Investigation of congeneric hybridization in and stock structure of weakfish (*Cynoscion regalis*) inferred from analyses of nuclear and mitochondrial DNA loci*

Jan F. Cordes
John E. Graves
School of Marine Science
Virginia Institute of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062
Present address (for J. F. Cordes): Department of Animal Science
University of California
Davis, California 95616
E-mail address (for J. F. Cordes): jfcordes@ucdavis.edu

The weakfish (*Cynoscion regalis*) is distributed along the east coast of the United States from Massachusetts to eastern Florida and is most abundant from New York to North Carolina (Bigelow and Schroeder, 1953). Historically there has been some question as to the taxonomic relationship between weakfish and sand seatrout (*Cynoscion arenarius*); some suggest they may be separate populations of a single species (Moshin, 1973; Weinstein and Yerger, 1976; Cowan, 1985; Ditty, 1989), and others treat them as separate species (Schlossman and Chittenden, 1981).

Weakfish support substantial commercial and recreational fisheries throughout their range. Precipitous drops in total annual catches between 1980 and 1994 led to a temporary ban on commercial fishing in federal waters in 1995 (Anonymous, 1995), and there is concern that bycatch of juvenile weakfish by shrimp trawlers at the southern end of the species' range is adversely impacting abundance (Vaughan et al.[1]).

As water temperatures warm in the spring, weakfish move north and inshore into estuaries to spawn. When inshore temperatures cool in the fall, juveniles move south to overwinter off the coast of North Carolina, and older fish are thought to migrate south and offshore (Wilk[2]). Traditional studies based on tag and recapture data (Nesbit, 1954), scale structure (Perlmutter et al., 1956), morphometric data (Scoles, 1990), and various life history characters (Shepherd and Grimes, 1983, 1984) suggest two or more independent stocks of weakfish. These characters may be influenced by environmental differences, however (Shepherd and Grimes, 1983), and may not reflect genetically distinct (reproductively isolated) stocks. Genetic analyses of weakfish stock structure in the mid Atlantic Bight employing allozyme analysis (Crawford et al., 1989) and restriction fragment length polymorphism (RFLP) analysis of whole molecule mitochondrial (mt) DNA (Graves et al., 1992) were unable to reject the null hypothesis that weakfish along the U.S. east coast comprise a single, genetically homogeneous stock. However, the power of both the analyses was limited by low overall genetic variation.

Recent analyses of new molecular markers, including microsatellite DNA loci and nuclear gene intron regions, have revealed elevated levels of genetic variation in relation to traditional methods, such as allozymes or RFLP analysis of mtDNA (Miller and Kapuscinski, 1996; Brunner et al., 1998). Although higher levels of genetic variation do not necessarily provide greater stock resolution (Seeb et al., 1998), microsatellite loci have revealed stock structure for some species, where more traditional molecular markers have not (Bentzen et al., 1996; Ruzzante et al., 1996; Patton et al., 1997). Similarly, analyses of variable gene intron regions have revealed stock structure within several marine fishes (Palumbi and Baker, 1994; Moran et al., 1997; Leclerc et al., 1996; Chow and Takeyama, 2000). In this study we employed analyses of nuclear and mtDNA markers to evaluate stock structure in weakfish along the east coast of the United States and to investigate possible hybridization between weakfish and other *Cynoscion* species.

Materials and methods

Sample collections were restricted to young-of the-year (YOY) fish (less than 140 mm SL) that are reported to remain in their natal estuaries during the first several months of growth (Rowe and Epifanio, 1994). YOY were collected in the summers of 1996 and 1997 from five sites along the east coast of the United States (Fig. 1), maintained on ice after capture, transported to the laboratory, and frozen at –80°C. Muscle tissue was excised from each sample and either stored at –80°C or placed in DMSO buffer (25 mM EDTA, 20% DMSO, saturated NaCl) and stored at room temperature. Genomic DNA was isolated by following the protocol of Sambrook et al. (1989), as modified in Cordes (2000).

