Genetic Variation of Striped Bass in Lake Texoma

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Abstract: To determine if inbreeding was a cause of an apparent decline of large striped bass (*Morone saxatilis*) in Lake Texoma, striped bass sampled from four sites on the reservoir (n = 206), in the Red River below Denison Dam in 2001 (n = 58), and archived scale samples from Lake Texoma collections in 1978 (n = 44) were genotyped and evaluated at six microsatellite loci. There was evidence of weak population genetic structure among the collection sites. However, analyses of Hardy-Weinberg and linkage equilibrium within sites did not provide evidence of recent inbreeding within Lake Texoma. Consequently, recent declines in the number of large adult striped bass in Lake Texoma cannot be explained by inbreeding depression.

Key words: striped bass, inbreeding, Lake Texoma, Red River

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Lake Texoma, a 30,225-ha reservoir on the Texas-Oklahoma border, was constructed in 1944 by the U.S. Army Corps of Engineers (USACOE) by impounding the Red and Washita rivers in north-central Texas and south-central Oklahoma. Striped bass were first stocked into Lake Texoma in 1965 (Table 1) by the Oklahoma Department of Wildlife Conservation (ODWC; Harper and Namminga, 1986). Natural reproduction was first confirmed in 1973 and has occurred annually since then (Mauck 1991). By 1999, 66% of recreational angling on Lake Texoma was directed toward striped bass (Hysmith et al. 2000).

Fish stock assessments from 1985 through 1988 showed a decrease in the number of large striped bass (>508 mm) (Mauck 1988, Hysmith 1989, Mauck 1991). The proportion of large fish in the population remained unchanged thereafter until further declines were documented in 1998, 2002, and again after 2005 (Moczygemba and Hysmith 1994, Hysmith and Moczygemba 1998, Hysmith et al. 1999, Hysmith and Moczygemba 2005). Despite a series of drastic changes in the angler harvest regulations for striped bass beginning in 1982 and continuing to 1989, there was no increase in the numbers of striped bass >508 mm (Moczygemba and Hysmith 1994). Creel surveys conducted from 1987 through 1999 also indicated a decline in the average size of harvested striped bass (Hysmith et al. 2000).

Several factors potentially contributing to the decline of large striped bass were considered. They included over-fishing, poor recruitment, angling mortality (AM), inbreeding, or a combination of factors. Hysmith et al. (1992) estimated hooking mortality of striped bass >508 mm was 56%, which strongly suggested AM may have negated the intended benefits of a stricter harvest regulation. In an effort to address growth over-fishing and reduce the impact of AM on fish >508 mm, the bag limit for striped bass was changed to 10 per day with 2 fish >508 mm on 1 September 1996. As of 2005 there has been no increase in the number of striped bass >508 mm and some evidence of even fewer striped bass >508 mm in the population (Hysmith and Moczygemba 2005). However, these data did eliminate poor recruitment as a factor contributing to the decline of large striped bass in Lake Texoma.

Because regulations did not have a noticeable positive effect upon the fishery and subsequent fish stock assessments showed evidence of a dynamic striped bass fishery (Hysmith and Moczygemba 2005), we ruled out other potentially contributing factors and concentrated on inbreeding. Because there was a limited founder population, we thought inbreeding may explain the apparent decline in large fish;

Table 1. Summary of stocking records for Lake Texoma, Texas-Oklahoma.

Year	*n* stocked	Source
1965	138	Moncks Corner, SC
1967	200,000	Moncks Corner, SC and Brookneal, VA
1968	5,000	Moncks Corner, SC
1969	284,614	Moncks Corner, SC
1970	77,640	Moncks Corner, SC
1971	96,839	Moncks Corner, SC
1972	208,340	Moncks Corner, SC and Oklahoma
1973	141,612	Hudson River, NY and Oklahoma
1974	548,898	Hudson River, NY and Tishomingo National Fish Hatchery
1977	1,600	Oklahoma sources (Texoma or Keystone)
1984	490	Texoma
1985	500	Texoma

not all fish, but large fish only. The objectives of this study were to evaluate genetic variation of striped bass in Lake Texoma and Red River below Denison Dam, evaluate population genetic structure, and determine if inbreeding was occurring among striped bass in Lake Texoma and the Red River below Denison Dam.

