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Low mitochondrial genetic diversity in the Indian Ocean humpback dolphin *Sousa plumbea* in South African waters

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ABSTRACT: The Indian Ocean humpback dolphin *Sousa plumbea* has been described as South Africa's most endangered marine mammal due to its low abundance, reliance on coastal habitats with increasing anthropogenic threats and high rates of mortality from bycatch in bather protection nets (BPNs). Although the species has been well studied in South Africa, only a single study has examined its molecular ecology to date, and its population structure remains poorly understood. However, understanding population structure is vital for the conservation and management of a species. To address these research gaps for *S. plumbea* in South African waters, we analysed the mitochondrial D-loop of 157 museum skin and tooth samples collected between 1963 and 2017 from across the species' geographic range in South Africa. Our data show that the humpback dolphin has extremely low mitochondrial diversity (haplotype diversity, $H_D = 0.47$; nucleotide diversity, $\pi = 0.2\%$) with only 3 haplotypes identified, which is comparable to the Critically Endangered Māui dolphin *Cephalorhynchus hectori maui* and the Critically Endangered Mekong population of Irrawaddy dolphin *Orcaella brevirostris*. Mitochondrial genetic diversity has not changed significantly in the last 50 yr, despite the high levels of bycatch in BPNs over this time period. Furthermore, we found no evidence of differentiation between dolphins from the KwaZulu-Natal Coast and the Cape South Coast (Western Cape and Eastern Cape). The extremely low mitochondrial diversity we found adds to the growing body of evidence that the humpback dolphin is becoming increasingly vulnerable and that urgent conservation efforts are required for the survival of the species.

KEY WORDS: Conservation · Population structure · Mitochondrial DNA · mtDNA · Genetics · Cetaceans · *Sousa plumbea*

1. INTRODUCTION

The Indian Ocean humpback dolphin *Sousa plumbea* (hereafter humpback dolphin) is the largest of 4 dolphin species in the genus *Sousa* and was officially recognised as a separate species in 2014 based on morphological, molecular and biogeographical evidence (Jefferson & Rosenbaum 2014). Humpback dol-

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phins inhabit shallow coastal waters from False Bay in South Africa to the Bay of Bengal in south-east Asia (Mendez et al. 2013, Jefferson & Rosenbaum 2014). In South Africa, the species is found near rocky reefs and estuaries between False Bay (34° 11′ 37″ S, 18° 39′ 07″ E) and Kosi Bay (26° 53′ 41″ S, 32° 53′ 02″ E; Keith et al. 2002, Plön et al. 2016), in shallow waters to a maximum depth of 25 m (Durham 1994). Certain areas along the coastline, such as Richard's Bay, Algoa Bay and Mossel Bay, have high densities of humpback dolphins (Karczmarski 2000, Plön et al. 2016) due to favourable habitat conditions and good feeding opportunities. Whether this represents a patchy distribution or simply a lack of research effort between 'hotspots' remains unknown (Karczmarski 2000, Plön et al. 2016).

The Indian Ocean humpback dolphin was classified as 'endangered' in South African waters according to a local Red List assessment conducted in 2014 (Plön et al. 2016) and globally Endangered by the IUCN in 2017 (Braulik et al. 2015, 2017). However, more recent assessments suggest that the South African humpback dolphin population likely comprises fewer than 500 individuals (Vermeulen et al. 2017). In addition to a decline in abundance at some study sites (James et al. 2015, Vermeulen et al. 2017), average group size has decreased from 6 or 7 individuals to 3 individuals, and changes in behaviour and movement patterns, such as increased time spent travelling, have been observed in Algoa Bay (Koper et al. 2016, Bouveroux et al. 2018, 2019). These changes are likely a result of increasing anthropogenic threats (Plön et al. 2016) and suggest that a shift in Red List threat classification may be required for this population.

The biggest direct threat to humpback dolphins in South Africa is incidental bycatch in bather protection nets (BPNs) along the KwaZulu-Natal Coast (Cockcroft 1990, Atkins et al. 2013, Plön et al. 2015). While the gillnets used are designed to entrap and kill sharks, other marine animals including loggerhead turtles *Caretta caretta*, bottlenose dolphins *Tursiops aduncus* and humpback dolphins are incidentally caught in these nets (Cliff & Dudley 2011). Studies carried out between 1980 and 2012 estimate that BPNs in KwaZulu-Natal were responsible for an unsustainable mortality rate of 5−10 % of the dolphin population annually (Cockcroft 1990, Atkins et al. 2013). As of 2019, some of the gillnets have been replaced by drumlines, a safer alternative for marine animals (www.shark.co.za; accessed 21 May 2020), but it is not yet clear if this approach has been effective in reducing entanglement rates.

