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Source: Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science, 2009(2009) : 121-132

Published By: American Fisheries Society

URL: <https://doi.org/10.1577/C09-001.1>

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## A Genetic Assessment of Current Management Strategies for Spotted Seatrout in Texas

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**Abstract.**—The spotted seatrout *Cynoscion nebulosus* is one of the most intensively managed finfishes in the Gulf of Mexico, due primarily to its importance as a sport fish species throughout its range. As a result, genetic divergence and patterns of gene flow have previously been assessed among designated populations of spotted seatrout by using various types of genetic markers in an attempt to provide meaningful data for delineation of management units. However, genetic data can be influenced both by contemporary gene flow and historical demography, and these processes can often result in similar genetic signatures. In this study, a nested clade analysis (NCA) was used to disentangle the effects of historical and contemporary processes on the distribution of mitochondrial DNA sequence haplotypes of spotted seatrout in the western Gulf of Mexico. The NCA was coupled with traditional *F*-statistics and a Mantel matrix procedure to compare the results of multiple analytical frameworks. Overall, genetic divergence among populations was low but significant and was highest between populations that were far apart geographically. Correlation between genetic divergence and geographic distance was supported by a significant Mantel matrix correlation coefficient, as well as two nested clades, which had distributions that correlated significantly with latitude. All three statistical procedures suggest that the genetic structure of spotted seatrout in the western Gulf of Mexico can best be described by continuous change and isolation by distance rather than representing discrete populations. These results are compared to the results of several previous studies using alternative types of genetic data and analytical procedures and are also used to put current management strategies for spotted seatrout into a genetic context.

The spotted seatrout *Cynoscion nebulosus* is unequivocally the most popular recreational marine finfish in Texas. Spotted seatrout currently account for 36% of all landings by private boat anglers and 76% of party boat landings annually in nearshore marine waters of Texas (Green and Campbell 2005). The Texas Parks and Wildlife Department (TPWD) has undertaken intensive efforts towards marine enhancement (stocking) of the species since 1991, and current annual production results in an average release of approximately 3 million fingerlings into Texas bays and estuaries. More traditional strategies for management of spotted seatrout have included indirect efforts, such as a ban on gill nets in Texas bays and estuaries in 1988, and targeted efforts, including daily bag and possession limits, slotted size limits, closure of the commercial fishery, and in-state regionalization of recreational harvest restrictions. As a result, the spotted seatrout is one of the most heavily managed and monitored species in the western Gulf of Mexico (hereafter, Gulf).

Due to the intensive management of spotted seatrout, an effort has been made to identify various types of data that will provide a meaningful basis for designation of management units. Recently, population genetic approaches have emerged as particularly effective means of monitoring populations for conservation and management objectives. This is because the information that can be acquired via genetic monitoring is often otherwise unavailable, and acquisition and analysis of genetic data are often more feasible and reliable than data acquired via traditional monitoring approaches (Schwartz et al. 2006). As a result, population genetic data are being used more commonly in the identification of management units in natural populations. Palsbøll et al. (2006) describe a traditional definition of management units based on demographic connectivity, such that population growth is essentially independent from one deme to the next and is not overly influenced by migration between demes. Such a definition, by extension, may logically result in delineation of management units based on some analog of the “significant divergence” criterion of Moritz (1994), which is based upon the statistical rejection of panmixia (Palsbøll et al. 2006). However, the relationship between demographic connectivity and gross genetic divergence is complex. For instance, a major caveat in this relationship is that divergence is

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Received January 5, 2009; accepted March 26, 2009

Published online June 11, 2009

TABLE 1.—Sample sizes, location codes, and diversity indices for spotted seatrout population samples collected along the Texas coast. The code for each location is the same as that used in the text. Sample data include yearly (2003, 2004, and 2005) and overall sample sizes ( $N$ ), number of haplotypes recovered, haplotype diversity ( $H_d$ ), and nucleotide diversity ( $\pi$ ).

Location	Code	2003	2004	2005	$N$ (bay)	Haplotypes	$H_d$	$\pi$
Sabine Lake	SL	15	15	14	44	12	0.75	0.005
East Matagorda	EM	14	14	—	28	15	0.92	0.007
West Matagorda	MB	14	13	—	27	16	0.94	0.007
San Antonio Bay	SB	15	15	10	40	18	0.90	0.007
Aransas Bay	AB	13	14	15	42	17	0.89	0.006
Corpus Christi Bay	CC	11	15	16	42	16	0.89	0.007
Upper Laguna Madre	UL	—	16	14	30	12	0.87	0.006
Lower Laguna Madre	LL	—	12	15	27	12	0.89	0.007

influenced not only by gene flow but also by historical events occurring on evolutionary time scales, such as range expansion or population fragmentation (Templeton et al. 1995). If the goal of management or conservation is to preserve naturally occurring associations between demes, contemporary effects must be given priority over historical processes. Many traditional genetic statistical analyses, such as those involving population comparisons with  $F$ -statistics (Weir and Cockerham 1984), typically do not discriminate effectively between contemporary and historical processes (Templeton 1998).

The relationship between demographic connectivity and genetic divergence is also complicated by the fact that there are few practical guidelines for relating measured divergence directly to measures of connectivity. For instance, the difference between multiple distinctive subpopulations organized in a single dimension and a single, widely distributed population operating under isolation by distance can be extremely subtle. This has been a particular problem for previous spotted seatrout studies, which have used similar results to come to different conclusions. Ramsey and Wakeman (1987) and King and Pate (1992) used clinal variation in allozymes frequencies to suggest that spotted seatrout in the Gulf constitute a single population with limited long-distance dispersal, resulting in isolation by distance. In contrast, Weinstein and Yerger (1976) and Gold et al. (1999) suggested that spotted seatrout in the Gulf represented multiple demographically isolated subpopulations with limited gene flow even between adjacent estuaries. Microsatellite studies have added little resolution to the issue.

