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GENETIC MATCHES OF SOUTHERN RIGHT WHALES IN THE INDIAN SECTOR OF THE ANTARCTIC: A CONTRIBUTION TOWARDS UNDERSTANDING THEIR MOVEMENT AND SITE-FIDELITY

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Abstract

Genetic analyses were conducted to investigate the individual identification (and matching) of southern right whales (*Eubalaena australis*) from samples collected in the austral summer in the Indian Ocean sector of the Antarctic (between 80°–135°E, south of 60°S). The study was conducted to evaluate the utility of this approach for studies on site fidelity and range. In total, 157 skin biopsy samples were collected from free-ranging whales during fourteen summer surveys. The DNA was extracted from each biopsy sample, genotyped at fourteen microsatellite loci, sequenced for 381 nucleotides of the mtDNA control region, and the sex determined by the presence of a Y-chromosome specific locus. Eight matches were detected (four males and four females) using individual matching by multi-locus genotypes supported by mtDNA haplotype and sex determination. Where photographs were available, two matches were confirmed by photo-identification. These eight re-samples show that at least some males and females returned to the same feeding grounds across years. The average longitudinal dispersal ranges, latitudinal dispersal ranges and average direct distances between marks and recaptures were: 13°06′ and 7°15′; 1°23′ and 0°47′; and 361 n.miles and 199 n.miles for males and females, respectively. The time spans ranged from 3–13 years with an average of 6.7 and 7.8 years for males and females, respectively. Sampling and matching occurred in an area where visual surveys showed aggregations of southern right whales associated with high krill concentration. The study confirms the feasibility of the genetic approach, but more definitive inferences on site fidelity and movement ranges will require a large number of biopsy samples genotyped, from both south and north of 60°S.

Key words: southern right whales, Antarctic, genetic tagging, site fidelity, movement, distribution.

Introduction

Baleen whales are important components of the Antarctic marine ecosystem as top predators. Substantial changes in their abundance and distribution, for example due to past whaling or more recently climate change, affects the entire ecosystem. For this reason, systematic monitoring of baleen whale abundance, pattern of movement and distribution in Antarctic waters is important.

A substantial amount of biological and ecological information on large whales and their environment was obtained in the Indo-Pacific sector of the Antarctic (35°E–145°W) by the ‘Japanese Whale

Research Program under Special Permit in the Antarctic' Phases I and II (JARPA, JARPAII) and the subsequent 'New Whale Research Program in the Antarctic' (NEWREP-A). These were conducted annually under the auspices of the Government of Japan between the 1987/88 and 2018/19 austral summer seasons under Article VIII of the International Convention for the Regulation of Whaling (ICRW). Most of the demographic and ecological analyses conducted under these research programs targeted the Antarctic minke whale (*Balaenoptera bonaerensis*) and other Balaenopterid species (see summaries of results in Murase *et al.* (2020) and Fujise and Pastene (2021)). In the context of this paper, southern right whale (*Eubalaena australis*) sightings were recorded and where possible, photo-identification and genetic samples were collected.

A further important source of information from Antarctic waters is the sightings, photo-identification and biopsy data collected at the circumpolar level by the International Whaling Commission-International Decade for Cetacean Research/Southern Ocean Whale and Ecosystem Research (hereafter IWC-IDCR/SOWER) surveys conducted between the 1978/79 and 2009/10 austral summer seasons. Photo-identification data and biopsy samples of species including southern right whales were collected opportunistically during the IDCR/SOWER.

This study focuses on the southern right whales. The species has a circumpolar distribution. They spend the austral winter in inshore waters of South America, South Africa and Australasia; in spring whales move south to spend the austral summer feeding in waters around Antarctic before returning north in the autumn (Reeves *et al.*, 2002). They can reach a maximum body length of 17 m and the body length at birth is 4–4.6 m. They are believed to live more than 70 years and calves are produced every 3–5 years (Reeves *et al.*, 2002). The primary breeding grounds are located at South Africa, South West Australia, Argentina, and New Zealand Sub-Antarctic (IWC, 2001; 2013; Carroll *et al.*, 2016). Research effort to investigate stock structure, distribution and abundance trend has focused mainly on the breeding grounds, and limited information exists on the distribution, site fidelity and movements in the feeding grounds, and on the connection between breeding grounds in low latitude waters and higher latitude feeding grounds.

Migration patterns, movements and feeding ground destinations have been studied using direct approaches including photo-identification and more recently, telemetry as well as indirect approaches such as visual surveys and historic whaling records. Telemetry has been used to investigate movement patterns of South African right whales (Mate *et al.*, 2011) and western South Atlantic right whales (Zerbini *et al.*, 2016; 2018). The telemetry and photo-identification (e.g., Best *et al.*, 1993) studies in the Argentina and South African breeding grounds show that southern right whales are found throughout large areas of the South Atlantic Ocean and visit several potential feeding areas each season.

