Fisheries Session

Natural Reproduction of White Bass × Striped Bass Hybrids in a Texas Reservoir

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Abstract: Natural reproduction of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) was documented in Lake Palestine, Texas. Electrophoresis and isoelectric focusing analysis indicated 29% of the sampled *Morone* spp. were non F-1 hybrids. These individuals apparently resulted when F-1 hybrids reproduced with white bass and/or F-1 hybrids. Age analysis revealed the non F-1 hybrids were from years when hybrid striped bass were not stocked.

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Hybrid striped were first produced in a hatchery in April 1965 (Bishop 1967). The objective of this hybridization was to produce a fish similar to the striped bass but more adaptable to inland reservoirs. Initial evaluations of this hybrid indicated faster early growth than striped bass. Hybrids also attained larger sizes than white bass. In addition, survival of stocked fry was high, enabling fishery managers to create a fishery with less expense and effort than that required for striped bass (Bishop 1967). The need for a large predator which would provide a fishery and act as a biological control for gizzard shad *Dorosoma cepedianum* prompted the stocking of this hybrid in many southeastern states.

The ability of hybrid striped bass to successfully reproduce in reservoirs was concern to many early researchers. Williams (1971) conducted fecundity studies and reported that both sexes matured and participated in spawning activites at one year of age. However, he was unable to confirm successful natural reproduction. Bishop (1967) produced F-2 hybrids using hormone injected females and naturally ripened males. Back-crosses were also produced using hybrid males and striped

bass females. A high degree of deformity was reported in the back cross fry. He cautioned that natural reproduction and/or back-crossing could create a serious fishery management problem if stunting or a high percentage of deformity resulted.

Concern has developed in several states that hybrid striped bass were reproducing naturally in reservoirs. Occurrence of fish which were morphologically indistinguishable from hybrids and which belonged to year classes when hybrids had not stocked stimulated electrophoretic assessment of hybrid populations by Avise and Van Den Avyle (1984). They reported only 6 of 642 fish sampled from the Savannah River drainage as having non F-1 genotypes. Their analyses of specimens from other locales indicated a higher degree of admixture of white bass and striped bass stocks. They concluded this admixture was due to either the natural reproduction of hybrids or mistaken hatchery production and stocking of later-generation hybrids or back-crosses.

In Texas, hybrid striped bass were first introduced in Lake Bastrop in 1972. The success of this stocking stimulated interest in hybrids and the number of lakes stocked grew rapidly. To date, 87 public inland lakes have been stocked with 23.3 million hybrid striped bass.

Questions arose in 1985 when what appeared to be young hybrids were collected from Lake Palestine. Hybrids had not been stocked in this lake since 1982. The purpose of this study was to examine possible natural reproduction of hybrid striped bass in Lake Palestine, Texas.

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Methods

Lake Palestine is a 10,320-ha multi-purpose impoundment located on the Neches River in Henderson, Cherokee and Smith counties, Texas. Hybrid striped bass fingerlings were stocked in 1978, 1979, and 1982 at a rate of 2.1, 1.8 and 2.5 per ha, respectively. A substantial fishery developed for hybrids in 1984 when the annual harvest was estimated at 0.379 kg/ha (Inman 1985). When initially stocked, no other *Morone* species were present in the lake. However, in 1984 white bass were collected for the first time during a fishery management survey. These fish were reportedly introduced by fishermen (Inman 1985).

Multifilament gill nets were used to collect specimens for electrophoretic analysis. Nets were 61 m long and 2.4 m deep, and consisted of 7.6 m sections with mesh size increasing from 12.7 mm square mesh to 101.6 mm square mesh by 12.7 mm increments. Nets were fished overnight for 3 nights each month from December 1985 to February 1986. All *Morone* spp. were placed on ice and returned to the laboratory. Total length and weight were recorded for each fish. Otoliths were removed and stored in envelopes and aged according to methods described by Synder

et al. (1983). Liver and white muscle tissue were excised from the fish, placed in numbered vials, frozen, and transported to the electrophoresis laboratories where they were stored at -40° C.

