Population Structure of Spotted Seatrout, *Cynoscion nebulosus*, in Texas Bays and Estuaries as Revealed by Analysis of Microsatellite DNA

by

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Submitted to the
Office of Honors Programs and Academic Scholarships
Texas A&M University
in partial fulfillment of the requirements for

1998-99 UNIVERSITY UNDERGRADUATE RESEARCH FELLOWS PROGRAM

April 15, 1999

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Introduction

Spotted seatrout, *Cynoscion nebulosus*, are a popular recreational fish and a vital fisheries resource in estuaries and bays of the northern Gulf of Mexico (Gulf) and the southeastern (Atlantic) coast of the United States. Historically, spotted seatrout have been fished both commercially and recreationally. However, in the recent past, recreational harvests have been considerably larger than commercial harvests (Van Voorhees et al. 1992; NMFS 1993). Spotted seatrout are not endangered, but perceived declines of the species in the Gulf have been attributed to loss of key habitat and overfishing (Shipp 1986; Patillo et al. 1997). As a result, there have been closures or restrictions of commercial fishing in most Gulf Coast states, and recreational fishing in the Gulf is now controlled by bag limits and/or gear restrictions in almost every state (GSMFC 1997). Because spotted seatrout spend their entire life cycle in inshore waters, conservation and management of the species is the responsibility of state governments. Fishing regulations for spotted seatrout vary from state to state (GSMFC 1997) as a function of stock assessment in each state. Currently, Texas, like most Gulf and Atlantic coast states, manages spotted seatrout based on a single-stock model, where allocation regulations are the same across bays and estuaries within a state. In contrast, states such as Florida manage spotted seatrout on a regional basis (Muller et al. 1997).

Previous studies of spotted seatrout have not established firmly whether spotted seatrout in the Gulf comprise more than one stock (King and Pate 1992). It is generally thought (Lorio and Peret 1978; Helser et al. 1993) that movement of spotted seatrout is restricted largely to natal estuaries and is based primarily on salinity changes or spawning activity. Tagging studies (Moffett 1961; Overstreet 1983; Baker and Matlock 1993) have indicated very little to no movement of juveniles or adults, with almost all tag returns occurring within 25-30 miles of the

release site. However, one study did document a return over 300 miles from the release site (Moffett 1961). Alternatively, reported differences in growth rates between spotted seatrout samples from varying areas of the Florida Gulf Coast (Iverson and Tabb 1962) are consistent with the existence of discrete subpopulations. Murphy and Taylor (1994), however, suggested that differences among spotted seatrout in growth rates reflected environmental and fishing effects, not separate subpopulations.

Past genetic studies of spotted seatrout also have been equivocal with respect to the single versus multiple stock issue, although only a few of these studies have employed modern molecular approaches. Weinstein and Yerger (1976) found discrete protein banding patterns in spotted seatrout sampled from each of seven estuaries along the western coast of Florida and suggested the possibility of discrete subpopulations. Weinstein and Yerger (1976) also observed an inverse relationship between genetic similarity and increasing geographic distance between samples, suggesting the existence of an isolation-by distance effect. Ramsey and Wakeman (1987) compared 40 presumptive gene loci in spotted seatrout from 13 sampling areas in the northern Gulf and two along the eastern coast of Florida. No strong regional divergence was observed, and overall divergence between subpopulations was low (indicated by a low F_{ST} value). There was, however, a spatial partitioning of rare alleles, as well as an isolation-by-distance effect. The latter was suggested by an observed pattern of spatial autocorrelation of alleles where positive correlations of allele frequencies occurred in adjacent localities, and negative correlations occurred between distant localities. King and Pate (1992) observed the same phenomenon (i.e., a low F_{ST} and an isolation-by-distance effect) when they studied 44 presumptive loci in spotted seatrout from 12 localities in the western Gulf. They hypothesized that the exchange of spotted seatrout genes between estuaries along the Texas coast was facilitated by a westerly directed,

near-shore transport mechanism that primarily affected eggs and larvae. This hypothesis is consistent with the data and the fact that spotted seatrout eggs and larvae are largely pelagic, while juvenile and adults are mostly demersal (Patillo et al. 1997). More recently, Gold et al. (1999) assayed variation in mitochondrial (mt) DNA in spotted seatrout sampled from eight localities in the northern Gulf and southeastern Atlantic. They found significant heterogeneity in mtDNA haplotype frequencies between Gulf and Atlantic samples, and an isolation-by-distance effect across the northern Gulf. Minimally, this suggests that different subpopulations (stocks) of spotted seatrout exist in the Gulf and Atlantic.

