Using Mixed-Stock Analysis of Humpback Whales (*Megaptera novaeangliae*) to estimate migratory allocation from Antarctic Feeding Areas to South Pacific Breeding Grounds

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ABSTRACT

Very few migratory connections have been documented between humpback whale (*Megaptera novaeangliae*) breeding grounds and feeding areas in the South Pacific Ocean. Understanding these migratory connections is crucial to management strategies especially for the allocation of historical Antarctic catches in population dynamic models used to assess the current recovery of these small, isolated breeding stocks. Here we use mixed-stock analysis of mtDNA haplotypes as described in Olavarría *et al.* (2007) to apportion samples from four Antarctic feeding areas (IWC Areas IV, V, VI* and I*) (n = 142) to seven breeding grounds, including: Western Australia; Eastern Australia; New Caledonia; Tonga; Cook Islands; French Polynesia; and Colombia (n = 1,504). Assuming that the breeding grounds represent 'baseline stocks' and that each feeding area represents 'mixed-stocks', results showed Area IV apportioned primarily to Western Australia (71.6%, SE 1.1%), Area VI* apportioned primarily to Tonga (78.4%, SE 24.5%), and Area I* whales apportioned primarily to Colombia (76.5%, SE 8.1%). Although limited by small sample size, Area V apportionment was close to evenly split between New Caledonia (44.4%, SE 28.4%) and Eastern Australia (51.0%, SE 26.4%). These results agree with previous *Discovery* marking, photo-identification and genetic studies of individuals suggesting that mixed-stock analysis could prove to be a useful tool for modeling the apportionment of feeding areas to breeding stocks for the purpose of assessing recovery and evaluating the impact of any future Antarctic catches.

KEY WORDS: SOUTHERN HEMISPHERE; BREEDING GROUNDS; FEEDING GROUNDS; GENETICS; MIGRATION; MOVEMENTS

INTRODUCTION

Humpback whales in the Southern Hemisphere undertake the annual migration from tropical breeding grounds to Antarctic feeding areas (Mackintosh 1942). These breeding stocks

experienced severe population declines due to commercial whaling in their associated Antarctic feeding areas that occurred legally and illegally until the early 1970's (Clapham *et al.* 2009). The number of humpback whales killed in the Southern Hemisphere was more than 200,000 in the 20th century (Clapham & Baker 2008).

The International Whaling Commission (IWC) divided the tropical breeding grounds into several stocks for management purposes based on distribution and *Discovery* tag returns, and divided Antarctica into feeding Areas I-VI based on summer catch distributions in the feeding season (Mackintosh 1942, 1965, IWC 2006). In the South Pacific and eastern Indian Ocean, these breeding regions were assigned stock designation D, E, F and G (Figure 1). Severe reduction in most Southern Hemisphere whale populations prompted the IWC to offer protection for humpback whales from whaling in 1966, but illegal Soviet pelagic whaling continued until the early 1970's. Increase in the breeding stocks has been variable, and some stocks remain small despite decades of protection (Clapham & Baker 2008). The Eastern Australia humpbacks appear to have increased more quickly (Paterson *et al.* 1994) than humpbacks in Oceania. The Oceania breeding grounds are defined here to include the regions of New Caledonia, Tonga, Cook Islands and French Polynesia (Baker *et al.* 2006).

In the South Pacific Ocean very few connections have been documented for humpback whale migratory connections. Describing connections between their breeding grounds and feeding areas is crucial for management strategies, especially for historical catches and modeling of current population dynamics (e.g., Jackson *et al.* 2008). Genetic markers have been informative for estimating population differentiation and designation of subpopulation status. Recent analyses of mtDNA suggest that the stocks E and F are genetically differentiated and further divided, separating New Caledonia (Stock E2) and Tonga (E3) from Eastern Australia (Stock E1); and Cook Islands (Stock F1) from French Polynesia (Stock F2) (Garrigue *et al.* 2006, IWC 2006, Olavarría *et al.* 2007, Poole 2006). Comparison of photo-identification catalogues between breeding sub-stocks E1, E2, E3, F1 and F2 has also confirmed that these populations are

somewhat isolated, with limited movement between regions (Garrigue *et al.* 2002). Although the breeding stocks have shown significant genetic differentiation of mtDNA there still remains uncertainty in the feeding areas about genetic differentiation (Loo *et al.* 2006).

