



Original Article

Genetic Diversity and Connectivity of Southern Right Whales (*Eubalaena australis*) Found in the Brazil and Chile–Peru Wintering Grounds and the South Georgia (Islas Georgias del Sur) Feeding Ground

Emma L. Carroll, Paulo H. Ott, Louise F. McMillan, Bárbara Galletti Vernazzani, Petra Neveceralova, Els Vermeulen, Oscar E. Gaggiotti, Artur Andriolo, C. Scott Baker, Connor Bamford, Peter Best,* Elsa Cabrera, Susannah Calderan, Andrea Chirife, Rachel M. Fewster, Paulo A. C. Flores, Timothy Frasier, Thales R. O. Freitas, Karina Groch, Pavel Hulva, Amy Kennedy, Russell Leaper, Matthew S. Leslie, Michael Moore, Larissa Oliveira, Jon Seger, Emilie N. Stepien, Luciano O. Valenzuela, Alexandre Zerbini, and Jennifer A. Jackson

From the School of Biological Sciences, University of Auckland, Auckland, New Zealand (Carroll); School of Biology, University of St Andrews, St Andrews, UK (Carroll and Gaggiotti); Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul, Torres, RS, Brazil (Ott); Universidade Estadual do Rio Grande do Sul, Osório, RS, Brazil (Ott and Oliveira); School of Mathematics and Statistics, Victoria University of Wellington, PO Box 600, Wellington, New Zealand (McMillan); Centro de Conservación Cetacea—Casilla 19178 Correo 19, Santiago, Chile (Galletti Vernazzani and Cabrera); Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic (Neveceralova and Hulva); Ivanhoe Sea Safaris, 83 Vyfer Street, Gansbaai, South Africa (Neveceralova); Dyer Island Conservation Trust, Great White House, Kleinbaai, Gansbaai, South Africa (Neveceralova); Mammal Research Institute Whale Unit, Department of Zoology and Entomology, University of Pretoria, Hatfield, South Africa (Vermeulen); Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora, Campus Universitário, 36036-900 Juiz de Fora, MG, Brazil (Andriolo); Instituto Aqualie, Juiz de Fora, MG, Brazil (Andriolo); Marine Mammal Institute and Department of Fisheries and Wildlife, Oregon State University, Newport, OR 97365 (Baker); British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET, UK (Bamford and Jackson); University of Southampton, University Road, Southampton, SO17 1BJ, UK (Bamford); Scottish Association for Marine Science, Oban, Argyll, Scotland (Calderan); Instituto de Ciencias Biomédicas (ICB), Universidad Andrés Bello, Chile (Chirife); Department of Statistics, University of Auckland, Auckland, New Zealand (Fewster); Área de Proteção Ambiental (Environmental Protection Area) Anhatomirim, ICMBio, MMA, Florianópolis, SC, Brazil (Flores); Department of Biology, Saint Mary's University, Halifax, Nova Scotia, Canada, B3H 3C3 (Frasier); Programa de Pós-Graduação em Genética e Biologia Molecular- Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil (Freitas); Instituto Australis, Imbituba, Santa Catarina 88780-000, Brazil (Groch); Department of Biology and Ecology, University of Ostrava, Ostrava, Czech Republic (Hulva); Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way, NE, Seattle, WA 98115-6349 (Kennedy and Zerbini); IFAW. 87–90 Albert Embankment, London SE1 7UD, UK (Leaper); Biology Department, Swarthmore College, 500 College Avenue, Swarthmore, PA 19081 (Leslie); Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA

02543 (Moore); Laboratório de Ecologia de Mamíferos, Universidade do Vale do Rio dos Sinos, Centro de Ciências da Saúde Avenida Unisinos 950 Cristo Rei - Sao Leopoldo 93022-000, RS, Brazil (Oliveira); School of Biological Sciences, University of Utah, Salt Lake City, UT 84112 (Seger and Valenzuela); Section of Marine Mammal Research, Department of Bioscience, Aarhus University, Aarhus, Denmark (Stepien); Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Laboratorio de Ecología Evolutiva Humana, UNCPBA, Quequén, Buenos Aires Province, Argentina (Valenzuela); Instituto de Conservación de Ballenas, Ciudad Autónoma de Buenos Aires 1429, Argentina (Valenzuela); Marine Ecology and Telemetry Research, Seabeck, WA (Zerbini); and Joint Institute for the Study of the Atmosphere and Ocean (JISAO), University of Washington, John M. Wallace Hall, 3737 Brooklyn Ave NE, Seattle, WA 98105 (Zerbini)

Address correspondence to E. L. Carroll at the address above, or e-mail: carrollemz@gmail.com.

*Deceased.

Received December 1, 2019; First decision January 14, 2020; Accepted April 21, 2020.

Corresponding Editor: Kim Andrews

Abstract

As species recover from exploitation, continued assessments of connectivity and population structure are warranted to provide information for conservation and management. This is particularly true in species with high dispersal capacity, such as migratory whales, where patterns of connectivity could change rapidly. Here we build on a previous long-term, large-scale collaboration on southern right whales (*Eubalaena australis*) to combine new (n_{new}) and published (n_{pub}) mitochondrial (mtDNA) and microsatellite genetic data from all major wintering grounds and, uniquely, the South Georgia (Islas Georgias del Sur: SG) feeding grounds. Specifically, we include data from Argentina (n_{pub} mtDNA/microsatellite = 208/46), Brazil (n_{new} mtDNA/microsatellite = 50/50), South Africa (n_{new} mtDNA/microsatellite = 66/77, n_{pub} mtDNA/microsatellite = 350/47), Chile–Peru (n_{new} mtDNA/microsatellite = 1/1), the Indo-Pacific (n_{pub} mtDNA/microsatellite = 769/126), and SG (n_{pub} mtDNA/microsatellite = 8/0, n_{new} mtDNA/microsatellite = 3/11) to investigate the position of previously unstudied habitats in the migratory network: Brazil, SG, and Chile–Peru. These new genetic data show connectivity between Brazil and Argentina, exemplified by weak genetic differentiation and the movement of 1 genetically identified individual between the South American grounds. The single sample from Chile–Peru had an mtDNA haplotype previously only observed in the Indo-Pacific and had a nuclear genotype that appeared admixed between the Indo-Pacific and South Atlantic, based on genetic clustering and assignment algorithms. The SG samples were clearly South Atlantic and were more similar to the South American than the South African wintering grounds. This study highlights how international collaborations are critical to provide context for emerging or recovering regions, like the SG feeding ground, as well as those that remain critically endangered, such as Chile–Peru.

Subject: Population structure and phylogeography, Conservation genetics and biodiversity

Keywords: population structure, connectivity, migration, gene flow

Exploitation can impact multiple ecological facets of populations and species, from demographic composition, life-history traits, and evolutionary potential, to patterns of population subdivision and connectivity (Allendorf et al. 2008). Investigating contemporary patterns of connectivity is critical for ongoing conservation and management efforts (Waples et al. 2018). Mechanisms such as density-dependent movement patterns (Thomas and Harwood 2003), fluidity in social groupings (Clapham and Zerbini 2015), and social transmission of migratory traditions (Clapham et al. 2008; Carroll et al. 2014) can influence patterns of connectivity during recovery from exploitation. Additionally, recovering populations may not reflect pre-exploitation patterns of connectivity and/or genetic diversity, due to changes in habitat quality, species' dispersal capacity, demography, social structure, and exploitation-induced evolutionary change (Blundell et al.

2002; Tinker et al. 2008; Mysterud 2011; Servanty et al. 2011; Uusi-Heikkilä et al. 2015).

The high dispersal capacity of marine mammals means that they can rapidly change their distribution. For example, humpback whales (*Megaptera novaeangliae*) are thought to have colonized Hawaii during the past 200 years, possibly linked to changing environmental conditions (Herman 1979). The southern right whale (*Eubalaena australis*), which underwent a dramatic demographic bottleneck due to whaling (IWC 2001; Jackson et al. 2008), has a historical and contemporary circumpolar distribution from around 12°S to 65°S, albeit with a discontinuity between Chile and New Zealand (Figure 1). However, various recent changes in distribution have been described during their recovery, apparently driven by spatially variable, and sometimes rapid temporal changes, in habitat use

