

Preliminary mitochondrial DNA analysis of low and high latitude humpback whales of Stocks D, E and F

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ABSTRACT

Genetic samples from 575 humpback whales obtained in the Antarctic during surveys of the JARPA/JARPA II and IDCR/SOWER, and from 768 whales from low latitude localities of the South Pacific and eastern Indian Ocean were analyzed to describe the genetic population structure, distribution and mixing of breeding stocks in the Antarctic feeding grounds. Genetic samples from breeding grounds were obtained mainly by biopsy sampling but also from sloughed skin and beachcast whales: Western Australia (n= 167), New Caledonia (n= 243), Tonga (n= 240), Cook Islands (n= 56) and French Polynesia (n= 62). In the Antarctic feeding grounds, samples were obtained only by biopsy sampling: Areas IIIE (n= 106), IV (n= 231), V (n= 171) and VI (n= 67). Genetic samples of both data sets were examined for approximately the first half of the mtDNA control region. Duplicated samples were excluded from the analysis. In the case of mother/calf pairs only one sequence was used. Sequences from both data sets were aligned to produce a single data set comprising 126 haplotypes. Three kinds of analyses were conducted to examine the distribution and mixing of breeding stocks in the Antarctic under hypotheses on stock structure agreed by the IWC SC previously for Stock D, E and F: analysis of *F_{st}*, mixing proportion and maximum likelihood comparison. In general results from the three approaches were consistent, although some disagreements were found. The most parsimonious interpretation of results is that the Western Australia whales (D) distributes mainly in Area IV, in the Antarctic sector comprised between approximately 80°E and 125°E and its occurrence decrease to the west and east of that sector. The French Polynesia whales (F2) do not occur in the Antarctic research area examined (e.g. from approximately 35°E to 120°W). The New Caledonia whales (E2) distribute in Areas VE (from approximately 150°E) and VI. Results for Tonga (E3) and Cook Island (F1) whales were similar. They distribute mainly in Area VI. The pattern is similar when Tonga and Cook Islands whales are treated together. Signals are clearer when Cook Islands, New Caledonia and Tonga are treated together. In this case whales distribute in Area VI and V. Results for Western Australia and French Polynesia are clear in that whales from those localities belong to different stocks with particular patterns of distribution. Results for the other three localities are consistent with the scenario Tonga and Cook Islands whales belonging to a same stock (Hypothesis 3) as well with the scenario New Caledonia, Tonga and Cook Islands whales belonging to a same stock (Hypothesis 4). Results of these analyses are preliminary and several future works were identified.

KEYWORDS: ANTARCTIC, FEEDING GROUNDS, BREEDING GROUNDS, GENETICS, HUMPBACK WHALE

INTRODUCTION

Humpback whales, *Megaptera novaeangliae*, are found worldwide in all major oceans. Like other Balaenopterid species, humpback whales migrate between summer feeding grounds in mid- and high latitudinal waters and winter breeding grounds in tropical or subtropical waters. Animals occur primarily in coastal and continental shelf waters.

Regarding the Southern Hemisphere, Mackintosh (1965) showed that humpback whales tend to gather into five or six distinct feeding concentrations in the Antarctic during the austral summer season. These

feeding concentrations were denominated as Groups I-V (with a Group IIa and IIb) corresponding roughly to IWC Management Areas I-VI. The Groups most documented are Groups IV and V. Omura (1953) examined the distribution of humpback whale in the feeding grounds of Areas IV and V based on catch data. He suggested that two populations occur in these Areas with a boundary around 130°-142°E. He did not discard the possibility of intermingling between these two populations in the feeding ground. He also examined the pattern of distribution by month and suggested that for the month where more data were available (November-March) the boundary between these two populations changed from 120°-130°E in November to eastside of 140°E in December and to 120°-140°E in January. Dawbin (1966) summarize the distribution and seasonal migratory movement of humpback whales from Groups IV and V, as demonstrated by mark-recapture data (Discovery-type marks). Whales from Group IV move mainly between Antarctic Area IV and Western Australia while whales from Group V move between Antarctic Area V and Eastern Australia and along the coast of New Zealand and southwest Pacific islands. Interchange of a few individuals between Groups IV and V was reported. Dawbin (1966) also reported that the boundary of Groups IV and V in the Antarctic do not correspond to the actual boundary between Areas IV and V and that some whales marked in Area VI were recovered in eastern Australia.

More recently the IWC SC described the hypothetical stock structure and migratory corridors for Southern Hemisphere humpback whales based mainly on information such as Discovery tags, photo-id, genetics and satellite track (IWC, 2005). There are seven breeding stocks named from A to G. Some of which (B, C, E and F) are further subdivided into sub-stocks.

The IWC SC has recommended genetic analyses that include samples from both low and high latitude areas. Samples from low latitude breeding grounds represent signal of the 'pure' stock, which can be 'monitored' in the Antarctic feeding grounds where a degree of spatial and temporal mixing is expected. The focus of the present genetic analyses is on Stocks D, E and F. We made use of a substantive number of genetic samples available from low and Antarctic latitudes to understand the structure, distribution and mixing of those breeding stocks in the Antarctic.

For this aim we used mtDNA control region sequences from one locality of Stock D, two of Stock E and two of Stock F. These data were already used in a study on stock structure in the breeding grounds (Olavarria *et al.*, 2007), which showed significant differences among those localities. Details of those data were provided to us through the IWC Secretariat under data access Protocol B (courtesy of Dr. C.S. Baker, acting Data Administrator, on behalf of the South Pacific Whale Research Consortium).

In the Antarctic mtDNA control region sequences were available from Area IIIE (related to Stock C), IV (related to Stock D), V (related to Stock E) and VI (related to Stock F). Part of these data were already used in a study on stock structure in the feeding grounds (Pastene *et al.*, 2006), which showed significant differences among those Areas.