Methods

Striped bass used in the study were collected from Lake Texoma, Texas-Oklahoma, (Figure 1) with experimental 25.4- to 77.0-mm mesh monofilament gill nets. Sampling occurred over a four-year period from the Red River arm, Washita River arm, and main pool of Lake Texoma. Striped bass from a fourth site, the Red River below Denison Dam, were collected with a boat mounted electrofisher. Samples were collected in February 1997 from the Red River arm (n = 34) and the main pool (n = 53), in February 2000 from the Red River arm (n = 38) and Washita arm (n = 81), and in May 2001 below Denison Dam (n = 58). Fish samples were representative of the population susceptible to the sampling gear and no attempt was made to separate individuals into age classes for this study. Length and weight was recorded for each fish and a fin clip was preserved in 70% ethanol and transported to the laboratory for genetic analyses. Additionally, striped bass scale samples collected from Lake Texoma in 1978 (n = 44) were analyzed with the following protocol.

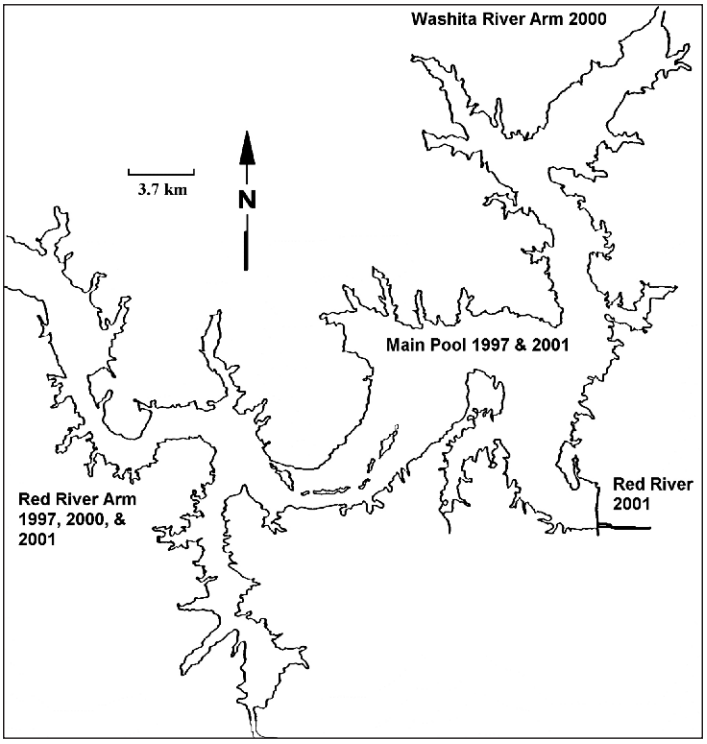

Figure 1. Four geographic areas of sampling for striped bass in Lake Texoma and Red River; 1997, 2000, and 2001.

Table 2. Primer sequence (reverse on the second line), PCR conditions, microsatellite loci characterization, and observed heterozygosity (H_o) used to evaluate inbreeding and population structure among striped bass collected in Lake Texoma, Texas-Oklahoma in 1997–2001.

Locus	Primer sequence	Annealing temperature (C)	Cycles	Size range (bp)	*n* of alleles	H_o
SB6	see Han et al. (2000)	55	35	197–259	21	0.73
SB20	see Roy et al. (2000)	55	30	116–120	2	0.11
SB91	see Roy et al. (2000)	58	35	141–177	17	0.82
Msa5–11	5′-CAAAAGACCATGCTAGAATCCA 5′-CCTATTTAGCAGCCACCATTG	57	30	167–168	2	0.37
Msa5–71	5′-TCACCTTGAACAAGAACCGA 5′-TATCTGCAAACCCCACACAA	57	30	181–182	2	0.53
Msa5–190	5′-GATCGATATGCAGCCCTTCTA 5′-AATGCGTTATTATGGCCATGGTA	58	40	346–352	4	0.39