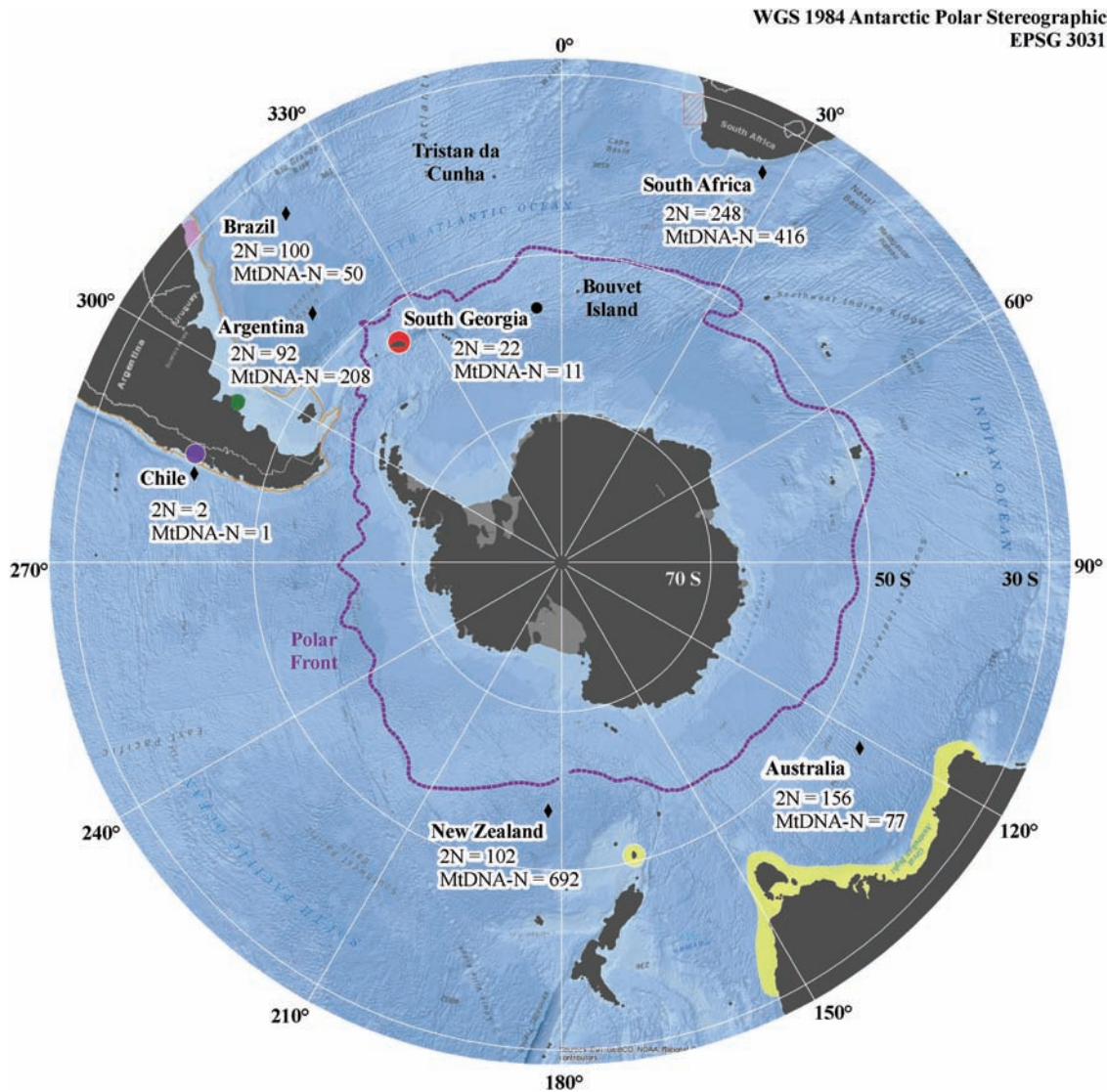


Figure 1. Location of southern right whale sampling sites including wintering grounds and the South Georgia (Islas Georgias del Sur) foraging ground; 2N and mtDNA-N are the sample sizes for the microsatellite loci and mitochondrial haplotype datasets, respectively. Also shown are putative foraging grounds, including Patagonian Shelf, Polar Front, and Tristan da Cunha, and a region off the west coast of South Africa indicated by red cross-hatching.

patterns (Harcourt et al. 2019). For example, southern right whales have recently been observed wintering in the Falkland Islands (Islas Malvinas: FI) (Weir and Stanworth 2019) and are thought to be re-colonizing mainland New Zealand (Carroll et al. 2014). Therefore, as the species recovers from exploitation, it is important to monitor patterns of connectivity for indications of change that could inform or impact management.

As with many other baleen whale species, assessment of contemporary connectivity of southern right whales requires large-scale, long-term international collaborations. This is because southern right whales are highly mobile, often crossing international boundaries when migrating between sheltered coastal areas that are used as socializing and calving grounds in winter and offshore, high-latitude foraging grounds typically used in summer. These coastal wintering grounds are the best-characterized habitat for the species, with the strongest recovery demonstrated by South Africa, Argentina and Brazil in the South Atlantic (Cooke et al. 2001; Groch et al. 2005; Brandão et al. 2018), and sub-Antarctic New Zealand and Southwest

Australia in the Indo-Pacific (Bannister 2009; Carroll et al. 2013). In contrast, historical calving grounds in Namibia, Mozambique/Madagascar, Tristan Da Cunha, Southeast Australia, and Chile-Peru show very little recovery (IWC 2001; Best et al. 2009; Galletti Vernazzani et al. 2014; Carroll et al. 2015; Roux et al. 2015).

Long-term photo-identification and genetic monitoring studies have revealed that both male and female southern right whales (IWC 2001; Rowntree et al. 2001; Carroll et al. 2013) have high levels of fidelity to nursery areas within well-studied, recovering wintering grounds. Females calve on average once every 3 years, and photo-identification studies estimate weaning to be up to 12 months (Burnell 2001). Thus, during the first year of life, a calf completes its first migratory cycle by being born in its mother's preferred wintering ground, traveling with its mother to her preferred summer feeding ground and potentially returning with her to its natal wintering ground the following year (Burnell 2001; Best et al. 2015). This vertical transmission of migratory preferences is called migratory culture and could lead to genetic structuring depending on the

proportion of each generation that adheres to parental migratory routes (Harrison et al. 2010).

Both philopatry and migratory culture have been inferred as drivers of population structure and recovery patterns in southern right whales (Clapham et al. 2008; Valenzuela et al. 2009; Carroll et al. 2015; Roux et al. 2015; Harcourt et al. 2019). A recent example of this was a circumpolar study that assessed genetic diversity and differentiation at 4 major extant southern right whale wintering grounds and found that there was hierarchical genetic structure in both mitochondrial DNA (mtDNA) and microsatellite loci amongst ocean basins (Carroll et al. 2019). The level of differentiation was higher in mtDNA than microsatellite loci, suggesting female philopatry was a strong driving factor, although sex-biased dispersal was not detected.

As the estimated gestation period for southern right whales is 10–13 months (Lockyer 1984; Best 1994), breeding is expected to occur along migratory corridors en route to wintering grounds during the austral autumn, or in the wintering grounds themselves. Due to this, it has been hypothesized that those wintering grounds from which the whales share a common feeding ground, and hence to some extent a common migratory route, could have higher levels of genetic connectivity (Carroll et al. 2015).

However, southern right whale summer foraging areas are little studied, and migratory routes or corridors are poorly defined (see Harcourt et al. 2019 for a recent review). Feeding grounds are typically offshore and logistically difficult to access directly, meaning that there is little published information on the genetic identity and connectivity of contemporary foraging areas. Instead, linkages between winter and summer habitats have been inferred using stable isotope, photo-identification and telemetry data (Best et al. 1993; Rowntree et al. 2001; Patenaude et al. 2007; Mate et al. 2011; Carroll et al. 2015; Valenzuela et al. 2018; Zerbini et al. 2018). These findings indicate that whales from the Argentinean wintering grounds use foraging grounds on the Patagonian Shelf, South Georgia (Islas Georgias del Sur: SG) and south of the Polar Front (Rowntree et al. 2001; Zerbini et al. 2015, 2018; Valenzuela et al. 2018), and whales from the South African wintering grounds use foraging grounds off the coast of South Africa, Bouvet Island and south of the Polar Front (Best and Schell 1996; Mate et al. 2011) (Figure 1).

Here, we present an analysis of genetic samples from 3 locations whose positions in the broader migratory network are not yet known: SG feeding grounds, and the Brazil and Chile–Peru wintering grounds.

SG is one of the few Southern Hemisphere summer foraging grounds for baleen whales to which researchers can gain direct access. Southern right whales are the most commonly seen whale species in SG during the austral summer (Moore et al. 1999; Richardson et al. 2012). The movement of whales between the Argentinean nursery area at Península Valdés and SG (Figure 1) has been shown using photo-identified individuals (Best et al. 1993; Moore et al. 1999) and satellite telemetry work (Zerbini et al. 2015, 2018). Previous genetic analyses suggested that the SG summer feeding ground was not significantly genetically differentiated from the Brazilian and Argentinean wintering grounds (unpublished: 10 microsatellite loci and 495 bp of the mtDNA control region; Ott 2002; Ott et al. 2011); nor was it different from the South African wintering ground (based on 275 bp of the mtDNA control region; Patenaude et al. 2007). Therefore, SG could show linkages to southern right whale wintering grounds on both sides of the South Atlantic.

In Chile–Peru, the southern right whale subpopulation is considered Critically Endangered by the International Union for

the Conservation of Nature (Cooke 2018) and is subject to an International Whaling Commission (IWC) Conservation and Management Plan (Galletti Vernazzani et al. 2016), as it appears isolated and small in size. Between 1964 and 2011, there were 108 opportunistic sightings of 179 southern right whales off the coasts of Chile and Peru (Galletti Vernazzani et al. 2014), including 39 calves. Given that southern right whales show fidelity to wintering grounds, it is possible that Chile–Peru represents a distinct genetic population. Alternatively, the population might have been extirpated during the whaling era and it could now be undergoing recolonization from elsewhere. The geographic position of the Chile–Peru population makes this particularly interesting in light of recent genetic analyses showing significant genetic differentiation, particularly between the South Atlantic (Argentina, South Africa) and the Indo-Pacific (Australia, New Zealand) ocean basins (Carroll et al. 2019). The Southeast Pacific Chile–Peru subpopulation is separated from the Southwest Pacific New Zealand subpopulation by a large discontinuity in mid-to-low latitude distribution (approximately 90° to 180°W) and from the Southwest Atlantic subpopulation by the South American continent. Little is known about the historical or contemporary feeding grounds of the Chile–Peru population to provide insight into which of these neighboring populations it might mix with: for example, whether it mixes with Southwest Atlantic whales on shared feeding grounds in summer.

The final location for which we provide the first published genetic assessment is Brazil. Of particular interest here is the genetic connectivity between the Brazilian wintering grounds and those off Argentina. While many whales that visit these wintering grounds show inter-annual fidelity to each location (Cooke et al. 2001; Rowntree et al. 2001; Groch et al. 2005), the Brazilian population has been growing at a rate faster than pure internal recruitment could permit. Groch et al. (2005) suggested that this elevated rate was partly driven by immigration from the wintering ground at Península Valdés, Argentina, given the latter's high estimated population growth rate in recent decades (e.g., Cooke et al. 2001) and previous direct matches between the 2 regions (Best et al. 1993). In this study, our new genetic samples enable us to investigate the genetic differentiation between these 2 wintering grounds for the first time.

Our study is the most comprehensive to date comparing the South Atlantic southern right whale wintering grounds together with the summer feeding ground off SG. As well as incorporating the first genetic samples from SG, Chile–Peru, and Brazil, we also augment the existing genotype databases for extant wintering grounds with over 70 new DNA samples from South Africa. This also provides a large genetic reference dataset for current and future assessments of the circumpolar southern right whale migratory network.

We establish the following specific hypotheses: 1) that Brazil and Argentina are not genetically distinct, due to their proximity and likely immigration from Argentina into Brazil; 2) that SG is genetically distinct from Indo-Pacific but not from South Atlantic wintering grounds, due to geographic proximity and previous genetic similarity with both South American and South African wintering grounds; and 3) that Chile–Peru is most closely related to the South Atlantic wintering grounds, given the potential overlapping foraging grounds and large discontinuity in distribution between South America and New Zealand.

Methods

Sample Collection: SG

Sampling in 2018 was conducted from the R/V *Song of the Whale*, which departed from Stanley, FI, on 22 January, sailed to the north

coast of SG and returned to Stanley on 21 February. Right whales were located using a combination of passive acoustics and visual observations (Jackson et al. 2018). All data collection was carried out under permit RAP/2017/017 issued by the Government of SG and the South Sandwich Islands.

Three skin biopsy samples were collected from southern right whales around SG using a stainless steel biopsy dart deployed from a modified veterinary capture rifle (Krützen et al. 2002) or from a crossbow (Lambertsen 1987) during the 2018 field season. Samples were stored in ethanol and frozen at -20°C . An additional 12 skin biopsy samples were available from a 1997 field expedition around SG, as previously described (Moore et al. 1999).

