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ORIGINAL PAPER

Temporal stability and mixed-stock analyses of humpback whales *(Megaptera novaeangliae)* in the nearshore waters of the Western Antarctic Peninsula

G. R. Albertson[1,12] · A. S. Friedlaender[1] · D. J. Steel[1,12] · A. Aguayo-Lobo[11] · S. L. Bonatto[4] · S. Caballero[6,12] · R. Constantine[8,12] · A. L. Cypriano-Souza[4] · M. H. Engel[5] · C. Garrigue[7,12,13] · L. Flórez-González[6,12] · D. W. Johnston[2] · D. P. Nowacek[2] · C. Olavarría[10,12] · M. M. Poole[3,12] · A. J. Read[2] · J. Robbins[9,12] · A. L. Sremba[1] · C. S. Baker[1,8,12]

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Abstract Southern Hemisphere humpback whales breed in tropical waters and migrate to Antarctica to forage. While the breeding grounds are well defined, the population structure on Antarctic feeding grounds is poorly described. The Western Antarctic Peninsula (WAP) is of particular interest, where rapidly changing environmental conditions could alter prey distribution or migration pathways. To examine changes in the population of whales around the WAP, we used mitochondrial DNA (mtDNA) and 15 microsatellite loci. We compared our WAP dataset to a dataset collected 18 years earlier, and identified new haplotypes for the region, but found no significant difference between the datasets. We compared whales from the WAP to breeding populations in Oceania, Colombia, and Brazil. We used an Analysis of Molecular Variance to confirm significant genetic differentiation between the WAP and each breeding ground (overall $F_{ST} = 0.035/0.007$ mtDNA/microsatellite, $p < 0.001$) except Colombia. Bayesian mixed-stock analyses showed a large apportionment to Colombia (mtDNA 93.0%; CL 91–99%; microsatellites 86%; CL 72–93%) and a small apportionment to French Polynesia/Samoan Islands (mtDNA 2.9%; CL 0.0–11.5%; microsatellites 8.9%; CL 0–22%), supporting the strong connection between Colombia and the WAP. Assignment tests allocated 81 individuals to Colombia and two to French Polynesia/Samoan Islands. No other breeding grounds had significant apportionments. Direct connectivity of French Polynesia to the WAP was confirmed with the first genotype match of French Polynesia to a feeding area. Continued genetic monitoring will highlight the complex patterns of humpbacks in this rapidly changing climate. Our results serve as a baseline for humpback whale population structure, illustrate mixed-stock analysis as a useful tool for

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✉ G. R. Albertson
renee.albertson@oregonstate.edu

[1] Marine Mammal Institute and Department of Fisheries and Wildlife, Oregon State University, Newport, OR 97365, USA

[2] Division of Marine Science and Conservation, Duke University Marine Laboratory, Beaufort, NC 28516, USA

[3] Marine Mammal Research Program, BP 698 Maharepa, Mo'orea, French Polynesia

[4] Faculdade de Biociencias, Pontificia Universidade Catolica do Rio Grande do Sul, Porto Alegre, RS, Brazil

[5] Projeto Baleia Jubarte/Instituto Baleia Jubarte Caravelas, Bahia, Brazil

[6] Laboratorio de Ecología Molecular de Vertebrados Acuáticos LEMVA, Departamento de Ciencias Biologicas, Universidad de los Andes, Carrera 1 no. 18A-10, Bogotá, Colombia

[7] Operation Cétaces, BP 12827, 98800 Nouméa, New Caledonia

[8] School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand

[9] Center for Coastal Studies, 5 Holway Avenue, Provincetown, MA 02657, USA

[10] Centro de Estudios Avanzados en Zonas Aridas – CEAZA, Raul Bitran 1305, La Serena, Chile

[11] Instituto Antartico Chileno, Punta Arenas, Chile

[12] South Pacific Whale Research Consortium, PO Box 3069, Avarua, Rarotonga, Cook Islands

[13] IRD UMR Entropie, BPA5 98848, Noumea, New Caledonia

migrating wildlife, and aid in future management considerations for humpbacks.

Keywords Humpback whales · Population structure · Climate change · Mixed-stock analysis · DNA markers · Antarctic Peninsula

Introduction

Humpback whales *(Megaptera novaeangliae)* have one of the longest seasonal migrations of any mammalian species (Rassmussen et al. 2007; Robbins et al. 2011; Stevick et al. 2011). The amount of energy required for this migration is substantial, especially considering there are little to no prey resources once the whales leave a feeding area. Therefore, whales must maximize foraging while in the feeding areas (Friedlaender et al. 2011). In the Southern Hemisphere around the Western Antarctic Peninsula (WAP), high-density foraging areas of humpback whales have been correlated to areas with high-density aggregations of krill (Nowacek et al. 2011). Humpback whale distribution (Friedlaender et al. 2006, 2011) and movement patterns around the Antarctic Peninsula at fine (Friedlaender et al. 2013) and broad spatial scales (Dalla Rosa et al. 2008) are highly dependent on krill distribution patterns. Climate change has an impact on the timing and distribution of many migratory species (Robinson et al. 2009; Charmantier and Gienapp 2014). The WAP has been identified as one of the most rapidly changing environments due to climate change trends, and these changes in the ocean affect krill distribution (Atkinson et al. 2004). With the rapid warming near the WAP, new migratory behavior patterns may emerge as whales adapt to the changing habitat (Robinson et al. 2009). Although protection from commercial whaling has clearly aided in the recovery of humpback whales, populations may face an additional challenge as climate change continues to alter sea ice habitat and krill densities around Antarctica (Robinson et al. 2009).

Like most baleen whales in the Southern Hemisphere, humpbacks are still recovering from intense twentieth-century whaling when over 200,000 humpback whales were killed, leaving the species near the brink of extinction (Clapham and Baker 2008; Clapham et al. 2009). The recovery of humpback whales in the Southern Hemisphere has been variable, with high levels of population growth in some areas and relatively slow recovery in other areas such as Oceania (Constantine et al. 2012). For conservation purposes, recent studies have elucidated gene flow and migratory patterns in order to evaluate stock structure and habitat use. Humpback whale breeding stocks are composed of whales returning each year to the location of their birth, and these patterns of maternal fidelity and natal philopatry are thought to shape genetic structure across each hemisphere and within each ocean basin (Olavarría et al. 2007; Baker et al. 2013; Jackson et al. 2014).

The breeding stocks on the Pacific and Atlantic sides of South America (stocks G and A respectively) and in Oceania in the South Pacific (stocks E and F; Fig. 1) are genetically differentiated by mitochondrial DNA (mtDNA) (Olavarría et al. 2007; Engel et al. 2008). These stock divisions have been supported by photo-identification and satellite tag studies, and are defined by the International Whaling Commission (IWC) as follows: E2 New Caledonia, E3 Tonga, F French Polynesia, G Colombia, and A Brazil (Fig. 1) (International Whaling Commission 2005, 2011). Moreover, originally based on whaling records, the Antarctic feeding areas have been divided into six management Areas (I–VI) by the IWC, where the WAP is considered part of Area I (Mackintosh 1965). The National Oceanic and Atmospheric Administration (NOAA) has proposed to reclassify the humpback whale into 14 distinct population segments under the US Endangered Species Act, citing this will provide a more "tailored conservation approach" (Bettridge et al. 2015). It is noted in the report by Bettridge et al. (2015, p. 122) that there are no trend data for smaller breeding grounds such as Oceania (New Caledonia, Tonga, and French Polynesia), and therefore it is imperative to continue to monitor these vulnerable populations.

