Genetic identity of humpback whales migrating past New Zealand

DEBBIE STEEL[1], NADINE GIBBS[2], EMMA CARROLL[3], SIMON CHILDERHOUSE[4], CARLOS OLAVARRÍA[3], C. SCOTT BAKER[1] AND ROCHELLE CONSTANTINE[3]

[1] *Marine Mammal Institute, Oregon State University, Newport OR 97365, USA*
[2] *Cook Strait Whale Project, Wellington, New Zealand*
[3] *School of Biological Sciences, University of Auckland, Auckland, New Zealand*
[4] *Blue Planet Marine, PO Box 3639, Richmond, Nelson, 7050, New Zealand*

ABSTRACT

Historically humpback whales (*Megaptera novaeangliae*) migrating past New Zealand have been linked to the east Australia migratory corridor, western South Pacific breeding grounds and IWC Antarctic Area V feeding grounds. Due to the largely opportunistic nature of sightings, to date most studies have analysed small datasets. Here we genetically analyse 211 samples (193 biopsy samples as part of a dedicated Cook Strait survey of whales on their northern migration, and 18 from dead, beachcast whales) largely collected between 2003 and 2010 (n=210). The 190 DNA profiles that passed quality control represented 167 unique whales. The majority of whales (n=164) were sampled in a single year. Comparison of the 167 whales to the Oceania (n=1,052 individuals) and east Australia (n=865 individuals) DNA registers revealed six matches to New Caledonia and five matches to east Australia; there were no matches to any other Oceania region. This study shows that humpback whales passing New Zealand on their northern migration show the least genetic difference to New Caledonia. However, they don't appear to show the same fidelity to the migratory corridor as they do to the breeding grounds. The low rate of between-year resightings and matches to east Australia suggests more variability in the use of migratory corridors. Possible connections to an east Australian breeding ground in the Great Barrier Reef could not be explored fully due to a lack of data from this area but given the level of matches to the east Australian migratory corridor this would be of interest in the future.

KEYWORDS: HUMPBACK WHALES, GENOTYPING, NEW ZEALAND, MIGRATION

INTRODUCTION

The breeding and feeding ground destinations of humpback whales (*Megaptera novaeangliae*) migrating past mainland New Zealand (NZ) have been described previously through *Discovery* tags (Chittleborough 1959, Dawbin 1964), photo-identification of flukes (Constantine *et al.* 2007; Garrigue *et al.* 2011a; Franklin *et al.* 2012), satellite tags (Garrigue *et al.* 2010) and genetic identification (Olavarría *et al.* 2006a SC/A06/HW31, Constantine *et al.* 2012). All studies have shown links to the International Whaling Commission (IWC) breeding stocks E1 and E2 and Antarctic feeding ground V. Historically, NZ whales were linked to the migratory corridors of Norfolk Island and east Australia and breeding grounds in Fiji, but more recently individuals have been linked primarily to New Caledonia and east Australia with only a single photo-ID match to Tonga and none to Fiji. Apart from Garrigue *et al.* (2011a), most analyses have used small sample sizes largely due to the low number and opportunistic nature of sightings of humpback whales as they migrate past coastal NZ.

The humpbacks migrating past NZ are considered part of the endangered breeding stock of Oceania whales (e.g. comprising IWC Breeding Stocks E2, E3 and F) with post-whaling recovery rates significantly lower than the east Australian (E1) humpbacks to the west of NZ (Childerhouse *et al.* 2008, Noad *et al.* 2011, Constantine *et al.* 2012). The exact reasons for the slow recovery are unknown but the illegal Soviet takes south of NZ in the late 1950s and early 1960s almost certainly had a major impact on this stock (Clapham *et al.* 2009).

Opportunistic records of humpback sightings around mainland NZ have been collected since 1970, with the majority of the 157 sightings reported from 1970-1999 occurring on the whales' northern migration (Gibbs and Childerhouse 2000). Humpback carcasses are occasionally found beachcast around NZ, providing another source of genetic material (Thompson *et al.* 2013). In addition to the

mainland sightings, around ten years ago there were sporadic reports of humpback whales swimming past Raoul Island (the only inhabited island in the Kermadec group, approximately 1,100 km northeast of mainland NZ) on their southern migration to Antarctic feeding grounds. Land-based surveys have involved a single four-hour survey at seven sites each year from 2008 – 2012 with a change in effort in 2013 where five surveys were conducted from 21 September – 17 November. The early surveys estimated up to 153 whales migrating past this remote island during a four-hour period with between six and 25 whales observed at the single site surveys in 2013 (e.g., Brown 2010, Potier and Shanley 2011, Gibson 2013).