Specific identification of individuals was determined by using a molecular key for 16 species of Chesapeake Bay

* Contribution 2532 of the Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062.

[1] Vaughan, D. S., R. J. Seagraves, and K. West. 1991. An assessment of the Atlantic weakfish stock, 1982–1988. Atl. States Mar. Fish. Comm. Spec. Rep. 21. 29 p. + tables. Atlantic States Marine Fisheries Commission, 1444 Eye St., NW 6th Floor, Washington, D.C. 20005.

[2] Wilk, S. J. 1976. The weakfish—a wide ranging species. Atl. States Mar. Fish. Comm, Mar. Resourc. Atl. Coast Fish. Leaflet 18, 4 p. Atlantic States Marine Fisheries Commission, 1444 Eye St., NW 6th Floor, Washington, D.C. 20005.

Manuscript accepted 21 October 2002.
Manuscript received 9 January 2003 at NMFS Scientific Publications Office.
Fish. Bull. 101:443–450 (2003).

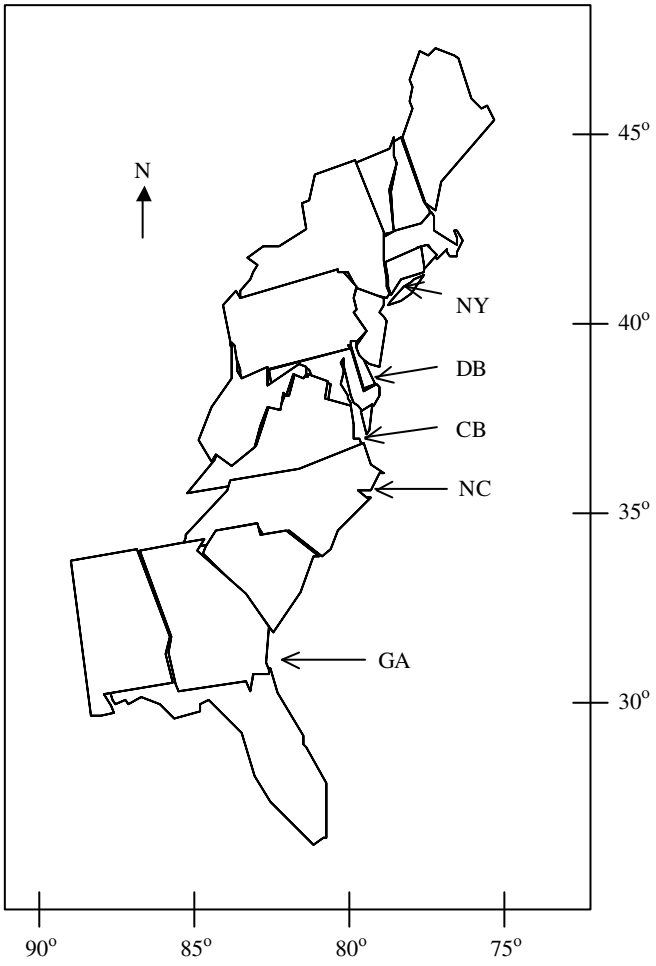

Figure 1

Sampling locations for young-of-the-year (YOY) weakfish (*Cynoscion regalis*) in the summers of 1996 and 1997. Sites are Peconic Bay, New York (NY); Delaware Bay, Delaware (DB); Chesapeake Bay, Virginia (CB); Pamlico Sound, North Carolina (NC); and Doboy Sound, Georgia (GA).

sportfishes (including eight species of sciaenids) based on a 12S/16S mtDNA gene region digested with *Rsa* I (Cordes et al., 2001). Additional 12S/16S mtDNA/*Rsa* I patterns were generated for silver seatrout (*Cynsoscion nothus*) and sand seatrout (*C. arenarius*) from the Gulf of Mexico, as well as for banded drum (*Larimus fasciatus*), gulf kingfish (*Menticirrhus littoralis*), and star drum (*Stellifer lanceolatus*) from the South Atlantic Bight following procedures in Cordes et al. (2001).