Here, mitochondrial DNA (mtDNA) sequence haplotypes recovered from samples of spotted seatrout on the coast of Texas were assayed and compared to previous population genetic examinations using allozymes (Weinstein and Yerger 1976; Ramsey and Wakeman 1987; King and Pate 1992), mtDNA restriction digest haplotypes (Gold et al. 1999), and microsatellites (Gold et al. 2003; Ward et al. 2007). A

traditional analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to demonstrate gross divergence among populations. Additionally, a nested clade analysis (NCA; Templeton 1998) was employed to disentangle the historical and contemporary effects on contemporary patterns of genetic divergence. These data were gathered with the goal of addressing two important questions regarding spotted seatrout in the northwestern Gulf: (1) how is genetic variation organized among Texas populations of spotted seatrout and (2) are current units of management as defined by TPWD supported by genetic data? To address the first objective, the organizational criteria of Laikre et al. (2005) were used. Laikre et al. (2005) described three basic types of marine population structure: distinct populations, continuous change, and no differentiation. For operational purposes, no differentiation was assumed in the case where there was a lack of significant genetic heterogeneity in haplotype distributions, although a conservative approach dictated that this conclusion must be repeatable across independent studies. Distinctive populations were indicated in the case in which significant structure was found among subpopulations (via AMOVA), which were defined a priori based on residents occurring in the eight major bays or inlets sampled. The reliability of such a result was tested by removing the most extreme sampling areas and repeating the AMOVA. Continuous change was indicated in the case where there was a lack of evidence for distinctive populations but there was significant genetic structure in the form of isolation by distance. The second objective relied on a qualitative analysis of whether the population structure of spotted seatrout on the coast of Texas is effectively supported by current TPWD enhancement practices.

## Methods

*Sample collection and laboratory techniques.*—Young-of-the-year spotted seatrout were collected from eight major bay systems (see Table 1 for location abbreviations) on the Texas coast over 3 years (Figure

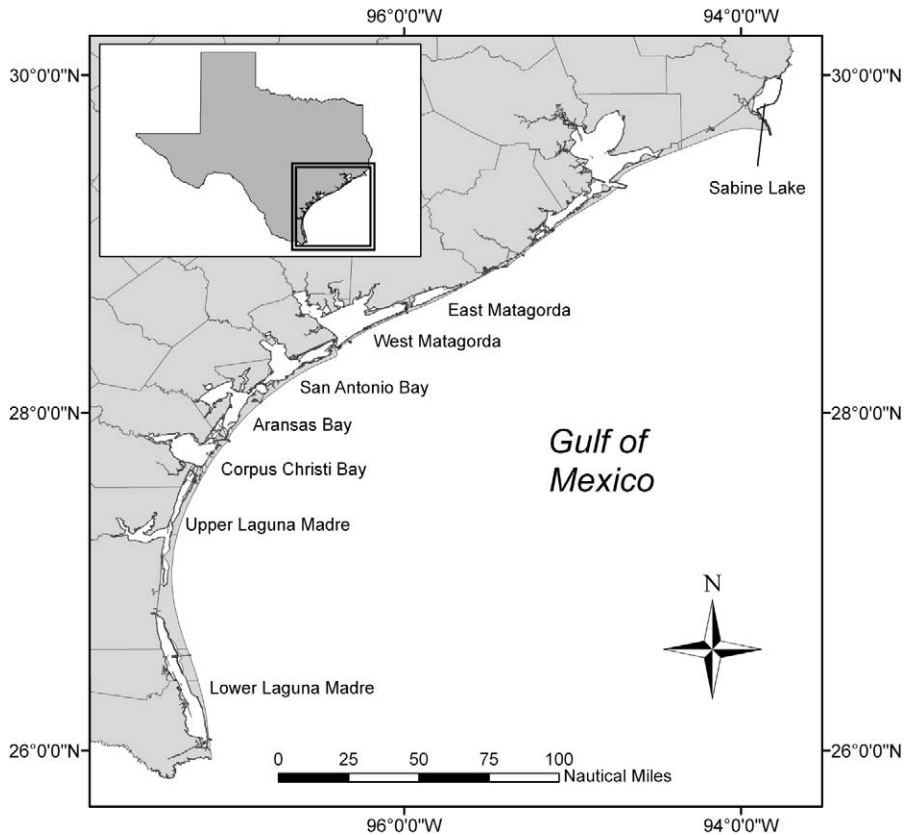


FIGURE 1.—Map of sampling sites for spotted seatrout sequence samples.

1) during the course of routine monitoring by TPWD. Within each bay, care was taken to avoid sampling possible family groups by taking individuals from multiple sampling locales on multiple dates. Samples consisted of either (1) 200-mg or larger fin clips or (2) whole fish in the case of small individuals. Samples were preserved immediately in vials containing a 95% solution of ethanol and were stored for various time periods prior to transport to the laboratory. Total genomic DNA was isolated by using PUREGENE DNA isolation kits (Gentra Systems, Inc., Minneapolis, Minnesota), with a final rehydration volume of 75  $\mu$ L. Finished (extracted DNA) samples were stored at  $-20^{\circ}\text{C}$ .

