

Characterization of Red Drum Microsatellite Markers in Spotted Seatrout

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Abstract.—Polymerase chain reaction primers for a total of 132 nuclear-encoded microsatellites originally developed from genomic libraries for red drum *Sciaenops ocellatus* produced reliable and consistent amplifications in the closely related spotted seatrout *Cynoscion nebulosus*. Thirty-three of the primers amplified microsatellites that were monomorphic among a sample of 30 individuals, while 12 of the remaining 99 polymorphic loci exhibited possible null alleles. This provides a set of 87 microsatellite loci that will be highly useful in future genetic studies related to the stock enhancement and culture of spotted seatrout. Spotted seatrout comprise an important recreational fishery in the bays and estuaries in U.S. waters of the Gulf of Mexico and western Atlantic Ocean, and stock enhancement via supplementation with hatchery-reared individuals is ongoing, planned, or under consideration in several southern states.

Spotted seatrout *Cynoscion nebulosus* comprise an important recreational fishery in the bays and estuaries in U.S. waters of the Gulf of Mexico and western Atlantic Ocean (Vanderkooy and Muller 2003). One management strategy to protect this fishery is stock enhancement via artificial hatchery spawning and the subsequent release of offspring (Blankenship and Leber 1995). Presently, approximately 3.5 million spotted seatrout fingerlings from two hatcheries are supplemented in Texas bays and estuaries by the Texas Parks and Wildlife Department (TPWD; D. Abrego and R. Vega, personal communication); similar programs are either planned or under consideration in other southern states (T. Bert, Florida Fish and Wildlife Conservation Commission, personal communication; W. Hawkins, University of Southern Mississippi, personal communication). Assessment of genetic diversity within both the hatchery broodstock and recipient populations and unequivocal identification of hatchery-raised fish in the wild are critical to the successful implementation of stock enhancement (Ward et al. 2006; Bert et al. 2007).

Nuclear-encoded microsatellites (abundant short stretches of DNA composed of di-, tri-, or tetranucleotide arrays embedded in unique DNA; Weber and

May 1989; Weber 1990) are ideal genetic tools for assessing these issues because of their generally high level of polymorphism and codominant Mendelian inheritance (Liu and Cordes 2004). In addition, microsatellites are useful in estimating the genetic parameters for traits such as growth rate, thermal tolerance, and disease resistance that are important to fish culture. To date, microsatellites have been used successfully to provide critical data relative to the TPWD stock enhancement program for red drum *Sciaenops ocellatus* (Gold et al. 2008; Karlsson et al. 2008c) and to assess the heritability of juvenile growth traits and thermal tolerance (Ma et al. 2007; Saillant et al. 2007).

A certain number of microsatellites generally are necessary for (1) unequivocal identification of hatchery-raised fish in the wild (Renshaw et al. 2006; Karlsson et al. 2008c), and (2) determination of the pedigree of offspring and estimation of genetic parameters when multiple families are generated via spontaneous group spawning (Saillant et al. 2007). Recently, a summary of 269 microsatellite PCR primers, developed in our laboratory from genomic libraries of red drum, was made available (Karlsson et al. 2008a). Red drum and spotted seatrout are both members of the family Sciaenidae, and microsatellite polymerase chain reaction (PCR) primers often amplify across members of the same family (Renshaw et al. 2007). The goal of this study was to identify PCR primers for red drum microsatellites that would reliably amplify microsatellites in spotted seatrout and be useful in spotted seatrout stock enhancement and culture.

Methods

Thirty spotted seatrout from the Lower Laguna Madre, Texas were provided by TPWD personnel. Genomic DNA from each individual was extracted using a standard phenol–chloroform protocol (Sambrook et al. 1989). A total of 132 of the PCR primers available for red drum microsatellites provided reliable and consistent amplifications of spotted seatrout DNA. The PCR protocols followed those outlined by Saillant et al. (2004) for red drum microsatellites *Soc9* to *Soc445* and those outlined by Karlsson et al. (2008b) for microsatellites *Soc500* to

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TABLE 1.—Summary data for red drum microsatellites characterized for spotted seatrout. The fluorescently labeled primer is in bold text with the appropriate label signified by one (6-Fam), two (Hex), or three (Ned) plus signs. Information taken from earlier descriptions of these microsatellites (Saillan et al. [2004] for *Soc9–Soc445* and Karlsson et al. [2008a] for *Soc508–Soc738*) is indicated by asterisks.