Despite the known history of humpback whales in the South Pacific, few direct connections between breeding stocks and their Antarctic feeding areas have been documented. Limited migratory information comes from early *Discovery* tag records (Dawbin 1966), photo-identification matches (Franklin *et al.* 2008, Rock *et al* 2006) and recent genotype matches (Steel *et al.* 2008). In the absence of direct observations of migratory pathways, molecular genetic markers have proven useful for inferring migration patterns by matching start and end point observations with population identity (Bowen *et al.* 2007). Mixed-stock analysis (MSA), originally developed for salmon management programs (Grant *et al.* 1980), provides an estimate of the most likely proportion of 'source populations' represented in a 'mixed population' sample using the frequencies of genetic markers (Bass *et al.* 2004). In fisheries, MSA is used to estimate what proportion of a population in a mixture came from each of a number of most likely source populations (Manel *et al.* 2005).

We used MSA with mtDNA haplotypes to examine what proportion of a humpback whale population in an Antarctic feeding mixture came from each of the breeding stock source populations. MtDNA haplotypes were derived from skin biopsy samples from seven breeding grounds (IWC breeding stocks D, E1, E2, E3, F1, F2 and G) and four IWC Antarctic feeding Areas (Areas IV, V, VI* and I*). The asterisk of Area VI and I reflect geographic distributions of humpback whales instead of the IWC boundaries and is discussed in greater detail in the Results section. This dataset revises and extends that reported previously in Olavarría *et al.* (2007), removing samples identified as replicates by genotyping (Steel *et al.* 2008) and including 392 previously unanalyzed samples from Oceania as well as 142 individual whales from four Antarctic Areas. An Analysis of Molecular Variance (AMOVA) of this dataset first confirmed differentiation between breeding grounds and between the four feeding areas. This was followed

by mixed-stock analysis (MSA) with a Bayesian baseline to estimate the migratory apportionment of each feeding area to the seven breeding grounds. The Bayesian approach allows for the uncertainty of rare haplotypes that may not be detected in source population samples (Pella & Masuda 2001).

METHODS

Field collection

A total of n = 1,713 samples were collected from live whales in the seven breeding grounds (Table 1). In addition to the samples described in Olavarría *et al.* (2007) (n = 1,112), biopsy and sloughed skin tissue samples (n = 392) were collected from humpback whales on breeding grounds of the South Pacific during the Austral winters of 2003 through 2007. Western Australia samples (biopsy only) were collected from North West Cape in 2002 as described in (Brasseur 2007) with additional samples collected in 1990, 1993 and 1994 (Baker *et al.* 1994; 1998). Eastern Australia samples were collected from humpback whales off Byron Bay and Ballina (sloughed skin only) as described in Olavarría *et al.* (2006). Oceania samples were collected primarily by members of the South Pacific Whale Research Consortium during synoptic surveys from 1999-2007 but also include smaller numbers of samples collected during surveys of some regions dating back to 1991. Samples collected from the Colombian breeding grounds were collected by members of Project Yubarta from 1991 to 1998 (Flórez-González 1991; Baker *et al.* 1998; Caballero *et al.* 2001; Steel *et al.* 2008). Sampling on breeding grounds was carried out aboard dedicated small boat surveys. Biopsy samples were collected using a stainless steel biopsy dart deployed from a crossbow (Lambertsen 1987) or a modified veterinary capture rifle (Krutzen *et al.* 2002).

