

Comparison of Relative Contribution, Growth, and Vulnerability to Angling of Triploid Florida Largemouth Bass and Diploid Northern Largemouth Bass Stocked in a Newly-renovated Reservoir

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Abstract: An evaluation was conducted to compare relative contribution, growth, and vulnerability to angling of triploid Florida largemouth bass (*Micropterus salmoides floridanus*) and diploid northern largemouth bass (*M. s. salmoides*) when stocked together in a newly-renovated reservoir. Triploid Florida largemouth bass were stocked in Lake Balmorhea, a 213-hectare reservoir in West Texas, for five consecutive years and failed to recruit in all years except the initial stocking year. Diploid northern largemouth bass were stocked only in the first year and produced significant year classes in years 4 and 5 of the study. Northern largemouth bass grew faster initially, but triploid Florida largemouth bass were similar in size by age 3. Diploid northern largemouth bass were more vulnerable to angling than triploid Florida largemouth bass through the first 3 years of life. Failure of triploid Florida largemouth bass to recruit into existing fish populations makes their value questionable until the reason for these failures is resolved.

Key words: largemouth bass, triploid, contribution, growth, angling vulnerability

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Genetic manipulation of largemouth bass (*Micropterus salmoides*) populations is sometimes used by fisheries managers in an effort to improve sport fisheries. This practice has normally taken the form of Florida largemouth bass (*M. s. floridanus*) stockings designed to increase the influence of that genotype on the population. Florida largemouth bass have a greater potential to reach trophy size (Bottroff and Lembeck 1978, Mauck 1984, Horton and Gilliland 1993); however, introgression with northern largemouth bass (*M. s. salmoides*) populations may reduce trophy potential of subsequent generations of bass. While Florida largemouth bass (FLMB) reach a larger maximum size, northern largemouth bass (NLMB) have been reported to be more susceptible to angling (Zolczynski and Davies 1976, Bottroff and Lembeck 1978, Kleinsasser et al. 1990). If NLMB are more susceptible to angling, this trait may also be reduced following the introgression of FLMB genes into a population. Garrett (2002) suggested the use of sterile triploid FLMB as a means to preserve the most desirable traits of each subspecies within the same water body. He theorized that a fishery could be developed where angling opportunities are maximized by creating a largemouth bass population in which a subset was highly vulnerable to angling and another portion had high growth potential and could avoid being caught long enough to reach large size. He suggested this could be accomplished by creating fisheries using sterile triploid FLMB in conjunction with native NLMB. Triploid largemouth bass may also have the potential benefit of increased growth over diploid bass (Wolters et al. 1982, Parsons and Meals 1997, Garrett 2002). According to Simon et al. (1993), suppressed gonad-

al development in triploids may allow metabolic energy and nutritional resources normally used for development of sexual characteristics and reproduction to be directed into somatic growth.

A total lake renovation project at Lake Balmorhea in the Trans Pecos area of West Texas in 1998 offered the opportunity to investigate the use of triploid FLMB in conjunction with diploid NLMB. The fish population was eradicated in 1998 using rotenone in an effort to eliminate introduced sheepshead minnows (*Cyprinodon variegatus*) that posed a threat to endangered Comanche Springs pupfish (*C. elegans*) in the area (Garrett 1998). Another objective of the renovation was to improve the sport fishery in Lake Balmorhea by eliminating a large population of undesirable species such as common carp (*Cyprinus carpio*) as well as introducing a balanced sportfish population. Triploid FLMB and diploid NLMB were first stocked into Lake Balmorhea in 1999, and this study was conducted to determine if sterile triploid FLMB could be used in conjunction with diploid NLMB to provide a bass fishery with both high catch rate NLMB and trophy FLMB within the same water body. The specific objectives were to compare 1.) the relative contribution of stocked triploid FLMB and diploid NLMB to each year class, 2.) the growth of stocked triploid FLMB and diploid NLMB, and 3) the relative vulnerability of triploid FLMB and diploid NLMB to angling.

Methods

Lake Balmorhea (Reeves County, Texas) is a 213-ha reservoir constructed in 1917 by the Reeves County Irrigation District for

the purpose of storing water for irrigation. It was constructed on Toyah Creek in the Pecos River watershed. The primary water source, other than infrequent rainfall in the semi-arid region, is from San Solomon Springs through a system of canals. The water level is lowered each summer as water is used for irrigation, reaching a low point in early fall, with the reservoir refilling throughout the fall and winter from San Solomon Springs during periods of low irrigation demand. The reservoir is shallow with a mean depth of 1 m.