Genomic DNA was isolated using a protein and polyester coprecipitation method (Dixson 2005) and a 5-µL aliquot of the resulting product was evaluated via electrophoresis in a 1% agarose gel, pre-stained with 2% ethidium bromide (w/v), to confirm its presence. Three previously published striped bass microsatellite primer pairs, SB6 (Han et al. 2000), SB20, and SB91 (Roy et al. 2000), and three primer pairs designed from Genbank (http://www.ncbi.nlm.nih.gov) entries, Msa5–11, Msa5–71, and Msa5–191 (Table 2), were used in this study. Two additional primer pairs designed from Genbank entries, Msa5–72 and Msa5–99, and one previously published primer pair, SB83 (Han et al. 2000) were monomorphic and not used in the analyses. One primer from each pair was tailed with the viral M13 (-29) F sequence (i.e., 5'-CACGACGTTGTAAAACGAC-3') at the 5'-end to allow fluorescent detection of polymerase chain reaction (PCR) products. The PCR reactions consisted of 1 µL template DNA, 6.0 µL sterile dH2O, 0.2µL 10mM dNTP, 0.4µL 10mM IRD700 dye-labeled M13(-29)(LI-COR Biosciences, Lincoln, Nebraska), 0.4 µL each of 10mM forward and reverse primer, 2.0 µL buffer (300mM Tris, 17.5mM MgCl2, 75mM (NH4)2SO4), and 0.5 units Taq DNA polymerase (Promega, Madison, Wisconsin). Reactions were optimized for each primer pair using an MJ Research PTC-200 thermalcycler (MJ Research, Waltham, Massachusetts). All reactions used 1-minute denaturation at 94 C, 45-second annealing, and 2-minute extension at 72 C. Annealing temperatures and number of cycles for each primer pair used in the study are described in Table 2. A final extension for 10 minutes at 72 C was followed by an indefinite soak at 4 C. Samples were stored at –20 C until they were analyzed.

DNA fragments were separated using a LI-COR 4200 DNA sequencer and standard electrophoresis protocols for fragment analysis (LI-COR Biosciences, Lincoln, Nebraska). A fluorescently labeled 350-b ladder and SAGA v. 3.2.0 software (LI-COR

Table 3. Probability values for the likelihood ratio test (first row) and Chi-square test (second row) for Hardy-Weinberg equilibrium and the exact *P*-values estimated when the alternate hypothesis is heterozygote deficiency (third row) for striped bass collected in four geographic locations in Texoma Reservoir on the Texas–Oklahoma border. Sample size is smaller than the number of collected fish since some samples did not amplify. Probabilities ≤ 0.05 are considered significant.

Population	*n*	*SB6*	*SB20*	*SB91*	*Msa5–11*	*Msa5–71*	*Msa5–190*
Red River	67	0.28	0.07	0.67	0.16	0.43	0.11
		< 0.01	0.01	0.03	0.16	0.43	0.10
		< 0.01	0.11	0.57	0.13	0.30	0.09
Washita	74	0.99	0.21	0.70	0.49	< 0.01	0.53
		< 0.01	0.11	0.78	0.49	< 0.01	0.61
		< 0.01	0.22	0.37	0.34	1.00	0.95
Main pool	52	0.61	0.58	1.00	< 0.01	0.53	0.71
		0.02	0.69	0.88	< 0.01	0.53	0.76
		< 0.01	1.00	0.54	< 0.01	0.36	0.25
Below dam	55	0.61	0.40	0.98	0.95	0.61	0.08
		< 0.01	0.53	0.99	0.95	0.61	0.10
		0.09	1.00	0.94	0.64	0.80	0.99

Biosciences, Lincoln, Nebraska) were used to size each fragment. Samples that did not amplify at all loci were excluded from the analysis. The actual sample size for each population is shown in Table 3.