Humpback dolphins demonstrate high site fidelity to certain areas, but periods of residency are brief, and the species has been known to travel up to 500 km between sites across their South African distribution range (Atkins et al. 2016, Vermeulen et al. 2017). The rocky, exposed coastlines along the Eastern Cape east of Algoa Bay may present an unfavourable habitat for the species since they prefer nearshore reefs and estuaries due to the higher density of prey species (Karczmarski 2000). The exposed coastlines have therefore been suggested to form a distribution break (Smith-Goodwin 1997, James et al. 2015, Vermeulen et al. 2017), which may isolate populations that are already small in size. If this is the case, we expect that individuals found west of Algoa Bay would form a subpopulation (which we refer to as the Cape South Coast subpopulation), and individuals found east of Mzamba Beach would form a separate subpopulation (which we refer to as the KwaZulu-Natal Coast subpopulation); see Fig. 1 for the delineation of these putative subpopulations. Under this assumption, humpback dolphins would not be found along the coastline between Algoa Bay and Mzamba Beach unless travelling between the 2 putative subpopulations. Understanding the population structure of this endangered species is necessary for the identification of appropriate conservation management units (Plön et al. 2016).

The KwaZulu-Natal Sharks Board (KZNSB) has been responsible for the implementation and management of BPNs off the KwaZulu-Natal coastline since the mid-1970s. In a long-standing agreement between KZNSB and the Port Elizabeth Museum (PEM), data and samples collected from the dolphins incidentally caught in the BPNs have been accessioned to the Graham Ross marine mammal collection at the PEM. Such museum collections of historical cetacean material offer a source of DNA from which changes in population identity and genetic diversity over time can be monitored (Wandeler et al. 2007, Foote et al. 2012, Meager & Sumpton 2016, Plön et al. 2019). Our data set offers a unique opportunity to investigate the impact of BPNs on the genetic diversity of the humpback dolphin between 1979 and 2017.

The aim of our study was to investigate the population structure and genetic diversity of the humpback dolphin in South African waters over the past 54 yr, to determine if there is any evidence to support a distribution split between the Cape South Coast and the KwaZulu-Natal Coast and whether the pressures faced by humpback dolphins in KwaZulu-Natal from incidental bycatch in BPNs resulted in changes in genetic diversity between 1979 and 2017.

2. MATERIALS AND METHODS

2.1. Sample collection and storage

A total of 157 samples (37 skin, 120 teeth), each originating from a different dolphin, were obtained from 2 museum collections in South Africa: the Graham Ross marine mammal collection at the PEM/Bayworld and the Iziko South African Museum in Cape Town (see Table S1 in the Supplement at www.int-res.com/articles/suppl/n046 p091_supp.pdf for sample details). These samples originated from humpback dolphins caught in BPNs or from beach strandings between 1963 and 2017. Of these 157 samples, 143 originated from KwaZulu-Natal and the North-Eastern Cape (assigned to the putative KwaZulu-Natal Coast subpopulation) and 14 originated from the Western Cape and Eastern Cape (assigned to the putative Cape South Coast subpopulation) (Fig. 1). Skin samples were stored in 70% (v/v) ethanol at room temperature, and tooth samples were stored dry at room temperature.

2.2. DNA extractions from teeth and tissue

Tooth samples were processed using one of 2 protocols. Teeth with open pulp cavities from juvenile dolphins were processed using a demineralisation protocol adapted from Pimper et al. (2009). Following the protein digestion step, samples were incubated for an additional 1 h at 50°C and then centrifuged at 1530 × g for 5 min. DNA was then extracted from 400 µl of the supernatant using the DNeasy Blood & Tissue Kit (Qiagen), following the Qiagen user-developed protocol DY01 (purification of total DNA from compact animal bone using the DNeasy Blood & Tissue Kit). Teeth with occluded pulp cavities from older dolphins were drilled and DNA extracted from the resulting tooth powder as previously described (Plön et al. 2019).

Mummified gum tissue was present on a small number of tooth samples (15 of 120) and used in place of tooth powder for a greater DNA yield. Mummified gum tissue was removed from the tooth with a sterile scalpel blade. Samples of 25–30 mg of mummified gum tissue or skin tissue were incubated at 55°C for 18–24 h in Buffer ATL (Qiagen) and Proteinase-K (0.2 mg ml^{-1}; New England Biolabs) with shaking. DNA was then extracted using the DNeasy Blood & Tissue Kit according to the manufacturer's instructions.

2.3. DNA amplification

A 550 bp region from the 5′ end of the mitochondrial D-loop was amplified from DNA samples using primers dlp1.5 and dlp5 (Dalebout et al. 1998). For DNA samples for which it was not possible to amplify the 550 bp D-loop product, a 400 bp fragment was amplified using primers dlp1.5 and dlp4 (Baker et al. 1998). Approximately 60–200 ng of DNA template was added to each 50 µl reaction containing a final concentration of 5 mM MgCl$_2$, 0.4 µM of each primer and 1× KAPA Taq ReadyMix + dye (Kapa Biosystems). The thermal profile was set up using the SimpliAmp Thermal Cycler (Applied Biosystems) with the following steps: an initial denaturation step at 95°C for 3 min, 30 cycles (tissue samples) or 35 cycles (tooth samples) of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 60 s followed by a final extension step at 72°C for 2 min.