In the feeding areas, biopsy samples (n = 214) were collected from living whales during the Austral summers of 1989 to 2005, during surveys by the International Decade of Cetacean Research and Southern Ocean Whale and Ecosystem Research Cruise (IDCR/SOWER) under supervision of the IWC (Report 2006), and during more localized surveys of the Antarctic Peninsula by the Chilean Antarctic Institute (INACH).

MtDNA amplification and sequencing

As described in detail by Olavarría *et al.* (2007) genomic DNA was extracted from tissue samples using a standard phenol/chloroform extraction protocol (Sambrook *et al.* 1989) modified for small skin samples by Baker *et al.* (1994). An approximately 800 base-pair (bp) fragment of the 5'-end of mtDNA control region (i.e. D-loop) was amplified via the Polymerase Chain Reaction (PCR) using the primers, light-strand tPro-whale Dlp1.5 and heavy-strand Dlp8G as reported in Garrigue *et al.* (2004). Amplification and temperature profiles were followed as documented in Olavarría *et al.* (2007). Unincorporated primers and nucleotides were removed from PCR products using exonuclease (Exo I) and shrimp alkaline phosphatase (SAP) and sequenced on an ABI3730xl DNA sequencer (Applied Biosystems) using the primer M13Dlp-1.5.

Data Analyses

Sequences were aligned and edited using SEQUENCHER™ (version 4.1.2, Gene Codes Co.) and checked visually by comparison to other chromatographs. Unique haplotypes were defined by 71 variable sites resolved from the 470 bp consensus region as discussed in Olavarría *et al.* (2007). The potential for replicate samples of individual whales was considered for each regional sample set. Replicates were removed where microsatellite genotyping allowed for individual identification (Steel *et al.* 2008). After sequencing corrections five of the original 115 haplotypes were removed from Olavarría *et al.* (2007) (Final n = 110).

A pair-wise comparison at the haplotype level was performed using the program ARLEQUIN (version 3.1, Schneider *et al.* 2000) to compare the degree of genetic diversity between breeding and feeding regions. An Analysis of Molecular Variance (AMOVA) was performed in using 10,000 permutations to measure the differentiation between breeding stocks at the haplotype level.

MSA was conducted using the Statistical Program for Analyzing Mixtures (SPAM; version 3.7b; Alaska Department of Fish and Game (2003)) for haplotype data with 10,000 iterations and 1,000 bootstrap resamples. For the future purposes of allocating historical catches to breeding stocks, we assumed the breeding ground samples represented the 'source stocks' in the MSA program and the

feeding area samples represented the 'mixed-stocks'. In this framework, each feeding area was analyzed separately, and component estimates were apportioned to the seven breeding grounds.

Given the large number of haplotypes (Olavarría *et al.* 2007) a Bayesian method was implemented in the estimation mode of SPAM. The Bayesian approach allows for the uncertainty of rare haplotypes that are actually present in a breeding ground, but not detected in a small sample (Pella & Masuda 2001). Thus the Bayesian method has the potential to correct for small samples or rare haplotypes better than the standard Maximum Likelihood methods (Luke *et al.* 2004). Previous studies (Pella & Masuda 2000, Antonovich 2003) emphasized that Bayesian modeling of baseline frequencies is an acceptable way to account for any negative bias in analyses caused by sparse data.

The simulation mode in SPAM was used to assess whether differences in haplotype frequencies among breeding regions were large enough to estimate the origins of feeding areas. The simulation mode assigns one breeding region 100% apportionment as a possible mixture scenario to evaluate performance for a given source stock or breeding region (Alaska Department of Fish &Game 2003), and then reports what percentage could actually be detected by the program. These simulations work similarly to a jack-knife estimate evaluating how well the program can assign haplotypes correctly back to their respective breeding ground. Typically results of 90% or better have been used in fisheries studies to indicate sufficient power among data to differentiate between each of the source stocks and to determine relative mixture proportions reasonably accurately (Antonovich 2003).