Migratory movements of humpback whales were originally documented using Discovery marking and recovery tags (Rayner 1940; Dawbin 1997; International Whaling Commission 2011). Despite tags revealing some variation in migratory routes, for management purposes, humpback whales were still considered to migrate in a more or less direct route to a feeding area from the corresponding breeding ground (Mackintosh 1942; Chittleborough 1965). More recently non-lethal methods using photo-identification (Stone et al. 1990; Stevick et al. 2004; Engel and Martin 2009; Robbins et al. 2011; Constantine et al. 2012; Franklin et al. 2012), genotype matching (Constantine et al. 2012, 2014), and satellite tags (Zerbini et al. 2006; Gales et al. 2010; Garrigue et al. 2010; Hauser et al. 2010; Zerbini et al. 2011; Constantine et al. 2016) showed more complex connections between breeding grounds and feeding areas in the Southern Hemisphere. Humpback whales that breed near Colombia and Ecuador on the west coast of South America (breeding stock G) were documented foraging around the WAP (Stone et al. 1990; Olavarría et al. 2000; Stevick et al. 2004; Albertson-Gibb et al. 2008; Steel et al. 2008). Moreover, the Antarctic Peninsula (Area I) represents one of the most genetically different Antarctic feeding areas (Amaral et al. 2016). This genetic difference suggests that a large number of the Colombia and Ecuador

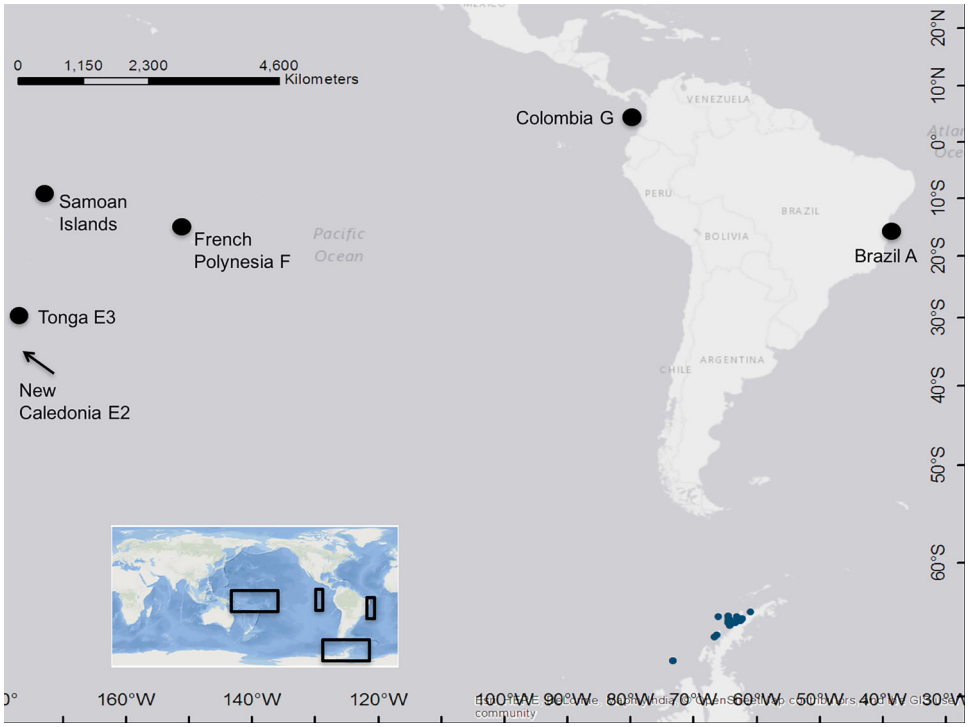

Fig. 1 Humpback whale breeding grounds New Caledonia, Tonga, Samoa, French Polynesia, Colombia, Brazil, and feeding area, the Western Antarctic Peninsula. The *letters* represent the breeding ground stock or sub-stock designations from the IWC. The Samoan Islands does not have an IWC designation since data collection from the region is relatively new. Therefore, the IWC has not reviewed this region for designation. *Circles* near the Antarctic Peninsula represent sample locations in the feeding area for the WAP2014 dataset

breeding populations may feed mainly in this area, whereas humpback whales that breed off the coast of Brazil on the east coast of South America (breeding stock A) were documented foraging near South Georgia/South Sandwich Islands (Zerbini et al. 2006; Engel et al. 2008; Engel and Martin 2009; Cypriano-Souza et al. 2010; Zerbini et al. 2011). Although the distance between the WAP and Brazil breeding grounds is less than the distance between the WAP and Colombia, there is no evidence of whales migrating from the WAP to breeding stock A or anywhere else in the Atlantic. A study in Oceania revealed extended migratory movements of whales from the breeding ground of American Samoa to feeding areas near the WAP, a round trip distance of at least 18,840 km. The authors suggest these extended movements could be motivated by reliable concentrations of prey near the WAP (Robbins et al. 2011). A study where humpback whales were tagged near the Kermadec Islands, North of New Zealand, found whales ranged over 3500 km in the Antarctic, from southeast of New Zealand in Area V to Bellingshausen Sea in Area I (Constantine et al. 2016).

To identify the current genetic composition of humpback whales in the WAP and to provide a framework for future studies of humpback whales in a changing environment, we use here a large sample collection of mitochondrial DNA (mtDNA) control region haplotypes and genotype information from 15 microsatellite loci of individual humpback whales from six breeding grounds to compare to humpback whales sampled in the WAP in 2014. Our main objectives are to (1) identify the current population structure and genetic diversity of the WAP2014 dataset, (2) compare this WAP 2014 dataset to a WAP dataset collected from 1996 to 1999 in order to document any temporal changes in population composition thus far, and (3) evaluate mixed-stock apportionments to South Pacific and South Atlantic breeding grounds E2, E3, F, G, and A to determine the origin of the WAP whales. We include the breeding ground of Brazil despite no previous connection between these two regions (Stevick et al. 2004), and despite recent evidence that Brazil and the WAP are genetically different (Cypriano-Souza et al. 2017). Our reasons were to provide baseline information in order to assess any future changes of population composition around the WAP where melting sea ice may be creating new ocean habitat and changing krill densities (Quetin and Ross 2003).

Methods

Sample collection

WAP2014 sampling

Surveys of humpback whales were conducted near the Gerlache Strait in the WAP (Fig. 2) during the Austral summer (January–March) of 2014 by the Palmer Long-Term Ecological Research (LTER) team (this dataset

referred to as WAP2014 from here forward). The Gerlache Strait includes the Palmer Archipelago and surrounding waters (Anvers, Wiencke, and other minor islands), and is bound by the Bransfield Strait in the northeast and the Bismark Strait to the southwest. Biopsy samples of adult whales ($n = 139$) were collected and stored in 70% ethanol. This WAP2014 dataset was collected from 16 different 'feeding aggregations,' where a feeding aggregation was defined as a geographically separate group of foraging whales, where little or no interchange occurred between aggregations (Witteveen 2008). Within these feeding aggregations whales were observed foraging as singles, pairs, or groups of three of more.

WAP1996 sampling

For a temporal comparison to a dataset near the Gerlache Strait, we used samples collected from 1996 to 1999 (referred to as WAP1996) by the Instituto Antarctico Chileno (Olavarría et al. 2000, 2006). Sampling for the WAP1996 dataset was conducted during surveys in the Austral summers of 1996, 1997, 1998, and 1999 near the Gerlache Strait (Fig. 2) by the Instituto Antartico Chileno (INACH) as part of the Scientific Antarctic Expeditions. Details of sampling collection and storage are described in Olavarria et al. (2000).