During the Workshop on the Comprehensive Assessment of Southern Hemisphere humpback whales (IWC, 2006), animals from Western Australia were considered as part of a single stock (D). Regarding to Stocks E and F the Workshop listed a total of six stock structure hypotheses and assigned them different ranks of plausibility (Figure 1). In the present study we did not attempt to carry out additional analyses on stock structure on breeding grounds samples (see section Discussion). Rather 'baseline' breeding ground samples in our analyses were defined according those hypotheses considered high and medium ranks by the Workshop (Figure 1).

Previous studies on baleen whales have used 'baseline' stock samples to estimate the mixing proportion of stocks in the feeding grounds or migratory corridors (Pastene *et al.*, 1998; Albertson-Gibb, *et al.*, 2008). The latter study was focused on the southern humpback whales and used a similar mtDNA data set from low latitude waters. However their sample size and sampling coverage in the Antarctic was limited.

In the present study we used a comprehensive mtDNA data set from low and Antarctic latitude to investigate the structure, distribution and mixing of Stocks D, E and F in the feeding grounds of Areas IIIE, IV, V and VI using a variety of methods.

MATERIALS AND METHODS

Samples

Skin biopsy samples were obtained from free-ranging whales along the sighting surveys of the JARPA and JARPA II and IDCR/SOWER surveys in Areas IIIE, IV, V and VI, on an opportunistic basis. Biopsy samples in JARPA were collected using an air gun described in Kasamatsu *et al.* (1991) and more recently using a Paxarm system. Biopsy samples in IDCR/SOWER were collected using several methods including Paxarm system and crossbows. At the laboratory all biopsy samples were checked for the possibility of re-sampling (two or more samples taken from a same individual) by comparing the genotype profiles produced by a set of six microsatellites. When mother/calf pairs were sampled, only the genetic information of the mother was used for the analysis (nine cases in Area V).

Genetic samples from breeding grounds were obtained mainly by biopsy sampling but also from sloughed skin and beachcast whales (see details in Olavarria *et al.*, 2007). At the laboratory the biopsy samples were checked for the possibility of re-sampling.

Table 1 shows the number of samples used in the present analysis by sampling locality. A total of 768 samples were used from the breeding grounds and 575 from the feeding grounds. Figure 2 shows the breeding ground localities and the geographical distribution of samples in the feeding grounds of Areas IIIE, IV, V and VI.

Molecular genetic analysis

Extraction of DNA and mtDNA control region sequencing

Details of the genomic DNA extraction and sequencing of samples from breeding and feeding grounds are given in Olavarria *et al.* (2007) and Pastene *et al.* (2006), respectively.

All sequences were aligned by eye. The aligned sequences involved a common segment of 335bp of the mtDNA control region. A total of 126 unique sequences (haplotypes) were determined in the common data set of 1,343 whales.

Data analysis

Samples from Western Australia were considered a baseline sample for Stock D. Baseline samples for Stocks E and F were defined according to the specifications of the high and medium rank plausibility hypotheses in Figure 1, (IWC, 2006), adapted to the availability of samples:

Hypothesis 1 (medium): New Caledonia (E2), Tonga (E3), Cook Islands (F1) and French Polynesia (F2) are separate stocks.

Hypothesis 3 (high): New Caledonia (representative sample for New Caledonia+Chesterfield Reef), Tonga+Cook Island and French Polynesia are separated stocks

Hypothesis 4 (medium): New Caledonia+Tonga+Cook Islands, French Polynesia are separated stocks.

Three kinds of analyses were conducted.

Fst analysis

The first analysis involved the estimation of *Fst* between the baseline stocks and samples of humpback whales in Areas IIIE, IV, V and VI. *Fst* values were estimated using the AMOVA (Excoffier *et al.*, 1992). The significance of the *Fst* values was estimated using 10,000 random permutations of the data matrix.

Mixing proportion analysis

The second analysis involved the estimation of mixing proportion of the baseline stocks in the Antarctic Areas. For this aim we used a maximum likelihood procedure (e.g. Fournier *et al.*, 1984), implemented in a computer program developed by one of us (TK). Variance of these estimates consisted of the variance from the sampling from the mixed population but do not consider the variance from the baseline stocks.

In likelihood analysis

The third analysis involved a finer grouping of the samples in the feeding grounds. Antarctic samples were arranged from east to west. The baseline stocks were compared using InML, with samples 1-15, 2-16, 3-17, etc. until reach the most western sample. A high In likelihood means that samples are alike. The 95% confidence intervals were obtained by creating samples randomly from all the Antarctic samples 1,000 times and estimated the InML in each case. This analysis was implemented using a computer program developed by one of us (PP).

RESULTS

Fst analysis

Table 2 shows the estimates of *Fst*. The Western Australia whales differed significantly from all Antarctic Areas except Area IV. The New Caledonia, Tonga and Cook Islands differed significantly from all Areas except Area VI. The French Polynesia differed significantly from all Areas.

Tonga+Cook Islands and New Caledonia+Tonga+Cook Islands showed a pattern similar when those localities were analyzed individually (differed significantly from all Areas except Area VI).

There is a good correlation between *Fst* and geography. For example for Area III E and IV the *Fst* values increase with distance (Table 2). For Area III E the smallest *Fst* value was in the comparison with Western Australia and the largest value in the comparison with the French Polynesia. The higher *Fst* values were found in the comparisons between the Antarctic Areas and the French Polynesia whales.

In summary the *Fst* analysis suggest that Western Australia whales are closely related to Area IV while New Caledonia, Tonga and Cook Islands whales are closely related to Area VI. The French Polynesia whales are not related to any of the Antarctic Areas examined.

Mixing proportion analysis

Table 3 shows the results of mixing proportion. The Western Australia whales are highly represented in Areas IV and III E, much less in Area V and absent in Area VI. With the exception of Area III E results are consistent with the results of the *Fst*.

