markers will prove invaluable for the description of genetic population structure, connectivity and demographics of dab around the British Isles. Furthermore, they will enable more accurate interpretation of biomonitoring data, and provide a neutral genetic background with which adaptive genetic markers can be compared.

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New microsatellite loci for the endangered scalloped hammerhead shark, *Sphyrna lewini*

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Abstract

We isolated 15 microsatellite markers for the scalloped hammerhead shark, *Sphyrna lewini*. Loci were tested on 80 specimens of *S. lewini* from four Eastern Pacific samples. The number of alleles per locus ranged from 6 to 31 (mean = 14). Observed and expected levels of heterozygosity per locus ranged from 0.39 to 0.91 (mean = 0.70) and from 0.54 to 0.90 (mean = 0.76), respectively. No pairs of loci were in gametic disequilibrium after Bonferroni correction of α. One locus showed significantly lower heterozygosity than expected under Hardy–Weinberg proportions in two populations, possibly caused by null alleles.

Keywords: conservation genetics, elasmobranch, fisheries, migration, population structure

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The scalloped hammerhead, *Sphyrna lewini*, is a circumtropical species (Compagno 1984) listed as 'endangered globally' on the IUCN Red List. Although landings of *S. lewini* have decreased in recent years (e.g. Vooren *et al*. 2005; Dudley &

Table 1 Primer sequences for new *Sphyrna lewini* microsatellite loci. T_a is annealing temperature. H_E and H_O are averaged expected and observed heterozygosities across all four Eastern Pacific samples, and *P*-values are given as the range across all four samples*

Locus	Primer sequence (5′– 3′)	Accession no.	Alleles	Range (bp)	T_a (°C)	H_E	H_O	*P* value
SLE013	F: ATGTTTATGACCATACGTGCG R: TTGATTGGCATTCAGTGACC	FJ236873	10	302–350	60	0.67	0.55	0.05–0.91
SLE018	F: ACAGAAACAGAACGAGGGACA R: TGGGTTGGCATTGAACAGAA	FJ236878	6	208–258	60	0.65	0.40	0.00–0.90
SLE025	F: CTCAGGCTAGTTGCACAGAAA R: TCAACTCCCCACAATCCCAT	FJ236874	31	234–398	57	0.90	0.91	0.96–0.10
SLE027	F: GAGACCAGCCAAAGGAAAAA R: ATGCCATATTCATCCAGGCAC	FJ236879	13	420–459	60	0.70	0.61	0.03–0.84
SLE028	F: TTTGGAGACATTGCAGAAAG R: CACTTGGGACTACACACACTG	FJ236875	25	226–281	55	0.84	0.88	0.06–0.83
SLE033	F: TTGGTCAATGTCCTCTTGCA R: CCCATGCTGTTTTGTTCTTTG	FJ236876	11	261–299	57	0.79	0.80	0.16–0.60
SLE038	F: AGCCTACTTCTGCCACATTTT R: AATCAAAGTTCCTGCAGTCCT	FJ236877	14	419–475	60	0.80	0.73	0.06–0.63
SLE045	F: AGGATGGGATTCAGTGACAGA R: AATAAGCTCAAAGGGCTGGA	FJ236880	5	403–411	60	0.66	0.57	0.01–0.96
SLE053	F: AAGTCAAAAGCTGTGTGCGA R: ATTCCCCACATACATTCCCCA	FJ236881	21	407–457	57	0.84	0.79	0.06–0.79
SLE054	F: CTGACACTGCCAATTTGCAT R: CCAACTGGAGTTGTCAATCCA	FJ236882	9	186–206	60	0.54	0.39	0.06–0.78
SLE071	F: TCAGACGGTGGTACGTACACA R: TGACCCTTTTGGATTGAAGGA	FJ236883	13	236–285	60	0.75	0.58	0.01–0.54
SLE077	F: TTCCCTCTCAGAGTGACATTG R: CCTTTCCTCCATACACAAACA	FJ236884	29	218–317	55	0.90	0.91	0.53–0.78
SLE081	F: ATGTTCATCATCCGAGACAGG R: CCAAACACACGTATCTGCACCCA	FJ236885	8	384–402	60	0.80	0.81	0.62–0.99
SLE086	F: TACAGACAGATTTCAGTGTGT R: ACGAATACGCATTCATACAC	FJ236886	6	350–361	60	0.71	0.67	0.18–0.62
SLE089	F: TTACCACAGTTTGTGTGGGTG R: AAGTTTCAGTGTCAGTGTGC	FJ236887	13	172–204	60	0.85	0.91	0.25–0.85

*Heterozygosities were calculated individually for each of the four Eastern Pacific samples and averaged across those four samples. However, their associated *P* values were not averaged, and are presented here as the range from lowest to highest.