Breeding grounds sampling

To apportion the WAP2014 dataset to breeding grounds in the South Pacific and South Atlantic, we used humpback whale samples collected from six breeding grounds. Humpback whale samples (sloughed skin and biopsy samples) currently archived at the University of Auckland, Oregon State University, or Pontificia Universidade Catolica do Rio Grande do Sul were analyzed from six winter breeding grounds: New Caledonia, Tonga, the Samoan Islands (including Independent and American Samoa, at the boundary between breeding stocks E and F), French Polynesia, Colombia, and Brazil (Table 1). There was a total of $n = 1262$ individuals after replicates were deleted. Samples from Oceania (New Caledonia, Tonga, Samoan Islands, and French Polynesia) were collected by members of the South Pacific Whale Research Consortium, primarily during synoptic surveys from 1999 to 2005, but also include a small number from before and

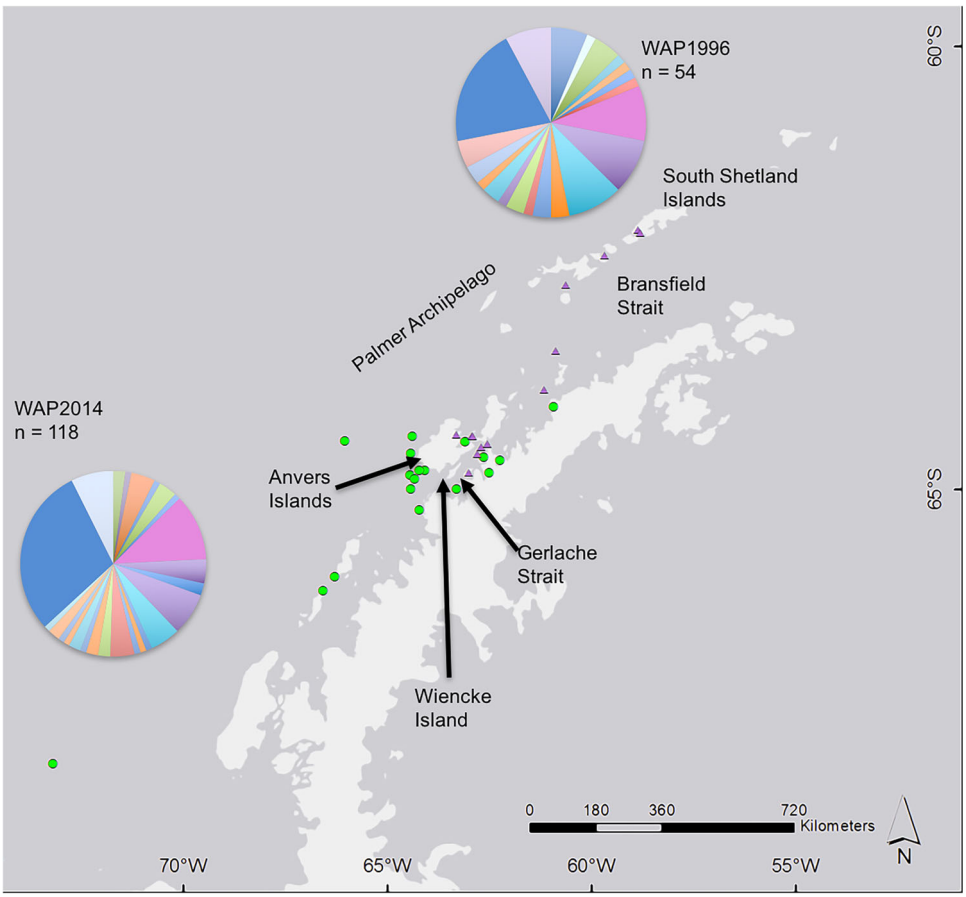

Fig. 2 Humpback whale sample locations for the WAP1996 dataset (*triangles*), and the WAP2014 dataset (*circles*). Pie charts represent haplotype frequencies, where each color is a separate haplotype

after this time ($n = 172$). Samples from Colombia were collected from 1991 to 1998 around Gorgona Island and coastal Colombia (Table 1) as described in Caballero et al. (2001). Samples from Brazil were collected by Instituto Baleia Jubarte from 1997 to 2011 in the areas of Abrolhos Bank and Praia do Forte as described in Engel et al. (2008), and are archived at the Pontificia Universidade Catolica do Rio Grande do Sul in Porto Alegre. Table 1 provides a complete description of which samples have been used previously in publications.

DNA extraction, sequencing, and genotype profiling of humpback whales

Total cellular DNA was isolated from skin tissue by digestion with Proteinase K followed by a standard phenol:chloroform extraction method (Sambrook et al. 1989) modified for small skin samples (Baker et al. 1994). Genetic sex was identified by amplifying the SRY (sex determining region of the Y chromosome) and ZFX/ZFY genes according to Gilson et al. (1998). An 800 bp

Table 1 The number of individual humpback whales from six Southern Hemisphere breeding grounds and the WAP feeding area used in this study

Region	Years	Individuals	F/M	Number of haplotypes	Previous studies	Haplotype diversity (*h*) (±SD)	Nucleotide diversity (π) (±SD)
New Caledonia	1995–2005	369	144/203	67	Garrigue et al. (2004); (Hap/Geno) Garrigue et al. (2006) ((Hap/Geno) Olavarría et al. (2007) (Hap) Steel et al. 2008 (Hap/Geno) Constantine et al. (2012) (Geno)	0.9727 (0.0023)	0.0209 (0.0106)
Tonga	1991–2003 2005	337	111/224	54	Olavarría et al. (2007) (Hap) Steel et al. (2008) Constantine et al. (2012) (Geno)	0.9647 (0.0028)	0.0203 (0.0103)
French Polynesia /Samoa	1997–2007 (FP) 2003–2011 (SA)	193 (FP) 110 (SA)	86/104 (FP) 30/76 (SA)	31 (FP) 37 (SA) 42 (FP + SA)	Olavarría et al. (2007) (Hap) Steel et al. (2008) (Hap/Geno) Constantine et al. (2012) (Geno)	0.9305 (0.0073)	0.0182 (0.0093)
Colombia	1991–1993 1995–1998	95	25/63	25	Olavarría et al. (2000, 2006, 2007) (Hap) Steel et al. (2008) (Hap/Geno)	0.8853 (0.0238)	0.0189 (0.0097)
Brazil	1997–2011	158	52/51	50	Engel et al. (2008) (Hap) Rosenbaum et al. (2009) (Hap) Cypriano-Souza et al. (2017) (Hap/Geno) Cypriano-Souza et al. (2010) (Geno)	0.9733 (0.0035)	0.0196 (0.0047)
Breeding Ground Total		1207	448/721	109		0.9110 (0.0107)	0.0194 (0.0097)
Western Antarctic Peninsula	1996–1999	54	33/20	19	Olavarria et al. (2000, 2006) (Hap)	0.9089 (0.0164)	0.0186 (0.0092)
Western Antarctic Peninsula	2014	118	57/60	25		0.8990 (0.0193)	0.0193 (0.0099)
Feeding area total		172	90/80	25		0.9003 (0.0151)	0.0188 (0.0094)

FP/SA represents French Polynesia (FP) and Samoa (SA) combined for analyses. The number of females (F) and males (M) does not add to the total number of individuals due to failure of some samples to amplify for sex identification. The number of haplotypes is the number of haplotypes out of 134 total haplotypes found in that region. Previous studies identify if and when data were used in previous studies, i.e., Hap refers to haplotypes only, Geno refers to Genotype only, and Hap/Geno refers to both haplotypes and genotype data used in the study. Noted exception, Samoan Islands samples were used previously through 2005. Samples collected after 2005 have not been analyzed previously. The haplotype (*h*) and nucleotide (π) diversity for each region and total for the breeding grounds and feeding areas was calculated in *ARLEQUIN*

fragment of the mtDNA control region was amplified and sequenced, as described in Olavarría et al. (2007). A consensus sequence of the mtDNA fragment, 470 bp, was reviewed using the program *SEQUENCHER v5.0* (Genes Code Co.). The program *ARLEQUIN v.3.5* (Excoffier and Lischer 2010) was used to determine haplotype (*h)* and nucleotide (π) diversity. Significant differences in haplotype diversity between each region were determined using the program *PERMUT v.2.0* (Petit 2010).