RESULTS

Revised Antarctic Areas

After reviewing sample locations of Antarctic samples, we modified the boundaries of Area VI and Area I to reflect apparent geographic groupings of samples (Figure 2). For Area VI*, we included three samples from the eastern edge of Area VI and included seven samples collected in the western edge of Area I (n = 27). For Area I*, we excluded one additional sample in the central

region and included all samples from the Antarctic Peninsula (n = 68). The four samples from the western boundary of Area V and the four samples from the eastern boundary of Area V were not considered sufficient for statistical analysis, but are included here for an initial assessment of Area V. Although we did not attempt to adjust for differences in sample size due to very limited numbers from the Antarctic region, the number of haplotypes was similar in each of the four areas except for Area V (Table 1).

Population Diversity and Differentiation

After removal of replicates within regions, a total of n = 1,713 samples representing 1,504 individual humpback whales from 7 discrete breeding regions and n = 214 samples representing 142 individuals from four feeding areas were available to determine genetic diversity and differentiation. Of the 110 haplotypes observed in this study, only four occurred in all seven of the breeding grounds, and 41 occurred in only one breeding ground. The number of haplotypes was greatest for New Caledonia (55), as reported previously in Baker *et al.* (1998) and Olavarría *et al.* (2003). However, based on increased samples size, this study found Colombia to have the lowest number of haplotypes (25) (Table 1) contrary to previous studies sighting French Polynesia having the lowest. Within the feeding areas, the number of haplotypes was similar, ranging from 18 haplotypes in Area VI (sample size n= 27) to 27 haplotypes in Area IV (sample size n= 39); the exception being Area V (seven haplotypes represented) where only eight samples were analyzed.

Differentiation between breeding grounds and feeding areas was quantified by an AMOVA. The proposed subdivisions of IWC breeding stock E into E1, E2, and E3, as described previously, and all pair-wise comparisons were supported by significant overall differences (F_{ST} = 0.027; P < 0.001). For breeding grounds, pair-wise F_{ST} values (Table 2) illustrated a greater difference between Colombia and the Oceania breeding grounds than between Western Australia and Oceania as shown in Olavarría *et al.* (2007). For feeding areas all pair-wise comparisons were supported by significant overall differences (F_{ST} = 0.041; P < 0.001). For feeding areas all pair-wise F_{ST} values (Table 2) illustrated the greatest difference between Area I* and Area VI*. Pair-

wise comparisons of feeding areas to breeding grounds showed significant F_{ST} values for all but three cases: Area IV to Western Australia, Area VI* to Tonga, and Area I* to Colombia. The exception was Area V which was only significant with French Polynesia and Colombia (Table 2).

Migratory Apportionments

Apportionments to breeding grounds from feeding areas were inferred from the MSA program. Area IV was apportioned primarily to Western Australia (71.6% SE 10.8%) with a small percentage to Tonga (28.8% SE 13.4%). Although sample size was small, there was an almost equal split of Area V between New Caledonia (44.1% SE 28.3%) and Eastern Australia (51.1% SE 26.5%) and a small contribution to Western Australia (4.6% SE 9.0%). Area VI* was apportioned primarily to Tonga (81.4% SE 22.5%), with a smaller percentage apportioned to the Cook Islands (5.0% SE 15.9%). A large percentage of Area I* was apportioned to Colombia (88.3% SE 7.5%) with a smaller percentage (8.8% SE 7.4%) to the Cook Islands. French Polynesia had very low apportionments from any of these areas (< 3%). The mixed-stock simulation in the MSA program indicated that the population of each breeding ground could be correctly re-assigned with at least 83% accuracy, with the exception of the Cook Islands (73%) (Table 3).

DISCUSSION

The management of whale stocks becomes more effective if it can be determined how stocks assemble on the breeding grounds especially if less abundant stocks can be protected in both breeding and feeding areas. Mixed-stock analysis provides insight into this organization provided that all breeding grounds are genetically differentiated. The MSA strengthened results from previous photo-identification and genotype studies by providing general population apportionments instead of individual connections. This analysis has shown that the prevailing trends identified here are that each feeding area is apportioned to one primary breeding region.