Electrophoresis

Liver and white muscle tissue samples were individually prepared for electrophoretic analysis by homogenizing in an equal volume of TRIS-HCL (pH 7.0) and centrifuging at 4° C for 20 minutes at 12,500 rpm. The supernatants were frozen at -60° C prior to evaluation. Horizontal starch gel electrophoresis was performed using the techniques of Selander et al. (1971), as modified by Harvey et al. (1980).

Three diagnostic enzyme loci were evaluated: superoxide dismutase (SOD) and esterase (EST) in the liver tissue and phosphoglucoisomerase (PGI) in the white muscle tissue. Descriptions of the banding patterns at these 3 loci are found in Avise and Van Den Avyle (1984). Allele designations at each loci also follow Avise and Van Den Avyle (1984) with white bass alleles designated as A, striped bass alleles designated as B, and hybrid genotypes designated as AB.

Isoelectric Focusing

White muscle tissue was homogenized in an equal volume of distilled water and centrifuged at 2000 rpm for 10 minutes. Supernatants were pipetted and frozen until thawed for analysis.

Gels were prepared according to LKB Application Note 2217 (LKB-Produkter AB) with some modifications Radola (1980). Gels were run using the LKB Multiphor II electrofocusing unit, the LKB Multitemp II circulating water bath, and the LKB 2117 power supply. The temperature of the water bath was held at 10° C during the entire focusing period.

While the anolytes, catholytes, and running conditions outlined in the LKB Application Note appear to give good results, we found that modifications of various components of the procedure produced better protein resolution. We chose to use a catholyte solution (pH 11.5) of 2.0 M ethylenediamine, 0.025 M argine, and 0.025 M lysine. The anolyte solution (pH 2.8) consisted of 0.025 M aspartic acid and 0.025 M glutamic acid.

Running conditions were modified from those in the Application Note. The power was set at 4.0W and current was adjusted until the initial voltage was 200. Corresponding current value was usually between 10.0 and 15.0 milliamps. Voltage was limited to a maximum of 2,000 and samples were run until the current remained constant for 10 to 15 minutes. This usually took place 2 to 3 hours after the start of the run. The sample application mask was removed after the gel had focused for 30 minutes.

After focusing, gels were removed from the Multiphor II unit and "fixed" for 5 minutes in 200 ml of 20% trichloroacetic acid. Gels were then washed for 200 ml of destaining solution which consisted of a solution of 35% ethanol and 10% acetic

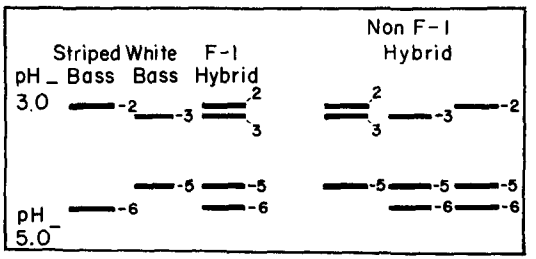

Figure 1. Typical isoelectric focusing bands of striped bass, white bass, F-1 hybrids, and non F-1 hybrids. Note absence of band in non F-1 hybrids.

acid. Proteins were stained with a 300 ml volume of staining solution (0.5% Coomassie Blue R-250 in destaining solution). Gels were destained with several changes of destaining solution and then allowed to air dry.

White bass and striped bass can be discriminated using a series of protein bands that focus in the pH 3 to pH 5 area of gel (Harvey and Fries 1985). Each species is characterized by a pair of proteins unique to that individual species. The F-1 hybrid demonstrates all 4 of these protein bands while demonstrable non F-1 hybrids lack 1 or more of the 4 proteins (Fig. 1). Fish were classified as white bass, striped bass, F-1 hybrid, or non F-1 hybrid using these criteria.

Result and Discussion

A total of 41 *Morone* spp. were collected and analyzed by isoelectric focusing of discriminatory sarcoplasmic proteins and electrophoresis of known discriminatory isozymes. Electrophoresis indicated the sample consisted of 21 white bass, 11 F-1 hybrids, and 9 non F-1 hybrids. Isoelectric focusing indicated the sample consisted of 21 white bass, 13 F-1 hybrids, and 7 non F-1 hybrids. Eight individuals were "misclassified" using 1 technique or the other. Of these, 3 fish identified as being either an F-1 hybrids or white bass by electrophoresis were identified as being non F-1 hybrids by isoelectric focusing. Conversely, 5 fish identified by isoelectric focusing as either F-1 hybrids or white bass were identified as non F-1 hybrids by electrophoresis. Either technique is adequate for screening *Morone* populations as both will yield a conservative estimate of the percentage of non F-1 individuals in that population. If a fish was identified as a non F-1 by either technique, then it was characterized as a non F-1 hybrid. When results from both techniques are combined the total number of non F-1 hybrids in the sample is 12.