Sequencing was carried out on a portion of the mtDNA control region. Template DNA samples were amplified via the polymerase chain reaction (PCR) under a modified touchdown protocol by using Ready-To-Go PCR beads (GE Healthcare, Piscataway, New Jersey) on a Techne Genius thermocycler (Techne, Inc., Princeton, New Jersey). Reactions consisted of 1  $\mu$ L of template DNA (50 ng/ $\mu$ L), one Ready-To-Go

bead, and 24  $\mu$ L of forward and reverse primer cocktail (0.4- $\mu$ M standard concentration of each primer), for a total of 25  $\mu$ L. The touchdown PCR protocol utilized for all reactions consisted of the following: an initial single denature period of 2 min at  $95^{\circ}\text{C}$ , 10 cycles of initial amplification ( $95^{\circ}\text{C}$  for 30 s;  $55^{\circ}\text{C}$  for 30 s, lowering  $1^{\circ}\text{C}$  each cycle; and  $72^{\circ}\text{C}$  for 1 min), 20 cycles of primary product amplification ( $95^{\circ}\text{C}$  for 30 s;  $50^{\circ}\text{C}$  for 30 s; and  $72^{\circ}\text{C}$  for 1 min, adding 3 s of extension per cycle), and a single final extension period of 7 min at  $72^{\circ}\text{C}$ . Each sample was amplified twice, and the amplifications were combined to assure a high concentration product following purification. The primer sequences used for PCR (DLoop3: TCAC-CYTRRCTNCCAAAGC, F1: TCACCYTRRCTNCCAAAGC) amplified an approximately 1,000-base-pair (bp) fragment. The PCR products were purified by using QIAquick PCR purification kits (Qiagen, Inc., Valencia, California) and were eluted in 30  $\mu$ L of buffer EB (provided in kit). The DNA fragment concentration was estimated by running purified PCR products out on a 1.5% agarose gel with molecular

mass standards, and approximately 50 ng of DNA were used for sequencing. Sequencing reactions were carried out in 10- $\mu$ L volumes by using Genomelab Quick Start Master Mix DTCS (Beckman Coulter, Inc., Fullerton, California) according to the manufacturer's instructions. Primers specific to an approximately 420-bp internal fragment of the original PCR product were used for sequencing (250F: TAAGATATACCATGCACTC, 670R: GTCATGTATTATGCACTTGA-TATCC). Sequencing reactions resulted in fragments that overlapped one another for 373 bp. The sequencing regime was 30 cycles of (1) denature (96°C for 20 s), (2) annealing (50°C for 20 s), and (3) extension (60°C for 4 min). Sequencing samples were precipitated with a 1/20 volume of sodium acetate-EDTA and glycogen and 2 volumes of a 95% solution of ethanol and were spun at 3,700 rotations/min for 30 min to form pellets. Pellets were rinsed twice with a 70% solution of ethanol, were dried, and then were rehydrated by using sample loading solution (Beckman Coulter, Fullerton, California). Finally, sequences were electrophoresed and analyzed on a Beckman CEQ8000 capillary sequencer (Beckman Coulter) using default sequencing module parameters. Raw sequences were examined for base-calling errors and trimmed manually. Forward and reverse traces for each sequence were aligned by using Sequencher version 4.2 (Gene Codes Corp., Ann Arbor, Michigan), and haplotypes were developed only by sequence that contained overlapping forward and reverse reads. All unique haplotypes have been uploaded to the GenBank database as batch submissions (accession numbers FJ816795-FJ817074) by using Sequin freeware (National Center for Biotechnology Information, Bethesda, Maryland).

*Sequence characteristics and genetic diversity.*—The averaged frequency of each nucleotide and tests for heterogeneity in base frequencies between haplotypes were assessed by using the program DAMBE (Xia and Xie 2001). Additionally, the rate of transitions and transversions between pairwise haplotypes were assessed and used in a substitution saturation test. In this analysis, the rate of transitions and transversions are each plotted relative to pairwise genetic distance in an effort to identify whether transversions approach or outnumber transitions at higher genetic distances, indicating substitution saturation. The genetic distance of Kimura (1980) was employed for saturation testing in DAMBE software. Haplotype diversity ( $H_d$ ) and nucleotide diversity ( $\pi$ ) were assessed for each population and overall by using the program DNAsp (Rozas et al. 2003).

*AMOVA and isolation by distance.*—Correlations between geographic and genetic distances were examined by using two traditional methods. First, a

hierarchical AMOVA procedure (Excoffier et al. 1992) was employed to look for significant associations within populations and within cohorts. Individuals from the same sample site were pooled into separate year-classes, and variance in mtDNA haplotype association was partitioned into between-individual, between-cohort, and between-population effects by using Arlequin version 3.0 (Excoffier et al. 2005). As the AMOVA procedure of Excoffier et al. (1992) requires information regarding the number of substitutions between haplotypes, the genetic distance between pairwise haplotypes was estimated by using the Tamura-Nei distance (Tamura and Nei 1993) with gamma-distributed rate heterogeneity. The distance metric and shape parameter ( $\alpha = 0.8139$ ) of the gamma distribution were chosen by using the program Modeltest version 3.7 (Posada and Crandall 1998) following systematic evaluation of 56 models of sequence evolution versus raw sequence data using PAUP version 4.0b1 (Phylogenetic Analysis Using Parsimony; available from Sinauer Associates, Inc., Sunderland, Massachusetts).