Simpfendorfer 2006; Martínez-Ortíz *et al*. 2007), the frequency of *S. lewini* and *Sphyrna zygaena* fins auctioned off in Hong Kong fish markets remains high, comprising nearly 5% of the total market fin weight (Clarke *et al*. 2006). Accordingly, data regarding stock structure, migration rates, and estimates of population size are needed to design effective conservation strategies. Species-specific microsatellite markers provide a means of obtaining these data for threatened and endangered taxa. We have therefore isolated and characterized 15 microsatellite loci for *S. lewini* using library-enrichment protocols of Clark & Brazeau (2005), modified from strategies developed by Kandpal *et al*. (1994).

The library was constructed using genomic DNA extracted from two juvenile sharks from Kaneohe Bay, Hawaii. DNA was digested using the *Sau*3 A enzyme, and fragments corresponding to 400–1500 bp were selected by cutting digested DNA out of a 3% agarose gel. *Sau*3 A linkers were ligated to size-selected DNA, and these fragments were enriched by polymerase chain reaction (PCR) using the DNA Engine DYAD Peltier Thermal Cycler (MJ Research Inc.). The enriched DNA was hybridized to a biotin-labelled probe containing a CA-repeat sequence. Enrichment of these probe-targeted fragments was done using Vectrex Avidin D matrix (Vector Laboratories). Non-specifically bound fragments were removed through a series of stringent washes, followed by elution and PCR amplification of CA-enriched fragments.

Fragments from the CA-enriched library were cloned by transforming *Escherichia coli* with product from the enriched library using the TOPO TA Cloning Kit (Invitrogen Corp.). Colonies were submitted to the Clemson University Genomics Institute (CUGI) for sequencing on an ABI 3130 (Applied Biosystems), and subsequently screened for CA-repeat motifs using Sequencher (GeneCodes Corp.). Ninety pairs

of primers were designed for sequences containing repeat regions, using Web Primer (Stanford University).

Loci yielding amplification products of expected size on agarose gels were amplified using fluorescent dye-labelled primers. A three-primer protocol was used (Hauswaldt & Glenn 2003; R. Toonen, personal communication.) in which the following sequences were attached to reverse primers corresponding to dye-labelled tags incorporated into the PCR: 6-FAM-GGAAACAGCTATGACCAT, VIC-CAGTC-GGGCGTCATCA, NED-ACCAACCTAGGAAACACAG, PET-GGCTAGGAAAGGTTAGTGGC. These loci were optimized on a larger sample of individuals. In general, PCRs were 12 μL total volume, with 1× GoTaq buffer, 0.5 μL forward primer, 0.05 μL unlabelled reverse primer, 0.45 μL dye-labelled reverse tag (primers at 5 μM concentration), 0.125 μL of 10% Triton X-100, 40 μM dNTPs, 0.35 U GoTaq polymerase (Promega), and 0.5 μL DNA. Thermocycling profiles for each locus differed in annealing temperature (Table 1), but all started with denaturing at 95 °C (4 min), 30–40 cycles of 95 °C (1 min), 55–60 °C (1 min), and 70 °C (1 min), followed by a final extension at 70 °C (10 min).

Fluorescently labelled products were run on an ABI 3130 capillary sequencer, using LIZ600 size standard (Applied Biosystems) and analysed with GeneMapper (Applied Biosystems). Loci that amplified consistently were further analysed in Micro-Checker (Van Oosterhout *et al*. 2004) to test for genotyping errors and to aid in identification of possible null alleles. Arlequin (Schneider *et al*. 2000) was used to test for deviations from Hardy–Weinberg (HW) proportions and to calculate observed and expected heterozygosities. GenePop (Raymond & Rousset 1995) was used to test for gametic-disequilibrium between all pairs of loci.

A total of 15 loci were characterized and found to be polymorphic among 80 individuals of *S. lewini* from four different Eastern Pacific samples. Data from these loci, including primer sequences, number of alleles, range of alleles, annealing temperature, observed and expected heterozygosities, and *P* values are listed in Table 1. All motifs were interrupted or imperfect CA repeats. Sequences of each microsatellite can be viewed on GenBank (Accession numbers in Table 1).

Seven loci (SLE013, SLE018, SLE027, SLE045, SLE053, SLE054, and SLE071) had significantly different values of observed and expected heterozygosities. However, departures from HW proportions at these loci were found in two of four populations at most, suggesting demographic factors may be responsible. Only one locus (SLE018) showed evidence of null alleles in two populations. No significant gametic disequilibrium was detected across all pairs of loci after Bonferroni correction of α. We plan to use these markers to analyse populations of *S. lewini* at large, global scales and along smaller, continental margins.

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