Microsatellite	Primer sequence (5'–3') ^{a*}	GenBank accession number ^{b*}	Repeat sequence ^{c*}	T_A ^c	N/N_A ^d	Size range ^e	H_E/H_O ^f	P_{HW} ^g
<i>Soc9</i>	AACATTTCCATCAGTATTTATCT ⁺⁺ TCCACATGAACACCAGTGCAGTTC		(AT) ₂₇	51	14/2	248–252	0.138/0.143	1.000
<i>Soc11</i>	GCCGAGTCACGAAGGAACAGAGAA ⁺ TGTCGTCTCATCTATCTCCATCTC	AF73258	(GA) ₁₁	62	21/4	216–242	0.331/0.238	0.138
<i>Soc44</i>	GAGGGTGACGCTAACAGTTGA CACAGCTCCACTCTGATATG ⁺⁺	AF73264	(CA) ₂₂ (GT) ₅	62	25/2	202–204	0.040/0.040	1.000
<i>Soc50</i>	CCCGTGATTTAGGCTCAGATA ⁺⁺ CCTTTAGAGTGCAGTAAAGTGATTT	AF73266	(GT) ₇	58	25/5	183–197	0.586/0.520	0.788
<i>Soc83</i>	TGCTGTAATTGAAAAGCAGTGTC AGCGAACTAGAATTTGGTTTTATA ⁺⁺	AF73269	(TG) ₁₉	56	12/2	113–115	0.391/0.167	0.093
<i>Soc99</i>	CACCCACTGACACACATAAC ⁺⁺⁺ GGAACCAATATGCTGCCATGAT	AF73272	(CA) ₂₉	62	28/9	151–169	0.771/0.750	0.052
<i>Soc133</i>	CATTGGACCATCGCTACTGCTG CTTGGCATTTCCAGACATCACTG ⁺	AF73276	(TGC) ₁₀	56	27/4	193–202	0.592/0.741	0.457
<i>Soc140</i>	GGTGCAAAACACAGCCATACAGT GCAAATCGAAGCCGAGTTTAG ⁺⁺⁺	AF73277	(CTGT) ₈	56	29/2	130–134	0.034/0.034	1.000
<i>Soc243</i>	GACGGGGATGCCATCTGC AATGCGAAAAAGACGAAACAGT ⁺	AF73283	(CCT) ₉	56	29/4	101–110	0.533/0.483	0.705
<i>Soc402</i>	CATATTTAACGAGCGACATAGC ⁺⁺ AAACAGATGAAGCACCTGGACT	AY161012	(CA) ₂₀	52	29/5	126–134	0.618/0.690	0.924
<i>Soc407</i>	AAAGTCTGCCTCTTACAGCTTC ⁺ GAGTTAAAGCGTGTGCTAGTCC	AY161016	(CA) ₁₃	56	30/2	130–132	0.033/0.033	1.000
<i>Soc409</i>	TTTATCTGCTCTGTGTGGAAGT ⁺⁺ ATCTATTTGTCCGTTTCTCTGC	AY161017	(TG) ₁₁	52	26/6	283–297	0.526/0.423	0.104
<i>Soc410</i>	GTACCAAGTCAGCCAGTGTACG ⁺ TCTCTGTGTCCTCTGTGTTTG	AY161017	(TG) ₁₇	56	27/3	290–298	0.073/0.074	1.000
<i>Soc412</i>	CACAGAAACTCAGCTCGAGACC ⁺⁺ AGGAAGAATGTACAAGGTGTTT	AY161019	(AC) ₁₃	49	30/6	121–131	0.382/0.400	0.628
<i>Soc415</i>	CTCAGCACCCCTCAGACATATGG CACAAGTTAAGTGGTATCGAGT ⁺⁺	AY161020	(TG) ₁₅	52	30/16	186–246	0.911/0.867	0.007