Stocking after renovation began in fall 1998 when 128 adult bluegill (*Lepomis macrochirus*), 144 adult channel catfish (*Ictalurus punctatus*), 28,000 advanced fingerling (239 mm mean total length) channel catfish, and 850 adult blue catfish (*I. furcatus*) were stocked. In spring 1999, 210,000 fingerling bluegill and 47,300 NLMB (mean total length 38 mm; range 29–46 mm) were stocked along with triploid FLMB (Table 1). Florida bass used in this study were genetically manipulated to induce triploidy according to procedures described by Garrett et al. (1992). The original plan called for stocking bass at a rate of 250/ha with a 70/30 ratio of diploid NLMB and triploid FLMB. The actual stocking rate for triploid FLMB in all years was based on the number of triploids that were produced. Triploid FLMB were stocked in 1999–2003 with numbers ranging from 7,125 (34/ha) to 37,255 (177/ha). Northern largemouth bass were only stocked in 1999 and made up 87% of the largemouth bass stocked that year. Northern largemouth bass were not stocked in subsequent years as natural reproduction was verified each spring beginning in 2000.

Sampling was conducted each spring from 2000 through 2004. Boat-mounted, pulsed direct current electrofishing equipment was used at five-minute random shoreline stations during daylight hours and all bass encountered were collected until a target sample size was achieved or the entire shoreline was sampled. Target sample size was 100 largemouth bass for each year class expected to be in the reservoir at the time of sampling (i.e., 100 bass for 2000, 200 bass for 2001, etc.). Prior to electrofishing each year, a sample of bass was collected on the same day by angling using

15–25 anglers. All anglers fished for largemouth bass using a variety of gears and methods, and retained all bass caught. The target sample size for angling was the same as for electrofishing and angling continued until that sample size was obtained or catch rates declined to a point indicating the target would not be achieved within 1 day. All anglers recorded time spent fishing and number of bass caught.

Each bass collected by either method was weighed and measured, and otoliths were removed to verify age. Tissue samples for genotype and ploidy determinations were collected from each fish. Genotype was determined for each and ploidy was determined periodically to verify that FLMB in Lake Balmorhea were triploid. Ploidy was not determined in 2000 or 2002 due to the unavailability of equipment. In 2001 ploidy was checked on all bass from the electrofishing sample that were determined to be FLMB by electrophoresis. In 2003 and 2004 ploidy was verified on any bass identified as FLMB from electrophoresis with either gear. Approximately 0.5 mm³ of liver tissue for genotype determination was excised from each fish and placed in an individual cryotube. Blood for ploidy determination was obtained from individual fish following the method described by Gold et al. (1991) by first puncturing a branchial arch and then collecting approximately 40 µL of blood using a heparinized microcapillary pipette. The blood sample was then aspirated into a 2.0-mL cryotube containing 40 µL of storage solution (Gold et al. 1991). Tissue samples were placed in liquid nitrogen and transported to the lab where they were stored at –80 C until analyzed. Subspecific genotypes of individual fish were inferred following electrophoresis of diagnostic loci (i.e., *sAAT-2** and *IDHP-1**; Philipp et al. 1983). Two-flow cytometry methods were used to determine ploidy. Some were thawed and stained in propidium iodide and analyzed on a Coulter Elite flow cytometer as described in Wickliffe et al. (1998). The others were stained with 0.5 mL of DAPI/detergent/DMSO (Eudeline et al. 2000), homogenized, and passed through a 30-µm screen just prior to analysis with a Partec PAS-II flow cytometer. Blood from normal diploid largemouth bass served as controls for flow cytometry. For the purpose of this paper, FLMB will be used to describe fish of the Florida largemouth bass genotype based on electrophoresis, regardless of ploidy, and FLMB verified as triploid will be designated accordingly. Electrophoretic analysis revealed the presence of a small percentage of intergrade largemouth bass in the samples taken from the reservoir after stocking, indicating probable contamination of the hatchery broodstock used to produce triploid FLMB. Intergrades were treated as stocked FLMB in calculating stocked fish contribution but excluded from growth analysis.