The New Caledonia whales are mainly represented in Areas V and VI and less represented in Areas IV and III E. From the geographical point of view the figure of 17.4% in Area III E is surprisingly high.

The Tonga whales are mainly represented in Area VI and much less in the other Areas, which is consistent with the results of *Fst*.

The Cook Island whales are poorly represented in the Areas examined. It is represented in Area VI with a 19.6% and is almost absent in other Areas. This result is consistent with that of *Fst*.

The French Polynesia is absent in all Areas, which is consistent with the *Fst* results.

When we treated Tonga+Cook Islands whales together, they are represented in higher percentage in Area VI, result consistent with that of *Fst*. When we treated New Caledonia+Tonga+Cook Islands whales together, they are highly represented in both Areas VI and V.

In likelihood analysis

Figure 3 show the results of the In likelihood for Western Australia whales. The highest In likelihood are found in the Antarctic sector comprised between 80°E and 125°E approximately, corresponding roughly with the limit of Area IV. Then there is concordance in these results with the geography. The values become smaller toward Area III E and much smaller toward Areas V and VI, which is consistent with the results found by the *Fst* (Table 2) and mixing proportion analyses (Table 3).

There is also some signals in the plotting for New Caledonia whales (Figure 4) where the maximum values are found in the sector comprised between 122°W and 150°E approximately (Area VI and east part of Area V). Values become much smaller to the west of that longitude. Then there is concordance in these results with the geography. This result do not agree with the *Fst* analysis, which was small but significant

in the comparison between New Caledonia and Area V (Table 2), but it is consistent with the results found by the mixing proportion analysis (Table 3).

The signal for the Tonga whales (Figure 5) is in the sector comprised between 122°W and 176°W (Area VI) and become smaller to the west of that longitude. Again there is concordance with geography and results are consistent with the *Fst* (Table 2) and mixing proportion analyses (Table 3).

The signal for the Cook Island whales is less clear. Higher values are found in the sector 122°W-177°W (Area VI), which is consistent with geography, and consistent with the results found in the *Fst* (Table 2) and mixing proportion (Table 3) analyses. However another peak is observed in Area IVE.

There is no signal for the French Polynesia whales, which is consistent with geography, and also consistent with the results of the *Fst* (Table 2) and mixing proportion (Table 3) analyses. Considering the results of the three analyses whales from the French Polynesia are probably distributed outside of our research area in the Antarctic.

For the combination Cook Islands+Tonga (Figure 8) the results are consistent with geography and consistent with the results of *Fst* (Table 2) and mixing proportion (Table 3) (but in this case the contribution in Area VI is higher, 65.2%, Table 3), as the signal in Figure 8 is from 122°W to 174°E approximately (almost Area VI).

The combination Cook Islands+New Caledonia+Tonga shows a clearer signal (Figure 9). Larger In likelihood values are in the sector comprised between 122°W and approximately 137°E (Areas VI and V). Values become lower in Areas IV and IIIIE, which is consistent with geography. Results are consistent with the high mixing proportion of this baseline stock in Areas V and VI (Table 3) but do not agree with the *Fst* results regarding Area V which showed a small but significant *Fst* value in the comparison between New Caledonia+Tonga+Cook Islands and Area V (Table 2).

DISCUSSION

Following previous recommendations from the IWC SC we conducted a mtDNA analyses on samples from low and high latitudes of Breeding Stocks D, E and F. The main objective of this analysis was to investigate the distribution and mixing of those stocks in the feeding grounds of Areas IIIIE, IV, V and VI under different grouping of the samples from the breeding grounds as defined in stock structure hypotheses listed previously by the IWC SC.

For this objective we used a variety of approaches, which were in general consistent in results, although some differences were found. Our most parsimonious interpretation of the results is as follow.

- 1) Results regarding the Western Australia whales (Stock D) were clear and consistent among the approaches used, and support the previous view that the core of this stock distributes mainly in Area IV, in the Antarctic sector comprised between 80°E and 125°E. This stock distributes and mixes with a different stock in Area IIIIE and to a lesser extent with other in Area VW.
- 2) Results regarding the French Polynesia baseline stock (F2) were clear and consistent among the approaches used. Whales from this putative stock do not occur in the Antarctic research area examined (e.g. from 35°E to 122°W). This was the stock most strongly differentiated from all Areas examined.
- 3) The New Caledonia baseline stock seems to distribute in Area VE and VI. There are some discrepancy for this pattern in the results found for the *Fst* that found significant differences between New Caledonia and Area V. This discrepancy could be elucidated perhaps by conducting analyses in Area V at smaller longitudinal sectors.
- 4) Results found for Tonga and Cook Island were similar and consistent among the approaches. They seem to distribute mainly in Area VI. The pattern is similar when Tonga and Cook Islands samples are pooled. It should be noted that the sample size in Area VI is still small to allow definitive conclusions.
- 5) Signals are clearer when Cook Islands, New Caledonia and Tonga are treated together. Whales distributes in Areas VI and V.

In summary results for Western Australia and French Polynesia are clear in that whales from those localities belong to different stocks with particular patterns of distribution in the feeding grounds. For the other three localities results are consistent with the scenario Tonga and Cook Islands whales belonging to a same stock (Hypothesis 3) as well with the scenario New Caledonia, Tonga and Cook Islands whales belonging to a same stock (Hypothesis 4).

Results regarding Western Australia/Area IV were similar to those of Albertson-Gibbs *et al.* (2008) but the allocation to Area IV in the present study was much higher. Results were also similar regarding Tonga/Area VI. Discrepancies between both studies are probably due to different sample sizes used for the Antarctic Areas, which were much higher in the present study.

Our results should be considered as preliminary pending further analyses. The following future works were identified.