The following microsatellite primers (Table 1) developed for red drum (*Sciaenops ocellatus*) and spotted seatrout (*Cynoscion nebulosus*) loci were used to amplify weakfish DNA: SOC050 and SOC044 (Turner et al., 1998), SOC014 (Chapman[3]), and CNE612 (Chapman et al., 1999). Amplifications of all microsatellite loci were carried out in 10 µL reactions containing 8.30 µL sterile dH_2O, 1.0 µL 10× PCR buffer with 15 mM $MgCl_2$, 0.20 µL 10 mM dNTP mixture, 0.05 µL forward primer (100 pm/µL) labeled with a fluorescent dye (Licor), 0.20 µL reverse primer (100 pm/µL), 0.05 µL *Taq* I polymerase (5 U/µL), and 0.20 µL weakfish DNA. Samples were first denatured for 4 min at 95ºC, followed by 32 cycles of PCR amplification performed under the following conditions: 1 min. at 94°C, 1 min. at 50°C, and 1 min. at 72°C. Reactions were given a final 7 min. extension at 72°C. PCR product alleles were separated electrophoretically on a 6% Long Ranger™ polyacrylamide gel with a model 4000 automated DNA infrared sequencer from Li-Cor (Lincoln, NE).

Universal actin gene primers developed by G. Warr and M. Wilson (cited in Reece et al., 1997) were used to identify and refine an approximately 800-bp actin intron region locus (CRESIA1) in weakfish (Cordes, 2000). Weakfish PCR amplification products obtained with S7 ribosomal protein intron 2 (RP2) primers originally developed from swordfish *Xiphius gladius* (Chow and Hazama, 1998) were cloned and sequenced as described in Cordes (2000) and checked against sequences published in Genbank to confirm their identity. The original RP2 primers were then used without modification for amplification of all samples. Both the CRESIA1 and RP2 amplifications were carried out under the same conditions outlined above for the microsatellite loci, with the exception that the annealing temperature was lowered to 45°C. CRESIA1 and RP2 amplification products from a subset of each weakfish collection were screened for polymorphisms with a panel of restriction endonucleases and the resulting digestions were separated on 2.5% agarose gels with 1% NuSieve and 1.5% agarose in 1×TBE buffer (Cordes, 2000). Gels were stained in 1×TBE buffer containing 30 µL (5 mg/mL) ethidium bromide (EtBr), visualized on a Spectroline model TR-302 transilluminator, and photographed with a Polaroid CU-5 land camera. *Dra* I was the only enzyme that revealed polymorphic restriction sites within CRESIAI, and only *Hinf* I revealed reliably scored polymorphisms in RP2. All YOY weakfish samples were subsequently screened for variation at CRESIAI/*Dra* I and RP2/*Hinf* I.

Microsatellite gel images and restriction enzyme digestion patterns for CRESIA1 and RP2 were analyzed by using the software program RFLPScan Plus 3.0 (CSPI-Scanalytics, 1996). Statistical analyses for all loci were performed with the Arlequin 1.1 software program of Schneider et al. (1997). Nonparametric, exact-significance tests (exact θ significance tests and exact probability tests) were used to evaluate sample genotype distributions for departures from Hardy-Weinberg expectations. Unbiased estimators of exact significance probabilities for the Hardy-Weinberg equilibrium tests were calculated by using the Markov chain algorithm of Guo and Thompson (1992) with a Markov chain length of 100,000 steps. Patterns of genetic diversity and divergence within and between populations were evaluated by using the analysis of molecular variance (AMOVA) of Excoffier et al. (1992), which generates F-statistics analogous to the θ values of Weir and Cockerham (1984). Significance of F-statistics was evaluated with exact F permutation procedures (Excoffier et al.,

[3] Chapman, R. W. 1998. Unpubl. data. Marine Resources Research Institute, Department of Natural Resources, Charleston, SC 29422.