<i>Soc416</i>	CTCGATACCACTTAACCTGT ⁺⁺⁺ ATCGACATAATCTGGCACCA	AY161020	(GA) ₃₈	49	30/17	139–191	0.909/0.833	0.082
<i>Soc417</i>	CTTACGTGATAAAAGTGGGTGA ⁺ ATATGCCAGTAATCCACCGAAG	AY161020	(AC) ₂₄	47	15/6	86–96	0.713/0.667	0.808
<i>Soc418</i>	GTTTTTCTGGCATTATGGATG TGAGGTTATCAAACACCTGCCACT ⁺	AY161021	(TG) ₂₄	52	29/2	253–255	0.267/0.310	1.000
<i>Soc419</i>	ATTAGCCAACCTGCTCCGCTCA ⁺ GAGTCCGTGGTGTAGGGGGGTA	AY161022	(AC) ₂₀	56	26/12	237–269	0.858/0.846	0.603
<i>Soc423</i>	GTCACGCCACTGATGGAGAT ⁺ TACCACTTACACTCAGCAGGTG	AY161025	(CA) ₂₆	51	30/7	183–223	0.420/0.433	0.178
<i>Soc426</i>	GAGAGGACGTGAGCTGCTGA ⁺ TGAGAAACAGAAACAGAAGGT	AY161028	(CA) ₁₁	52	30/14	121–159	0.876/0.767	0.042
<i>Soc431</i>	GACACGCTGTGGTAGATGAAAACG ⁺⁺ TGTATATTAGTTGGCAAGGCAGAG	AY161032	(TG) ₂₉	53	30/11	125–155	0.648/0.667	0.602
<i>Soc432</i>	TTAGGCTACCTCTGGAGGCACA ⁺⁺ GTGTGTTTGAGGGTTCAGCGTAC	AY161033	(AC) ₁₆	52	29/12	90–118	0.727/0.690	0.165
<i>Soc434</i>	GACTCTCCAGATATGCTGA ⁺⁺ TCCTTGTTTATCTTGGTGTCTGT	AY161034	(CA) ₂₃	52	28/9	127–183	0.695/0.750	0.124
<i>Soc439</i>	ACTCTCGTCCCACTTACCACA ⁺⁺ TATGTTTGCATATAAGCTCA	AY161037	(TG) ₁₇	49	30/4	87–101	0.437/0.467	0.701
<i>Soc508</i>	GCAGCACATTTTCAGCACAC TAATGCCCTGTTATCTATCTA	EF609022	(GATA) ₁₈	15/17		242–230	0.943/0.933	0.651
<i>Soc510</i>	AGATGCCAACACCTCTAAAAC ACAGACCACTCCGACTCAAA	EF609024	(GATA) ₈	18/6		264–316	0.470/0.389	0.232
<i>Soc516</i>	GCAGACACAAAATGTTCAAAGCA GGAAGACTCAGGAGCAGGTT	EF609027	(CA) ₁₁	23/2		178–182	0.507/0.478	1.000
<i>Soc522</i>	CTAGTGGATCTGCTTGATGCTTT GCACACGCTGAGGAGTGAA	EF609029	(CA) ₁₃	29/6		125–141	0.572/0.482	0.111
<i>Soc525</i>	CACTGTGGGGACATTTAGAGG GGGTGTGACAAAACCTCAGGGAC	EF609032	(CA) ₁₄	30/2		111–113	0.033/0.033	1.000
<i>Soc527</i>	CCAAATGTCAAGCTGCTC GCTGTGAAAATCTCACCACACTT	EF609034	(TG) ₁₆	27/2		195–203	0.140/0.148	1.000
<i>Soc532</i>	GAGTGCTCACAAGTGCCAG GTGCCAGATAGATGCTGACG	EF609037	(CA) ₁₄	22/11		134–166	0.867/0.864	0.873

