

## Using Genetics in the Design of Red Drum and Spotted Seatrout Stocking Programs in Texas: A Review

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**Abstract.**—In response to stock identification and hatchery-product evaluation concerns, the Texas Parks and Wildlife Department conducted extensive genetic surveys on populations of red drum *Sciaenops ocellatus* and spotted seatrout *Cynoscion nebulosus* to estimate baseline levels of genetic variation, delineate population structure, and identify rare genes that could be useful as genetic markers for assessing supplemental stocking programs. Significant clinal variation at the locus that codes for aspartate aminotransferase-2 and in average individual heterozygosity was observed in spotted seatrout. Allozyme analyses suggest that both spotted seatrout and red drum within the areas surveyed are single, randomly mating populations. Rare alleles that could function as genetic markers were identified in both species. These genetic surveys have led to modifications of the respective species' supplemental stocking programs. For example, presence of clinal genetic variation in spotted seatrout allele frequencies has resulted in the development of three distinct broodstocks representative of the upper, middle, and lower Texas coasts. To evaluate stocking success, marker alleles were chosen for red drum and spotted seatrout. Research was conducted to test inheritance and viability of the selected alleles. No differences in growth were observed among fish of various ages possessing all possible marker allele genotypes, and the alleles appeared to be inherited according to simple Mendelian expectations.

Prior to 1981, red drum *Sciaenops ocellatus* in Texas supported commercial and recreational fisheries. Declining populations in the late 1970s, attributable to growth and recruitment overfishing, resulted in a ban on commercial sale of red drum and strict harvest regulations on sportfishing (TPWD 1985). Additional regulations were imposed throughout the 1980s to increase population abundance in the coastal bays and to augment recruitment into the Gulf of Mexico spawning population.

The spotted seatrout *Cynoscion nebulosus* is a vital recreational resource along much of the U.S. Atlantic coast and throughout the Gulf of Mexico. In addition to experiencing high fishing harvest, geographic populations throughout the spotted seatrout's range are susceptible to environmental perturbations. Recently, spotted seatrout abundance on the Texas coast has been reduced by severe freezes (McEachron et al. 1984) and red tide *Ptychodiscus brevis* (Hammerschmidt 1987). These in-

cidents, combined with increased recreational fishing demand (Meador and Green 1986), have led to changes in management policies, such as restricted size, bag, and possession limits (TPWD 1985).

The Texas Parks and Wildlife Department (TPWD) has initiated marine stocking programs to reverse the decline of red drum and spotted seatrout populations. Many of the immediate effects of the red drum stocking program have been assessed by TPWD (Bumgardner et al. 1990). Survival of stocked red drum has been evaluated by stocking out of season and tagging (Matlock et al. 1984). Due to intrinsic variability in growth, the former method is useful in identifying the contribution of hatchery-produced individuals for only about 1 year. The latter method is generally cost prohibitive because of low recapture rate and poor tag retention (Bumgardner et al. 1990).

An effective marking method is needed to determine the contribution of the stocked fish to the red drum population and recreational fishery; this tool

also will be needed to determine the contribution of stocked spotted seatrout. Furthermore, extensive knowledge of the population structure of red drum and spotted seatrout inhabiting the Texas coast, including the identification of stocks, is needed to facilitate prudent long-range management decisions.

Gene marking, which has proven successful in evaluating stocking programs of reservoir walleye *Stizostedion vitreum* (Murphy et al. 1983) and anadromous salmonids (Seeb et al. 1986) may be a viable method for assessing red drum and spotted seatrout stocking programs. Using allozymes as genetic markers can provide all the information (e.g., growth, movement, survival, population estimation, and stock contribution) commonly obtained using artificial and other biological tagging methods. Furthermore, selectively bred genotypes (i.e., fish with genetics markers) mark a large number of individuals without the handling stress and mortality associated with other tagging methods. In addition, the mark will endure for the life of the fish and constitutes a heritable trait. Therefore, gene marking also allows estimation of reproductive contributions of stocked fish.