Area IV

Historically Western Australia (breeding stock D) was associated with Antarctic Area IV stock for management purposes (Mackintosh 1942). Here using mtDNA data and MSA, the larger apportionment of Area IV to Western Australia supports these previous findings that linked individuals using historical whaling data (*Discovery* tags) and more recent photo-identification (Dawbin 1964; Gill & Burton *et al.* 1995; Franklin *et al.* 2008). However, the smaller apportionment to Tonga and the absence of any apportionment to Eastern Australia is puzzling. Olavarría *et al.* (2007) found low level haplotype frequency matches between Western Australia and breeding Stock E (Tonga and New Caledonia) which they used to predict possible movements between Western Australia and Stock E despite the large geographic distance between them. Chittleborough (1965) concluded that Western and Eastern Australia populations remained separated in most years, but mixed periodically on the feeding grounds as shown with *Discovery* tags. Recently Gales *et al.* (unpublished data) elucidated with satellite-tagging that whales from Eastern Australia either migrated directly south to Area V, migrated by the west coast of New Zealand continuing down to mid-region of Area V or migrated to the most eastern region of Area V. The exception was one individual who migrated from Eastern Australia directly to central Area IV. Photo-identification studies comparing breeding stocks D and E have shown Tonga to be a possible stopover for other breeding grounds to the west (Eastern Australia, Stock E1 and New Caledonia, Stock E2) and to the east (Cook Islands, Stock F1) (SPWRC 2009, Garrigue et al. In Review). In addition, there was a *Discovery* tag found in a whale from Tonga on the border of Area IV/V (Dawbin 1966), suggesting some of these whales may feed in the eastern portion of Area IV. All of this evidence suggests that the possibility exists that periodically there may be some overlap in the feeding regions of Area IV and V, but essentially Western Australia is somewhat isolated from the South Pacific. Therefore, the small apportionment of Area IV animals to Tonga suggests Tonga may be an occasional stopover for some whales headed to other areas in Stock E and these animals may be periodically using the Area IV feeding area. Another possibility is that the smaller apportionments indicated by the MSA may have higher degrees of uncertainty as shown by the higher standard errors associated with these estimates.

Area V

The sample size for this region was too small to provide significant statistical power. Despite this statistical limitation these samples provide intriguing insight into possible migratory destinations. Our results show almost equal apportionment to Eastern Australia and New Caledonia. This strengthens previous individual connection studies including *Discovery* tags and photo identification matches linking Eastern Australia to Antarctic Area V (Dawbin 1964; Olavarría *et al.* 2006; Franklin *et al.* 2008; Rock *et al.* 2006), and the genotype match linking Antarctic Area V and New Caledonia (Steel *et al.* 2008). The illegal Soviet Whaling from 1959/60-1961/62 (Clapham *et al.* 2009; Berzin 2008) that targeted Area V so intensely making this arguably the most important area for apportionment of historical catches. Even more when it is considered that this is the likely location for some of Japan's hunting grounds for the so-called scientific whaling of humpback whales under their program JARPA II. Small breeding stocks of humpback whales in the South Pacific could be at risk from hunting events in Area V and stress the need for additional Antarctic samples from this area to help clarify migration destinations.

Area VI*

A close connection between Area VI* and Tonga is indicated by the lack of significance in the AMOVA, and the large apportionment of Area VI* to Tonga in the MSA analysis. These results agree with recent genotype matching and limited *Discovery* tags, which have provided evidence of connections between the two regions (Dawbin *et al.* 1964; Steel *et al.* 2008). There was a 5.0% apportionment from Area VI* to the Cook Islands which has also been proposed as a component of the Area VI stock (Hauser *et al.* 2000). Recently a humpback whale satellite-tagged in the Cook Islands in September 2006 was located on the border of Area VI and Area I in December 2006. This implies that at least some humpbacks wintering in the Cook Islands feed in Area VI with perhaps some interchange between Area VI and Area I (Hauser *et al.* In Press), or what is referred to here as Area VI*. In addition, the whale was recorded heading southeast from the Cook Islands suggesting areas to the east of the Cook Islands, possibly French Polynesia, may host some whales that feed primarily in the western part of Area I (Hauser *et al.* In press).