Up to 15 microsatellite loci were amplified using previously published primers, EV1, EV14, EV21, EV37, EV94, EV96, EV104, GATA28, GATA417, RW31, RW410, RW48, GT23, GT211, and GT575 (Valsecchi and Amos 1996; Palsboll et al. 1997; Waldick et al. 1999; Berube et al. 2000), and followed protocols previously described by Constantine et al. (2012). Alleles were sized with *GENEMAPPER v.4.0* (Applied Biosystems) and all automated calling was confirmed by visual inspection (Bonin et al. 2004). All samples from New Caledonia, Tonga, Samoan Islands, French Polynesia, Colombia, and the Antarctic Peninsula were genotyped at Oregon State University (OSU). The Brazil dataset was originally genotyped in Brazil and Seoul, South Korea. To calibrate microsatellite allele size between OSU and the Brazil dataset for this study, 40 Brazil samples were genotyped onsite at OSU (see Cypriano-Souza et al. 2017 for complete description of Brazil sample genotyping). The program MICRO-CHECKER (Van Oosterhout et al. 2004) was used to identify genotyping errors due to non-amplified alleles (null alleles), short allele dominance (large allele dropout), and the scoring of stutter peaks. As a precaution against poor DNA quality, only those samples that amplified at a minimum of 10 microsatellite loci were retained for further analyses. Data compilation and initial analyses of microsatellite alleles, sex identification, and mtDNA haplotypes were conducted with the program *GenAlEx v.6.5* (Peakall and Smouse 2012). Replicate genotypes were identified with the program *CERVUS v.3.0* (Kalinowski et al. 2007b) and excluded from further analyses. To evaluate the confidence in matching, we calculated the probability of identity (PI), or chance that a pair of randomly selected individuals will have matching genotypes. We also calculated the PIsib, the probability of identity among siblings, and a conservative upper bound of the number of loci necessary to distinguish individuals (Waits et al. 2001). To avoid false exclusion due to potential dropout and genotyping errors of mismatching loci, the electropherograms were reviewed and either corrected based on visual inspection or repeated for confirmation. We required a minimum overlap of 10 matching loci to accept samples as replicates from the same individual. For the WAP1996 dataset we used sequence and sex identification information from Olavarria et al. (2000) and the completed genotypes of these samples as described in Constantine et al. (2012).

The DNA extraction, mtDNA sequencing, genetic sex identification, and microsatellite genotyping for the breeding grounds samples have been described fully elsewhere. See Engel et al. (2008) and Cypriano-Souza et al. (2010) for samples from Brazil and Constantine et al. (2012) for samples from Oceania. Following a Quality Control protocol (Morin et al. 2010), unique genotypes within breeding grounds and the WAP feeding area were compared and identified.

Diversity and tests of differentiation

For mtDNA, the program *ARLEQUIN v.3.5* (Excoffier and Lischer 2010) was used to calculate haplotype diversity and indices of genetic differentiation (pairwise F_{ST} and ϕ_{ST}) between each region, including all breeding grounds and both WAP datasets. An Analysis of Molecular Variance (AMOVA) tested the significance of regional differentiation using 10,000 random permutations. For microsatellite loci, we used the program *GENEPOP* (*v*.4.2) (Raymond and Rousset 1995) to evaluate deviations from Hardy–Weinberg Equilibrium and calculate pairwise F_{ST}. The program *GENODIVE* (Meirmans and Van Tienderen 2004) was used to calculate G" $_{ST}$, an unbiased F'$_{ST}$ estimator which corrects for within population diversity and differences in population sizes (Meirmans and Hedrick 2011). To evaluate any sex bias, analyses were repeated for each sex separately.

To evaluate temporal changes within a season, we performed a within season comparison of genetic differentiation using the WAP2014 dataset. For mtDNA and microsatellite markers, we calculated the F_{ST} between the sampling periods 'early season' (11 days of sampling: 21 January–02 February, 2014; $n = 86$) and 'late season' (4 days of sampling: 25 February, 19–22 March, 2014; $n = 29$).

Mixed-stock analysis and assignment tests

Mixed-Stock Analysis (MSA) has been used successfully in several migratory species including salmon (Bowen et al. 2007; Griffiths et al. 2010), turtles (Bowen et al. 2007), and more recently humpback whales (Albertson-Gibb et al. 2008; Schmitt et al. 2015). Based on haplotype and microsatellite allele frequencies, MSA assigns individuals or apportionments in a population from a 'mixed-stock' to migratory locations of a 'pure stock' based on haplotype and microsatellite allele frequencies (Pella and Masuda 2001). Specific to humpback whales in the

Southern Hemisphere, the 'mixed-stock' is the Antarctic feeding area, and the 'pure stocks' are the tropical breeding grounds near the equator.

Stock apportionments to the breeding grounds from the WAP2014 dataset, and the combined WAP datasets, were estimated using mtDNA with the program *BAYES* (Pella and Masuda 2001). We ran six independent Markov Chain Monte Carlo (MCMC) chains of 40,000 iterations with different starting values and discarded the first 20,000 iterations as burn-in. The posterior distribution incorporates the information about genetic characters in the baseline samples, including relatedness of stocks. The Bayesian approach incorporates uncertainty where haplotypes may have a very low frequency, i.e., it accommodates the possibility of rare haplotypes that are actually present but not detected in a small sample (Pella and Masuda 2001; Antonovich and Templin 2003). To assess convergence we used the Raftery–Lewis and Gelman–Rubin diagnostics, where a value greater than 1.2 illustrated convergence of the chains. However, if one of the diagnostics showed the chains had not converged, we reanalyzed the mixture running six chains with 60,000 iterations following the protocol described in Dann et al. (2012).

The program *ONCOR* (Kalinowski et al. 2007a) was used to estimate stock apportionments and individual assignment to the breeding grounds from the WAP2014 and the combined WAP genotype datasets. Based on the allele frequencies in the breeding grounds, and using microsatellite data, stock apportionments were estimated using allele frequencies from the WAP2014 dataset. In addition to the population level stock apportionments, assignment tests estimated the origin of each individual in the WAP2014 dataset. *ONCOR* assigns individuals in the WAP dataset to the breeding grounds that would have the highest probability of producing the given genotype in the WAP dataset. Simulations were run to assess the power of the program to correctly assign individuals. In these simulations, a sample is simulated in which all the individuals are from the same population. *ONCOR* uses the resampling method "Leave-one-out cross validation" to simulate mixture genotypes and to estimate their probability of occurrence in baseline populations. This resampling method yields essentially unbiased estimates of genetic stock identification (Anderson et al. 2008).

Additionally, the Bayesian assignment method in *GENECLASS 2.2.2* was implemented (Piry et al. 2004) to assign individuals from the WAP2014 dataset to breeding grounds. This program is similar to *STRUCTURE 2.3.3* in that it uses Bayesian clustering to assign individuals to populations. The Bayesian method of Rannala Mountain was used with Monte Carlo sampling computation and the Paetkau et al. (2004) simulation algorithm. We ran 10,000 simulated individuals with the Type I error set at 0.01 to determine the highest probability of assignment to the breeding grounds. Each individual was assigned to the population with the highest likelihood.

Results

After quality control, there were 136 samples of humpback whales from the WAP2014 dataset that included microsatellite genotypes (10 or more loci), mtDNA haplotypes, of which 135 also included genetic sex information. Genotype matching yielded 118 individuals from the 136 samples. The WAP2014 dataset showed an overall 60:57 male:female ratio (Table 1). Replicate samples identified by genotype matches also matched at sex and haplotype and were excluded from further analyses. All whales that were genetically recaptured were in groups composed of at least one different individual than when they were first sighted. Although not all whales in each group were sampled each time, there was at least one new individual identified with the whale that was recaptured in each new group.

mtDNA haplotype and microsatellite diversity in the WAP2014 dataset

Haplotypes new to the South Pacific (SP) dataset

The haplotypes in this study represented a 470 bp section of the mtDNA control region, defined by 79 variable sites. Within the WAP2014 dataset, two haplotypes not previously found in the WAP, Oceania/South Pacific, or Brazil were identified, bringing the total of Oceania/South Pacific haplotypes to 112, and the combined breeding grounds of Oceania/South Pacific and Southwestern Atlantic datasets to 132 haplotypes. The WAP2014 dataset included 25 of the 133 haplotypes. One of the two newly identified haplotypes in the WAP matched one originally sampled in Ecuador (SP123, Accession number HQ241481.1). The one whale with this haplotype in the WAP2014 dataset was a male. The second newly identified haplotype matched one sampled in the Southeast Atlantic originally sampled in breeding stock B (SP124, Accession number GQ913713.1), representing the only haplotype between the Southeast Atlantic and our WAP datasets. This individual was a female.

