EVALUATION OF STOCKING SUCCESS IN MARINE WATERS - GENE-MARKING OF RED DRUM AND SPOTTED SEATROUT FINGERLINGS RELEASED INTO TEXAS BAYS

by

Rocky Ward, William J. Karel, and Ivonne R. Blandon

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Coastal Fisheries Division

4200 Smith School Road

Austin, Texas 78744

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A debt of gratitude is owed to the personnel of the Coastal Fisheries Division of Texas Parks and Wildlife Department. The staff of the Enhancement Branch was instrumental in the care of broodfish and in the production and stocking of gene-marked fingerlings. Field crews stationed at East Matagorda Bay, West Matagorda Bay, Galveston Bay, and the upper and lower Laguna Madre collected and documented samples coming from routine resource monitoring and creel surveys over a number of years. Members of the technical staff at the Perry R. Bass Marine Fisheries Research Station were involved in initial work on gene-marked broodfish and fingerlings and were instrumental in cataloging, storing, and processing samples. Dr. Tim L. King (now of the Biological Resources Division of the U.S. Geological Survey) designed the studies and implemented the early stages. Larry McEachron, Robert L. Colura, and Paul Hammerschmidt provided support and direction for this research. Funding was provided by Texas Parks and Wildlife Department and by the U.S. Fish and Wildlife Service Sportfish Restoration Program (project F-36-R, CR012).

ABSTRACT

From 1993-1997, Texas Parks and Wildlife Department (TPWD) released approximately 30 million hatchery-spawned red drum fingerlings (*Sciaenops ocellatus*) and 5 million spotted seatrout (*Cynoscion nebulosus*) fingerlings each year into Texas' bays and estuaries. A number of studies have been employed by TPWD to evaluate the success of these stockings including long-term research utilizing gene-marking. During the years 1993, 1994, and 1995 a total of about 2 million red drum fingerlings were stocked into East Matagorda Bay; of these, 50% were heterozygous and 25% were homozygous for an uncommon allele of a dimeric esterase locus. Subsequent electrophoretic examinations of 6,081 red drum collected from 1993-1997 in TPWD routine resource monitoring or in creel surveys in this bay and in adjacent reaches of neighboring bays found no evidence of increases in frequency of the marker-allele in supplemented year-classes.

During the summer of 1994, over 1.3 million gene-marked spotted seatrout fingerlings were stocked into 3 sites in the lower Laguna Madre and smaller stockings (totaling approximately 82,500) were stocked into 3 sites during the summer of 1995. The percentage of individuals homozygous for the marker allele (Peptidase-B) ranged from 50-60% of the total stocking and the percentage of heterozygous individuals from about 27-50% of the total stocking. Electrophoretic examinations of approximately 7,000 samples collected in the upper and lower Laguna Madre from 1994-1997 suggest spotted seatrout stockings have limited and localized effects on natural populations. Frequencies of marker alleles and individuals homozygous for the marker allele increased only in areas of the lower Laguna Madre adjacent to stocking sites.

INTRODUCTION

Red drum (*Sciaenops ocellatus*) and spotted seatrout (*Cynoscion nebulosus*) have supported recreational and commercial fisheries in Texas since the late 1800s. Management of these fisheries traditionally utilized size limits, bag limits, and closures to protect stocks and reduce conflict between commercial and recreational interests (Heffernan and Kemp 1980, Matlock 1980). Following a decline in population levels during the 1970s, use of nets and sale of wild red drum and spotted seatrout were prohibited in Texas and bag limits and size restrictions were strengthened. As an adjunct to these traditional management actions, the feasibility of stocking as an enhancement tool was considered and spawning and pond culture techniques were developed (Colura et al. 1976, Arnold et al. 1977, Roberts et al. 1978, McCarty et al. 1986, McCarty 1990). Following successful culture trials, over 140 million red drum fingerlings were released into Texas bays from 1983 to 1993 (McEachron et al. 1995). Stockings continue with current yearly goals of approximately 30 million red drum fingerlings released (C. Thibodeaux, personal communication). Spotted seatrout culture techniques (Colura 1974; Colura et al 1990a) and pond protocols were developed in the early 1970s (Colura et al. 1976; Colura et al. 1990b). Spotted seatrout stocking in Texas is limited (about 5 million/year) and considered experimental.

Although stocking efforts in marine waters are controversial (Courtenay and Moyle 1992; Grimes 1998), increasing numbers of marine organisms are being stocked world-wide (e.g., Richards and Edwards 1986; Bartley and Kent 1990; Polovina 1991; Sproul and Tominaga 1992; Smith et al. 1992, 1997; Wespestad et al 1994; Roberts et al. 1995; Fujii and Maruyama 1997; Leber et al. 1995, 1998; Masuda and Tsukamoto 1998; Blaxter 2000). The most common criticisms of these programs have been that they are

ineffective or that they endanger the genetic resources of the species being enhanced (Philipp et al. 1993). The Coastal Fisheries Division of TPWD has been sensitive to these criticisms in designing and evaluating the red drum and spotted seatrout enhancement programs, utilizing the findings of a number of investigations of population structures of red drum and spotted seatrout in the Gulf of Mexico (Weinstein and Yerger 1976; Ramsey and Wakeman 1987; King and Pate 1992; Gold et al. 1993; Gold and Richardson 1994; Gold et al. 1999; Gold et al. 2001) to set guidelines for TPWD marine efforts.

Efforts to document the success of TPWD stockings have employed a number of techniques including out-of-season stockings, statistical analyses of life history and population characters, and stocking of marked individuals (McEachron et al. 1995). Out-of-season stockings were used to investigate survival of hatchery-produced red drum larvae (Matlock 1988; Holt et al. 1994) and fingerlings (Dailey and McEachron 1986). No evidence was found for survival of larvae but fingerlings were recovered up to 45 days post-release. Matlock (1990) compared red drum catch rates in routine resource surveys and in angler landings in a stocked versus an unstocked bay and concluded stockings had been effective.

A number of physical and chemical 'tags' have been used to assess survival of stocked red drum (Buckley and Blankenship 1990; Younck and Cook 1991). In Texas, Matlock et al. (1986) documented survival of out-of-season stocked fingerlings and fingerlings marked with coded-wire tags, finding growth rates equal to naturally-spawned red drum. Exposure to oxytetracycline-HCl (OTC) produced marks in red drum which were identifiable up to 10 months post-release (Bumguardner 1991) and in a second study (McEachron et al. 1998) OTC-marked red drum were recovered up to 598 days

following stocking. However, OTC has potentially deleterious sublethal and lethal effects (Tsukamota et al. 1989; Bumguardner and King 1996) and physical tags may also have adverse effects (Johnsen and Ugedal 1988; Serafy et al. 1995). Bumguardner et al. (1990) studied effects of coded wire tags on survival and growth. Tagged fish grew significantly less than control fish and survival was reduced. Additionally, tag retention was less than 50% after 23 days. The small size at release (approximately 30 mm) may explain the poor results obtained using this technique with red drum in Texas since anchor tags were used successfully with 160-200 mm (TL) red drum stocked in South Carolina (Smith et al. 1997).

Efforts have been made to utilize marks which do not require differential treatment of marked fish. Differences in position of the first annulus of the sagitta otolith may represent a method of differentiating between naturally-spawned and hatchery-produced red drum (Jenkins et al. 1997). Similar phenotypic traits involving color or pattern have been used to evaluate stocking success with *Paralichthys olivaceous* in Japanese waters (Sproul and Tominaga 1992; Kitada et al. 1992) and have been suggested to be potentially useful markers to evaluate a proposed enhancement program for summer flounder (*P. dentatus*) in the northeast United States (Duffy and Nardi 1997).

