Genetic variability in spotted seatrout (*Cynoscion nebulosus*), determined with microsatellite DNA markers

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Abstract—Variation in the allele frequencies of five microsatellite loci was surveyed in 1256 individual spotted seatrout (*Cynoscion nebulosus*) obtained from 12 bays and estuaries from Laguna Madre, Texas, to Charlotte Harbor, Florida, to St. John's River on the Florida Atlantic Coast. Texas and Louisiana collection sites were resampled each year for two to four years (1998–2001). Genetic differentiation was observed. Spotted seatrout from Florida waters were strongly differentiated from spotted seatrout collected in Louisiana and Texas. The greatest genetic discontinuity was observed between Tampa Bay and Charlotte Harbor, and Charlotte Harbor seatrout were most similar to Atlantic Coast spotted seatrout. Texas and Louisiana samples were not strongly structured within the northwestern Gulf of Mexico and there was little evidence of temporal differentiation within bays. These findings are contrary to those of earlier analyses with allozymes and mitochondrial DNA (mtDNA) where evidence of spatial differentiation was found for spotted seatrout resident on the Texas coast. The differences in genetic structure observed among these markers may reflect differences in response to selective pressure, or may be due to differences in underlying genetic processes.

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Spotted seatrout (*Cynoscion nebulosus*) support an important recreational fishery in the northern Gulf of Mexico and along the U.S. Atlantic Coast. Management of this fishery is multijurisdictional, employing a variety of strategies including reduction or elimination of commercial exploitation, adjustments of recreational fish-size limits and bag limits, closed seasons, and artificial spawning and stocking of fish (Vanderkooy and Muller, 2003). Effective management requires an understanding of the ecology, life history, and genetic structuring of a species. Understanding genetic population structure is important to every aspect of fishery management but is especially critical when stocking fish is the chosen management strategy. Stocking without regard for existing genetic variability within and among populations places the genetic integrity of the targeted species at risk (Allendorf et al., 1986). Genetic population structuring may be evidence of adaptation to past environmental differences, whereas genetic variability may enable a population to meet future environmental challenges.

Evidence of population structuring in spotted seatrout has been gained through morphological, physiological, and genetic examinations. Regional differences have been found in otolith and scale structure, growth rate (Iverson and Tabb, 1962; however, see Murphy and Taylor, 1994 for a different interpretation), and in reproductive physiology (Brown-Peterson et al., 2002). Each of these studies found evidence of biologically significant regional differentiation—a finding consistent with observations of limited movement within and between bays (Music, 1981; Overstreet, 1983; Baker and Matlock, 1993) and between bays and adjacent nearshore waters (Baker et al., 1986).

Studies of genetic markers generally support the existence of population structuring among spotted seatrout in the northern Gulf of Mexico. Studies examining protein variation found evidence of weak (Ramsey and Wakeman, 1987; King and Pate, 1992) to strong (Weinstein and Yerger, 1976) population subdivision. The King and Pate (1992) study found indications of clinal variation in mean heterozygosity and in alleles of the aspartate aminotransferase-2 locus, indicating possible adaptation to environmental gradients on the Texas coast (King and Zimmerman, 1993). In each of the allozyme studies indications of isolation by distance were found, as was found in a survey in which mitochondrial DNA was used (mtDNA; Gold et al., 1999). In the

mtDNA study, significant heterogeneity was found in haplotype frequencies among collection sites, indicating that spotted seatrout were spatially differentiated. In contrast, Gold et al. (2003) found no significant differences in microsatellite DNA allele frequency among spotted seatrout inhabiting Texas bays. A similar study of microsatellite variation among spotted seatrout of the U.S. Atlantic and Florida Gulf coasts (employing different loci from those of Gold et al., 2003) found extensive spatial differences that coincided with known zoogeographic barriers (Wiley and Chapman, 2003). Surprisingly, the Indian River spotted seatrout sample on Florida's Atlantic coast was genetically more similar to the Choctawhatchee sample from the Florida Panhandle than to more northerly Atlantic Coast samples. The overall pattern that emerges from applications of molecular markers to the examination of spatial genetic variability in spotted seatrout is mixed. Most studies have found limited gene flow between adjacent bays and moderate population subdivision, and patterns of differentiation that may be described as "isolation by distance." The failure of the Gold et al. (2003) to find statistically significant genetic subdivision is surprising, given that studies employing allozymes and mtDNA have been successful in discerning genetic structuring and given that microsatellites are considered to be among the most capable genetic markers at resolving population level differentiation (Wright and Bentzen, 1994). Gold et al. (2003) focused on the northwestern Gulf of Mexico, and their geographically limited examination may have restricted the ability of the marker to discern patterns in the spatial variability of spotted seatrout. It is also possible that underlying genetic properties of the markers accounted for the differences in observed variation, or that some markers (i.e., allozymes) were able to detect genetic adaptation to environmental gradients which microsatellites, assumed to be selectively neutral, were not.

The present study is an attempt to re-examine genetic variability in spotted seatrout in the northern Gulf of Mexico. As did Gold et al. (2003) (who also examined a portion of the present data), we used microsatellite markers. Samples from Louisiana and the Gulf and Atlantic coasts of Florida were added in an attempt to give a greater spatial perspective that may be useful in evaluating the observed genetic variability among populations. In addition, samples from multiple years were included for Texas and Louisiana bays, allowing for evaluation of the stability and robustness of detected genetic structure.