Amplicons were visualised on a 1% (w/v) agarose gel, and PCR products were purified using the Wizard SV Gel & PCR Clean-Up system (Promega). Purified PCR products were sequenced with the 5′ primer dlp1.5 (all samples) and 3′ primer dlp4 (104 tooth samples) at Stellenbosch University Central Analytical Facilities or LGC Genomics. Haplotype sequences have been deposited in GenBank (see Table 1 for accession numbers).

2.4. Quality control procedures for working with historical samples

To minimize the contamination risks associated with DNA from historical samples, dedicated labora-
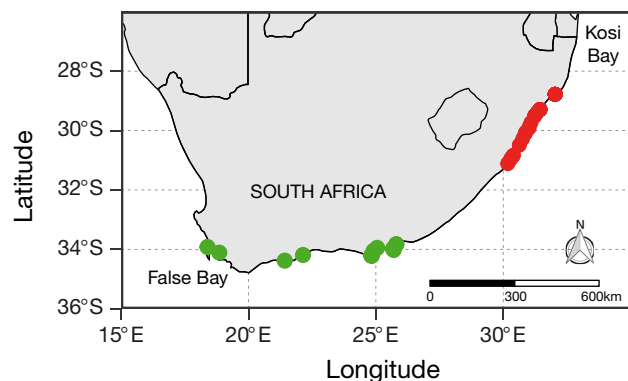

Fig. 1. Distribution of sampling locations along the South African Coast. Each circle represents a sampling location and could represent one or more samples. Red dots: sampling sites from the KwaZulu-Natal Coast; green dots: sampling sites from the Cape South Coast. False Bay and Kosi Bay represent the range ends of the species' distribution in South Africa

tories, equipment and reagents were used for (1) drilling/sample preparation, (2) DNA extractions and (3) pre-PCR setup, respectively. None of the laboratories in the department had ever been used to process samples from any marine mammal, so extraction blanks were not performed. However, independent replicate DNA extractions were performed on several samples, and they all yielded the same DNA sequences. All workbenches were decontaminated with a 10% (v/v) bleach solution followed by 70% (v/v) ethanol prior to any work with the samples or DNA. Before drilling and between samples, the drill and drill bits were also decontaminated with 10% (v/v) bleach solution. A new pair of gloves and a fresh drill bit were used for each sample; used drill bits were soaked in 70% (v/v) ethanol for 1 h and decontaminated with 10% (v/v) bleach prior to reuse. A maximum of 6 samples were drilled or prepared at a time to prevent cross-contamination. Teeth and gum tissue on teeth were lightly sanded to remove exogenous DNA and briefly wiped down with 70% ethanol (v/v) to ensure removal of any residue. A no-template control (NTC) was included in all PCRs performed, and DNA from a single tissue sample was used as a positive control in all PCRs throughout the study. Amplified products were only purified and sequenced from experiments where there was no amplification in the NTC. Forward and reverse sequences were aligned to ensure that replicate samples produced identical sequencing results for the same individual. Sequences were only included if there was minimal background noise and the length of good quality base calling encompassed the length of the consensus sequence.

2.5. Data analyses

DNA sequences were reviewed using BioEdit (Hall 1999), and poor-quality base calls were manually edited. Sequences were then aligned using the ClustalW function. Relationships among haplotypes were investigated using parsimony median-joining networks (Bandelt et al. 1999) in the program POPART (Leigh & Bryant 2015). MEGA-X (Tamura et al. 2011) indicated that the best-fitting nucleotide substitution model was Tamura, 1992 (T92) with no gamma correction. The nucleotide substitution model was used to calculate Φ_{ST}, an F-statistic. F-statistics considered were F_{ST} which is a measure of population differentiation due to genetic structure, with an F_{ST} value of 0 indicating no genetic differentiation between 2 populations, and Φ_{ST} which is the same measure but with

the nucleotide substitution model considered in the calculation. Arlequin v.3.5.2.2 (Excoffier & Lischer 2010) was used to calculate measures of genetic diversity (haplotype diversity [H_D] and nucleotide diversity [π]) and F-statistics (F_{ST} and Φ_{ST}); negative values of F_{ST} and Φ_{ST} are presented as zero. Coalescent simulations were calculated from 10 000 replicates in DNASP v.5.10 (Librado & Rozas 2009) to estimate the 95% CIs for H_D and π. DNASP v.5.10 uses the average number of nucleotide differences per locus (k) to calculate the CIs for nucleotide diversity. To adjust these intervals for π (which is a per-base measure), interval limits were divided by the length of the locus (322 bp).

To investigate whether there is a distribution break along the South African coastline, samples were divided into 2 putative subpopulations, namely the KwaZulu-Natal Coast subpopulation (n = 143 samples) and the Cape South Coast subpopulation (n = 14 samples) (Fig. 1). Pairwise genetic differentiation analyses using F-statistics were performed between the Cape South Coast and the KwaZulu-Natal Coast samples to investigate patterns in population genetic structure. This comparison was restricted to samples obtained up to 2005 (n = 118), as this was the last year that samples were collected from the Cape South Coast and there were far more samples from the KwaZulu-Natal Coast which could bias the comparison.

