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Contribution to the Theme Section 'Marine functional connectivity'

Mitochondrial haplotypes reveal low diversity and restricted connectivity of the critically endangered batoid population in a Marine Protected Area

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ABSTRACT: Stability and long-term persistence of a species rely heavily on its genetic diversity, which is closely allied to its capacity for adaptation. In threatened species, population connectivity can play a major role in maintaining that diversity, and genetic assessments of their populations can be crucial for the design of effective spatial conservation management. Not only is it worth evaluating the amount of diversity in a candidate population for protection, but the magnitude of outgoing gene flow can provide insight into its potential to replenish others via emigrants. The critically endangered flapper skate *Dipturus intermedius* receives protection in the Loch Sunart to the Sound of Jura Marine Protected Area (MPA) in Scotland. However, there is insufficient knowledge of genetic diversity and connectivity across its range. Recent tagging studies in the MPA suggest the presence of animals with high levels of site fidelity and residency, as well as transient individuals, raising concerns of limited connectivity to populations beyond the MPA. In this study, a newly developed mitochondrial haplotype marker allowed use of DNA sourced from fin clips, mucus and egg cases to investigate population structure and mitochondrial variability across several sites around the British Isles, including the MPA. Unfortunately, results characterized the MPA as having particularly low haplotype diversity and significant population differentiation from other sample sites. More than a quarter of its individuals carry a haplotype rarely observed elsewhere, leaving outgoing gene flow questionable. The MPA appears unlikely to sustain the species' existing mtDNA genetic diversity or act as an effective source population.

KEY WORDS: Conservation · Connectivity · Population genetics · MPA · Mitochondrial haplotypes · Endangered · Flapper skate · Batoid · Elasmobranch · *Dipturus intermedius*

1. INTRODUCTION

Genetic diversity is of paramount importance for the long-term persistence of a species coping with changing environmental pressures (Hughes & Stachowicz 2004, Agashe et al. 2011, Epstein et al. 2016). Lack of genetic diversity is often associated with higher levels of inbreeding and reduced fitness (Brook et al. 2002, Keller & Waller 2002, Reed & Frankham 2003, Acevedo-Whitehouse et al. 2006, Kyriazis et al. 2021, Hasselgren et al. 2021), raising concern that a consequential loss of genetic diversity may impair

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adaptive potential (Nei et al. 1975, Corti et al. 2011, Abascal et al. 2016, Parra et al. 2018). Severe population declines, due to anthropogenic pressures for example, can precipitate genetic bottlenecks (Leigh et al. 2019), with the resulting small, fragmented populations likely to become genetically depauperate, more susceptible to genetic drift and often lacking incoming gene flow that may help to maintain genetic diversity (Willi et al. 2006, DiBattista 2008, Robinson et al. 2016). This situation can be exacerbated by harvesting (Allendorf et al. 2008), habitat degradation (Xu et al. 2019) and philopatric behaviours such as residency (Flowers et al. 2016, Monti et al. 2018), thereby minimising dispersal success, reducing the number of migrants and ultimately leading to local extirpation.

Recently, many species of elasmobranchs (sharks, skates and rays) have experienced population collapses and local extinctions due to indirect and direct fishing pressure (Stevens et al. 2000). They are also threatened with a further decrease in numbers due to the indirect impacts of habitat loss and degradation (Dulvy et al. 2014). The absence of complete landing records of species and species complexes with unresolved taxonomy has complicated the traceability of stock declines (Iglésias et al. 2010, Stevens et al. 2000, Naylor et al. 2012). One family especially negatively affected by this are the skates (Rajidae), as aggregated historical catch records of batoids, along with unreliable landing records, have been shown to mask local extinctions, e.g. the long-nose and white skates (Dulvy et al. 2000).

One rajid species of conservation concern is the flapper skate *Dipturus intermedius*, recently recognised as a species and part of the common skate (*Dipturus batis*) species complex (Griffiths et al. 2010, Iglésias et al. 2010, Last et al. 2016). *D. intermedius* is listed as Critically Endangered by IUCN due to severe population declines (Ellis et al. 2021). This reclassification means there is a lack of catch data pertaining specifically to this species (Iglésias et al. 2010). Like many other elasmobranchs, the flapper skate is K-selected with slow growth, late maturity and long life (Stevens et al. 2000, Cailliet et al. 2005, Régnier et al. 2021). Recently, a hatching in captivity confirmed that incubation lasts approximately 18 mo (Benjamins et al. 2021). Due to its life history strategy, the flapper skate can sustain only low levels of fishing mortality (Walker & Hislop 1998, Myers & Worm 2005), leaving the remaining populations highly vulnerable. Currently, retention on board, transshipping and landing is prohibited in most of the species' range [S.S.1. 2012/63 (2012), EU (2019), Simpson & Sims (2016)].

To facilitate additional protection of the common skate species complex, Scotland established the Loch Sunart to Sound of Jura Marine Protected Area (hereafter the MPA) in 2014 (Loch Sunart to the Sound of Jura Marine Protection Order 2014), a 741 km^2 area off the west coast, known to harbour the flapper skate *D. intermedius*. In 2016, the Loch Sunart to the Sound of Jura Marine Conservation Order 2016 was enacted to enforce regional and temporal bans on several mobile fishing activities, such as beam and demersal trawling, suction and mechanical dredging, as well as longline fishing in the MPA, to remove the threat of accidental by-catch. The recent discovery of a flapper skate egg laying site near the Isle of Skye, west coast of Scotland, and its designation as an emergency marine protected area (The Red Rocks and Longay urgent Marine Protected Area) in March 2021, affords temporary protection for a breeding aggregation and their egg cases.