1992). Type-I error was controlled for all multiple testing with the sequential Bonferroni method of Rice (1989).

Results and discussion

Inclusion of nontarget species in weakfish samples

Initial analysis of the SOC050 microsatellite data revealed a significant departure of genotypic frequencies from expectations of Hardy-Weinberg equilibrium for the Georgia 1997 sample, even after correction for multiple tests (α=0.005). Similarly, initial SOC050 AMOVA results indicated a significant within-population variance (P=0.031), and exact F permutation tests of population pairwise F_{ST} values resulted in a number of near-significant corrected P values, all involving the Georgia 1997 sample. Inspection of the Georgia 1997 SOC050 alleles revealed a bimodal size distribution due to the presence of several unusually small alleles less than 187 bp in size. It was suspected that alleles in the smaller mode might be the result of misidentified individuals, hybridization, or introgression.

Analysis of putative weakfish with small SOC050 alleles with the 12S/16S marker of Cordes et al. (2001) resulted in three distinct restriction digestion patterns. One pattern matched that reported for weakfish, and the other two did not match any of the 16 species surveyed by Cordes et al. (2001). To determine the identity of the unknown patterns, voucher samples of five additional sciaenid species (listed in the "Materials and methods" section above) were analyzed with the 12S/16S mitochondrial marker. The two unknown patterns matched those of silver seatrout (*Cynoscion nothus*) and sand seatrout (*C. arenarius*) (Table 2). The SOC050 locus was subsequently amplified for all silver seatrout (n=13) and sand seatrout (n=15) samples, produ-

Table 1

Primer sequences for amplifying microsatellite, actin gene intron, and ribosomal protein 2 gene intron loci in weakfish (*Cynoscion regalis*).

Locus	Primer sequence (5′–3′)	Length (bp)	Repeat motif in weakfish	Annealing temperature	Original reference
Microsatellites					
CRE66	CRE66F: TGGTCTGTTAGTCCACAGTGTTG CRE66R: CGTTGCCTTCATTACAGGAGAC	251	$[GATA]_{25}$	40°C	This study
CRE80	CRE80F: ACAGCATGTGAGGGTTAAGGAT CRE80R: TACAGCTCTCTGACTGATGTAGTTGA	136	$[GATA]_9$	40°C	This study
SOC050	SOC050F: CCCGTGATTTTAGGCTCATCAGATA SOC050R: CCTTTAGAGTGCAGTAAGTGATTT	193	$[GT]_4n_3[GT]_{10}$ $n_7[GT]_9$	50°C	(Turner et al., 1998)
SOC044	SOC044F: GAGGGTGACGCTAACAGTTGA SOC044R: CACAGCTCCACTCTGATATG	202	$[CA]_3n_{39}[GT]_3$ $n_5[GT]_2n_2[GT]_2$	50°C	(Turner et al., 1998)
SOC014	SOC014F: GTATGTATTAAGGGCACAAGGTG SOC014R: GATTGCTGCTGGACAGACTG	114	$[CA]_5$	50°C	(Chapman[1])
CNE612	CNE612F: CAAGTGCACGGTATGTGATG CNE612R: AGGAACCTGACCAATCCAAA	131	$[GT]_5n_{10}[GT]_{11}$		(Chapman et al., 1999)
Introns					
CRESIA1	CRESIA1F: ATGCCTCTGGTCGTACCACTGG CRESIA1R: CAGGTCCTTACGGATGTCG	545	—		This study
RP2	RP2F: AGCGCCAAAATAGTGAAGCC RP2R: GCCTTCAGGTCAGAGTTCAT	731	—		(Chow and Hazama, 1998)

[1] Chapman, R. W. 1998. Unpubl. data. Marine Resources Research Institute, Department of Natural Resources, Charleston, SC 29422.