Most of the samples (88%) were obtained from animals incidentally caught in BPNs off the KwaZulu-Natal Coast; thus, we used this subset of samples to investigate temporal trends in genetic diversity and identify any changes in population structure over time (for example, to determine whether bycatch in BPNs has caused a decrease in genetic diversity or population structure has changed due to an influx of immigrants, either of which could change the observed haplotype frequencies). Samples were first divided into overlapping 4 yr 'bins', and F-statistics were calculated for adjacent bins within a sliding window analysis (Table S2). This fine-scale temporal analysis indicated no significant shift in genetic structuring over time; therefore, subsequent pairwise genetic differentiation analyses using F-statistics were conducted on non-overlapping 8 yr bins. These provided good sample sizes for each bin while allowing us to assess genetic diversity and conduct temporal comparisons over the sample collection period. To investigate changes in genetic diversity more sensitively, we used the 'genetic_diversity_diffs' method (Alexander et al. 2016) on the non-overlapping 8 yr bins in R v.4.02; this script calculates the differ-

ences in H_D and π between groups using 10 000 permutations.

The estimated mitochondrial diversity of 13 threatened coastal or riverine dolphin species or populations was compiled from the published literature to evaluate how the levels of genetic diversity observed for the humpback dolphin in South African waters in this study compared to other endangered delphinids. These species encompassed the full range of IUCN Red List threat levels (IUCN 2020). In addition, 2 highly threatened delphinid populations were included: the pantropical spotted dolphin *Stenella attenuata* in Hawaii and the spinner dolphin *S. longirostris* in Hawaii and Brazil, although the global status of these 2 species is listed as Least Concern (Braulik & Reeves 2018, Kiszka & Braulik 2018).

3. RESULTS

3.1. Genetic diversity and population structure

The mitochondrial control region was successfully amplified from all 157 samples (143 from the KwaZulu-Natal Coast and 14 from the Cape South Coast), and after editing, a sequence alignment of 322 bp was created. This alignment identified 3 mitochondrial haplotypes (SA1–SA3) based on 3 variable sites (Table 1). All variable sites were C/T transitions and all 3 haplotypes have previously been reported (Frère et al. 2008, Mendez et al. 2013).

A relatively high level of heteroplasmy was observed among the sequences, with 11 samples (6.9% of all samples; 9 from the KwaZulu-Natal Coast and 2 from the Cape South Coast) showing clear double peaks at the same nucleotide position (268Y) on the sequence chromatograms. To verify the occurrence of heteroplasmy, DNA was re-extracted from 3 samples, the control region was re-amplified and sequenced and the same double peak was observed in the resulting chromatograms. This indicated that the observed heteroplasmy was not due to contamination during the extraction/PCR steps or mutations introduced through PCR or base miscall during sequencing. Since a single allele at this position could not be determined, and there were instances of both alleles in homoplasmic sequences, both possible alleles (268C and 268T) and their respective haplotypes (SA1 and SA2) were included for all 11 samples in subsequent analyses, increasing the total number of control region sequences (shown in Table 1) to 168 sequences (152 from the KwaZulu-Natal Coast, 16 from the Cape South Coast).

All 3 of the South African haplotypes (SA1, SA2 and SA3) were observed in samples from both the KwaZulu-Natal Coast and the Cape South Coast (Fig. 2). The 2 putative subpopulations revealed similar levels of mitochondrial genetic diversity, with the Cape South Coast subpopulation having an H_D of 0.43 and a π of 0.22%, and the KwaZulu-Natal Coast subpopulation having an H_D of 0.44 and a π of 0.18%. No evidence of genetic differentiation between these 2 regions was found (F_{ST} = 0.0018, p = 0.33; Φ_{ST} = 0, p = 0.66).

3.2. Temporal analysis of dolphins from the KwaZulu-Natal Coast

Temporal analyses were conducted on non-overlapping 8 yr bins over the entire collection period. Levels of haplotype and nucleotide diversity did not vary strongly across the 8 yr time periods (Table 2). While the most recent period (2013–2017) showed low diversity, values were similar to diversity levels from previous time periods, with overlapping 95% CIs, and no significant reduction in diversity was seen. The relative abundance of each of the 3 haplotypes fluctuated over time, with the most abundant haplotype, SA1, decreasing in frequency, accompanied by a concurrent increase in the frequency of SA2 (Fig. 3). Pairwise F_{ST} comparisons among the 8 yr periods found animals from the first period (1979–1986) to be genetically differentiated from all subsequent periods (Table 3). A similar pattern was found with pairwise Φ_{ST} comparisons, except that the comparison between 1979–1986 and 2013–2017 was not significant, perhaps due to the small sample size from the 2013–2017 period. Haplotype diversity differences among the 8 yr time periods found animals from the first period (1979–1986) to have a significantly

Table 1. Positions of the 3 variable sites within the 322 bp fragment of the mitochondrial D-loop, which define the 3 South African haplotypes of humpback dolphins found in this study (SA1–SA3) for all 168 control region sequences. Dots: no variation from the top sequence (SA1); asterisk: nucleotide position where heteroplasmy was observed

	Frequency	Nucleotide position 213	267	268*	GenBank accession number
SA1	114	T	T	C	MZ493184
SA2	44	•	•	T	MZ493185
SA3	10	C	C	•	MZ493186

higher haplotype diversity compared to animals caught between 2004 and 2012 (Table 4); however, no difference in nucleotide diversity was seen between any of the time periods.