Surprisingly, Area VI* showed no apportionment to French Polynesia (<3.0%) despite the geographic proximity of these seasonal habitats and the large sample size of French Polynesia. This suggests the whales in French Polynesia may be traveling to an as of yet unsampled component of Area VI or Area I, or that breeding stocks like Tonga and Colombia, the main apportionment from Area VI* and I* respectively, have sufficiently larger proportions of haplotype frequencies to these feeding areas masking what would be otherwise a small apportion to French Polynesia. Despite the fact that French Polynesia had very low apportionments from Area VI*, these recent findings reveal an interesting combination of results that should be explored further.

Area I*

The large apportionment from Area I* to Colombia confirms a strong association between Colombia and the Antarctic Peninsula. These differences support earlier conclusions that Antarctic Peninsula and Colombia are somewhat isolated from Oceania (Garrigue *et al.* 2002, Olavarría *et al.* 2007). Genetic markers and naturally marked individuals have confirmed that there is a connection between Colombia and Area I around the Antarctic Peninsula (Stevick *et al.* 2004, Stevick *et al.* 2006, Olavarría *et al.* 2000, Caballero *et al.* 2001, Stone *et al.* 1990). The AMOVA here and in Olavarría *et al.* (2007) suggest these regions are somewhat isolated from Oceania and Antarctic Areas V and VI as shown in the higher degree of differentiation in the pair-wise F_{ST} values (Table 2).

Implications of the Study

Mixed-Stock Analysis proved to be a useful method for assisting the proportional component estimates of feeding grounds to breeding stocks. Sample sizes from most breeding grounds were considerably larger ($n > 100$) and more representative of the known distribution of humpbacks in the South Pacific. Unfortunately, sample sizes for the feeding areas were relatively small resulting in large standard errors for some apportionments implying results for the smaller apportionments

should be approached with caution. A more concerted and systematic sampling of feeding areas, especially in Area V, is needed to complement the coordinated sampling of whales from Oceania breeding grounds.

South Pacific humpback whale data has three primary shortfalls: 1. a large number of different haplotypes, 2. variation in population size, and 3. generally sparse data. Although Maximum Likelihood methods have previously been used for data with these limitations in MSA, contributions from abundant stocks are underestimated and those from less common or even absent stocks are overestimated (Pella & Masuda 2001; Bolker *et al.* 2003). Previous studies (Antonovich 2003) emphasized that Bayesian modeling of baseline frequencies is an acceptable way to account for any negative bias in analyses caused by sparse data.

Other limitations of this study included the exclusion of less studied South Pacific breeding grounds where sample sizes were too small ($n < 5$) for consideration in this analysis, (Samoa, American Samoa, Vanuatu and Fiji) and the impact their absence might have on apportionments from feeding areas. Despite these limitations, MSA provided the first population analysis of South Pacific humpback whales providing additional inference to associate feeding areas and breeding grounds.

The future of MSA for migratory connections

Although the simulation mode in the MSA program is intended to assess power among specific data more concise methods are beginning to be developed. These show that 100% simulation algorithms of current MSA programs tend to have a positive bias in the simulation results overstating the power of assignment (Anderson *et al.* 2008). This bias is thought to be exacerbated when F_{ST} values indicate that relationships are relatively close among breeding populations as shown here. This is something that should be considered in future MSA studies as more samples become available.

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Table 1. Humpback whale samples collected including the number of individuals, the number of haplotypes in each region and the number of unique haplotypes relative to seasonal habitats. Individual samples were collected from 1991-2005 and replicates within regions were deleted. Area I* is solely the Antarctic Peninsula, and Area VI* includes 7 individuals from Area I/VI border (Figure 2).