TABLE 1.—Continued.

Microsatellite	Primer sequence (5'–3') ^a *	GenBank accession number ^b *	Repeat sequence*	T_A^c	N/N_A^d	Size range ^e	H_E/H_O^f	P_{HW}^g
<i>Soc538</i>	CCAGCATATTTTGTAGCAGC TGAAGGTTTTCCCGTAGT	EF609041	(CA) ₁₇		15/6	208–222	0.694/0.733	0.753
<i>Soc548</i>	CGCACACACAAAAACAGTGAG CCATCGGTCCAGTATGAAGTC	EF609048	(CA) ₁₃		25/7	166–188	0.500/0.400	0.230
<i>Soc550</i>	CGCAGACAGACAGCCTCTAT CATCCTCTCCTCAGTGTAGCC	EF609050	(CA) ₂₆		16/9	217–255	0.782/0.813	0.344
<i>Soc551</i>	TCAACCACACTCAGGTCCAG GCAACACAAAGCACTCACAAA	EF609051	(CA) ₁₀		27/7	257–277	0.576/0.444	0.110
<i>Soc558</i>	CAGTGAACAGGAACGACTTTTAGA GACCCATCAACCTCCAGCA	EF609058	(CA) ₁₈		28/11	185–223	0.805/0.714	0.617
<i>Soc559</i>	TGTGGGACAAGATGTGAAAT TTACCTTGGAAACGGTGTGA	EF609059	(CA) ₃₁		22/3	201–209	0.251/0.272	1.000
<i>Soc564</i>	TGCCAGCATCCGTCTACTCA AAGAGCCGACCAATCAGAGAG	EF609063	(CA) ₁₅		28/14	172–210	0.879/0.786	0.512
<i>Soc566</i>	GGACAAGAGAAGCAGCAGACG TCATCAGTCCAAAGCTACG	EF609065	(GA) ₁₃		30/4	171–179	0.555/0.433	0.166
<i>Soc567</i>	ACACACACTCCACAGATGAAT CGCTGCCCAAAATAGAAT	EF609066	(CA) ₃₁		17/3	236–240	0.469/0.353	0.225
<i>Soc571</i>	CGTGAACCTGAGCGGAGACA GGGAGTGTGTGAGAATGTGGA	EF609070	(CA) ₂₀		28/9	234–252	0.851/0.821	0.580
<i>Soc575</i>	GGACGCACCATCTCTCCATCT AGGCTTTGCTCTTTTCAGACG	EF609074	(CA) ₁₁		19/10	246–276	0.809/0.895	0.103
<i>Soc580</i>	CTGAGCCTGAACGCACATCAT GAACAAACAACCTGTCACCTGCTG	EF609079	(CA) ₂₅		15/2	301–309	0.331/0.400	1.000
<i>Soc586</i>	GGGAAATGGACACAAAAGAAT CACCTGGGACCTTAGTCACTT	EF609082	(CA) ₃₅		25/6	151–161	0.740/0.800	0.348
<i>Soc588</i>	TGAATGACTTGCTTTGCTGAA AATAACCCCACTCTCCCT	EF609084	(GA) ₂₂		30/13	180–212	0.875/0.900	0.224
<i>Soc590</i>	CAATGGACAGTTTGTAGAGTTC GAAACCCACCAATCACT	EF609086	(CA) ₁₈		28/4	187–193	0.733/0.821	0.656
<i>Soc592</i>	AGAAGGAGGTCAGGAGGCATT TCCCATTCACAAACAAGCA	EF609087	(CA) ₁₃		28/5	125–133	0.631/0.607	0.144