Sample Collection: Chile

A southern right whale was seen alive on 9 February 2017 in Cocotue Bay, Chile, with clear entanglement scars and lesions, but poor weather prevented further at-sea observations (Galletti Vernazzani et al. 2017). Subsequently, a southern right whale carcass was found at Playa Mar Brava, Carelmapu ($41^{\circ}42'S$, $73^{\circ}42'W$, see Figure 1), on 16 February, and reported on 18 February 2017. It corresponded to the same individual seen at sea previously and entanglement lesions were confirmed. Skin and blubber samples were collected from the carcass during a necropsy on 19 February 2017 (Galletti Vernazzani et al. 2017). Samples were given to Centro de Conservación Cetacea under the certificate of delivery and custody of marine protected species 2017-54-4 granted by National Fisheries and Aquaculture Service. Samples were transported on dry ice from Chile to the University of St Andrews, Scotland, for analysis.

Sample Collection: South Africa

A total of 88 skin samples were available from right whales sampled on the South African wintering ground that had not previously been analyzed. This included 50 skin samples archived at the University of Pretoria and 38 sloughed skin samples collected by P. Neveceralova and P. Hulva from Charles University. The 50 samples held by the University of Pretoria were collected between 1990 and 2013 under permits RES2009/06, RES2011/24, RES2012/89, RES2013/58, V1/9/3/1, VI/19/5/1, and VI/9/5/1. The samples held by Charles University were collected during the austral winter of 2016 and 2017 under research permits RES2016/99 and RES2017/89. These samples are in addition to the previously published mtDNA

control region sequence and microsatellite genotype data from 350 and 46 South African right whales, respectively (Carroll et al. 2019).

Sample Collection: Brazil

Skin samples ($n = 60$) were collected from southern right whales along the Brazilian coast, primarily at Santa Catarina and Rio Grande do Sul States (between $27^{\circ}50'S$ and $29^{\circ}20'S$) (Ott et al. 2011) using a dart projection system (Brown et al. 1991; Ott 2002). Samples were collected from a small inflatable boat operating from and around the coastline, usually up to 3–5 km and rarely as far as 10 km distance from shore, during fieldwork conducted between 1998 and 2007. Fieldwork was undertaken by GEMARS in collaboration with the Projeto Baleia Franca under permits IBAMA #039/98, 1803/98-23, CMA 004-02, CMA 011-03, SISBIO 12022-1 and exported under CITES permit No. 15BR018985/DF. Samples were placed in 20% DMSO and frozen at -20°C .

Published Data Included in the Analysis

We augmented the data generated in this study with mtDNA and microsatellite genotype data from Argentina and the Indo-Pacific using published data archived on dryad (Carroll et al. 2015, 2019) (see Table 1: <https://doi.org/10.5061/dryad.vv5347p>).

DNA Extraction and DNA Profile Construction

DNA was extracted using standard phenol/chloroform protocols, modified for small tissue samples (Sambrook et al. 1989). We constructed DNA profiles, comprising genetically identified sex, mtDNA haplotype (381 bp) and microsatellite genotype (up to 17 loci) for each sample, as previously described (Carroll et al. 2015, 2019). Each tray of up to 96 samples was run with between 4 and 7 control samples (New Zealand and/or Australian samples previously genotyped) to ensure consistent binning of alleles and a negative control to detect contamination. In previous work, no significant deviations from Hardy-Weinberg equilibrium were detected using these loci (Carroll et al. 2011, 2019). However, the overall dataset and dataset partitioned by sampling location were investigated for null alleles and deviations from Hardy-Weinberg equilibrium using CERVUS (Kalinowski et al. 2007). We also tested for linkage disequilibrium using GENEPOP (Rousset 2008), with a P -value adjusted for multiple comparisons. CERVUS was also used to identify matches and calculate the probability of identity (Waits et al. 2001) for replicate samples within and between sampling locations as previously

Table 1. Summary of microsatellite and mtDNA diversity statistics for southern right whale winter nursery/socializing (W) and summer feeding (S) grounds

	Microsatellites					Mitochondrial DNA					Source
	2N	$n_{\text{alleles}} (\pm\text{SD})$	$\text{Effn}_{\text{alleles}} (\pm\text{SD})$	$H_{\text{Obs}} (\pm\text{SD})$	$H_{\text{Exp}} (\pm\text{SD})$	N	n_{hap}	$h \pm \text{SD}$	$n (\%) \pm \text{SD}$		
South Africa-W	246	10.53 ± 1.28	5.16 ± 0.48	0.74 ± 0.03	0.77 ± 0.03	416	39	0.94 ± 0.01	2.4 ± 1.2	Carroll et al. (2019); this study	
Argentina-W	92	8.23 ± 0.84	4.73 ± 0.47	0.71 ± 0.04	0.76 ± 0.03	208	28	0.94 ± 0.01	2.3 ± 1.2	Carroll et al. (2019)	
Brazil-W	100	8.88 ± 0.97	4.85 ± 0.46	0.74 ± 0.04	0.76 ± 0.03	50	21	0.94 ± 0.02	2.6 ± 1.4	This study	
South Atlantic-W	440	11.94 ± 1.38	5.11 ± 0.49	0.73 ± 0.03	0.77 ± 0.03	674	57	0.97 ± 0.02	2.4 ± 1.2	Carroll et al. (2019); this study	
SG-S	22	6.63 ± 0.72	4.60 ± 0.56	0.75 ± 0.06	0.77 ± 0.06	11	10	0.98 ± 0.05	2.2 ± 1.3	This study	
Indo-Pacific-W	258	9.41 ± 0.83	4.98 ± 0.41	0.76 ± 0.02	0.77 ± 0.02	769	13	0.71 ± 0.01	1.6 ± 0.8	Carroll et al. (2015)	

Sample size (2N), mean number of alleles (n_{alleles}), effective number of alleles ($\text{Effn}_{\text{alleles}}$), observed (H_{Obs}) and expected (H_{Exp}) heterozygosity is reported for the microsatellite loci used in the analysis. Sample size (N), number of haplotypes (n_{hap}), haplotype diversity (h), and nucleotide diversity (n) are reported for the 381 bp fragment of the mtDNA control region analyzed. South Africa, Argentina and Brazil are pooled for the South Atlantic dataset.

described (Carroll et al. 2011, 2019). Briefly, as a precaution against false exclusion due to allelic dropout and other genotyping errors (Waits and Leberg 2000; Waits et al. 2001), the initial screening for replicates investigated every pair of genotypes that matched at 7 loci and mismatched at up to 3 loci. The electropherograms for any mismatching alleles were scrutinized for possible genotyping error (Bonin et al. 2004), and the locus was re-amplified and re-scored in both samples if necessary.

Estimating Genetic Diversity and Differentiation

Genetic diversity was calculated for all sampling locations except for Chile–Peru, because we had only 1 sample from this area. For the microsatellites, we estimated the actual and effective number of alleles and observed and expected heterozygosity using the program Genodive (Meirmans and van Tienderen 2004). For the mtDNA data, we report the number of haplotypes, haplotype diversity, and nucleotide diversity, calculated using Arlequin v3.5 (Excoffier and Lischer 2010). To visualize the placement of the mtDNA haplotypes found in SG and Chile–Peru in the broader dataset, we created a median-joining haplotype network (Bandelt et al. 1999) using PopART (Leigh and Bryant 2015).

We compared the SG dataset to the wintering ground samples in a hierarchical manner, starting by assessing the level of differentiation between SG and the ocean basin datasets (Indo-Pacific: comprising Australia and New Zealand; and South Atlantic: comprising Argentina, Brazil, and South Africa). The SG samples were significantly different from the Indo-Pacific samples, so we then compared the SG dataset to the South Atlantic samples grouped by wintering ground. This was done in a pairwise manner, and also included assessments of genetic differentiation between each pair of wintering grounds, in particular Brazil and Argentina.

For microsatellites, differentiation was estimated using F_{ST} calculated in GENEPOP and Jost's D (Jost 2008) calculated in Genodive. Significance was assessed using the exact G test in GENEPOP with 100K dememorizations, 1K batches and 10K iterations per batch. For mtDNA, differentiation was estimated using F_{ST} and ϕ_{ST} calculated in Arlequin, the latter using the Kimura 2-Parameter evolutionary model (Kimura 1980). Significance was assessed using permutation tests and, given the small sample size from SG, the exact test of differentiation (1,000,000 Markov chain steps; 1,000,000 dememorization steps, with significance set at $\alpha = 0.05$).

Population Structure and Assignment

We conducted 2 distinct population structure and assignment analyses to investigate how the Chile–Peru and SG samples sat within the broader migratory network. The first analysis used the program STRUCTURE (Pritchard et al. 2000), which clusters samples into a specified number of populations, k , and estimates the proportion of each sample's genotype assigning to each cluster. We first used all data (published and newly generated) and ran the analysis in admixture mode with and without a location prior (sampling location). Ten replicates of $k = 1$ to 5 were conducted each with burn-ins of 1 million iterations and runs of 10 million Markov chain Monte Carlo (MCMC) iterations, and convergence was assessed by visually inspecting the summary statistics (e.g., F_{ST}). Where structure was detected, the dataset was split and reanalyzed with the same settings to detect any further substructure. We assessed the optimal value of k using the mean log-likelihood from across the 10 runs summarized with STRUCTURE HARVESTER (Earl and VonHoldt 2012). We used CLUMPAK (Kopelman et al. 2015) to summarize the modes or distinct solutions for each value of k .

We also used the population visualization and assignment program GENELOT (McMillan and Fewster 2017) to determine the ability of the data to assign SG samples to reference wintering ground datasets. Given the low level of differentiation seen between wintering grounds, this population assignment method is preferable to a mixed stock analysis (MSA) such as ONCOR (Kalinowski et al. 2008). This is because when F_{ST} is low, MSA analyses are biased towards estimating that each of the k reference populations contributes proportion $1/k$ to the stock composition (ONCOR manual). GENELOT calculates the fit of the reference samples, and the fit of the query samples, into reference populations by calculating the log genotype probability (LGP) of the individual's genotype arising in each population using the method of Piry et al. (2004), approximated for individuals with missing data using the method of McMillan and Fewster (2017). If an individual has a high LGP for a given reference population, it indicates that the genotype of that individual has a high probability of arising from that population, though the individual may have high LGP for more than 1 population. We used the leave-one-out setting, which removes each individual from its sampling population before calculating its LGP fit to that population. The program also calculates the "home assignment probability" (HAP) for each pair of reference populations. The HAP is the probability that a random genotype arising from reference population A will be correctly assigned to its source population A, rather than to the alternative reference population B.

