Manchester Metropolitan University

Fox, G and Darolti, I and Hibbitt, J-D and Preziosi, RF and Fitzpatrick, JL and Rowntree, JK (2018)*Genetic assessment of ex situ populations to aid species conservation and maintain heterozygosity in non-model species.* Journal of Zoo and Aquarium Research, 6 (2). pp. 50-56. ISSN 2214-7594

https://e-space.mmu.ac.uk

Research article

Bespoke markers for ex-situ conservation: application, analysis and challenges in the assessment of a population of endangered undulate rays

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Abstract
Genetic data are important and informative in the management of ex-situ populations. Where the risk of inbreeding is particularly great, it is critical that tools are employed that allow for the quantification of genetic variation and to identify potential breeding pairs. This study demonstrates the rapid application of laboratory and bioinformatics techniques to develop a novel microsatellite marker panel for use with a population of the endangered undulate ray (*Raja undulata*) and shows how a minimally invasive sampling method can be used with aquarium-dwelling individuals. The study assesses the population and investigates how informative a small microsatellite marker panel is to the conservation of a restricted ex-situ group. It was found that after a single captive generation of *R. undulata* there is no detectable evidence of reduced heterozygosity and no observable aquaria effects or differences between the generations. In conclusion, the study demonstrates that it is practical, quick and informative to develop a bespoke panel of markers to aid ex-situ conservation efforts of non-model species and make recommendations that these processes should constitute the minimum effort required in managing such a population.

Introduction

The elasmobranchii are a subclass of carnivorous, cartilaginous fish, including the sharks, rays, skates and sawfish. These species are found extensively in coastal, demersal and pelagic marine habitats and an additional minority inhabit freshwater systems (Compagno 1990). Common traits include slow growth and low productivity (Frisk et al. 2001; Walker 1998), resulting in high vulnerability and slow response to overexploitation from fishing activities (Ferretti et al. 2010; Smith et al. 1998). Recorded declines in elasmobranch populations over recent decades are typically associated with increasing fishing effort; an effect which can be seen in oceans the world over, for example in the Gulf of Mexico (Shepherd and Myers 2005); the Northwest Atlantic (Baum et al. 2003); the Mediterranean Sea (Ferretti et al. 2008); the Sea of Japan (Nakano 1999) and

the Indian Ocean (Appukuttan and Nair 1988). Whether fishing effort targets elasmobranchs specifically (Rose 1998; Stevens et al. 2000) or they are a common feature of bycatch (Oliver et al. 2015), with the majority of global fisheries at risk of overexploitation (Botsford et al. 1997) the long-term effect on elasmobranch populations is largely unknown (Baum et al. 2003).

The undulate ray (*Raja undulata*) is an endangered skate often present in bycatch of commercial trawl fishing operations off the south coast of England, France, western Ireland and southern Portugal (Coehlo et al. 2009). Existing in small isolated populations, the species has recorded declines of up to 80% in some areas since the early 1980s, which has been directly attributed to fishing activities (Ellis et al. 2012). In 2009, the species was classified as endangered by the IUCN (Gibson et al. 2008). A managed breeding and monitoring programme

(Mon-P) was established in 2010 by the European Association of Zoos and Aquaria (EAZA) in response to the new IUCN classification and a European Union ban on the landing of this skate species was put in place. Currently, 36 aquaria across nine countries hold *R. undulata*. As part of the larger European breeding program, a small captive group is maintained across several UK aquaria, comprising a mixture of wild-caught and captive-bred individuals. Very little is known about the genetic diversity or population genetic structure of this species either in captivity or in the wild. The elasmobranchii are a charismatic focal point of interest for the general public in aquaria and are the subject of intense conservation effort to manage their ex-situ conservation. With >100 chondrichthyan species present in European zoos and aquaria (8.6% of all known elasmobranch species), there is great interest in the community for methods and techniques for sustainable conservation of these animals (Janse et al. 2017).