In 2004, systematic surveys we established in the main 'hotspot' for humpback whale sightings around mainland NZ; at the Tory Channel entrance to Cook Strait (Gibbs and Childerhouse 2004) (Fig. 1). These land based surveys were based near the historic land-based spotting site used by the Tory Channel whalers (Dawbin 1964) but boat-based researchers were directed by the land-based observers to collect photo-ID and biopsy tissue samples to individually identify the whales. A preliminary study using only 30 genetic samples from NZ suggested that whales migrating northward through Cook Strait were most closely related to whales on the New Caledonia breeding ground (Olavarría *et al.* 2006a SC/A06/HW31). Since the initial Olavarría *et al.* (2006a) study, the collection of additional tissue samples from both stranded and living whales has greatly increased the number of samples available. This allows for an expanded investigation using genetically identified individuals to investigate the likely breeding ground destinations of these whales. In addition, we were able to investigate between- and within-year fidelity of individual whales to the NZ migratory corridor.

METHODOLOGY

Sample collection and DNA profiling

Dedicated surveys for humpback whales were conducted at Tory Channel, Cook Strait, NZ (Fig. 1) during the austral winters (i.e. July-August) of 2004-2013. Land-based surveys were conducted by four or more dedicated observers from a site ($41^0$12.45'S $174^0$19.43'E) 127 m above sea level. Surveys were conducted in favourable weather conditions, typically up to Beaufort 5. When a pod of whales was sighted, a research vessel was launched to attempt to collect photo-ID images of the underside of the whales' flukes (Katona *et al.* 1979) and collect a biopsy sample. Where possible, the whales were identified using both methods. Skin biopsy samples were collected using a modified veterinary device (Krützen *et al.* 2002).

In addition, we accessed humpback whale samples held by the NZ Cetacean Tissue Archive from 1998-2012 (Thompson *et al.* 2013). Skin samples in the Archive were preserved in 70% ethanol on location and curated at the University of Auckland for storage at -20°C. Total genomic DNA was extracted from skin biopsy samples either using standard proteinase K digestion and phenol/chloroform methods (Sambrook *et al.* 1989), as modified for small samples by Baker *et al.* (1994), or the Puregene DNA isolation kit (Gentra).

DNA profiles for each sampled whale included mtDNA control region haplotypes (470 bp), sex and up to 15 microsatellite loci. Molecular identification of sex and sequencing of the mtDNA control region (470 bp) followed methods previously described by Olavarria *et al.* (2007). Microsatellite genotyping at up to fifteen microsatellite loci (EV1, EV14, EV21, EV94, EV96 and EV104; Valsecchi and Amos 1996; GATA28 and GATA417; Palsbøll *et al.* 1997; RW18, RW31, RW410 and RW48; Waldick *et al.* 1999; GT23, GT211 and GT575; Bérubé *et al.* 2000) followed methods previously described by Constantine *et al.* (2012). Amplicons from 4-6 loci were co-loaded for capillary electrophoresis with an ABI3730 or an ABI 3130. Alleles were sized with Genemapper v4.0 (Applied Biosystems) and all automated calling was confirmed by visual inspection (Bonin *et al.* 2004). As a precaution against poor DNA quality, only those samples that amplified at a minimum of 11 microsatellite loci were retained for further analyses (QC11 dataset).

Data management, analyses of microsatellite allele frequency and analysis of probability of identity for each microsatellite locus and mtDNA were conducted using the program GenAlEx (Peakall and Smouse 2005).

Genotype matching and genetic diversity

We used CERVUS v3.0 to compare QC11 DNA profiles between- and within-years (Kalinowski *et al.* 2007). Within-year matches provide an indication of residency times between sampling events and

locations and between-year matches give an indication of migratory fidelity to NZ. For the final DNA register, only one copy of the DNA profile for each individual whale was retained and was assigned a unique genetic ID.

Confidence in matches was given by the average probability of identity (PID: Paetkau and Strobeck 1994), calculated in GENAlEx. As a precaution against false exclusion due to allelic dropout and other genotyping errors (Waits and Leberg 2000; Waits *et al.* 2001), the initial comparison allowed for mismatches at up to three loci. The electropherograms of the mismatching loci were then reviewed and either corrected based on this visual inspection or repeated. Because of some incomplete genotypes, we required a minimum overlap of 8 loci to identify replicate samples of individual. Where a pair-wise comparison did not meet this minimum overlap, the missing loci for the limiting genotype were repeated or the sample was removed from the data set.

Genetic diversity was investigated using both mitochondrial and microsatellite markers derived from the DNA profiles. We used Arlequin v3.1 (Excoffier *et al.* 2005) to estimate haplotype and nucleotide diversity of the mitochondrial control region. Observed and expected heterozygosity of microsatellite loci were calculated in GenAlEx, and we used GENEPOP v4.2 (Raymond and Rousset, 1995) to test for deviations from the Hardy-Weinberg equilibrium.

Connectivity and differentiation of New Zealand with Oceania and East Australia
We used individual-based and population-level approaches to investigate connectivity of NZ with Oceania and east Australia. First, the NZ DNA register was compared with a curated database of DNA profiles from 1,052 humpback whales sampled in three wintering grounds of Oceania (i.e. New Caledonia, Tonga, American Samoa-French Polynesia; Fig. 1) from 1995-2012 (Steel *et al.*2008 SC/60/SH13; Constantine *et al.* 2012) to assess the movement of individuals between these regions. In addition, as a proxy for the presumed breeding ground on the Great Barrier Reef in Australia, we compared the NZ DNA register with two DNA registers from the migratory corridor of east Australia (Anderson *et al.* 2010 SC/62/SH7, Schmitt *et al.* 2014).