We used GENELOT to assess the genetic distinctiveness of the South Atlantic wintering grounds by visually inspecting the displays and calculating pairwise HAPs. Concurrently, we investigated whether the SG samples demonstrated preferential fit to either of the wintering grounds in each pair. GENELOT comparisons were made between Argentina versus Brazil, and between the latter 2 combined into Southwest Atlantic versus South Africa.

Results

Genotyping Success

DNA profiles were constructed for all 3 SG samples collected in 2018 and 9 samples collected in 1997 (average of 15.7 loci). Comparison of genotypes revealed that 1 whale was sampled on 2 occasions in 1997, based on a DNA profile that matched at sex, mtDNA haplotype and microsatellite genotype (Supplementary Material 1). PCR inhibition was a problem for the 1997 samples, so samples were cleaned using the Zymo 1-step PCR inhibitor removal kit and re-genotyped. The Chile–Peru sample was also re-extracted and re-genotyped. Of the 162 loci re-amplified across these 12 samples, there was only 1 allelic mismatch, giving a per-allele error rate of 0.003, comparable with the previous right whale genotyping research error rate of 0.007 per allele (Carroll et al. 2019).

Of the 60 Brazilian samples, 59 produced genotypes that passed quality control (QC) and comprised an average of 16.6 loci. Comparison of the genotypes showed that 59 genotypes represented 50 unique individuals (Supplementary Material 1). Of the 88 South African samples, 86 produced genotypes that passed QC and comprised an average of 15.5 loci. Comparison of the genotypes showed that the 86 genotypes represented 77 unique individuals (Supplementary Material 1). This included a match between the new South African genotypes and the existing dataset, which matched at 15 loci for a PID of 1.10E-18. Only 1 copy of this genotype was retained for analyses.

Comparison of genotypes across all sampling locations revealed 1 potential recapture between wintering grounds. This was a female

with a calf sampled in southern Brazil (~28°S) in 2000 and then in Peninsula Valdés in 2006. These samples matched at 16 available loci, genetically identified sex, and mtDNA haplotype; the probability of identity ($PID = 6.55E-21$) gives high confidence in the match (Supplementary Material 1). The sample was retained in both Argentinean and Brazilian datasets for wintering-ground based comparisons, but only a single copy was retained for the Southwest Atlantic-based analyses.

Overall, 361 unique genotypes, assumed to represent unique individuals, were used in the analysis. These were genotyped at an average of 16.1 loci per individual: 48% of genotypes were complete, and 44% missing 1–2 loci, and overall the dataset had 5.5% missing data. No pairs of loci were found to be in significant linkage disequilibrium. Analysis of the overall dataset showed deviation from Hardy-Weinberg in 3 loci: CA232, GT122, and TR3G1, which all indicated the presence of null alleles. The first 2 loci did not show a consistent pattern across sampling partitions (some showed excess while others deficiency of heterozygotes; all nonsignificant), suggesting that this may be an artifact of pooling sub-populations. The third locus, TR3G1, did show significant deviation from the Hardy-Weinberg equilibrium with null alleles detected in the overall and South African dataset, but this was not significant in other partitions. All population structure analyses were run with and without TR3G1, but no substantive differences were found, so results from all loci are reported.

Genetic Diversity

As previously described (Carroll et al. 2019), the microsatellite diversity was comparable but mtDNA diversity was higher in the South Atlantic compared with the Indo-Pacific. All South Atlantic wintering grounds had very similar levels of genetic diversity (Table 1) for both mtDNA and microsatellite data. Chile was not included in this analysis, given the sample size of 1.

Chile–Peru Sample Assignment

The Chile–Peru sample had an mtDNA haplotype previously only found in the Indo-Pacific (BakHapC; see Figure 2). The Chile–Peru sample appeared to be admixed between the Indo-Pacific and South Atlantic populations, with a higher proportion from the Indo-Pacific, based on the STRUCTURE analysis with and without location

prior settings (see Figure 3 and Supplementary Figures S1 and S2 in Supplementary Material 2). The Chile–Peru sample also had a strong fit (high LGP) to both oceans using GENELOT (Figure 4).

Genetic Population Structure of South Atlantic Wintering and SG Feeding Grounds

As would be expected from geographic proximity, the SG samples were clearly South Atlantic (Argentina, Brazil, and South Africa) in origin, based on significant differentiation seen in both mtDNA ($F_{ST} = 0.287$, $\phi_{ST} = 0.081$, $P < 0.001$) and microsatellite markers ($F_{ST} = 0.026$, Jost's $D = 0.026$, $P < 0.001$) when compared to the Indo-Pacific dataset, and the lack of significant differentiation when compared to combined South Atlantic samples (Table 2). Of the 10 mtDNA haplotypes found in SG based on the 381 bp fragment, 3 were found in all 3 South Atlantic wintering grounds, 6 were shared with only the Southwest Atlantic wintering grounds, and 1 (BakHapE) was shared with only the South African wintering ground (Figure 2).

The South Atlantic wintering grounds showed statistically significant levels of genetic differentiation based on both mtDNA and microsatellite markers (all comparisons $P < 0.05$). The Brazilian wintering ground showed higher levels of microsatellite differentiation from South Africa (Jost's $D = 0.010$, $P < 0.001$) compared with Argentina (Jost's $D = 0.001$, $P < 0.05$). The highest levels of differentiation were between Argentina and South Africa from the mtDNA data ($F_{ST} = 0.048$, $\phi_{ST} = 0.081$, $P < 0.001$).

The SG feeding ground appeared most genetically similar to the Argentina and Brazilian wintering grounds, with no statistically significant genetic differentiation detected (Table 3, $P > 0.05$). In contrast, there was significant mtDNA differentiation ($F_{ST} = 0.018$, $\phi_{ST} = 0.071$, $P < 0.001$) between SG and South Africa. The microsatellite loci also showed a substantively higher degree of differentiation between SG and South Africa (Jost's $D = 0.019$, $F_{ST} = 0.005$) compared with the Southwest Atlantic wintering grounds, but neither comparison was statistically significant.

Overall, the STRUCTURE analysis supported previous findings of weak differentiation between ocean basins (Supplementary Material 2). When using a standard admixture model, the mean log-likelihood was highest at $k = 3$ and under the location prior setting, the mean log-likelihood was highest at $k = 2$ (Figure 3,

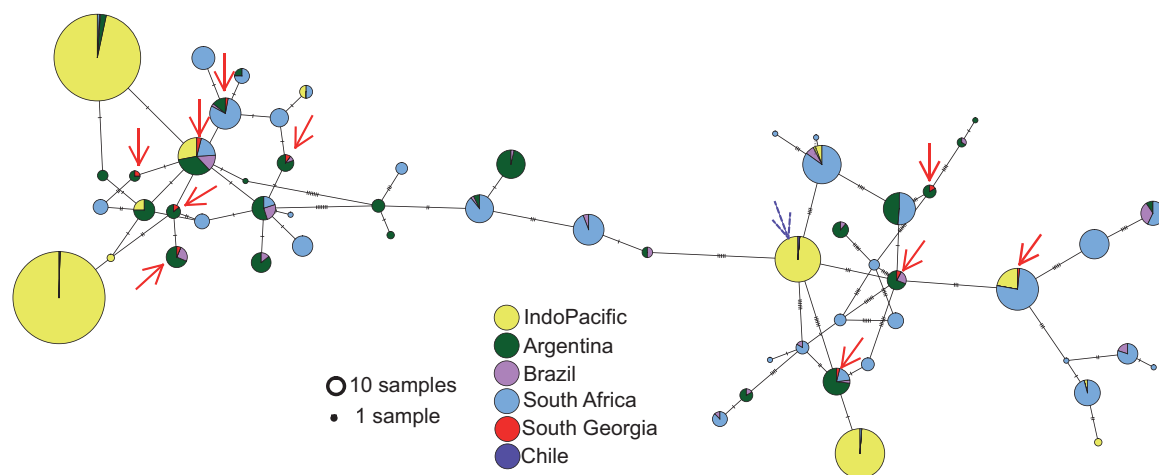


Figure 2. Haplotype network of southern right whale mtDNA control region (381 bp) showing the position of Chile–Peru sample (indicated by dashed purple arrow) and South Georgia (Islas Georgias del Sur) samples (red block arrows) in the broader southern right whale haplotype network.

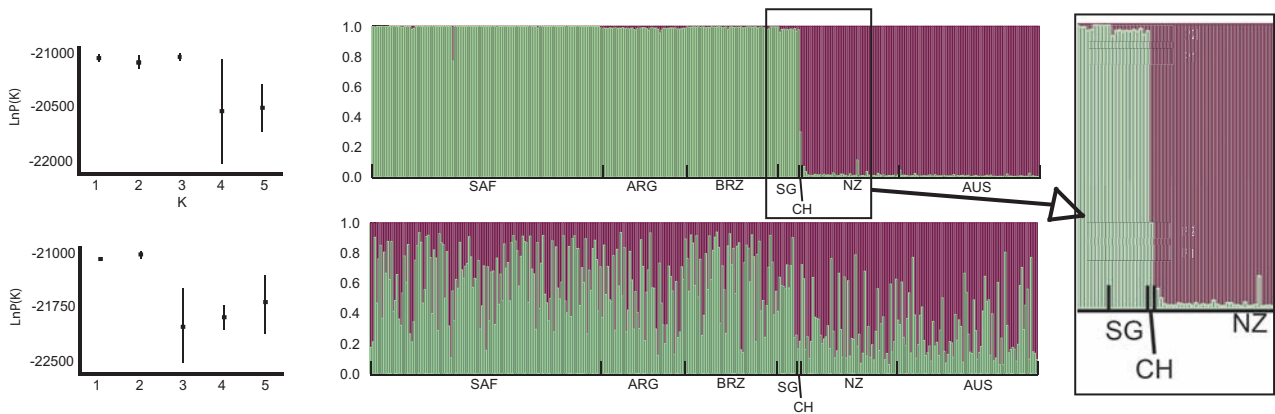


Figure 3. Mean log-likelihood ($\text{LnP}(K)$), for $K = 1$ to 5 (left), and the proportion of each individual's genome that is assigned to each cluster when $K = 2$ (right) for location prior with admixture setting (top) and standard admixture setting (below) implemented. SAF = South Africa; ARG = Argentina; BRZ = Brazil; SG = South Georgia (Islas Georgias del Sur); CH = Chile-Peru; NZ = New Zealand; AUS = Australia. Inset shows fine detail for Chile-Peru sample.

Supplementary Table S1). However, visual inspection of the ancestry proportion plot for $k = 3$ revealed admixture differences only between the South Atlantic and the Indo-Pacific, as also observed for $k = 2$ (Supplementary Figure S1). Therefore, $k = 2$ is presented in Figure 3, and $k = 3$ in Supplementary Material 2 (Supplementary Figures S1 and S2). This pattern is consistent with previous findings based on 222 samples (Carroll et al. 2019).