Haplotype diversity in feeding aggregations

The WAP2014 samples were collected from 16 different feeding aggregations (Table 2). Each feeding aggregation of the WAP2014 dataset contained multiple haplotypes (Table 2), and within feeding aggregations all groups

consisted of whales with different haplotypes. With one exception of a group with two females, all feeding groups containing adult whales included both males and females.

Haplotype diversity within the 2014 season

When the WAP2014 season was delineated by 'early' and 'late' (see Methods section for these delineations), there were 22 and 17 of the 25 total haplotypes found in the early and late season, respectively. Of the newly identified haplotypes, SP123, first identified in Ecuador, was only observed in the early season (21 January). SP124, first identified in the Southeast Atlantic, was only observed late season (21 March).

Haplotype diversity within the WAP

Between the two WAP datasets, 15 haplotypes overlapped. The WAP1996 dataset contained a total of 19 haplotypes, three of which were not found in the WAP2014. The WAP2014 contained a total of 25 haplotypes, nine of which were not found in the WAP1996 dataset.

Haplotype diversity of the WAP and breeding grounds

All of the 132 haplotypes in this study are published on GenBank (see Engel et al. 2008 for Brazil haplotypes and Olavarria et al. 2007 for Oceania/South Pacific haplotypes and accession numbers for two additional haplotypes in the text above). The number of sampled individuals in the analyses was delineated by region, haplotype, and sex. The number of haplotypes in each region ranged from 19 to 67. Of the 19 haplotypes that had been previously identified in the WAP, three private haplotypes from Colombia were found in the WAP2014 dataset. Seven of the 112 South Pacific haplotypes found in our WAP2014 dataset were shared with Brazil. Three haplotypes were found in all regions (WAP2014, WAP1996, New Caledonia, Tonga, Samoan Islands, French Polynesia, Colombia, and Brazil). The haplotype diversity (h) for each region was high, ranging from 0.8853 to 0.9733, and nucleotide diversity π, from 0.021 to 0.018 (Table 1). Colombia had the lowest haplotype diversity ($h = 0.8853$), which was significantly different from the other breeding grounds ($p = 0.215$), but was not significantly different from WAP1996 ($h = 0.9089$) or WAP2014 ($h = 0.8990$) (Table 3).

Microsatellite diversity

Diversity of microsatellite loci was relatively high, with an average of 12.8 alleles per locus and average observed heterozygosity of 0.719 (Online Resource 1). Only two loci, GATA417 and GT211, showed a significant deviation from Hardy–Weinberg ($p < 0.001$). When these loci were removed the significance of results was not changed, so they were retained.

Individual recaptures identified by genotyping

The genotype profiles were used to identify recaptures of individuals (i.e., when whales were inadvertently

Table 2 Feeding aggregations of humpback whales from the WAP2014 dataset

Date	Location	Number of whales in feeding aggregation (M/F)	Number of haplotypes in feeding aggregation
21 January	Station 100.10	10 (5/5)	5
23 January	Armstrong Reef	11 (7/4)	4
24 January	Cape Leblond	5 (4/1)	4
26 January	Flandres Bay	13 (7/6)	11
27 January	South of Anvers Island	6 (0/6)	5
28 January	Joubin Islands	(3/3)	6
30 January	Palmer Deep Basin	25 (17/8)	13
31 January	West of Anvers Island	2 (2/0)	2
01 February	West of Anvers Island	8 (5/3)	4
02 February	Joubin Islands	12 (7/5)	5
02 February	Cape Monaco	3 (1/2)	3
25 February	East of Peterman Island	8 (4/4)	4
19 March	Cuverville Island	6 (6/0)	4
21 March	Andvord Bay	3 (2/1)	3
21 March	Wilhelmina Bay	12 (5/7)	7
22 March	Cierva Cove	3 (0/3)	2

The total number of whales in each group is shown with males and females in parentheses

resampled). Although most of the genotype recaptures within the WAP2014 dataset were from the same day, one was recaptured two days apart, and two individuals were recaptured up to six weeks apart. A female sampled on 30 January and 25 February and a male sampled on 02 February and 19 March were sampled each time within the Gerlache Strait (Fig. 3). The third whale was recaptured two days apart (28 January and 30 January), once south of Anvers Island and then in the Palmer Basin. There was one genotype recapture within the Antarctic feeding area between the WAP1996 dataset and the WAP2014 dataset (sample codes: Mno96AP009 and Mno14AP049, a female, Table 4).

Comparison between the unique genotypes from South Pacific and South Atlantic breeding grounds ($n = 1262$) and the WAP feeding areas ($n = 172$) revealed one match representing a migratory connection between French Polynesia and the WAP2014 dataset (sample codes: Mno07FP031 and Mno14AP008, a female, Table 4). All matches were supported by at least 10 loci as well as matching sex and mtDNA haplotype (Table 4), with maximum Probability of Identity, $PI < 1.1 \times 10^{-14}$ and a maximum $PI_{sib} < 4.1 \times 10^{-5}$.

Genetic differentiation among regions

One assumption of MSA is that each breeding stock (or source stock) is genetically differentiated, therefore pairwise comparisons were evaluated for each breeding ground. A test of differentiation for both mtDNA and microsatellites revealed significant differences for all pairwise comparisons of breeding grounds, with the exception of French Polynesia and the Samoan Islands. Therefore, French Polynesia and the Samoan Islands were combined for analyses and are referred to from here forward as FP/SA.

Pairwise comparisons for the mtDNA dataset showed significant differentiation between each of the populations except Colombia and each of the WAP1996 and WAP2014 datasets, as well as between the WAP1996 to WAP2014 datasets (Table 3). These results were similar to the measurement of haplotype distance, ϕ_{ST} as well (Online Resource 2). The low ϕ_{ST} values illustrate the similarities between haplotypes. In addition, the pie charts illustrate strong similarities in the haplotypes and the haplotype frequencies between the WAP feeding area and Colombia breeding ground (Fig. 4). When a separate test of genetic differentiation was performed for each sex, the pattern of genetic differentiation in the mtDNA was similar to the combined male and female dataset (Online Resource 3).

The microsatellite dataset showed weak, but significant genetic differentiation between each of the populations except Colombia and each of the WAP1996 and WAP2014 datasets, as well as between the WAP1996 to WAP2014 datasets (Table 3). The G''_{ST} results showed a similar pattern (Online Resource 2). This pattern of genetic differentiation was similar for males, but for female whales, there was no significant genetic differentiation in the pairwise test between FP/SA and WAP1996 (Online Resource 4).

Temporal differentiation within the WAP

Five sampling locations in the WAP2014 dataset overlap with the sampling locations of the WAP1996 dataset in the northernmost part of the WAP (Fig. 2). When the two datasets were compared, the pie charts show similar

Table 3 Pairwise F_{ST} comparison of humpback whale breeding and feeding populations in the southeast Pacific, southwest Atlantic, and WAP; mtDNA analysis is shown below diagonal, and microsatellite analysis above diagonal

Region	New Caledonia	Tonga	FP/SA	Colombia	Brazil	WAP1996	WAP2014
New Caledonia		0.002*	0.004**	0.008**	0.015**	0.008*	0.007*
Tonga	0.008**		0.002**	0.008**	0.017**	0.006*	0.008*
FP/SA	0.027**	0.013**		0.008**	0.017*	0.005*	0.006*
Colombia	0.061**	0.063**	0.077**		0.018**	0.000	0.000
						($p = 0.492$)	($p = 0.163$)
Brazil	0.032**	0.036**	0.052**	0.084**		0.017**	0.007**
WAP1996	0.054**	0.005**	0.060**	0.000	0.070**		0.000
							($p = 0.104$)
WAP2014	0.051**	0.055**	0.067**	0.000	0.074**	0.000	

WAP1996 and WAP2014 represent the dataset from the 1996 and 2014 WAP datasets, respectively