Table 2

Restriction digestion patterns of the 12S/16S mitochondrial DNA region for putative weakfish (*Cynoscion regalis*) individuals in the Georgia 1997 sample, sand seatrout (*C. arenarius*) and silver seatrout (*C. nothus*) digested with the enzyme *Rsa* I. *n* = number of individuals exhibiting the adjacent pattern. Apparent total size differences may be gel artifacts due to unresolved bands <100 bp in one or more of the species.

Species	*n*	Restriction	Fragment	Sizes	(bp)	Total size (bp)
Georgia 1997 Sample						
weakfish	3	461	300	200	167	1128
unknown A	7	413	300	200	167	1080
unknown B	5	461	300	256	167	1184
Known standards						
Cynoscion arenarius	15	461	300	256	167	1184
Cynoscion nothus	13	413	300	200	167	1080

cing allele sizes of 175–181 bp for silver seatrout and 175–193 bp for sand seatrout. Amplification of the silver seatrout and sand seatrout samples with the remaining three microsatellite and two intron loci did not provide further evidence of hybridization. SOC044 and CNE612 allele size ranges for both species fell within the range exhibited by the weakfish samples, and the SOC014 and both intron loci did not amplify in either the silver seatrout or sand seatrout samples.

Individuals with unusually small SOC050 alleles from the Georgia 1997 sample fell into one of four general classes. Seven individuals had silver seatrout mtDNA and two small SOC050 alleles and were presumably pure silver seatrout. The inclusion of these individuals in the collection may not be surprising because both weakfish and silver seatrout are common in the South Atlantic Bight (Bigelow and Schroeder, 1953; Hildebrand, 1955) and are difficult to distinguish during their early life history stages. Although the latter species is known to inhabit deeper waters as adults (Ginsburg, 1931), both species are inshore summer spawners (Devries and Chittenden, 1982; Shepherd and Grimes, 1984).

Three individuals possessed sand seatrout mtDNA and two small SOC050 alleles and were presumably pure sand seatrout. Some researchers have suggested that weakfish and sand seatrout represent separate populations of a single species (Moshin, 1973; Weinstein and Yerger, 1976; Cowan, 1985; Ditty, 1989), and others treat them as separate species (Schlossman and Chittenden, 1981) with distributions confined to the western Atlantic (weakfish) and the Gulf of Mexico (sand seatrout). Paschall (1986) was unable to distinguish between the two species using allozyme electrophoresis. In contrast, results presented here are consistent with the existence of two distinct species, with weakfish and sand seatrout co-occurring off the east coast of the United States at least as far north as Doboy Sound, Georgia. This distribution pattern is consistent with the phylogeographic patterns of 19 freshwater, coastal, and marine species distributed along the U.S. East Coast and the Gulf of Mexico that exhibited geographically concordant forks in their intra- or interspecific mtDNA phylogenies (or in both phylogenies) (Avise, 1992). In the present situation, apparently distinct Gulf (sand seatrout) and Atlantic (weakfish) species may have reestablished contact in a hybrid zone (see below) through movement of the Gulf species into the Atlantic.

Three individuals had weakfish mtDNA and a single small SOC050 allele and were presumably hybrids of weakfish and sand seatrout or silver seatrout (with female weakfish parentage). In addition, two individuals possessed sand seatrout mtDNA and a single small SOC050 allele and were presumably hybrids of weakfish and sand seatrout with female sand seatrout parentage. These data suggest that hybridization occurs between weakfish and sand seatrout and that the genetic exchange is not gender restricted. Because of the overlap in microsatellite allele sizes seen between silver seatrout and sand seatrout, hybridization between weakfish and silver seatrout could not be excluded. The lack of suspected hybrids with silver seatrout mtDNA, however, suggests that hybridization did not involve this species. The possibility exists that the putative hybrids are in fact weakfish with rare mtDNA haplotypes common to the three *Cynoscion* species studied here. This seems unlikely because only one 12S/16S mtDNA/*Rsa* I pattern was noted among 40 weakfish in the species identification study of Cordes et al. (2001). Furthermore, analysis of 20 weakfish taken from each of the four locations outside of Georgia with the 12S/16S marker revealed no new mtDNA patterns. Also, the mtDNA haplotypes seen in sand seatrout and silver seatrout seem to vary in size and can not be clearly related to the weakfish haplotype by the addition or deletion of presumed restriction sites. This condition is more in keeping with mtDNA of different species, although the apparent size differences may be gel artifacts due to unresolved bands <100 bp in one or more of the species.

