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Characterization and evaluation of polymorphic microsatellite markers in the anadromous fish *Spirinchus thaleichthys*

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Abstract We describe seventeen microsatellite loci isolated from longfin smelt, *Spirinchus thaleichthys*, which is an anadromous fish inhabiting estuarine and nearshore marine waters along the western Pacific coast. Five to 16 alleles per locus were detected, the expected heterozygosity ranged from 0.33–0.93, and moderate levels of differentiation were found between collections. These polymorphic microsatellites will provide useful tools for studying population genetic structure at ecological scales within wild and propagated populations.

Keywords Longfin smelt · Conservation · Propagation · Pelagic Organism decline

While longfin smelt, *Spirinchus thaleichthys*, remain among one of the more common pelagic native species in the San Francisco Bay-Delta, CA, a recent significant population decline at the southern edge of its distribution has been observed (Sommer et al. 2007). This species was recently listed as Threatened under the California Endangered Species Act (CDFG 2009) although a petition for listing under the U.S. Endangered Species Act remains undetermined. Longfin smelt show a diversity of migratory patterns and anadromous and resident populations are native to the Pacific coast of North America. These fish are

semelparous, typically undergo a two-year life cycle and spawn in freshwater and the low salinity zone before larval and juvenile fishes are transported downstream to nursery habitats (Hobbs et al. accepted). Juveniles move out of the San Francisco Bay into nearshore coastal waters, while others seem to reside in the Bay-Delta (Moyle 2002). Little is known about San Francisco longfin smelt spawning habitats and ecology, although adults presumably migrate back to the low salinity zone immediately prior to spawning (Rosenfield and Baxter 2007). A better-studied resident population inhabits Lake Washington, WA (Chigbu 2000; Chigbu and Sibley 1994; Chigbu et al. 1998). While longfin smelt are reported to be widespread in nearshore coastal regions and estuaries, few fish have been observed and fewer spawning populations have been identified between San Francisco Bay and Lake Washington (USFWS 1994, CDFG 2009).

Whole genomic DNA was extracted from fin tissue of longfin smelt from Suisun March, CA using the PureGene Genomic DNA Purification Kit. Six libraries enriched for one of four tetranucleotide repeat motifs [AAAC, CAGA, CATC, TAGA] were constructed, screened, and sequenced by Genetic Identification Services according to Børk et al. (2008). Sequences were analyzed with SEQUENCHER version 4.7 (Gene Code Corporation) to remove duplicate sequences. Repeat regions in sequences were identified with MREPS version 2.5 (Kolpakov et al. 2003) and primer pairs flanking these regions were created with PRIMER 3 (Rozen and Skaletsky 2000) for 200 of 227 novel sequences. For screening, one of four 5' modifications (universal primers: T7T, T7P, M13, SP6) was added to the end of each forward primer using the method of Schuelke (2000), which incorporated a 5' fluorophore during polymerase chain reaction (PCR). Each primer set was initially tested on eight longfin smelt samples to determine

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Table 1 Characterization of microsatellite loci: locus, motif, accession number, primer sequence, tail sequence, annealing temperature (T_a), sample size (n), number of alleles (N_A), size range of observed allelic variation, H_O , and H_E . Significant deviations from Hardy–Weinberg expectations are in bold