The second method for examining the relationship between geographic and genetic distance was a Mantel matrix correlation analysis. This test allows for direct examination of isolation-by-distance effects. Geographic association between sampling areas was represented by a distance matrix calculated between pairwise sites. A midpoint for each sample site (bay) was identified and linear distances between each adjacent midpoint were calculated. Distances between nonadjacent sites were calculated by summing the distances between each site that occurred in succession between them. Genetic divergence between pairwise populations ( $F_{ST}$ ) was estimated by using the method of Reynolds et al. (1983) and subsequently compared to geographic distance in a matrix correspondence test as implemented in Arlequin. Significance of pairwise divergence estimates ( $F_{ST} > 0$ ) was assessed with 100 data permutations; significance was assessed before and after Bonferroni adjustment for multiple tests. The significance of the regression coefficient of matrix correspondence ( $r$ ) was assessed by 1,000 iterations of a randomization procedure as implemented in Arlequin. In order to determine whether AMOVA and Mantel results were biased by observations on the extreme ends of the sampling area, both analyses were repeated with the northernmost and southernmost samples removed from the data set.

*Nested clade analysis.*—The NCA method of Templeton et al. (1995) was used to disentangle the historical (evolutionary) versus contemporary (demographic) effects on genetic divergence. Recent evaluations of the NCA technique have indicated that it

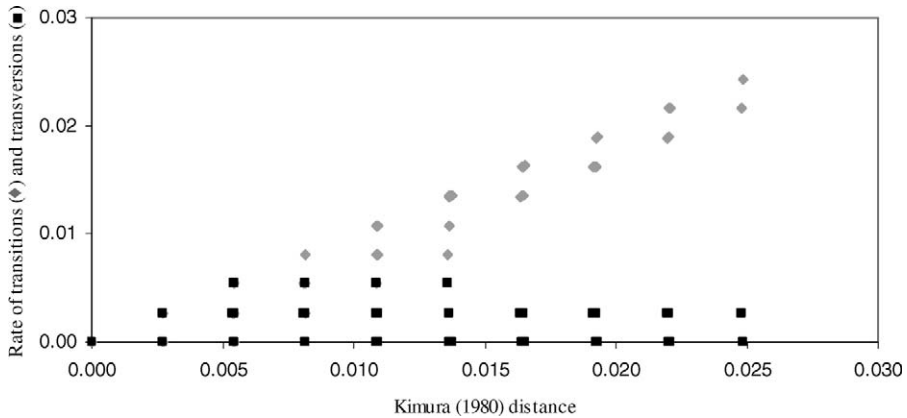


FIGURE 2.—Mutation saturation test of the mtDNA control region locus for spotted seatrout. The x-axis is the calculated genetic distance (Kimura 1980) between pairwise haplotypes, and the y-axis is the frequency of transition and transversion substitutions occurring between the same pair.

results in an unreasonably high rate of type I error (false positives; see Petit 2008 for a review of these critiques). However, Garrick et al. (2008) suggest that NCA is an appropriate tool for formulating hypotheses regarding evolutionary history, and these hypotheses can then be tested by using complementary analyses. In the present case, NCA is justified for two reasons. First, strong prior expectations of population structure exist due to the similarity among findings from previous literature. Second, complimentary analyses (AMOVA, Mantel matrix) were conducted and were compared directly to NCA results. Templeton (2004) suggests that NCA can be strengthened in the presence of complimentary analyses and strong prior expectations.

A set of equally parsimonious haplotype networks were constructed from sequence data by using the median-joining method of Bandelt et al. (1999) as configured in Network version 4.2 (Fluxus Engineering, Suffolk, UK). A 95% consensus network was then estimated by using the maximum likelihood method of Polzin and Daneschmand (2003). Reticulations or “loop” structures in this network were resolved by using the systematic criteria of Pfenninger and Posada (2002). Briefly, all ambiguities were resolved by favoring links towards (1) high-frequency haplotypes, (2) interior haplotypes, and (3) haplotypes sampled from the same population. The resulting haplotype network was then subjected to the nesting criteria of Templeton et al. (1988), resulting in a nested clade design in which each  $n$ -step clade resided within an  $(n + 1)$ -step clade until all inferior clades fell into a single inclusive clade. Finally, a systematic contingency analysis of each nested clade was performed by using GEODIS version 2.0 (Posada et al. 2000). Following

contingency analysis, the decision-making criteria of Templeton (1998) were followed in order to assign significant patterns of clade association to either contemporary or historical effects.

## Results

### *Genetic Diversity of the mtDNA Control Region*

The overall sequence set included 280 examined individuals (Table 1) sequenced over 373 nucleotides and resulted in recovery of 60 novel haplotypes. Single-base insertion–deletions (indels) were present at four sites, and sites with indels were treated as missing data. Otherwise, 41 sites were variable and 328 sites were invariant. The control region locus was A–T rich, with pooled base frequencies of 0.28 for A, 0.32 for T, 0.20 for C, and 0.20 for G. There was no evidence for heterogeneity in base frequencies among haplotypes ( $\chi^2 = 3.39$ ,  $df = 231$ ,  $P \approx 1.000$ ), and the rate of transitions or transversions across a range of pairwise genetic distances indicated no evidence for mutation saturation (Figure 2). Five major haplotypes, each with a frequency of greater than 0.05, accounted for 70.4% of all individuals sampled. All other haplotypes occurred at a rate less than 2% in the overall sample (the haplotype was present in four or fewer individuals in each case). Among sampling sites,  $H_d$  ranged from 0.754 (SL) to 0.940 (MB; Table 1). Values of  $\pi$  ranged from 0.005 (SL) to 0.007 (EM, WM, SB, CC, LL).