Variant DNA or DNA products (proteins) provide a wealth of potentially useful markers that may be used to examine the success of fish stockings and to assess interactions of hatchery-produced fish with wild fish (Ferguson 1994; Wilson and Donaldson 1998). Nuclear and mitochondrial DNA markers (Danzmann et al. 1991) have been used effectively to assess stocking success, but the most heavily utilized molecular markers have been electrophoretically detectable protein variants (Allendorf and Utter 1979; Murphy et al. 1983; Seeb et al. 1986; Altukhov and Salmenkova 1990;

Koppelman et al. 1992; Jordan and Youngson 1992; King et al. 1995). Genetic-marks are inexpensive in terms of the time and resources needed to produce large numbers of marked fish, do not require special or differential handling of marked fish prior to release, are not lost over time, and may be transmitted to the next generation of individuals.

Effective gene-marking studies involve a number of steps (Gharrett and Seeb 1990; King et al. 1993). First, a comprehensive survey should be completed to determine the distribution of allelic variation across the range of the species. This survey will also identify potential marker alleles. Second, marker allele candidates must be shown to exhibit inheritance patterns which are predictable, which usually means yielding ratios approximating Mendelian expectations. Third, the candidate alleles must be shown to confer no obvious advantage or disadvantage in survival or growth. Selection for such traits is possible in fish intended for stocking (Taniguchi et al. 1997) and may be an unintended outcome of retention of broodstock heterozygous or homozygous for a marker-allele. Finally, a monitoring program must be in place to evaluate changes in the frequency of the marker-allele following stocking.

The studies described in this manuscript incorporated the guidelines suggested by Gharrett and Seeb (1990) and King et al. (1993) to assess survival of hatchery produced red drum and spotted seatrout in Texas marine waters. Broodfish were selected for production of offspring bearing a genemark with no apparent effects on growth or survival. Fingerlings (F_1) were stocked into a primary bays. Individuals subsequently collected in routine resource monitoring programs or creel surveys were electrophoretically screened for homozygous or heterozygous expression of the marker allele to test the null hypothesis of no enhancement of year-class strength. Rejection of the null hypothesis required statistically significant increases in frequency of the marker-

allele in enhanced year-classes compared to expectations based on non-enhanced year-classes from the same or comparison bays.

METHODS

Red drum

Initial screening of allele frequencies. The frequency and temporal stability of allozymes in red drum collected along the Texas coast were evaluated from published surveys (Gold et al. 1993; Gold et al. 1994). Alleles from polymorphic loci identified in these studies were considered as candidates for a genetic marker. The *ESTD*95* allele of the dimeric esterase locus (E.C. number 3.1.1..) was chosen based on frequency (about 6%), ease of staining, and because it could be resolved from fin-clips allowing non-lethal sampling (King et al. 1995).

Relationship of phenotype to survival and growth. Broodfish scored as heterozygous for the *ESTD*95* allele were maintained in groups of eight to ten individuals in indoor 5,000 l circular tanks equipped with biofiltration systems. Efforts were made to maintain equal sex ratios in each tank. Spawning was effected through manipulation of temperature and photoperiod (Arnold et al. 1977). Following spawning, fertilized eggs and larvae were maintained for 48 hours in 400 l incubators. Two-day-old larvae were stocked into 0.1 ha earthen ponds at the rate of about 75,000/pond. This procedure was repeated for eight separate spawns. Temperature in the ponds averaged 28^{o}C and salinity averaged 29 ‰. Thirty days after each spawn fingerlings were harvested and 100 individuals were sacrificed for determination of size and genotype. Total length (nearest mm) and weight (1/100 g) were determined for each fingerling. Muscle tissue was then excised from the posterior body of each fish and ground in an equal volume of buffer (Selander et al. 1971). Homogenized samples were centrifuged at

10,000 rpm for 10 min. Supernatant was absorbed onto filter papers which were placed onto gels prepared as 12% suspensions of hydrolyzed starch (Starch Art Corporation, Smithville, Texas). Gel and electrode buffers were tris-citrate, pH-8.0 (Selander et al. 1971). Gels were electrophoresed at 60 mA for approximately two hrs. Following electrophoresis, a 1.5 mm thick slice was taken from the middle of the gel. The slice was covered with a solution composed of 4-Methylumbelliferyl acetate dissolved in acetone then mixed in a 50 mM sodium acetate solution (Manchenko 1994) and incubated at room temperature for approximately 5 min. Resulting *ESTD** bands were visualized under long-wave UV light and immediately scored.

Approximately 2,000 30-day-old fingerlings from spawn 1 were stocked into a 0.2 ha earthen pond for long-term grow-out. Seventy-five fish were collected by rod and reel 320 days post-stocking. Each fish was individually measured and weighed. Fin tissue was removed from each fish and assayed to determine allelic variation at the *ESTD** locus using the same histochemical techniques as employed with muscle. Differences in length and weight were examined using a χ^2 approximation of the Kruskal-Wallace test (SAS Institute 1985). Contingency χ^2 was used to test for deviations of observed phenotypic ratios from ratios expected based on Mendelian inheritance (1:2:1).

Stocking gene-marked red drum. Gene-marked broodfish were maintained at the TPWD/Coastal Conservation Association/Central Power and Light Marine Development Center. Maintenance of broodfish, inducement of spawning, and treatment of eggs, fry, and fingerlings followed established guidelines (McCarty 1990). A total of 247,931 F_1 fingerlings were stocked into East Matagorda Bay in 1993. This effort was followed by an additional 682,649 F_1 fingerlings in 1994 and 1,119,167 F_1 fingerlings in 1995. Releases were carried out by TPWD Coastal Fisheries Division hatchery personnel and

followed established TPWD procedures for red drum stocking. A sample of 100 fingerlings from each harvest were retained to determine genotypic ratios of stocking cohorts.

Collection and treatment of samples. Beginning in August 1993, fin-clips were taken from every subadult red drum caught during the routine resource monitoring program in East Matagorda Bay, Galveston Bay west of the Galveston Island Causeway, and West Matagorda Bay east of Palacios Point. All juvenile red drum from these areas caught during routine resource monitoring bag seine efforts were retained whole on ice until frozen (-20^{o} C) upon return to the field station. Fin clips were taken from red drum encountered in creel surveys if they were reported to have been captured in the target areas. Special efforts were made to meet anglers returning from fishing in East Matagorda Bay and fin clips were taken from their red drum catch. Total length was recorded for each red drum sampled. Fin clips were frozen in water (-20^{o} C) upon return to the field station. All samples were transported to the Perry R. B. Marine Fisheries Research Station at the earliest possible date where they were stored at -85^{o}C until processed. Juvenile red drum and fin clips were processed and electrophoretically examined as described above. Fin clips were homogenized in a lesser volume of buffer (approximately 1:2).

Analysis of stocking success. Age of individual fish were assigned from age-length keys (based on TPWD analyses of otolith age determinations) assuming a biologically realistic hatching date of 1 October (Murphy and Taylor 1990). Age-length keys were applied to observed total length to assign individuals to that year-class with the highest probability of correct assignment.

Some variables generated by genetic analyses are unlikely to meet assumptions of parametric tests of inference. To combat this, tests of difference between means (paired-sample *t*-tests; SAS Institute 1985) were conducted on the ranks of the variables. Strength of relationships between stocking intensity and subsequent estimates of marker-allele frequencies were explored using Spearman correlation analysis. The probability that r_S is significantly greater than 0 was tested by routines resident in the Proc Corr program (SAS Institute 1985).

Frequencies of *ESTD** alleles in East Matagorda Bay, West Matagorda Bay, and Galveston Bay were examined for conformance to Hardy-Weinberg expectations using the computer program BIOSYS I (Swofford and Selander 1981). This program was also used to estimate mean heterozygosity (*H*-bar) and heterogeneity of allele frequencies among bay/year-class groupings.

Spotted seatrout

Initial screening of allele frequencies. The frequency and temporal stability of allozymes in spotted seatrout collected along the Texas coast were evaluated from published surveys (King and Pate 1992). Alleles from polymorphic loci identified in these studies were considered as candidates for a genetic marker. The *PEPB*A* allele of the peptidase-B locus (E.C. number 3.4.11..), utilizing leu-gly-gly as substrate, was chosen based on frequency (about 7%), ease of staining, and because it could be resolved from fin-clips allowing non-lethal sampling (King et al. 1995).