Genetic approaches to assessment of population structure and gene flow have been used previously with considerable success (Avise 1986; Ovenden 1990; Ramsey and Wakeman 1987). Recently, microsatellites have been the molecular marker of choice for studies on population structure and/or species conservation (Fitzsimmons et al. 1995; Ruzzante et al. 1996; Heist and Gold 1999; Van Treuren et al. 1999; Piertney et al. 1998). Microsatellites are short, repetitive stretches of DNA composed of an array of di-, tri-, or tetranucleotide repeats. Microsatellites are present in all eukaryotic species, found within unique sequence DNA, inherited according to Mendelian principles, and distributed evenly within euchromatic areas of chromosomes (Weber 1990; Wright 1993; Weber and May 1989; Vaiman et al. 1994). These characteristics, along with the high degree of polymorphism observed at microsatellite loci, make microsatellites useful genetic markers for studies of population structure.

Several studies have employed microsatellites to examine population structure in marine species (Ruzzante et al. 1996; Heist and Gold 1999; Tessier and Bernatchez 1999). Ruzzante et al (1996) used microsatellite DNA to show that Atlantic cod overwintering in inshore Newfoundland can be distinguished genetically from those overwintering offshore. Heist and

Gold (1999) used five microsatellite loci to examine red snapper from three locations in the northern Gulf and one near the northern Yucatan Peninsula. Analysis of these loci yielded population subdivision (i.e., F_{ST} and R_{ST}) estimates that did not differ significantly from zero, consistent with the hypothesis that red snapper comprise a single stock in these regions.

This study was designed to address population structure of spotted seatrout in Texas bays and estuaries via an analysis of allele distributions at nuclear-encoded microsatellite loci. The null hypothesis is that spotted seatrout from the Texas Gulf coast comprise a single stock or homogeneous Mendelian population. To test this hypothesis, allele distributions of five microsatellite loci from three estuaries along the Texas Gulf coast, and from one estuary on the east coast of Florida were examined. The goal of the project was to provide additional information on spotted seatrout population structure in order to enhance conservation and management of the species.

Materials and Methods

A total of 162 spotted seatrout was collected by angling and gill netting between 1989 and 1991. Geographic samples included three localities from the Texas Gulf coast and one locality from the east coast of Florida (Figure 1). Heart and/or spleen tissue was removed from each individual and frozen immediately in liquid nitrogen for transport to College Station, where they were transferred to -80°C until processed. Genomic DNA was isolated from frozen tissue as described in Gold and Richardson (1991). The polymerase chain reaction (PCR) was used to amplify products from five nuclear-encoded microsatellite loci. Primers used to amplify each locus are given in Table 1, and were developed initially by Turner et al. (1998) for the red drum, *Scieanops ocellatus*. DNA amplifications were carried out in 10 µl reactions and in general

followed conditions described in Turner et al. (1998). Each reaction contained 1 μl sample DNA (200 ng), 1 μl 10X reaction buffer (10% Triton-X 100, 500 mM KCl, 100mM Tris [pH9.0]), 200 μM of each dNTP, 1 mM $MgCl_2$, 5 pmols of each PCR primer, and 0.1 μl of *Taq* DNA polymerase (purified in our lab following Engelke et al. 1990). The forward primer of each pair was end-labeled with [γ^{32}P]-dATP. Amplification reactions were performed in an Omn-E thermal cycler (Hybaid), and consisted of 25 cycles of denaturation (94°C for 30s), annealing (56°C for 30s), and extension (72°C for 30s), with an initial denaturation of 94°C for two minutes. PCR products for each locus were separated on 6% denaturing polyacrylamide gels (Sequagel, National Diagnostics) and visualized by autoradiography. The repeat length of each allele was scored by side-by-side comparison to a DNA base-pair ladder and clones of microsatellites of red drum of known repeat length isolated from an existing genomic library (Turner et al. 1998). Allele frequencies for each sampling area are listed by locus in Appendix 1. Representatives of each available homozygous genotype at each locus were sequenced and compared to the corresponding red drum clone. This was necessary to confirm the presence of a microsatellite and to determine the repeat type and length (Appendix 2). Alleles were sequenced as follows: *Soc* 12, alleles 10 and 12; *Soc* 50, alleles 18 and 19; *Soc* 133, alleles 10 and 12; and *Soc* 201, alleles 10 and 11. Alleles at *Soc* 243 were not sequenced successfully. Nucleotide sequencing was carried out using PCR product from 100 μl reactions (conditions as described above but with 3 μl sample DNA used) that was then purified with isopropanol. Sequencing reagents and parameters were derived from the *fmol* Sequencing System (Promega) for 10 μl reactions. Reactions were then amplified using an Omn-E thermal cycler (Hybaid) for 25 cycles of denaturation (95°C for 30s), annealing (65°C for 30s), and extension (72°C for 30s), with an initial denaturation of 95°C for

two minutes. Sequencing products were run on 6% denaturing polyacrylamide gels (Sequagel, National Diagnostics) and visualized with autoradiography.