Avise and Van Den Avyle (1984) reported some limitations associated with the use of allozymes to identify later-generation hybrids or backcrosses. They reported that expected frequencies of "misclassification" can be calculated based on the expected probabilities that first-generation backcrosses, and that F-2 hybrids will exhibit parental or F-1 hybrid genotypes. For later-generation backcrosses the problem of distinguishing hybrid genotypes become far more severe. Even though some misclassification of individual fish can occur using electrophoresis techniques, extensive hybrid reproduction can be demonstrated (Avise and Van Den Avyle 1984).

Table 1. Numbers and comparisons of genotypes using three marker loci and sarcoplasmic proteins in samples of white bass, striped bass, and their hybrids from Lake Palestine. A = white bass alleles: B = striped bass alleles; Protein numbers are: 3,5 = white bass proteins, 2,6 = striped bass proteins.

N	Protein system (Electrophoresis)			Taxon	Banding patterns (Isoelectric	Taxon
41	SD	EST	PGI	assignment	focusing)	assignment
20	AA	AA	AA	white bass	#3,5	white bass
1	AA	AA	AA	white bass*	#2,3,5	non F-1
9	AB	AB	AB	F-1 hybrid	#2,3,5,6	F-1 hybrid
1	AB	AB	AB	F-1 hybrid*	#2,5,6	non F-1
1	AB	AB	AB	F-1 hybrid*	#2,3,6	non F-1
1	AB	AB	AA	non F-1	#2,3,5	non F-1
1	AB	AB	AA	non F-1	#2,3,5,6	F-1 hybrid*
1	AB	AA	AB	non F-1	#3,5	white bass*
1	AB	AA	AA	non F-1	#3,5,6	non F-1
1	AB	AA	AA	non F-1	#2,3,5,6	F-1 hybrid*
1	BB	AB	AB	non F-1	#2,3,5,6	F-1 hybrid*
1	BB	AB	AB	non F-1	#2,3,5	non F-1
1	AB	BB	AB	non F-1	#2,5,6	non F-1
1	AB	AA	BB	non F-1	#2,3,5,6	F-1 hybrid*

Asterisk (*) indicates non-agreement in techniques.
Underlined individuals are homozygous for striped bass alleles at one discriminatory isozyme locus.

In Lake Palestine, 29% of the *Morone* spp. sampled were identified as non F-1 hybrids. These individuals were the result of F-1 hybrids reproducing with white bass and/or F-1 hybrids. Four fish demonstrated isozyme loci that were homozygous for a characteristic striped bass allele (Table 1). This indicates they were likely produced by F-1 hybrids reproducing with F-1 hybrids.

Ages indicated non F-1 hybrids were from 1983, 1984, and 1985 year classes. F-1 hybrid striped bass were from the 1979 and 1982 year classes. Stocking records were reviewed and no F-1 hybrid striped bass were assigned to a year class which did not correspond to a documented stocking. In addition, no non F-1 hybrids were old enough to have been accidentally stocked by hatcheries. Avise and Van Den Avyle (1984) reported that mistaken hatchery production and stocking of later-generation hybrids or backcrosses could occur because most stocking programs depend on procurement of brood fish from "wild" stocks.

Management Implications

The extent of hybrid striped bass reproduction in Lake Palestine was not determined. However, the fact that it has occurred is of importance to fishery managers who have or will use this fish in fishery management programs. Later-generation hybrids or backcrosses could compete with white bass, F-1 hybrid striped bass, and/or striped bass. Deformed progeny, as reported by Bishop (1967) and Ware (1970), could occur. Additionally, there is the threat of gene-pool contamination.

Since most striped bass and hybrid striped bass programs procure broodfish from wild stocks, contamination may result in the use of backcrossed or later generation hybrids as brood fish. This could change philosophies on stocking rates and frequency.

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