Helser and Lai (2004) showed compelling evidence from across North America that, at the population level, fast growth in LMB may result in reduced maximum size. This could either represent a trade-off between growth and reproduction (von Bertalanffy 1957) or between growth and mortality (Mangel and Stamps 2001, Metcalfe and Monaghan 2003, Lee et al. 2013). One possible issue with the Helser and Lai (2004) analysis is they apparently used data that pooled fish by sex and genetic background. If the fastest-growing fish are primarily fish at southern latitudes, and if southern populations have a shorter lifespan than northern counterparts (Beamesderfer and North 1995), it could be that these confounding factors may have induced the inverse relation between growth and lifespan detected by Helser and Lai (2004). In contrast, in both natural and aquaculture settings, Legendre and Albaret (1991) found strong positive relationships between overall maximum size and growth at

one and two years of age for 69 species of tropical fish. It is well documented that FLMB can grow faster than NLMB at adult life stages ( $\geq$ age 3; Botttroff and Lembeck 1978, Wright and Wigtil 1980, Maceina et al. 1988) and also attain a larger maximum size (Maceina et al. 1988, Buckmeier and Betsill 2002). Results of the current study suggest ShareLunker offspring were heavier by age 4, but the effectiveness of this program to increase production of trophy size LMB in a system remains equivocal. What is still needed is an age and growth comparison of sufficient trophy-sized fishes with differing molecular histories; however, it may be difficult to convince anglers or biologists that the information gained is sufficiently valuable to justify the sacrifice of such individuals to obtain accurate ages.

Many states and private hatcheries have integrated FLMB into hatchery programs. Given appropriate environmental conditions, this practice can be expected to produce larger fish. While subsequent work is needed to control for levels of introgression among resident LMB populations and evaluate growth differences between ShareLunker offspring and FLMB reared under similar hatchery conditions, this study suggests that selectively breeding large individuals may be a useful tool to increase numbers of larger fish within a population. This method should be investigated by other agencies as a means to increase the trophy potential of fisheries for a variety of species, including LMB.

## Acknowledgments

This study was funded through the Federal Aid in Sport Fish Restoration Program, Project F30-R and Toyota ShareLunker. The Freshwater Fisheries Center staff conducted all rearing and stocking procedures; J. Mathews, T. Owens, and D. Wade oversaw operations and provided logistical support. Inland Fisheries Management staff assisted with ShareLunker offspring tagging and performed all LMB collections throughout the study; D. Buckmeier, J. Hernandez, and B. Wienecke provided practical instruction and guidance on tagging procedures. B. Van Zee, C. Bonds, S. Dumont, R. Myers, R. Betsill, and D. Daugherty provided helpful reviews on this manuscript.

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