Materials and methods

A total of 1256 individuals were examined. Sample collection sites are shown in Figure 1. All Texas sites were sampled in three consecutive years, 1998–2000, except Corpus Christi Bay which was sampled across four years, 1998–2001. The Louisiana site was sampled in 1999 and in 2000, and the Florida sites were sampled only in 2000. Texas and Florida samples were obtained through routine resource sampling efforts of Texas Parks and Wildlife Department and Florida Department of Natural Resources personnel, respectively, and Louisiana samples were donated by licensed recreational anglers. Soft dorsal-fin tissue was removed from the fish, placed in 95% ethanol, and stored at room temperature until processed. Genomic DNA was extracted by using the PureGene DNA isolation kit and protocols (Gentra Systems, Inc., Minneapolis, MN).

Primers designed to amplify three microsatellites (*Soc*12, *Soc*50, and *Soc*243) originally developed for red drum (*Sciaenops ocellatus*) by Turner et al. (1998) were employed. Two additional primer pairs (*Cne*133 and *Cne*133′) were designed by sequencing products of the *Soc*133 (Turner et al., 1998) forward and reverse primers and then identifying internal primer sequences that amplified separate repeat regions within the original *Soc*133 amplicon. Primer sequences for *Cne*133 and *Cne*133′ and protocols for amplification and interpretation are discussed in Gold et al. (2003).

Summary statistics were generated by using the Microsoft Excel add-on Microsatellite Toolkit (Park, 2001) and ARLEQUIN, version 2.001 (Schneider et al., 2000), which was also employed to test frequencies for deviations from Hardy-Weinberg equilibrium by using exact tests performed with Markov-chain randomization (Guo and Thompson, 1992). Permutations with 1000 resamplings (Manly, 1991) were used to generate probability values (P) for each test of Hardy-Weinberg equilibrium for each microsatellite locus in each sample. ARLEQUIN was also used to test for linkage between microsatellite loci, and significance of P-values was estimated by 1000 resamplings. Critical values for interpreting significance levels for simultaneous inferential comparisons were adjusted by using the sequential Bonferroni approach (Rice, 1989).

Allelic distribution homogeneity of each microsatellite was assessed with exact tests implemented with the statistical package GENEPOP, version 3.4 (Raymond and Rousset, 1995), and significance was estimated by permutation with 1000 resamplings for each comparison. Population subdivision was estimated with Weir and Cockerham's (1984) theta (θ) as generated in FSTAT, version 2.9.3 (Goudet, 1995), and a bootstrap procedure in FSTAT was employed to calculate a 95% confidence interval (CI). Although the use of theta is contigent on the assumption of an infinite-alleles mutation model (Kimura and Crow, 1964), it has been shown to compare favorably with other measures of genetic subdivision when employed with microsatellite data (Ruzzante, 1998). The significance of population differentiation across all loci was estimated as the combined probability of P-values for Fisher's exact tests for individual loci. Separate analyses were made for data sets that comprised 13 samples defined by site and collection date and 13 samples defined by site alone, with date combined across all year classes.

A hierarchical analysis of gene diversity was performed by using the analysis of molecular variance

model (AMOVA; Michalakis and Excoffier, 1996) in ARLEQUIN (Excoffier et al., 1992). The components of genetic diversity attributable to variance between regions (Atlantic versus Gulf of Mexico), variance among sampling sites within regions, temporal variance among years within sampling sites, and variance among individuals within samples were estimated. The significance of each variance component was tested with nonparametric permutation procedures (~1000 permutations; Excoffier et al., 1992). In addition, genetic differentiation among all collection sites for each sampling year and between pairs of populations within sampling years was estimated by using the theta statistic of Weir and Cockerham (1984) accessed on FSTAT (Goudet, 1995).

Cavalli-Sforza and Edwards' chord distance (D_C; Cavalli-Sforza and Edwards, 1967) was used to reconstruct phylogenetic relationships among collection sites. Estimations of D_C were obtained with the statistical package NJBPOP (Cornuet et al., 1999). Takezaki and Nei (1996) found D_C to be a better estimate of genetic divergence with microsatellite DNA data than with measures based on the step-wise mutation model. This estimate is not based on the assumption of a constant population size or a constant mutation rate among loci (Takezaki and Nei, 1996) and appears to accurately resolve closely related populations (Paetkau et al., 1997; Angers and Bernatchez, 1998). A phenogram was generated from the chord-distance matrix with the neighbor-joining (N-J) algorhithm. Robustness of each node was evaluated by bootstrapping over loci for 2000 replications (Hedges, 1992) with the SEQBOOT program on PHYLIP, version 3.5c (Felsenstein, 1995). The PHYLIP program CONSENSE then was used to generate a consensus tree which was drawn with the program TREEVIEW (Page, 1996).