The focus of the present paper is the Indian Ocean sector of the Antarctic, between 80°–135°E, south of 60°S. Previous studies have shown that animals from that sector are associated with breeding grounds in the Australasian region (e.g., IWC, 2001; 2013). For example, Bannister (2001) reviewed the distribution and movement of 'Australian' southern right whales based on historical whaling data, recent sighting surveys, 1960s Soviet catch data and photo-identification data. The review confirmed the traditional view of seasonal movements to and from coastal breeding grounds in warm waters and feeding grounds in colder waters. In terms of direct evidence, he presented two photo-identification matches, the first made between whales identified of either Western Australia or South Australia in winter/spring and waters around 40°–44°S; 116°–125°E where a sighting of 35 animals had been made in December–January 1995–96 (Bannister *et al.*, 1997). The other evidence was a southern right whale photographed at 64°26′S; 114°54′E in February 1996, which had been identified over a period of 18 years on the coast of Western Australia (Bannister *et al.*, 1999). These data suggest that at least some of the summer feeding aggregation of southern right whales in the Antarctic Indian Ocean sector belong to the South West Australian population.

Most recently, Mackay *et al.* (2020) presented telemetry information of six animals tagged in Australasian wintering grounds. They suggested at least three probable foraging grounds: to the southwest

of Western Australia, the Subtropical Front and Antarctic waters—the Subtropical Front appearing to be a feeding ground for animals from both New Zealand and Australian waters. They also suggested that the observed variable population growth rates between wintering grounds in Australasia might reflect fidelity to different quality feeding grounds. Thus, similar to results from the South African and Argentina breeding grounds, Australasian animals appear to visit multiple potential feeding areas each season.

A high concentration of sightings of southern right whales was observed in the Indian sector (80°–135°E) of the Antarctic Ocean during various austral summer season cruises of JARPA and JARPAII (Matsuoka and Hakamada, 2020) and the IWC-IDCR/SOWER cruises. Biopsy samples collected opportunistically during the IDCR/SOWER and JARPA/JARPAII surveys in that sector were used in the present study on genetic matching based on microsatellite DNA (msDNA). The main objective of the individual identification based on genetic matching was to evaluate the utility of this approach to study site fidelity and ranges in this species. The use of genetic markers ('tags') has long been shown to represent a viable alternative/supplement to photographic methods of individual recognition—such markers are permanent in all individuals (Palsbøll *et al.*, 1997a).

Materials and methods

Samples

A total of 157 skin/blubber biopsy samples were obtained opportunistically from free-ranging southern right whales along the sighting surveys of JARPA/JARPAII (n=108) and IWC-IDCR/SOWER (n=49) in the Antarctic Indian sector (80°–135°E), south of 60°S from the 1993/94 to 2015/16 austral summer seasons. A variety of collection systems was used including crossbows, air guns and modified shot guns, all using modified collection darts that took a small sample of skin and blubber. The geographical distribution of the southern right whales sampled is shown in Fig. 1.

DNA extraction

Genomic DNA was extracted from approximately 0.05g of the outer epidermal layer of the skin tissue using standard phenol/chloroform protocols (Sambrook *et al.*, 1989) or using Gentra Puregene kits (QIAGEN). Extracted DNA was stored in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0).

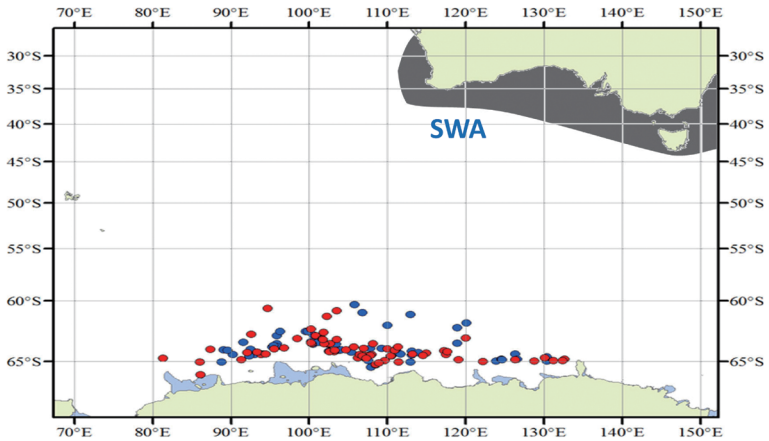

Fig. 1. Geographical distribution of southern right whales in the Indian Ocean sector of the Antarctic examined in this study. Red: females; Blue: males. SWA: South West Australia. Light blue shading shows the ice configuration.