Genotype frequencies at each locus were tested for deviation from Hardy-Weinberg equilibrium frequencies by using Fisher's exact test provided in GENEPOP 3.1 under option 1 (Raymond and Rousset 1995). Each locus was tested for independence of genotypes. This was done by first creating contingency tables for all pairs of loci in each population, and then performing a probability test (or Fisher exact test) for each table by using a Markov chain method (GENEPOP, option 2.1). Homogeneity of allele frequencies at each locus among sample localities was tested by using (i) the randomization procedure of Roff and Bentzen (1989) provided in the MONTE program in the Restriction Enzyme Analysis Package (REAP; McElroy et al. 1992), and (ii) Fisher's exact test (GENEPOP, option 3.1 and 3.2). Additional estimates of population subdivision included Weir and Cockerham's (1984) θ, an unbiased estimator of Wright's F_{ST} (Wright 1969), and Slatkin's (1995) R_{ST}, estimated using RST CALC (Goodman 1997). R_{ST} is thought to be a more appropriate estimator for microsatellites than F_{ST} in that it assumes a stepwise mutation model rather than an infinite allele theory. Statistical significance of θ and R_{ST} was assessed using AMOVA and 1000 random permutations. Genetic distance, $(\delta\mu)^2$, was estimated after Goldstein et al. (1995) to determine if genetic distances between localities differed significantly.

Results

Nucleotide sequencing of PCR amplification products from spotted seatrout confirmed homology of microsatellite loci in spotted seatrout with those identified in red drum (Turner et al. 1998; Appendix 2). At locus *Soc* 12, allele 10 in spotted seatrout has the same repeat type as the

red drum clone, a compound CA dinucleotide repeat, and an increase of one repeat unit relative to the red drum clone [$(CA)_7CT(CA)_3$ vs. $(CA)_7CT(CA)_4$]. Allele 15 is identical to allele 10 in repeat length but contains an additional insertion of 10 base pairs after the sixth CA repeat [$(CA)_6N_{10}CACT(CA)_5$ vs. $(CA)_7CT(CA)_5$] (Appendix 2.1). At *Soc* 50, allele 18 in spotted seatrout has the same repeat type as the red drum clone, a compound TG dinucleotide repeat, but contains an additional repeat unit in the first section and a deletion of one repeat unit in the third section [$(TG)_5N_3(TG)_4N_5(TG)_6TA(TG)_3$ vs. $(TG)_4N_3(TG)_4N_5(TG)_7TA(TG)_3$]. Allele 19 is identical to allele 18 except that it does not have the deletion of one repeat unit in the third section [$(TG)_5N_3(TG)_4N_5(TG)_7TA(TG)_3$ vs. $(TG)_5N_3(TG)_4N_5(TG)_6TA(TG)_3$] (Appendix 2.2). At locus *Soc* 133, allele 10 in spotted seatrout has the same repeat type as the red drum clone, a TGC trinucleotide repeat, but is shorter and imperfect [$C(TGC)_4$ vs. $(TGC)_8$]. Allele 12 also is imperfect but contained two additional repeat units relative to allele 10 [$C(TGC)_6$ vs. $C(TGC)_4$] (Appendix 2.3). At locus *Soc* 201, length variation between alleles 10 and 11 in spotted seatrout is not due to a change in microsatellite repeat number. The repeat is present in both alleles and in the same copy number as in red drum. Allele 10 is two base pairs longer than the red drum clone, and allele 11 is two base pairs longer than allele 10. These differences in size, however, stem from two insertion/deletion changes; there is also a base substitution difference between the two alleles (Appendix 2.4).