For comparison with this individual-based approach, we used Arlequin v3.1 to test for differentiation in mtDNA haplotypes between NZ and wintering grounds in Oceania and the east Australia migratory corridor (Olavarría *et al.* 2006b SC/58/SH25 with 160 additional sequences Anderson pers. comm.). Specifically, we estimated pairwise F-statistics (F_{ST}), Φ_{ST}. The significance of these differences was tested with a permutation procedure in Arlequin. Differentiation in microsatellite allele frequencies between NZ and wintering grounds in Oceania and the east Australia migratory corridor was tested with GENEPOP v4.2 (Raymond and Rousset, 1995).

RESULTS

New Zealand humpback whale DNA profiles
Between 1998 and 2012, 211 samples from humpbacks were collected in NZ: 193 from dedicated Cook Strait surveys and 18 from beachcast samples held in the NZ Cetacean Tissue Archive. The majority of samples (n = 210) were collected between 2003 and 2012. The number of samples per year ranged between one (1998 and 2003) and 52 (2012; Table 1). Of these 211 samples, 190 (90%) were successfully passed the quality control criteria of genotyping at a minimum of 11 loci.

After reconciling matches between- and within-years, these 190 DNA profiles represented 167 unique whales: 164 whales sampled one or more times within the same year and three sampled at least once in two or more years. All matches were supported by an average of 13 matching microsatellite loci (range 10-14), genetically identified sex plus mitochondrial DNA haplotype. The probability of a match by chance (P_{ID}) ranged from 2.0×10^{-9} to 8.3×10^{-17} depending on the number and variability of the loci in a given match. This means we are confident the markers used are able to differentiate individuals in a population of a few thousand whales (Constantine *et al.* 2012).

All three of the between-year recaptures were males: one was seen in 2004, 2005 and 2007, another in 2010 and 2011, and the third male was seen in 2011 and 2012. There was a male bias in the sex ratio of sampled whales in NZ, consistent with findings from the east Australia migratory corridor (Brown *et al.* 1995) and winter breeding grounds in Oceania (Constantine *et al.* 2012), but differing from the unbiased sex ration reported on the Area V feeding grounds (Constantine *et al.* 2014).

Genetic diversity of the New Zealand population
Sequences of the mtDNA control region were available for 150 of the 167 unique individuals. The 470 bp consensus sequence revealed 51 variable sites that defined 41 haplotypes; 40 of which were previously described by Olavarría *et al.* (2007; Olavarría *et al.* 2006b SC/58/SH25).The haplotype diversity was 0.969 and nucleotide diversity was 0.023. The microsatellite loci showed a range of observed and expected heterozygosity (Table 2), but the markers showed reasonable diversity with an average of 10 alleles per locus. Average observed and expected heterozygosity was 0.68 and 0.73, respectively. All loci except GATA28 showed a positive F_{IS} (average 0.073), but only three loci (EV37, rw48 and GT23) showed a significant deviation from HW (overall p = 0.0016)

Connectivity of New Zealand with Oceania and East Australia
Comparison of the 167 unique whales in the NZ humpback whale DNA register with the 1,052 in the Oceania DNA register (1995-2012) revealed six matches to New Caledonia. Of the six matches, two were females and four were males (Table 3). There were no matches between NZ and any other wintering ground in Oceania.

Comparison of the NZ humpback whale DNA profile catalogue with the 865 individuals in the east Australia humpback whale DNA register revealed five matches: three males and two females (Table 3).

Between-region DNA profile recaptures matched at an average of 11.9 microsatellite loci, genetically identified sex plus mtDNA control region haplotype. The probability of identity ranged between 5.06×10^{-8} to 1.77×10^{-19} for the individual matches.

Differentiation of New Zealand with Oceania and East Australia
Using the mtDNA haplotypes of all individuals in the sample, New Zealand was weakly but significantly differentiated from all three Oceania breeding grounds and the migratory corridor of east Australia at the haplotype level and to all regions except New Caledonia at the sequence level (Table 4). At the nuclear level New Zealand was weakly but significantly different to all breeding grounds and the migratory corridor of east Australia.

DISCUSSION

This study shows that humpback whales passing NZ on their northern migration show the least genetic difference to New Caledonia. However, they do not appear to show the same fidelity to the migratory corridor as they do to the breeding grounds. The low rate of between-year resights reported in our study suggests more variability in the use of migratory corridors. As the majority of samples were collected from whales during the austral winter months when they are on their northern migration, the findings do not necessarily reflect the movement patterns of whales on their southern migration. To date, there is no place around mainland NZ where whales are frequently sighted on the southern migration, and thus no dedicated surveys that could be compared to Cook Strait. There are, however, reports of large numbers of humpback whales migrating south past Raoul Island in the Kermadecs, to the northeast of mainland NZ. These are reported primarily during September – November with an estimate of up to 153 whales during a single four-hour period in October 2010 (Brown 2010). Whales are rarely sighted at Raoul during the austral winter months during the northern migration which suggests that the whales use different migration corridors at different times of the year.