Stock structure genetic analyses have been carried out for the breeding grounds samples based on hypothesis testing and results showed significant differences among all localities (Olavarria *et al.*, 2007). Further analyses should include samples from Eastern Australia (E1). Apart from hypothesis testing, estimates of the migration rates among putative breeding stocks using IM or Mdiv would be useful. It would also be useful to investigate possible yearly variation in the pattern of structure.

Previous genetic analyses in the feeding grounds have shown different pattern of distribution by sex with males being more mobile than females (Pastene *et al.*, 2006). Future analyses of distribution and mixing in the feeding grounds similar to those conducted in this study should be conducted separately for males and females.

Distribution of stocks in the feeding grounds could change yearly according to oceanographic conditions which in turn determine the distribution of krill. Analyses on a year basis (or groups of years) in the Antarctic samples would be useful. This could be possible for Areas where sample sizes are larger (e.g. Area IV).

The analytical tool used for estimating mixing proportion does not consider variance attributed to the haplotype frequency in the baseline stocks. The program used should be modified to take this into account and the analyses repeated.

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Table 1. Number of samples of the humpback whale used in the present mtDNA analysis.

Breeding grounds						Feeding grounds				
WA	NC	TG	CI	FP	Total	A IIIE	A IV	A V	A VI	Total
167	243	240	56	62	768	106	231	171	67	575

WA= Western Australia; NC= New Caledonia; TG= Tonga; CI= Cook Islands; FP= French Polynesia

Table 2. *Fst* values between breeding ground samples grouped under different stock structure hypotheses (see text for explanation) and feeding grounds sample from Antarctic management Areas (significant *p* values are shown in parenthesis).

	WA	NC	TG	CI	FP	TO+CI	NC+TO+CI
A IIIE	0.0089	0.0213	0.0245	0.0379	0.0452	0.0245	0.0211
A IV	-0.0018	0.0135	0.0128	0.0195	0.0336	0.0115	0.0105
A V	0.0144	0.0058	0.0113	0.0279	0.0422	0.0119	0.0072
A VI	0.0093	0.0003	0.0010	0.0107	0.0320	0.0001	-0.0017

WA= Western Australia; NC= New Caledonia; TG= Tonga; CI= Cook Islands; FP= French Polynesia

Table 3. Mixing proportion of breeding ground samples grouped under different stock structure hypotheses (see text for explanation) in the Antarctic management Areas (standard errors are shown in parenthesis).

	WA	NC	TG	CI	FP	TO+CI	NC+TO+CI
A IIIE	0.791 (0.059)	0.174 (0.098)	0.005 (0.096)	0.000 (0.000)	0.030 (0.045)	0.000 (0.000)	0.187 (0.068)
A IV	0.828 (0.034)	0.064 (0.029)	0.065 (0.036)	0.042 (0.031)	0.000 (0.000)	0.107 (0.032)	0.169 (0.033)
A V	0.142 (0.041)	0.832 (0.043)	0.000 (0.000)	0.026 (0.023)	0.000 (0.000)	0.032 (0.030)	0.847 (0.044)
A VI	0.000 (0.000)	0.371 (0.129)	0.433 (0.154)	0.196 (0.097)	0.000 (0.000)	0.652 (0.122)	1.000 (0.000)

WA= Western Australia; NC= New Caledonia; TG= Tonga; CI= Cook Islands; FP= French Polynesia

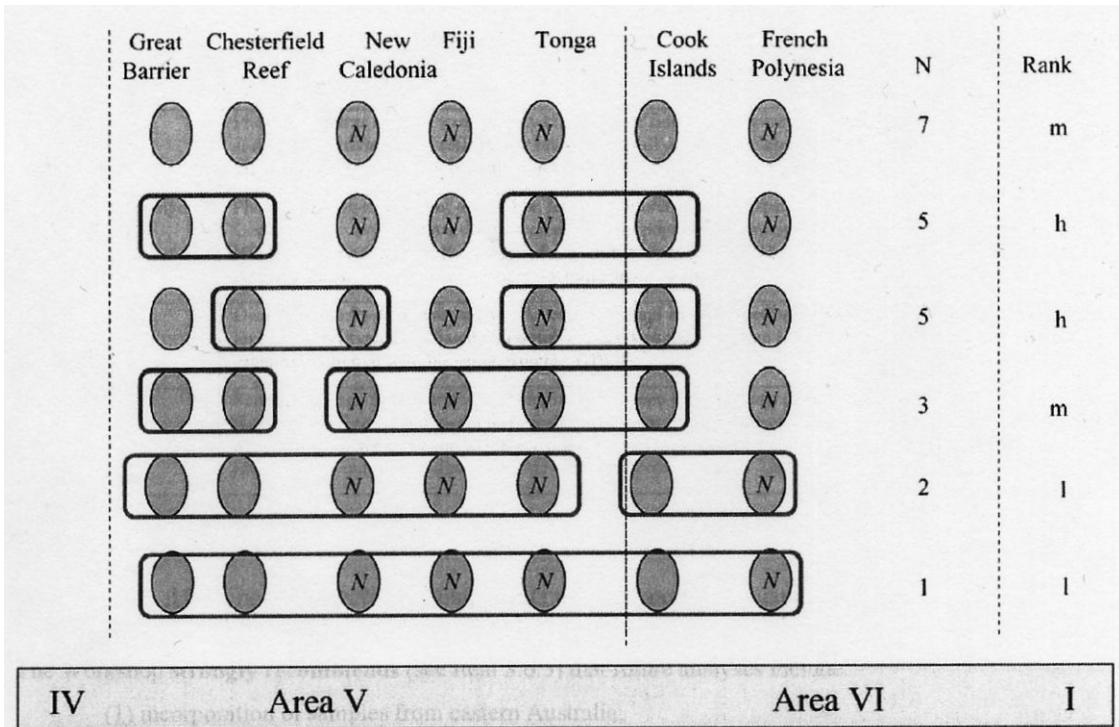


Figure 1. Schematic diagram showing possible models for stock structure in the South Pacific. N= number of breeding stocks and Rank, l= low, m= medium and h= high (taken from IWC, 2006). Samples from the New Caledonia, Tonga, Cook Islands and French Polynesia (in addition to Western Australia), were available for the present mtDNA study.