Region	**Years Samples Collected**	**Individuals**	**Haplotypes**	**Unique Haplotypes by seasonal habitat**
Breeding Regions				
Western Australia	1990, 1993, 1994, 2002	174	53	21
Eastern Australia	2002, 2003	156	38	0
New Caledonia	1995-2005	367	55	10
Tonga	1991, 1994-1996, 1998-2003, 2005	355	51	2
Cook Islands	1998-2003, 2005	101	29	1
French Polynesia	1997-2007	247	30	2
Colombia	1991-1998	104	25	9
Total Breeding regions		**1504**		
Feeding Areas				
Antarctic Area IV	1999	39	27	16
Antarctic Area V	1991, 1999, 2001	8	7	4
Antarctic Area VI*	1990, 2001	27	18	5
Antarctic Area I*	1990, 1994, 1996, 1997, 1998, 1999	68	20	14
Total Feeding Areas		**142**		

Table 2. Updated South Pacific humpback whale F_{ST} values corrected for replicate samples. Pair-wise test of differentiation between all areas for mtDNA control region sequences. Values in bold represent a significant difference based on 10,000 random permutations of the data matrix ($P < 0.05$). Symbols above matrix represent significant difference (+) between regions or no significant difference (-) between regions.

Region	WA	EA	NC	Tg	CI	FP	Col	Area IV	Area V	Area VI*	Area I*
Western Australia D		+	+	+	+	+	+	-	-	+	+
Eastern Australia E1	**0.019**		+	+	+	+	+	-	-	+	+
New Caledonia E2	**0.013**	**0.010**		+	+	+	+	+	-	-	+
Tonga E3	**0.013**	**0.018**	**0.007**		+	+	+	+	-	-	+
Cook Islands F1	**0.028**	**0.043**	**0.033**	**0.015**		+	+	+	-	-	+
French Polynesia F2	**0.029**	**0.046**	**0.032**	**0.021**	**0.005**		+	+	+	+	+
Colombia G	**0.060**	**0.047**	**0.056**	**0.057**	**0.080**	**0.078**		+	+	+	-
Area IV	0.001	**0.005**	**0.006**	**0.007**	**0.027**	**0.029**	**0.060**		-	-	+
Area V	0.024	0.001	0.005	0.014	0.047	**0.047**	**0.070**	0.014		-	+
Area VI*	**0.007**	**0.012**	0.001	0.001	0.014	**0.022**	**0.060**	0.001	0.009		+
Area I*	**0.040**	**0.045**	**0.038**	**0.038**	**0.054**	**0.052**	0.001	**0.041**	**0.052**	**0.039**	

Table 3. Percent apportionments using SPAM 3.7 of South Pacific humpback whale breeding populations including (standard errors) from the feeding areas IV, V, VI* and I*. Listed in the Simulation Apportionment column is the actual apportionment the program could assign given the baseline data using the simulation mode of SPAM 3.7. The simulation mode assigns one breeding region 100% apportionment as a possible mixture scenario using 10,000 iterations.

Region	AREA IV	AREA V	AREA VI*	AREA I*	Simulation Apportionment
Western Australia Sub-stock D	71.1 (10.8)	4.64 (9.0)	0.00 (.000)	0.00 (.000)	95.0%
Eastern Australia Sub-stock E1	0.00 (.000)	51.1 (26.5)	0.00 (.000)	0.00 (.000)	89%
New Caledonia Sub-stock E2	0.00 (.00)	44.1 (28.3)	12.9 (13.7)	0.00 (.000)	91%
Tonga Sub-stock E3	28.8 (13.4)	0.02 (8.0)	81.4 (22.5)	0.00 (.000)	83%
Cook Islands Sub-stock F1	0.00 (8.0)	0.01 (2.4)	5.0 (15.9)	8.8 (7.4)	73%
French Polynesia Sub-stock F2	0.00 (.000)	0.03 (3.2)	0.71 (12.3)	2.7 (8.2)	85%
Colombia Sub-stock G	0.00 (.000)	0.04 (2.2)	0.01 (0.6)	88.3 (7.5)	95%
Unknown	0.03	0.01	1.16	0.66	

Figure 1. South Pacific humpback whale breeding grounds (Stocks D, E, F, G) and feeding areas (Area IV, V, VI* and I*). Arrows show the results from the South Pacific humpback whale mixed-stock apportionments greater than 10% for breeding grounds from the feeding areas. Included in the boxes is the number (n) of individuals from each region.