When analyzing the South Atlantic dataset, both the standard admixture and location prior setting returned an optimum of $k = 1$ using the mean log-likelihood method (Supplementary Table S2, Supplementary Figures S3 and S4). However, there was an evident structure at $k = 2$ and $k = 3$ in the location prior analyses, with distinct admixture patterns visible between the South African and Southwest Atlantic samples (Supplementary Figure S4).

The GENEPLLOT analyses demonstrated that the SG samples were more closely associated with the South Atlantic wintering grounds than those in the Indo-Pacific (Figure 4). Within the South Atlantic, some genetic distinctiveness was apparent amongst the South Atlantic wintering grounds, but the resolution was not sufficient to assign any individual SG sample with high confidence. This is shown visually in Figure 5. All SG samples had high LGPs for Brazil, Argentina, and South Africa, meaning the sampled genotypes had a high probability of arising in any of these regions. However, when considering the 11 SG samples as a group, there was reasonable evidence they were more closely associated with the Southwest Atlantic wintering ground than South Africa, with 9 of the 11 samples yielding a higher LGP for Southwest Atlantic than South Africa. Based on the corresponding HAPs (Supplementary Table S1), which give the per-sample probability that the LGP is higher in the source population, this presents significant evidence against the hypothesis that all 11 samples originated from South Africa (HAP 0.66; $P = 0.002$), but no evidence against the hypothesis that all 11 samples arose from the Southwest Atlantic (HAP 0.62; $P = 0.96$). In general, HAPs within the region were low, with the greatest differentiation being a modest 0.79 for correct assignment of South African genotypes against an Argentinean alternative (Supplementary Table S3).

Discussion

Here we have used an expanded genetic dataset (361 vs. 222 microsatellite genotypes previously used) to investigate the position of a

southern right whale foraging ground, SG, and 2 wintering grounds (Brazil and Chile-Peru) in the species' circumpolar migratory network using both microsatellite and mtDNA data from across the Southern Hemisphere. Differentiation indices, STRUCTURE and GENEPLLOT analyses strongly suggested that the SG feeding ground is associated with wintering grounds in the South Atlantic, rather than Indo-Pacific. Within the South Atlantic, differentiation indices suggest a closer association of SG to the Southwest Atlantic wintering grounds (Argentina and Brazil) than to the South African wintering ground. However, the assignment method did not exclude the possibility of occasional movement of individuals from South Africa to SG. Brazil shows a small but statistically significant level of genetic differentiation from its neighboring nursery ground in Argentina, and the Chile-Peru sample appeared admixed between the South Atlantic and the Indo-Pacific Ocean basins.

This work analyzed nearly 140 new samples to augment an existing circumpolar dataset (Carroll et al. 2019), showing the utility of long-term international collaborations to provide information useful for conservation and management of exploited marine species. The analyses conducted here also highlight parallels with the well-studied humpback whale, which also shows hierarchical population structure with greater differentiation between than within ocean basins (Kershaw et al. 2017; Rosenbaum et al. 2017). For example, genetic studies conducted to date in humpback whales show statistically significant genetic nuclear and mitochondrial differentiation between the Southeast Pacific population (wintering in central America and Colombia, and summering in the Antarctic Peninsula) and the Brazilian Southwest Atlantic population (Cypriano-Souza et al. 2017). This suggests a similar historic pattern of long-term differentiation between the Atlantic and Pacific applies to both species.

For humpback whales within the South Atlantic, wintering grounds in the Southwest and Southeast Atlantic (Brazil and Gabon, respectively) are weakly differentiated by microsatellites, suggesting regular gene flow between these populations (Kershaw et al. 2017). Our patterns show slightly stronger wintering ground differentiation by southern right whales between the Southwest (Brazil and Argentina) and Southeast Atlantic (South Africa). Increased connectivity in humpback whales compared with southern right whales could be due to greater overlap of foraging areas in humpback whales (Zerbini et al. 2006, 2011; Seakamela et al. 2015) compared with southern right whales. It could also be related to recovery and density-dependent

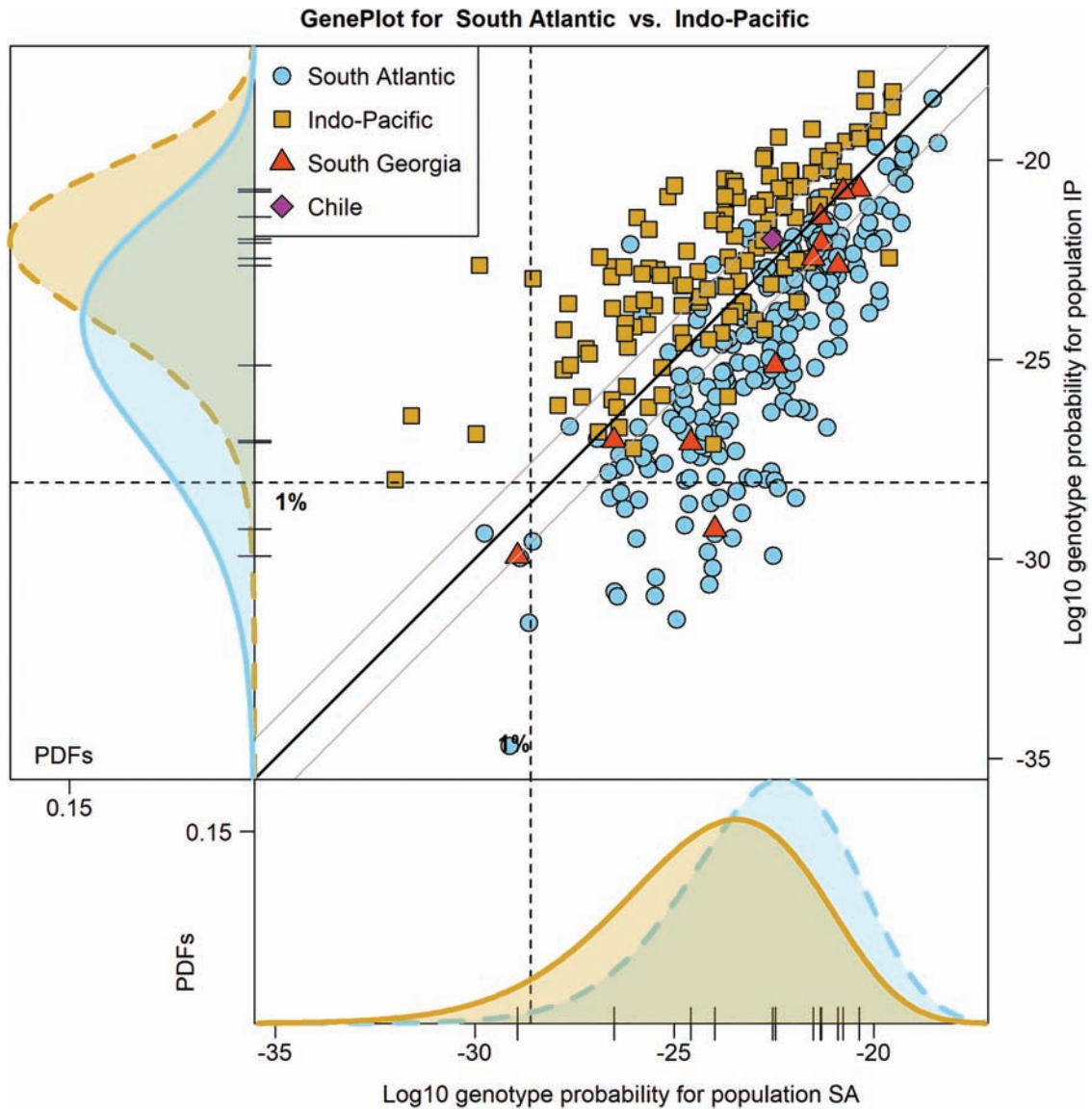


Figure 4. GENEPLLOT of southern right whale microsatellite genotypes showing LGP for South Atlantic and Indo-Pacific reference populations, with South Georgia (Islas Georgias del Sur) and Chile-Peru query samples. The thick central diagonal line indicates an equal posterior probability for both reference populations; a point lying on that line has the same LGP with respect to both reference populations. The thin diagonal lines indicate where the genotype probability (the inverse-log of the LGP) for one population is 10 times greater than it is for the other population. Also shown are the 1% quantiles: only 1% of genotypes that could arise from the given population are estimated to have LGPs below this line. The marginal plots show the home (dashed line) and away (solid line) profiles of LGPs within each reference population, with rug plots indicating the LGPs of the query samples on each axis. Bottom margin – home: South Atlantic; away: Indo-Pacific. Left margin – home: Indo-Pacific; away: South Atlantic.

processes; the Southwest Atlantic humpback whale population is approaching pre-whaling abundance (Zerbini et al. 2019), and while such information is not available for Southwest Atlantic southern right whales, comparable work done in New Zealand (Jackson et al. 2016) and at a global scale (Jackson et al. 2008) suggests southern right whales are still far from pre-exploitation levels.

Within the Southwest Atlantic, we see subtle but statistically significant genetic differentiation between southern right whales sampled in Brazil and Argentina in both mtDNA ($F_{ST} = 0.024$, $\phi_{ST} = 0.028$, $P < 0.001$) and microsatellite markers ($F_{ST} = 0.001$, Jost's $D = 0.001$, $P < 0.05$). This magnitude of differentiation is lower than that used to define southeast and southwest Australian right whale wintering ground as distinct stocks (mtDNA $F_{ST} = 0.098$,

microsatellite $F_{ST} = 0.013$, $P < 0.05$, Carroll et al. 2019). Thus, we believe the difference between Brazil and Argentina likely reflects differences in the demographic aggregation of whales using each region, as also seen in humpback whales and linked to female philopatry (Rosenbaum et al. 2017). However, the changing distribution of southern right whales as this population recovers (Crespo et al. 2019), and likely immigration from Argentina into Brazil (Groch et al. 2005), suggest that patterns of local fidelity and movement are complex and do not currently suggest multiple distinct populations calving in the Southwest Atlantic. Further analyses of movements between the 2 regions via photo-ID, genotyping, and satellite tracking are expected to shed more light on patterns of exchange of whales across this region.