Annual estimates of stocked fish contribution to the popula-

Table 1. Stocking summary for Florida triploid bass in Lake Balmorhea, 1999–2003, and proportion (%) of stocked Florida largemouth bass in the overall largemouth bass spring electrofishing sample in Lake Balmorhea. Ranges in parentheses.

Year class	Florida triploid		Sample year				
	N	Mean total length	2000	2001	2002	2003	2004
1999	7,125	38 (29–46)	23.0	16.0	19.0	14.0	9.0
2000	12,860	59 (45–70)		0.0	1.8	3.5	0.0
2001	15,203	55 (43–84)			0.0	0.7	0.0
2002	12,123	62 (34–112)				0.0	0.0
2003	37,255	69 (53–95)					0.8

tion by genotype were determined from electrofishing samples. Electrofishing was assumed to provide a non-biased sample of the population accurately representing the actual genetic composition. The number of fish by genotype (NLMB and FLMB) was divided by total number of fish collected to estimate the proportion of fish in the population by genotype. For fish caught by angling, annual proportion estimates by genotype were also made. Upper and lower 95% confidence intervals were computed according to Gustafson (1988) for proportion estimates used to compare angling and electrofishing samples. Vulnerability to angling was compared between NLMB and FLMB each year of the study. The number of fish collected angling and electrofishing was compared using two-way Chi square (X^2) tests (SAS 2005, Zar 1984) to determine if proportions of NLMB and FLMB differed significantly ($P \leq 0.05$) by method of capture. A Student's t -test was used to compare the mean total length (TL) of each genotype within an age class for each sample year. For growth analysis, electrofishing and angling samples were combined since no differences between the two gears were detected for any year using the t -test ($P \leq 0.05$). The study was terminated after the 2004 sample when a severe fish kill occurred in Lake Balmorhea due to toxic golden alga (*Prymnesium parvum*) in late spring.

Results

2000 Sample

The angling sample yielded 104 largemouth bass with very high catch rates of 5.75 bass/angler hour. Electrofishing catch rates were also relatively high at 244/hour. These fish were all verified as age 1 from the original stocking in 1999. Florida largemouth bass made up 23 % of the electrofishing sample (Table 1) even though only 13% of the bass stocked in 1999 were FLMB. The proportion of FLMB in the angling sample was significantly different from that in the electrofishing sample ($X^2=21.10$, $df = 1$, $P < 0.0001$) with 99 % of the bass caught by anglers being NLMB (Table 2). Mean TL of NLMB (227 mm) was significantly longer ($P < 0.05$) than mean TL of FLMB (157 mm) at age 1 (Table 3). Since NLMB were slightly longer than FLMB when stocked, mean growth increments were compared and the difference between mean TL from stocking to age 1 was also greater for NLMB (176 mm) than FLMB (119 mm) during the first year in Lake Balmorhea.

2001 Sample

Ploidy analysis revealed that 10 of the 33 FLMB collected by electrofishing were diploid indicating the hatchery procedures used to induce triploidy were not completely successful. Angler catch rates were high (2.3 bass/angler hour) yielding a sample of

Table 2. Summary of Florida largemouth bass (FLMB) contribution to the 1999 year class, as estimated by two sampling techniques and electrophoretic analysis, Lake Balmorhea. Values for % FLMB in rows for a particular sample year followed by the same letter are not significantly different (Two-way Chi square test; $P \leq 0.05$). CI = confidence interval

Year	Electrofishing			Angling		
	N	% FLMB	95% CI	N	% FLMB	95% CI
2000	104	23 z	14–32	100	1 y	0–4
2001	193	17 z	11–21	200	4 y	1–7
2002	104	20 z	14–26	191	10 y	5–15
2003	63	32 z	19–45	33	15 z	0–31

Table 3. Mean total lengths of largemouth bass from 1999 year class collected from Lake Balmorhea by electrofishing and angling combined. NLMB = northern largemouth bass, FLMB = Florida largemouth bass, N = sample size. Values for mean length in rows for a particular sample year followed by the same letter are not significantly different (t-test; $P > 0.05$)

Sample year	Age	NLMB (N)	FLMB (N)
2000	1	227 z (179)	157 y (20)
2001	2	268 z (352)	229 y (41)
2002	3	277 z (254)	269 z (23)
2003	4	345 z (71)	342 z (22)
2004	5	391 z (23)	406 z (7)

200 largemouth bass. Catch rates also were high for electrofishing at 196/hour. Analysis of otoliths revealed that almost all bass collected in 2001 were age 2 and thus from the original stocking in 1999. Only three of 396 bass collected were from the 2000 year class, two NLMB and one triploid FLMB.