Laboratory procedure for msDNA

Genetic variation was examined at 14 msDNA loci: EV1, EV14, EV21, EV37, EV94 (Valsecchi and Amos, 1996), GT23, GT211, GT310 (Bérubé *et al.*, 2000), GATA28 (Palsbøll *et al.*, 1997b), DlrFCB17 (Buchanan *et al.*, 1996), TR3G2, TR2G5, TR2F3 and TR3F2 (Frasier *et al.*, 2006). Details of the laboratory work for msDNA are provided in Pastene and Goto (2016).

Laboratory procedure for mtDNA

Mitochondrial DNA (mtDNA) control region sequences were used as one way to confirm individual identification by nuclear markers. Samples sharing a same genotype should also share a same haplotype.

The first 470 base pairs (bp) at the 5′ end of the mtDNA control region were amplified by polymerase chain reaction (PCR). Details of the laboratory work for mtDNA sequencing are provided in Pastene and Goto (2016).

Laboratory procedure for sex determination

Sex determination is important both to examine potential sex-specific patterns of movement as well as an additional way to confirm individual msDNA matching. Samples sharing the same genotype should be of the same sex. The SRY locus located on the Y chromosome was used for sex determination following the method of Abe *et al.* (2001) with a slight modification (see Milmann *et al.*, 2021).

Analytical procedure

MtDNA

Variable sites and unique sequences (haplotypes) were identified using the program MacClade (Maddison and Maddison, 1989).

MsDNA

The computer program MICRO-CHECKER (van Oosterhout *et al.*, 2004) was used to check for null alleles and reading/typing errors. The probability that two unrelated individuals have identical genotype (i.e., an identical genetic 'tag') is negatively correlated with the number of loci analysed and the degree of variation at each locus (Palsbøll, 1999). For this reason, it is important the estimations of the nuclear DNA diversity of southern right whales at each msDNA locus, as well the probability of identity (*I*). The latter is the probability that two unrelated individuals from the same panmictic population have an identical composite genotype (see Paetkau and Strobeck, 1994). The *I* was estimated per locus and across loci. The number of alleles per locus, inbreeding coefficient (F_{IS}) and expected heterozygosity (H_E) per locus was calculated using FSTAT 2.9.3 (Goudet, 1995). Statistical tests for the deviations from expected Hardy–Weinberg genotypic proportions were conducted using GENEPOP 4.0 (Rousset, 2008), both by each locus and for all loci combined. Individuals with identical multi-locus genotypes sampled during the same sighting (duplicates) were investigated, and one from each duplicate was removed for subsequent analyses.

Results

Final dataset

There were four cases of biopsies with identical multi-locus genotypes sampled during the same sighting (duplicates). There were two cases of mother-offspring pairs, which were deduced from observations at the field and from genetic data. After removing four duplicates, the sample sizes for the msDNA analyses on individual identification was 153 (76 males and 77 females).

Level of msDNA diversity and probability of identity

No evidence of null alleles and typing/reading errors was found. Table 1 shows the estimated msDNA diversity indices. The number of alleles per locus ranged from 2 to 14 (average 7.5), and the H_E ranged from 0.07 to 0.89 (average 0.65). The F_{IS} in each locus ranged from −0.035 to 0.162 (average 0.007). There was no significant deviation from the Hardy–Weinberg genotypic proportion. *I* for each locus ranged from 0.022 at DlrFCB17 to 0.861 at EV21, which was estimated to be 1.95×10^{-10} when all loci were combined.

Genetic matching

As shown in Table 2, eight matches were detected (four males and four females). The multi-locus genotype matches were supported by mtDNA (same haplotype), sex determination (same sex), and, in the two cases where pictures were available, by photo-identification matches (Fig. 2).

Site-fidelity and movement range

Figs. 3A–B show the geographical distribution of the matches of female and male southern right whales, respectively. Table 2 summarises the matches and associated data. The elapsed time between sample and re-sample ranged between 3 and 13 years (average 7.25 years). In the case of females, the range was 3–11 years (average 7.75 years) and, in the case of males 4–13 years (average 6.75 years). The average longitudinal dispersal ranges were 13°06′ and 7°15′ in males and females, respectively. The average latitudinal dispersal ranges were 1°23′ and 0°47′ in males and females, respectively (Table 2). The average direct distances between sample and re-sample positions were 361 n.miles and 199 n.miles for males and females, respectively (Table 2). A statistical test showed no significant differences between females and males in the direct distances between samples and re-samples (W = 14, Mann-Whitney, p = 0.114).