<i>Soc594</i>	TCGTGCTCTGTCTCCGTTT TGACTATTTTGTCTTTTACTTC	EF609089	(CA) ₁₃		16/2	206–208	0.417/0.563	0.250
<i>Soc601</i>	CTTTGGGACAACAGAAATGC TCCAGCAAGCAGACAACAAT	EF609094	(GA) ₂₂		25/4	166–172	0.626/0.560	0.535
<i>Soc602</i>	AGCAACCATTTCTCCACAC CCATCACACACACAGGTTAA	EF609095	(CA) ₁₂ CG(CA) ₅		28/11	153–189	0.728/0.821	0.762
<i>Soc609</i>	CCCGCATTAGACAGAAAAC ATGGGTATGTGTGGTTACAG	EF609099	(CA) ₂₃		26/8	269–285	0.762/0.846	0.579
<i>Soc616</i>	TTCTCTCTCCGTGTTTGTGTT ACTGGGCAGGTTTCTCTGAC	EF609104	(CA) ₃₂		26/7	307–329	0.434/0.385	0.387
<i>Soc618</i>	ACCCGATAAGGAAACAAGCAGA CATCACCGTCCAACCCAGAGAA	EF609106	(GA) ₁₇		27/2	118–120	0.107/0.111	1.000
<i>Soc619</i>	GCGTTCTCTCTCTCGTGACAA GCTCTCTCGCTCTCGTCTT	EF609107	(CA) ₁₉		23/3	171–183	0.357/0.261	0.337
<i>Soc620</i>	CGTGTACGCCACAATCTCCA AGCCAGCTAAACCTCTCA	EF609108	(CA) ₁₃		15/4	149–165	0.628/0.733	0.351
<i>Soc623</i>	CACTTTCACTTTCTGCCCTCA TCTGGTTTCTGGCTTTCATACA	EF609111	(CA) ₁₄		21/9	127–147	0.757/0.810	0.770
<i>Soc624</i>	CACGCTGGTCTTTTCTCA CTGGGGTTATTTGTGTGTGC	EF609112	(CA) ₂₈ AA(CA) ₇		15/8	125–151	0.802/0.667	0.192
<i>Soc625</i>	TACCCCTCAGAAATGGTCGCTT GAGAGTGTACCCCGTGTGC	EF609113	(CA) ₁₂		27/2	115–117	0.037/0.037	1.000
<i>Soc626</i>	ACTTTGAGCCAATGCTTTCC GTGAGCTGTGTATCCCTGTGC	EF609114	(CT) ₆ (CA) ₉		29/5	184–194	0.655/0.759	0.877
<i>Soc635</i>	CATCAGCACGGTTATTTCTTG CCTCTCTCTTTCTTCCTCG	EU015887	(CA) ₂₃		25/14	239–269	0.918/0.920	0.697
<i>Soc640</i>	AGGACATTTGGAGTGGAAAGAGTA ATGGGGACAGGAGTTTCTA	EU015892	(CA) ₁₄		23/7	163–181	0.670/0.565	0.219
<i>Soc645</i>	GAGTTGGTCAATAGCCACAGG ATCTGAAAGGGCAGGTGTTT	EU015896	(CA) ₂₀		29/3	150–154	0.163/0.172	1.000
<i>Soc646</i>	GGGAAAGTAGATAGGGGCACA AGAGGTCAGGTTGAGCAGAGT	EU015897	(CA) ₁₆		19/7	120–140	0.657/0.579	0.241
<i>Soc654</i>	CTCCGCTGCCAAACTGAC TGTGCTCTACATCCTCTCT	EU015905	(CA) ₁₃		14/4	162–178	0.558/0.500	0.555
<i>Soc657</i>	GGAAAGCAAAGCAAAAGAACT AGCCGAATGAGACAGAGGAAA	EU015908	(CA) ₃₄		30/2	195–201	0.127/0.133	1.000