Protected areas are often a favoured strategy to nurture genetic diversity (Laikre et al. 2016, Sandström et al. 2019). However, to be effective this requires gene flow between protected and non-protected areas (Allendorf et al. 2008). To-date there has been no assessment of flapper skate genetic population structure, and the MPA was established without knowledge of its genetic diversity or genetic connectivity. Monitoring of tagged flapper skate within the MPA indicates both a high degree of site attachment, particularly by females, as well as transient individuals, although reconstructed movements of electronically tagged skate suggested that most transients may remain in the vicinity of the MPA (Neat et al. 2015, Pinto et al. 2016, Lavender et al. 2021). As movement studies covered a relatively short proportion of the species' potential lifespan, estimated to be up to 47 yr for females and 28 yr for males (Régnier et al. 2021), there remains uncertainty about the extent of lifetime movements and philopatric tendencies. Nevertheless, based on research trawl data from around the British Isles, the aggregation of flapper skate within the MPA appears isolated from other high-density areas to the north and west (Neat et al. 2015, Pinto et al. 2016, Rindorf et al. 2020, Frost et al. 2020). This isolation, coupled with the high levels of site attachment observed, may mean connectivity between this aggregation and other areas is restricted. Poor genetic connectivity between the MPA and other populations raises concerns of potential isolation and depauperate genetic variability, reducing the benefit of this spatial protection for the species. Hence, there is a need to investigate genetic connectivity between the MPA and other populations

to determine the sufficiency of current conservation management.

Many population genetic studies have relied on mitochondrial derived markers to determine the presence or absence of gene flow among populations of a species (Moritz 1994, Petit & Excoffier 2009). Estimates of diversity and population structure using mitochondrial markers have informed conservation management for threatened animals, including several batoid species (Sandoval-Castillo et al. 2004, Phillips et al. 2011, Castillo-Páez et al. 2014, Tikochinski et al. 2018, Cruz et al. 2021, Garrigue et al. 2022). Yet, gene flow between elasmobranch populations has often been investigated using only a part of the mtDNA control region (Knaus et al. 2011, Domingues et al. 2018). While established mitochondrial markers allow comparability between studies, they can lead to cryptic haplotypes being overlooked, meaning the ability to detect discrete populations within a species is often strongly affected by the choice of marker (Knaus et al. 2011, Castillo-Páez et al. 2014, Feutry et al. 2014). Exploration of the whole mitogenome to inform selection of molecular markers can greatly improve the resolution and detection of population genetic structure (Shamblin et al. 2012, Feutry et al. 2014, 2015).

Our study aimed to compare the mitochondrial diversity of flapper skate populations found in the MPA with those from other locations and investigate their genetic connectivity. An earlier study sequenced the partial mtDNA control region of flapper skate, revealing a maximum of 3 haplotypes and potential structure between the Azores and the British Isles (Griffiths et al. 2010, Bache-Jeffreys et al. 2021). We compared full mitochondrial genomes of 4 individuals from different geographic locations to identify more variable areas of the mitogenome suitable for analysis of population structure. The derived marker can offer insights into the flapper skate's current spatially protected diversity and indicate possible implications for adaptation of future management approaches.

2. MATERIALS AND METHODS

2.1. Sampling and DNA extraction

Samples were collected from *Dipturus intermedius* individuals caught and released during Marine Scotland Science (MSS) and the Centre for Environment Fisheries and Aquaculture Science (CEFAS) surveys, as part of an acoustic and archival tagging programme (Thorburn et al. 2021, Lavender et al. 2021), and from sampling charters. Fin clips (preserved in either 100% ethanol or RNAlater) were collected under UK Home Office licenses between 2011 and 2020 at 8 sampling sites located around the British Isles (Fig. 1). Of the 153 animals sampled in the MPA, 139 possessed either a dart tag (Scottish Shark Tagging Programme) or PIT tag (NatureScot/MSS), lowering the risk of double sampling significantly, and allowing use of the SkateSpotter database (https://skatespotter.sams.ac.uk/) to provide some insights into the movement of individuals.

To explore potentially less invasive methods of DNA sampling than fin clips, 36 mucus samples from animals residing in the MPA, and 15 spent egg cases (found washed ashore but not fully dried) were trialled as DNA sources, the latter having the potential to allow identification of the provenance of females using egg laying sites. Isolation of DNA from skin mucus is an efficient method of obtaining good-quality DNA from basking sharks (Lieber et al. 2013, 2020), suggesting that such methodology could be utilised in other vulnerable elasmobranchs. Mucus samples were taken from landed skate by wiping/scraping the skin with either a swab, a wooden stick, or a cotton cloth. These utensils were conserved in 100% ethanol and kept at either +4 or −20°C.

Genomic DNA was isolated by proteinase K digestion and phenol extraction protocols (Sambrook et al. 1989, Lieber et al. 2020) from either a 2 mm^2 piece of tissue, a 1 cm^2 piece of the innermost layer of an egg case, a swab, a wooden stick, or 1 cm^2 of cloth.