Non-random mating and genetic drift are major concerns for small populations and can have devastating implications for the evolutionary potential of the group. The small size of the population limits potential reproductive pairings, as inbreeding becomes a risk with the increased probability of a pair of individuals being related to one another (Witzenberger and Hochkirch 2011). Prolonged inbreeding in a closed population increases the probability of progeny being homozygous at a given locus, resulting in the overall reduction of heterozygosity of the group after successive generations. Genetic drift and adaptation to captivity can also contribute to the loss of rare alleles and overall reduction in heterozygosity (Price and Hadfield 2013; Willoughby et al. 2014). It is widely recognised that the fitness of a population is inversely related to allelic homozygosity, and severe effects, such as loss of viability or infertility, can present after just a few generations of close inbreeding (Frankham et al. 2004). These detrimental effects are cumulative as they are amplified by successive generations in captivity (Christie et al. 2012). As a result, the longer it has been in isolation, the less-well suited a captive population becomes to providing individuals for release (Earnhardt 2010; Lacy 2012). It is imperative, therefore, that the genetic variation present at the founding of the ex-situ population be carefully retained and inbreeding avoided through strategic genetic management of the population (Fernández et al. 2004; Frankham et al. 2010; Pelletier et al. 2009).

Under ideal conditions, during the establishment of a new ex-situ population, the entire group should be assessed using genetic markers to estimate the diversity of the cohort and help establish a baseline of genetic diversity, to identify any genetic similarity of founding individuals and to support future management. In the case of an existing population, genetic markers should be used even in the presence of detailed keeper reports and pedigrees; whilst these resources contain valuable information, they are limited in scope to the time that the individuals (or their ancestors) have been known to the relevant managers. The most common genetic marker used in analyses of this type is the microsatellite; short, repetitive, hypervariable regions of DNA that appear to be a feature universal to all genomes. Microsatellite marker panels are available in online databases for many species and published, optimised methodologies are available for developing novel sets of markers (Castoe et al. 2012; Griffiths et al. 2016). As the rate of species extinction is elevated above the background rate (Pimm et al. 2014) and there is potential for an unprecedented increase in the number of ex-situ populations being managed across a wide range of taxa (Dawson et al. 2011), it is imperative that general best practice guidelines in genetic management are established now. In line with the recommendations of Witzenberger and Hochkirch (2011) and Janse et al. (2017), the current best practice is argued to be the use genetic markers to characterise the diversity and relatedness of individuals in a captive breeding program and this should be the minimum standard required for the establishment, or maintenance, of any ex-situ conservation programme.

When sampling for the collection of DNA, the aim should be to minimise stress or discomfort experienced by the subject whilst collecting high-quality genomic template, especially in the case of an endangered or threatened species. Tissue sampling or destructive biopsy is clearly counterproductive in some cases, therefore the development and testing of non- or minimally invasive sampling methods is paramount. Here, a minimally invasive sampling method, developed for wild elasmobranchs by Lieber et al. (2013), is tested on aquarium specimens and found to be highly successful when combined with an off-the-shelf DNA extraction kit that enables isolation of high-purity DNA from the mucus layer.

In this investigation, bioinformatics techniques are used to develop a novel microsatellite marker panel suitable for use in *Raja undulata*, using Illumina shotgun next-generation sequencing data. These markers are then optimised in the laboratory and used to characterise a small ex-situ population. The viability and confidence with which the small marker panel can be used for population management is assessed, whilst providing a snapshot of the diversity contained within this population of captive elasmobranchs.