Allele frequencies at each locus across all localities are given in Appendix 1. All five loci exhibited two or three common alleles, with the total number of alleles at each locus ranging from four to six. Additional summary statistics for each locus are listed in Table 2. Observed heterozygosity for all loci ranged from 0.22 to 0.59 with a mean of 0.426. Almost all loci had frequencies of heterozygotes and homozygotes that matched Hardy-Weinberg expected

proportions, with the exception of locus *Soc* 201 in the sample from east Florida ($P_{HW} = 0.01$). This result was non-significant when probability values were corrected for multiple tests by using the sequential Bonferoni method (Rice 1989). None of the pairwise comparisons of genotypes across populations were significantly non-random (Table 3), suggesting that genotypes at one locus are independent of genotypes at other loci.

Allele frequency homogeneity among samples was tested at each locus. Comparison groups were (i) among all samples, (ii) among Gulf samples only, and (iii) between Atlantic and pooled Gulf samples. For all loci except *Soc* 201, the Roff-Bentzen test revealed significant heterogeneity among all samples and between samples from the Atlantic and Gulf (Table 4). No significant heterogeneity was found among samples from the Gulf. For locus *Soc* 201, the Roff-Bentzen test revealed significant heterogeneity only among all samples (P=0.027). Results of Fisher's exact test paralleled results from the Roff-Bentzen tests for all five loci.

Two estimates of population structure, θ and R_{ST}, also were computed for the same group comparisons. Because size variation at two of the loci, *Soc* 12 and *Soc* 201, was not attributable to changes in the number of repeat units, these two loci were excluded from the R_{ST} calculations. Estimates of θ and R_{ST} (Table 5) also indicated significant population structure among all samples and between samples from the Atlantic and Gulf. Probability tests for θ at each locus and R_{ST} over all suitable loci were significant (i.e., significantly different from zero) for each of these comparisons except for locus *Soc* 133, where the θ value was non-significant (after a Bonferoni correction) between the Atlantic and Gulf samples. Genetic distances between pairwise comparisons of all localities, measured as $(\delta\mu)^2$, are listed in Table 6. The distances between the Atlantic sample and the Gulf samples are five to ten fold larger than the genetic distances among samples from the Gulf, suggesting that the Atlantic sample differs more significantly from the Gulf

samples than the samples in the Gulf differ from one another.

Discussion

Results of this study are consistent with the hypothesis that spotted seatrout comprise a single, Mendelian population in the northern Gulf of Mexico. None of the tests of homogeneity of allele frequency or population structure revealed significant differences among samples from the Gulf. The sample from east Florida differed significantly from the pooled Gulf samples. The distinction of the Atlantic sample from the Gulf samples was reinforced by the difference in magnitude of $(\delta\mu)^2$ values in comparisons of Atlantic versus Gulf samples relative to comparisons among Gulf samples. These results are consistent with those from a survey of variation in restriction sites of mitochondrial DNA among samples of spotted seatrout from the northern Gulf and southeastern Atlantic (Gold et al. 1999), where significant differences were found between samples from the Gulf and samples from the Atlantic but not among samples from the western Gulf. Overall, genetic divergence of spotted seatrout into distinct subpopulations in the Atlantic and Gulf regions is similar to patterns of population structure in several other marine species. Several species of marine finfish, including red drum, black drum, black sea bass, toadfish, and greater amberjack, are known to have distinct subpopulations in the Gulf and Atlantic (Avise 1992; Gold and Richardson 1998). It has been suggested that this shared pattern developed as a result of changes in climate during glacial times (Avise 1992), but it also may be influenced by ocean currents, lack of suitable habitats between the two regions, and differences in the available fauna in the Atlantic and the Gulf (Gold and Richardson 1998).

The absence of discrete subpopulations of spotted seatrout in the northern Gulf is concordant with previous studies of spotted seatrout where little genetic divergence was observed

between samples of spotted seatrout from the Gulf (Ramsey and Wakeman 1987; King and Pate 1992). Both of these latter studies, however, documented an isolation-by-distance effect based on spatial autocorrelation of alleles. King and Pate (1992) suggested this was due to a westerly-directed, near shore transport mechanism for spotted seatrout eggs and larvae. The absence of population structure found in this study does not falsify an isolation-by-distance hypothesis, as the geographic distances among sample localities were to small to permit a robust spatial autocorrelation analysis. It also may be that divergence among samples of spotted seatrout in the Gulf is too recent to be reflected by the microsatellite loci employed in this study. However, while the null hypothesis cannot be rejected in this study, it also cannot be proven. Additional microsatellite studies with larger samples and more microsatellite loci could conceivably provide a more rigorous test of the null hypothesis.

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Table 1 PCR primers developed for red drum (*Sciaenops ocellatus*) and used to amplify microsatellite loci in spotted seatrout (*Cynoscion nebulosus*).