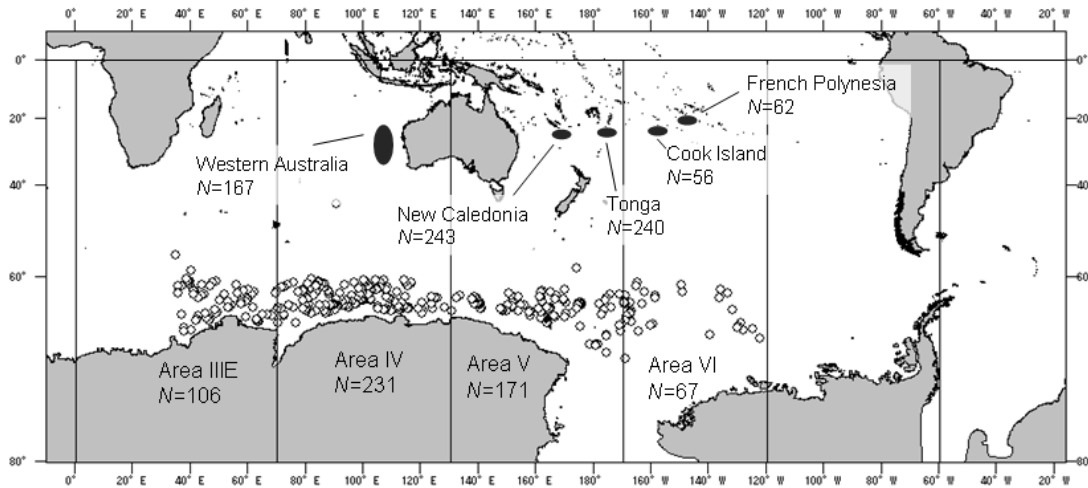


Figure 2: Geographical distribution of humpback whale samples from breeding and feeding grounds analyzed in this study.

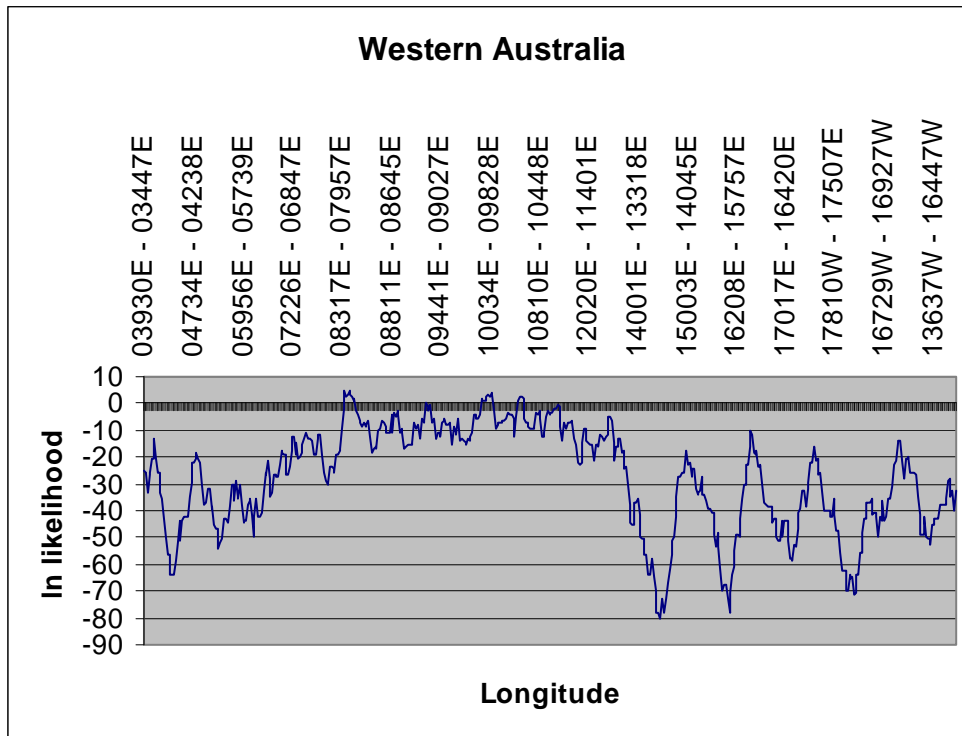


Figure 3. Comparison of samples from Western Australia and samples from the feeding grounds of management Areas VI-III E ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= -3.568; 0.025 CI= -50.619).