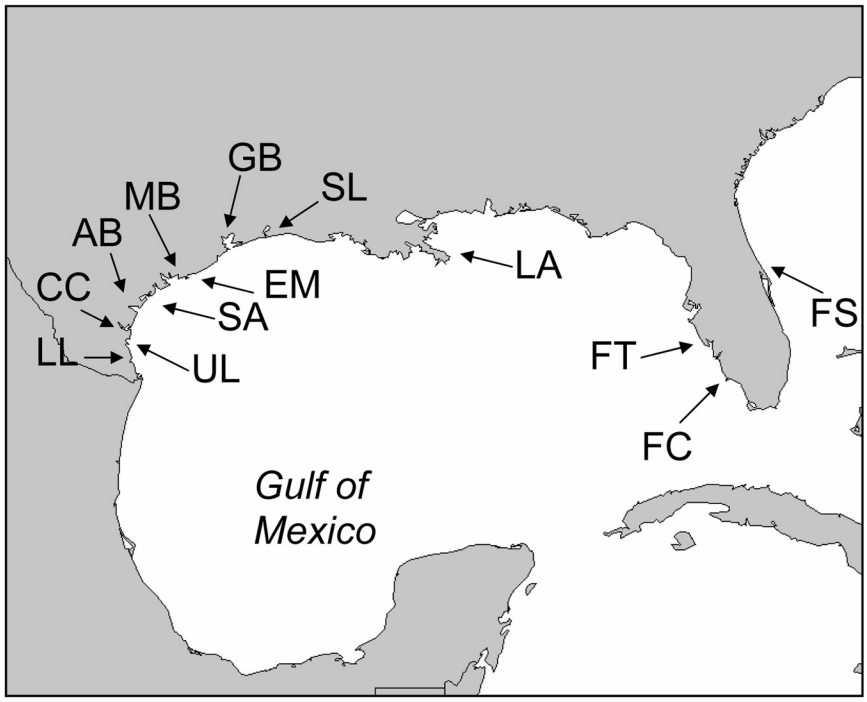

Figure 1

Sampling localities for spotted seatrout (*Cynoscion nebulosus*) examined from the northern Gulf of Mexico and the Atlantic Coast of Florida. LL = lower Laguna Madre; UL = upper Laguna Madre; CC = Corpus Christi Bay; AB = Aransas Bay; SA = San Antonio Bay; MB = Matagorda Bay; EM = east Matagorda Bay; GB = Galveston Bay; SL = Sabine Lake; LA = Grand Isle, Louisiana; FT = Tampa Bay, Florida; FC = Charlotte Harbor, Florida; FS = St. John's River, Florida.

Results

The number of alleles per sample (Table 1) exceeded those reported for the same loci in red drum (*Sciaenops ocellatus*), the species of origin for the markers (Turner et al., 1998). Mean observed heterozygosity (H_O) ranged from 0.21 to 0.39, and there were no statistically significant deviations from Hardy-Weinberg expectations at any locus and sample combination after Bonferroni adjustment. Without the Bonferroni correction, allele frequencies at 16 of 165 comparisons would have failed to meet expectations. This included all loci except *Soc*243, which was observed to be in Hardy-Weinberg equilibrium in all samples. *Soc*012 most often failed to meet Hardy-Weinberg expectations; six of 33 samples were out of equilibrium before Bonferroni adjustment. Observed heterozygosity was lower than expected in 14 of the 16 locus-and-sample combinations that failed to meet Hardy-Weinberg expectation before adjustment. Statistically significant linkage disequilibrium was noted for one pair of loci in one sample (*Cne*133′ with *Soc*12 in sample GB99) after Bonferroni adjustments. If the unadjusted critical value (α=0.05) was applied, 15 of 330 comparisons were statistically significant. Interestingly, the associated loci *Cne*133 and *Cne*133′ did not exhibit linkage disequilibrium for any sample.

After Bonferroni adjustment, exact tests for allele distribution homogeneity across all 33 samples demonstrated statistically significant differentiation for all microsatellite loci except *Soc*50, which approached statistical significance (P=0.05). All theta estimates were significantly greater than zero after Bonferroni adjustments, as was the overall theta of 0.116 (95% CI, 0.007–0.073; P<0.001), indicating significant genetic differentiation across both the spatial and temporal dimensions sampled in this study. When spatial samples were collapsed to form 13 spatial samples, all loci except *Soc*50 exhibited statistically significant deviations from

Table 1

Summary statistics for spotted seatrout (*Cynoscion nebulosus*) samples included in a survey of microsatellite variation. n = number of individuals in sample; n_a = number of alleles; P_{HW} = probability of meeting Hardy-Weinberg expectations (with Bonferri-corrected critical value of $\alpha = 0.0003$.