3.3. Comparative genetic diversity of threatened delphinids

The estimated mitochondrial diversity of 13 threatened coastal or riverine dolphin species or populations was compiled (Table 5) to compare the mitochondrial diversity calculated in this study with other threatened delphinids from similar environments. Our study provided a significantly (8×) larger sample size for humpback dolphins compared to previous

work but showed haplotype and nucleotide diversity levels ($H_D = 0.47$; $\pi = 0.2\%$) that were similar to those previously reported for this species in South African waters ($H_D = 0.58$–0.65; $\pi = 0.21$–0.25%; Frère et al. 2008, Mendez et al. 2013; Table 4). This finding indicates that additional sampling effort is unlikely to reveal further unsampled mitochondrial genetic diversity in this population.

The other species and populations with levels of mitochondrial diversity most similar to the humpback dolphin in this study are the Vulnerable Australian humpback dolphin *Sousa sahulensis*, 2 populations of spinner dolphins *Stenella longirostris* in Hawaii and Brazil, a population of pantropical spotted dolphins *Stenella attenuata* from Hawaii and the Endangered Hector's dolphin *Cephalorhynchus hectori.* Although the spinner dolphin and pantropical spotted dolphin are classified as Least Concern at the species level under the IUCN Red List criteria

Fig. 2. Median-joining haplotype network for all control region sequences (n = 168). Circle size corresponds to the frequency of the haplotype. Number of intersecting lines between haplotypes corresponds to the number of mutations separating each haplotype
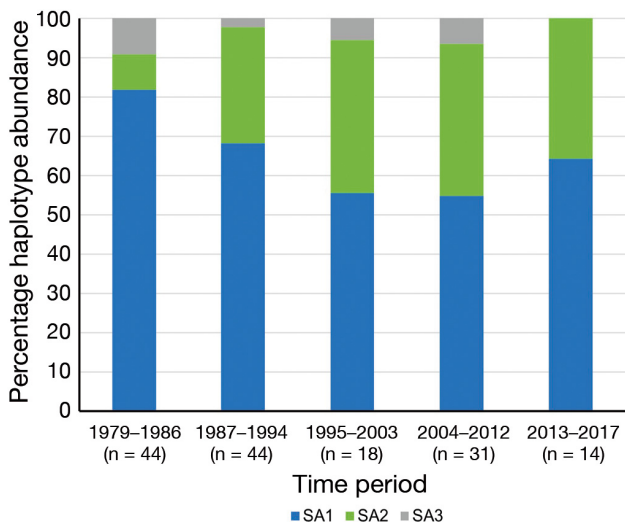

Fig. 3. Percentage haplotype abundance (SA1–SA3) in humpback dolphins from the KwaZulu-Natal Coast only (n = 151 control region sequences), grouped in 8 yr time periods. n: number of control region sequences in each time period

Table 2. Haplotype abundance and measures of genetic diversity of humpback dolphins from the KwaZulu-Natal Coast between 1979 and 2017 (n = 151 control region sequences). n: number of control region sequences; H_D: haplotype diversity; π: average per site nucleotide diversity (as a percentage)

	n	Haplotype abundance			Measures of genetic diversity			
		SA1	SA2	SA3	H_D	95% CI	π	95% CI
1979–1986	44	36	4	4	0.32	0.13–0.71	0.16	0.06–0.42
1987–1994	44	30	13	1	0.46	0.13–0.71	0.17	0.06–0.42
1995–2003	18	10	7	1	0.57	0.22–0.75	0.23	0.10–0.47
2004–2012	31	17	12	2	0.56	0.18–0.72	0.23	0.06–0.44
2013–2017	14	9	5	0	0.50	0.14–0.54	0.15	0.04–0.17

(Braulik & Reeves 2018, Kiszka & Braulik 2018), individual populations may be considered more vulnerable. The number of haplotypes identified among the humpback dolphins in this study is remarkably low, especially considering the large sample size. The only 2 species reported to have fewer mitochondrial haplotypes are the Critically Endangered Māui dolphin *Ephalorhynchus hectori maui* (n = 1 haplotype) and the Critically Endangered Mekong population of Irrawaddy dolphin *Orcaella brevirostris* (n = 3 haplotypes), and both studies used smaller sample sizes (n = 70 and 42, respectively; Hamner et al. 2012, Krützen et al. 2018).