Locus	Primer sequences (5'-3')	Length (bp)*	Repeat Sequence*
Soc 12	GCACCATCTTGCCACTGATGAATT GGGCTCTTACAACTCGTTTCAGAT	187	$[GT]_7$
Soc 50	CCCGTGATTTTAGGCTCAGATA CCTTTAGAGTGCAGTAAGTGATTT	183	$[GT]_7$
Soc 133	CATTTGGACCATCGCTACTGCTG CTTGGCATTTCCAGACATCACTG	205	$[TGC]_8$
Soc 201	GGAGGAACTGATGAGGGCAGTGT GCACAACACACCTCGCTATATC	229	$[CCT]_6$
Soc 243	GACGGGGATGCCATCTGC AATGCGAAAAAGACGAAACAGT	106	$[CCT]_9$

* Length indicates size in bp of PCR product amplified from the red drum clone. Repeat sequence indicates the repeat motif [in brackets], and number of uninterrupted copies observed in the red drum cloned allele. See Appendix 2 for length and repeat sequence comparisons to spotted seatrout.

Table 2 Summary statistics for each microsatellite locus scored in spotted seatrout.

Locus	Sample			
	LLM	TPB	SAB	EFL
Soc 12				
n_i	55	35	31	41
n_a	6	3	4	2
H_O	0.546	0.362	0.410	0.221
P_{HW}	0.504	0.230	0.470	1.000
Soc 50				
n_i	55	35	31	39
n_a	4	4	4	3
H_O	0.582	0.590	0.563	0.407
P_{HW}	0.503	0.781	0.116	0.664
Soc 133				
n_i	55	35	31	41
n_a	4	4	4	3
H_O	0.374	0.338	0.232	0.434
P_{HW}	0.320	1.000	0.290	0.073
Soc 201				
n_i	55	35	31	40
n_a	4	4	4	4
H_O	0.467	0.449	0.310	0.330
P_{HW}	0.425	0.378	1.000	0.010*
Soc 243				
n_i	55	33	31	39
n_a	3	3	3	4
H_O	0.489	0.502	0.444	0.460
P_{HW}	0.793	0.870	0.804	1.000

Sample acronyms are as given in Figure 2. n_i = number of individuals scored; n_a = number of alleles; H_O = observed heterozygosity; P_{HW} = probability of conforming to Hardy-Weinberg proportions.

* Non-significant ($P > 0.05$) when corrected for multiple tests.

Table 3 Probability of genotypic equilibrium among loci for combined samples ($\alpha = 0.05$).

	Locus				
Locus	*Soc* 12	*Soc* 50	*Soc* 133	*Soc* 201	*Soc* 243
Soc 12					
Soc 50	0.546				
Soc 133	0.682	0.445			
Soc 201	0.893	0.197	0.210		
Soc 243	0.090	0.181	0.516	0.810	

Table 4 Probability of allele-frequency homogeneity among comparison groups.

	Locus				
Group Comparison	*Soc* 12	*Soc* 50	*Soc* 133	*Soc* 201	*Soc* 243
P_{RB}					
All samples	0.005	0.021	0.002	0.027	0.000
Gulf samples	0.324	0.878	0.472	0.087	0.827
Atlantic vs Gulf	0.006	0.001	0.000	0.141	0.000
$P_{\text{Exact Test}}$					
All samples	0.002	0.011	0.004	0.017	0.000
Gulf samples	0.305	0.878	0.362	0.035*	0.285
Atlantic vs Gulf	0.003	0.002	<0.000	0.177	0.000

* Non-significant ($P > 0.05$) when corrected for multiple tests.

Table 5 Estimates of population subdivision as determined by θ (F_{ST}) and R_{ST}. The probability that a θ or R_{ST} value differs from zero is in parentheses.

	Locus					
Group Comparison	*Soc* 12	*Soc* 50	*Soc* 133	*Soc* 201	*Soc* 243	Mean
θ						
All samples	0.050 (<0.001)	0.033 (<0.001)	0.019 (0.013)	0.010 (0.128)	0.028 (0.006)	---
Gulf samples	0.015 (0.103)	-0.011 (0.981)	0.000 (0.331)	0.005 (0.180)	-0.012 (0.936)	---
Atlantic vs Gulf	0.078 (<0.001)	0.071 (<0.001)	0.036 (0.043)*	0.013 (0.139)	0.063 (<0.001)	---
R_{ST}						
All samples	---	0.026	0.002	---	0.068	0.032 (0.000)
Gulf samples	---	-0.012	0.007	---	-0.009	-0.005 (0.689)
Atlantic vs Gulf	---	0.073	-0.007	---	0.130	0.066 (0.000)

* Non-significant ($P > 0.05$) when corrected for multiple tests.