Sample location	Sample acronym	n		*Soc* 12	*Soc* 50	*Soc* 243	*Cne* 133	*Cne* 133′
St. Johns River, Florida 2000	FS00	26	n_a	3	2	3	3	3
			P_{HW}	0.32	0.19	0.13	1.00	0.11
Charlotte Harbor, Florida 2000	FC00	22	n_a	3	2	2	3	2
			P_{HW}	0.18	1.00	1.00	1.00	1.00
Tampa Bay, Florida 2000	FT00	40	n_a	3	2	2	4	2
			P_{HW}	0.18	1.00	1.00	0.23	0.25
Grand Isle, Louisiana 1999	LA99	60	n_a	3	2	3	4	3
			P_{HW}	0.46	1.00	0.86	1.00	0.05
Grand Isle, Louisiana 2000	LA00	39	n_a	3	3	2	3	2
			P_{HW}	0.69	0.21	0.51	1.00	1.00
Sabine Lake, Texas 1998	SL98	23	n_a	3	2	2	3	2
			P_{HW}	0.157	1.00	1.00	0.04	1.00
Sabine Lake, Texas 1999	SL99	36	n_a	3	3	3	3	2
			P_{HW}	0.61	0.07	1.00	0.30	1.00
Sabine Lake, Texas 2000	SL00	30	n_a	3	3	3	3	2
			P_{HW}	1.00	0.22	0.34	1.00	1.00
Galveston Bay, Texas 1998	GB98	28	n_a	4	2	2	3	2
			P_{HW}	0.03	1.00	1.00	1.00	1.00
Galveston Bay, Texas 1999	GB99	37	n_a	3	3	3	3	2
			P_{HW}	0.61	0.07	1.00	0.30	1.00
Galveston Bay, Texas 2000	GB00	40	n_a	3	5	3	3	3
			P_{HW}	0.03	0.32	0.39	0.80	1.00
E. Matagorda Bay, Texas 1998	EM98	33	n_a	4	2	2	3	2
			P_{HW}	0.91	0.29	0.72	0.27	1.00
E. Matagorda Bay, Texas 1999	EM99	40	n_a	4	2	2	3	2
			P_{HW}	0.47	0.02	0.15	0.01	1.00
E. Matagorda Bay, Texas 2000	EM00	40	n_a	3	2	2	3	2
			P_{HW}	0.33	1.00	0.72	0.21	0.25
Matagorda Bay, Texas 1998	MB98	40	40	3	3	3	3	2
			P_{HW}	0.22	0.01	0.67	1.00	1.00
Matagorda Bay, Texas 1999	MB99	40	40	3	2	2	3	2
			P_{HW}	0.36	0.25	0.51	1.00	1.00
Matagorda Bay, Texas 2000	MB00	40	40	3	2	3	4	2
			P_{HW}	0.36	1.00	0.56	1.00	1.00
San Antonio Bay, Texas 1998	SA98	40	n_a	3	3	3	3	2
			P_{HW}	0.03	0.12	0.37	1.00	1.00
San Antonio Bay, Texas 1999	SA99	40	n_a	3	3	3	3	3
			P_{HW}	0.84	0.02	0.39	0.68	1.00
San Antonio Bay, Texas 2000	SA00	40	n_a	4	4	2	3	2
			P_{HW}	0.01	0.39	1.00	1.00	1.00
Aransas Bay, Texas 1998	AB98	40	n_a	3	2	3	3	3
			P_{HW}	1.00	0.18	0.67	0.16	1.00
Aransas Bay, Texas 1999	AB99	39	n_a	4	3	3	4	2
			P_{HW}	0.43	1.00	0.67	0.57	1.00
Aransas Bay, Texas 2000	AB00	40	n_a	3	2	3	4	2
			P_{HW}	0.04	0.06	0.39	0.19	1.00
Corpus Christi Bay, Texas 1998	CC98	40	n_a	3	2	3	3	2
			P_{HW}	0.61	1.00	0.57	1.00	1.00
Corpus Christi Bay, Texas 1999	CC99	50	n_a	3	4	3	3	2
			P_{HW}	0.04	<0.01	0.81	0.17	1.00

continued

Table 1 (continued)

Sample location	Sample acronym	n		*Soc* 12	*Soc* 50	*Soc* 243	*Cne* 133	*Cne* 133′
Corpus Christi Bay, Texas 2000	CC00	40	n_a	3	2	4	3	3
			P_{HW}	0.10	0.33	0.25	1.00	0.19
Corpus Christi Bay, Texas 2001	CC01	35	n_a	3	4	4	4	3
			P_{HW}	0.59	0.02	0.71	0.74	1.00
Upper Laguna Madre, Texas 1998	UL98	40	n_a	3	2	3	3	2
			P_{HW}	0.05	1.00	0.33	1.00	1.00
Upper Laguna Madre, Texas 1999	UL99	40	n_a	3	3	4	3	3
			P_{HW}	0.07	0.05	0.16	0.04	1.00
Upper Laguna Madre, Texas 2000	UL00	40	n_a	3	4	4	4	2
			P_{HW}	0.31	1.00	0.36	1.00	1.00
Lower Laguna Madre, Texas 1998	LL98	38	n_a	4	3	2	3	2
			P_{HW}	0.06	0.13	1.00	0.79	0.01
Lower Laguna Madre, Texas 1999	LL99	40	n_a	3	3	5	4	2
			P_{HW}	0.55	1.00	1.00	0.04	1.00
Lower Laguna Madre, Texas 2000	LL00	40	n_a	3	2	3	3	2
			P_{HW}	0.84	1.00	0.31	1.00	0.39

homogeneous allele distributions and the overall test was significant (P_{exact}<0.001). Estimated theta values were statistically significant after Bonferroni adjustment, except *Cne*133′. The overall estimate of theta (θ=0.057, 95% CI, 0.005–0.062) was lower than the estimate including both spatial and temporal dimensions (θ=0.116), indicating that temporal differences likely contributed to overall population differentiation in spotted seatrout.

Analysis of molecular variance indicated a statistically significant 4.11% of the among-sample genetic variance was attributable to differences between Gulf and Atlantic samples (P=0.04). Statistically significant variance was also detected among sampling sites within the Gulf of Mexico (0.49% of the total variance, P<0.001). No significant genetic variance attributable to temporal differences was found within bays (P=0.42). An overall F_{ST} of 0.046 was estimated, which was statistically significant (P<0.001).

We found no significant differences among Texas bays in the 1998 collections (Table 2). In 1999, spotted seatrout from Texas and Louisiana were not differentiated except for the Galveston Bay samples that differed significantly from all other samples. Analyses of year 2000 collections, which included samples from Texas, Louisiana, and Florida, revealed differences between Florida samples and all samples from Louisiana and Texas. The Louisiana sample differed from most Texas samples, and within Texas most samples were genetically undifferentiated, except for samples Galveston Bay which were significantly different from those of all bays, except Sabine Lake and Corpus Christi. Finally, the upper and lower Laguna Madre samples were statistically different from each other. Temporally, the only statistically significant differences among years were seen among Louisiana and Galveston Bay samples. The overall theta within sampling years ranged from less than 0.006 in 1998 to 0.080 in 2000, perhaps reflecting the increased genetic variability introduced by the Florida samples in the 2000 data set. The statistically insignificant differentiation among sampling years within bays, with the exception of the Louisiana and Galveston Bay, supports the notion that temporal differentiation, at least on the limited scale reported in our study, may be ignored and temporal samples can be collapsed within bays. The genetic structure of spotted seatrout in Galveston Bay is highly differentiated, both temporally and spatially, showing significant differences in 1999 and 2000 with almost all other bays.