### *Population Structure of Spotted Seatrout*

The AMOVA indicated no significant genetic divergence among cohorts within estuaries ( $P = 0.878$ , Table 2). However, significant genetic divergence ( $F = 0.016$ ,  $P = 0.046$ ) was indicated among pooled populations between estuaries. Examination of

TABLE 2.—Analysis of molecular variance (AMOVA) results for spotted seatrout, including degrees of freedom, sum of squares (SS), and overall variance component results. The  $F$ -statistics were estimated from the AMOVA procedure and include  $F_{CT}$  (divergence between sampling sites),  $F_{SC}$  (divergence between cohorts), and  $F_{ST}$  (overall divergence due to geographic and temporal variation). The first AMOVA was performed with all samples included. The second AMOVA was performed after the northernmost (SL; code defined in Table 1) and southernmost (LL) sample sites had been removed.

	df	SS	Variance component	$F$ -statistic	$P$
<b>All Samples Included</b>					
Among populations	7	14.73	0.03	$F_{CT} = 0.016$	0.046
Within populations, among years	12	14.42	-0.03	$F_{SC} = -0.019$	0.878
Within years, among individuals	260	422.00	1.62		
Total	279	451.11	1.62	$F_{ST} = -0.003$	0.579
<b>Six Populations (Extreme Removed)</b>					
Among populations	5	1.90	~0.0	$F_{CT} = -0.000$	0.493
Within populations, among years	9	3.50	~0.0	$F_{SC} = -0.010$	0.812
Within years, among individuals	194	87.83	0.45		
Total	279	451.11	1.62	$F_{ST} = -0.010$	0.899

individual pairwise  $F_{ST}$  values suggested that divergent populations in the northern (SL) and southern (UL, LL) sample sites were driving the significance of the overall AMOVA (Table 3). Comparisons between SL and both UL and LL were significant, and all four significant comparisons involved one of these three populations. Interestingly, the comparison between UL and the adjacent CC population also resulted in a significant genetic divergence estimate. However, after correction for multiple tests, only a single comparison (UL versus SL) was significant. When the northernmost (SL) and southernmost (LL) sample sites were removed from the analysis, the AMOVA was no longer significant. Overall sample divergence as measured via isolation by distance was highly significant. The Mantel matrix correspondence analysis recovered an  $r$ -value of 0.717, and permutation analysis found a larger value for  $r$  in only 10 of 1,000 comparisons ( $P = 0.01$ ). However, after removal of the extreme sample sites, the Mantel test was not significant ( $P = 0.430$ ).

The mtDNA haplotype network resulted in eight ambiguous loop structures, all of which were resolved by using systematic criteria (Figure 3a). Haplotype sampling was relatively inclusive; only a single expected median haplotype was unsampled. The clade

nesting procedure resulted in 12 one-step clades, 5 two-step clades, and 2 three-step clades (Figure 3b). Contingency analysis of nested clades resulted in five significant clade distances (Table 4). Two of these were significantly small distances at tip clades (clades 1–5 and 2–5), and the remaining three were significantly large values of interior-tip clade distance. In each case, the inference procedures of Templeton (1998) indicated clade distributions that followed patterns expected under an isolation-by-distance scenario. In particular, clades 1–5 and 1–9 showed significantly strong frequency gradients on a north-to-south basis (Figure 4).

**Discussion**

The estimates of intrapopulation  $H_d$  recovered in this study were exceptionally high. For instance, the coastwide  $H_d$  ( $\pm$ SE) of  $0.88 \pm 0.04$  contrasts with the data of Gold et al. (1999), which demonstrated a range of 0.240–0.506 mtDNA diversity. There are two interrelated differences between studies that can account for this difference. First, the previous study used restriction fragment length polymorphisms (RFLPs) in mtDNA, whereas the current study examined direct sequence data. Second, the current

TABLE 3.—Pairwise estimates of spotted seatrout genetic divergence ( $F_{ST}$ ) between sampling sites (below diagonal; site codes defined in Table 1) and the corresponding  $P$ -value for each estimate (above diagonal) (boldface = significant; \* Significant before Bonferonni correction; \*\* Significant after Bonferonni correction).

Site code	SL	WM	EM	SB	AB	CC	UL	LL
SL	0	<b>0.018*</b>	0.063	0.090	0.198	0.288	<b>0.000**</b>	<b>0.009*</b>
WM	0.060	0	0.765	0.729	0.315	0.297	0.468	0.369
EM	0.041	-0.017	0	0.936	0.261	0.351	0.180	0.180
SB	0.030	-0.015	-0.022	0	0.378	0.423	0.198	0.243
AB	0.012	0.001	0.005	-0.005	0	0.541	0.306	0.621
CC	0.004	0.004	-0.002	-0.003	-0.006	0	<b>0.018*</b>	0.090
UL	0.081	-0.002	0.014	0.006	0.004	0.030	0	0.963
LL	0.066	0.002	0.026	0.011	-0.007	0.025	-0.025	0