Relationship of phenotype to survival and growth. Over 1,300 potential broodfish were collected from the vicinity of Port Mansfield in the lower Laguna Madre using rod and reel, fin clipped, lip tagged, and transported in hauling trailers to the Perry R. Bass Marine Fisheries Research Station. Histochemical techniques followed

Manchenko (1994) and tissue preparation and electrophoretic techniques were identical to that utilized in the red drum. Individuals scored as homozygous or heterozygous for the *PEPB*A* allele were maintained in groups of eight to ten individuals in indoor 5,000 l circular tanks equipped with biofiltration systems. Efforts were made to maintain equal sex ratios in each tank. Spawning was effected through manipulation of temperature and photoperiod (Colura et al. 1990c, 1991). Following spawning, fertilized eggs and larvae were maintained for 48 hours in 400-l incubators. Two-day-old larvae from 14 separate spawns were stocked into separate 0.1 ha earthen ponds at a rate of about 65,000/pond and maintained according to protocols described by Colura et al. (1976). Thirty days after each spawn fingerlings were harvested and 100 individuals were sacrificed for determination of size and genotype. Total length (nearest mm) and weight (1/100 g) were determined for each fingerling. Genotype for each individual was determined in the manner described above. Fingerlings from several spawns were stocked into a 0.2 ha earthen pond for long-term grow-out. A sample of 100 fish was collected from the pond by rod and reel 180 days post-stocking and approximately 400 fish at 360 days post-stocking. Each fish was individually measured and weighed. Fin tissue was removed from each fish and assayed to determine allelic variation at the *PEPB** locus. Differences in length and weight were examined using a χ^2 approximation of the Kruskal-Wallace test (SAS Institute 1985). Contingency χ^2 was used to test for deviations of observed phenotypic ratios from ratios expected based on Mendelian inheritance (1:2:1). Individuals determined to be heterozygous or homozygous for the *PEPB*A* allele were retained for use as broodfish.

Stocking gene-marked spotted seatrout. Gene-marked broodfish were maintained at the PRBMFRS hatchery facility for the 1994 stocking season and transferred to the

TPWD/Coastal Conservation Association/Central Power and Light Marine Development Center for the 1995 season. Maintenance of broodfish, inducement of spawning, and treatment of eggs, fry, and fingerlings followed established guidelines (McCarty 1990). Approximately 1,300,000 gene-marked fingerlings were stocked into three sites in the lower Laguna Madre in the summer of 1994 (Table 7; Figure 3). This effort was followed by an additional 82,500 fingerlings stocked in three sites in 1995. Releases were carried out by TPWD Coastal Fisheries Division hatchery personnel and followed established TPWD procedures for spotted seatrout stockings. A sample of 100 fingerlings from each harvest was retained to determine genotypic ratios of stocking cohorts.

Collection and treatment of samples. Beginning in August 1994, fin-clips or muscle plugs were taken from spotted seatrout encountered during the routine resource monitoring and harvest programs in the lower and upper Laguna Madre. Total length was recorded for each spotted seatrout sampled. Sample preparation, electrophoresis, and visualization were similar to that described for red drum except for differences in stain preparation.

Analysis of stocking success. Statistical analysis followed that of the red drum study.

RESULTS

Red drum

Survival and growth. Among the eight spawns maintained for a 30-day period, six exhibited phenotypic ratios that were significantly different from the 1:2:1 ratio expected with a Mendelian system of inheritance (Table 1). Of those six, two had heterozygote deficiencies and four had heterozygote excesses. Overall, an excess of

heterozygotes was noted. Five of the six spawns not exhibiting Mendelian ratios had excesses of the *ESTD*100* allele, surpassing the *ESTD*95* allele overall by a ratio of 1.24:1. Red drum sampled at day 320 were found to have phenotypic ratios not significantly different from Mendelian predictions (χ^2=4.65, $p < 0.01$). Excesses in the *ESTD*100* allele were still evident, with a ratio of 1.34:1.

Length and weight of fingerlings at 30 days varied among the eight spawns (Table 2) with mean lengths for some samples (e.g., spawn 4; $\overline{X}$ = 44.66 mm, *SD* = 47.66) being more than twice that observed in other samples (e.g., spawn 8; $\overline{X}$ = 21.20 mm, *SD* = 75.98). Mean weight was found to be even more variable, with spawn 4 ($\overline{X}$ = 1.22 g, *SD* = 47.84) being many times greater than spawn 8 ($\overline{X}$ = 0.09 g, *SD* = 76.56).

Statistically significant differences in length among phenotypes were observed in two of the eight spawns examined (Table 2). In both cases, heterozygotes were found to be of greater length than homozygotes for either allele. Four spawns exhibited statistically significant differences in weight among phenotypes. As with length, heterozygotes were consistently heavier than homozygous siblings. No statistically significant differences among phenotypes in length or weight were observed at 320 days (Table 3).

Evaluation of stocking success. Frequencies of the marker allele (*ESTD*95*) in East Matagorda Bay ranged from 0.056 in year-class 1993 to 0.10 in year-class 1990 (Figure 1) with a mean of 0.062. In Galveston and West Matagorda Bays, the frequency of the marker-allele ranged from 0.041 to 0.070 and 0.0541 to 0.127 respectively, with means of 0.065 and 0.068. Mean heterozygosity increased during each year of stocking

(Figure 2), however the overall correlation between stocking rate and mean heterozygosity for East Matagorda Bay was negative (r_S = -0.30, p = 0.47).

All but 3 of 20 bay-year-class combinations were found to be in Hardy-Weinberg equilibrium (Table 4). Of those three, only one was from a stocked year-class (1993 in East Matagorda Bay) and in that comparison the number of heterozygotes observed was less than expected.

Frequency of the marker allele across bays and year-classes did not differ significantly from expected frequencies (χ^2 = 23.466, p = 0.22). Year-classes receiving supplemental stockings did not exhibit significantly greater frequencies of the marker allele than the same year classes in comparison bays (East Matagorda compared with Galveston Bay, t = 0.50, p = 0.667; East Matagorda compared with West Matagorda Bay, t = 1.00, p = 0.423). Significant differences in marker-allele frequency were observed between enhanced and non-enhanced year classes in East Matagorda Bay (t = 4.38, p = 0.005), however differences in means were in the opposite direction from that predicted by the one-tailed hypothesis. A positive correlation was found between stocking rate and marker-allele frequency but the correlation coefficient was not significantly different from zero (r_S = 0.50, p = 0.667).

Spotted seatrout.

Survival and growth. Over 120 potential broodfish were identified that were either homozygous or heterozygous for the *PEPB*A* allele. The 14 ponds stocked with larvae produced by these broodfish were harvested at 30 days post-stocking. No statistically significant differences in length between the three genotypes were observed (Table 5), suggesting comparable growth. No deviations from Hardy-Weinberg expectations were noted, indicating survival was not related to genotype. The three

genotypes demonstrated no differences in growth at 180 and 360 days (Table 6) and Hardy-Weinberg equilibrium was met at these sampling dates.

Evaluation of stocking success. Gene-marked broodfish produced an estimated 1,275,000 fingerlings (Table 7) that were stocked into 3 sites in the lower Laguna Madre in 1994 (Table 8). Of these, 59.4% were homozygous for the marker allele, 27.1% were heterozygous, and 13.5% were homozygous for the alternate allele. In 1995, poor pond survival resulted in the stocking of an estimated 82,565 fingerlings into 3 lower Laguna Madre sites. Approximately half of these stocked fingerlings were homozygous for the marker allele and half were heterozygous.

Approximately 7,000 samples were taken from the lower and upper Laguna Madre Fall and Spring resource and creel surveys from spring 1994 through spring 1997 (Table 9). Each sample was classified as adjacent (if taken from resource samples in or adjacent to a stocked grid), distant (taken from resource samples in lower Laguna Madre not adjacent to a stocked grid), creel (taken during a creel survey), or ULM (taken from the upper Laguna Madre). An additional classification of LLM combined adjacent, distant, and creel samples from the lower Laguna Madre. Of the 35 total frequency distribution groupings, one failed to meet Hardy-Weinberg expectations.