The topology of the neighbor-joining tree based on D_C for 34 site and year groups (Fig. 2) was poorly supported by bootstrap replications and demonstrated little correspondence to geographic and temporal patterns. An exception was the separation of samples FC00 and FS00 (Charlotte Bay and St. John's River, FL, respectively; acronyms are defined in Table 1) from samples collected in the northern and western Gulf. When groupings were collapsed across years (Fig. 3), the distinctiveness of the Florida samples from the southern Gulf Coast and the Atlantic Coast continued to be supported. Spotted seatrout from Florida's Tampa Bay (FT00) were found to be more closely related to spotted seatrout from Louisiana and Texas than to spotted seatrout in the other Florida samples. Texas and Louisiana samples formed a well-differentiated grouping; however, within that grouping, there was little correspondence between genetic differentiation and geographic location. One exception was the south coast of Texas (Corpus Christi Bay and the upper and

lower Laguna Madre), which formed a weakly supported clade.

A statistically significant ($P<0.001$) correlation between D_C and geographic distance was observed ($r=0.90$) and thus supported the isolation-by-distance hypothesis. The greatest geographic distance between adjacent collection sites was observed between Charlotte Harbor and St. John's River; however, when the St. John's River sample was excluded from the analysis, a significant positive relationship was still evident ($r=0.80$, $P<0.001$). The correlation between D_C and geographic distance within the western Gulf of Mexico (Texas and Louisiana) remained positive ($r=0.42$) and statistically significant ($P=0.05$).

Discussion

Spotted seatrout inhabiting a series of sites from the lower Laguna Madre of Texas to St. John's River on the Atlantic Coast of Florida were genetically differentiated by analyses of allele frequencies of five microsatellite markers. Samples from Florida waters were strongly differentiated from spotted seatrout from Louisiana and Texas, and the statistically significant correlation between geographic distance and genetic distance was due primarily to these differences. Within Florida, the Charlotte Harbor spotted seatrout is genetically more similar to the Atlantic Coast spotted seatrout from St. John's River than to the neighboring Tampa Bay spotted seatrout, which is genetically more similar to Texas and Louisiana than to other Florida fish of the same species. Differences in allele frequency between Charlotte Harbor and Tampa Bay samples represented the greatest discontinuity observed in this study. This putative population structure is congruent with Wiley and Chapman's (2003) findings of distinct population subdivision among Atlantic Coast spotted seatrout, although details of that structure may be difficult to reconcile. Wiley and Chapman (2003) found spotted

Table 2

Results of tests for homogeneity in allele distributions among samples of spotted seatrout (*Cynoscion nebulosus*). Pairwise-θ estimates for 1998 samples (above diagonal), 1999 samples (below diagonal), and 2000 samples (lower matrix). θ^T is θ calculated across all sampling periods within a bay. * indicates statistical significance ($\alpha=0.05$) after adjustment for multiple comparisons (Rice, 1989). Acronyms for within-bay samples collapsed across years are the two letters (e.g., LA99+LA00=LA). Definitions for abbreviations for sample locations (LA, SL, etc.) are given in Table 1.

Sample location	LA	SL	GB	EM	MB	SA	AB	CC	UL	LL
SL	−0.007	0.000	0.026	−0.011	−0.010	−0.003	0.011	0.015	0.012	0.002
GB	0.092*	0.070*	0.000	0.019	0.010	0.009	0.006	0.009	0.004	0.029
EM	0.009	0.002	0.053*	0.000	−0.007	0.006	−0.003	0.019	0.018	0.020
MB	0.007	0.007	0.083*	0.000	0.000	−0.008	−0.001	0.002	−0.001	0.005
SA	−0.004	−0.006	0.078*	−0.001	−0.006	0.000	0.008	−0.002	−0.005	0.000
AB	0.008	0.001	0.089*	−0.004	−0.000	−0.001	0.000	0.009	0.008	0.024
CC	−0.006	−0.008	0.079*	0.001	−0.004	−0.011	−0.001	0.000	0.000	0.010
UL	0.003	−0.002	0.081*	−0.001	0.011	−0.002	0.000	0.001	0.000	0.003
LL	0.005	0.002	0.072*	−0.009	−0.006	−0.003	−0.006	−0.003	0.000	0.000

	FC	FT	LA	SL	GB	EM	MB	SA	AB	CC	UL	LL
FS	0.007	0.326*	0.108*	0.061*	0.110*	0.039*	0.077*	0.053*	0.053*	0.0532*	0.031*	0.081*
FC		0.341*	0.136*	0.077*	0.145*	0.031*	0.085*	0.054*	0.059*	0.068*	0.043*	0.081*
FT			0.245*	0.185*	0.257*	0.263*	0.195*	0.229*	0.219*	0.236*	0.288*	0.136*
LA				0.057	0.011	0.084*	0.067*	0.058*	0.039	0.029	0.093*	0.063*
SL					0.065	0.006	0.012	−0.006	0.003	−0.001	0.016	0.003
GB						0.105*	0.104*	0.083*	0.072*	0.050	0.115*	0.083*
EM							0.011	−0.003	0.004	0.004	−0.007	0.019
MB								0.009	−0.004	0.013	0.016	0.001
SA									−0.006	−0.005	0.004	0.013
AB										−0.004	0.008	0.008
CC											0.013	0.016
UL												0.034*
θ^T			0.065*	0.004	0.049*	<0.001	−0.004	−0.002	<0.001	0.013	0.001	0.013

seatrout from Indian River, Florida (which is near St. John's River), to be genetically more similar to spotted seatrout from Choctawhatchee Bay in the Florida Panhandle than to other Atlantic Coast fish of the same species. The analyses of the two studies, taken together, indicate at least two distribution breaks in the eastern Gulf and the Atlantic, the first between Georgia and the upper Atlantic Coast of Florida and a second between Charlotte Harbor and Tampa Bay. The clustering of populations observed by Wiley and Chapman (2003) between Indian River and Choctawhatchee Bay may, in light of our finding of a genetic discontinuity between the intervening Charlotte Harbor and Tampa Bay, reflect relative differences in genetic affinity discerned by the two data sets. Resolution of this possible incongruence will require examination of numerous sampling sites collected from both the Atlantic and Gulf coasts of Florida.