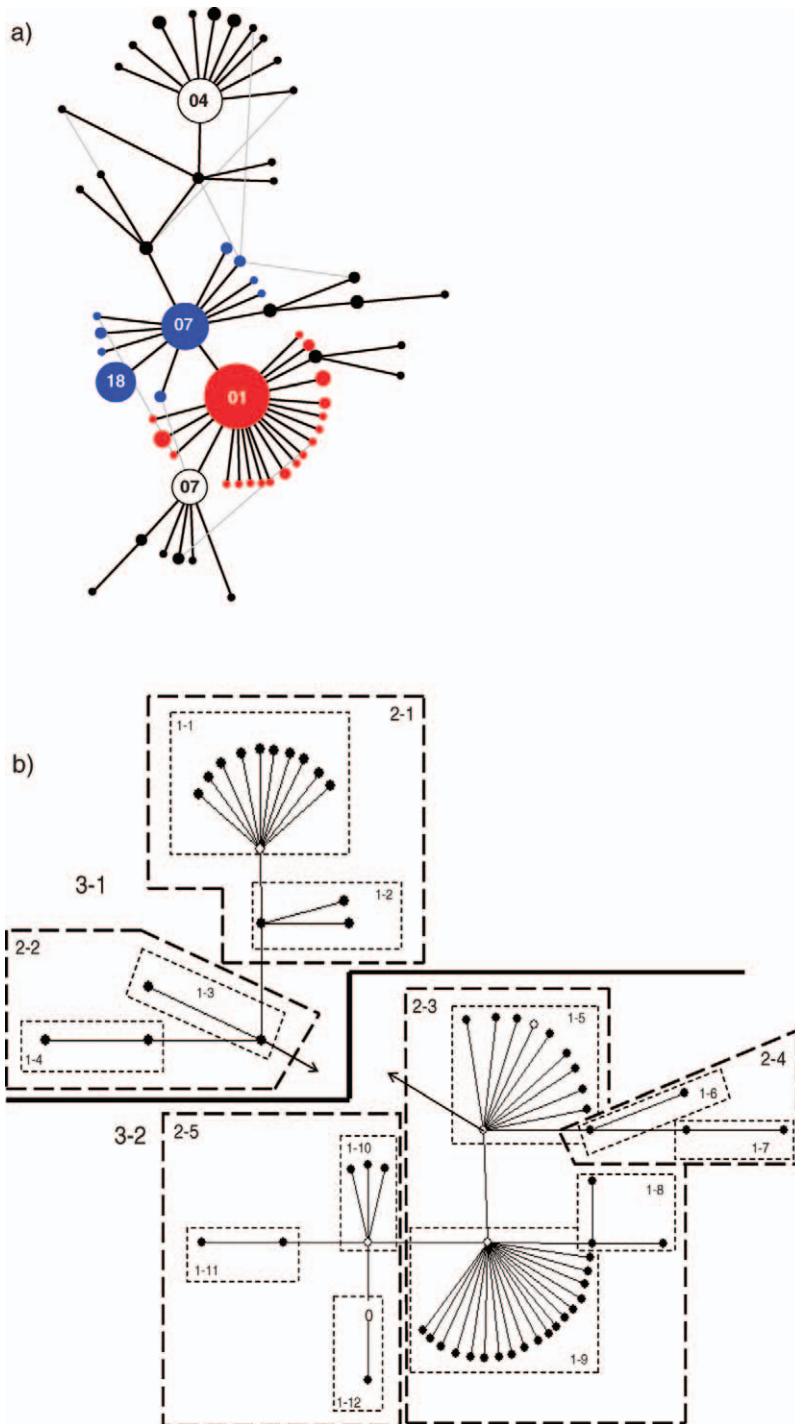


FIGURE 3.—Evolutionary reconstruction of mtDNA haplotypes recovered from eight populations of spotted seatrout, including (a) the 95% consensus haplotype network, with the five most common haplotypes labeled (01 corresponds to haplotype SST01 as discussed in the text, and so forth). Unresolved loop structures are represented by light grey lines; the size of nodes demonstrates the frequency of a particular haplotype. Clades with significant distances are colored (clade 1–5, blue; clade 1–9, red). (b) The nesting structure of haplotypes following Templeton et al. (1987), with one-step clades represented by light dashed lines, two-step clades represented by bold dashed lines, and three-step clades separated by a single, solid line. Numbers (x–y) associated with each clade represent that it is the yth clade residing in an x-step clade level.



TABLE 4.—Significant clades from nested clade analysis of spotted seatrout, including the type of distance that was significant ( $D_c$  = clade distance,  $D_n$  = nested clade distance), the value of the clade distance, whether the distance was significantly small or large (S or L), the  $P$ -value for significance testing, the inference steps used from Templeton (1998), and the inferred evolutionary mechanism. I-T = interior-tip distance; (t) = tip clade.

Clade	Nested clade	Distance	Value (S or L)	$P$	Inference key steps	Inference
1-5	47 (t)	$D_c$	125.22 (S)	0.031	1, 2, 3, 4 (no)	Restricted gene flow with isolation by distance
1-9	I-T	$D_c$	168.55 (L)	0.006	1, 2, 3, 4 (no)	Restricted gene flow with isolation by distance
	I-T	$D_n$	50.59 (L)	0.000		
2-5	1-11 (t)	$D_c$	0.00 (S)	0.039	1, 2, 3, 4 (no)	Restricted gene flow with isolation by distance
	I-T	$D_c$	223.26 (L)	0.005		

study examined a portion of the mtDNA control region, which has exceptionally high rates of base substitution relative to other mtDNA loci (Lopez et al. 1997). For instance, the control region of menhadens *Brevoortia* spp. is so highly substituted that Anderson (2007) found 106 haplotypes in 113 individuals assayed across four species. Thus, the difference between the current data and the earlier RFLP study is likely a sampling effect brought about by a higher probability of

detecting haplotypes that are undetectable with restriction enzymes. There are two other notable differences between these studies that may be accounted for by sampling effects. First, the data of Gold et al. (1999) indicated that the LL site had lower intrapopulational mtDNA diversity than other sites in the western Gulf. This finding contrasts with that of King and Pate (1992), who found no such pattern in allozyme diversity, and with results of various other studies