2.2. Marker development and amplification

Earlier studies utilizing only part of the mitochondrial control region (Griffiths et al. 2010, Bache-Jeffreys et al. 2021) did not show sufficient variability to allow detailed analysis of population structure, with only 3 haplotypes revealing no structure in the British Isles. Consequently, there was a need to identify a new variable marker region. To achieve this, 4 complete mitochondrial genomes were analysed: 1 from North Scotland (Schwanck et al. 2022, Genbank MT890688) and 3 further individuals from Ireland (n = 1), the Celtic Sea (n = 1) and the MPA (n = 1), and amplified using the 23 overlapping primers designed for the genus *Dipturus* as detailed in Schwanck et al. (2022) (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m731p279_supp.pdf). Briefly, fragments obtained from Sanger sequencing at Genewiz (https://www.genewiz.com, Essex, UK)

were assembled using Geneious V. 11.1.5 (Kearse et al. 2012) and annotated based on the available complete mitogenome from a North Scotland individual (Genbank MT890688). All 4 complete mitochondrial genomes (GenBank accession numbers MT890687–MT890690) were aligned and the most variable region identified that includes partial cytochrome *b* gene, tRNAThr, tRNAPro, and partial control region. Primers were designed using Primer3 V. 2.3.7 (Untergasser et al. 2012) to obtain an 862 bp fragment: FlappVarF 5′-TGC CTT TAC TCC ACA CGT CT-3′ and FlappVarR 5′-TGG GTG CGT CAG ATT TAT GGT-3′. The PCR reaction to obtain the targeted marker contained 5.9 µl H_2O, 1 µl Buffer (10×), 1 µl dNTP mixture (2 mM each), 0.6 µl $MgCl_2$, 0.2 µl of each primer (10 µM), 0.1 µl *Taq* polymerase and 1 µl genomic DNA (20 ng μl^{-1}). Initial denaturation of 5 min at 95°C was followed by 40 cycles of 95°C for 30 s, annealing at 59°C for 30 s and an extension at 72°C for 1.5 min, with a final extension at 72°C for 10 min. PCR products were purified using the enzymes *Exo*I and rSAP (New England Biolabs) following manufacturer's protocols and sequenced with the forward primer using Sanger sequencing at Genewiz. The resulting sequences were trimmed to 715 bp in Geneious V. 11.1.5, retaining only high-quality nucleotide sites.

2.3. Analysis

To visualise overall diversity, mutations and possible mitochondrial divergence, a minimum spanning network was created with PopART V. 1.7 (Bandelt et al. 1999). The following measures of genetic diversity were calculated for the whole data set and sample sites with more than 10 individuals using the R package 'pegas' (Paradis 2010, R Core Team 2022): overall and site-specific haplotype and nucleotide diversity, Tajima's *D* and Theta *S* (a measure of the number of segregating sites per nucleotide weighted by sample size, hereafter indicated by θ). Since $\theta = 4N_e\mu$ (N_e = effective population size, µ = mutation rate), θ is positively correlated with effective population size. Differences in diversity between sample sites were tested for significance with a permutation test of 10 000 replicates, using the script genetic_diversity_diffs (Alexander et al. 2016) and corrected with the Benjamini-Hochberg FDR adjustment to control the False Discovery Rate (FDR) (Benjamini & Hochberg 1995). Furthermore, randomised haplotype accumulation curves with 1000 permutations were calculated to compare and evaluate sampling effort and diversity at each site using the R package 'spider' (Brown et al. 2012, R Core Team 2022), which used rarefaction curves to quantify the relationship between haplotype richness and sample size (Gotelli & Colwell 2001). Using Arlequin version 3.5 (Excoffier & Lischer 2010), pairwise Φ_{ST} (Excoffier et al. 1992) was computed between sample sites of n ≥ 10 based on pairwise differences. Negative Φ_{ST} values were rounded to zero. Significance was calculated using 10 000 permutations to estimate population differentiation and corrected with the Benjamini-Hochberg FDR adjustment (Yekutieli & Benjamini 1999).

3. RESULTS

Comparison of the 3 mitochondrial genomes sequenced in this study and 1 available from Genbank revealed 14 polymorphic nucleotide positions (Fig. S1 in the Supplement). A marker region encompassing 3 variable positions and spanning from the cytochrome *b* gene into the control region was chosen. The primers designed in this study amplify a highly variable region, producing a 715 bp sequence suitable for the analysis of mitochondrial diversity and population connectivity in the flapper skate. The derived mitochondrial DNA marker was reliably amplified with DNA extracted from 307 tissue samples. Success was lower with DNA isolated from spent egg cases and skin mucus, with only 2 of 15 egg cases (1 from North Scotland and 1 from the MPA) and 12 of 36 (all MPA) skin mucus samples producing the full target sequence.

The final data set of 321 sequences from 8 sample sites around the British Isles revealed 16 variable nucleotide positions and 16 unique haplotypes (Table 1; Genbank Accession numbers OM338058–OM338073). One additional nucleotide position resulted in ambiguous sequencing results, showing either a clear signal for adenine or overlapping signals of both adenine and guanine. Though this position might be another indication of mitogenomic variability and a possible case of heteroplasmy, it was regarded as not truly variable as there was no sequence with an unambiguous guanine signal. This base position was omitted from the analysis.