Methods

Microsatellite marker development
High-throughput, shotgun genomic sequencing can be used in order to identify microsatellite regions in the target genome. High quality, large molecular weight, genomic DNA is essential for successful next-generation sequencing and can be collected in a variety of ways, often using a species-specific method. Samples of blood, tissue or buccal swabs (Dunn et al. 2010) are also commonly used for genetic sampling. In this instance, tissue samples were obtained from a female ray that had been euthanised due to terminal ill health resulting from a severe fungal infection of the lateral line system. A range of tissue samples were taken from the animal post euthanasia under the guidance of Mark F. Stidworthy, veterinary pathologist at International Zoo Veterinary Group (IZVG). DNA was extracted from 25 mg heart tissue using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol and checked for quality on a NanoDrop ND-1000 spectrophotometer (260/280 >1.4) and on a 1% agarose electrophoresis gel. A sequencing library was prepared using 50 ng genomic DNA and analysed on an Illumina MiSeq platform at the University of Manchester (UK) Genomics Facility using a shotgun, paired-end 2*250 sequencing methodology (Nextera DNA Library Preparation Kit, Illumina, San Diego, USA). In total, 11,019,590 raw sequencing reads were produced from the MiSeq run. Low quality regions were removed from each end of the reads, reads were trimmed using the average quality score over a sliding-window of 4 nt and a quality threshold of 20, and a minimum length of 50 nt was applied using Trimmomatic v0.0.4 (Bolger et al. 2014). If either of the paired-end reads failed a quality check, both reads were discarded, thus maintaining parity in the paired-end data. A majority (92%) of reads successfully passed quality filtering and were subsequently screened for potential microsatellite loci using pal_finder v0.02.04 software (Castoe et al. 2012). Non-perfect repeat loci were discarded and a minimum motif size of 3 nt was implemented (Griffiths et al. 2016).

Primer sequences were designed using Primer3 v.4.0.0 (Koressaar and Remm 2007; Untergrassar et al. 2012) using conditions optimised for the Qiagen Type-it microsatellite PCR kit (Qiagen, Hilden, Germany) (optimum length: 25 nt, minimum length: 18 nt, maximum length: 30 nt, minimum GC%: 45%, maximum GC%: 65%, minimum melting temperature: 62°C, maximum melting

temperature: 75°C, optimum melting temperature: 68°C, with remaining options set to the Primer3 default values); a set of PCR reagents designed specifically for amplification of microsatellite loci. The pal_finder process produced 698 potential loci that were ranked by predicted utility as a microsatellite marker (larger motifs preferred) and the primer sequences from the first 24 results were used to purchase DNA oligos from Sigma Aldrich (Missouri, USA) (scale: 0.025 µmole, purification: DST).

Sampling

For characterisation of the microsatellite loci, the 35 captive *R. undulata* (17 wild caught, 18 captive bred) were sampled using a modified form of the minimally invasive sampling method developed for wild elasmobranch sampling by Lieber and colleagues (2013), a method not known to have been previously demonstrated on captive animals. Small (1.5 cm x 2.5 cm), autoclaved sections of kitchen scouring pad (Vale Mill Ltd., Rochdale) were used to gently scrub the pectoral fin of the rays against the direction of the scales removing epidermal mucous secretions. Inter-species contamination was controlled, to the best of our ability, through the use of the species-specific PCR primers. As the markers were designed in a sample taken from excised heart tissue of an undulate ray (low risk of contamination), successful marker amplification implies a lack of contamination as the target DNA was of the same taxa as the heart sample. Intra-species contamination is more difficult to control for; however, it appears not to have been an issue, as microsatellite peak traces did not show multiple banding. The pads were immediately placed into individual tubes of absolute ethanol and stored at −80°C. During DNA extraction, extraneous pad was removed and DNA was extracted using the E.Z.N.A. Mollusc DNA Kit (Omega Bio-Tek, Norcross, USA); the use of chloroform:isoamyl alcohol (24:1) successfully isolating the mucus, precipitating proteins and producing high quality DNA extract. Elution was performed in 100 uL MilliQ water and used in downstream PCR for genotyping. This sampling technique reduces stress and damage to the animal as it minimises, or eliminates in some cases, the time the specimen spends out of the water during sampling. The technique could potentially be applicable to any captive elasmobranch with a mucus layer on the skin. A total of 35 animals were sampled from 10 different aquaria. More details as to the provenance of the samples are given in Table 1. Samples were also taken from several related *Raja* species (*R. microcellata*, *R. brachyura*, *R. montagui* and *R. clavata*) in order to test the cross-compatibility of the primers.

Marker amplification

Twenty-four potential markers were tested in the laboratory, of which eight successfully amplified.