In response to stock identification and hatchery-product evaluation concerns, TPWD conducted extensive allozyme surveys on red drum and spotted seatrout populations. Ancillary to determining stock structure, the investigations were designed to estimate baseline allele frequencies and to identify rare alleles that could be used as genetic markers for assessing supplemental stocking programs. The objectives of this paper are to (1) review the population structure of red drum and spotted seatrout inhabiting the Texas Gulf Coast as suggested by allozyme analysis, (2) review the gene marking experimentation, and (3) briefly summarize the influence of genetic analyses on present management strategies such as broodstock management and on the development of stock assessment programs using gene marking.

#### Allozyme Surveys

##### Red Drum

An extensive survey of allozyme variation in adult (offshore) and juvenile (inshore) red drum from North Carolina to southern Texas suggested that genetically differentiated subpopulations occur in the northern Gulf of Mexico and along the southern Atlantic coast (Gold et al. 1993). Gold et al. (1994) analyzed allele frequencies for nine polymorphic loci by year-class and reported an absence of tem-

poral and spatial allelic heterogeneity among inshore and offshore year-classes and among all geographic populations surveyed in the northern Gulf of Mexico. Similarly, five geographic populations of juvenile (1986 and 1987 year-classes) red drum from Texas exhibited an effective absence of genetic subdivision, a high degree of genetic similarity, and homogeneous allele frequencies.

##### Spotted Seatrout

Spotted seatrout from Texas bays and estuaries demonstrated levels of genetic variability, gene flow, and population differentiation similar to that reported in other portions of the species range (King and Pate 1992; Ramsey and Wakeman 1987). Examination of spotted seatrout allelic and genotypic frequencies at seven polymorphic loci revealed low levels of genetic variability and clinal patterns of geographic variation in *sAAT-2\** (which codes for aspartate aminotransferase, enzyme number 2.6.1.1. [IUBNC 1984]) allele frequencies and average individual heterozygosity (King and Pate 1992). A statistically significant association (cline) existed in the frequency of the *sAAT-2\*80* allele with respect to degrees north latitude and west longitude. Frequency of the *sAAT-2\*80* allele increased from 0.9% in fish from east Texas to 17.1% in fish from Mexico. A statistically significant association also existed in average individual heterozygosity with respect to degrees north latitude and west longitude. Tests of heterogeneity indicated allele counts at *sAAT-2\** were distributed heterogeneously among the 12 localities.

#### Gene Marking

Both allozyme surveys identified gene loci with allele frequencies favorable for producing a selectively bred gene mark. Moreover, selected loci were resolvable using noninvasive sampling (e.g., fin tissue). The frequency of the selected alleles was sufficiently low that individuals homozygous for the marker would be rare in the wild population. Based on these criteria, the *PEPB\*85* allele (tripeptide aminopeptidase, 3.4.-.-), which averaged 7% coast-wide, was chosen for the spotted seatrout gene marking study. In red drum, four loci (*ESTD\** [esterase-D, 3.1.-.-], *PEPB\**, *PEPD\** [proline dipeptidase, 3.4.13.9], and *PEPS\** [peptidase-S, 3.4.-.-]) were identified as potential markers. The dimeric esterase locus *ESTD\**, was chosen primarily because stain ingredients are less toxic, less costly, and the products can be resolved much more quickly than can peptidase stains. The *ESTD\*95* allele av-

eraged 6% along the Texas Gulf Coast. Both target loci were di-allelic (*PEPB*\*-100 and \*-85; *ESTD*\*100 and \*95).

From fall 1991 to spring 1993, more than 700 red drum and 600 spotted seatrout from the entire Texas coast were screened for rare alleles. Approximately 50 mature *ESTD*\*100/95 and one mature *ESTD*\*95/95 red drum were collected. Nearly 120 mature *PEPB*\*100/85 and 3 mature *PEPB*\*85/85 spotted seatrout were obtained. These fish were placed into large indoor tanks and subjected to a temperature and photoperiod cycle that induced red drum spawns beginning in late June 1992 and spotted seatrout spawns in August 1992. Research was initiated at the onset of spawning to test basic biological assumptions concerning inheritance and viability of the selected allele.