Our findings suggest that it is more likely that SG is visited by whales from the Southwest Atlantic than from South Africa. The connection between SG and the Southwest Atlantic is consistent with several lines of existing evidence. Firstly, previous unpublished analyses of 10 microsatellite loci from the samples collected in SG in 1997 together with samples from the Southwest Atlantic wintering grounds did not find significant differentiation (Ott 2002; Ott et al. 2011). Secondly, there have been photo-ID matches between whales in SG and Península Valdés (Best et al. 1993; Moore et al. 1999). In contrast, comparison of photos collected during the SG surveys of 1997 (Moore et al. 1999) and 2018 (J. Jackson et al. in preparation) and the South African wintering ground catalog did not produce any matches (E. Vermeulen, unpublished data). Finally, satellite tagging studies have shown movement from Península Valdés, Argentina (Zerbini et al. 2015, 2018), but not from South Africa, to SG (Mate et al. 2011), although the latter study had a small sample size (5 whales were tracked offshore). Southern right whales wintering in South Africa appear to feed mainly to the east of SG (20°W–20°E), including Bouvet Island (Mate et al. 2011) (Figure 1), so might not visit SG (Best 1970, 1981; Best and Schell 1996).

Despite these findings, there could be connectivity between South Africa and SG. One sample collected from SG had an mtDNA haplotype only found in South Africa to date. Additionally, our analysis might underestimate connectivity between South Africa and SG due to the timing of our sampling surveys. Stable isotope data from the contemporary population and historical whaling data suggest that South African right whales might not feed below the sub-tropical

convergence until the late austral summer and autumn (February to May). Thus, it could be that we are only able to sample a subset of the whales that feed at SG during our summer expedition (Best 1970, 1981; Best and Schell 1996). However, bearing in mind that the degree of genetic differentiation was higher but non-significant between South Africa and SG (Jost's $D = 0.019$, $P = 0.13$) than it was between South Africa and the Southwest Atlantic wintering grounds (Jost's $D = 0.008$ – 0.010 , $P < .001$), and the overall HAP was consistent with all SG samples originating from the Southwest Atlantic, it could simply be that the small SG sample size and generally weak differentiation seen in southern right whales means that we lack adequate power to rule out South Africa as a migratory connection to SG. Further analysis could help to improve the resolution of this pattern, including more samples, additional loci, and inclusion of samples collected from whales feeding at SG during the autumn and winter.

Here we also analyzed the first genetic sample from the Chile–Peru southern right whale wintering ground. We are unable to make any firm statements about the genetic identity of this population, owing to the sample size of 1. However, our findings suggest hypotheses that could be tested with additional data. Surprisingly, the single microsatellite genotype appears to be admixed between the South Atlantic and Indo-Pacific, whilst having a maternal lineage (BakHapC) previously only found in the Indo-Pacific. Thus, the Chile–Peru population could have historically been a “stepping stone” between the South Atlantic and Indo-Pacific, rather than more closely linked to the Southwest Atlantic wintering grounds as we had initially hypothesized. The limited observational data show no trend in the number of sightings or individuals in the Chile–Peru stock over time, although there has been a small increase in the number of calves between 1975 and 2010 (Galletti Vernazzani et al. 2014, 2016). These findings highlight the importance of continuing to monitor the region and collect additional samples as the species recovers from whaling.

Overall this study yields patterns of genetic differentiation, which are similar to those previously observed for humpback whales (Schmitt et al. 2014; Kershaw et al. 2017; Rosenbaum et al. 2017), where neighboring breeding grounds show weak but statistically significant differentiation. We suggest that this is probably driven by a combination of philopatry and isolation by distance, with shared foraging grounds increasing the chance of genetic connectivity. While stronger divergence in mitochondrial compared with nuclear markers seen in both species is consistent with female philopatry, both sexes show fidelity to natal wintering grounds and sex-biased dispersal has not been detected in either species (Kershaw et al. 2017; Carroll et al. 2019), suggesting that gene flow occurs in both sexes.

Table 2. Estimates of genetic differentiation between the Indo-Pacific and South Atlantic wintering grounds and the SG feeding ground, with (A) mtDNA table showing ϕ_{ST} (top right), F_{ST} (bottom left quadrant) and (B) microsatellites showing Jost's D (top right) and F_{ST} (bottom left quadrant)

A.mtDNA	South Atlantic	SG	Indo-Pacific
South Atlantic		0.026	0.189***§
SG	0.000		0.081*§
Indo-Pacific	0.159***§	0.287***§	
B.Microsatellites	South Atlantic	SG	Indo-Pacific
South Atlantic		0.007	0.035***
SG	0.002		0.026***
Indo-Pacific	0.011 ***	0.007***	

Significance: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ from permutation test; § $P < 0.001$ from exact test of differentiation for mtDNA data.

Table 3. Pairwise genetic differentiation indices between South Atlantic sampling sites with (A) mtDNA table showing ϕ_{ST} (top right), F_{ST} (bottom left quadrant) and (B) microsatellites showing Jost's D (top right) and F_{ST} (bottom left quadrant)

A.mtDNA	Argentina	Brazil	South Africa	SG
Argentina		0.028*§	0.081***§	0.000
Brazil	0.024***§		0.022*§	0.010
South Africa	0.048***§	0.034***§		0.071***§
SG	0.000	0.001	0.018*§	
B.Microsatellites	Argentina	Brazil	South Africa	SG
Argentina		0.001*	0.008***	0.000
Brazil	0.001*		0.010**	0.000
South Africa	0.002***	0.003**		0.019
SG	0.000	0.000	0.005	

Significance: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$ from permutation test; § $P < 0.001$ from exact test of differentiation for mtDNA data.

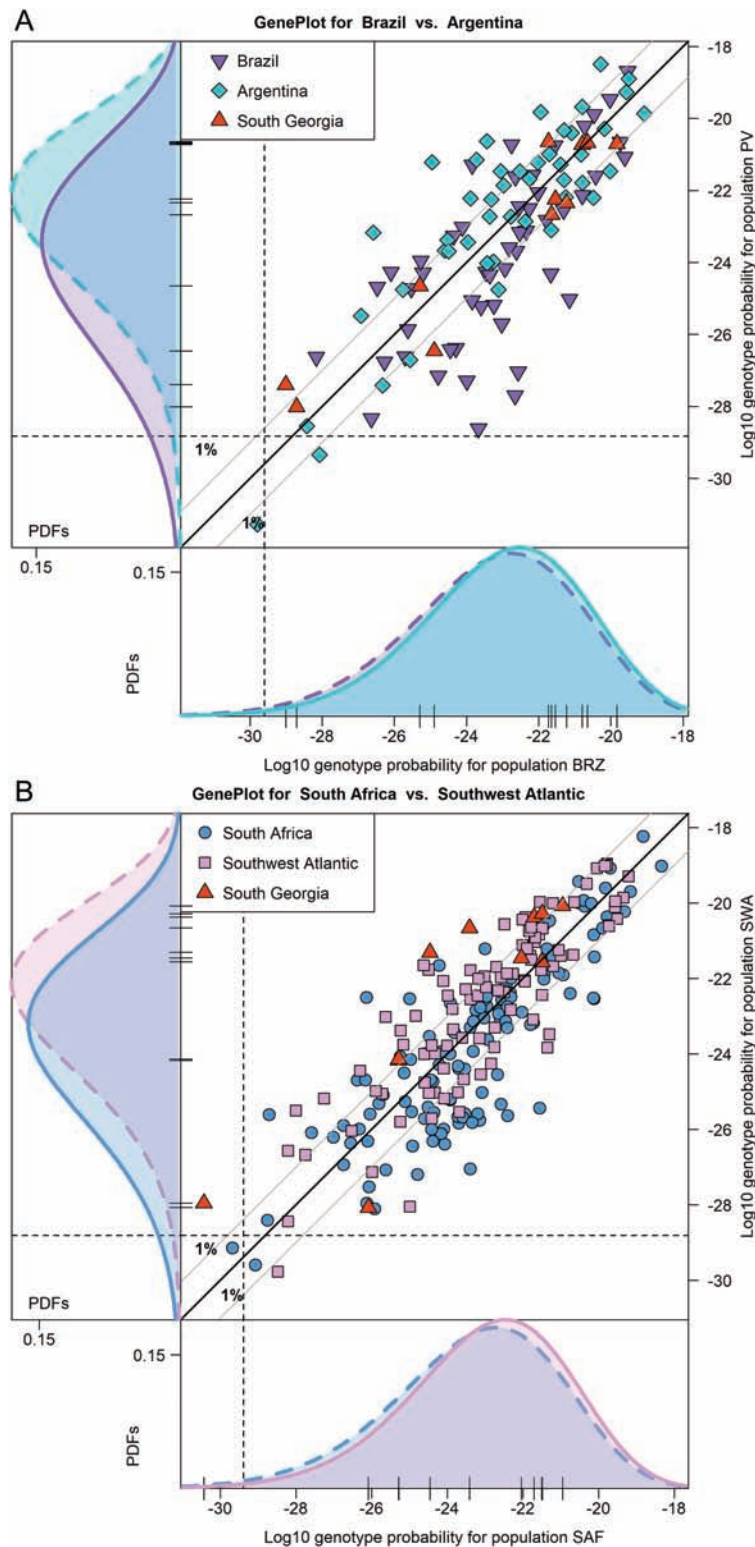


Figure 5. GENEPLots of southern right whale microsatellite genotypes showing LGPs of South Georgia query samples plotted against reference populations of: **(A)** Brazil and Argentina, with dashed lines in the marginal plots corresponding to home populations of Brazil in the bottom margin and Argentina in the left margin; **(B)** South Africa and Southwest Atlantic (Brazil and Argentina combined), with dashed lines in the marginal plots corresponding to home populations of South Africa in the bottom margin and Southwest Atlantic in the left margin. Other plotting details are the same as those in Figure 4.

In contrast to humpbacks, southern right whales have suffered an extended bottleneck period of about 150 years, and virtual local extirpation in some former wintering areas, including the Chile/

Peru, Namibia, and Tristan Da Cunha (IWC 2001). Right whale population growth is also slower as they calve less frequently than humpbacks (Zerbini et al. 2010), and as a consequence, the latter is

likely much further from recovery (IWC 2012; Jackson et al. 2015). Additionally, southern right whales show an interesting dichotomy of long-term fidelity to wintering grounds and sudden shifts in range (Carroll et al. 2019), epitomized by their apparent sudden reappearance in FI waters (Weir and Stanworth 2019). Consequently, we conclude that patterns of population connectivity are likely to continue to change as populations recover. Genetic monitoring of population connectivity patterns over this time will provide important insights into changes in whale movements and connectivity in relation to recovery, ensuring that appropriate conservation management measures can be put in place. Such work is only possible through long-term collaborations that use standardized approaches, to ensure comparability across time of data, regardless of population and laboratory of origin.

Supplementary Material

Supplementary material is available at *Journal of Heredity* online.