The relative contribution of 1999 year class FLMB in the electrofishing sample was 17% (Table 2) and closely approximated the original percentage stocked. The angling sample was significantly different from the electrofishing sample ($X^2=16.61$, $df = 1$, $P < 0.0001$) with only 4% of the 200 bass caught angling being FLMB.

Growth of the 1999 year class to age 2 indicated NLMB were larger (mean = 268 mm TL) than FLMB (mean = 229 mmTL). Although this difference was less pronounced than at age 1, it was statistically significant ($P < 0.05$).

2002 Sample

Angler catch rates were 1.7 bass/angler hour, and electrofishing catch rates were 82/hour in 2002. As in previous years, the 1999

year class dominated the sample with 105 of 109 bass collected by electrofishing being age 3; the remaining fish were age 2 (2000 year class). No age 1 bass were collected with either gear.

Differences in angling vulnerability of the 1999 year class appeared to be less pronounced than in previous samples but were still significantly different ($X^2=5.19$, $df=1$, $P=0.0228$) with FLMB making up 20% of the electrofishing sample, but only 10% of the angling sample (Table 2).

Growth of NLMB and FLMB from the 1999 year class was similar by age 3 with NLMB reaching 277 mm TL and FLMB reaching 269 mm TL (Table 3). No growth comparisons were made for the other ages due to small sample sizes.

2003 Sample

Ploidy analysis revealed that 18 (82%) of 22 age 4 FLMB were triploid. Angling catch rates were only 0.5 bass/angler hour yielding a sample of 52 bass, while electrofishing catch rates were 72/hour. The 2003 sample indicated a strong year class was produced in Lake Balmorhea in 2002 for the first time since the original stocking in 1999. Electrofishing resulted in a catch of 141 largemouth bass of which 67 (47.5%) were age 1 and 63 (44.7%) were age 4. The remaining 11 bass were from the 2000 (age 3) and 2001 (age 2) year classes and accounted for only 4.3% and 3.5%, respectively, of the total electrofishing sample. The strong year class produced in 2002 appeared to be made up entirely of fish spawned in Lake Balmorhea. Of 83 age 1 bass collected using both gears, 79 were diploid NLMB and 4 were diploid F_1 bass. No age 1 triploid FLMB were collected.

The percentage of FLMB in both the angling and electrofishing samples was similar at 12% and 17%, respectively, in 2003 when all ages were included. Even when looking at only age 4 bass (1999 year class), the percentages were not significantly different ($X^2=2.29$, $df=1$, $P=0.1298$). Florida largemouth bass comprised 32% of the electrofishing sample and 15% of the angling sample (Table 2). Growth of the 1999 year class of FLMB and NLMB to age 4 was almost identical, with FLMB reaching 342 mm TL and NLMB reaching 345 mm TL (Table 3).

2004 Sample

Catch rates declined severely in 2004 due to effects of toxic golden alga in Lake Balmorhea. Only one largemouth bass was caught in 54 angler hours. Electrofishing over a two-day and one-night period yielded only 120 bass. The majority of all bass collected were from the spring inflow area of the lake that was serving as a refuge from the toxic alga.

Of the 120 bass captured by electrofishing, 33 were age 5 from the original stocking in 1999. The strong year class produced in

2002 accounted for 76 of the 120 bass (63.3%); 10 age 1 bass were also collected. Only one bass from the 2000 year class was collected, and none were collected from the 2001 year class. Growth of NLMB and FLMB from the original stocking to age 5 appeared to be similar, although sample size was low (Table 3). Low sample size in 2004 also precluded comparisons of angling vulnerability.

Discussion

While the current study was terminated prematurely due to problems associated with golden alga, several inferences can be drawn concerning use of sterile triploid bass to create fisheries with both a component of trophy fish and easy-to-catch fish. The first objective was to compare relative contribution of stocked triploid FLMB and diploid NLMB in the reservoir. With the exception of the initial stocking, no subsequent stockings of triploid FLMB made a substantial contribution to the bass population in Lake Balmorhea. Despite four years of stocking with rates up to 177/ha and fingerlings up to almost 70 mm TL, no significant number of triploid FLMB were collected from subsequent year classes. The only successful stocking occurred in 1999, when the fish were stocked into the reservoir with no predators present and an abundance of available forage.