Table 3. Genetic differentiation of humpback dolphins among 8 yr time periods based on pairwise F_{ST} (bottom diagonal of matrix) and Φ_{ST} (top diagonal of matrix) comparisons, with their corresponding p-values in parentheses. Samples included are from the KwaZulu-Natal Coast only (n = 151 control region sequences). **Bold**: statistically significant (p < 0.05)

	1979–1986 (n = 44)	1987–1994 (n = 44)	1995–2003 (n = 18)	2004–2012 (n = 31)	2013–2017 (n = 14)
1979–1986 (n = 44)	–	**0.057** (0.04)	**0.14** (0.013)	**0.14** (0.003)	0.095 (0.07)
1987–1994 (n = 44)	*0.072* (0.01)	–	0 (0.48)	0.00039 (0.35)	0 (0.81)
1995–2003 (n = 18)	**0.11** (0.01)	0 (0.60)	–	0 (1.00)	0 (0.88)
2004–2012 (n = 31)	**0.11** (0.009)	0 (0.47)	0 (1.00)	–	0 (0.74)
2013–2017 (n = 14)	**0.11** (0.02)	0 (0.78)	0 (1.00)	0 (0.83)	–

Table 4. Genetic diversity differences in humpback dolphins between 8 yr time periods based on 'genetic_diversity_diffs' (Alexander et al. 2016), with haplotype diversity differences between time periods (top diagonal of matrix) and nucleotide diversity differences (as a percentage) between time periods (bottom diagonal of matrix), and their corresponding p-values in parentheses. Samples included are from the KwaZulu-Natal Coast only (n = 151 control region sequences). n: number of control region sequences. **Bold**: statistically significant (p < 0.05)

	1979–1986 (n = 44)	1987–1994 (n = 44)	1995–2003 (n = 18)	2004–2012 (n = 31)	2013–2017 (n = 14)
1979–1986 (n = 44)	–	0.136 (0.15)	0.247 (0.06)	**0.242** (0.03)	0.173 (0.21)
1987–1994 (n = 44)	0.003 (0.96)	–	0.111 (0.37)	0.106 (0.32)	0.037 (0.79)
1995–2003 (n = 18)	0.068 (0.38)	0.065 (0.41)	–	0.005 (0.97)	0.074 (0.62)
2004–2012 (n = 31)	0.072 (0.24)	0.069 (0.28)	0.004 (0.96)	–	0.069 (0.63)
2013–2017 (n = 14)	0.004 (0.96)	0.007 (0.93)	0.072 (0.46)	0.076 (0.40)	–

4. DISCUSSION

The humpback dolphin in South African waters exhibits very low levels of genetic diversity, with only 3 mitochondrial haplotypes identified in 157 individuals. This level of genetic diversity is comparable to Critically Endangered coastal and estuarine dolphin species, such as the Māui dolphin *Cephalorhynchus hectori maui* (Hamner et al. 2012) and the Mekong population of Irrawaddy dolphin *Orcaella brevirostris* (Krützen et al. 2018). As highlighted by the Red List assessment of the humpback dolphin in 2014 (Plön et al. 2016), genetic studies on the species in South Africa are lacking, and the findings of this study will be extremely beneficial to our understanding of the species, which in turn will help us improve conservation measures. Additionally, information regarding its genetic population structure is necessary to motivate changes in conservation management. South Africa is the western end of the distribution range for the Indian Ocean humpback dolphin *Sousa plumbea*, and our results suggest the population using these waters is panmictic. Coupled with recent estimates of low abundance (fewer than 500 animals; Vermeulen et al. 2017), low levels of mitochondrial diversity provide crucial information about a highly vulnerable population and highlight the urgent need for increased conservation management (Plön et al. 2016).

4.1. Low genetic diversity in *S. plumbea*

Our study identified similarly low genetic diversity in the South African Indian Ocean humpback dolphin population compared to that obtained from previous studies, despite our substantially larger sample size (approximately 8× larger). Frère et al. (2008) identified 4 South African haplotypes among 23 individuals for a 335 bp fragment of the mitochondrial control region. Three are identical to the haplotypes identified in this study, while the fourth, present in only a single

Table 5. Estimated levels of mitochondrial diversity for threatened coastal and riverine dolphin species and populations. Populations and species with a haplotype diversity (H_D) < 0.5 are shaded in grey. π: average per site nucleotide diversity (as a percentage); KZN: KwaZulu-Natal; SA: South Africa