* Indicates significant F_{ST} $p < 0.01$

** Indicates significant F_{ST} $p < 0.001$

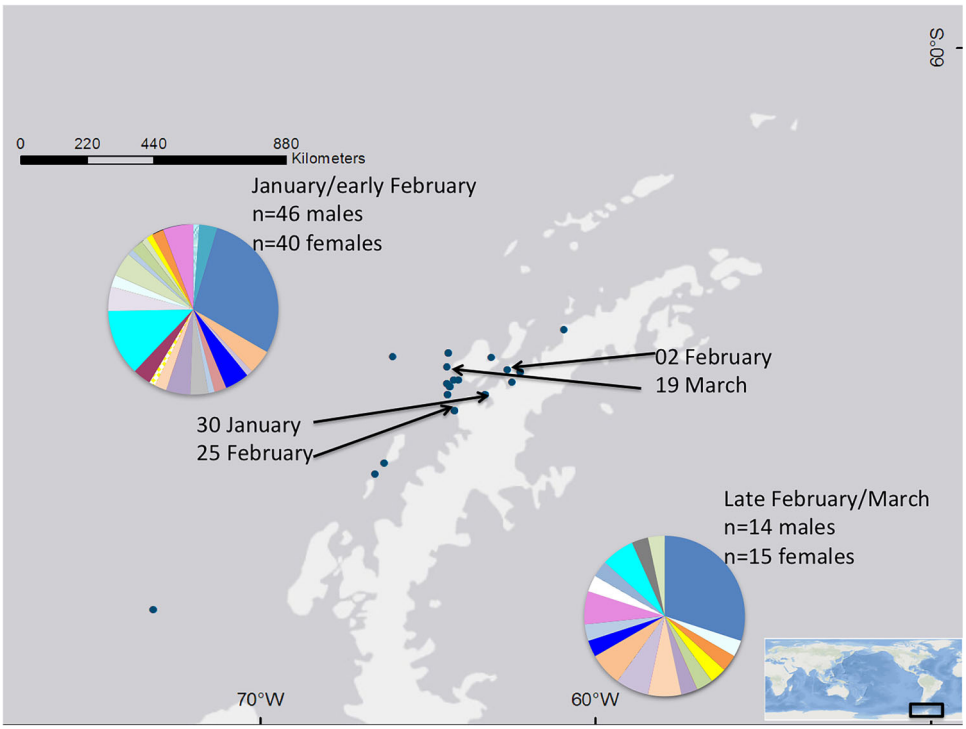

Fig. 3 Humpback haplotype temporal frequency comparisons from the WAP2014 dataset early in the feeding season (21 January–02 February, 2014) and late in the feeding season (25 February, 19–22 March, 2014). The number of males and females is shown for each time period. The arrows with the two dates represent 2 individual whales that were resighted using genotype matches at the locations where the arrows point on the dates given. One was a female, first sighted on 30 January and resighted 25 February. The other was a male, first sighted 25 February and resighted 22 March. Pie charts represent haplotype frequencies, where each color is a separate haplotype

Table 4 Summary of migratory connections from genotype matches of humpback whales in the breeding grounds of Oceania, Colombia, and the WAP. WAP1996 and WAP2014 represent the WAP 1996–1999 and the WAP 2014 datasets, respectively

Sample codes	Location	Date	Sex	mtDNA haplotype	Number of matching loci
1	WAP2014	20 Jan14	Female	SP1	15
Mno14AP008	−67.700S −73.200W	06 Sep 07	Female	SP1	
Mno07FP031	French Polynesia				
	−17.500S				
	−149.764 W				
2	WAP 2014	26 Jan 14	Female	SP68	13
Mno14AP049	−64.857S −64.441 W	25 Jan 96	Female	SP68	
Mno96AP009	WAP 1996				
	−63.983S				
	−61.067 W				
3	WAP	1994	Male	SP8	15
(Steel et al. 2008) MnoIWC94H101	−67.050S	1991	Male	SP8	
Mno91Co011	−71.300 W				
	Colombia				
	2.960 N				
	−78.180 W				

Individuals 1 and 2 were first identified in this study, and Individual 3 was first identified in Steel et al. (2008)

haplotypes and haplotype frequencies (Fig. 2). A test of genetic differentiation between the two datasets showed no significant differences ($p = 0.104$).

When the WAP2014 dataset was divided into temporal sampling periods 'early season' and 'late season,' no significant differences were found ($p = 0.531$) in haplotype frequencies between the two time periods (Fig. 3).

Mixed-stock apportionments of WAP2014 dataset

Simulation chains run in the program *BAYES* confirmed the independence of each breeding ground (New Caledonia, Tonga, FP/SA, Colombia, and Brazil) and the ability of the program to apportion breeding regions from the WAP with at least 80% certainty with the mtDNA dataset. *BAYES*

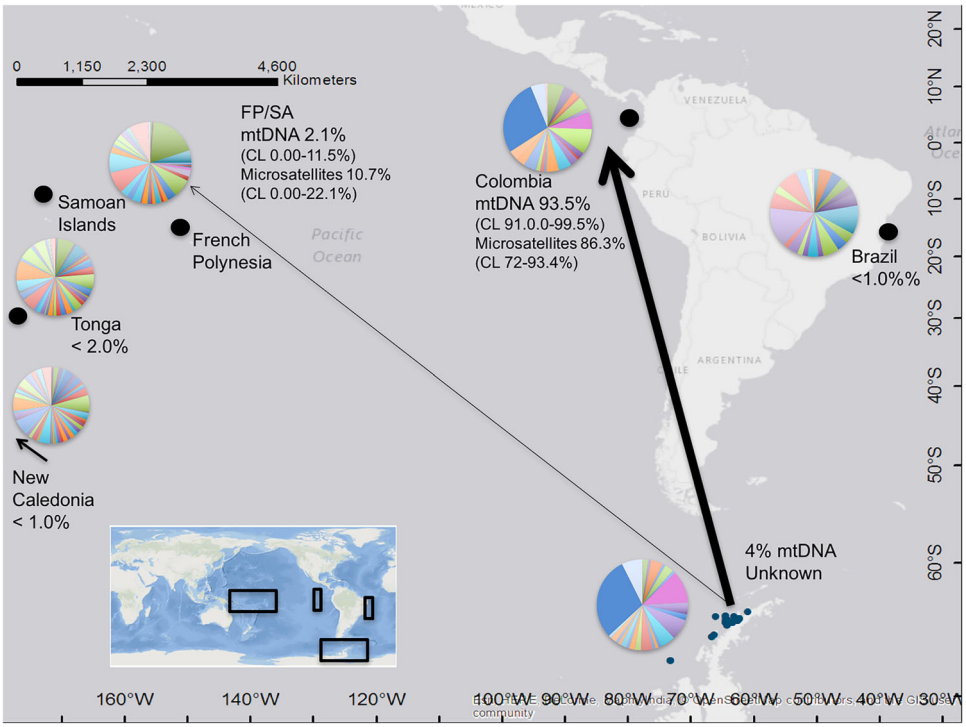

Fig. 4 Map of humpback whale breeding grounds New Caledonia, Tonga, Samoa, French Polynesia, Colombia, Brazil, and feeding area, the Western Antarctic Peninsula. The pie charts represent haplotype frequencies from each breeding region and both WAP datasets in the Antarctic feeding area. The *arrows* depict the qualitative mixed-stock apportionments derived from the program BAYES using mtDNA and ONCOR using microsatellites from the WAP datasets. FP/SA represents the French Polynesia and Samoa breeding regions combined for the haplotype frequency pie chart and mixed-stock apportionment. An additional 4% of the mtDNA could not be accounted for due to new haplotypes in the WAP datasets that are not in the breeding stock datasets

apportioned 93.5% of the WAP2014 dataset to the Colombia breeding ground, and a very small apportionment with relatively large confidence intervals to FP/SA (2.9%, CL 0.0–11.5%) (Fig. 4). There was less than 1% apportionment each to New Caledonia, Tonga, and Brazil. These results are illustrated by the haplotype frequency pie charts and relative size of the arrows (Fig. 4). The program *ONCOR,* using 15 microsatellites, apportioned 86.1% to Colombia (CL 72–93.4%), 8.9% to FP/SA (CL 0.00–22.1%), and 1.7% to Tonga (CL 0.0–28.1) (Fig. 4). Due to finding no significant genetic differentiation between the WAP1996 and WAP2014 datasets, these two datasets were also combined for MSA analysis. The results of the combined WAP datasets were similar to the WAP2014 dataset, and are shown in Online Resource 5.