Table 6 Genetic distances between localities measured as $(\delta\mu)^2$.

		Locality	
Locality	LLM	TPB	SAB
LLM			
TPB	0.015		
SAB	0.024	0.008	
EFL	0.126	0.108	0.128

Appendix 1

Table A1.1 Allele frequencies at *Soc* 12 among samples of spotted seatrout (*Cynoscion nebulosus*) from the Gulf of Mexico and eastern coast of Florida. Allele number represents an estimated number of repeat units relative to the red drum (*Sciaenops ocellatus*) clone.

	Locality			
Allele	LLM	TPB	SAB	EFL
7	0.018	0.000	0.000	0.000
10	0.609	0.771	0.726	0.902
11	0.036	0.000	0.048	0.000
12	0.036	0.029	0.048	0.000
13	0.009	0.000	0.000	0.000
15	0.291	0.200	0.177	0.098

Table A1.2 Allele frequencies at *Soc* 50 among samples of spotted seatrout (*Cynoscion nebulosus*) from the Gulf of Mexico and eastern coast of Florida. Allele number represents an estimated number of repeat units relative to the red drum (*Sciaenops ocellatus*) clone.

	Locality			
Allele	LLM	TPB	SAB	EFL
17	0.100	0.129	0.081	0.049
18	0.355	0.314	0.371	0.171
19	0.536	0.543	0.532	0.780
20	0.000	0.014	0.000	0.000
21	0.009	0.000	0.016	0.000

Table A1.3 Allele frequencies at *Soc* 133 among samples of spotted seatrout (*Cynoscion nebulosus*) from the Gulf of Mexico and eastern coast of Florida. Allele number represents an estimated number of repeat units relative to the red drum (*Sciaenops ocellatus*) clone.

	Locality			
Allele	LLM	TPB	SAB	EFL
10	0.173	0.129	0.065	0.061
11	0.045	0.043	0.081	0.207
12	0.773	0.800	0.839	0.732
13	0.009	0.029	0.016	0.000

Table A1.4 Allele frequencies at *Soc* 201 among samples of spotted seatrout (*Cynoscion nebulosus*) from the Gulf of Mexico and eastern coast of Florida. Allele number represents an estimated number of repeat units relative to the red drum (*Sciaenops ocellatus*) clone.

	Locality			
Allele	LLM	TPB	SAB	EFL
4	0.000	0.000	0.000	0.013
9	0.009	0.014	0.032	0.000
10	0.236	0.157	0.161	0.138
11	0.691	0.714	0.790	0.825
12	0.064	0.114	0.000	0.025
20	0.000	0.000	0.016	0.000

Table A1.5 Allele frequencies at *Soc* 243 among samples of spotted seatrout (*Cynoscion nebulosus*) from the Gulf of Mexico and eastern coast of Florida. Allele number represents an estimated number of repeat units relative to the red drum (*Sciaenops ocellatus*) clone.

	Locality			
Allele	LLM	TPB	SAB	EFL
5	0.019	0.044	0.016	0.122
8	0.620	0.632	0.629	0.744
11	0.361	0.324	0.355	0.122
14	0.000	0.000	0.000	0.012

Appendix 2

Table A2.1 Alignment of spotted seatrout DNA sequences with that of red drum for locus *Soc* 12. Bases in bold indicate primer and repeat regions.

```
Consensus[a] ------------------------CTTAATGGATTTCGATGC-TTCGGGGAGCCACCA
   Soc 12 GCACCATCTTGCCACTGATGAATTCTTAATGGATTTCGATGC-TTCGGGGAGCCACCA
allele 10 ------------------------CTTAATGGATTTCGATGCCTTCGGGGAGCCACCA
allele 15*-----------------------CTTAATGGATTTCGATGCCTTCGGGGAGCCACCA

Consensus CATGTGTCTGCTGGCCTATAAACAGAAAGCCAGGAAGAGGCTGGGGA--CCATAAACA
   Soc 12 CATGTGTCTGCTGGCCTATAAACAGAAAGCCAGGAAGAGGCTGGGGAAACCATAAACA
allele 10 CATGTGTCTGCTGGCCTATAAACAGAAAGCCAGGAAGAGGCTGGGGAGCCCATAAACA
allele 15*CATGTGTCTGCTGGCCTATAAACAGAAAGCCAGGAAGAGGCTGGGGAGCCCATAAACA

Consensus CACACACACA----------CACTCACACACA--CCATGC-ACTGGTTTGTGATAACA
   Soc 12 CACACACACA----------CACTCACACACA--CCATGCCACTGGTTTGTGATAACA
allele 10 CACACACACA----------CACTCACACACACACCATGCGACTGGTTTGTGAT----
allele 15*CACACACACATTCACACTTGCACTCACACACACACCATGCGACTGGTTTGTGAT----

Consensus ------------------------
   Soc 12 CATCTGAAACGAGCTGTAAGAGCCC
allele 10 ------------------------
allele 15*------------------------
```