Other studies have suggested that there may be sex specific variation in migration path even though humpback whales typically have a high degree of natal site fidelity to breeding grounds (Brown *et al.* 1995, Craig and Herman 1997, Valesecchi *et al.* 2010). Whales have been tracked migrating from New Caledonia past Raoul Island and mainland NZ, showing a link between New Caledonia and NZ whales on the southern migration, but samples sizes are still small (Garrigue *et al.* 2010, Garrigue *et al.* 2011a, b).

Despite almost two-thirds of the individuals in the Oceania DNA catalogue being sampled on breeding grounds to the east of New Caledonia, the fact that there were no matches is interesting as places such as Fiji, Vanuatu and Tonga are almost due north of NZ; although there are very few samples from Fiji or Vanuatu so conclusions are difficult to make. The results we present here are broadly consistent with earlier studies that matched NZ, east Australia and Oceania humpback photo-ID catalogues and found links to a single Oceania breeding ground; New Caledonia (Garrigue *et al.* 2002, Garrigue *et al.* 2011a; Franklin *et al.* 2008). This study, while confirming a lack of significant difference between NZ and

New Caledonian whales from sequences (but not mtDNA haplotypes), does provide new evidence that NZ has direct linkages with east Australia. This is supported by five individuals that were seen in both NZ and east Australia which is similar to the six individuals seen in both NZ and New Caledonia. This new evidence suggests that whales migrating through NZ may head to either New Caledonia (E2) or east Australia (E1) breeding grounds. Only one study found a match between NZ and another Oceania breeding ground, Tonga (Constantine *et al.* 2007).

Most (91%) of the samples collected from NZ are from Cook Strait during the northward migration. It is known that the whales that migrate up the east coast of the South Island split at the bottom of the North Island with some whales heading north through Cook Strait and others heading north up the east coast of the North Island (Dawbin 1964, Gibbs and Childerhouse 2000). It is possible that the whales migrating through Cook Strait could be heading to a different tropical breeding ground (e.g. E1 or E2) to those whales migrating up the east coast of the North Island (e.g. E3) and that by sampling at Cook Strait we are not getting a representative sample from all whales migrating past NZ. This could be investigated by sampling southeast of Cook Strait (e.g. Kaikoura) where whales from both migratory paths occur together and/or from sampling on the east coast of the North Island.

The E1 breeding grounds in the Great Barrier Reef – Coral Sea region are not as well studied as the east Australian migratory corridors, with no genetic results from the region which limits our understanding of linkages between breeding grounds. Surveys (Chaloupka and Osmond 1999) and satellite tag studies (Smith *et al.* 2012) have identified these calving grounds but systematic surveys including genetic sampling techniques would be valuable to help understand how the increase in east Australia humpback whales influences neighbouring regions. With the east Australian whales recovering rapidly, there may be an increased rate of interchange with other regions in the western South Pacific e.g., New Caledonia, Fiji and NZ. The waters of Fiji have extremely low numbers of humpback in comparison with the mid-20th century (Gibbs *et al.* 2004) and the Chesterfield Reef to the west of New Caledonia and in the Coral Sea region also has shown little increase in numbers post-whaling (Oremus and Garrigue 2014). Whether the recovery of the E1 stock of whales has an effect on population increase in other areas has yet to be seen. Our study has shown that there is a low resight rate of whales migrating through Cook Strait, but this may be a consequence of the relatively short survey season. The resight rate of whales is low (<2%) and the photo-ID matches show similarly low rates (Gibbs unpub. data). It could be that the survey season is too short to account for variation in migration times, or that whales use different routes to migrate past NZ.

Overall, the genetic sampling of humpbacks in NZ suggests that the destination for whales migrating past NZ is to both the New Caledonia (E2) and the east Australian (E1) breeding grounds. Further sampling on the east Australian breeding ground will be necessary to characterise the genetic identity of that breeding stock which will help in better defining the likely stock structure of whales migrating past NZ.

ACKNOWLEDGEMENTS

We thank our colleagues in the South Pacific Whale Research Consortium, Australian Antarctic Division and Southern Cross University for the use of their data throughout the region. The International Fund for Animal Welfare, Department of Conservation, OMV Ltd, Dolphin Watch Ecotours Picton, WWF-New Zealand, Heberley family and many field volunteers generously supported this research. Thanks to Department of Conservation staff who continue to collect stranding samples. The collection of biopsy samples was conducted under Marine Mammal and Animal Ethics permits to the Department of Conservation, New Zealand.

REFERENCES

Anderson, M., Steel, D., Franklin, W., Franklin, T., Paton, D., Burns, D., Harrison, P., Baverstock, P.R., Garrigue, C., Olavarría, C., Poole, M.M., Hauser, N., Constantine, R., Thiele, D., Clapham, P., Donoghue, M. and Baker, C.S. (2010). Microsatellite genotype matches of eastern Australian humpback whales to Area V feeding and breeding grounds. Report SC/62/SH7 to the Scientific Committee of the International Whaling Commission, Agadir, Morocco.