The median-joining network analysis showed low levels of divergence as most connections between haplotypes were characterised by 1 or 2 substitutions, the maximum being 3 (Fig. 1). In all sample sites with >10 individuals, the most common haplotypes I and V are present. Also prominent in this data set is haplotype VIII, which makes up 27.3% of the

Table 1. Polymorphic positions in 16 mitochondrial haplotypes of *Dipturus intermedius* found in a 715 bp marker. Mitogenome column lists the nucleotide position of each polymorphism within the full mitochondrial genome and marker column lists the position within the 715 bp marker. Roman numerals represent the haplotypes

Mitogenome	Marker	Haplotype															
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
15 411	34	C	T	.	T	T	.	.	T	T	.	T	T	.	.	.	.
15 433	56	A	.	.	G	.	.	.	.	.	.	.	G	.	.	.	.
15 475	98	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
15 580	203	G	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
15 670	293	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
15 671	294	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
15 692	315	G	A	.	.	.	.	.	.	A	.	.	.	.	.	.	.
15 725	348	G	C	.	.	.	.	.	.	.	.	.	.	A	.	.	.
15 734	357	T	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.
15 742	365	G	.	.	A	.	.	.	.	.	.	A	.	.	.	.	.
15 798	421	T	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
15 823	446	A	G	.	.	.	.	.	.	G	.	.	.	.	.	.	.
15 882	505	G	.	.	.	.	A	.	.	.	.	.	.	A	.	.	.
15 907	530	T	C	.	C	C	.	C	C	C	.	C	C	.	.	.	.
16 038	661	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
16 041	664	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.

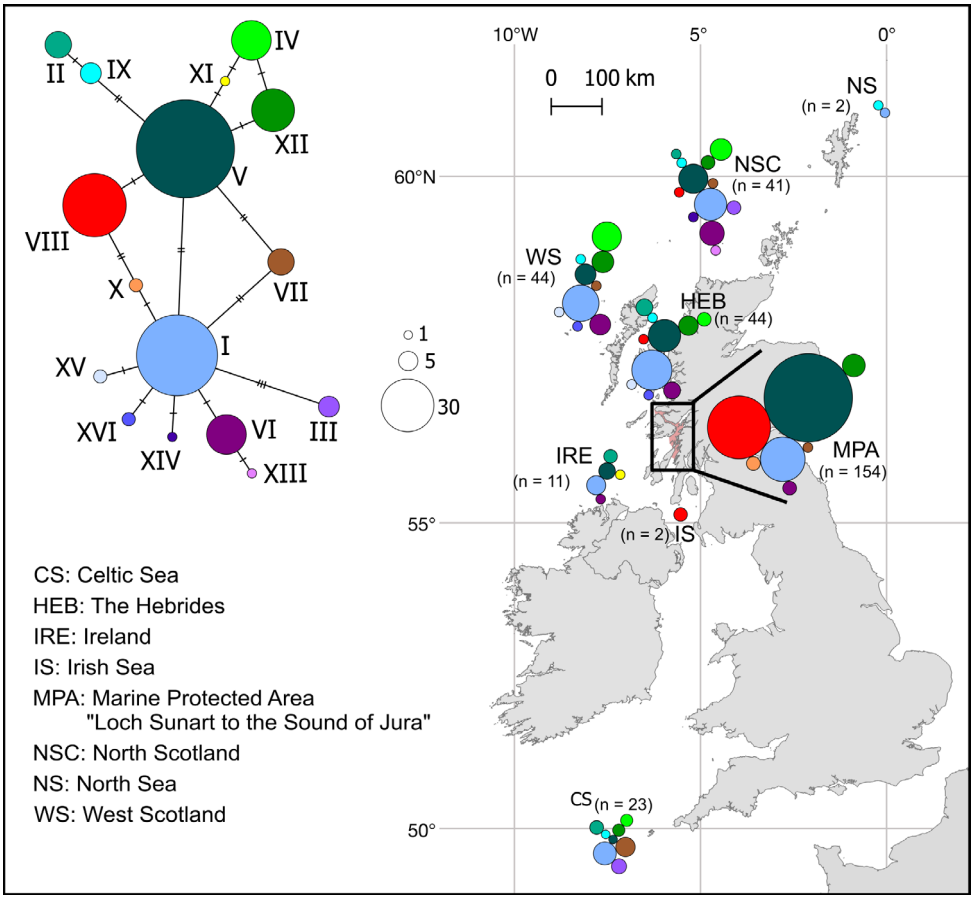

Fig. 1. Haplotype network and geographic distribution of haplotypes. Network in the upper left corner displays 16 haplotypes in *Dipturus intermedius*, hatch marks indicate the number of substitutions between haplotypes. Map on the right displays the haplotype representation and frequency at each sample site. Black box highlights the MPA. Circle size indicates sample number, colours indicate haplotype I–XVI

MPA population but occurs only sparsely in other areas, namely North Scotland (n = 1), the Hebrides (n = 1), and the Irish Sea (n = 2).

Overall haplotype diversity was 0.8, and most sample sites approximated to that value (Table 2). Three sample sites showed 1 or 2 exclusive haplotypes that did not appear elsewhere, the MPA (X), North Scotland (XIII, XIV) and Ireland (XI). The MPA showed 7 haplotypes and a haplotype diversity of 0.646, an outlier in comparison to other sample sites, with significantly less diversity than all sample sites except Ireland (Fig. 2, p-values in Table S2). While the nucleotide diversity of the MPA was also significantly less than at the other sites, it contrasts with the Celtic Sea, which was significantly more diverse than 2 of the 5 other populations (Fig. 2). Values of Tajima's *D* were approximately 0 in all sample sites and none was statistically significant. The value of θ, which is positively correlated with effective population size, was also low in the MPA (θ = 1.069), whilst North Scotland had the highest value (θ = 3.272). Haplotype accumulation curves indicate haplotype richness of the MPA was represented accurately by the present data set and that the haplotype richness of the other sites would potentially increase with more samples (Fig. 3). The haplotype accumulation curve for all sites combined nears an asymptote (Fig. S2), suggesting sampling to-date has provided representative relative haplotype diversity estimates.