* Allele 15 possesses a 10 base pair insertion, not an increase of five repeat units.

[a] Consensus sequence determined using GeneTool v1.1 (LifeScience Software Resource)

Table A2.2 Alignment of spotted seatrout DNA sequences with that of red drum for locus *Soc* 50. Bases in bold indicate primer and repeat regions.

```
Consensus[a] ----------------------AATGAGGTGTGCATGAAGTCCGCTCCTTCTACAAAT
   Soc 50 CCCGTGATTTTAGGCTCAGATAAATGAGGTGTGCATGAAGTCCGCTCCTTCTACAAAT
allele 18 ----------------------AATGAGGTGTGCATGAAGTCCGCTCCTTCTACAAAT
allele 19 ----------------------AATGAGGTGTGCATGAAGTCCGCTCCTTCTACAAAT

Consensus ATTTGTGTGTG--CAGTGTGTGTGCA-TATGTGTGTGTGTG--TATGTGTGTATCTT-
   Soc 50 ATTTGTGTGTG--CAGTGTGTGTGCAGTATGTGTGTGTGTGTATGTGTGTATCTT-
allele 18 ATTTGTGTGTGTGCAGTGTGTGTGCAATATGTGTGTGTGTG--TATGTGTGTATCTTT
allele 19 ATTTGTGTGTGTGCAGTGTGTGTGCAATATGTGTGTGTGTGTATGTGTGTATCTTT

Consensus GTGTCTGTCTCCAGTATTTCGAACAGACAGCAGTCTGCAAGCTGGT------------
   Soc 50 GTGTCTGTCTCCAGTATTTCGAACAGACAGCAGTCTGCAAGCTGGTAAATCACTTACT
allele 18 GTGTCTGTCTCCAGTATTTCGAACAGACAGCAGTCTGCAAGCTGGT------------
allele 19 GTGTCTGTCTCCAGTATTTCGAACAGACAGCAGTCTGCAAGCTGGT------------

Consensus ----------
   Soc 50 GCACTCTAAAG
allele 18 ----------
allele 19 ----------
```

[a] Consensus sequence determined using GeneTool v1.1 (LifeScience Software Resource)

Table A2.3 Alignment of spotted seatrout DNA sequences with that of red drum for locus *Soc* 133. Bases in bold indicate primer and repeat regions.

```
Consensus[a] ----------CTGCTGCTGCTGC---------ACTCTCAACTCTCCAT
  Soc 133 CATTTGGACCATCGCTACTGCTGCTGCTGCTGCTGCTGCACTCTCAACTCTCCAT
allele 10 ----------CTGCTGCTGCTGC---------ACTCTCAACTCTCCAT
allele 12 ----------CTGCTGCTGCTGCTGCTGC---ACTCTCAACTCTCCAT

Consensus TTTGATGCTTTTATTCTT-CTCT-CGAGCCGAGCTGAAACACATTCTTGCCCGTCATC
  Soc 133 TTTGATGCTTTTATTCTTGCTCTACGAGCCGAGCTGAAACACATTCTTGCCCGTCATC
allele 10 TTTGATGCTTTTATTCTTCCTCTCCGAGCCGAGCTGAAACACATTCTTGCCCGTCATC
allele 12 TTTGATGCTTTTATTCTTCCTCTCCGAGCCGAGCTGAAACACATTCTTGCCCGTCATC

Consensus ATCATCATCATCATCAAAAA-AAGAAAAA-AAAAAAA--GAAAAGGCAGCAGATTCCC
  Soc 133 ATCATCATCATCATCAAAAAAAAGAAAAAAAAAAAAAAAGAAAAGGCAGCAGATTCCC
allele 10 ATCATCATCATCATCAAAAA-AAGAAAAAGAAAAAAA--GAAAAGGCAGCAGATTCCC
allele 12 ATCATCATCATCATCAAAAA-AAGAAAAAGAAAAAAA--GAAAAGGCAGCAGATTCCC

Consensus GCTGATG----------
  Soc 133 GCTGATGCAGTGATGTCTGGAAATGCCAAG
allele 10 GCTGATG----------
allele 12 GCTGATG----------
```