Baker, C.S., Slade, R.W., Bannister, R.W., Abernethy R.B. et al. (1994). Hierarchical structure of mitochondrial DNA gene flow among humpback whales, *Megaptera novaeangliae*, world-wide. *Mol Ecol* 3: 313-327.

Bérubé, M., Jørgensen, H., McEwing, R. and Palsbøll, P.J. (2000). Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale, *Megaptera novaeangliae*. *Mol Ecol* 9: 2181-2183.

Bonin, A., Bellemain, E., Bronken Eidesen, P. et al. (2004). How to track and assess genotyping errors in population genetic studies. *Mol Ecol* 13: 3261-3273.

Brown, M., Corkeron, P., Hale, P., Schultz, K., and Bryden. M. (1995). Evidence for a sex-segregated migration in the humpback whale (*Megaptera novaeangliae). Proc R Soc B* 259:229-234.

Brown, N. (2010). Raoul Island Whale Survey. Unpublished Department of Conservation Report, Warkworth, New Zealand.

Chaloupka, M. and Osmond, M. (1999) Spatial and seasonal distribution of humpback whales in the Great Barrier Reef region. *American Fish Soc Symp* 23:89-106.

Childerhouse, S., Jackson, J., Baker, C.S., Gales, N., Clapham, P.J., and Brownell Jr, R.L. (2008). *Megaptera novaeangliae* (Oceania subpopulation). IUCN 2012. IUCN Red List of Threatened Species. Available at http://iucnredlist.org

Chittleborough, R.G. (1959) Australian marking of humpback whales. *Norsk Hvalf Tid* 48: 47-55.

Clapham, P., Mikhalev, Y., Franklin, W., Paton, D., Baker, C.S., Ivashchenko, Y.V., Brownell Jr., R.L. (2009). Catches of humpback whales, *Megaptera novaeangliae*, by the Soviet Union and other nations in the Southern Ocean, 1947-1973. *Mar Fish Review* 71: 39-43.

Constantine, R., Russell, K., Gibbs, N., Childerhouse, S. and Baker, C.S. (2007). Photo-identification of humpback whales (*Megaptera novaeangliae*) in New Zealand waters and their migratory connections to breeding grounds of Oceania. *Mar Mamm Sci* 23: 715-720.

Constantine, R., Jackson, J.A., Steel, D., Baker, C.S., Brooks, L., Burns, D., Clapham, P., Hauser, N., Madon, B., Mattila, D., Oremus, M., Poole, M., Robbins, J., Thompson, K. and Garrigue, C. (2012). Abundance of humpback whales in Oceania using photo-identification and microsatellite genotyping. *Mar Ecol Prog Ser* 453: 249-261.

Craig, A.S., Herman, L.M. (1997). Sex differences in site fidelity and migration of humpback whale (*Megaptera novaeangliae*) to the Hawaiian Islands. *Can J Zool* 75:1923–1933

Dawbin, W.H. (1964) Movements of whales marked in the south west Pacific Ocean 1952 to 1962. *Norsk Hvalf Tid 53*:68-78

Excoffier, L., Laval, G. et al. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol Bioinf Online* 1: 47-50.

Franklin, W., Franklin, T., Brooks, L., Gibbs, N., Childerhouse, S., Smith, J., Burns, D., Paton, D., Garrigue, C., Constantine, R., Poole, M.M., Hauser, N., Donoghue, M., Russell, K., Mattila, D., Robbins, J., Oosterman, A., Leaper, R., Baker, C.S. and Clapham, P. (2008) Eastern Australia (E1 breeding grounds) may be a wintering destination for Area V humpback whales (*Megaptera novaeangliae*) migrating through New Zealand waters. Report SC/60/SH3 to the Scientific Committee of the International Whaling Commission, Santiago, Chile.

Garrigue, C., Aguayo, A., Amante-Helweg, V.L.U., Baker, C.S., Caballero, S., Clapham, R., Constantine, R., Denkinger, J., Donoghue, M., Flórez-González, L., Greaves, J., Hauser, N., Olavarría, C., Pairoa, C., Peckham, H. and Poole, M. (2002). Movements of humpback whales in Oceania, South Pacific. *J Cetacean Res Manage* 4: 255-260.

Garrigue, C., Zerbini, A.N., Geyer, Y., Heide-Jørgensen, M.-P., Hanaoka, W. and Clapham, P. (2010). Movements of satellite-monitored humpback whales from New Caledonia. *J Mammal* 91: 109-115.

Garrigue, C., Franklin, T., Constantine, R., Russell, K., Burns, D., Poole, M., Paton, D., Hauser, N., Oremus, M., Childerhouse, S., Mattila, D., Gibbs, N., Franklin, W., Robbins, J., Clapham, P. and Baker, C.S. (2011a). First assessment of interchange of humpback whales between Oceania and the East coast of Australia. *J Cetacean Res Manage (Special Issue)* 3: 269-274.