Table 1. Indices of microsatellite DNA diversity in southern right whales from the Indian sector of the Antarctic: A: number of alleles; H_E: expected heterozygosity; HW: *P*-value for the test of Hardy-Weinberg equilibrium; F_{IS}: inbreeding coefficient; and *I*: probability of identity.

Microsatellite loci	A	H_E	HW	F_{IS}	*I*
EV1	14	0.87	0.25	0.010	0.025
GT310	6	0.62	0.05	0.057	0.205
GT23	8	0.81	0.96	0.024	0.059
EV94	5	0.41	0.78	−0.021	0.401
EV14	10	0.76	0.14	0.006	0.076
GT211	10	0.82	0.08	0.077	0.055
EV37	11	0.82	0.95	−0.017	0.046
GATA28	10	0.77	0.28	0.006	0.090
EV21	3	0.07	0.10	0.162	0.861
DlrFCB17	13	0.89	0.54	−0.019	0.022
TR2F3	2	0.49	0.62	0.044	0.379
TR3G2	8	0.77	0.52	−0.035	0.093
TR2G5	2	0.50	0.14	−0.123	0.376
TR3F2	3	0.48	0.68	0.055	0.361
Overall		0.65	0.21	0.007	1.95e-10

Table 2. Cases of sample/re-sample in southern right whales from the Indian sector of the Antarctic determined by microsatellite DNA genotyping. All genotype matches were supported by the mtDNA analysis (same haplotypes) and by the results of sex determination (same sex). Cases 2 and 3 (the only ones for which photographs were available) were confirmed by photo-identification, SR: sample/resample; ID: label of the whale; S: sex; BL: body length determined visually by researchers from the vessels (in meters); ET: elapsed time (year) between samples and re-samples; DD: direct distance between the positions of samples and re-samples (in n. miles); LAR: latitudinal dispersal range between samples and re-samples; LOR: longitudinal dispersal range between samples and re-samples.

	SR	ID	S	BL	Date (D/M/Y)	Position		ET	DD	LAR	LOR
1	Sample	93IVR004	M	14.3	05/03/1994	64°14′S	113°04′E	5	377	1°34′	13°34′
	Re-sample	98SWR046	M	14.3	31/01/1999	62°40′	99°30′				
2	Sample	97IVR007	M	11.9	15/01/1998	62°55′	100°28′	4	241	1°41′	8°13′
	Re-sample	01IVR023	M	12.9	15/02/2002	64°36′	92°15′				
3	Sample	99IVR012	F	16.4	10/02/2000	64°35′	114°31′	8	303	0°19′	11°46′
	Re-sample	07IVR56	F	15.3	01/03/2008	64°54′	126°17′				
4	Sample	98SWR140	F	13.4	05/02/1999	63°19′	103°28′	3	265	1°11′	9°38′
	Re-sample	01IVR018	F	13.3	14/02/2002	64°30′	93°50′				
5	Sample	01IVR024	M	13.2	15/02/2002	64°36′	92°15′	13	490	0°31′	18°47′
	Re-sample	14AJ4R036	M	13.7	28/02/2015	64°05′	111°02′				
6	Sample	98SWR137	F	13.1	02/02/1999	63°00′	100°50′	9	157	1°03′	5°21′
	Re-sample	07IVR49	F	12.8	22/02/2008	64°03′	95°29′				
7	Sample	98SWR141	F	12.8	07/02/1999	63°55′	105°42′	11	69	0°36′	2°16′
	Re-sample	09SWR011	F	13.2	29/01/2010	64°31′	107°58′				
8	Sample	09SWR008	M	13.2	24/01/2010	64°27′	111°38′	5	335	1°47′	11°51′
	Re-sample	14AJ4R004	M	12.7	22/02/2015	62°40′	99°47′				

Discussion

Previously Carrol *et al.* (2016) carried out successfully a genetic matching study in southern right whales of the New Zealand nursery ground. The study presented here is the first of such kind in southern right whales of the Indian sector of the Antarctic. Results of the Hardy–Weinberg equilibrium test suggest that whales in this sector belong to the single putative 'South Western Australia' population earlier identified by the IWC Scientific Committee (IWC, 2001; 2013) based upon *inter alia* results of photo-identification matches (Bannister *et al.*, 1999; Bannister, 2001). Individual identification by genetic matching has the potential to contribute to studies on movement and site fidelity in this population. Here, some relevant aspects of this approach and its utility for southern right whales are discussed.