Values of pairwise Φ_{ST} indicate strong differentiation ($p < 0.01$) between the MPA and all other sample sites (Table 3): the smallest differentiation being with

Table 2. Haplotype details by sample site. MPA: Loch Sunart to the Sound of Jura Marine Protected Area; NCS: North Scotland; WS: West Scotland; CS: Celtic Sea; IRE: Ireland; HEB: the Hebrides; NS: North Sea; IS: Irish Sea; n: number of individuals. –: No analysis performed due to small sample size

	n	Haplotypes (exclusive)	Haplotype diversity	Segregating sites	Theta S (θ)	Tajima's *D*	Nucleotide diversity
Total	321	16	0.8	16	2.521	–0.532	0.00276
MPA	154	7(1)	0.646	7	1.069	0.047	0.00153
NSC	41	12(2)	0.856	14	3.272	–0.702	0.00356
WS	44	9	0.818	10	2.299	–0.04	0.00318
HEB	44	10	0.784	11	2.529	–0.520	0.00292
CS	23	8	0.854	11	2.980	0.209	0.00443
IRE	11	5(1)	0.818	7	2.390	0.079	0.00329
NS	2	2	–	4	–	–	–
IS	2	1	–	0	–	–	–

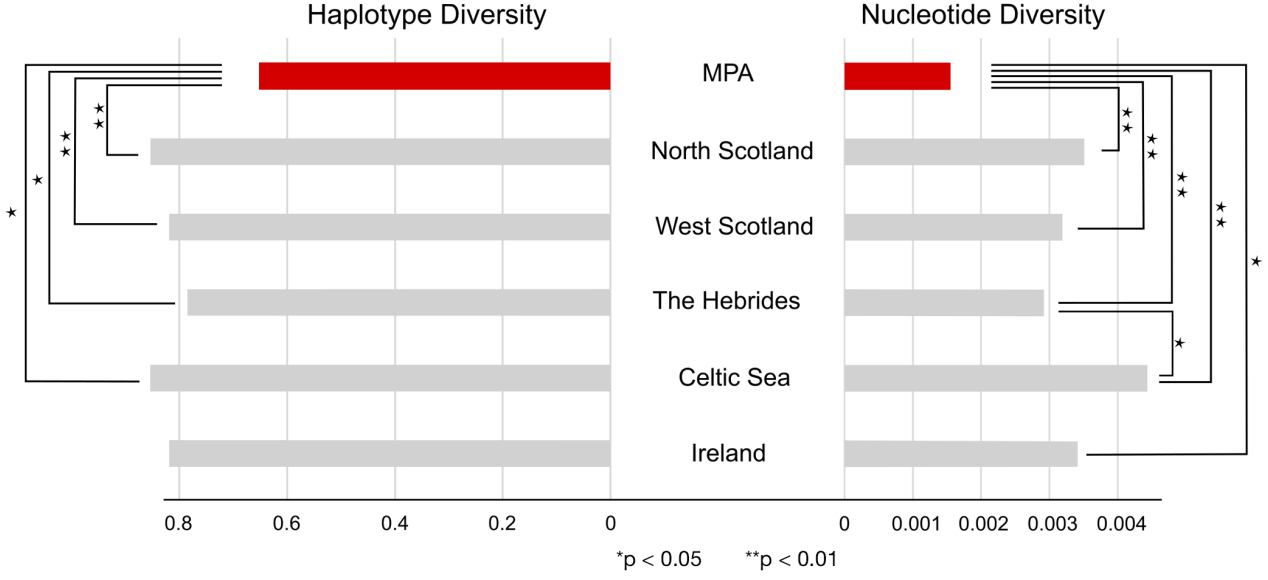

Fig. 2. Comparison of haplotype and nucleotide diversity of the 6 sample sites with n > 10. Bars of the MPA highlighted in red, asterisks indicate significant difference between sample sites *p ≤ 0.05; **p ≤ 0.01 (Benjamini-Hochberg adjusted)

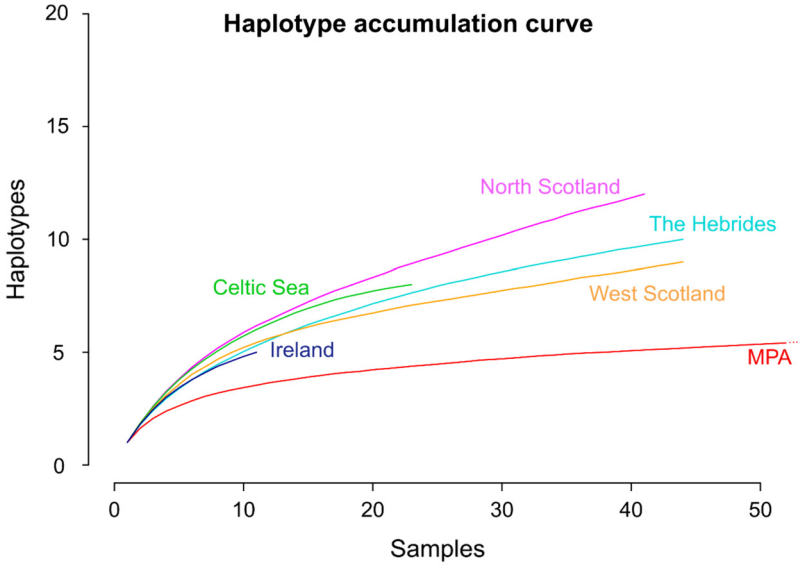

Fig. 3. Haplotype accumulation (rarefaction) curves of sample sites with n > 10 based on random subsampling of sequences, 1000 permutations