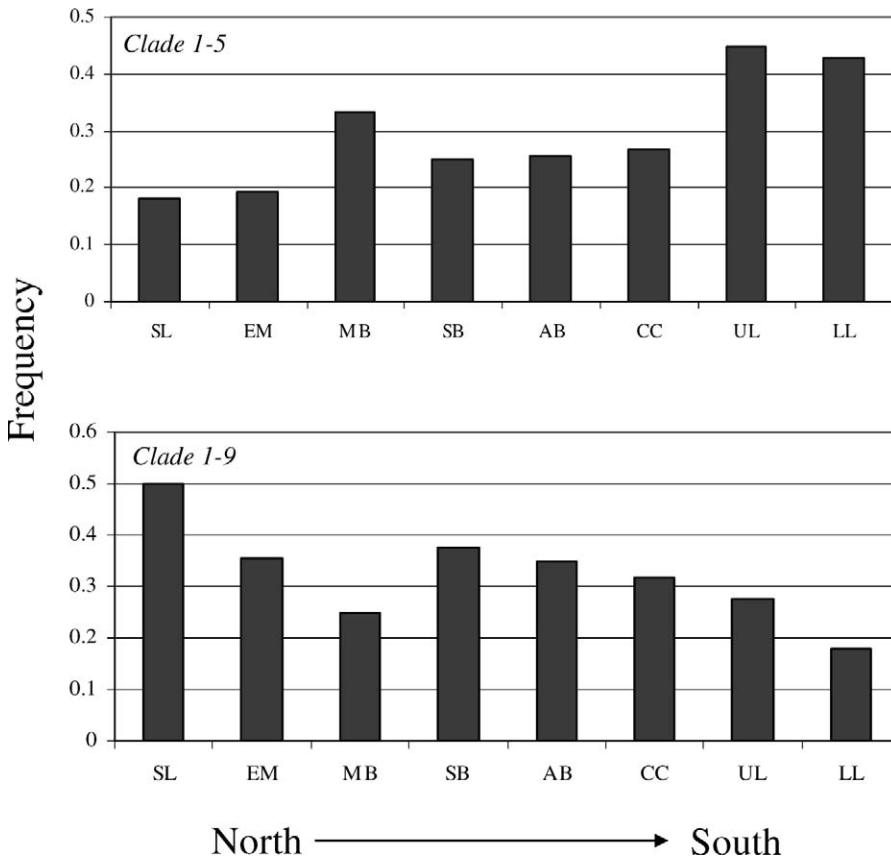


FIGURE 4.—The frequency of significant tip-clades from nested clade analysis of spotted seatrout mtDNA haplotypes, arranged in order from northern to southern sampling sites along the coast of Texas. The sample site labels are consistent with those found in Table 1 and the text.

using microsatellites (Gold et al. 2003; Ward et al. 2007). Such a pattern (low mtDNA diversity, high nuclear diversity) might indicate site fidelity of females, coupled with male-mediated gene flow whereby nuclear diversity is maintained near coastwide levels through immigration of males. This is supported by the finding that estimates of  $F_{ST}$  for allozymes ( $F_{ST} = 0.012$ ; King and Pate 1992) and microsatellites ( $F_{ST} = 0.013$ ; Gold et al. 2003) are approximately half of the  $F_{ST}$  estimates recovered with mtDNA ( $F_{ST} = 0.025$ ; Gold et al. 1999). However, the current mtDNA data seem to contradict lower diversity in LL and conform to the nuclear-based studies in finding that this sample seems to be similar in genetic diversity to other western Gulf populations of spotted seatrout. Moreover, the difference between mtDNA and nuclear DNA estimates of  $F_{ST}$  might merely reflect differences in the genetic effective size of each genome.

Second, the current data indicate that the SL site had the lowest spotted seatrout diversity of the eight sampled sites in this study, owing mainly to an elevated frequency of the most common haplotype (haplotype SST01; Figure 3). This finding is worth noting because the SL site is separated from the Gulf by a single channel of approximately 10 km and is farther away from adjacent estuaries than any other system sampled. Such a remote setting may result in restricted gene flow into the estuary, particularly in the case of passive transport of eggs and larvae from adjacent estuaries. However, the pattern of low diversity indicated in SL is not supported by the Gold et al. (1999) study. Additionally, reduced variation due to restricted gene flow would be expected to impact both mtDNA and nuclear genomes. Previous studies using nuclear microsatellites suggest no such reduction of variation at this site (Gold et al. 2003; Ward et al. 2007).

Thus, genetic variability as measured by mtDNA  $H_d$  seems to be transient in western Gulf spotted seatrout populations, and seemingly low estimates of diversity for any given sampling period and in any given estuary may reflect a sampling bias rather than the result of some biological mechanism, such as long-term restrictions of gene flow, natural selection, or inbreeding. A good example of this sampling effect is the SL samples from three consecutive years examined in this study. Haplotype SST01 was recorded in 9 of 15 SL samples (60% of fish) collected in 2003 and 9 of 14 samples (64%) obtained in 2005 but only 3 of 15 samples (20%) collected in 2004.

The first objective of this study was to determine how populations of spotted seatrout are organized along the Texas coast. To address this objective, the criteria of Laikre et al. (2005) will be used here to

characterize the general pattern of population structure in spotted seatrout. Briefly, coastal zone population structure can be broken down into three basic types (distinct populations, continuous change, and no differentiation). Each type carries subtle differences in implication for management. For instance, the case of distinct populations suggests that management should take place wholly on local scales, whereas continuous change suggests that management boundaries might be set to account for the migratory capacity of individuals between adjacent areas. There has been some disagreement on this point in the previous literature concerning spotted seatrout. For instance, Ramsey and Wakeman (1987), and later King and Pate (1992), concluded that spatial patterns among spotted seatrout population samples could best be described as isolation by distance and that there was not sufficient evidence for demographically independent subpopulations among Gulf areas. In contrast, Gold et al. (1999) suggested that there was evidence of distinct subpopulations organized by bay, supporting an assertion made first by Weinstein and Yerger (1976). In the former case, Gold et al. (1999) argued that mtDNA were more sensitive markers for population studies than the previously used allozyme markers, and indeed the divergence recovered among groups in the Gold et al. (1999) study was highly significant.