Re-evaluation of the remaining 1996-97 SOC050 data revealed occasional occurrences of small alleles in individual fish in all but the New York samples (Table 3). Examination of the 12S/16S mtDNA region of these individuals identified a single silver perch (*Bairdiella chrysoura*) in the Chesapeake Bay 1997 sample (silver perch mtDNA

Table 3

Frequencies of unusual alleles in four geographical samples of weakfish (*Cynoscion regalis*) taken in 1996 and 1997. The number of individuals with anomalous alleles that were subsequently eliminated from the population structure analysis is given in parentheses after the sample names.

Sample	Allele (bp)	Frequency
Georgia 1996 (4)	175	0.018
	177	0.009
	179	0.009
North Carolina 1996 (1)	177	0.010
Chesapeake Bay 1996 (1)	177	
Delaware Bay 1996 (2)	179	0.011
	181	0.011
Georgia 1997 (15)	171	0.021
	173	0.010
	175	0.125
	177	0.094
	179	0.010
North Carolina 1997 (1)	177	0.009
Chesapeake Bay 1997 (3)	171	0.009
	177	0.009
	179	0.009
Delaware Bay 1997 (5)	177	0.023
	181	0.034

and two alleles 171 bp in size). All other individuals were putative hybrids with weakfish mtDNA and a single small SOC050 allele characteristic of silver and sand seatrout. As mentioned previously, subsequent analysis of 20 weakfish taken from each of the four locations outside of Georgia with the 12S/16S marker revealed only weakfish mtDNA. If the small SOC050 alleles found in the more northern populations are not simply rare weakfish alleles shared in common with the other two *Cynoscion* species, they may indicate that introgressive hybridization is responsible for the migration of the smaller alleles into northern waters (although the northward movement of hybrid fish out of the contact zone cannot be excluded). As a result of these findings, all individuals in the 1996 and 1997 collections exhibiting at least one small SOC050 allele less than 183 bp in length were eliminated from the population structure analyses.

Stock structure analysis

All four microsatellite loci were polymorphic in all sampled locations in both years. Allele frequency distributions for each locus are available from the authors upon request. Sample sizes (n), number of alleles (N), expected heterozygosities (gene diversities), and significance test results for Hardy-Weinberg equilibrium are provided in Table 4. Levels of variation differed greatly among the four microsatellite loci. The number of alleles ranged from two (SOC014) to 37 (CNE612), and average expected heterozygosities ranged from 0.085 (SOC014) to 0.928 (CNE612). These values are consistent with heterozygosity ranges reported in other multilocus microsatellite studies on species including Atlantic cod (Bentzen et al., 1996), northern pike (Miller and Kapuscinski, 1996), pink and sockeye salmon (Seeb et al., 1998), and Arctic char (Brunner at al., 1998). In contrast, Crawford et al. (1989) and Graves et al. (1992) found very low levels of genetic variation in an analysis of weakfish populations using allozymes and mtDNA restriction fragment-length polymorphism (RFLP) analyses, respectively. None of the genotypic distributions for any of the four microsatellite loci at any of the collection locations in either year differed significantly from Hardy-Weinberg expectations after correcting for multiple tests (Table 4).