Comparisons of observed genotypic frequencies with baseline (1994) frequencies (Table 10; Figure 4) found three statistically significant differences. One comparison, ULM in Fall 1996 ($\chi^2 = 8.377$, $P < 0.05$), had an paucity of heterozygotes relative to baseline. The other two statistically significant comparisons, adjacent in Fall 1995 ($\chi^2 = 18.915$, $P < 0.05$) and adjacent in Spring 1996 ($\chi^2 = 14.062$, $P < 0.05$), had excesses of the genotype homozygous for the marker allele. Comparisons of marker-allele

frequencies among the different distance classes (Figure 5) demonstrates the same increase in the Fall 1995 sampling season.

DISCUSSION

Gene-marking results provide evidence that stockings of red drum fingerlings into East Matagorda Bay during the years 1993, 1994, and 1995 were ineffective. No effect was seen for three stocking efforts, at three different stocking rates, made across three years. Failure to find evidence of enhancement following stocking of over 1.5 million gene-marked red drum fingerlings may be due to several factors. First, it's possible that the stockings were effective but the technique employed to assess enhancement, allozyme electrophoresis, was unable to resolve relevant changes in marker-allele frequency. This possibility assumes error in technique or interpretation of sufficient magnitude to render the results unreliable. This is unlikely given the correspondence between allele frequencies observed in preliminary surveys (about 6%; King et al. 1995) and the frequencies described in this report. Additionally, the finding of adherence to Hardy-Weinberg expectations of a majority of bay/year-class combinations (17 of 20) is evidence that misinterpretation of gels was not a major factor.

A second possibility is that stockings were successful but increases in marker-allele frequencies were masked by emigration from stocked regions. This is unlikely since the entire stocked bay and adjacent bays were monitored and no obvious increases were seen in marker-allele frequency in any area, though movement to the Gulf of Mexico would not have been detected.

A more likely possibility is that some portion of the gene-marked red drum did survive, but the contribution of this cohort to the total population was masked by the numbers of naturally-spawned red drum present at stocking sites. It is also conceivable

that full or nearly full utilization of available habitat by naturally-spawned red drum precluded enhancement by stocking. Routine resource monitoring surveys found East Matagorda Bay to have among the highest catch-rates of red drum during the three years in which stocking of gene-marked red drum occurred (Unpublished TPWD data), suggesting that habitat availability may have limited the success of these stocking efforts.

It does not necessarily follow from these results that red drum fingerling stockings will be ineffective under all conditions. In a review of a variety of studies directed at determining the effectiveness of red drum enhancement efforts in Texas, McEachron et al. (1998) found indications that stocking effectiveness varied widely. Multiple regression analyses found strong correspondence between stocking rate and estimated population size for two Texas bays (including East Matagorda Bay); however, three other bays showed distinctly weaker correspondence and four bays demonstrated no evidence of enhancement. Perhaps the strongest evidence McEachron et al. (1998) report for the effectiveness of enhancement of red drum in Texas waters emerged from length-frequency analyses comparing an unstocked area, Cedar Lakes, with a number of bays which had received Spring (out-of-season) stockings. Not all stocked bays showed this effect, suggesting that some enhancement efforts were effective while others were not. Additional studies are needed to determine the factors which predict stocking success with red drum.

Results of the spotted seatrout gene-marking study suggested that this fishery can be enhanced using large-scale stocking efforts. Increases in the incidence of the homozygous genotype in the lower Laguna Madre provided evidence that 30-day-old fingerlings can survive the stocking process and positively impact the total population for months afterward. The presence of individuals homozygous for the marker allele in the

Fall of 1996 suggests the effect may persist for at least two years since the 1995 stockings were so limited.

Tagging studies have documented spotted seatrout movement between bays and between a bay and the Gulf of Mexico (Marwitz 1989), however such movements may be limited in frequency and distance (e.g., Iverson and Tabb 1962) and may represent seasonal migration to and from spawning and foraging sites (Baker and Matlock 1993). In light of this, it is interesting that increases in numbers of homozygous genotypes were only noted in individuals obtained from grids adjacent to stocking sites. This suggests enhancement effects of stockings may be localized.

Failure to observe bay-wide enhancement may be due to a number of factors. The intensity of the stockings may have been insufficient to increase allele frequencies in non-adjacent sites, suggesting that increased stocking rates may result in greater enhancement. It is also possible that spotted seatrout exhibit marked site fidelity, reducing bay-wide enhancement effects to non-detectable levels. If this is proven to be true then efforts will need to be directed at dispersing stocked spotted seatrout fingerlings as much as possible.

Demonstrations of successful spotted seatrout stocking makes efforts to reduce competition between hatchery-spawned and naturally-spawned conspecifics critical. Stocking efforts must be shown to enhance the total population, not replace naturally produced individuals, if stockings are to be an effective part of a management program.

The critical next stage in the scientific evaluation of spotted seatrout stockings is to determine the degree to which hatchery-reared individuals compete with naturally spawned individuals. Failure to make this evaluation leaves the enhancement program

open to serious criticisms of its effectiveness and potential detrimental effects on the ecology and genetics of the impacted population.

Gene-marking studies utilizing different molecular markers to assess stocking success in other bays are warranted. Comparisons of year-class strength in unstocked, Spring-stocked, and Fall-stocked bays may provide valuable data concerning stocking success, and correspondence between these measures of success and measures of habitat availability may be crucial to designing an effective enhancement program.

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Table 1. Analysis of allele frequency distributions for eight spawns of gene-marked red drum sampled at 30 days of age. Chi-square test of deviation from frequencies expected from conformation to Mendelian ratios (1:2:1).

Spawn	N	*ESTD** Phenotype			χ^2
		100/100	100/95	95/95	
1	91	22	50	19	1.09
2	21	13	6	2	15.38**
3	82	28	46	8	10.97**
4	55	10	40	5	12.27**
5	84	34	48	2	26.10**
6	89	16	55	18	5.05
7	61	11	43	7	10.79**
8	68	14	27	27	7.85*
Overall	551	148	315	88	24.39**

Table 2. Kruskal-Wallis Test (Chi-square approximation) for differences in length and weight between *ESTD** phenotypes of 30-day-old gene-marked red drum. Standard deviations of length and weight are presented parenthetically. Degrees of freedom = 2 for all comparisons.

Spawn	Genotype (N)	Length			Weight		
		Mean	χ^2	*P*	Mean	χ^2	*P*
1	100/100 (22)	35.59 (107.2)	6.17	0.046	0.40 (107.8)	6.17	0.046
	100/95 (50)	37.52 (124.5)			0.47 (125.2)		
	95/95 (19)	36.21 (101.7)			0.43 (102.3)		
2	100/100 (13)	25.08 (12.8)	1.46	0.481	0.16 (13.5)	1.73	0.422
	100/95 (6)	24.67 (11.9)			0.14 (12.6)		
	95/95 (2)	26.00 (7.7)			0.16 (8.2)		
3	100/100 (29)	31.52 (103.8)	5.96	0.051	0.34 (104.3)	6.22	0.045
	100/95 (46)	31.57 (108.2)			0.33 (108.7)		
	95/95 (8)	27.87 (64.3)			0.21 (64.6)		
4	100/100 (9)	40.33 (43.0)	1.10	0.576	0.68 (43.1)	2.23	0.327
	100/95 (40)	46.07 (50.5)			1.40 (50.7)		
	95/95 (5)	41.20 (33.4)			0.76 (33.5)		
5	100/100 (34)	37.73 (108.7)	2.59	0.275	0.41 (109.6)	2.43	0.296
	100/95 (48)	37.60 (109.6)			0.42 (110.5)		
	95/95 (2)	40.00 (33.8)			0.51 (34.0)		
6	100/100 (16)	36.37 (92.7)	2.71	0.258	0.39 (93.5)	2.27	0.072
	100/95 (55)	37.02 (117.3)			0.44 (118.3)		
	95/95 (18)	36.06 (96.9)			0.39 (97.8)		
7	100/100 (11)	28.82 (52.9)	15.46	0.001	0.23 (53.2)	14.19	0.001
	100/95 (43)	31.65 (62.8)			0.30 (63.1)		
	95/95 (7)	29.43 (43.9)			0.26 (44.1)		
8	100/100 (14)	21.86 (65.1)	1.73	0.422	0.11 (65.6)	0.57	0.751
	100/95 (27)	21.59 (78.8)			0.09 (79.4)		
	95/95 (27)	20.48 (78.8)			0.08 (79.4)		
Totals	100/100 (148)	32.93			0.34		
	100/95 (315)	35.25			0.49		
	95/95 (88)	30.19			0.29		

Table 3. Kruskal-Wallis Test (Chi-square approximation) for differences in length and weight between phenotypes of 320-day-old gene-marked red drum. Standard deviations of length and weight are presented parenthetically. Degrees of freedom = 2 for all comparisons.