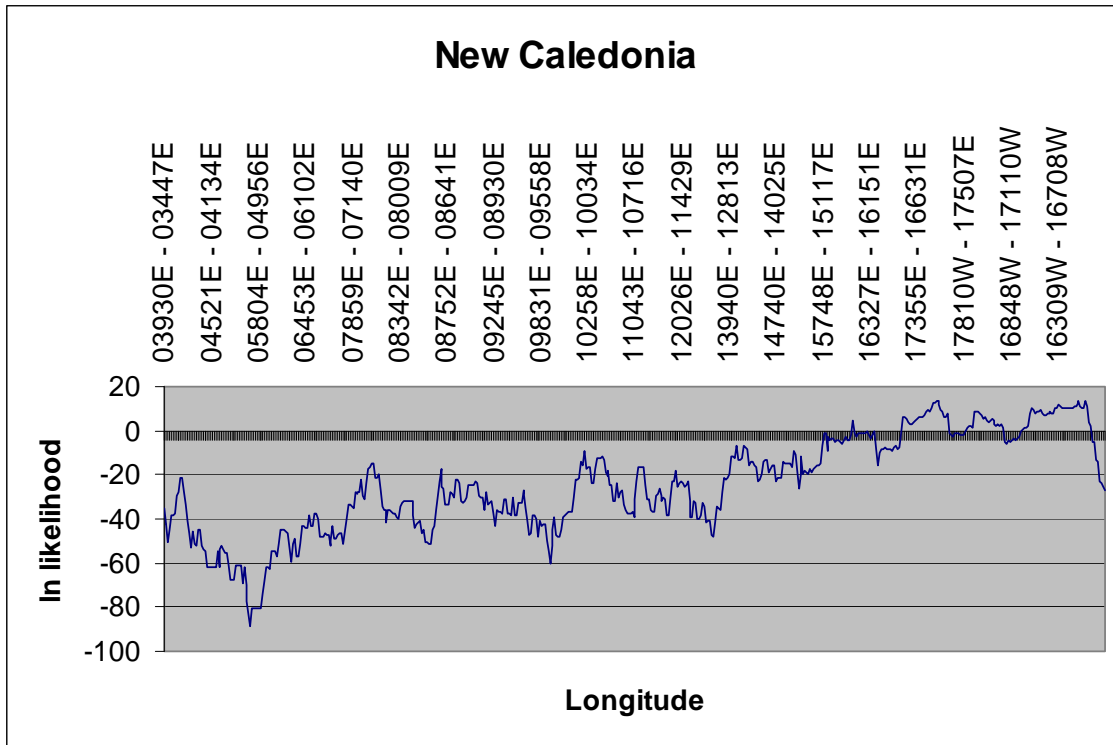


Figure 4. Comparison of samples from New Caledonia and samples from the feeding grounds of management Areas VI-IIIIE ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= -3.893; 0.025 CI= -48.752).

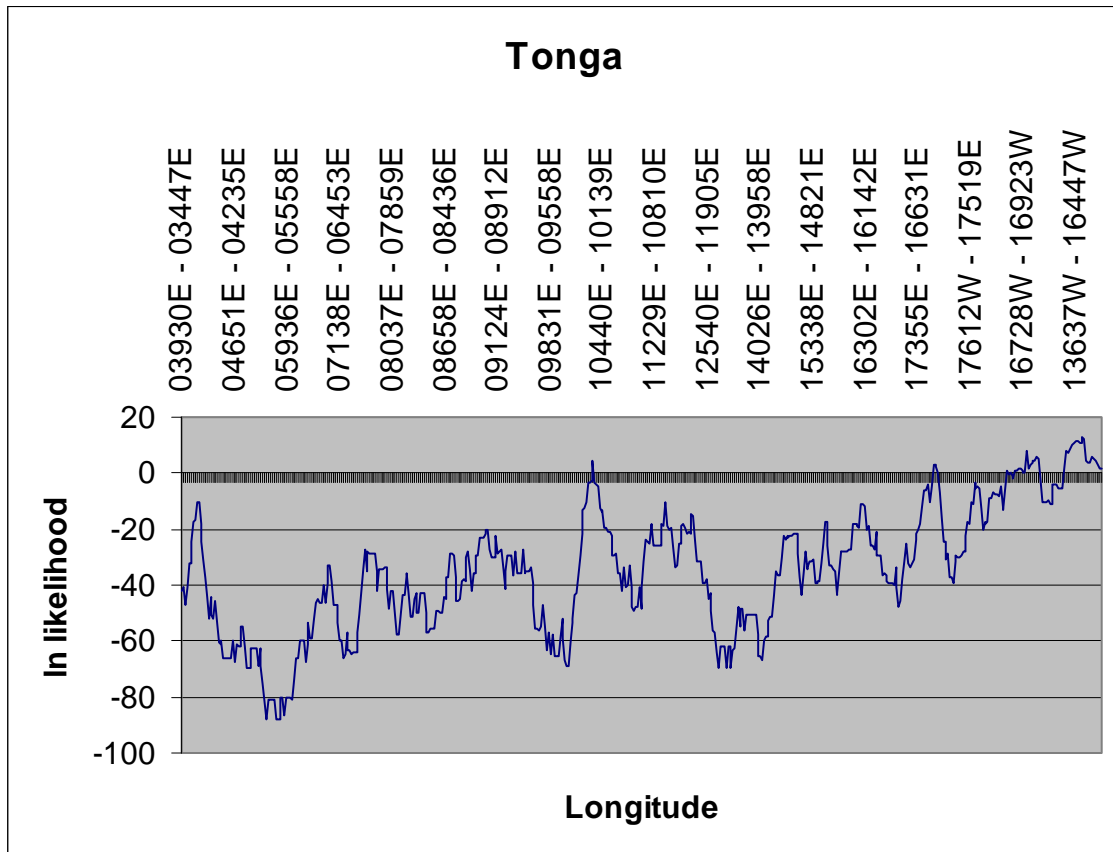


Figure 5. Comparison of samples from Tonga and samples from the feeding grounds of management Areas VI-IIIIE ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= -8.753; 0.025 CI= -59.704).