Utility of genetic matching for individual identification of southern right whales

The number (n=14) of msDNA loci used in the present study and the degree of polymorphism (see Table 1) proved to be appropriate for the purpose of individual identification in southern right whales. There was a very low estimated probability of identity i.e., two unrelated individuals having an identical composite genotype. The eight cases of genotyping matching were supported by the mtDNA analyses (same haplotype) and sex determination (same sex). In addition, for the two cases where photographs were available, genotyping matching was supported by photo-identification. The authors conclude that the eight cases of genotyping matching corresponded to matching of the same individual whales and thus confirms the utility of genetic matching to identify individuals (see also Carrol *et al.*, 2016).

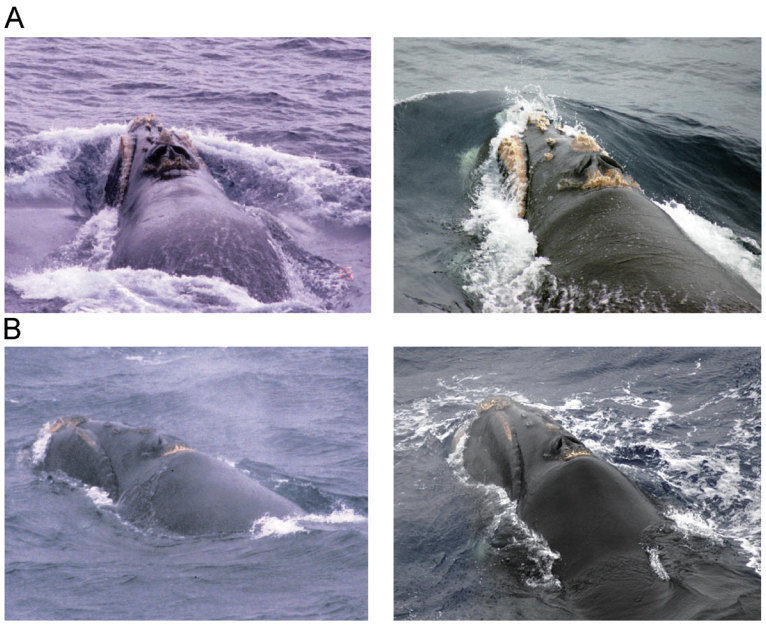

Fig. 2 **A**: A southern right whale sighted in the Indian sector of the Antarctic on 15 January 1998 (left) and re-sighted in the same sector on 15 February 2002 (right) (Matching No. 2 in Table 2). **B**: A southern right whale sighted in the Indian sector of the Antarctic on 10 February 2000 (left) and re-sighted in the same sector on 1 March 2008 (right) (Matching No. 3 in Table 2).

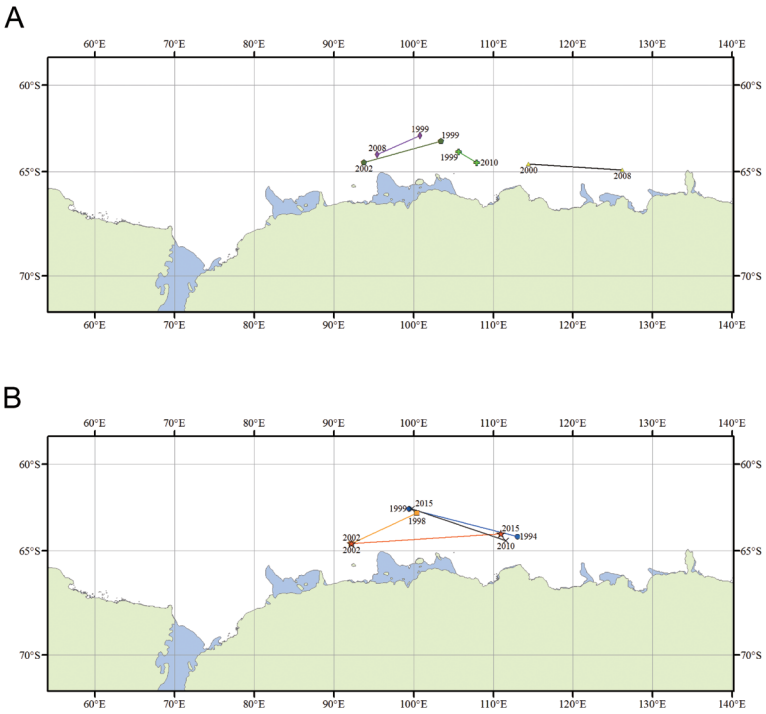

Fig. 3. Maps showing sample/re-sample positions referred to in the text. **A**: females–note that match 2002–2008 was also confirmed by photographs. **B**: males–note that match 1998–2002 was also confirmed by photographs. Light blue shading shows the ice configuration.