PCR amplifications of 5 uL total volume were performed using the Qiagen Type-it Microsatellite PCR Kit (Qiagen, Hilden, Germany). Reactions consisted of 2.5 µL Type-it mastermix, 1.5 µL PCR grade H_2O, 0.5 µL genomic DNA at 20 ng/µL and 0.5 µL primer pair at 2 µM. This 5 µL reaction was amplified under the conditions specified by the PCR kit (5 min 95°C, 28x {30 sec 95°C, 90 sec 60°C, 30 sec 72°C}, 30 min 60°C) and successful amplifications were confirmed by the presence of bands on a 1% agarose electrophoresis gel. A three-primer universal-tailed approach was used to label amplicons with fluorescent moieties (Blackett et al. 2012) and fragment length reported using an Applied Biosystems 3730 DNA analyser capillary sequencer (Applied Biosystems, Foster City, California, USA) and GeneScan 500 LIZ dye size standard (Thermo Fisher Scientific, Carlsbad, USA) at the University of Manchester DNA Sequencing Facility.

Population genetic analysis

Raw data analysis was performed using GeneMapper 5.0 (Thermo Fisher Scientific, Carlsbad, USA) and confirmed that loci were scoreable and polymorphic. The novel markers were analysed for evidence of linkage disequilibrium and for Hardy–Weinberg

Table 1. Sample numbers taken from each of the 10 UK aquaria. The provenance of each sample is given as well as the number of private alleles detected at each aquarium.

| Aquarium	Total N	Wild Caught	Captive Bred	Private Alleles
Sea Life London Aquarium	9	4	5	2
Weymouth Sea Life Adventure Park	5	1	4	2
Sea Life Blackpool	4	2	2	0
Sea Life Chessington	5	1	4	0
Sea Life Adventure, Southend	3	2	1	1
Sea Life Great Yarmouth	2	2	0	2
Sea Life Loch Lomond	1	1	0	0
Blue Reef Aquarium, Portsmouth	3	3	0	1
National Marine Aquarium, Plymouth	2	0	2	3
Marine Biology Association, Plymouth	1	1	0	0
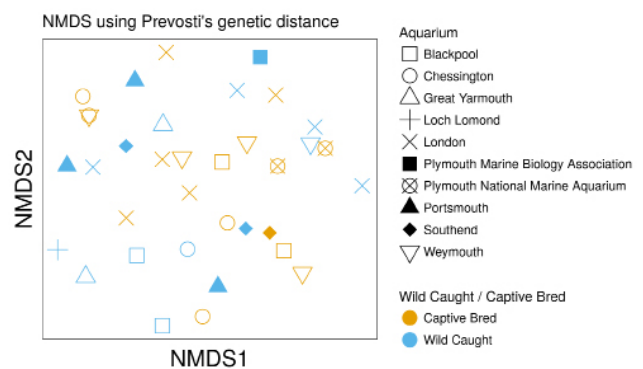
Figure 1. Ordination of Prevosti's genetic distance between individuals derived via non-metric multidimensional scaling (NMDS). Each point represents an individual, point shapes are aquaria and point colour represents whether the individual is wild caught or captive bred. The stress value of the NMDS was 0.17, demonstrating reasonable confidence in the ordination whilst maintaining a minimum number of dimensions.

Table 2. Locus ID, nucleotide motif, number of alleles (NA), size range of fragments (SR), PCR annealing temperature (TA), expected (Hexp) and observed (Hobs) heterozygosity, number of individuals tested (N), P-value from testing for Hardy–Weinberg equilibrium (PHWE) and primer nucleotide sequences (5' to 3' orientation). *Marker RU13 not used in this study due to deviation from expected HWE values.