Year class failures in 2000 and 2001 could be attributed to competition and/or predation by the dominant 1999 year class that exhibited extremely high survival rates indicated by high catch rates in 2000 and 2001. However, successful recruitment by bass spawned within the reservoir in both 2002 and 2003 would indicate that environmental conditions in those years were favorable for the recruitment of largemouth bass. One explanation for the lack of triploid FLMB recruitment could be a reduced ability of triploid fish to compete and survive in a natural environment compared to that of diploid fish. Czesny et al. (2002) found diploid saugeyes (female walleye *Sander vitreum* x male sauger *S. canadensis*) foraged more successfully than triploid saugeyes. They found that diploid saugeye captured significantly larger prey items, had fewer unsuccessful attempts, and reached satiation quicker than triploids. They theorized that the inferior foraging skills of triploid fish may lead to poor growth and perhaps lower survival in reservoirs. Triploid fish may be more susceptible to predators if they spend more time foraging (Milinski 1984). Wolters et al. (1991) also found decreased survival of triploid channel catfish compared to diploids when they were grown together in earthen ponds. Additional research would be necessary to determine if triploid FLMB are less adapted to compete in natural environments than either diploid FLMB or NLMB.

Growth data for this study was inconclusive due to the fish kill before bass reached trophy size. For the first four years, bass

growth was relatively slow in Lake Balmorhea but generally followed a trend expected from past studies comparing FLMB and NLMB. Northern largemouth bass were significantly longer at age 1; however, the genotypes were similar in size by age 3. This agrees with previous studies on diploid FLMB compared to diploid NLMB (Zolczynski and Davies 1976, Bottroff and Lembeck 1978). Most studies of other species have failed to demonstrate a growth advantage of triploid fish compared to diploids (Benfey 1999).

This study demonstrated substantial differences between triploid FLMB and diploid NLMB regarding vulnerability to angling, especially when the fish were relatively small. The dramatic difference at age 1 may be partially explained by size differences between the genotypes; however, the differences persisted at significant levels through year 3 of the study when mean total lengths were similar between FLMB and NLMB. Whether these differences are a result of subspecies differences or ploidy differences can not be determined from this study; but past studies indicate it could be a combination of the two factors. Zolczynski and Davies (1976) and Bottroff and Lembeck (1978) found higher catchability in NLMB than in FLMB. Czesny et al. (2002) found a decreased foraging ability in triploid saugeye and cited a reduced aggressiveness in triploids (Cassani and Caton 1986, Tave 1993) as a possible reason for reduced survival. Benfey (1999) also found that triploids often display reduced aggressiveness and inferior performance when reared under sub-optimal conditions. Reduced aggressiveness or inferior foraging ability could certainly be expected to influence angling vulnerability. In the current study, it appeared that catchability differences may have been decreasing as the fish grew larger; however, this may have been due to changes in the fish population resulting from angler harvest. By age 4, many bass in Lake Balmorhea had reached the legal minimum length of 354 mm TL, and differential harvest by anglers may have been a factor in removing faster growing individuals as well as those most susceptible to angling. This possible differential harvest may have confounded data interpretation on both growth and angling vulnerability.

Management Implications

The current study revealed several factors that would have to be addressed before use of triploid largemouth bass could be considered a viable management tool. In order for the management practice to work as proposed by Garrett (2002), triploid FLMB would have to be pure to avoid contamination by diploid FLMB leading to production of intergrades within the population. Overall, only 73% of FLMB checked in this study proved to be triploid. Currently, hatchery production techniques are not capable of 100% triploid induction, and raising largemouth bass to a size where individuals could be tested would be expensive and impractical.

Literature on other species (Cassani and Caton 1986, Parsons and Meals 1997) also indicates triploid induction rates are often below 100% on a production basis.

The reason for the failure of triploid FLMB to recruit after the first year in Balmorhea also would have to be investigated before this technique could be considered practical. The failure of four separate stockings of triploid FLMB in Balmorhea indicates serious problems may exist with their ability to compete in a natural environment. Further research to determine the ability of triploids to survive when stocked into existing fish populations would be necessary before they could be considered a viable management tool. At the current time, the expense of producing triploid FLMB, their failure to recruit, and failure of the present study to demonstrate any distinct growth advantage of triploids, would suggest little practical benefit in terms of sport fish management in reservoirs.

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