Funding

This work was supported by the EU BEST 2.0 medium grant 1594 and UK DARWIN PLUS grant 057 and additional funding from the World Wildlife Fund GB107301. The collection of the Chile–Peru sample was supported by the Global Greengrants Fund and the Pacific Whale Foundation. The collection of the Brazilian samples was supported through grants by the Brazilian National Research Council to Paulo H. Ott (CNPq proc. n° 144064/98-7) and Paulo A.C. Flores (CNPq proc. n° 146609/1999-9) and with support from the World Wildlife Fund (WWF-Brazil). The collection of the South African samples was supported by the Global Greengrants Fund, the Pacific Whale Foundation and Charles University Grant Agency (1140217). E.L.C. was partially supported by a Rutherford Discovery Fellowship from the Royal Society of New Zealand. This study forms part of the Ecosystems component of the British Antarctic Survey Polar Sciences for Planet Earth Programme, funded by the Natural Environment Research Council.

Acknowledgments

For the SG samples, we thank Marine Conservation Research International for vessel charter support and Steve Dent and the Veterinary Department in Stanley. For the Chile–Peru sample, the authors thank Jose Aviles for his contribution to the sighting, reporting of the stranding, and the necropsy work; the National Fisheries and Aquaculture Service for assistance with the necropsy; Antonio Palma, Mauricio Ulloa, Marisol Romero and especially Hans Ossvald; and the Southern Right Whale Health Monitoring Program in Argentina for necropsy protocols. The Chile–Peru sample was transferred from Chile to the UK under Chilean CITES export permit 18000007WS and European Union CITES import permit 569116/01. The authors would like to express the sincere gratitude to Dr. Bradley White and all staff members of the Natural Resources DNA Profiling and Forensic Centre, Trent University, for the opportunity, support and invaluable contributions in the pioneering genetic analyses of the Brazilian southern right whales. For the South African samples, we thank Christopher Wilkinson, Meredith Thornton, Wilfred Chivell, Jason Keith Stafford, Dyer Island Conservation Trust, Dyer Island Cruises and Ivanhoe Sea Safaris for help and support. Samples were transferred to the UK under CITES permits: South African export permit 207896 and UK import permit 569921/01 (University of Pretoria samples) and under South African export permit 171354 and UK import permit 550270/01 (Charles University samples). Ethics and acknowledgements for the New Zealand and Australian datasets can be found in the original publication detailing the generation of these genetic data (Carroll et al. 2015). Figure 1 was made with ArcGIS Online

ocean basemap, with the following sources: Esri, GEBCO, NOAA, National Geographic, DeLorme, HERE, and Geonames.org.

Data Availability

We have deposited the primary data underlying these analyses as follows: Microsatellite data: genotypes of all samples used in analysis archived in genepop format at Dryad <https://doi.org/10.5061/dryad.pg4f4qrk8>; DNA sequences: Mitochondrial DNA haplotype frequencies for each region and linked Genbank Accession numbers in excel file, along with example Arlequin file, submitted to dryad <https://doi.org/10.5061/dryad.pg4f4qrk8>.

References

- Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N. 2008. Genetic effects of harvest on wild animal populations. *Trends Ecol Evol.* 23:327–337.
- Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol.* 16:37–48.
- Bannister JL. 2009. Southern right whale aerial survey, southern Australian coast, 2009. Final report on work funded by the Island Foundation. Massachusetts (MA), pp. 17.
- Best P. 1970. Exploitation and recovery of right whales *Eubalaena australis* off the Cape Province. *Investigational Report (Sea Fisheries Institute, South Africa)*. 80:1–20.
- Best P. 1981. The status of right whales (*E. glacialis*) off South Africa, 1969–1979. *Investigational Report (Sea Fisheries Institute, South Africa)*. 123:1–44.
- Best P. 1994. Seasonality of reproduction and the length of gestation in southern right whales *Eubalaena australis*. *J Zool (Lond)*. 232:175–189.
- Best P, Elwen SH, Palsbøll PJ, Thornton M, Austin E, Vinding K. 2015. Possible non-offspring nursing in the southern right whale, *Eubalaena australis*. *J Mammal.* 96:405–416.
- Best P, Glass J, Ryan P, Dalebout M. 2009. Cetacean records from Tristan de Cunha, South Atlantic. *J Mar Biol Assoc U K.* 89:1023–1032.
- Best P, Payne R, Rowntree VJ, Palazzo J, Both M. 1993. Long-range movements of South Atlantic right whales *Eubalaena australis*. *Mar Mamm Sci.* 9:227–234.
- Best P, Schell D. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements feeding and growth. *Mar Biol.* 124:483–494.
- Blundell GM, Ben-David M, Groves P, Bowers RT, Geffen E. 2002. Characteristics of sex-biased dispersal and gene flow in coastal river otters: implications for natural recolonization of extirpated populations. *Mol Ecol.* 11:289–303.
- Bonin A, Bellemain E, Bronken Eidesen P, Pompanon F, Brochmann C, Taberlet P. 2004. How to track and assess genotyping errors in population genetics studies. *Mol Ecol.* 13:3261–3273.
- Brandão A, Vermeulen E, Ross-Gillespie A, Findlay K, Butterworth DS. 2018. Updated application of a photo-identification based assessment model to southern right whales in South African waters, focussing on inferences to be drawn from a series of appreciably lower counts of calving females over 2015 to 2017. Report SC/67B/SH22 presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK. Available from: <https://iwc.int/home>
- Brown M, Kraus S, Gaskin DE. 1991. Reaction of North Atlantic right whales (*Eubalaena glacialis*) to skin biopsy sampling for genetic and pollutant analysis. *Report of the International Whaling Commission Special Issue.* 13:81–90.
- Burnell SR. 2001. Aspects of the reproductive biology, movements and site fidelity of right whales off Australia. *J Cetacean Res Manag Spec Issue.* 2:89–102.
- Carroll EL, Alderman R, Bannister JL, Bérubé M, Best PB, Boren L, Baker CS, Constantine R, Findlay K, Harcourt R, et al. 2019. Incorporating non-equilibrium dynamics into demographic history inferences of a migratory marine species. *Heredity.* 122:53–68.

- Carroll EL, Baker CS, Watson M, Alderman R, Bannister J, Gaggiotti OE, Gröcke DR, Patenaude N, Harcourt R. 2015. Cultural traditions across a migratory network shape the genetic structure of southern right whales around Australia and New Zealand. *Sci Rep.* 5:16182.
- Carroll EL, Childerhouse SJ, Fewster R, Patenaude N, Steel DJ, Dunshea G, Boren L, Baker CSS. 2013. Accounting for female reproductive cycles in a superpopulation capture–recapture framework. *Ecol Appl.* 23:1677–1690.
- Carroll EL, Patenaude NJ, Alexander A, Steel DJ, Harcourt R, Childerhouse SJ, Smith S, Bannister JL, Constantine R, Baker CS. 2011. Population structure and individual movement of southern right whales around New Zealand and Australia. *Mar Ecol Prog Ser.* 432:257–268.
- Carroll EL, Rayment WJ, Alexander AM, Baker CS, Patenaude NJ, Steel D, Constantine R, Cole R, Boren LJ, Childerhouse S. 2014. Reestablishment of former wintering grounds by New Zealand southern right whales. *Mar Mamm Sci.* 30:206–220.
- Clapham P, Aguilar A, Hatch LT. 2008. Determining spatial and temporal scales for management of cetaceans: lessons from whaling. *Mar Mamm Sci.* 24:183–201.
- Clapham P, Zerbini AN. 2015. Is social aggregation driving high rates of increase in some Southern Hemisphere humpback whale populations? *Mar Biol.* 162:625–634.
- Cooke J. 2018. *Eubalaena australis* (Chile-Peru subpopulation). The IUCN Red List of Threatened Species 2018: e.T133704A50385137. Available from: <https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T133704A50385137.en>.
- Cooke J, Rowntree VJ, Payne R. 2001. Estimates of demographic parameters for southern right whales (*Eubalaena australis*) observed off Península Valdés, Argentina. *J Cetacean Res Manag Spec Issue.* 2:125–132.
- Crespo EA, Pedraza SN, Dans SL, Svendsen GM, Degradi M, Coscarella MA. 2019. The southwestern Atlantic southern right whale, *Eubalaena australis*, population is growing but at a decelerated rate. *Mar Mamm Sci.* 35:93–107.
- Cypriano-Souza AL, Engel MH, Caballero S, Olavarria C, Flórez-González L, Capella J, Steel DJ, Sremba AL, Aguayo A, Thiele D, et al. 2017. Genetic differentiation between humpback whales (*Megaptera novaeangliae*) from Atlantic and Pacific breeding grounds of South America. *Mar Mamm Sci.* 33:457–479.
- Earl DA, VonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Resour.* 4:359–361.
- Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour.* 10:564–567.
- Galletti Vernazzani B, Arroyo P, Goya E, Palma A. 2016. Revised conservation management plan for eastern South Pacific southern right whale population (*Eubalaena australis*) - submitted by Chile and Peru April 2016. Report SC/66b/BRG/23 submitted to the Scientific Committee of the International Whaling, Cambridge, UK. Available from: <https://iwc.int/home>
- Galletti Vernazzani B, Cabrera E, Brownell RL. 2014. Eastern South Pacific southern right whale photo-identification catalog reveals behavior and habitat use patterns. *Mar Mamm Sci.* 30:389–398.
- Galletti Vernazzani B, Chirife A, Cabrera E, Sironi M, Brownell RL Jr. 2017. Entanglement and death of a critically endangered eastern South Pacific southern right whale (*Eubalaena australis*) in Chile. Report SC/67A/HIM/14 presented to the Scientific Committee of the International Whaling Commission. Cambridge (UK): Scientific Committee of the IWC. Available from: <https://iwc.int/home>
- Groch K, Palazzo J, Flores P, Ardlor F, Fabian M. 2005. Recent rapid increase in the right whale (*Eubalaena australis*) population off southern Brazil. *Latin American Journal of Aquatic Mammals.* 4:41–47.
- Harcourt R, van der Hoop J, Kraus S, Carroll EL. 2019. Future directions in *Eubalaena* spp.: comparative research to inform conservation. *Front Mar Sci.* 5:530.
- Harrison XA, Tregenza T, Inger R, Colhoun K, Dawson DA, Gudmundsson GA, Hodgson DJ, Horsburgh GJ, McElwaine G, Bearhop S. 2010. Cultural inheritance drives site fidelity and migratory connectivity in a long-distance migrant. *Mol Ecol.* 19:5484–5496.
- Herman L. 1979. Humpback whales in Hawaiian waters: a study in historical ecology. *Pacific Sci.* 33:1–15.
- IWC. 2001. Report of the workshop on the comprehensive assessment of right whales. *J Cetacean Res Manag Spec Issue.* 2:1–60.
- IWC. 2012. Report of the workshop on the assessment of southern right whales, Buenos Aires, Argentina 13–16 September 2011. Report SC/64/Rep5 presented to the Scientific Committee of the International Whaling Commission. Cambridge (UK): Scientific Committee of the IWC. Available from: <https://iwc.int/home>
- Jackson JA, Carroll EL, Kennedy A, Leaper R, Calderan S, Leslie MS, Andriolo A, Stepien E, Zerbini AN, Miller BS, et al. 2018. Sightings and acoustic records of right whales collected in South Georgia (Islas Georgias del Sur) waters January–February 2018. Report SC/67B/SH/20 submitted to the Scientific Committee of the International Whaling Commission. Cambridge (UK): Scientific Committee of the IWC. Available from: <https://iwc.int/home>
- Jackson JA, Carroll EL, Smith TD, Zerbini AN, Patenaude NJ, Baker CS. 2016. An integrated approach to historical population assessment of the great whales: case of the New Zealand southern right whale. *R Soc Open Sci.* 3:150669.
- Jackson JA, Patenaude NJ, Carroll EL, Baker CS. 2008. How few whales were there after whaling? Inference from contemporary mtDNA diversity. *Mol Ecol.* 17:236–251.
- Jackson JA, Ross-Gillespie A, Butterworth D, Findlay K, Holloway S, Robbins J, Rosenbaum H, Weinrich M, Baker CS, Zerbini AN. 2015. Southern hemisphere humpback whale comprehensive assessment - a synthesis and summary: 2005–2015. Report SC/66a/SH/03 to the Scientific Committee of the International Whaling Commission, Cambridge, UK. Available from: <https://iwc.int/home>.
- Jost L. 2008. G(ST) and its relatives do not measure differentiation. *Mol Ecol.* 17:4015–4026.
- Kalinowski S, Manlove K, Taper M. 2008. ONCOR: a computer program for genetic stock identification. Bozeman (MT): Department of Ecology, Montana State University. Available from: <http://www.montana.edu/kalinowski/Software/ONCOR.html>
- Kalinowski S, Taper M, Marshall T. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol.* 9:801–888.
- Kershaw F, Carvalho I, Loo J, Pomilla C, Best PB, Findlay KP, Cerchio S, Collins T, Engel MH, Minton G, et al. 2017. Multiple processes drive genetic structure of humpback whale (*Megaptera novaeangliae*) populations across spatial scales. *Mol Ecol.* 26:977–994.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 16:111–120.
- Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Mol Ecol Resour.* 15:1179–1191.
- Krützen M, Barré L, Möller L, Heithaus M, Simmer C, Sherwin WB. 2002. A biopsy system for small cetaceans; darting success and wound healing in *Tursiops* spp. *Mar Mamm Sci.* 18:863–878.
- Lambertsen R. 1987. A biopsy system for large whales and its use for cytogenetics. *J Mammal.* 68:443–445.
- Leigh JW, Bryant D. 2015. Popart: full-feature software for haplotype network construction. *Methods Ecol Evol.* 6:1110–1116.
- Lockyer C. 1984. Review of baleen whale (Mysticeti) reproduction and implications for management. *Report of the International Whaling Commission Special Issue.* 6:27–50.
- Mate B, Best P, Lagerquist B, Winsor M. 2011. Coastal, offshore and migratory movements of South African right whales revealed by satellite telemetry. *Mar Mamm Sci.* 27:455–476.
- McMillan LF, Fewster RM. 2017. Visualizations for genetic assignment analyses using the saddlepoint approximation method. *Biometrics.* 73:1029–1041.