Table 3. Population differentiation Φ_{ST} including sample sites with >10 individuals. Lower-left matrix displays pairwise Φ_{ST} values, while upper-right matrix displays pairwise p-values. Asterix indicates significant p-values, $p < 0.01$ (Benjamini-Hochberg adjusted). MPA: Loch Sunart to the Sound of Jura Marine Protected Area; NSC: North Scotland; WS: West Scotland; CS: Celtic Sea; IRE: Ireland; HEB: the Hebrides

	MPA	NSC	WS	HEB	CS	IRE
MPA		<0.001*	<0.001*	<0.001*	<0.001*	0.003*
NSC	0.212*		0.617	0.695	0.341	0.594
WS	0.244*	0		0.317	0.156	0.294
HEB	0.191*	0	0.013		0.282	0.749
CS	0.276*	0.011	0.041	0.023		0.568
IRE	0.207*	0	0.033	0	0	

the Hebrides (Φ_{ST} = 0.19) and the largest with Celtic Sea (Φ_{ST} = 0.276). Excluding the MPA, other sample sites did not show significant differentiation between them ($\Phi_{ST} \leq 0.041$).

4. DISCUSSION

This study aimed to provide insight into the mitochondrial diversity of *Dipturus intermedius* and genetic connectivity of populations between the MPA and other localities. In the context of current conservation management, our results display a striking pattern of mitogenomic variability and differentiation in the investigated flapper skate populations, revealing reduced diversity and restricted connectivity in the only adult population benefitting from permanent spatial protection. As the North and Irish Sea are represented by only 2 individuals, it is not appropriate to discuss diversity and differentiation in these locations.

4.1. Genetic diversity

In comparison to most other sites, the haplotype and nucleotide diversities of skate in the MPA are significantly lower. The non-significant discrepancy in haplotype diversity between the MPA and Ireland may be an artefact of the latter's small sample size (n = 11). The MPA's haplotype richness (7) is the smallest, despite its sample size exceeding all others (n = 154). This unbalanced diversity is more apparent when comparing the haplotype accumulation curves, which show an asymptote is reached much earlier in the MPA than at sites that have the potential to reveal increased diversity with larger sample numbers (Fig. 3). Three haplotypes represent about 92% of the MPA population, the 4 less common haplotypes being present in only 12 animals. While skates in the MPA were sampled in 9 different years, the 4 rarer haplotypes were found only in samples taken in 2014 (XII), 2019 (VI, VII, X, XII) and 2020 (X, XII) (Fig. 4), which might indicate recent immigration of more diverse haplotypes. However, due to the small sample sizes for each of these years, this temporal perspective should be interpreted with caution.

The reduced mitogenomic diversity and skewed distribution of haplotypes found in the MPA could be attributed to a range expansion and subsequent founder effect, in which a few animals of the more common haplotypes colonised the region around the Isle of Mull and the Sound of Jura. Similar processes have been suggested to explain low mitogenomic variation in Australian grey nurse sharks (Stow et al. 2006) and bottlenecks in several species of sawfishes (Phillips et al. 2017). However, the history of the fisheries affecting this critically endangered species suggests that the subsequent drastic decrease in population size might have precipitated a local bottleneck,

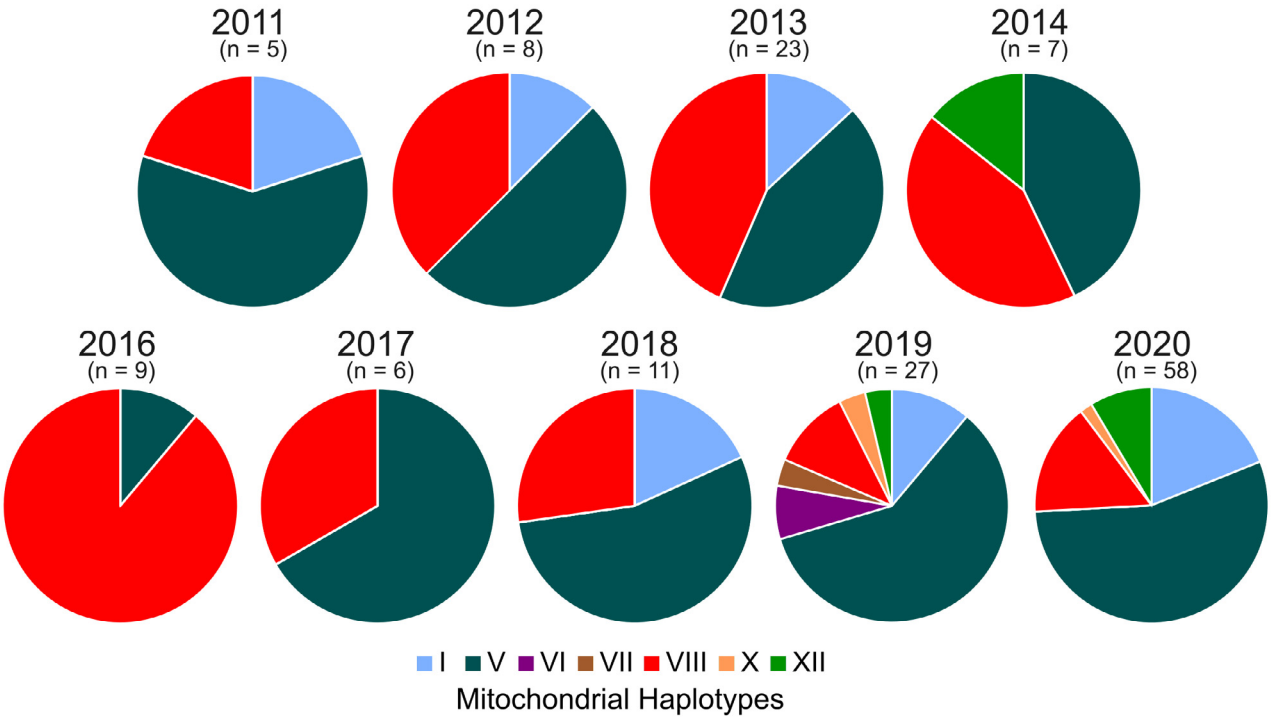

Fig. 4. Haplotype frequencies in annual sample sets of *Dipturus intermedius* between 2011–2014 and 2016–2020 in the Loch Sunart to the Sound of Jura Marine Protected Area