Locus Name	Motif	NA	SR (bp)	TA (°C)	Hexp	Hobs	PHWE	N	Primer Sequences (5' - > 3' orientation)	GenBank Accession
Ru02	AAGAGG	10	347 - 419	60	0.808	0.800	0.0180	35	CCCTGTTCTCCTGCTCTCCATTACC CTCTCCCTATAGCTCAGGCCTTCGG	MH049873
Ru03	ACTGCC	10	412 - 463	60	0.827	0.882	0.0694	34	CATTCACAACTGCAGTCCAATGTCC TCTGCTGTCAAGCTGTTGTGTCAGG	SRP134840
Ru08	AGGTG	13	351 - 415	60	0.887	0.800	0.0113	35	TGAGGAATTCATTGCCACAAACTGC TCCTCTCACATAACCCTGTGTATGCC	MH049874
Ru09	ATAG	22	209 - 385	60	0.945	0.939	0.1463	33	TCTTTGCTCCTACCGGTTCTTCTCG CAGAACAAGGCTTGGTGGTCTTGG	MH049875
Ru13*	ACAG	9	317 - 373	60	0.787	0.313	0	32	CATTCTTAACAGGGCAGCTACTTGTGG AAAGATTGGTAGGAAGATGGATCGG	MH049876
Ru14	AGGC	8	277 - 313	60	0.754	0.882	0.7937	34	ACCTCGAAACCGCCATTAAGAATCC CTGCATGTTATCGAGCAATCAGTCG	MH049877
Ru20	ACAG	9	374 - 407	60	0.846	0.886	0.1317	35	GGACACTTGACACAGCTTTGGTCTCC GGGAGTTACCTTCATGGTGAGACAGG	MH049878
Ru21	AAT	5	373 - 388	60	0.682	0.543	0.1631	35	CATGACTGGGGCTAGAAGGTGTTGC GTTAGAGCAGTCCGCCATGAAGGG	MH049879

equilibrium using GenePop v.4.2 online (Raymond and Rousset 1995; Rousset 2008). Estimates of pairwise relatedness were calculated for every pair of individuals using the triadic likelihood estimator of relatedness, a measure suited to a relatively small number of markers, implemented in Coancestry using the R (R Core Team, 2017) package "related" (Pew et al. 2015). The rate of heterozygosity, inbreeding coefficient and measures of genetic distance were calculated using the "adegenet" package in R (Jombart 2008; Jombart and Ahmed 2011; Rogers 1972). Rates of allelic richness and private alleles were identified using the R package "PopGenReport" (Adamack and Gruber 2014). The data were split by generation, and comparisons were drawn between each generation. In this instance, all wild-caught individuals were compared to all captive-bred offspring, as at the time of sampling there was only a single generation captive population (F1 generation).

Results

Eight polymorphic microsatellite markers were initially characterised and every marker demonstrated to amplify consistently at an annealing temperature of 60°C, advantageous for multiplex PCR. These novel markers were used to genotype 35 captive *R. undulata* individuals at the eight loci. GENEPOP results for linkage disequilibrium (LD) showed that 48% of total marker pairs exhibited significant evidence of LD; however, when just the wild-caught individuals were tested, this percentage was reduced to zero. GENEPOP was also used to check for deviation from the expected allele frequencies of Hardy–Weinberg. Three markers showed significant deviation in the total population and a single marker (Ru13) showed deviation from expected frequencies in the wild-caught animals only. This marker (Ru13) was subsequently removed from the analysis. Summary statistics for the remaining seven markers are given below in Table 2. A success rate of 98%

was achieved in obtaining genotypic data. Average allelic richness was 7.0 in the wild-caught group, 6.4 in the captive-bred group and 1.7 per aquarium. The average observed rate of heterozygosity at each marker was 0.81. Observed heterozygosity (Hobs) and the average estimated inbreeding coefficient (r) were calculated for the wild-caught animals (Hobs=0.80, r=0.21±0.003) and the first generation, captive-bred individuals (Hobs=0.83, r=0.18±0.005).

Table 3. Microsatellite markers tested in several other *Raja* species. Size ranges in a limited number of samples.