*ESTD** Phenotypes	Length			Weight		
	Mean	χ^2	*P*	Mean	χ^2	*P*
100/100 (N=25)	305.00	0.18	0.91	307.20	1.20	0.55
	(19.82)			(63.67)		
100/95 (N=29)	301.17			293.45		
	(31.28)			(71.11)		
95/95 (N=15)	304.27			314.00		
	(18.14)			(85.76)		

Table 4. Frequencies of genotypes for the *ESTD** locus among sampled red drum in three Texas bays. Deviation from Hardy-Weinberg excpectations are examined using chi-square (probability is in parentheses).

	Galveston				E. Matagorda				W. Matagorda			
	100/100	100/95	95/95	χ^2 (*P*)	100/100	100/95	95/95	χ^2 (*P*)	100/100	100/95	95/95	χ^2 (*P*)
1990					4	1		0.000 (1.000)	2			
1991	34	3		0.043 (0.835)	205	31		0.577 (0.448)	18	4		0.162 (0.688)
1992	117	17		0.577 (0.448)	537	68	2	0.006 (0.937)	356	48	1	0.198 (0.656)
1993	111	9		0.161 (0.688)	750	79	7	8.623 (0.003)	456	49	3	4.067 (0.044)
1994	213	29		0.947 (0.330)	1188	140	4	0.002 (0.965)	210	27	2	1.228 (0.268)
1995	227	35		1.302 (0.254)	517	67	1	0.569 (0.450)	44	10		0.502 (0.479)
1996	116	19		0.730 (0.393)	208	34	2	0.251 (0.616	39	11	1	0.087 (0.769)
1997					22	2	1	7.146 (0.008)				

Table 5. Analysis of genotypic frequency distributions from 14 gene-marked spotted seatrout spawns sampled at 30 days of age and combined spawns sampled at 180 and 360 days of age by chi-square test of deviation from Hardy-Weinberg equilibrium as determined from observed allele frequencies. Spawn, expected frequency (parenthetical), partial, and total χ^2 values[a] are given.

Spawn	N	χ^2_{AA}		χ^2_{AB}		χ^2_{BB}		$\chi^2_{Tot.}$
1	96	0.01	(51)	0.061	(39)	0.092	(6)	0.163
2								
3	71	0.595	(21)	1.19	(29)	0.595	(21)	2.38
4								
5	157	0.112	(117)	1.238	(33)	3.411	(7)	4.761
6	134	0.294	(77)	1.621	(43)	2.238	(14)	4.154
7	104	0.003	(36)	0.01	(51)	4.251	(17)	4.264
8								
9	100	0.001	(63)	0.005	(33)	2.438	(4)	2.444
10	97	0.116	(24)	0.259	(52)	0.145	(21)	0.52
11	131	0.881	(66)	0.351	(53)	1.926	(6)	3.158
12	78	0.63	(27)	2.161	(45)	1.855	(6)	4.646
13	99	0.152	(48)	0.649	(38)	0.69	(13)	1.491
14								
180 day	96	0.406	(31)	0.672	(42)	0.278	(23)	1.356
360 day	112	0.03	(36)	0.079	(49)	0.051	(27)	0.16
360 day	304	0.342	(87)	0.792	(148)	0.459	(69)	1.593

[a] No statistically significant values at the 0.05 level.

Table 6. Kruskal-Wallis Test (χ^2 approximation) for differences in length and weight between phenotypes of gene-marked spotted seatrout 30 day spawns and combined spawns of 180 and 360 days of age. Spawns, genotype, sample size, mean length, mean weight, χ^2 values[a], and *P* values are given.

			Length			Weight		
Spawn	Genotype	N	Mean	χ^2	*P*	Mean	χ^2	*P*
1	AA	10	33.2	2.22	0.137	0.35	3.04	0.081
	AB	10	35.5			0.27		
2								
3	AA	16	45.06	1.97	0.374	0.83	2.89	0.236
	AB	21	46.57			0.84		
	BB	15	44.8			0.70		
4								
5	AA	81	35.41	0.72	0.697	0.36	0.69	0.708
	AB	16	34.88			0.33		
	BB	2	42.0			0.62		
6	AA	42	28.1	3.69	0.158	0.21	0.06	0.972
	AB	27	27.3					
	BB	11	28.0					
7	AA	29	36.1	1.52	0.469	0.39	1.5	0.473
	AB	42	37.29			0.45		
	BB	14	36.07			0.038		
8								
9	AA	21	34.43	0.37	0.542	0.35	0.77	0.381
	AB	2	34.0			0.32		

Table 6. (Continued)

			Length			Weight		
Spawn	Genotype	N	Mean	χ^2	*P*	Mean	χ^2	*P*
10	AA	23	42.65	0.89	0.642	0.91	1.63	0.444
	AB	52	45.4			1.16		
	BB	22	44.14			1.07		
11	AA	34	31.62	2.99	0.225	0.25	1.75	0.417
	AB	34	32.71			0.27		
	BB	10	31.7			0.25		
12	AA	21	27.0	5.16	0.076	0.17	0.03	0.987
	AB	40	26.43			0.17		
	BB	5	25.6			0.17		
13	AA	46	30.52	1.67	0.433	0.21	0.76	0.683
	AB	38	30.16			0.2		
	BB	15	30.53			0.2		
14								
180 day	AA	31	171.2	1.59	0.452	42.19	2.0	0.362
	AB	42	166.6			39.09		
	BB	23	175.0			47.36		
360 day	AA	7	291.9	4.836	0.089			
	AB	9	304.8					
	BB	6	327.0					

[a] No statistically significant values at the 0.05 level.

Table 7. Production data on gene-marked spotted seatrout stocked in 1994[a] and 1995[b] in the lower Laguna Madre, Texas. Stocking date, parental genotype, number of fry stocked, harvest date, fingerlings harvested, fingerling size, and percent return are given.

Stocking Date	Parental Genotype	Fry Stocked	Harvest Date	Fingerlings Harvested	Size (mm)	Percent Return
7/24/94	BBxBB	126,825	8/16/94	8,341	33.0	6.6
7/23/94	BBxBB	221,825	8/16/94	115,726	26.0	52.2
7/23/94	BBxBB	266,000	8/16/94	255,437	26.0	96.0
7/25/94	ABxAB	515,375	8/18/94	542,375	28.0	105.4
7/30/94	BBxBB	109,725	8/22/94	36,598	29.7	33.4
7/24/94	BBxBB	283,575	8/22/94	85,371	21.5	30.1
7/30/94	ABxAB	142,500	8/25/94	75,466	23.7	53.0
7/29/94	ABxAB	197,125	8/25/94	139,146	24.2	70.6
7/30/94	BBxBB	98,325	9/1/94	32,592	25.8	33.1
8/3/94	BBxBB	229,900	9/1/94	77,104	20.9	33.5
6/12/95	ABxBB	188,238	7/12/95	28,232	29.7	15.0
7/2/95	ABxBB	303,654	7/27/95	36,515	28.2	12.0
8/5/95	ABxBB	150,000	8/23/95	24,318	30.0	16.2

[a] One additional pond stocking gave zero return.

[b] 12 additional pond stockings gave zero returns.

Table 8. Summary data on gene-marked spotted seatrout stocked in 1994 and 1995 in the lower Laguna Madre, Texas. Stocking date, estimated number stocked, parental genotypes, and stocking sites are given.