Digestion of actin intron (CRESIA1) amplifications with the restriction endonuclease *Rsa* I revealed a single polymorphic restriction site that produced two alleles. Expected heterozygosities ranged from 0.000 for the monomorphic Georgia 1997 sample to 0.096 for the Chesapeake Bay 1996 sample. Digestion of the RP2 amplifications with the restriction endonuclease *Hinf* I also resulted in two alleles. Expected heterozygosities ranged from 0.194 in the Delaware Bay 1997 sample to 0.370 in the Georgia 1997 sample. Levels of genetic variation within the two nuclear gene intron regions were low in relation to three of the four microsatellite loci and were more similar to those found in the polymorphic allozyme loci of Crawford et al. (1989). In another study where nuclear intron RFLP analysis was used, similar levels of heterozygosity in Pacific salmon were found (Moran et al., 1997), as in RFLP studies of anonymous single copy nuclear (ascn) DNA loci in Atlantic cod (*Gadus morhua*) (Pogson et al., 1995) and blue marlin (*Makaira nigricans*) (Buonaccorsi et al., 1999). In contrast, higher heterozygosities (44–58%) were reported in an ascnDNA/RFLP analysis of striped bass (*Morone saxatilis*) by Leclerc et al. (1996). None of the sample genotype distributions for either locus differed significantly from Hardy-Weinberg expectations after correcting for multiple tests (Table 4).

To test for population structure, microsatellite loci were analyzed individually and as a combined data set. AMOVA results did not reveal significant differences between sample locations or years for any of the four loci or for the combined data (all $P>0.05$). Single-locus population pairwise F_{ST} values were relatively low, and mean F_{ST} values ranged from 0.002 (SOC050 and CNE612) to 0.018 (SOC044). Exact F permutation tests were not significant for any of the four loci or the combined data set after correction for multiple testing.

AMOVA results for both the actin and RP2 loci indicated no significant differences between sample locations or years (all $P>0.05$). Single-locus population pairwise F_{ST} values for the actin locus were consistently low (mean=0.005), ranging from $F_{ST} < 0.000$ for most of the comparisons to an F_{ST} of 0.035 between Georgia 1996 and Georgia 1997 and between Chesapeake Bay 1996 and Georgia 1997. A single exact F permutation test, between Delaware 1996

Table 4

Sample sizes (n), number of alleles (N), expected heterozygosities (H_{exp}), and P values for tests of Hardy-Weinberg equilibrium for four microsatellite loci, the actin intron (CRESIA1), and the ribosomal protein 2 intron (RP2) gene regions. GA = Georgia, NC = North Carolina, CB = Chesapeake Bay, DB = Delaware Bay, NY = New York. NT = monomorphic sample not tested.

	GA 1996	NC 1996	CB 1996	DB 1996	NY 1996	GA 1997	NC 1997	CB 1997	DB 1997	NY 1997
SOC050										
n	51	49	64	46	46	33	52	55	42	54
N	7	6	6	5	7	6	6	6	8	7
H_{exp}	0.741	0.702	0.694	0.731	0.724	0.758	0.712	0.740	0.737	0.722
P[1]	0.067	0.542	0.507	0.130	0.959	0.566	0.577	0.721	0.349	0.174
SOC044										
n	47	46	63	55	55	36	60	56	52	56
N	2	3	2	2	2	2	2	2	2	2
H_{exp}	0.362	0.434	0.374	0.251	0.416	0.407	0.302	0.350	0.203	0.419
P[1]	1.000	0.019	0.496	0.303	0.512	0.010	0.669	0.116	0.0.512	0.198
SOC014										
n	43	48	64	52	54	39	56	55	52	57
N	2	2	2	2	2	2	2	2	2	2
H_{exp}	0.090	0.081	0.046	0.075	0.170	0.144	0.053	0.088	0.038	0.068
P[1]	1.000	1.000	1.000	1.000	1.000	1.000	0.027	1.000	1.000	1.000
CNE612										
n	39	43	62	50	46	33	54	52	50	56
N	17	23	20	20	22	19	25	23	22	23
H_{exp}	0.916	0.943	0.928	0.912	0.916	0.935	0.934	0.934	0.923	0.936
P[1]	0.050	0.113	0.898	0.530	0.238	0.752	0.522	0.419	0.060	0.290
CRESIA1										
n	40	42	40	42	40	36	51	54	45	55
N	2	2	2	2	2	1	2	2	2	2
H_{exp}	0.096	0.089	0.031	0.055	0.096	0.000	0.025	0.053	0.047	0.020
P[1]	0.076	0.091	1.000	0.036	0.078	NT	1.000	1.000	1.000	1.000
RP2										
n	48	45	45	42	41	29	48	49	42	41
N	2	2	2	2	2	2	2	2	2	2
H_{exp}	0.237	0.200	0.217	0.230	0.253	0.373	0.237	0.201	0.194	0.253
P[1]	0.184	0.432	0.104	0.120	0.180	0.298	0.189	0.465	0.052	0.179