Spotted seatrout inhabiting the northwestern Gulf of Mexico from the Laguna Madre to Grand Isle, Louisiana, were not found to be subdivided into discernible stocks or populations and there was little indication of temporal differentiation within bays. Exceptions to this lack of temporal differentiation were seen in Galveston Bay and in Louisiana. Temporal differences in these two sites may be due to sampling error (although the *n* for each year's sample in the two sites appear to be adequate), or these two large regions may harbor populations that are temporally or spatially genetically structured. Some indications of geographically coherent spatial patterns were observed among spotted seatrout in the northwestern Gulf of Mexico. For example, there was an indication that this species on the lower coast is genetically differentiated, albeit weakly, from conspecifics inhabiting bays on the middle and upper Texas coast. This finding is similar to that found in allozyme (King and Pate, 1992) and mtDNA data (Gold et al., 1999) where differences between Laguna Madre samples and more northerly bays were observed. Galveston Bay spotted seatrout were also found to be genetically divergent, being genetically distinct from spotted seatrout in all other bays in 1999 and most bays in 2000. Galveston Bay was, in addition, one of two sites where the genetic structure of spotted seatrout was found to be temporally heterogeneous. Gold et al. (2003) found the upper Texas Coast to be a region of genetic transition; a notable shift in allele frequencies of the *Soc*201 locus was evident between Matagorda Bay and Sabine Pass—a span that includes Galveston Bay.

The lack of genetic population subdivision in the northwestern Gulf is consistent with the observed decrease in heterozygosity in relation to Hardy-Weinberg expectations. Similar heterozygote deficiencies in white seabream (*Diplodus sargus*) (Lenfant and Planes, 2002) were hypothesized to represent mixing of genetically disparate individuals during some stage of recruitment (the Wahlund effect). This phenomenon may be characteristic of many marine fishes, especially those with local populations recruited from a larval stage with highly dispersive capabilities. Spotted seatrout, due to its unique life history characteristics, is not an obvious example of a marine species expected to exhibit high gene flow. Spotted seatrout are confined to nearshore waters, spend most of their life within an estuarine habitat, and spawn and select their nursery area within the estuary (McMichael and Peters, 1989). Results of tagging studies support the hypothesis of a natal bay affinity based on spotted seatrout life history (e.g., Baker and Matlock, 1993). Stock structure among spotted seatrout was detected by the studies of morphology, physiology, (Iverson and Tabb, 1962), and genetics (Gold et al., 1999, and references therein). This population structure was not detected in the Gold et al. (2003) or the present analyses of variation in microsatellite DNA loci—markers expected to yield a high-resolution analysis of population-level genetic variability. The differences observed between microsatellite variability and that seen in allozymes (Weinstein and Yerger, 1976; Ramsey and Wakeman, 1987; King and Pate, 1992) and mtDNA (Gold et al., 1999) may reflect different evolutionary processes (Gold et al., 2003).

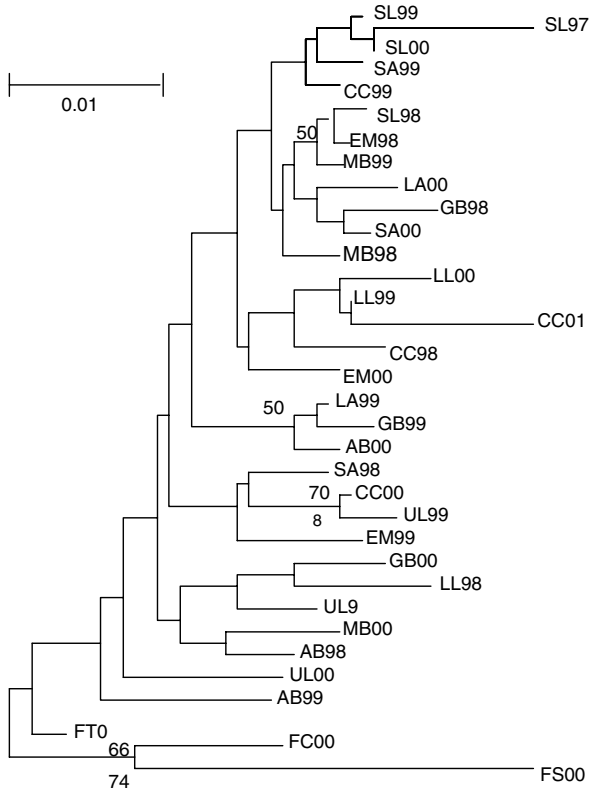

Figure 2

Cavalli-Sforza and Edwards (1967) chord distance neighbor-joining phenogram calculated for 33 spatial and temporal samples of spotted seatrout (*Cynoscion nebulosus*). Values along branches indicate bootstrap values as percentages of replicates, based on locus (above line) or individual (below line). Letter codes are defined in Table 1.