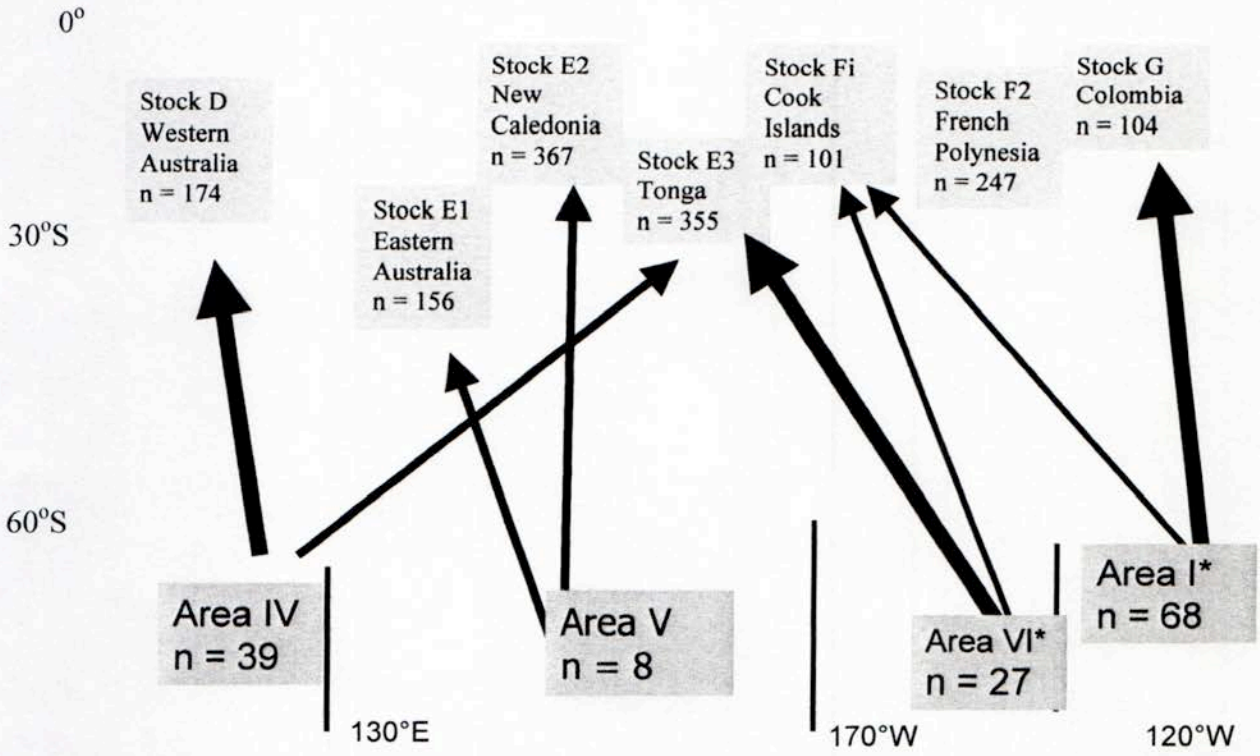

Figure 2. Distribution in the Antarctic feeding grounds of humpback whale samples taken during the IWC-SOWER cruises (1989, 1994, 1996-1999, 2001, 2002, 2003-2005), and INACH Antarctic Peninsula cruises. Individuals outside a circle were not used in analyses after regrouping of available samples. Actual Area boundaries as implemented by the IWC are shown with lines and labeled with degrees. Circled portions designate samples used in analysis.