TABLE 1.—Continued.

Microsatellite	Primer sequence (5'–3')*	GenBank accession number ^b *	Repeat sequence*	T_A ^c	N/N_A ^d	Size range ^e	H_E/H_O ^f	P_{HW} ^g
<i>Soc658</i>	AATCTCCCAGTGCCTTTGA CTGCTTTTCCCTCTATTTCTC	EU015909	(GA) ₁₄	28/10	165–191	0.800/0.821	0.461	
<i>Soc660</i>	TTGCCAATGTTCTTTCTCTCT ATTCTACTCCTGCCAAGAT	EU015911	(CA) ₁₂ CT(CA) ₂ AA(CA) ₅	30/18	115–167	0.902/0.867	0.193	
<i>Soc661</i>	ACCGCTCAAACAACACA AGGAGATTGGGAGTGGAGATA	EU015912	(CA) ₁₃	30/6	155–165	0.749/0.800	0.761	
<i>Soc662</i>	CGTCTTTAGGAAGGTGTGGC CCTGTCTGGAGGGGAAAC	EU015913	(CA) ₂₀ CC(CA) ₁₆ AA(CA) ₃	30/6	89–121	0.641/0.700	0.779	
<i>Soc666</i>	TAATCTCTGTGTGTCCAGGTG GACGCAAGGCTGAGGCATA	EU015917	(CA) ₄ CG(CA) ₂₁	25/6	194–208	0.416/0.400	0.286	
<i>Soc667</i>	TAACGCTCTGCCACTG CATCTACGAATGCCCAACA	EU015918	(CA) ₁₄ CCTA(CA) ₅	27/11	219–249	0.851/0.778	0.169	
<i>Soc670</i>	TTCGTCCCGTCACAGCACA CAAAGAGAGATGAATAAACCCAAAG	EU015921	(CA) ₃₀	29/3	220–224	0.132/0.138	1.000	
<i>Soc671</i>	CGCCTCTCTCTCAGATGT ACAGTGGGCAAAATCCATACA	EU015922	(CA) ₃₀ TACG(CA) ₄	30/5	187–199	0.499/0.433	0.126	
<i>Soc672</i>	CGTATGGTGAGTGTGGCA TGTCGTCTCTGAATGTGTCTCT	EU015923	(CA) ₂₂	14/2	139–141	0.254/0.286	1.000	
<i>Soc675</i>	TGTCCCCATAAAGAAACAAGG ACACAACGTCTACAGGAAGGC	EU015925	(CAGA) ₄ CAGG (CAGA) ₂ CAGG(CAGA) ₂	30/2	92–96	0.097/0.100	1.000	
<i>Soc683</i>	TTCGCACACATACATAACTAACT AGCGTCATAATCCAACCTGTCA	EU015932	(CA) ₆ CTGA(CA) ₇	29/3	185–195	0.132/0.138	1.000	
<i>Soc685</i>	TCAAACAGGGTCAATTGGTGA AGGAGAAACGCAGGGAAGA	EU015934	(CA) ₁₄	29/3	220–226	0.605/0.517	0.666	
<i>Soc692</i>	TGCTGCCATTGAGAAGAGA TTTGTATGTTAGGGGTTGTGT	EU015941	(CA) ₁₀	28/3	121–131	0.137/0.143	1.000	
<i>Soc694</i>	CTCGCTCCCATCGTGACT TCCTGAAAGTTGTCTGTGTC	EU015943	(CA) ₁₁	15/9	175–193	0.857/1.000	0.717	
<i>Soc695</i>	TCTGGAGGGATGATGTGTTT CCTGTTTCACTGCTACTCGC	EU015944	(CA) ₂₁ CT(CA) ₅	16/3	129–133	0.123/0.125	1.000	
<i>Soc696</i>	GAAAAATGGTGA AACCTGA CAAAAATGGAGAACCTGAAG	EU015945	(CA) ₃₁	15/3	185–193	0.191/0.200	1.000	
<i>Soc706</i>	ACTCTGTTGCTCCACTACCCA GCTCTTCTCTGTTGTGTA	EU015954	(CA) ₅ AA(CA) ₈	14/4	162–170	0.267/0.214	0.206	
<i>Soc708</i>	TTCCCACTAGAGCTGTGATTGA TCTGACTTCTCTGCCATT	EU015956	(CA) ₁₇	15/10	148–174	0.823/0.800	0.766	
<i>Soc713</i>	AATAGTTTCTCTGGAATTGACG TGGCTTAGACAAGTGGTGCT	EU015961	(CA) ₁₈	15/3	184–188	0.591/0.533	0.712	
<i>Soc730</i>	GCACAGGGAGATAAACACAG CTGAAGAAAAGCCAGAGTGAA	EU015974	(CA) ₁₄	29/2	101–130	0.034/0.034	1.000	
<i>Soc738</i>	TGTAACAGCAGAGACTGAAGC CTGGTGAAAGGCAGAGTA	EU015981	(CA) ₂₈	28/3	94–98	0.105/0.107	1.000	

^a Primer sequences are forward (top) and reverse (bottom).