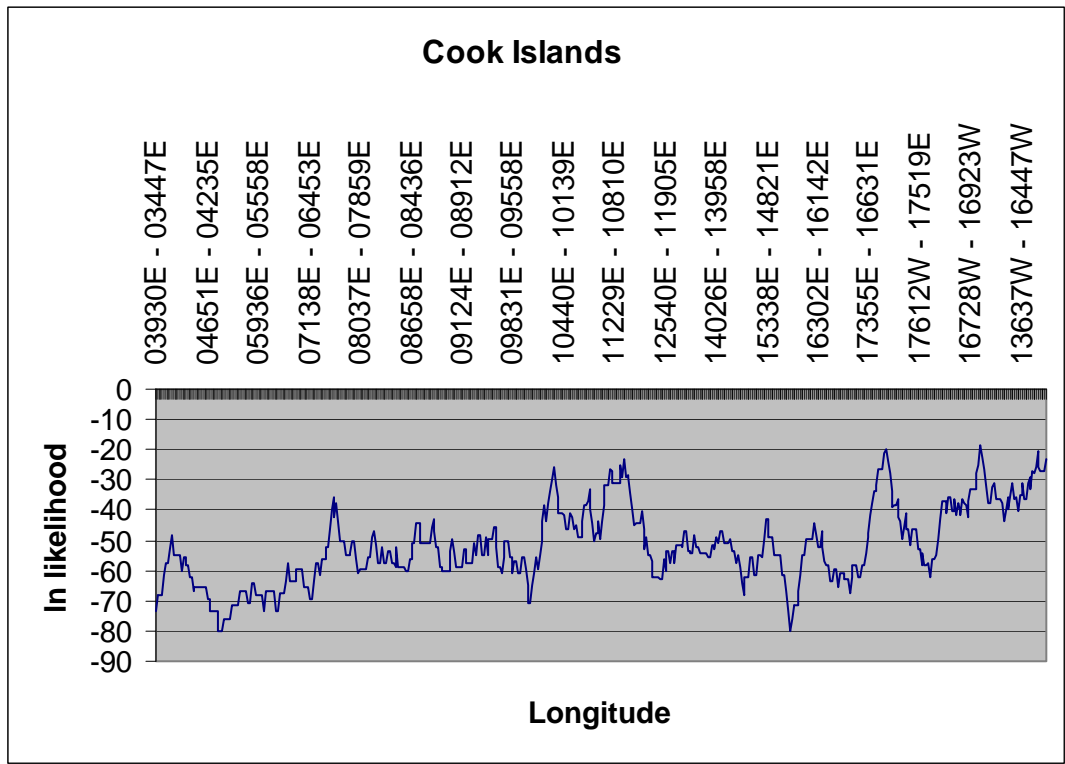


Figure 6. Comparison of samples from Cook Islands and samples from the feeding grounds of management Areas VI-IIIIE ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= -32.114; 0.025 CI= -69.565).

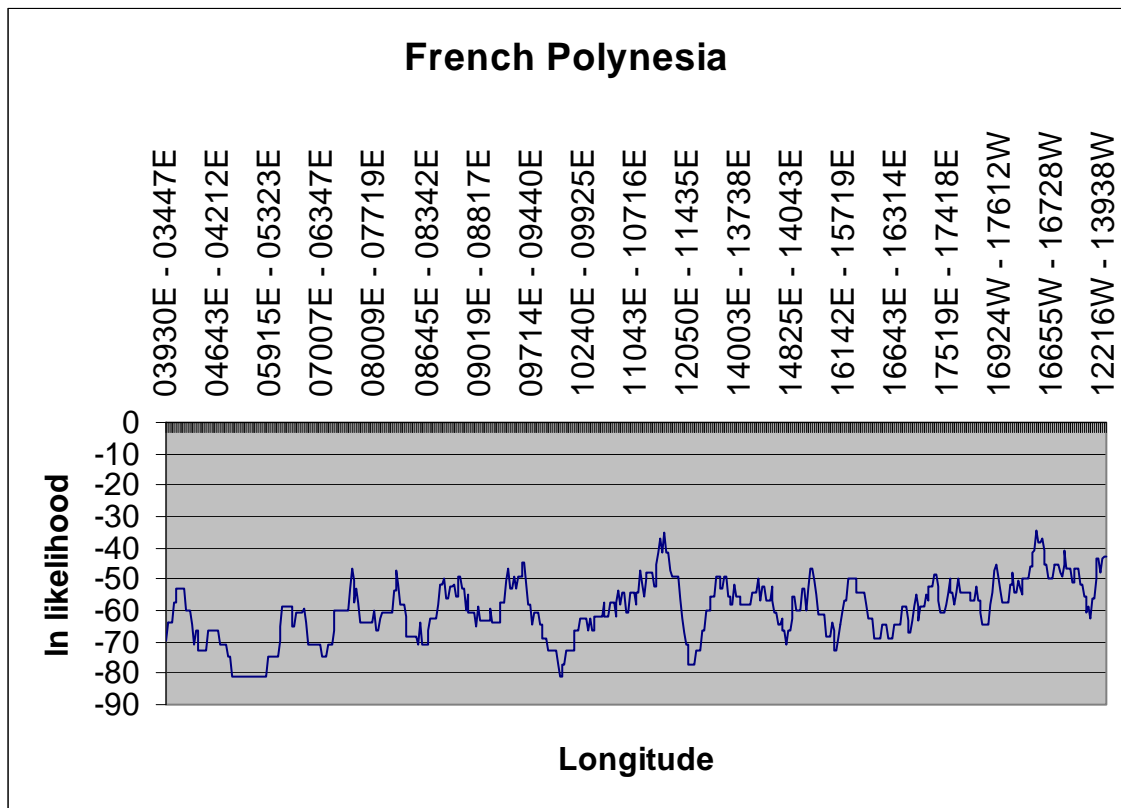


Figure 7. Comparison of samples from French Polynesia and samples from the feeding grounds of management Areas VI-IIIIE ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= -42.408; 0.025 CI= -75.414).