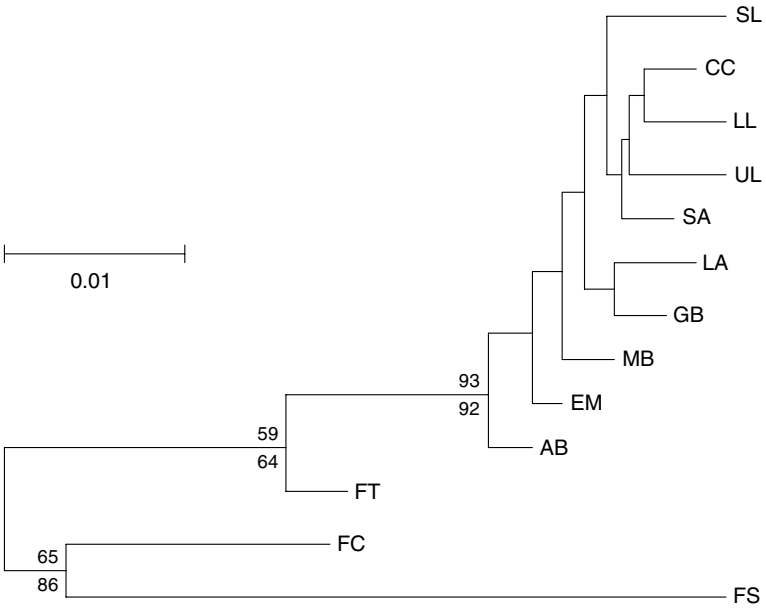

Figure 3

Cavalli-Sforza and Edwards (1967) chord distance neighbor-joining phenogram calculated for 13 spatial samples of spotted seatrout (*Cynoscion nebulosus*). Values along branches indicate bootstrap values as percentages of replicates, based on locus (above line) or individual (below line). Letter codes are defined in Table 1.

Microsatellites are assumed to be selectively neutral, whereas the allozyme and mtDNA markers used in earlier studies are potentially subject to selection and thus may present different patterns for the same region (Hellberg et al., 2002). King and Zimmerman (1993) suggested the cline in *AAT*-2 observed by King and Pate (1992) may reflect adaptation to temperature or salinity gradients along the Texas Coast. Microsatellite markers would not, in the absence of linkage to loci affected by selection, be subject to such processes. It is also possible, as Gold et al. (2003) suggested, that the earlier allozyme and mtDNA studies provided evidence, not of genetic differentiation of populations inhabiting neighboring bays, but rather of a general confirmation of the isolation-by-distance model, where greatest genetic differences are found between the most peripheral sampling sites.

Currently, about 5 million fingerling spotted seatrout are stocked per year into Texas bays and estuaries. Neither Florida nor the other states of the eastern Gulf of Mexico have implemented large-scale spotted seatrout stocking programs; however such efforts are being considered. The genetic population structure observed in studies of allozymes (Weinstein and Yerger, 1976; Ramsey and Wakeman, 1987; King and Pate, 1992) and mtDNA (Gold et al., 1999) argue for a cautious policy concerning the stocking of spotted seatrout. Gold et al. (2003) suggested the gene flow observed in microsatellite markers argued against the current Texas policy of stocking only into the bay from which broodfish were procured. Allowing stocking into both the bay of broodfish origin and into adjacent bays would meet this suggestion of simulated gene flow and still protect the putative population subdivision detected by the earlier studies. Should stocking programs in Florida or elsewhere in the northeastern Gulf be implemented, it is critical, considering the level of population subdivision observed in the present study and that of Wiley and Chapman (2003), that fine-scale genetic surveys in the eastern Gulf be accomplished. It is also obvious that inter-regional transfers of spotted seatrout should be strictly avoided.

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Literature cited

Allendorf, F., N. Ryman, and F. Utter.
1986. Genetics and fishery management: past, present, and future. *In* Population genetics and fishery management (N. Ryman, and F. Utter, eds.), p 1–19. Washington Sea Grant Program, Seattle, WA.

Angers, B., and L. Bernatchez.
1998. Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. Mol. Biol. Evol. 15:143–159.

Baker, W. B., Jr., and G. C. Matlock.
1993. Movement of spotted seatrout in Trinity Bay, Texas. Northeast Gulf Sci. 13:29–34.

Baker, W. B., Jr., G. C. Matlock, L. W. McEachron, A. W. Green, and H. E. Hegen.
1986. Movement, growth, and survival of spotted seatrout tagged in Bastrop Bayou, Texas. Contr. Mar. Sci. 29:91–101.

Brown-Peterson, N. J., M. S. Peterson, D. L. Nieland, M. D. Murphy, R. G. Taylor, and J. R. Warren.
2002. Reproductive biology of female spotted seatrout, *Cynoscion nebulosus*, in the Gulf of Mexico: differences among estuaries? Environ. Biol. Fishes 63:405–415.

Cavalli-Sforza, L. L., and A. W. F. Edwards.
1967. Phylogenetic analysis models and estimation procedures. Am. J. Human Gen. 19:233–257.

Cornuet, J.-M., S. Piry, G. Luikart, and M. Solignac.
1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000.

Excoffier, L., P. E. Smouse, and J. M. Quattro.
1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Felsenstein, J.
1995. PHYLIP (Phylogeny Inference Package), version 3.57c. Univ. Washington, Seattle, WA.

Gold, J. R., L. R. Richardson, and C. Furman.
1999. Mitochondrial DNA diversity and population structure of spotted seatrout (*Cynoscion nebulosus*) in coastal waters of the southeastern United States. Gulf Mex. Sci. 1999:40–50.