- Meirmans P, van Tienderen P. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes*. 4:792–794.
- Moore MJ, Berrow SD, Jensen BA, Carr P, Sears R, Rowntree VJ, Payne R, Hamilton P. 1999. Relative abundance of large whales around South Georgia (1979–1998). *Mar Mamm Sci*. 15:1287–1302.
- Mysterud A. 2011. Selective harvesting of large mammals: how often does it result in directional selection? *J Appl Ecol*. 48:827–834.
- Ott PH. 2002. *Diversidade genética e estrutura populacional de duas espécies de cetáceos do Atlântico Sul Ocidental: Pontoporia blainvillei e Eubalaena australis* [Ph.D. dissertation]. Porto Alegre (Brazil): Universidade Federal do Rio Grande do Sul.
- Ott PH, Flores PAC, Freitas TRO, White BN. 2011. Genetic diversity and population structure of southern right whales, *Eubalaena australis*, from the Atlantic coast of South America. Report SC/12/RW25 presented to the Scientific Committee of the International Whaling Commission. Cambridge (UK): Scientific Committee of the IWC. Available from: <https://iwc.int/home>
- Patenaude NJ, Portway VA, Schaeff CM, Bannister JL, Best PB, Payne RS, Rowntree VJ, Rivarola M, Baker CS. 2007. Mitochondrial DNA diversity and population structure among southern right whales (*Eubalaena australis*). *J Hered*. 98:147–157.
- Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. *J Hered*. 95:536–539.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*. 155:945–959.
- Richardson J, Wood A, Neil A, Nowacek D, Moore M. 2012. Changes in large whale distribution, relative abundance, and species composition around South Georgia from opportunistic sightings: 1992–2011. *Endangered Species Research*. 19:149–156.
- Rosenbaum HC, Kershaw F, Mendez M, Pomilla C, Leslie MS, Findlay KP, Best PB, Collins T, Very M, Engel MH, et al. 2017. First circumglobal assessment of Southern Hemisphere humpback whale mitochondrial genetic variation and implications for management. *Endangered Species Research*. 32:551–567.
- Rousset F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour*. 8:103–106.
- Roux JP, Braby RJ, Best P. 2015. Does disappearance mean extirpation? The case of right whales off Namibia. *Mar Mamm Sci*. 31:1132–1152.
- Rowntree VJ, Payne R, Schell D. 2001. Changing patterns of habitat use by southern right whales (*Eubalaena australis*) on their nursery ground at Península Valdés, Argentina, and in their long-range movements. *J Cetacean Res Manag Spec Issue*. 2:133–143.
- Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Schmitt NT, Double MC, Jarman SN, Gales N, Marthick JR, Polanowski AM, Baker CS, Steel D, Jenner KCS, Jenner MNM, et al. 2014. Low levels of genetic differentiation characterize Australian humpback whale (*Megaptera novaeangliae*) populations. *Mar. Mammal Sci*. 30:221–241.
- Seakamela S, Findlay K, Meyer M, Kirkman S, Venter K, Mdokwana B, Tsanwani M, Shabangu F, Wilke C, Ismail H. 2015. Report of the 2014 cetacean distribution and abundance survey of South Africa's west coast. Report SC/66a/SH30 presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.
- Servanty S, Gaillard JM, Ronchi F, Focardi S, Baubet É, Gimenez O. 2011. Influence of harvesting pressure on demographic tactics: implications for wildlife management. *J Appl Ecol*. 48:835–843.
- Thomas L, Harwood J. 2003. Estimating grey seal population size using a Bayesian state-space model. SCOS Briefing paper 03/3, Sea Mammal Research Unit, University of St Andrews. Available from: <http://www.smru.st-andrews.ac.uk/research-policy/scos/>.
- Tinker MT, Doak DF, Estes JA. 2008. Using demography and movement behavior to predict range expansion of the southern sea otter. *Ecol Appl*. 18:1781–1794.
- Uusi-Heikkilä S, Whiteley AR, Kuparinen A, Matsumura S, Venturelli PA, Wolter C, Slate J, Primmer CR, Meinelt T, Killen SS, et al. 2015. The evolutionary legacy of size-selective harvesting extends from genes to populations. *Evol Appl*. 8:597–620.
- Valenzuela LO, Rowntree VJ, Sironi M, Seger J. 2018. Stable isotopes in skin reveal diverse food sources used by southern right whales (*Eubalaena australis*). *Mar Ecol Prog Ser*. 603:243–255.
- Valenzuela LO, Sironi M, Rowntree VJ, Seger J. 2009. Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). *Mol Ecol*. 18:782–791.
- Waits LP, Leberg PL. 2000. Biases associated with population estimation using molecular tagging. *Anim Conserv*. 3:191–199.
- Waits LP, Luikart G, Taberlet P. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol*. 10:249–256.
- Waples RS, Hoelzel RA, Gaggiotti OE, Tiedemann R, Palsbøll PJ, Cipriano F, Jackson JA, Bickham JW, Lang A. 2018. Guidelines for genetic data analysis. *J Cetacean Res Manag*. 18:33–80.
- Weir CR, Stanworth A. 2019. The Falkland Islands (Malvinas) as sub-Antarctic foraging, migratory and wintering habitat for southern right whales. *J Mar Biol Assoc U K*. 100:1–11. doi:10.1017/S0025315419001024.
- Zerbini AN, Ajó AF, Andriolo A, Clapham PJ, Crespo E, González R, Harris G, Mendez M, Rosenbaum H, Sironi M, et al. 2018. Satellite tracking of Southern right whales (*Eubalaena australis*) from Golfo San Matías, Rio Negro Province, Argentina. Report SC/67B/CMP17 to the Scientific Committee of the International Whaling Commission. Cambridge (UK): Scientific Committee of the IWC. Available from: <https://iwc.int/home>
- Zerbini AN, Adams G, Best J, Clapham PJ, Jackson JA, Punt AE. 2019. Assessing the recovery of an Antarctic predator from historical exploitation. *R Soc Open Sci*. 6:190368.
- Zerbini AN, Andriolo A, Heide-Jørgensen M, Pizzorno J, Maia Y, VanBlaricom G, DeMaster DP, Simoes-Lopes P, Moreira S, Bethlem C. 2006. Satellite-monitored movements of humpback whales *Megaptera novaeangliae* in the southwest Atlantic Ocean. *Mar Ecol Prog Ser*. 313:295–304.
- Zerbini AN, Andriolo A, Moreira S. 2011. Migration and summer destinations of humpback whales (*Megaptera novaeangliae*) in the western South Atlantic Ocean. *Journal of Cetacean Research and Management (Special Issue)*. 3:113–118.
- Zerbini AN, Clapham P, Wade P. 2010. Assessing plausible rates of population growth in humpback whales from life-history data. *Mar Biol*. 157:1225–1236.
- Zerbini AN, Mendez M, Rosenbaum H, Sucunza F, Andriolo A, Harris G, Clapham P, Sironi M, Uhart M. 2015. Tracking southern right whales through the southwest Atlantic: new insights into migratory routes and feeding grounds. Report SC/66b/BRG26 presented to the Scientific Committee of the International Whaling Commission. Cambridge (UK): Scientific Committee of the IWC. Available from: <https://iwc.int/home>