eradicating less common mitochondrial haplotypes. The reduction in distributional extent that accompanied the decline in common skate abundance (Walker & Hislop 1998, Rindorf et al. 2020), and their disappearance from nearby areas, e.g. the Irish Sea (Brander 1981), supports this hypothesis. The MPA provides a suitable habitat for the flapper skate, offering core and home ranges of preferred depth for several life stages (Thorburn et al. 2021) and tagging data indicates residency of some individuals (Neat et al. 2015, Lavender et al. 2021). Egg cases of flapper skate found in the MPA suggest the area supports reproduction, perhaps allowing it to be (re-)populated by remaining animals following a bottleneck. Nevertheless, values of Tajima's *D* in this study were close to zero and non-significant, indicating the observed variation is similar to that expected under the mutation-drift equilibrium model. There is no support for a reduction in genetic variation caused by a bottleneck or less extreme population contraction (as might be indicated by a positive Tajima's *D*), or a subsequent population expansion (a negative Tajima's *D*). However, as the overall diversity of the mitochondrial haplotypes is not high (only 16 haplotypes at 14 segregating sites), eradication of a few haplotypes might be insufficient to produce detectable disequilibrium between segregating sites and haplotypes.

Considering the large number of samples used in this analysis, the reduced mitogenomic diversity of this sampled subset remains remarkable, despite lack of support from Tajima's *D*, perhaps indicating a genetic bottleneck or more pertinently a lack of connectivity with other populations. Gene flow with other populations might be crucial for increasing the genetic diversity of the MPA stock.

4.2. Connectivity between the MPA and other populations

Gene flow between populations is influenced by, for example, dispersal ability of a species, geographic barriers, the distance between population fragments, and philopatric tendencies, like residency and natal homing (Frankham et al. 2010, Flowers et al. 2016, Hirschfeld et al. 2021). Based on the variability and low differentiation of most sites in this study with n > 10, lack of dispersal ability and subsequent admixture can be ruled out as a species-specific trait of flapper skate. However, the presence of exclusive haplotypes and differences in haplotype frequency between locations suggest some population structure, rather than complete panmixia between the sampled locations. This is supported by

the haplotype accumulation curves, which show different trajectories for each location, reflecting differences in haplotype diversity (Fig. 3). Though gene flow across sample sites around the British Isles is apparent, the MPA appears to be isolated. Significant differentiation between the MPA and other sample sites can be attributed to differences in haplotype richness, unequal frequencies and the dominance of haplotype VIII, found in 27.3% of the MPA population but rarely elsewhere, supporting the possibility of both restricted recruitment from outside or limited emigration from the MPA. So, the question remains why MPA population admixture is restricted.

The MPA appears as an outlier compared to the admixed sites, suggesting a site-specific explanation for this isolation is plausible. Whilst oceanographic barriers have the potential to fragment populations by blocking movement (Hirschfeld et al. 2021), the presence of the common haplotypes V and I within the MPA rejects any suggestion of a more permanent barrier isolating this population. Also, the almost exclusive haplotype VIII seems not to be derived from a historic mitochondrial divergence but from a recent mutational event, as its sequence is only one substitution different from the most common haplotype V. There is currently no evidence that connectivity between the MPA and other areas has been obstructed by barriers. Instead, it might be more likely that behavioural idiosyncrasies, like partial migration, could have caused the differing pattern in connectivity. Tracking flapper skates inside the MPA identified a high likelihood of partial migration, with some individuals using the same sites repeatedly, whilst others exhibited more extensive, less repetitious movements (Neat et al. 2015, Pinto et al. 2016, Lavender et al. 2021). It is conceivable for animals across the metapopulation to differ in their migratory strategies, which may explain differences in gene flow between the populations.

If a disproportionately large proportion of animals remain resident and reproduce inside the MPA, it would explain the dominance of haplotypes derived from founder animals after either a colonization or a bottleneck, and why haplotype VIII is rarely found elsewhere. While this protected area was established to protect the present seemingly largely site-attached population, it is of interest to see if it could potentially act as a source population which has a surplus of individuals that leave the area to stay and reproduce elsewhere. This is especially relevant since protecting a source population is considered more effective than protecting a sink population when conserving endangered species (Dias 1996). Based on the perceived pattern, it seems unlikely that the MPA would act as a source population in the wide metapopulation.