Species	Locus Name							
	Ru02	Ru03	Ru08	Ru09	Ru13	Ru14	Ru20	Ru21
Raja microcellata								
	341-419	412-463	351-432	209-385	317-373	277-376	374-407	373-388
Raja brachyura								
	347-377	408-463	351-428	204-385	317-419	277-391	374-407	373-388
Raja montagui								
	347-364	412-483	351-415	209-385	317-373	277-313	374-422	373-388
Raja clavata								
	343-419	412-463	351-415	209-385	285-373	277-313	374-407	373-388

There was no significant difference in either heterozygosity (two sample t-test, t=0.52644, df=10.171, P=0.6099) or the average inbreeding coefficient (two sample t-test: t=−1.0356, df=14.225, P=0.3177) between wild-caught and captive-bred individuals. One to three private alleles were discovered in six of the 10 aquaria (aquarium population size ranging from 1–9 individuals). A nonmetric multidimensional scaling (NMDS) analysis of Provesti's genetic distance among individuals (Figure 1), calculated using the R (R Core Team, 2018) package "vegan" (Oksanen et al. 2017), provides a visual interpretation of the genetic similarity of individuals. The calculated stress value of the NMDS was 0.17, the lowest stress value of each of the measures of genetic distance calculated using the "adegenet" (Jombart 2008; Jombart and Ahmed 2011) package in R. A stress value of <0.2 indicates a fair fit of the data in the NMDS analysis (Kruskal 1964).

The minimally invasive extraction method and the seven primer pairs were tested with several other species of the *Raja* genus (species listed previously) and were demonstrated to successfully amplify polymorphic loci in every species tested, suggesting good cross-species compatibility of the primers and sampling technique. Allelic range in these species very closely matched those discovered in *R. undulata* (Table 3). Four or fewer samples from each species were tested and, therefore, more detailed locus statistics are not provided here.

Discussion

The goal of this study was to develop and optimise a novel set of microsatellite markers for the endangered undulate ray (*Raja undulata*) and subsequently assess their power and informativeness for ex-situ conservation of this species. Genomic DNA, extracted from a tissue sample, was successfully used to generate a sequencing library, and bioinformatics and laboratory techniques were employed to discover and optimise seven microsatellite markers from the resulting next-generation sequencing (NGS) dataset. In order to undertake genetic analyses of this nature, a reliable source of DNA is required, but often this can come at the cost of distress or harm to the subject. Therefore, non-invasive genetic sampling methods are preferable to invasive tissue, blood or biopsy sampling, particularly for threatened species. Although an initial tissue sample was used for the development of the markers, a minimally-invasive sampling method for the collection of the remaining samples from the captive animals (Lieber et al. 2013) was tested. This technique takes advantage of the mucus secreted by the skin of many elasmobranchs and this study demonstrates the successful isolation of high-quality, amplifiable DNA from captive animals. The new markers were used to genotype a small captive population of 35 animals, across 10 UK aquaria, demonstrating that the minimally-invasive sampling methodology was suitable for a study of this nature. Several quality-checking procedures were applied to the markers themselves, such as tests for linkage disequilibrium (LD) or deviations from Hardy–Weinberg Equilibrium (HWE). Evidence of both LD and deviation from HWE was observed in some markers. The deviation from expected HWE can be attributed to the fact that the test population breaks many of the underlying assumptions of HWE, mainly that one should consider a large, unrelated population, which is not the case here. Several statistical analyses of the data were performed, making routine measurements of heterozygosity of the population at these loci, calculating inbreeding coefficients and genetic distance, for example.

The results show rates of heterozygosity at each marker ranging from 0.54–0.94 (average 0.81), implying that when all markers are taken into account, the rate of genetic variation in the captive population is not likely to be significantly lower than the wild population from which it was founded. For comparison, Chapman et al. (2011) used seven microsatellite markers to measure heterozygosity in an elasmobranch population consisting of 104 individuals of the critically-endangered smalltooth sawfish (*Pristis pectinata*) and discovered an average rate of heterozygosity of 0.83. Heterozygosity rates in wild-caught animals and captive-bred, F1 generation individuals did not show any significant difference, demonstrating that a high proportion of genetic variation has been carried into this generation. Data reporting the proportion of wild-caught individuals that successfully contributed to the F1 generation are unfortunately not available. These measures should be repeated at each new generation and can be interpreted as a proxy for the measure of total variation in the group. The captive-bred *R. undulata* of the present study had an average rate of heterozygosity of 0.83. It is important to note, however, that these results on the captive-bred population only take into account the F1 generation and that any decrease in the rate of heterozygosity will likely become apparent over subsequent generations (Willoughby et al. 2017). Continued monitoring via the methods explained in this study will be critical to continue to evaluate the genetic diversity of the population and to continue to monitor for inbreeding depression. Several aquaria housing private alleles within their cohort have been identified, and this information may be useful for maintaining genetic variation when the breeding plan is developed.