Locus	Sequence	Accession no.	Primer sequence	Primer tail sequence name	T_a (°C)	N_A	Size range (bp)	Lake Washington		Bay-Delta			
								H_O	H_E	n	H_O	H_E	n
SptB12	(CTGT) ₁₅	GU946459	F: TGTCTTTGGGTCACATTTTC R: GAACAAGTCTGCCACCAG	M13	54	12	157–272	0.75	0.69	12	0.50	0.79	12
SptC3	(GTAT) ₂ (GGAT) ₄ GTAT(GGAT) ₃	GU946466	F: GCCGTTCCTTGAGACTAA R: TGCTCTGGGAATTCAGTG	T7P	55	10	287–373	0.60	0.56	15	0.54	0.85	13
SptC5	(CATC) ₂ (ACTT(CATC) ₂ CACC(CATC) ₃) ₅	GU946467	F: TGACTCCCTCCTTTCCTC R: GGGTAGAGGGAAACCATGT	T7P	55	5	238–258	0.47	0.38	15	0.64	0.66	14
SptC6	(CA) ₄ CC(CA) ₇ AA(CA) ₂ CC(CA) ₄	GU946468	F: GTTCGGCTGCTCCTCTAT R: TGGGTGTGTTTGTGTGTG	T7P	55	6	122–142	0.66	0.54	15	0.82	0.75	11
SptC10	(GGAT) ₆ (GAAT) ₂ GGACGAAT(GGAT) ₃ (GAAT(GGAT) ₂) ₂	GU946463	F: ATTTACGGACGGATGGAT R: GCCATTTTCTTGGCTTTCA	T7P	55	9	202–232	0.53	0.63	15	0.79	0.87	14
SptB130	(CC(TGTC) ₂ (TCGT) ₂) ₇	GU946462	F: TGGACTGGTGTGACCATCTG R: GCCAGACAGATATAGACGAACAAA	T7T	60	10	490–542	0.77	0.67	13	0.70	0.86	10
SptC114	(CA) ₄ CC(CA) ₁₀ CC(CA) ₄	GU946465	F: TCGCTGCTCCTCTATGACT R: GGGTGTGTGTGTGTGTGT	M13	60	6	95–130	0.53	0.39	15	0.71	0.68	14
SptB104	((GACA) ₂) ₁₇	GU946461	F: CAGCTGCCAACTGATGATA R: GCTTTGCCTCCTTCATTGAC	T7P	59	11	201–291	0.53	0.79	15	0.90	0.86	10
SptB108	(ATGG) ₁₃	GU946464	F: TCCTTGTGAAAACGCTAATCC R: GAATGTCACCCACCCACAGTA	M13	60	8	121–148	0.50	0.61	14	0.43	0.78	14
SptD9	(GT) ₁₃	GU946474	R: CCCCACACCCACTAC R: CCCCACACCCACTAC	M10	62	11	190–201	0.57	0.62	14	0.93	0.82	14
SptD3	(ATCT) ₂ (CTATCTGT) ₃ CTAT(CTATCTGT) ₂ CTAT(CTATCTGT) ₄ (CTATCCGT) ₂ (CTAT) ₃	GU946473	F: TCGTTTTCCACAAAAGGTC R: CGGGAGAGGAATACAACA	M10	59	9	200–316	0.75	0.69	12	0.50	0.80	12
SptD269	(GTCT) ₉	GU946469	F: TGCTCCTCTAAGTACAGGAAAGG R: AGGCAGGCAGAGACAGAT	M13	62	10	124–174	0.60	0.76	15	0.50	0.61	15
SptD206	((TGTC) ₃ TGCA) ₄ (TGTC) ₆ TGCA(TGTC) ₆	GU946460	F: TGCCTCGTCTACTACTACC R: AGCCAAGTGTCCAGACAAAGG	T7T	59	16	204–372	0.93	0.57	14	0.57	0.93	14
SptD220	(CTGT) ₃ CTACAACCCTA(CTGT) ₇	GU946475	F: TACAACATCGCCAGACTGCT R: TGTGACAGACAAGACGGAGAG	M13	62	13	297–432	0.31	0.76	13	0.93	0.90	14
SptD292	(ACAG) ₉	GU946472	F: TGGGATGGGATTTTAGGATG	SP6	60	5	146–166	0.27	0.33	15	0.85	0.76	13

Table 1 continued

Locus	Sequence	Accession no.	Primer sequence	Primer tail sequence name	T _a (°C)	N _A	Size range (bp)	Lake Washington		Bay-Delta			
								H _O	H _E	n	H _O	H _E	n
SptD277 (GGGAGACAGAC(C/G)A) ₁₃		GU946470	R: CTCCTCTGCAACCCTGTGAT	SP6	59	16	218–397	0.40	0.67	15	0.71	0.93	14
	F: TTCGATTGGGAGAGAGAGA												
	R: TGAGACATGATCCCCTCCTT												
SptD289 (TC) ₂₄		GU946471	F: GACGCCCTATTTCTGGTCTTC	SP6	60	14	182–296	0.60	0.76	15	0.64	0.91	14
	R: TGGACGGACAAACTAGACAGG												

microsatellite amplification and polymorphism. Based on allelic diversity and clean scoring, nineteen primer sets were selected for amplification in a total of 12–15 individuals per collection from the San Francisco Delta, CA and Lake Washington, WA.