Species	IUCN status	Sampling location	Sample size	No. of haplotypes	H_D	π	Control region length (bp)	Sampling time period	Reference
Australian humpback dolphin *Sousa sahulensis*	Vulnerable	Queensland, Australia	159	8	0.52	0.7	500	2006–2011	Parra et al. (2018)
Humpback dolphin *Sousa* spp.	Endangered	Bangladesh	16	9	0.88	2	380		Amaral et al. (2017)
Indian Ocean humpback dolphin *Sousa plumbea*	Endangered	South Africa	157[a]	3	0.47	0.2	322	1979–2017	Present study
		South Africa (KZN)	20	3	0.65	0.23	456		Mendez et al. (2013)
		South Africa (KZN)	23	4	0.58	0.21	335	1987–1997	Frère et al. (2008)
		South East Africa (SA, Mozambique, Tanzania and Madagascar)	39	8	0.82	4	456		Mendez et al. (2013)
Indo-Pacific humpback dolphin *Sousa chinensis*	Near Threatened	Hong Kong	19	4	0.61	0.67	338	1993–1996	Frère et al. (2008)
		China	91	8	0.34	2	456		Mendez et al. (2013)
Indo-Pacific bottlenose dolphin *Tursiops aduncus*	Near Threatened	South Africa (KZN)	50	6	0.63	0.39	599	1994–2000	Natoli et al. (2008)
		Bangladesh	17	7	0.7	0.9	380		Amaral et al. (2017)
Heaviside's dolphin *Cephalorhynchus heavisidii*	Near Threatened	South Africa	395	51	0.93	0.65	580	2009–2012	Gopal et al. (2019)
Hector's dolphin *Cephalorhynchus hectori hectori*	Endangered	New Zealand (North Island)	13	3	0.41	0.44	206	1870–1987	Pichler & Baker (2000)
		New Zealand (South Island)	36	9	0.65	0.84	206	1870–1987	
		New Zealand (South Island)	46	5	0.35	0.3	206	1988–1998	Hammer et al. (2012)
		New Zealand	388	21	0.85	0.79	650	1988–2007	Hammer et al. (2012)
Māui dolphin *Cephalorhynchus hectori maui*	Critically Endangered	New Zealand (North Island)	70	1	0	0	650	1988–2007	
Franciscana dolphin *Pontoporia blainvillei*	Vulnerable	Argentina and Brazil	44	11	0.75	1.2–1.3	434	1998–2010	Negri et al. (2016)
Guiana dolphin *Sotalia guianensis*	Near Threatened	South America	91	27	0.52	0.39	500		Caballero et al. (2018)
Irrawaddy dolphin *Orcaella brevirostris*	Critically Endangered	Cambodia (Kratie)	42	3	0.26	0.1	412	2000–2009	Krützen et al. (2018)
		Cambodia (Stung Treng)	12	2	0.17	0.04	412		
Pantropical spotted dolphin *Stenella attenuata*	Least Concern	Hawaii	101	10	0.45	0.5	571	2002–2003	Courbis et al. (2014)
Spinner dolphin *Stenella longirostris*	Least Concern	Hawaii (Midway Atoll)	119	4	0.41	0.18	417	2001–2005	Andrews et al. (2010)
		Brazil	162	11	0.38	0.6	413	2004–2012	Faria et al. (2020)

individual, differs from SA2 by a single nucleotide. This variable site was outside the 322 bp region analysed for all 157 samples, but it was covered in the 104 samples that were sequenced using primer dlp4, and this haplotype was not detected. Mendez et al. (2013) also identified the same 3 haplotypes among 20 samples from South Africa; however, it is highly likely that all those samples were also included in the current study, as they originated mostly from the same sample collection (BPNs). Given that the same haplotypes have been identified in all 3 studies conducted on the South African population and that the present study covered a much larger number of individuals, it appears that the full extent of mitochondrial control region genetic diversity in this population has been captured.

The genetic diversity of the humpback dolphin in South African waters is comparable to other threatened populations of coastal and riverine dolphin species (Table 4); however, the total number of haplotypes identified for the sample size was comparatively low. This could be due to natural historical events (Attard et al. 2015, Morin et al. 2018, Parra et al. 2018), recent anthropogenic impacts (Pichler & Baker 2000, Pimper et al. 2010, Hamner et al. 2012) or a combination of factors; for example, due to being at the edge of a distribution range, which may have caused genetic isolation (Hamner et al. 2012, Attard et al. 2015, Faria et al. 2020) or directly reduced genetic diversity.

The demographic history of the humpback dolphin is not well understood; thus, one cannot exclude a natural historical event from being an underlying factor influencing the low genetic diversity of this population. Smith-Goodwin (1997) suggested, for example, that the low genetic diversity observed in humpback dolphins in South Africa could be the result of a genetic bottleneck during the Quaternary ice ages, with low sea levels resulting in a population decline. Parra et al. (2018) demonstrated that a population bottleneck during the late Holocene period is likely responsible for the low levels of mitochondrial and nuclear genetic diversity observed in the current Australian humpback dolphin *S. sahulensis* populations. Further genetic and environmental investigation into past demographic changes influencing the humpback dolphin in South Africa would be useful to evaluate whether there is a historical contribution to the low genetic diversity observed.

Considering the possible impact of anthropogenic disturbance on genetic diversity, coastal marine mammal populations are at a higher risk of declining due to their close proximity to human disturbances (Karczmarski 2000, Allen et al. 2012, Avila et al. 2018). Coastal dolphins that display high site fidelity are also at extremely high risk of habitat fragmentation and degradation due to their dependence on a small number of productive habitats (Karczmarski et al. 1998, Crain et al. 2009). Anthropogenic impacts, such as bycatch in BPNs (Cockcroft 1990, Atkins et al. 2013, Plön et al. 2015), pollution (Karczmarski et al. 1998, Gui et al. 2016, Aznar-Alemany et al. 2019) and boat traffic (Karczmarski et al. 1998, Koper et al. 2016) are some of the main threats to the humpback dolphin, both locally and globally (Plön et al. 2016). Additionally, the population is at the western edge of the species' range, a characteristic often associated with lower genetic variability and increased genetic isolation, as there are fewer nearby populations (Sagarin & Gaines 2002, Sexton et al. 2009, Nykänen et al. 2019). The level of genetic connectivity between this population and Mozambique is also unknown. Previous studies have suggested that movement of individuals between South Africa and Mozambique might be possible (Guissamulo & Cockcroft 2004, Mendez et al. 2011); however, there is insufficient genetic evidence to determine whether there is gene flow between the 2 regions. Additional investigation into the movement patterns of *S. plumbea* between these 2 regions and additional samples for genetic analysis from Mozambican waters are necessary to determine whether the South African population is genetically isolated.