[a] Consensus sequence determined using GeneTool v1.1 (LifeScience Software Resource)

Table A2.4 Alignment of spotted seatrout DNA sequences with that of red drum for locus *Soc* 201. Bases in bold indicate primer region and insertion/deletion events resulting in distinct alleles. The microsatellite in red drum and the corresponding region in spotted seatrout is underlined and one substitution is denoted in bold.

```
Consensus ----------------------TAAAAGACCAACACCTCC-CCTCCTCCTCCTTCTC
  Soc 201 GGAGGAACTGATGAGGGCAGTGTTAAAAGACCAACACCTCCTCCTCCTCCTCCTTCTC
allele 10 ----------------------TAAAAGACCAACACCTCCGCCTCCTCCTCCTTCTC
allele 11 ----------------------AAAAGACCAACACCTCCGCCTCCTCCTCCTTCTC
Consensus CTCCTGACTGGAAAATCATAATCCCTGCTGACAGGCTGAGAGGTGAAC-AATGAGAAA
  Soc 201 CTCCTGACTGGAAAATCATAATCCCTGCTGACAGGCTGAGAGGTGAACAAATGAGAAA
allele 10 CTCCTGACTGGAAAATCATAATCCCTGCTGACAGGCTGAGAGGTGAACGAATGAGAAA
allele 11 CTCCTGACTGGAAAATCATAATCCCTGCTGACAGGCTGAGAGGTGAACGAATGAGAAA

Consensus TAAGGG-GGTTAT-AA-TTTTT-TTTTT------CAATTTTC-G-----AAATGAAGA
  Soc 201 TAAGGGTGGTTATGAA-TTTTT-TTTTT-----CCAATTTTCAGTCAATAAATGAAGA
allele 10 TAAGGGAGGTTATCAAGTTTTTGTTTTTTTTCCCCAATTTTCTG-----AAATGAAGA
allele 11 TAAGGGAGGTTATCAAGTTTTTGTTTTTTTTA---CAATTTTCTGTCAGTAAATGAAGA

Consensus GCTAATTGAGCCACAGTCCACATGCAAAC-AG-AGAATTT-------------------
  Soc 201 GCTAATTGAGCCACAGTCCACATGCAAACTAGTAGAATTTGATATAGCGAGGTGTGTT
allele 10 GCTAATTGAGCCACAGTCCACATGCAAACCAGCAGAATTT-------------------
allele 11 GCTAATTGAGCCACAGTCCACATGCAAACCAGCAGAATTT-------------------

Consensus ---
  Soc 201 GTG
allele 10 ---
allele 11 ---
```

[a] Consensus sequence determined using GeneTool v1.1 (LifeScience Software Resource)

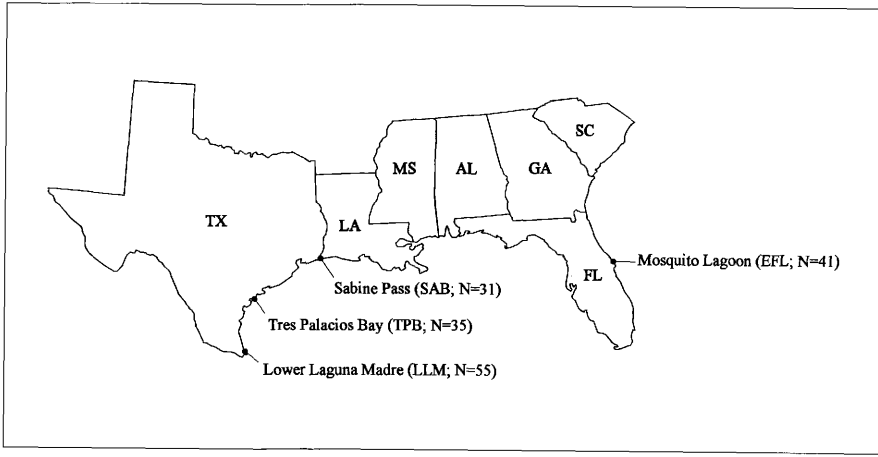

Figure 1 Sampling localities and sample sizes for spotted seatrout, *Cynoscion nebulosus*.