^b Sequence for *Soc9* is not available in GenBank.

^c Annealing temperature in °C.

^d N is the number of samples that were scored, and N_A the number of alleles detected.

^e Refers to alleles thus far uncovered; for *Soc508* to *Soc738*, size includes the 21-base-pair 5' tail sequence primer used for PCR amplification (Karlsson et al. 2008c).

^f H_E and H_O are expected and observed heterozygosities, respectively.

^g Probability of deviation from Hardy–Weinberg expectations.

Soc739. For the first group of markers (*Soc9–Soc445*), one of three fluorescent labels (6-Fam, Hex, or Ned) was attached to one of the primers in each pair (Table 1). For the second set of markers (*Soc500–Soc739*), the 5' tail sequence primer as described in Karlsson et al. (2008b) and the 6-Fam fluorescent label were used for each amplification. The amplified PCR products were run on an ABI-377 automated sequencer, and the alleles were sized using the Genescan-500 Rox size standard (Applied Biosystems); allele sizing and

calling were performed with Genescan 3.1.2 and Genotyper 2.5 software, respectively. The genetic variability of each microsatellite was measured by the number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Wright's F_{IS} , estimated as Weir and Cockerham's f in the software program GDA (Lewis and Zaykin 2001), was used to measure the departure of genotype proportions from Hardy–Weinberg expectations at each microsatellite. Fisher's exact test, as performed in GDA, was used to

test the significance of departures from Hardy–Weinberg equilibrium (genotype) expectations at each microsatellite and for departure from genotypic equilibrium at pairs of microsatellites. The effect of Hardy–Weinberg departures (within-locus disequilibrium) on the significance of between-locus linkage disequilibrium tests was removed by preserving genotypes in GDA (Lewis and Zaykin 2001). Evidence for the occurrences of null alleles, large-allele dropout, or both, was explored with Microchecker (Van Oosterhout et al. 2004).

Results and Discussion

Thirty-five of the PCR primers in the first set of markers (*Soc9–Soc445*) and 97 in the second set (*Soc500–Soc739*) generated microsatellite bands that could be scored easily. Summary data for all 132 microsatellites may be found at <http://wfsc.tamu.edu/doc>, under the file name Appendix 1, which is under the heading North American Journal of Aquaculture (2009). Of these, 33 of the PCR primers appeared to amplify monomorphic microsatellites, while 12 of the remaining 99 polymorphic microsatellites exhibited possible null alleles upon further evaluation with Microchecker: *Soc442*, *Soc445*, *Soc535*, *Soc589*, *Soc622*, *Soc628*, *Soc638*, *Soc642*, *Soc668*, *Soc715*, *Soc721*, and *Soc737*. Genotypes at two of these microsatellites, *Soc445* and *Soc628*, deviated significantly from Hardy–Weinberg expectations after Bonferroni correction (Rice 1989). Summary data for the remaining 87 microsatellite loci are presented in Table 1; the number of alleles detected per microsatellite ranged from 2 (17 loci) to 18 (*Soc660*). Expected heterozygosity ranged from 0.033 (*Soc407* and *Soc525*) to 0.943 (*Soc508*), while observed heterozygosity ranged from 0.033 (*Soc407* and *Soc525*) to 0.933 (*Soc508*). All pairwise comparisons of microsatellites did not deviate significantly from genotypic equilibrium after Bonferroni correction (Rice 1989). The microsatellites characterized in this study will prove highly useful for future genetic studies related to the stock enhancement and culture of spotted seatrout.

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