Garrigue, C., Clapham, P., Gales, N., Geyer, Y., Oremus, M. and Zerbini, A. (2011b) Oceanic seamounts: a new humpback whale (*Megaptera novaeangliae*) habitat discovered using satellite tagging. Fourth International Symposium on Bio-logging, 14-18 March 2011, Tasmania, Australia. [available from http://www.cmar.csiro.au/biologging4/documents/O5.14_Garrigue.pdf]

Gibbs, N., and Childerhouse, S. (2000). Humpback whales around New Zealand. Advisory Science Notes 287. Department of Conservation, Wellington, New Zealand.

Gibbs, N., and Childerhouse, S. (2004). First report for the Cook Strait humpback whale survey winter 2004. Unpublished report WGNCO-45765, Department of Conservation, Wellington, New Zealand.

Gibbs, N., Paton D., Childerhouse, S, McConnell, H., and Oosterman, A. (2004). A survey of whales and dolphins in the Lomaiviti Island group, Fiji, 2003. Unpublished Final Report. Department of Environment and Heritage, Canberra, Australia.

Gibson, T. (2013). Raoul Island Whale Survey. Unpublished Department of Conservation Report, Warkworth, New Zealand.

Kalinowski, S.T., Taper, M.L. and Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16: 1099-1106.

Katona, S., Baxter, B., Brazier, O., Kraus, S., Perkins, J. and Whitehead, H. (1979) Identification of humpback whales by fluke photographs. pp 33-44. *In*: H.E. Winn and B.L. Olla (eds.) *Behavior of Marine Animals*, Vol. Vol. 3. Plenum Press, New York.

Krützen, M., Barré, L., Möller, L.M., Heithaus, M.R., Simms, C., and Sherwin, W.B., (2002). A biopsy system for small cetaceans; darting success and wound healing in *Tursiops* spp. *Mar Mamm Sci* 18: 863-878.

Noad, M.J., Dunlop, R.A., Paton, D. and Cato, D.H. (2011). Absolute and relative abundance estimates of Australian east coast humpback whales (*Megaptera novaeangliae*). *J Cetacean Res Manage* 3: 243-252.

Olavarría, C., Childerhouse, S., Gibbs, N., and Baker, C.S. (2006a). Contemporary genetic diversity of New Zealand humpback whales and their genetic relationship with Breeding Stocks D, E, F and G. Report (SC/A06/HW31) to the Intersessional Workshop for the Comprehensive Assessment of Southern Hemisphere Humpback Whales, Scientific Committee of the International Whaling Commission, Hobart, Australia.

Olavarría, C., Anderson, M., Paton, D., Burns, D., Brasseur, M., Garrigue, C., Hauser, N., Poole, M., Caballero, S., Flóres-González, L. and Baker, C.S. (2006b). Eastern Australia humpback whale genetic diversity and their relationship with Breeding Stocks D, E, F and G. Report SC/58/SH25 to the Scientific Committee of the International Whaling Commission, St Kitts and Nevis.

Olavarría, C., Baker, C.S., Garrigue, C., Poole, M., Hauser, N., Caballero, S., Flórez-González, L., Brasseur, M., Bannister, J.L., Capella, J., Clapham, P.J., Dodemont, R., Donoghue, M., Jenner, C., Jenner, M.-N., Moro, D., Oremus, M., Paton, D., Rosenbaum, H. and Russell, K. (2007). Population structure of South Pacific humpback whales and the origin of the Eastern Polynesian breeding grounds. *Mar Ecol Prog Ser* 330: 257-268.

Oremus, M., and Garrigue, C. (2014). Humpback whale surveys in the Chesterfield Archipelago: A reflection using 19th century whaling records. *Mar Mamm Sci* 30: 827-834.

Paetkau, D. and Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Mol Ecol* 3: 489-495.

Palsbøll, P. J., Bérubé, M., Larsen, A.H. and Jørgensen, H. (1997). Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. *Mol Ecol* 6: 893-895.

Peakall, R. and Smouse, P.E. (2005). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* 6: 288-295.

Pompanon, F., Bonin, A., Bellemain, E. and Taberlet, P. (2005). Genotyping errors: causes, consequences and solutions. *Nat Rev Genet* 6: 847-859.

Potier, S. and Shanley, T. (2011). Raoul Island Whale Survey. Unpublished Department of Conservation Report, Warkworth, New Zealand.

Raymond, M. and Rousset, F. (1995). GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicism. *J Hered* 86: 248-249.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual 2nd ed.* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory Press.

Schmitt, N.T., Double, M.C., Jarman, S.N., Gales, N., Marthick, J.R., Polanowski, A.M., Baker, C.S., Steel, S., Jenner, K.C.S., Jenner, M-N.M., Gales, R., Paton, D., and Peakall, R. (2014). Low levels of geentic differentiation characterize Australia humpback whale (*Megaptera novaeangliae*) populations. *Mar Mamm Sci* 30:221-241.