In order to evaluate changes over time, average gene diversity as described by Weir (1996) was calculated for recently collected samples and those collected in 1978. A Student's *t*-test was used to detect significant differences between samples under two conditions, one in which all samples were used and another using a random sub-sample of the recent collections to account for differences in sample size. Given that inbreeding within populations as well as population genetic structure may lead to departures from Hardy-Weinberg equilibrium (HWE) or linkage equilibrium (LE), we first explored possible genetic structure in the dataset using pair-wise F_{ST}'s among sites and a standard AMOVA implemented in ARLEQUIN, and the Bayesian clustering method implemented in STRUCTURE (Pritchard et al. 2000). STRUCTURE analyses were performed under the admixture model for one to four possible clusters using 10,000 burn-in steps and 100,000 Markov chain Monte Carlo simulations. Following this we tested each site within Lake Texoma for deviations from HWE at all loci using "Genepop" exact tests (Raymond and Rousset 1995) and likelihood ratio and Chi-square tests using "POPGENE" (Yeh and Boyle 1997). We evaluated departures from LE using the likelihood ratio test implemented in ARLEQUIN version 3.1 (Excoffier et al. 2005).

Results

Mean gene diversity of the two most polymorphic loci, SB6 and SB91, was 0.77 for the historic samples (n = 44), 0.85 for the recent samples (n = 248), and 0.84 when using the subset of randomly selected recent samples (n = 44). In both comparisons, the differences were statistically significant with P = 0.02 and 0.01, respectively. We also observed a greater number of alleles at these loci among the recent samples compared to those collected in 1978, with a total of 25 alleles in the recently collected subset, 38 in the entire sample, and 17 among samples collected in 1978. Thus, each estimate of genetic diversity within Lake Texoma suggested that genetic diversity has not decreased and in fact has increased since 1978. Primer sequences, PCR conditions, microsatellite loci characterization, and observed heterozygosity (H_o) for striped bass collected in Lake Texoma, Texas-Oklahoma, are presented in Table 2.

Pair-wise F_{ST}'s among sites were small (mean 0.013 ± SD 0.008) but significant for four of six comparisons. Non-significant values were obtained for comparisons between the Red River arm and the main pool as well as the Washita River arm and the Red River below the dam. AMOVA analyses indicated that this population structure was significant overall (F_{CT} = 0.0127, P < 0.00001). Bayesian clustering analyses resulted in nearly equal probabilities of any individual within Lake Texoma originating from any geographic site (Table 4) and resolved a single panmictic population as the most likely structure of the data set. This was true when considering only the Washita and Red River arms of Lake Texoma or when using the four geographic sites (Figure 1).

Only sporadic departures from HWE or LE were observed and the majority of tests across all populations and loci suggested that allele frequencies and the association of alleles among loci conformed to HW and linkage expectations (Table 3). Of 72 total estimates for departures from HWE, two likelihood tests, eight Chi-square tests, and four exact tests for heterozygote deficiency demonstrated significant deviation. The main pool population at the Msa5–11 locus was the only instance in which all three tests suggested significant departure from HWE and the SB6 locus was

Table 4. Results of Bayesian clustering showing the proportion of assigned membership for each predefined population. Results showing proportions when considering only the Red River and Washita populations are in parentheses.

	Inferred cluster			
Population	I	II	III	IV
Red River	0.243 (0.500)	0.259 (0.500)	0.256	0.242
Washita	0.24 (0.501)	0.237 (0.499)	0.255	0.264
Main pool	0.260	0.265	0.246	0.229
Below dam	0.256	0.246	0.239	0.260

the one locus for which a majority of the tests indicated deviation from HWE. Of 60 total estimates for departures from linkage equilibrium seven were significant and a maximum of three significant departures were found within any single site (Red River arm).