Individual assignment tests of WAP2014 dataset

Simulations were run to assess the power of the MSA to assign the feeding area samples to the breeding stocks (i.e., the power of the program to assign to the correct breeding ground, expressed as percent accuracy). The simulations found the probability of occurrence of the mixture in the assigned population as follows: FP/SA 83.0% (CL 74–92%), New Caledonia 85.4% (CL 76–95%), Tonga 71.9% (CL 59–84%), Colombia 83.4% (CL 75–91%), and Brazil 99.8% (CL 99–100%).

Results from individual assignment tests with *ONCOR* assigned 81 individuals to Colombia and two individuals to French Polynesia/Samoan Islands with >95% certainty. Seven individuals had mixed assignment (Colombia and FP/SA), and the remaining 28 individuals had mixed assignment from three breeding grounds. These mixed assignments had the largest proportions to Colombia and FP/SA and a small proportion (~10% or less) assigned to Tonga. There were no assigned individuals to New Caledonia or Brazil. Of the two individuals assigned to FP/SA, one was a male with a haplotype that is shared with Brazil, and the other was a female with a haplotype common in FP/SA. The program *GENECLASS2* assigned the WAP2014 individuals with the highest probability to Colombia (113 individuals), FP/SA (one individual), and Tonga (four individuals).

Discussion

Population structure of WAP humpback whales

Our results provide insight into the population structure of humpback whales feeding around the WAP, near the Gerlache Strait. We found that the genetic composition of humpback whales near Gerlache Strait has not changed significantly over the past 18 years. The genetic composition of groups within feeding aggregations showed multiple haplotypes. Moreover, these groups did not remain stable, meaning that whales identified on multiple occasions were not observed with the same whales they were first sighted with. This suggests that the foraging groups are not generally composed of maternal kin and are fluid, similar to humpback whales in the North Pacific feeding grounds (Pierszalowski et al. 2016). The MSA using mtDNA and microsatellites confirmed the previously identified and stable connection between the WAP and Colombia along the Pacific coast of South America (Stone et al. 1990; Stevick et al. 2004; Albertson-Gibb et al. 2008; Engel et al. 2008; Steel et al. 2008; Cypriano-Souza et al. 2017).

Population level migratory connections reveal stability between WAP/Colombia

MSA can be a useful tool to evaluate wildlife migrating large distances. Three factors should be considered to effectively estimate stock proportions using MSA: the degree of differentiation among baseline or breeding populations, adequate sampling of contributing source populations (breeding grounds), and a sufficient number of genetic markers (Kalinowski 2004). Our study met these criteria and provided reasonable estimates of the apportionments for the WAP humpback whales. MSA of the WAP2014 mtDNA dataset yielded similar results to the WAP1996 dataset, with the major apportionment to Colombia (Albertson-Gibb et al. 2008). This is not surprising given the lack of genetic differentiation between the regions and the genotype recapture between the two WAP datasets, as well as photo-identification studies linking these migratory destinations. What is surprising, or unique for humpback whales, is the stability and continued fidelity to one feeding area at the population level. In other areas where humpback whales are found, including the North Pacific, Northwest Atlantic, and Southeast Atlantic, there is evidence of genetic structure, but the similarities between breeding grounds and one feeding area are not nearly as predictable (Rosenbaum et al. 2002, 2009; Baker et al. 2013). When Antarctic feeding areas adjacent to the WAP were evaluated in pairwise comparisons to other feeding areas in the Southern Hemisphere, Amaral et al. (2016) found the Antarctic Peninsula was highly differentiated from other Antarctic feeding areas. This contrasts with the pattern of low differentiation and higher levels of gene flow among adjacent Antarctic feeding areas. The authors attributed this to strong fidelity to the WAP. This could be due to the consistently high density of krill in this area, and the fact that humpback whales that feed here may stay in the general area of the WAP, because they do not need to travel great distances to find food once the feeding area has been reached (Dalla Rosa et al. 2008). The two genotype matches within the WAP2014 season illustrated whales were near the Gerlache Strait both times they were sampled (Fig. 3). Although it is unknown if they moved considerably in between sampling periods, they still returned to the Gerlache Strait area to forage. This movement pattern has been documented previously. In 2006, a humpback whale was first tagged in the Gerlache Strait, circled Anvers Island to the south, and came back to the Gerlache Strait about six weeks later (Dalla Rosa et al. 2008).

The microsatellites in the MSA showed a large apportionment to Colombia (86%), but also an apportionment to FP/SA. Although a genotype match was identified between the WAP1996 dataset and Colombia (Steel et al. 2008), we did not find a genotype match between the WAP2014 dataset and Colombia, despite the large apportionment from the MSA. This may be due to the whales in our Colombia dataset (last sampled in 1998) comprising a smaller percentage of Colombia whales as the population continues to grow, making them more challenging to recapture. The MSA showed a small apportionment to FP/SA. With the genotype match between French Polynesia and the WAP, as well as the photo-identification matches connecting American Samoa and the WAP (Robbins et al. 2011), we expected to find an even larger apportionment to FP/SA in the mtDNA dataset. Given the haplotype frequencies and haplotypes shared between Colombia and the WAP, it is possible that Colombia overwhelmed other regions with lesser apportionments, i.e., one of the shared haplotypes of Colombia and the WAP had the highest frequency (Fig. 4). This could be why a larger apportionment for FP/SA was observed in the microsatellite dataset since there appeared to be little differences between the male and female datasets. This is similar to findings in the North Pacific that found little evidence of male-biased dispersal in humpback whales (Baker et al. 2013). Therefore, the difference in mtDNA and microsatellite MSA analyses is probably not due to sex bias in dispersal.

Individual level migratory connections reveal exceptionally long distances traveled

Although the WAP appears to be the primary feeding area for whales that breed along the west coast of South

America, as shown by the genetic differentiation between this feeding area and other adjacent feeding areas (Amaral et al. 2016), there is increasing evidence that whales travel from the more distant eastern South Pacific breeding grounds to feed there. Our study identified the first genotype match connecting this breeding ground to a feeding area. In addition, there have been four individual migratory connections of humpback whales between French Polynesia and the WAP identified using photo-identification (Poole & Friedlaender, Unpublished). The migratory connection of the WAP to French Polynesia spans a minimum distance of 8100 km. In each case, this was a similar distance traveled by two individual humpback whales in the migratory connections of the WAP with American Samoa identified using photo-identification (Robbins et al. 2011). In the American Samoa connection, two individual whales sighted in American Samoa were seen on three occasions in the WAP. Therefore, the individual migratory connections of French Polynesia and the WAP, as well as the assignment tests linking FP/SA, provide additional evidence that some whales that breed in eastern Polynesia migrate to the WAP.