Utility for studies on site fidelity and movement ranges of southern right whales

The eight matches here show that at least some females and males visit the same feeding ground in the Indian sector of the Antarctic south of 60°S in summer. The total samples and the matches both were close to 1 : 1 males and females. Although the direct distances between sample and re-sample

by sex were statistically non-significant, this probably relates to the small sample size. Qualitatively (Table 2 and Fig. 3), the results are not in conflict with the findings of Carroll *et al.* (2011) that males are more mobile than females.

As explained earlier, telemetry data from southern right whales tagged in breeding grounds in Australasia (Mackay *et al.*, 2020), South Africa (Mate *et al.*, 2011) and Argentina (Zerbini *et al.*, 2016; 2018) suggested that individuals may visit several feeding grounds within the same season e.g., in middle latitudes and high latitudes, and this aspect of southern right whale's ecology should be considered when discussing site fidelity. The abundance of southern right whales in the Indian sector of the Antarctic was estimated as 910 in 1988/89 from sightings data (95% CI: 409–2,026) (Matsuoka and Hakamada, *in press*) and the number of individuals identified in this study was 145 (153 minus eight individuals re-sampled). This corresponds to about 16% (range between about 1–36% of the estimated total population). The low number of matches limits the applicability of inferences on relative site fidelity and the pattern of longitudinal dispersal to the total population. The comparison above should be seen with caution. The 1988/89 estimate has wide confidence intervals and the biopsy sampling is pretty wide and carried out after the abundance estimates.

Whilst the present study confirms the feasibility of the approach, it is clear that more definitive inferences on site fidelity and movement ranges will require a larger number of biopsy samples, from both south and north of 60°S (all the samples in this study were obtained south of this latitude).

Food availability in the area of genetic matching

Recognizing the limited number (and sampling distribution) of samples, the present results allow the formulation of the following ecological inferences for further investigation. The Indian Ocean sector of Antarctic waters south of 60°S represents one of the several possible feeding grounds for the South West Australia population (e.g., see Mackay *et al.*, 2020). Our results show that at least some southern right whales of both sexes return to the same sector at some point in their lives, presumably for feeding. Matches were found in a broad area that has shown consistent annual aggregations of southern right whales (e.g., Matsuoka and Hakamada, 2020) and that were seen in areas of high krill concentrations (e.g., Murase *et al*, 2002; Matsuoka *et al.*, 2003; Nicol, 2006).

Qualitative analyses of food consumption of southern right whales in the Antarctic Indian sector could be investigated by stable isotope analyses of the same biopsy samples used in the present study. Information on prey species consumed by southern right whales will assist in confirming the ecological inference aforementioned.

Finally, genotyping data in this study can be used for other purposes, for example to estimate abundance and other demographic parameters (e.g., survival rates) of this species within a mark-recapture context (e.g., Wade *et al.*, 2010).

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References

Abe, H., Goto, M., Pastene, L.A., Dewa, K. and Naito, E. 2001. Practical use of multiplex fluorescent PCR for cetacean sex identification. *Mar. Mammal Sci.* 17: 657–664. doi: 10.1111/j.1748-7692.2001.tb01011.x.

Bannister, J. L. 2001. Status of southern right whales (*Eubalaena australis*) off Australia. *J. Cetacean Res. Manage.* (Special