Date	Number stocked	Parental Cross	Locality
8/16/94	359,504	BBxBB	Laguna Atacosa Refuge, Cameron County, TX
8/18/94	515,375	ABxAB	East Ranch, Willacy County , TX
8/22/94	119,969	BBxBB	Willacy County Park, Willacy County, TX
8/25/94	214,612	ABxAB	East Ranch, Willacy County , TX
9/1/94	107,696	BBxBB	Willacy County Park, Willacy County, TX
7/12/95	25,232	ABxBB	Willacy County Park, Willacy County, TX
7/27/95	33,515	ABxBB	Queen Isabella Causeway, Cameron County, TX
8/23/95	23,818	ABxBB	East Ranch, Willacy County , TX

Table 9. Analysis of genotypic frequency distributions from spotted seatrout collected from Spring 1994 to Summer 1997 for conformance to Hardy-Weinberg expectations. Collection period, locality, sample size (parenthetical), partial, and total χ^2 values are given. LLM is the lower Laguna Madre and ULM is the upper Laguna Madre.

Collection Period	Locality	N	χ^2_{AA}		χ^2_{AB}		χ^2_{BB}		$\chi^2_{Tot.}$
Spring 1994	Adjacent	40	0.0007	(35)	0.0208	(5)	0.156	(0)	0.1775
Spring 1994	Creel	0[a]							
Spring 1994	Distant	294	0.0038	(259)	0.1268	(35)	1.044	(0)	1.1746
Spring 1994	LLM	334	0.0048	(294)	0.1511	(40)	1.198	(0)	1.3538
Spring 1994	ULM	667	0.0059	(590)	0.1682	(86)	1.2097	(1)	1.3836
Fall 1994	Adjacent	20	0.0001	(18)	0.0053	(2)	0.05	(0)	0.0554
Fall 1994	Creel	56	0	(53)	0.0021	(3)	0.04	(0)	0.0422
Fall 1994	Distant	395	0.0013	(352)	0.0458	(41)	0.4064	(2)	0.4535
Fall 1994	LLM	471	0.0011	(423)	0.0393	(46)	0.34	(2)	0.3805
Fall 1994	ULM	751	0.0083	(652)	0.2215	(93)	1.4809	(6)	1.7107
Spring 1995	Adjacent	24[b]		(24)		(0)		(0)	
Spring 1995	Creel	92	0.0003	(84)	0.0155	(8)	0.174	(0)	0.1898
Spring 1995	Distant	570	0.0003	(516)	0.0094	(53)	0.0793	(1)	0.0889
Spring 1995	LLM	686	0.0004	(624)	0.014	(61)	0.137	(1)	0.1514
Spring 1995	ULM	428	0.0002	(375)	0.0049	(51)	0.0299	(2)	0.035
Fall 1995	Adjacent	18	0.0319	(14)	0.3946	(3)	1.2288	(1)	1.6554
Fall 1995	Creel	130	0.0001	(123)	0.0055	(7)	0.094	(0)	0.0996
Fall 1995	Distant	465	0.0005	(415)	0.0143	(49)	0.1123	(1)	0.127
Fall 1995	LLM	613	0.0003	(552)	0.0101	(59)	0.0891	(2)	0.0995
Fall 1995	ULM	514	0.0061	(464)	0.2175	(47)	1.9431	(3)	2.1667

Table 9. (Continued)

Collection Period	Locality	N	χ^2_{AA}		χ^2_{AB}		χ^2_{BB}		$\chi^2_{Tot.}$
Spring 1996	Adjacent	214	0.0323	(196)	1.2426	(15)	11.9579	(3)	13.2327[c]
Spring 1996	Creel	63	0.0044	(52)	0.0918	(11)	0.48	(0)	0.5762
Spring 1996	Distant	582	0.0007	(521)	0.0273	(60)	0.258	(1)	0.286
Spring 1996	LLM	859	0.0026	(769)	0.0904	(86)	0.7957	(4)	0.8887
Spring 1996	ULM	469	0	(411)	0.0006	(56)	0.0032	(2)	0.0039
Fall 1996	Adjacent	82	0.0002	(76)	0.0006	(6)	0.116	(0)	0.1167
Fall 1996	Creel	89	0.009	(83)	0.4402	(5)	5.4363	(1)	5.8855
Fall 1996	Distant	200	0.0025	(185)	0.1204	(14)	1.445	(1)	1.5679
Fall 1996	LLM	371	0.0061	(344)	0.2971	(25)	3.6217	(2)	3.9249
Fall 1996	ULM	121	0	(115)	0.0037	(6)	0.074	(0)	0.0777
Spring 1997	Adjacent	200	0.0011	(180)	0.0405	(19)	0.3659	(1)	0.4075
Spring 1997	Creel	60	0	(58)	0.0004	(2)	0.017	(0)	0.0174
Spring 1997	Distant	453	0.0023	(413)	0.1006	(38)	1.0905	(2)	1.1934
Spring 1997	LLM	713	0.0037	(651)	0.1509	(59)	1.5518	(3)	1.7063
Spring 1997	ULM	66	0.0001	(61)	0.0073	(5)	0.095	(0)	0.1024

[a] No samples were taken.

[b] All samples were monomorphic for the A allele.

[c] Statistically significant at the 0.05 level.

Table 10. Analysis of genotypic frequency distributions from spotted seatrout finclips collected between spring 1995 and summer 1997 relative to 1994 genotypic frequency baseline[a]. Collection period, locality, sample size, observed N (parenthetical), and χ^2 values (partial and total) are given. LLM is the lower Laguna Madre and ULM is the upper Laguna Madre.

Collection Period	Locality	N	χ^2_{AA}	χ^2_{AB}	χ^2_{BB}	$\chi^2_{Tot.}$
Spring 1995	Adjacent	24	0.321 (24)	2.56 (0)	0.06 (0)	2.941
Spring 1995	Creel	92	0.081 (84)	0.341 (8)	0.23 (0)	0.652
Spring 1995	Distant	570	0.136 (516)	1.02 (53)	0.129 (1)	1.285
Spring 1995	ULM	428	0.021 (375)	0.133 (51)	0.005 (2)	0.158
Fall 1995	Adjacent	18	0.257 (14)	0.608 (3)	18.05 (1)	18.915[b]
Fall 1995	Creel	130	0.449 (123)	3.41 (7)	1.16 (0)	5.019
Fall 1995	Distant	465	0.002 (415)	0.009 (49)	0.022 (1)	0.033
Fall 1995	ULM	514	0.644 (464)	4.729 (47)	0.091 (3)	5.464
Spring 1996	Adjacent	214	0.152 (196)	2.703 (15)	11.21 (3)	14.062[b]
Spring 1996	Creel	63	0.301 (52)	2.709 (11)	0.16 (0)	3.17
Spring 1996	Distant	582	0.013 (521)	0.075 (60)	0.145 (1)	0.233
Spring 1996	ULM	469	0.024 (411)	0.134 (56)	0.039 (2)	0.197
Fall 1996	Adjacent	82	0.12 (76)	0.87 (6)	0.21 (0)	1.2
Fall 1996	Creel	89	0.176 (83)	2.139 (5)	2.766 (1)	5.081
Fall 1996	Distant	200	0.264 (185)	2.536 (14)	0.5 (1)	3.3
Fall 1996	ULM	121	2.244 (115)	5.543 (6)	0.59 (0)	8.377[b]
Spring 1997	Adjacent	200	0.019 (180)	0.198 (19)	0.5 (1)	0.717
Spring 1997	Creel	60	0.389 (58)	3.034 (2)	0.15 (0)	3.573
Spring 1997	Distant	453	0.224 (413)	2.227 (38)	0.67 (2)	3.121

Table 10. (Continued)

Collection Period	Locality	N	χ^2_{AA}	χ^2_{AB}	χ^2_{BB}	$\chi^2_{Tot.}$
Spring 1997	ULM	66	0.226 (61)	1.299 (5)	0.59 (0)	2.155

[a] 1994 observed genotypic frequencies: LLM AA - 0.8907; AB - 0.1068; BB - 0.0025

ULM AA - 0.8697; AB - 0.1254; BB - 0.0049

[b] Statistically significant at 0.05.