Smith, J.N., Grantham, H.S., Gales, N., Double, M.C., Noad, M.J. and Paton, D. (2012). Identification of humpback whale breeding and calving habitat in the Great Barrier Reef. *Mar Ecol Prog Ser* 447: 259-272.

Steel, D., Garrigue, C., Poole, M., Hauser, N., Olavarría, C., Flóres-González, L., Constantine, R., Caballero, S., Thiele, D., Paton, D., Clapham, P., Donoghue, M. and Baker, C.S. (2008).

Migratory connections between humpback whales from South Pacific breeding grounds and Antarctic feeding areas based on genotype matching. Report SC/60/SH13 to the Scientific Committee of the International Whaling Commission, Santiago, Chile.

Thompson, K.F, Millar, C.D., Baker, C.S., Dalebout, M., Steel, D., van Helden, A.L., and Constantine, R. (2013). A novel conservation approach provides insights into the management of rare cetaceans. *Biol Cons* 157: 331-340.

Valsecchi, E. and Amos, W. (1996). Microsatellite markers for the study of cetacean populations. *Mol Ecol* 5: 151-156.

Valsecchi, E. Corkeron, P.J., Galli, P., Sherwin, W., Bertorelle, G. (2010). Genetic evidence for sex-specific migratory behaviour in western South Pacific humpback whales. *Mar Ecol Prog Ser* 398: 275-286.

Waits, J. L. and Leberg, P.L. (2000). Biases associated with population estimation using molecular tagging. *An Conserv* 3: 191-199.

Waits, L. P., Luikart, G. and Taberlet, P. (2001). Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Mol Ecol* 10: 249-256.

Waldick, R. C., Brown, M.W. and White, B.N. (1999). Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale. *Mol Ecol* 8: 1763-1765.

Weir, B. and Cockerham, C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.

Figure 1. Map of the study region.

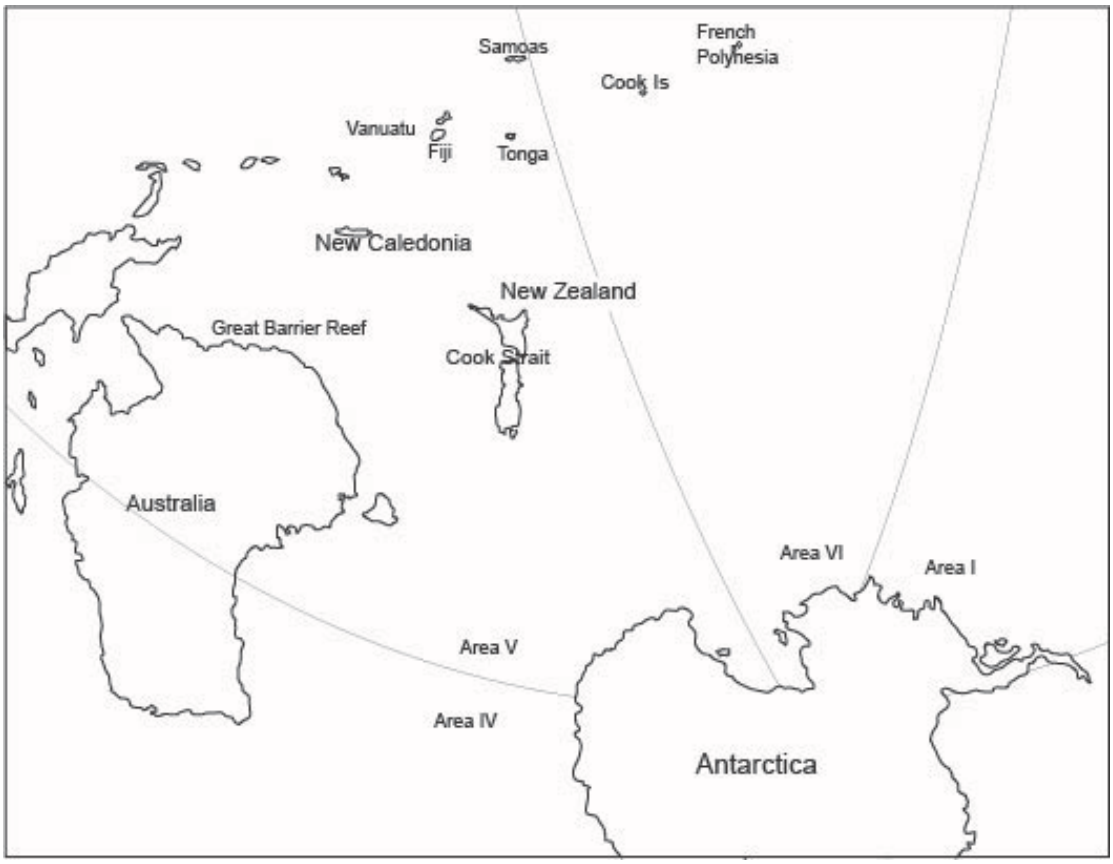

Table 1: Number of tissue samples collected from humpback whales from New Zealand during dedicated surveys in Cook Strait (SURVEY) and beachcast whales (STRANDING). The number of samples that generated DNA profiles that passed quality control are listed (N_{QC}), as is the number of unique DNA profiles per year (N_{UNIQUE}). The number of males, females and whales of unknown sex are listed by year also.