Polymerase chain reaction was performed with the following conditions: 20 ng DNA template, 1× GoTaq[®] Flexi Buffer, 1.875 mM MgCl₂, 0.2 mM of each dTNP, 0.1 μM of fluorescently labeled primer and reverse primer, 30 nM tailed forward primer, and 0.4U of Taq DNA polymerase (all reagents from Promega), for a total reaction volume of 10 ul. PCR was performed using a Bio-Rad DNA engine Dyad thermal cyclor under the following conditions: 95°C for 5 min; 5 cycles at 95°C for 45 s, 68°C for 5 min with 2°C decrease at each subsequent cycle, followed by 72°C for 1 min; 5 cycles at 95°C for 45 s, 58°C for 5 min with 2°C decrease at each subsequent cycle, followed by 72°C for 1 min; 30 cycles at 94°C for 45 s, °C for 2 min, followed by 72°C for 1 min; final extension at 72°C for 10 min; and held at 8°C. One microliter of fluorescently-labeled amplified product was run undiluted on an ABI 3130xl Genetic Analyzer with a LIZ600 size standard (ABI). GENEMAPPER version 4.0 (ABI) was used to analyze the electropherograms. All analyses were computed with the GENALEX software (Peakall and Smouse 2006) were estimated. This program calculated number of alleles, observed (H_O) and expected (H_E) heterozygosities, deviations from Hardy–Weinberg equilibrium (HWE), a pairwise *F_{st}* value, and genetic distance was calculated for Principal Component Analysis (PCA) to assess differentiation between collections.

Seventeen of the nineteen primers optimized in long-fin smelt were consistently scoreable (Table 1). The number of alleles per locus ranged from 5 to 16 with an average of 13.8 alleles per locus, and the observed and expected heterozygosities ranged between 0.267–0.929 and

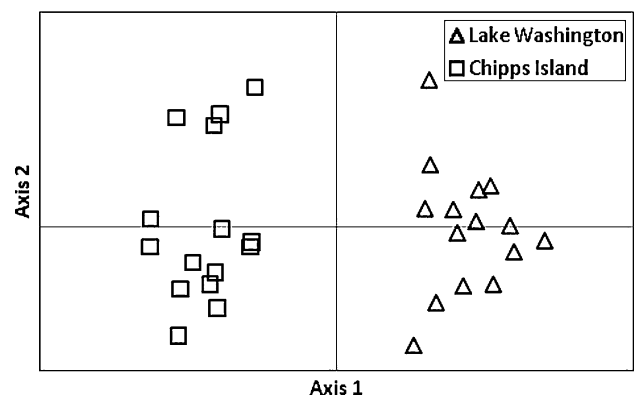


Fig. 1 Principal Component Analysis of two collections of longfin smelt (n = 15 each). Axis 1 accounts for 33.1% of the genetic covariance, while Axis 2 explains an additional 16.2% of the total variation

0.331–0.926, respectively. Seven loci deviated significantly from Hardy–Weinberg expectations. A pairwise F_{st} value of 0.091 was calculated between the two collections, and visualization of genetic distances using Principal Component Analysis supports moderate levels of differentiation between these two collection sites (Fig. 1). Genetic analysis of these collections suggested genetic isolation between locations, which are separated by over 800 miles of coast. These results are concordant with a previous allozyme study (Stanley et al. 1995), and support the distinctiveness and genetic isolation of the longfin smelt populations in these locations. These genetic markers will be useful for further evaluation of spatial and temporal genetic variation in *S. thaleichthys* and characterizing relationships among individuals in populations.

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