Satellite tag data from Oceania showed individual humpback whales in the South Pacific traveled in a southeast direction to feeding areas (Garrigue et al. 2015). Constantine et al. (2016) found that individual humpback whales tagged near the Kermadec Islands north of New Zealand traveled south to the Antarctic, with a distributional pattern spread over 3500 km in Areas V, VI, and I. This suggests whales from the same breeding ground may be feeding in different areas. Since whales are known to follow krill patches (Dalla Rosa et al. 2008; Friedlaender et al. 2013), this distribution pattern could be due to individuals following different krill patches, and therefore the feeding area groupings would have a greater emphasis on foraging and not individual associations. This hypothesis fits the pattern we found here. A continued study of the feeding grounds will be important in the future to determine how the mixing of whales on the feeding ground changes with shifting krill distributions in relation to climate change. Although our genotype match and the American Samoa photo-identification matches showed this trend, they also demonstrated a great distance traveled. Moreover, the American Samoa study showed an individual whale made a return trip to the WAP from American Samoa, and one other individual was identified in both locations, illustrating this migratory pattern is not anomalous. In support of this, other studies suggest the energy costs required to migrate a greater distance are outweighed by foraging in high-density krill areas such as the WAP (Atkinson et al. 2004; Friedlaender et al. 2006; Dalla Rosa et al. 2008; Nowacek et al. 2011; Robbins et al. 2011). This could be especially true for pregnant females. Our WAP2014 dataset showed almost a 50:50 sex ratio; however, the true sex ratio of the region remains unknown, and may change over the course of a season with a higher percentage of pregnant females. The pattern found in other baleen whales shows pregnant females are some of the last whales to leave a feeding area, presumably to maximize foraging time (Sumich 2014). Pregnant females would have a reason to seek out feeding areas with higher prey density to ensure they can sustain themselves and their developing fetus. These long distance movements by individuals may suggest that humpback whale migration to the feeding area is likely motivated by several factors including oceanographic changes, prey concentrations, and prey distribution (Atkinson et al. 2004; Friedlaender et al. 2006; Dalla Rosa et al. 2008; Nowacek et al. 2011).

In another study, a genotype match was identified between the breeding grounds of Colombia and French Polynesia (Steel et al., In Review). The whale, a male, was first observed in Colombia and then eight years later observed in French Polynesia, a distance of almost 8200 km. There is documentation of movement between adjacent breeding grounds for humpback whales, sometimes even within one breeding season (Garrigue et al. 2011). Less common is the movement of humpback whales between breeding grounds that are a large distance apart, i.e., between Japan and Hawaii (6000 km) in the North Pacific and the west and east coasts of Australia in the Southern Hemisphere (3000–5000 km) (Chittleborough 1962; Calambokidis et al. 2008). One of the greatest longitudinal movements between breeding grounds was a female humpback whale sighted off Brazil and two years later identified off the eastern coast of Madagascar, a distance of almost 10,000 km (Stevick et al. 2011). It is assumed in these cases that with greater distances the whales are using a feeding area accessible to both breeding grounds. It is plausible the motivation of movement between adjacent breeding grounds is to explore new regions (Olivieri et al. 1995; Clapham and Zerbini 2015), but with long distance movements between breeding grounds this seems less certain. Instead, long distance movements may be motivated by oceanographic shifts in the feeding area. It is likely in the case of the individual identified in French Polynesia and Colombia that the feeding area was the WAP, and this is what allowed the transition between breeding grounds. Large longitudinal movements by humpback whales have been documented within feeding areas, especially in unpredictable oceanographic circumstances (Stevick et al. 2006; Dalla Rosa et al. 2008), and it is not clear how humpback whales would select a breeding ground after these large-scale movements.

Immigration into the WAP in the future?

Two haplotypes were identified in the WAP2014 dataset that had not been found previously in the WAP. The haplotypes were identified in earlier studies that focused on humpback whale breeding grounds. These haplotypes could represent recent immigration into the WAP, although this could also be explained by the growing sample size. Continued sampling of the WAP in coming years, including the expansion of sampling areas to the north will be important to monitor any changes in the population. One of the two haplotypes previously described matched one originally sampled in Ecuador (Accession number HQ241481). Ecuador and Costa Rica are also breeding grounds for humpback whales, and there is evidence of interchange between Ecuador and Colombia (Felix et al. 2012). Although our breeding ground samples do not include Ecuador and Costa Rica, a hypothesis proposed for migratory connections suggests whales from Ecuador and the southern part of Colombia use the WAP feeding area, while whales from the northern part of Colombia and Costa Rica use the Strait of Magellan feeding area (Stevick et al. 2004; Acevedo et al. 2007). Moreover, it has been documented that the Magellan Strait is a separate feeding area from the WAP, and the two feeding areas are genetically differentiated (Olavarría et al. 2006; Acevedo et al. 2007). Our results support this hypothesis, as our Colombia samples are from Gorgona Island near southern Colombia and coastal southern Colombia. Due to the expansion of the Colombia population and possible exchange with Ecuador and Costa Rica (Acevedo et al. 2007), updated samples from Colombia and the inclusion of samples from Ecuador and Costa Rica would be useful to compare to the WAP sample collection. It is also possible that there are unsampled feeding areas where Colombia whales forage.

Another haplotype from our dataset (one female) matched one sampled originally in the Southeast Atlantic (Accession number GQ913713.1, Kershaw et al. 2017). Although it is not possible to compare our microsatellite genotypes to those of Kershaw et al. without standardizing allele sizes, the haplotype has not been identified in Brazil or any of the South Pacific breeding grounds. A haplotype first sampled in a region does not predict individuals with that haplotype are originally from that region. However, the fact that it has not been sampled previously in the WAP, South Pacific, or Brazil is intriguing. It would be useful in the future to standardize and compare the genotype information from the Eastern Atlantic and the WAP. Although not possible to determine without the genotype comparison of the regions, this could represent another example of an extraordinarily long distance migration at some point in time, similar to the individual female humpback whale that was sighted off Brazil and then again off Madagascar (Stevick et al. 2011).

There was no clear evidence of a migratory connection of the WAP whales to Brazil. This is consistent with previous genetic (Cypriano-Souza et al. 2017) and tagging studies (Zerbini et al. 2006, 2011) of whales in Brazil that migrated southeast of Brazil. Although the assignment tests did not show any individuals from Brazil migrating to the WAP, there has been documentation of migrants between Brazil and Colombia. Two individuals sampled in Brazil were considered migrants from Colombia, and one individual sampled in Colombia was considered a migrant from Brazil (Cypriano-Souza et al. 2017). Our study, and that of Cypriano-Souza et al. (2017), showed the F_{ST} between Colombia and Brazil was significant. Cypriano-Souza et al. (2017) also discuss the results from *BAYESASS*, a program that estimates the magnitude and direction of recent migration, and found the two breeding grounds have low enough levels of gene flow to be considered separate populations. However, when Colombia and the WAP were pooled and compared to Brazil, the number of migrants was estimated to be 43.8 per generation (based on F_{ST}). This suggests that some whales in Brazil may be using the WAP feeding area. Moving forward, these populations will continue to be monitored over the next several years, and will be compared to our growing WAP dataset to identify any changes. This is especially important considering climate change and the rapid warming around the WAP. We anticipate with changing climate conditions, whales from Brazil may begin to utilize the WAP, and we suggest sampling, especially on the northern end of the WAP, should increase each season to document any changes in the coming years.

Conservation and management implications

Climate change continues to alter patterns in migratory species (Robinson et al. 2009; Charmantier and Gienapp 2014). Although the 1986 moratorium on whaling has clearly aided in the recovery of humpback whales, populations may face an additional challenge as climate change continues to alter sea ice habitat and krill densities around Antarctica (Robinson et al. 2009). Previous studies have found that humpback whales in the Antarctic follow krill patches over large distances (Friedlaender et al. 2006, 2013; Dalla Rosa et al. 2008; Curtice et al. 2015), and it is well known that the WAP has one of the highest concentrations of krill in the Southern Ocean (Quetin and Ross 2003). Moreover, it was recently found that the direct hotspots of humpback whale activity are the same areas targeted for the most intense krill fishing effort (Weinstein et al. 2017). The authors suggested the krill fishery be re-evaluated to take into consideration humpback whale

foraging and climate change. The changes brought about by warming trends is already evident in this area (Atkinson et al. 2004), and knowledge of how humpback whales adapt to this changing environment will be imperative for future conservation. The concerted sampling effort of humpback whales around the WAP and tropical breeding grounds over the next decade will help illuminate any patterns linking climate change, oceanographic conditions, and movements of whales in the context of ecosystem structure and function. Continued genetic monitoring will highlight any changes in migratory connections or the composition of the populations. It will be important to collect hormone data as well to identify any emerging patterns in timing of the migration of pregnant females since energy demands are especially high for these females. Our study and others documenting extensive point-to-point migrations of humpback whales show that migration is not exclusively based on the shortest distance between the feeding and breeding areas, but include several factors that must be considered in future management decisions.

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