Figure Headings

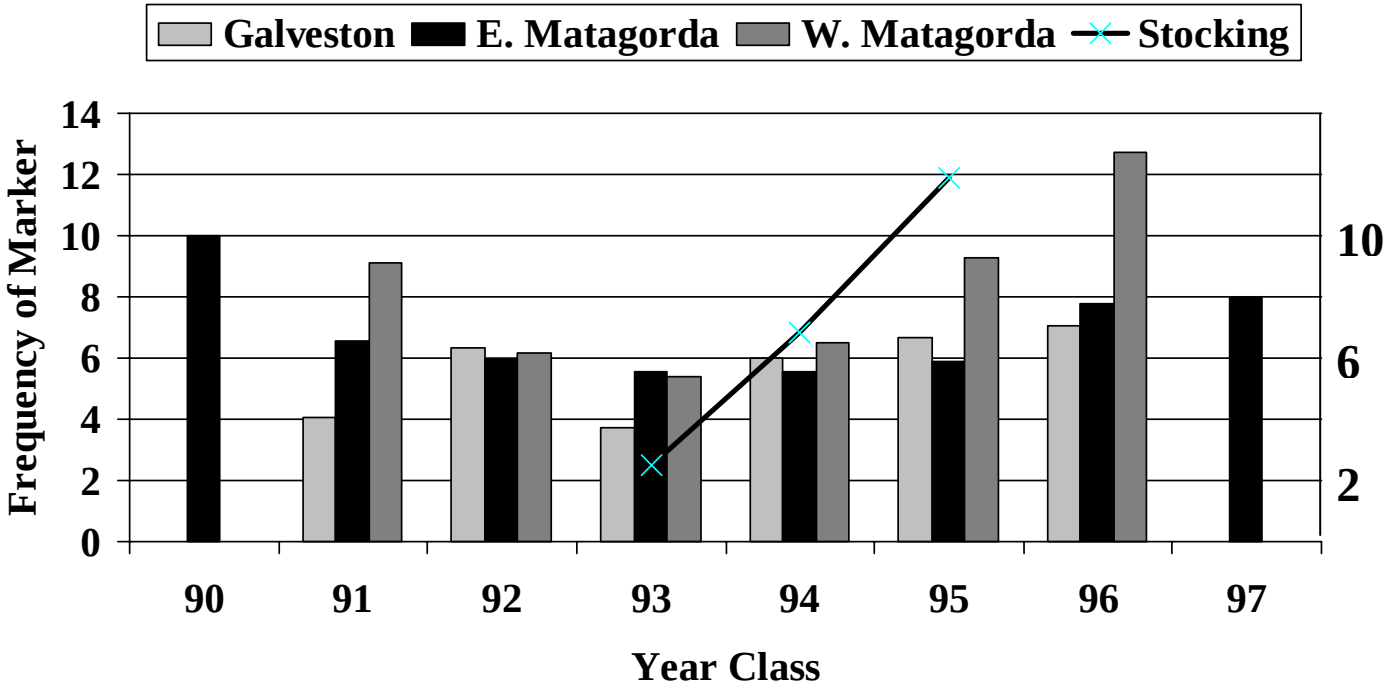
Figure 1. Observed frequency of dimeric esterase marker allele (*ESTD*95*) in Galveston Bay, East Matagorda Bay, and West Matagorda Bay red drum in year classes 1990-1997. Stocking rates of gene-marked red drum in East Matagorda Bay in years 1993, 1994, and 1995 is indicated by the line graph (x 1,000).

Figure 2. Mean heterozygosity of dimeric esterase locus (*ESTD**) in Galveston Bay, East Matagorda Bay, and West Matagorda Bay red drum in year classes 1990-1997. Stocking rates of gene-marked red drum in East Matagorda Bay in years 1993, 1994, and 1995 is indicated by the bar graph (x 1,000).

Figure 3. Map of the lower Laguna Madre system depicting the general locations of gene-marked spotted seatrout stocking sites. The 1994 stocking sites included: 1) East Ranch, 2) Willacy County Park, and 3) Laguna Atascosa. The 1995 stocking sites included : 1) East Ranch, 2) Willacy County Park, and 3) Queen Isabella Causeway.

Figure 4. Frequency of marker allele homozygotes (*f*) at different distances from stocking sites. Collection locales were classified as: 1) adjacent (samples taken within 1 sq. nautical mi. of stocking site), 2) creel (samples from creel surveys within 2 sq. nautical mi. of stocking site), 3) distant (all remaining samples from the lower Laguna Madre), 4) LLM (combined samples from the lower Laguna Madre); and 5) ULM (combined samples from the upper Laguna Madre).

Figure 5. Frequency of marker allele (*f*) at different distances from stocking sites. Collection locales were classified as: 1) adjacent (samples taken within 1 sq. nautical mi. of stocking site), 2) creel (samples from creel surveys within 2 sq. nautical mi. of stocking site), 3) distant (all remaining samples from the lower Laguna Madre), 4) LLM (combined samples from the lower Laguna Madre), and 5) ULM (combined samples from the upper Laguna Madre).



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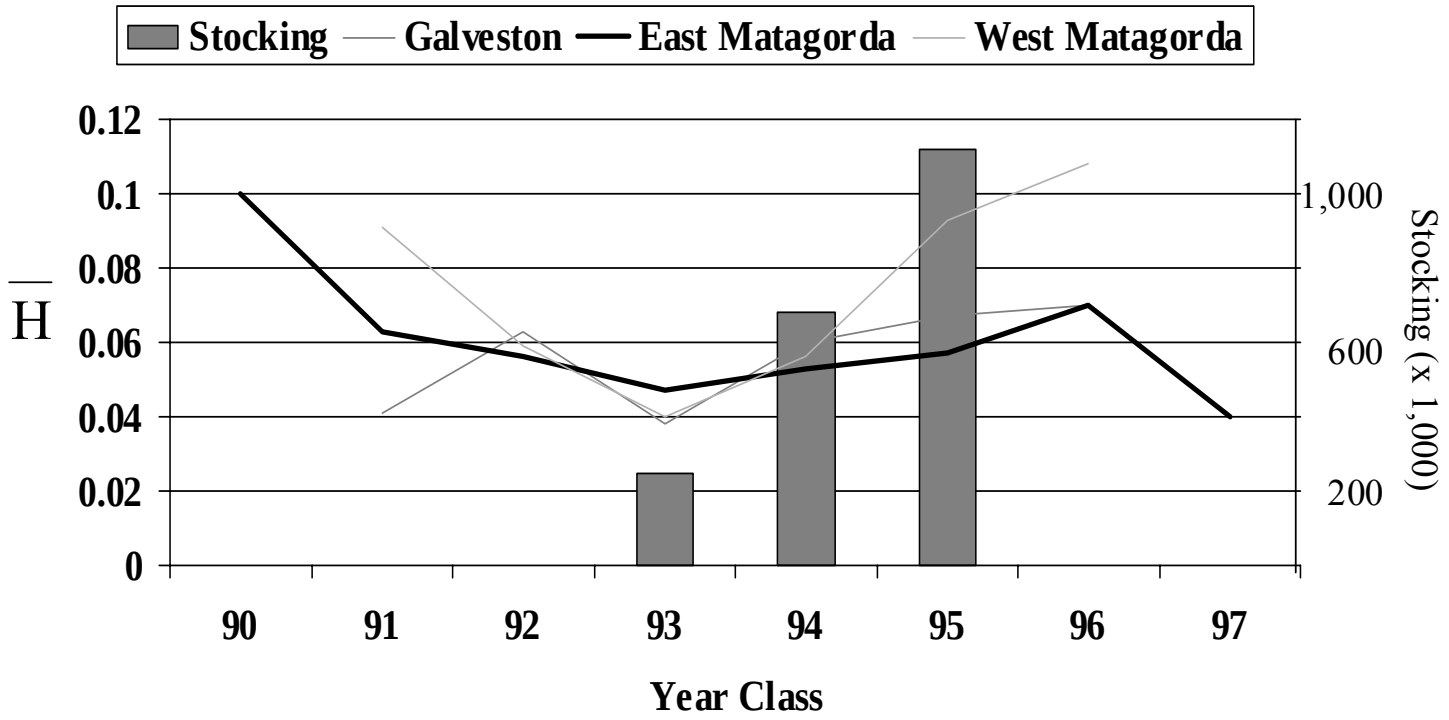