In the current study, two of three distinctive analytical methods (Mantel matrix and NCA) indicate that spotted seatrout on the Texas coast exhibit continuous change, with genetic isolation by distance, resulting in significant divergence between the northern and southernmost coastal sampling sites. The AMOVA indicated overall significant divergence among bays; the divergence was similar to that recorded in previous studies and was minimal (between-site divergence  $F_{CT} = 0.016$ ,  $P = 0.046$ ). Two lines of evidence suggest that this may be an artifact of the AMOVA procedure rather than evidence for distinct populations. First, tagging studies in Texas indicate that while spotted seatrout tend to utilize discrete areas and avoid movement among different areas for most of the year, a great deal of movement and mixing occurs during the spawning season near offshore passes (Baker and Matlock 1993). Although this movement appears to be limited in scale (mean = 31 km; Baker and Matlock 1993), it does not include migration that occurs at egg or larval stages, which can be important in fishes with pelagic offspring (Norcross and Shaw 1984). Moreover, inshore bays on the Texas coast often do not represent meaningful boundaries for dispersal. For instance, SB, AB, and CC are all connected by large natural waterways as well as by the Gulf Intracoastal Waterway (ICWW; Figure 1). It is unlikely that individual spotted seatrout recognize

distinction among these areas. With a few exceptions (LL, EM, SL), other adjacent bays are similarly connected by natural waterways, and all are connected by the ICWW.

Second, AMOVA can be expected to give false positive results in systems where a single, continuously changing population organized along a single dimension, such as a coastline, is arbitrarily cordoned into putative subpopulations. Specifically, the mechanics of isolation by distance dictate that individuals that are located in close proximity are likely to share more alleles in common with one another than are individuals from distant populations. Thus, if arbitrary boundaries are imposed upon such a genetic landscape (such as the case of segregating inlet bay systems), differences between subpopulations existing at the extreme boundaries of the sampling area are likely to bias standard tests, such as AMOVA. Removal of the extreme populations in this study resulted in both a nonsignificant AMOVA as well as a nonsignificant Mantel test. While such a result does not necessarily preclude isolation by distance (that is, the sampling range has merely been reduced to a size smaller than that at which migration is significantly restricted), it does make the conclusion of independent subpopulations organized by bay dubious.

A final concern regarding the genetic structure of spotted seatrout in the western Gulf is whether previous stock enhancement has influenced contemporary haplotype distributions. There is a distinctive possibility that this has been the case, given that large-scale stocking of spotted seatrout has occurred since 1991. Unfortunately, this is impossible to test with the present data set. However, assessments made prior to enhancement (Weinstein and Yerger 1976; Ramsey and Wakeman 1987) have resulted in divergence values that are similar to those made after enhancement (King and Pate 1992; Gold et al. 1999; this article), all of which fall into the  $F_{ST}$  range of 0.012–0.032. Future examinations should attempt to quantify this putative effect by examining archived samples and including historical data.

The second objective of this study was to determine whether current units of management as defined by TPWD are supported by genetic data. The TPWD has undertaken stock enhancement of spotted seatrout since 1991, and current estimates suggest that 3 million fingerlings are released each year into Texas bays and estuaries. A major concern in any intensive enhancement scenario is that the genetic identity of released individuals is complimentary to that of the natural population (Blankenship and Leber 1995). In particular, care must be taken that the released individuals do not alter the fitness of the natural population through

outbreeding depression and introduction of maladaptive alleles (Waples 1995). Although assessments based on neutral markers can be poor indicators of quantitative genetic variation (McKay and Latta 2002), the most efficient and accessible method to date of evaluating the risk of release on the fitness of natural populations is through an assessment of gene flow between adjacent populations. Based on previous findings of clinal variation in allozymes (King and Pate 1992; King and Zimmerman 1993), TPWD has adopted a policy of returning hatchery stock to the estuary from which broodstock were extracted or to an adjacent estuary. This is particularly important for spotted seatrout, as some evidence suggests that adaptive differences, organized longitudinally and related to salinity or temperature gradients, may occur on the Texas coast (King and Zimmerman 1993). Adaptive differences can occur even in the face of gene flow, and maintaining adaptive genetic variation should be a high priority in enhancement efforts because it relates directly to survival and reproduction of released individuals (Conover et al. 2006). The current findings reinforce the assertion that clinal variation exists among sampling sites, although in this case the variation is in neutral mtDNA rather than protein markers. The LL site in particular is significantly divergent from more northerly sites. Similarly, the SL site is divergent from sampling areas further south.

Thus, the results obtained here suggest that the current practice of returning hatchery-reared fish to areas in or directly adjacent to the site of broodstock origin should be continued. Such a strategy most accurately approximates the naturally restricted movement of genes into geographically distant estuaries and would also result in preservation of adaptive differences that may have gone undetected by this and other studies using wholly neutral genetic markers. The transient nature of dominant mtDNA haplotypes within estuaries and from year to year further strengthens support for this strategy, as it demonstrates some degree of demographic isolation over short time scales. The current enhancement strategy of TPWD is thus probably sufficient to limit detrimental impacts on natural populations insofar as release of wild genotypes is concerned, although this finding should be reevaluated as anthropomorphic forces (higher fishing effort, loss of habitat through development, reduction in freshwater inflow) continue to pressure this highly valuable fishery.

#### Acknowledgments

We thank the entire staff of TPWD's coastal fisheries monitoring program for providing the necessary samples to complete this work. M. Fisher, B.

Bumgardner, and D. McDonald improved the original draft with editorial comments. This research was funded in part by a Federal Aid in Sportfish Restoration (FASFR) Grant (F-143-R). Routine monitoring by TPWD is also funded by a FASFR Grant (F-34M).

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