Gene flow from immigration is similarly lacking. Successful recruitment from beyond the MPA's boundaries depends on immigrants being able to establish and breed (Frankham et al. 2010). In the case of the MPA, high density blocking by established individuals might be considered as new recruits have to compete with a comparatively dense population for resources. Consequently, they might not easily settle or are eradicated without contributing to the gene pool (Waters et al. 2013). A large proportion of residents in the MPA could enforce this. According to recapture records of our 139 sampled animals with tags from 2010 to 2020, 43 were not captured in another year (24 of these at the end of our sampling period). The other 62 animals were captured ≥2 yr in a row, 28 ≥3 yr in a row, and 17 ≥4 yr in a row (https://skatespotter.sams.ac.uk/). While these data are biased in terms of time of tagging and sampling/catching effort due to the method of collection (sea angling at preferred fishing marks) in different areas of the MPA, it indicates many of our samples include individuals exhibiting site attachment, possibly hindering settlement of immigrants. However, while most of the 12 animals with 1 of the rarer haplotypes have been captured only in 1 or 2 yr, the database revealed that 2 animals with rare haplotypes were recaptured over 4 yr (Di000446: 2016, 2018 and 2019; Di000318: 2017, 2019 and 2020) and 1 for 5 yr in a row (Di000414: 2016–2020), possibly an indication that these (immature) skates might be resident in or regularly returning to the MPA to eventually reproduce there.

4.3. Suitability of a mitochondrial DNA marker

Although our estimates of mitogenomic diversity in the MPA are not encouraging, it is appropriate to address whether the results may be an artefact of marker choice, masking potential divergence by not identifying more diverse haplotypes. Our alignment of 4 mitochondrial genomes showed scattered polymorphic nucleotide positions (Fig. S1), indicating that a comparison of full mitochondrial genomes might provide better resolution. However, the overall pattern of diversity and connectivity is likely appropriate, and a mitochondrial marker offers accessible and cost-effective population genetic analysis, which has been identified to be of importance when implementing long-term genetic monitoring to protect bio-

diversity (Hoban et al. 2021). It also offers the opportunity to use egg cases and mucus as additional sources of DNA, though their amplification has been less consistent, probably due DNA degradation (Lieber et al. 2013).

Despite the difference in mitogenomic variability between the MPA and other sites, animals residing in the MPA may exhibit more variability in their nuclear genomes. Although providing a rapid assessment of diversity and population structure, mitochondrial DNA may not represent patterns in the nuclear genome of animals since it is an organelle genome and maternally inherited. Therefore, nuclear markers (e.g. Single Nucleotide Polymorphisms) would complement this study, allowing a more comprehensive investigation of genetic diversity, connectivity and adaptive potential.

4.4. Implications for conservation management

This study shows the importance of linking genetic assessments to conservation management. Conserving genetic diversity in the wild not only decreases risks of extirpation, it also allows adaptation to changing environmental conditions (Hollingsworth et al. 2020). An MPA can be useful to protect representative genetic diversity of a species or unique adaptive variants (Beger et al. 2014). Conservation measures targeting the flapper skate, such as the landing ban and the protected areas, were not designed with conservation of genetic diversity in mind, which is why its value in protecting flapper skate diversity was estimated here.

Our analysis revealed that the Loch Sunart to the Sound of Jura MPA, designated to conserve the flapper skate, protects only about half of the species' mitogenomic diversity and shows indications of a relatively small population size, harboring a relatively small subset of the total haplotypic diversity. It seems genetically relatively isolated from the other locations, lacking signs of incoming or outgoing gene flow, a fragmentation possibly caused by a history of overfishing and behavioral idiosyncrasies. As a management measure, it currently does not appear sufficient to protect the genetic diversity of the flapper skate or to provide surplus individuals to other populations, so it must be considered a less effective MPA than is desirable. The rare haplotypes found in more recent years in the MPA may indicate more immigration from outside, which could be due to increasing population sizes and therefore, a higher number of migrant flapper skate in the Northeast Atlantic. In fact, there has been a marked rise in abundance of animals identified as part of the common skate complex in trawl surveys in the last 10–20 yr, providing evidence of regional recovery since the reduction in demersal fishing from 1990 onwards and the ban on landings (Rindorf et al. 2020, ICES 2021). A re-established connection might be of importance to the animals found inside the MPA, as their low mitogenomic diversity might place them at risk. Given the extent of movement indicated from tag-recapture experiments and electronic tagging (Little 1995, 1997, Neat et al. 2015, Lavender et al. 2021), re-colonisation rates may be slow. Therefore, recovery of diversity in the MPA could be a protracted process.

Fortunately, signs of recovery in other areas indicate that the landing ban has been successful in allowing populations throughout British waters to increase and should maintain the haplotype diversity shown in areas outside the MPA. If skate numbers do not continue to increase, additional protection to the landing ban outside the MPA might become necessary to protect more of the species' genetic diversity. These management measures could include further protected areas or measures to reduce bycatch, such as the removal of tickler chains from bottom trawl nets (Kynoch et al. 2015). Such management measures may be appropriate in areas such as North Scotland, which shows high haplotype and nucleotide diversity and is known to harbour egg laying sites of flapper skate (Phillips et al. 2021).

Continuous genetic monitoring of mitogenomic diversity should be conducted to learn more about the genetic connectivity between the MPA and other populations. This monitoring would allow observation of any potential increase in diversity of the MPA through incoming migrants, as well as an increasing abundance of haplotypes VIII or X outside the MPA if MPA individuals do indeed emigrate. Based on observed levels of bidirectional gene flow, we would be able to learn if the MPA more likely poses a source or sink population in the context of the wider metapopulation.

A more comprehensive investigation using nuclear genomic techniques would be useful to confirm if the mitogenomic composition of the MPA is represented by a similarly distinct pattern of nuclear diversity. Using nuclear markers, an analysis of inbreeding, relatedness and contemporary connectivity would further reveal if the assumed small founding population is putting the contemporary MPA population at risk. This would also provide further information on the efficacy of the MPA as a conservation management tool.

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