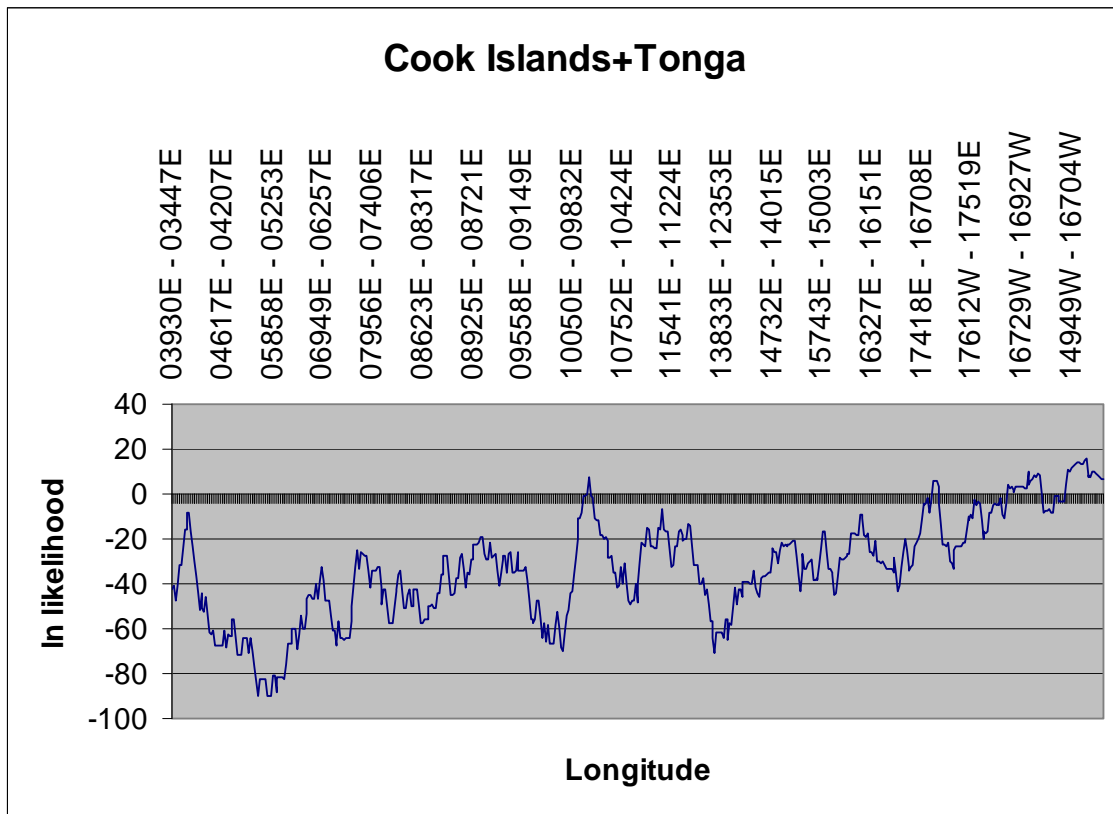


Figure 8. Comparison of samples from Cook Islands+Tonga and samples from the feeding grounds of management Areas VI-III E ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= -5.628; 0.025 CI= -60.040).

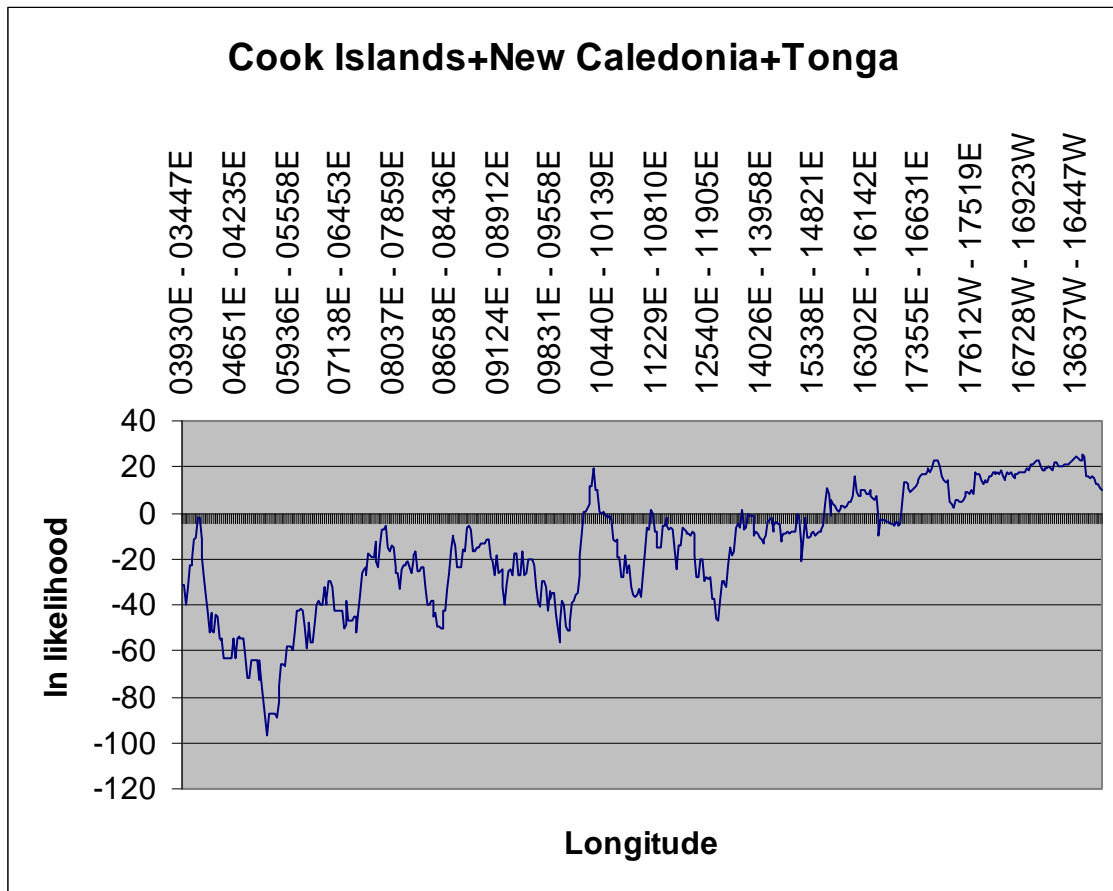


Figure 9. Comparison of samples from Cook Islands+New Caledonia+Tonga and samples from the feeding grounds of management Areas VI-IIIIE ordered from east to west, based on maximum likelihood analysis. A high ln likelihood means that samples are alike (0.975 CI= 9.873; 0.025 CI= -44.676).