Gold, J. R., L. B. Stewart, and R. Ward.
2003. Population structure of spotted seatrout (*Cynoscion nebulosus*) along the Texas Gulf Coast, as revealed by genetic analysis. *In* Biology of the spotted seatrout (S. A. Bartone, ed.), p. 17–29. CRC Press, Boca Raton, FL.

Goudet, J.
1995. F-STAT (version 1.2): A computer program to calculate F-statistics. J. Hered. 86:485–486.

Guo, S., and E. Thompson.
1992. Performing the exact test of Hardy-Weinberg proportion from multiple alleles. Biometrics 48:361–372.

Hedges, S. B.
1992. The number of replications needed for the accurate estimation of the bootstrap *P* value in phylogenetic studies. Mol. Biol. Evol. 9:366–369.

Hellberg, M. E., R. S. Burton, J. E. Neigel, and S. R. Palumbi.
2002. Genetic assessment of connectivity among marine populations. Bull. Mar. Sci. 70:273–290.

Iverson, E. S. and D. C. Tabb.
1962. Subpopulations based on growth and tagging studies of spotted seatrout, *Cynoscion nebulosus*, in Florida. Copeia 1962:544–548.

Kimura, M., and J. F. Crow.
1964. The number of alleles that can be maintained in a finite population. Genetics 49:725–738.

King, T. L., and H. O. Pate.
1992. Population structure of spotted seatrout inhabiting the Texas Gulf Coast: an allozymic perspective. Trans. Am. Fish. Soc. 121:746–756.

King, T. L., and E. G. Zimmerman.
1993. Clinal variation at aspartate aminotransferase-2 in spotted seatrout, *Cynoscion nebulosus* (Cuvier), inhabiting the northwestern Gulf of Mexico. Animal Gen. 24:59–61.

Lenfant, P., and S. Planes.
2002. Temporal genetic changes between cohorts in a natural population of a marine fish, *Diplodus sargus.* Biol. J. Linn. Soc. 76:9–20.

Manly, B. J. F.
1991. Randomization and Monte Carlo methods in biology, 281 p. Chapman and Hall, New York, NY.

McMichael, R. H., Jr., and K. M. Peters.
1989. Early life history of spotted seatrout, *Cynoscion nebulosus* (Pisces: Sciaenidae), in Tampa Bay, Florida. Estuaries 12:98–110.

Michalakis, Y., and L. Excoffier.
1996. A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. Genetics 142:1061–1064.

Murphy, M. D., and R. G. Taylor.
1994. Age, growth, and mortality of spotted seatrout in Florida waters. Trans. Am. Fish. Soc. 123:482–497.

Music, J. L., Jr.
1981. Seasonal movement and migration of spotted seatrout (*Cynoscion nebulosus*). Estuaries 4:280.

Overstreet, R. M.
1983. Aspects of the biology of the spotted seatrout, *Cynoscion nebulosus*, in Mississippi. Gulf Res. Rep. (suppl. 1):1–43.

Paetkau, D., L. P. Waits, P. L. Clarkson, L. Craighead, and C. Strobeck.
1997. An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. Genetics 147:1943–1957.

Page, R. D. M.
1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Comp. Appl. Biosci. 12:357–358.

Park, S. D. E.,
2001. Trypanotolerance in west African cattle and the population genetic effects of selection. Ph.D. diss., 241 p. Univ. Dublin, Dublin, Republic of Ireland.

Ramsey, P. R., and J. M. Wakeman.
1987. Population structure of *Sciaenops ocellatus* and *Cynoscion nebulosus* (Pisces: Sciaenidae): biochemical variation, genetic subdivision and dispersal. Copeia 1987:682–695.

Raymond, M., and F. Rousset.
1995. GENEPOP (vers. 1.2): population genetics software for exact tests and ecumenicism. J. Genet. 86:248–249.

Rice, W. R.
1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Ruzzante, D. E.
1998. A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Can. J. Fish. Aquat. Sci. 55:1–14.
Schneider, S., D. Roessli, and L. Excoffier.
2000. ARLEQUIN, vers. 2.000: a software for population genetics data analysis. Genetics and Biometrics Laboratory, Department of Anthropology and Ecology, Univ. Geneva, CP 511 1211 Geneva 24, Switzerland.
Takezaki, N., and M. Nei.
1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389–399.
Turner, T. F., L. R. Richardson, and J. R. Gold.
1998. Polymorphic microsatellite DNA markers in red drum (*Sciaenops ocellatus*). Mol. Ecol. 7:1771–1773.
Vanderkooy, S. J., and R. G. Muller.
2003. Management of spotted seatrout and fishery participants in the U.S. *In* Biology of the spotted seatrout (S. A. Bartone, ed.), p. 227–245. CRC Press, Boca Raton, FL.
Weinstein, M. P., and R. W. Yerger.
1976. Electrophoretic investigation of subpopulations of the spotted seatrout, *Cynoscion nebulosus* (Cuvier), in the Gulf of Mexico and Atlantic Coast of Florida. Comp. Biochem. Physiol. 54B:97–102.
Weir, B. S., and C. C. Cockerham.
1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.
Wiley, B. A., and R. W. Chapman.
2003. Population structure of spotted seatrout, *Cynoscion nebulosus*, along the Atlantic Coast of the U.S. *In* Biology of the spotted seatrout (S. A. Bartone, ed.), p. 31–40. CRC Press, Boca Raton, FL.
Wright, J. M., and P. Bentzen.
1994. Microsatellites: genetic markers for the future. Rev. Fish Biol. Fish. 4:384–388.