[1] None of the samples differed significantly from Hardy-Weinberg expectations after sequential Bonferroni corrections (α=0.005).

and Georgia 1997, was significant after correction for multiple testing (α<0.001). Single-locus population pairwise F_{ST} values for the RP2 locus were also low (mean=0.006), ranging from F_{ST} < 0.000 for most of the comparisons to a high of 0.050 between Georgia 1997 and Delaware Bay 1997. None of the exact F permutation tests were significant after correction for multiple testing.

From our results we were unable to reject the null hypothesis that weakfish comprise a single, genetically homogeneous stock. These results are consistent with those based on allozymes (Crawford et al., 1989) and RFLP analysis of mtDNA (Graves et al., 1992) and illustrate the point that increased genetic variability in microsatellites in relation to more traditional markers will not always provide greater stock resolution (Seeb et al., 1998). The amount of genetic exchange necessary to prevent the accumulation of significant genetic divergence between fish from different locations may be as little as a few individuals per generation (Allendorf and Phelps, 1981). Weakfish tagging data indicate that low levels of exchange occur between geographically distant populations of weakfish (Bain et al., 1998). Estimates of natal homing in yearling weakfish, calculated by Thorrold et al. (2001) using geochemical signatures in the otoliths of the same weakfish used in the present study, indicated spawning-site fidelity ranging from 61% to 81%, suggesting exchange rates sufficient to prohibit genetic divergence between locations.

The inclusion of nontarget species in our weakfish samples illustrates the advantages in using multiple marker systems. If only a single microsatellite locus had been used, or if the study had been restricted to nuclear intron markers alone, it is very likely that the sand seatrout and

silver seatrout specimens would have gone unnoticed. This could easily have resulted in a type-II error. Likewise, if nongenetic markers such as otolith microchemistry had been used exclusively, the analyses of Thorrold et al. (2001) would have been based on a mixed-species sample. Instead, it was possible not only to recognize the individuals as anomalous but also to identify them to species and provide evidence of hybridization between at least two of the *Cynoscion* congeners. It is hoped that further refinement of the inter- and intraspecific molecular markers developed here and in other studies will eventually be helpful in further clarifying the taxonomic status, population structure, and possible hybridization within the genus *Cynoscion*.

Acknowledgments

We would like to thank all those who supplied us with weakfish samples, including Louis Barbieri, Susan Lowerre-Barbieri, Christina Grahn, Patrick Geer, Mike Greene, and Simon Thorrold. We also appreciate the samples of banded drum, gulf kingfish, and star drum provided by Trey Knott and the silver seatrout and sand seatrout specimens provided by Bill Karel. We gratefully acknowledge Kim Reece, John Gold, Linda Richardson, and Robert Chapman for generously providing us with their published and unpublished primer sequences. Funding for this study was provided through the Virginia Marine Resources Commission.

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