issue) 2: 103–110.
Bannister, J. L., Burnell, S., Burton, C. and Kato, H. 1997. Right whales off southern Australia: direct evidence for a link between onshore breeding grounds and offshore probable feeding grounds. *Rep. int. Whal. Commn.* 47: 441–444.
Bannister, J. L., Pastene, L. A. and Burnell, S. R. 1999. First record of movement of a southern right whale (*Eubalaena australis*) between warm water breeding grounds and the Antarctic Ocean, South of 60°S. *Mar. Mammal Sci.* 15(4): 1337–1342. doi: 10.1111/j.1748-7692.1999.tb00895.x.
Bérubé, M., Jørgensen, H., Mcewing, R. and Palsbøll, P. J. 2000. Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. *Mol. Ecol.* 9(12): 2181–2183. doi: 10.1046/j.1365-294x.2000.105315.x.
Best, P. B., Payne, R., Rowntree, V., Palazzo, J. T. and Both, M. D. 1993. Long-range movements of South Atlantic right whales, *Eubalaena australis*. *Mar. Mammal Sci.* 9 (3): 227–234. doi: 10.1111/j.1748-7692.1993.tb00451.x.
Buchanan, F. C., Friesen, M. K., Littlejohn, R. P. and Clayton, J. A. 1996. Microsatellites from beluga whale *Delphinapterus leucas*. *Mol. Ecol.* 5: 571–575. doi: 10.1046/j.1365-294X.1996.00109.x.
Carroll, E. L., Patenaude, N. J., Alexander, A. M., Steel, D., Harcourt, R., Childerhouse, S., Smith, S., Bannister, J. L., Constantine, R. and Baker, C. S. 2011. Population structure and individual movement of southern right whales around New Zealand and Australia. *Mar. Ecol. Prog. Ser.* 432: 257–268. doi: 10.3354/meps09145.
Carroll, E. L., Fewster, R. M, Childerhouse, S. J., Patenaude, N. J., Boren, L. and Baker, C.S. 2016. First direct evidence for natal wintering ground fidelity and estimate of juvenile survival in the New Zealand southern right whale *Eubalaena australis*. *PLoS ONE* 11 (1): e0146590. doi:10.1371/journal.pone.0146590.
Frasier, T. R, Rastogi, T., Brown, M. W., Hamilton, P. K., Kraus, S. D. and White, B. N. 2006. Characterization of tetranucleotide microsatellite loci and development and validation of multiplex reactions for the study of right whale species (genus *Eubalaena*). *Mol. Ecol. Note* 6: 1025–1029. doi: 10.1111/j.1471-8286.2006.01417.x.
Fujise, Y. and Pastene, L. A. 2021. Whales as indicators of historical and current changes in the marine ecosystem of the Indo-Pacific sector of the Antarctic. pp. 85–103. *In*: Kanao, M., Godone, D. and Dematteis, N. (eds.): *Glaciers and Polar Environment.* IntechOpen, London, UK. I-XI+188 pp. doi: 10.5772/intechopen.94323.
Goudet, J. 1995. FSTAT, version 1.2: a computer program to calculate F-statistics. *J. Hered.* 86: 485–486. doi: 10.1093/oxfordjournals.jhered.a111627.
International Whaling Commission. 2001. Report of the workshop on the comprehensive assessment of right whales: a worldwide comparison. *J. Cetacean Res. Manage.* (Special issue) 2: 1–60.
International Whaling Commission. 2013. Report of the IWC workshop on the Assessment of Southern Right Whales. *J. Cetacean Res. Manage. (Suppl.)* 14: 437–462.
Mackay, A. I., Bailleul, F., Carroll, E. L., Andrews-Goff, V., Baker, C. S., Bannister, J., Boren, L., Carlyon, K., Donnelly, D. M., Double, M., Goldsworthy, S. D., Harcourt, R., Holman, D., Lowther, A., Parra, G. J. and Childerhouse, S. J. 2020. Satellite derived offshore migratory movements of southern right whales (*Eubalaena australis*) from Australian and New Zealand wintering grounds. *PLoS ONE* 15 (5): e0231577. doi: 10.1371/journal.pone.0231577.
Maddison, W. P. and Maddison, D. R. 1989. Interactive analysis of phylogeny and character evolution using the computer program MacClade. *Folia Primatol. (Basel)* 53 (1–4): 190–202. doi: 10.1159/000156416.
Mate, B. R., Best, P. B., Lagerquist, B. A. and Winsor, M. H. 2011. Coastal, offshore and migratory movements of South African right whales revealed by satellite telemetry. *Mar. Mammal Sci.* 27 (3): 455–476. doi: 10.1111/j.1748-7692.2010.00412.x.
Matsuoka, K., Watanabe, T., Ichii, T., Shimada, H. and Nishiwaki, S. 2003. Large whale distributions (south of 60°S, 35°E–130°E) in relation to the southern boundary of the Antarctic Circumpolar Current (A CC). pp. 26–30. *In*: Huiske, A. H. L., Gieskes, W. W. C., Rozema, J., Schrno, R. M. L., van der Vies, S. M. & Wolff, W.J. (eds.) *Antarctic Biology in a Global Context.* Backhuys Publishers, Leiden, The Netherlands. 338 pp.
Matsuoka, K. and Hakamada, T. 2020. Density distribution of several major whale species in the Indo-Pacific region of Antarctic using JARPA and JARPAII sighting data obtained through 1987/88–2008/09 seasons. *Cetacean Popul. Stud.* 2: 15–35. doi: 10.34331/cpops.2.1_15.
Matsuoka, K. and Hakamada, T. *in press*. Estimates of abundance south of 60°S for southern right whales (*Eubalaena australis*) in the Antarctic Area IV (80°–130°E) based on 1998/99 SOWER survey data. *J. Cetacean Res. Manage*.
Milmann, L., Taguchi, M., Siciliano, S., Baumgarten, J. E., Oliveira, L. R., Valiati, V. H., Goto, M., Ott, P. H. and Pastene, L. A. 2021. New genetic evidences for distinct populations of the common minke whale (*Balaenoptera acutorostrata*) in the Southern Hemisphere. *Polar Biol*. 44: 1575–1589. doi: 10.1007/s00300-021-02897-2.
Murase, H., Matsuoka, K., Ichii, T. and Nishiwaki, S. 2002. Relationship between the distribution of euphausiids and baleen whales in the Antarctic (35°E–145°W). *Polar Biol.* 25: 135–145. doi: 10.1007/s003000100321.
Murase, H., Palka, D., Punt, A. E., Pastene, L. A., Kitakado, T., Matsuoka, K., Hakamada, T., Okamura, H., Bando, T., Tamura, T., Konishi, K., Yasunaga, G., Isoda, T. and Kato, H. 2020. Review of the assessment of two stocks of Antarctic minke whales (eastern Indian Ocean and western South Pacific). *J. Cetacean Res. Manage*. 21: 95–122.
Nicol, S. 2006. Krill, currents, and sea ice: *Euphausia superba* and its changing environment. *BioScience* 56 (2):111–120. doi: 10.1641/0006-3568(2006)056[0111:KCASIE]2.0.CO;2.
Paetkau, D. and Strobeck, C. 1994. Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.* 3: 489–495. doi: 10.1111/j.1365-294x.1994.tb00127.x.
Palsbøll, P. J. 1999. Genetic tagging: contemporary molecular ecology. *Biol. J. Linn. Soc.* 68: 3–22. doi: 10.1111/j.1095-