Discussion

Variation at the microsatellite loci described herein differs from previous studies in terms of size, range, and numbers of alleles (Table 2). The size difference was partially attributed to the use of M13 (-29) F-tailed primers for fluorescent detection, which added 19 base pairs. We reported a larger size range for SB6 and SB91, as well as more alleles (21 and 17, respectively, vs. 9 each) than were previously described although the observed heterozygosities were similar to the original account (Han et al. 2000, Roy et al. 2000). In the case of SB91, the differences in alleles were attributed to our larger sample size than that of the initial description (n = 44; Roy et al. 2000). The original description for the SB6 locus was based upon a similar sample size (e.g., n = 256), although the samples were acquired from South Carolina rivers (Han et al. 2000) and may have represented a more homogeneous population than that of Lake Texoma which was stocked with fish derived from South Carolina, Virginia, and New York populations (Table 1). Conversely, for SB20 we report fewer alleles (2 vs. 5), a smaller size range, and a reduced heterozygosity of 0.11 compared to 0.46 despite a larger sample size than that described by Roy et al. (2000).

Temporal changes in allele frequencies among striped bass populations have been reported. Diaz et al. (1998) reported significant temporal changes in PCR-restriction fragment length polymorphism of the SB14 locus among annual spawning cohorts of striped bass in the Santee River sampled between 1992 and 1994, although this was not observed among other loci and populations within the Santee-Cooper system. Similarly, Rogier et al. (1985) found some evidence of temporal instability among striped bass populations in the spawning tributaries of Kerr Reservoir, Virginia. In contrast, Wirgin et al. (1993) reported temporal stability of mtDNA major length genotypes in striped bass populations of the Atlantic coast. In the present study, it is unclear what role hatchery stockings had in the apparent increase in genetic diversity. Records indicated the reservoir was stocked only twice since 1978, and both of those stockings involved relatively few fish, which were derived from broodfish collected from Lake Texoma (Table 1). On the other hand, the reservoir was stocked annually between 1967 and 1974 and again in 1977 with fish from transplanted populations within Oklahoma and from out-of-state sources. One or more of these stockings could have resulted in the introduction of new alleles that were not detected in the 1978 sample.

Population genetic structure among the four sites was significant but weak based on these analyses. Population subdivision was not indicated in a separate study of striped bass collected in 2000 from the main pool and the Red River and Washita arms (ODWC, unpublished data). Conversely, Diaz et al. (1998) reported significant differences in allele frequencies for one of three loci examined as strong evidence for population subdivision among striped bass populations in the Santee-Cooper system. However, the Santee-Cooper population was indigenous and derived from free-ranging striped bass (Scruggs 1957) whereas the Texoma population is allochtonous. Anadromous populations of striped bass exhibit seasonal migrations (Waldman et al. 1990) although some populations, especially those at the extreme of their range, remain near their natal streams (Dudley et al. 1977). Rogier et al. (1985) stated it unlikely that landlocked populations of striped bass would develop strong homing capabilities due to the relatively short residency time in the natal stream prior to deposition in downstream reservoirs. The degree of philopatry among the Texoma striped bass is unknown, but may account for the weak population structure resolved here.

Overall, sporadic departures from HWE (Table 3) and LE did not identify a clear pattern suggestive of recent inbreeding, and the levels of heterozygosity reported herein are similar to those reported in other studies (Han et al. 2000, Roy et al. 2000). Thus, it was more likely that departures from HWE were more likely due to genetic drift or sampling bias rather than inbreeding.

The Texoma striped bass population exhibited weak signals of population structure and no significant evidence of inbreeding. It is plausible that repeated stockings were beneficial in terms of gene diversity. Despite high fecundity, mortality rates for embryos and larvae are high with daily rates up to 19% for postlarvae (Uphoff 1989), and the probability of reproductive success for any individual striped bass is minimal on an annual basis (Secor 2000). However, hatchery practices are designed to protect young fish during their most vulnerable life stages. The significant change in gene diversity over time, as well as the increase in alleles among the recent collections compared to samples from 1978, was most probably the result of repeated stockings. While Diaz et al. (1998) cautioned against augmenting striped bass in the Santee-Cooper system with hatchery stocks, transplanted populations such as that in Lake Texoma may have benefited from supplementary stockings even if natural recruitment was sufficient to sustain populations.

In summary, genetic diversity among striped bass has actually increased as demonstrated by gene and allelic diversity. Population structure was weak to nonexistent. And finally, there was no significant evidence of recent inbreeding among striped bass.

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