4.2. A single population of humpback dolphins in South African waters?

Based on a large sample size from one of the 2 compared subpopulations, the KwaZulu-Natal Coast, our work finds no evidence of female-mediated population structure between the Cape South Coast and the KwaZulu-Natal Coast, suggesting that South African humpback dolphins form a single population. mtDNA can be a powerful marker for detecting female-mediated population structure, which is a common feature of cetacean populations (Hoelzel 2009, Pimper et al. 2010). However, we note that our sample sizes were highly imbalanced between the 2 regions, and additional sampling from the Cape South Coast is necessary to improve our capacity to detect population structure. Furthermore, Vermeulen et al. (2017) found no evidence for dolphin movement between the Cape South Coast and Richards Bay based on photo-identification data, suggesting separate populations may exist along the South African coast. Given the

possibility that fragmentation of this population is happening rapidly due to myriad coastal impacts, rapidly evolving markers, such as microsatellites, should be employed in future studies to examine whether populations have become fragmented more recently. Microsatellites are more difficult to amplify from bone or tooth samples due to DNA degradation (Morin et al. 2007, Allentoft et al. 2011, Foote et al. 2012) but would provide valuable insights (e.g. Nichols et al. 2007). Preliminary work by Smith-Goodwin (1997) suggested there may be microsatellite-mediated population structuring between the Cape South Coast and the KwaZulu-Natal Coast, albeit based on a small sample size, further supporting the idea that there may be local fragmentation. Both Smith-Goodwin (1997) and Vermeulen et al. (2017) examined animals beyond the edge of our sampling range; thus, we also recommend further sample collection through biopsies north of Durban and off the Cape South Coast, as well as analyses of our 157 DNA samples using microsatellite markers, to identify if any subtle population structuring is present across the region. For now, we advise that this population be managed as a single unit, but strongly recommend further genetic research to understand whether there is local fragmentation underway, given the conservation concerns.

4.3. No change in mitochondrial genetic diversity over time

Overall, we found no significant decline in genetic diversity over the time period covered by our study. The same haplotypes (SA1–SA3) are seen between 1979 and 2017, and the changes in relative abundance of each haplotype (and resulting changes in haplotype and nucleotide diversity) are likely due to genetic drift. Genetic drift (the changes in allele frequencies that occur randomly over time due to chance) affects smaller populations more dramatically than larger ones (Willi et al. 2007). This observation is supported by the significant genetic difference observed between samples collected in the earliest time period and subsequent periods, indicating changes in relative haplotype abundance over time. The difference in genetic diversity between the earliest time period and the period between 2004 and 2012 is further supported by the more sensitive 'genetic_diversity_diffs' method (Alexander et al. 2016).

The population of humpback dolphins off the South African coast is highly vulnerable due to their reliance on habitats with increasing human activity and high mortality rates from bycatch in BPNs (Plön et al. 2016).

In the late 1990s, the minimum population size of humpback dolphins in Algoa Bay was estimated at 466 (Karczmarski et al. 1999), while an abundance estimate for the KwaZulu-Natal Coast suggested a population of 81–240 individuals (Durham 1994). However, a more recent estimate suggested a total of 500 animals left in South African waters (Vermeulen et al. 2017), with a total of 247 unique individuals identified from 15 photo-identification catalogues between 2000 and 2016 across South Africa. Over the last 2 decades, there have been fewer sightings of humpback dolphins, group sizes have decreased, predominant behaviours have changed (Koper et al. 2016, Bouveroux et al. 2018) and maximum ranging distances have increased (Vermeulen et al. 2017). These observations are likely due to changes in food availability, anthropogenic disturbances, a possible decline in population numbers (Koper et al. 2016, Bouveroux et al. 2018) or a result of these cumulative impacts (Plön et al. 2021). A decline in population numbers can result in a concurrent decrease in genetic diversity (Frankham 1996, Willi et al. 2007, Banks et al. 2013), but when diversity levels are already low, the capacity to detect further reductions is reduced. The current low levels of mitochondrial genetic diversity may therefore make any future decline in genetic diversity difficult to detect since there is very little diversity left to lose. Our data provide additional evidence that conservation efforts should be prioritized to offer the Indian Ocean humpback dolphin in South African waters the best possible chance of survival.

4.4. Conservation implications

Our data suggest a single population of humpback dolphins exists off the coast of South Africa with extremely low genetic diversity. Although there is no evidence that mitochondrial genetic diversity is declining, such a change may be difficult to detect given the low level of genetic diversity found at all the time periods tested. Our study further highlights the vulnerability of this species and the necessity to protect their habitat, mitigate anthropogenic impacts and increase conservation efforts for their long-term survival. These data contribute important information towards a conservation and management plan for the species inhabiting South African waters.

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