8312.1999.tb01155.x.
Palsbøll, P. J., Allen, J., Bérubé, M., Clapham, P. J., Feddersen, T.P., Hammond, P. S., Hudson, R. R., Jorgensen, H., Katona, S., Larsen, A. H., Larsen, F., Lien, J., Mattila, D. K., Sigurjonsson, J., Sear, R., Smith, T., Sponer, R., Stevick, P. and Oien, N. 1997a. Genetic tagging of humpback whales. *Nature* 388: 767–769. doi: 10.1038/42005.
Palsbøll, P. J., Bérubé, M., Larsen, A. H., and Jørgensen, H. 1997b. Primers for the amplification of tri-and tetramer microsatellite loci in baleen whales. *Mol. Ecol.* 6: 893–895. doi: 10.1046/j.1365-294X.1997.d01-214.x.
Pastene, L. A. and Goto, M. 2016. Genetic characterization and population genetic structure of the Antarctic minke whale *Balaenoptera bonaerensis* in the Indo-Pacific region of the Southern Ocean. *Fish. Sci.* 82: 873–886. doi: 10.1007/s12562-016-1025-5.
Reeves, R. R., Stewart, B. S., Clapham, P. J. and Powell, J. A. 2002. *Guide to Marine Mammals of the World*. National Audubon Society. Chanticleer Press, Inc., New York. 527 pp.
Rousset, F. 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol. Ecol. Resour.* 8: 103–106. doi: 10.1111/j.1471-8286.2007.01931.x.
Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual.* 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, New York, USA.
Valsecchi, E., and Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 5: 151–156. doi: 10.1111/j.1365-294X.1996.tb00301.x.
van Oosterhout, C., Hutchinson, W. F., Wills, D. P. and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535–538. doi: 10.1111/j.1471-8286.2004.00684.x.
Wade, P. R., Kennedy, A., LeDuc, R. G., Barlow, J., Carretta, J., Shelden, K., Perryman, W. L., Pitman, R., Robertson, K. M., Rone, B., Salinas, J. C., Zerbini, A. N., Brownell, Jr R. L. and Clapham, P. J. 2010. The world's smallest whale population? *Biol. Lett.* 7 (1): 83–85. doi: 10.1098/rsbl.2010.0477.
Zerbini, A. N., Ajó, A. F., Andriolo, A., Clapham, P. J., Crespo, E., Gonzalez, R., Harris, G., Mendez, M., Rosenbaum, H., Sironi, M., Sucunza, F. and Uhart, M. 2018. Satellite tracking of southern right whales (*Eubalaena australis*) from Golfo San Matías, Rio Negro Province, Argentina. Paper SC/67B/CMP/17 presented to the IWC Scientific Committee, May 2018 (unpublished). 10 pp. [Available from the IWC Office].
Zerbini, A. N., Rosenbaum, H., Mendez, M., Sucunza, F., Andriolo, A., Harris, G., Clapham, P. J., Sironi, M., Uhart, M. and Ajó, A. F. 2016. Tracking southern right whales through the southwest Atlantic: an update on movements, migratory routes and feeding destinations. Paper SC/66b/BRG26 presented to the IWC Scientific Committee, June 2016 (unpublished). 16 pp. [Available from the IWC Office].

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