While it is common to calculate the likely pedigree (i.e. relatedness) from this type of genetic data, the power to correctly assign offspring to parents will be very low for captive populations with a limited captive population size. In these cases, it is far more informative to directly examine the genetic similarity of individuals. The calculation of Provesti's genetic distance (Prevosti et al. 1975) enabled the visualisation of a proxy measure of dissimilarity between individuals (see Figure 1) through calculating the absolute genetic distance between each pair of individuals. Figure 1 shows no clustering around a particular aquarium or between the wild caught or captive bred groupings, indicating the lack of an aquarium effect or differentiation of the F1 generation from the wild individuals. Rather, the individual genotypes suggest a homogenous mixture with no apparent groupings, or sub-structuring emerging. These results fall within expectations as ~50% of the total individuals were wild caught (17 of 35) and so can be expected to be reasonably unrelated to one another as they originate from a wild population. Progeny from relatively high admixture would be expected to maintain high levels of variation in the F1 generation and similarly be relatively unrelated to one another (with the exception of siblings, parents-progeny, etc.).

This study leads to the recommendation that similar analyses be performed as new individuals are caught, born or moved between aquaria to enable population managers to intervene should a particular group of individuals appear to become distinct from other groups, or when one of the measures, or proxy measures, of variation among individuals begins to fall. With a greater number of microsatellite markers, the work could be extended to include relatedness estimates of a much higher confidence and this would also lead to the production of accurate pedigrees—very useful tools to the community managing these animals, but beyond the scope of this piece of work.

Conclusion

Ex-situ conservation is a very important management tool and is likely to be increasingly used as the rate of anthropogenic-influenced species declines continues to climb (Ceballos et al. 2015). Captive populations must be carefully and strategically managed in order to successfully provide individuals for reintroduction, maintain genetic variation and reduce the negative effects of inbreeding (Frankham et al. 2004). Janse et al.

(2017) succinctly summarised the contemporary elasmobranch populations in European aquaria and identified the requirement for good programme management. This study demonstrates that researchers can move relatively quickly from collecting tissue/swab samples, through designing a novel marker panel to producing quantifiable, genetic data and drawing conclusions regarding the structure of a captive population (the majority of the work on this analysis was performed in a matter of a few months). In the absence of a good quality pedigree or studbook, these techniques should form the minimum requirement when working with ex-situ populations, and as NGS technologies continue to improve, the number and nature of available markers will also increase, leading to significant gains in the quality of the data available. The power of this particular study was limited by a lack of markers, thus preventing some analyses from being performed. However, from the data generated here, it is evident that the population of undulate rays in UK aquaria do not currently appear to be suffering from any malady resulting from their small population size, and the findings appear to fall in line with other managed groups of elasmobranchs. The results, however, constitute a time-bound observation and are therefore only representative of the population at the time the samples were taken. In conclusion, the study has shown that it is feasible and useful to design and optimise a panel of markers for a small, ex-situ population and that even with a small number of markers, the resulting data can be informative and help with the management of the population. With these markers available to the community, it is hoped that a better understanding of the captive population in UK aquaria in relation to individuals in European aquaria and in wild populations can be reached. This study forms the basis for further scope of greater scope, encompassing a greater sample size, more sampling sites (aquaria) and more microsatellite markers to increase the statistical power of the analyses.

Acknowledgements

Conflict of Interest

The authors declare that they have no conflict of interest.

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