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Lieber, L., Dawson, D. A., Horsburgh, G. J., Noble, L. R., & Jones, C. S. (2015). Microsatellite loci for basking shark (*Cetorhinus maximus*) monitoring and conservation. *Conservation Genetics Resources*, 7(4), 917–944 . <https://doi.org/10.1007/s12686-015-0493-8>

Published in:
Conservation Genetics Resources

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
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Microsatellite loci for basking shark (*Cetorhinus maximus*) monitoring and conservation

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Abstract

The filter-feeding basking shark (*Cetorhinus maximus*) is the largest fish found in the Northeast Atlantic, however there is little baseline ecological data despite its globally threatened status. In order to investigate the basking sharks' population connectivity, patterns of relatedness and effective population numbers over time, we developed a microsatellite marker set suitable for genetic monitoring. Forty-seven markers were screened, of which 24 were assessed in 20 unrelated individuals from the Isle of Man using DNA extracted from skin mucus swabs. We selected 19 polymorphic loci with a mean observed heterozygosity of 0.73 to create a marker set with sufficient power to unambiguously resolve between individuals ($PI_{sibs}=1.7 \times 10^{-7}$).

Keywords: Basking sharks, Cetorhinidae, individual identity, non-invasive sampling, microsatellite

Basking sharks (*Cetorhinus maximus*) are designated 'Endangered' (IUCN) in the Northeast Atlantic and little is known about their life history traits (Sims 2008). There are no reliable population number estimates, although seasonal aggregations in this region could represent a significant proportion of the global population. Individual identification is essential for basking shark monitoring and current management efforts. Here, we present a microsatellite marker set suitable for genotyping DNA obtained from non-invasive sampling using mucus swabs (Lieber et al. 2013) that will allow the first estimates of contemporary genetic diversity, connectivity and effective population size; parameters vital to the species' conservation.

Microsatellites were isolated from a female (BSUK05_17; sampled off Cornwall, UK, in 2004) and a male basking shark (BSUK05_35; Loch Maddy, Scotland, UK in 2005). BSUK05_17 was enriched for repeats (following Armour et al. 1994) and 64 clones Sanger-sequenced to reveal 35 that were

primer designable with >9 repeats. Additionally, Illumina-sequenced paired-end libraries were created from the repeat-enriched genomic DNA of BSUK05_17 or BSUK05_35 (not enriched) using the SureSelect Library Prep Kit, ILM (Agilent Technologies Inc., California) and a MiSeq Benchtop Sequencer. In total, 47 primer sets were designed from Sanger sequences or from a consensus of overlapping MiSeq sequences using PRIMER3 v0.4.0 (Rozen and Skaletsky 1999), (27 Sanger (five were homologous to other shark species) and 20 MiSeqs (15 enriched, 5 unenriched); Table 1 and Supplementary Table 1).

Products were amplified in a 2- μ l PCR reaction, including 1.0 μ l (30–80ng) DNA (air dried), 1 μ l primer mix (forward and reverse primer at 0.2 or 0.4 mM, Table 1) and 1 μ l Qiagen Multiplex Master mix with the following profile: 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 56°C for 90 s, 72°C for 60 s and finally 60°C for 6 min. PCR products were diluted (1:160) and separated on an ABI 3730 48-well capillary DNA analyser using ROX GS500 size standard (Applied Biosystems Inc.). Alleles were scored using the GENEMAPPER v3.7 software.

Initially, all 47 loci were tested in four individuals from different locations (Ireland, Scotland, England and New Zealand) using DNA isolated from muscle tissue. Twenty-four loci (12 Sanger and 12 MiSeq) that could be scored easily were then further tested in 20 individuals sampled in the seas around the Isle of Man (IoM, 54°N, 4°W) using DNA isolated from skin mucus swabs (sexing free swimming individuals is difficult but these were suspected to be 10 males and 10 females based on the presence/absence of claspers). Nineteen loci were amplified in eight fluorescent multiplexes (*Cmax07* was multiplexed with *BS1HF4* and *BS1HA5* from Noble et al. 2006); Table 1). Allele sizes observed in the library individual were very close to those expected, suggesting the correct loci were amplified (\pm 4bp; except *Cmax01* = -8bp; Table 1). The remaining 28 markers were dropped (see Supplementary Table 1). Both, Sanger- and MiSeq-sequenced microsatellite markers showed similar success rates (\approx 50%).

Assuming that sharks possess heterogametic sex determination (XY or ZW), the 19 selected loci were autosomal based on the presence of heterozygotes in a larger sample of males and females (displaying a minimum of 20 heterozygotes per locus in 78 males and a minimum of 15 heterozygotes per locus in 69 females; LL et al. unpublished data). Individuals were checked for repeat sampling (100% matching genotypes) and checked for relatives using the probability of identity option in CERVUS v3.0.3 (P_{ID}) and ML Relate. The 20 individuals genotyped were confirmed unique and unrelated ($r \leq 0.25$) and used to calculate observed and expected heterozygosities (CERVUS), and to test for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP v4.2. To correct p-values in multiple tests, the False Discovery Rate method was applied, after which no groups of loci showed significant LD, and none of the 19 selected loci deviated from HWE. Estimated

null allele frequencies were low (<0.1) and there was no evidence of large allele dropout using MICRO-CHECKER.

Scoring error rates were calculated for the nineteen reliable loci selected for further population genetic analysis (*Cmax01* – *Cmax19*; Table 1). For these loci, 25% of individuals were re-run (re-PCRing and re-genotyping) at least once from the same DNA stock. Mean allelic error rate was calculated as the number of mistyped alleles (n=2) divided by the total number of alleles (n=77), yielding a mean error rate of 0.026 per allele. Mismatches between repeat runs were assessed through re-genotyping. The probability of identity among siblings (PI_{sibs}) using these nineteen loci on 20 IoM individuals was 1.7×10^{-7} , demonstrating the low probability of randomly drawing two different individuals from this population with the same multi-locus genotype.

This microsatellite marker set will provide a powerful tool for basking shark monitoring and will be utilised in on-going research investigating global population structure, relatedness and effective population size.

Acknowledgements We thank David Sims, Jackie and Graham Hall (Manx Basking Shark Watch), and Malcolm Francis for providing swab/tissue samples. Shark tissue sampling was performed under licence (UK Home Office ASPA 1986 project license: PPL 30/2882 issued to David Sims). Microsatellite isolation and genotyping was performed at the NERC Biomolecular Analysis Facility at Sheffield and funded by the Natural Environment Research Council (NERC), UK (grant reference NBAF723 to CSJ and LRN). Jennifer Dawe and Darren Grafham (Sheffield Diagnostics Genetics Service) performed the MiSeq sequencing at The Children’s Hospital Sheffield supported by the Sheffield Children's NHS Trust, UK. LL was supported by funding from the MASTS (The Marine Alliance for Science and Technology for Scotland) pooling initiative and their support is gratefully acknowledged. MASTS is funded by the Scottish Funding Council and contributing institutions. Funding from Scottish Natural Heritage (SNH) (grant to LRN and CSJ) also supported this work.

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