Progeny of heterozygous red drum broodfish ( $N = 7$  spawns) were cultured to 30 d of age to detect fitness differences among genotypes and to confirm Mendelian inheritance in the  $F_1$  fish (*viz* 1:2:1 ratio of \*100/100:\*100/95:\*95/95). Samples of  $F_1$  progeny ( $N = 100$ ) from these trials revealed little growth differential (as measured by total length in millimeters) among red drum possessing any of the three genotypes (TPWD, unpublished data). Inheritance (i.e., survival) among the three genotypes was variable; however, there was no trend suggesting decreased survival of fish possessing the rare \*95 allele in either the homozygous or heterozygous genotype (TPWD, unpublished data). This preliminary investigation suggests the *ESTD*\*95 is a biologically sound gene marker in that red drum possessing the rare allele appear to perform equally well as fish possessing the common allele. See King et al. (1993) for an overview of the Texas red drum gene marking program. Fish sampled from spotted seatrout pond culture trials are awaiting electrophoretic analysis.

#### Management Implications

Genetic evidence presented in this study suggests that the biological and physical processes necessary for formation of discrete stocks are not present in red drum or spotted seatrout inhabiting the Texas Gulf Coast, and gene flow is sufficient to prevent genetic partitioning. These findings suggest that each of these species should be regulated as a single stock. However, spatial heterogeneity and clinal variation in allele frequencies at the *sAAT-2*\* locus and in average individual heterozygosity were observed in spotted seatrout. Regardless of the mechanism(s) creating the clinal variations, the finding

may indicate genetic or nonheritable variation in physiology and should be incorporated into any long-term management strategy of spotted seatrout populations in Texas.

In light of the clinal variation observed at the *sAAT-2*\* locus and in individual heterozygosity, and given the limited vagility of spotted seatrout, stocking success may be augmented if broodstock were collected at sites environmentally similar to those being rehabilitated. The TPWD's recently established (spring 1992) spotted seatrout population enhancement program has selected broodstock that will assist in preserving the genetic variability present. Specifically, three separate broodstocks from the upper, middle, and lower Texas coasts are maintained at TPWD's Gulf Coast Conservation Association-Central Power and Light-Marine Development Center near Corpus Christi, Texas. Every effort has been made to stock fingerlings produced by these broodstocks in bays and estuaries contiguous to broodstock collection sites. In addition to the stock identification efforts, research has identified an allozyme marker to assist in assessing the success of the supplemental stocking program. Broodstock homozygous for the rare *PEPB*\*85 allele have been spawned and are being reared to maturity. Genetically marked spotted seatrout could be stocked by fall 1994.

A red drum gene marking study, based on a rare allozyme, was implemented in fall 1993. Approximately 250,000, 30-d-old fingerlings were stocked into East Matagorda Bay, Texas. A choice was made by TPWD to stock offspring from heterozygous crosses rather than awaiting the development of homozygous rare (marked) fish. This plan consists of monitoring changes in *ESTD*\*95 frequency (and the absolute number of \*95/\*95 individuals) at the stocking site and adjacent bay systems. The recovery phase, which began immediately, consists of the removal of fin tissue from all fish collected in East Matagorda Bay bag-seine and gill-net samples by TPWD Resource Program personnel. Fin tissue will also be taken from fish during harvest surveys.

Gene marking projects using selectively bred characters raise concerns about potential deleterious effects on the red drum population. Texas Parks and Wildlife Department has gone to unprecedented lengths to assure the utility of the marker alleles and the success of the assessment procedures. The potential benefits of a successful stock assessment should overshadow negative effects the selectively bred fish might impart on the natural population (King et al. 1993).

In summary, allozyme surveys of spotted seatrout

and red drum populations have allowed identification of broodstock that better represent the extant genetic variation in targeted stocking areas. The same surveys have provided a robust mechanism (*viz*, gene mark) for direct estimates of stocking success, abundance, natural mortality, movement, recruitment into the natural population, and reproduction.

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