YEAR	SURVEY	STRANDING	TOTAL	N_{QC}	N_{UNIQUE}	M	F	U
1998	0	1	1	1	1	0	1	0
2003	0	1	1	1	1	0	0	1
2004	12	3	15	12	11	6	2	3
2005	11	1	12	11	11	7	3	1
2006	10	1	11	10	10	6	3	1
2007	10	1	11	11	11	6	2	3
2008	16	0	16	13	12	5	7	0
2009	20	4	24	16	14	8	3	3
2010	24	0	24	21	19	10	9	0
2011	41	3	44	42	36	24	11	1
2012	47	5	52	52	45	31	12	2
TOTAL	191	20	211	190	171	104	52	15

Table 2: Summary of microsatellite loci used to individually identify humpback whales sampled during dedicated surveys at Cook Strait, New Zealand or beachcast around New Zealand, between 1998 and 2012.The observed (H_O) and expected (H_E) heterozygosity and probability of identity (P_{ID}) were calculated with the program GENALEX (Peakall and Smouse 2006), Weir and Cockerham (1984) F_{IS} was calculated in GENEPOP. *indicates significance at 0.05.

Locus	N	Source	Repeat	Size range	n alleles	H_O	H_E	F_{IS}	Probability of Identity
EV1	167	(Valsecchi and Amos 1996)	2	123-129	4	0.497	0.536	0.0764	0.277
EV14	167	(Valsecchi and Amos 1996)	2	125-145	10	0.695	0.716	0.0333	0.119
EV21	164	(Valsecchi and Amos 1996)	2	109-119	6	0.628	0.667	0.0610	0.156
EV37	167	(Valsecchi and Amos 1996)	2	192-232	20	0.856	0.922	0.0739*	0.011
EV94	166	(Valsecchi and Amos 1996)	2	202-220	9	0.807	0.819	0.0175	0.056
EV96	160	(Valsecchi and Amos 1996)	2	147-173	13	0.806	0.862	0.0676	0.032
EV104	164	(Valsecchi and Amos 1996)	2	143-151	4	0.372	0.396	0.0642	0.401
GATA417	164	(Palsbøll, Bérubé *et al.* 1997)	4	187-274	18	0.866	0.909	0.0510	0.015
GATA28	167	(Palsbøll, Bérubé *et al.* 1997)	4	143-203	13	0.557	0.549	-0.0122	0.216
GT211	164	(Bérubé, Jorgensen *et al.* 2000)	2	100-120	10	0.738	0.822	0.1054	0.053
GT23	166	(Bérubé, Jorgensen *et al.* 2000)	2	101-123	9	0.705	0.783	0.1027*	0.073
GT575	143	(Bérubé, Jorgensen *et al.* 2000)	2	137-177	13	0.762	0.796	0.0459	0.066
rw31	36	(Waldick, Brown *et al.* 1999)	2	106-122	7	0.528	0.662	0.2167	0.149
rw4-10	166	(Waldick, Brown *et al.* 1999)	2	192-214	10	0.789	0.816	0.0354	0.055
rw48	156	(Waldick, Brown *et al.* 1999)	2	108-120	6	0.603	0.722	0.1687*	0.113
Average	154.5				10.1	0.681	0.732		0.120
									$3.4X10^{-17}$

Table 3: The sex, years of genotype capture and available additional information for humpback whales sampled in A) both New Caledonia and New Zealand and B) east Australia and New Zealand.

Year of Capture		Sex	Additional information
A) New Zealand	New Caledonia		
2007	2004; 2011	Female	-
2008	2002	Female	-
2009	1999; 2000; 2001	Male	Yearling in 1999
2012	2005	Male	-
2012	1997	Male	Escort in New Caledonia
2012	2000; 2001; 2004	Male	Calf in 2000
B) New Zealand	East Australia		
2004	2003	Male	Captured in Byron Bay
2005	2002	Male	Captured in Byron Bay
2001	2004	Female	Captured in Byron Bay
2011	2002	Male	Captured in Byron Bay
2012	2001	Female	Captured in Hervey Bay

Table 4: Population differentiation for mtDNA haplotype (F_{ST}) and nucleotide (Phi_{ST}) and microsatellite (uF_{ST}) between New Zealand and the migratory corridor of east Australia and Oceania breeding grounds. N indicates the number of individuals with haplotypes used for each population; there were 151 individuals with haplotypes and 167 with genotypes in the NZ population. *indicates significance at 0.05, ** at 0.01 and *** at 0.001.

	n haplotypes	F_{ST}	Phi_{ST}	n genotypes	uF_{ST}
East Australia	316	0.0028*	0.0094*	734	0.0012***
New Caledonia	367	0.0059***	0.0023	381	0.0006***
Tonga	337	0.0116***	0.0085*	347	0.0025***
French Polynesia/ American Samoa	302	0.0251***	0.0325***	325	0.0039***