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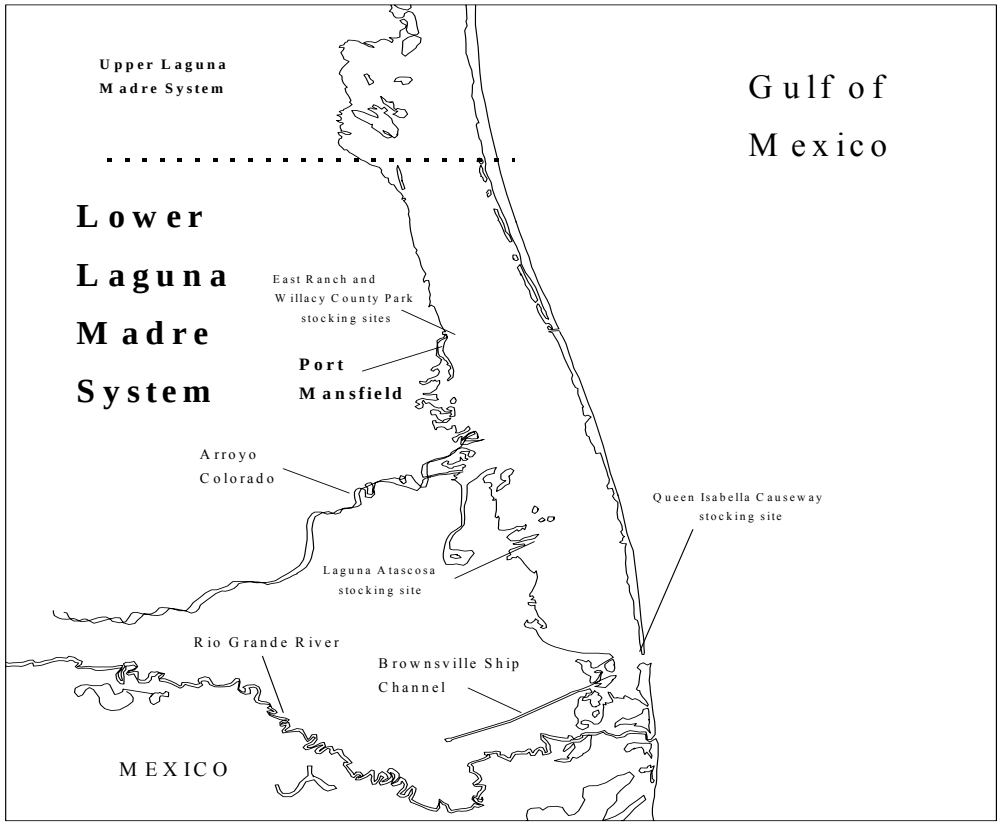

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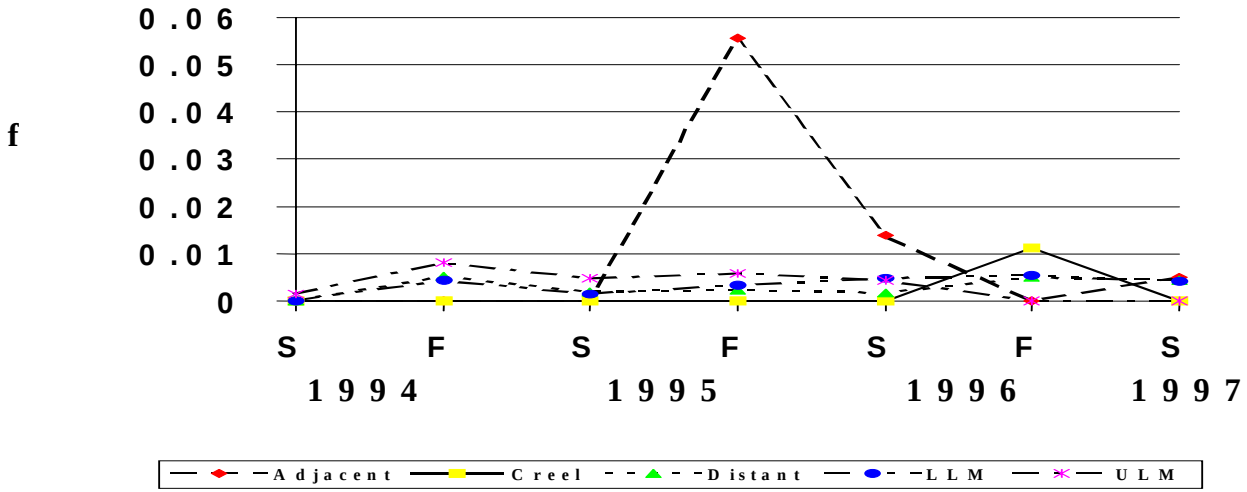

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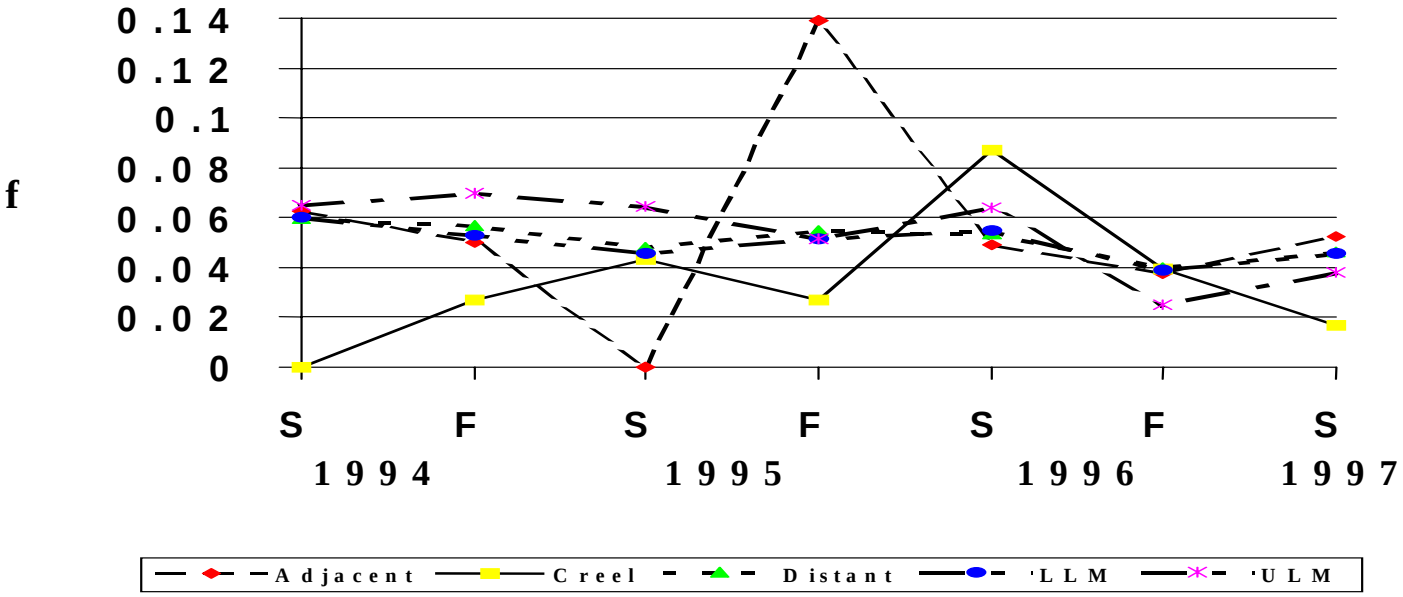

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