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Review of the studies on stock identity in the minke whale *Balaenoptera acutorostrata* from the Southern Hemisphere

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ABSTRACT

In response to a request from the SC/Steering Group on Stock Definition we conducted a review of the studies on stock identity in the Southern Hemisphere ordinary form minke whale. Studies on stock identity, which started at the end of the decade of the 70's, were revised by the SC during the CA of the species in 1990. Until that date, all the analyses were conducted using samples and data from commercial whaling operations in the Antarctic. Genetic studies were based mainly on allozyme although studies based on mitochondrial and nuclear DNA were also conducted. The latter, however, involved limited number of samples from only Areas IV and V. Non-genetic studies revised in 1990 involved morphology, catch and sighting distribution pattern, analysis of Discovery marks and ecological markers. Results from the different approaches failed to identify unambiguously any isolated population in the Antarctic. Ecological markers, however, were identified as a potential useful approach. Studies on stock identity under the JARPA started after the CA. They have been based mainly on analysis of mtDNA and in a lesser extent in morphometric. Results suggest considerable heterogeneity, but little geographic concordance with Areas IV and V. An extensive mtDNA analysis showed that the only significant source of heterogeneity was attributable to a group of whales in the western part of Area IV migrating early in the season of 1989/90 and 1991/92. Although no definitive conclusion on the stock structure in the feeding ground has been reached, it is noted that some studies provide evidence for certain degree of structuring. Particularly different studies conducted before and after the CA suggest a boundary or an interaction of stocks in the western part of Area IV and eastern part of Area III. It is possible that, by considering the behavior of the minke whale in the feeding ground, which is caused by the dynamic of prey species, a 'dynamic boundary' rather a fixed boundary among stocks could occur in these Areas. We identified and discussed several aspects of the work, which need to be addressed in order to clarify further the stock structure of this species, among others: the need for genetic sampling in areas of low latitude; the need for DNA-based genetic analysis in Areas other than IV and V; development and application of methods to estimate statistical power of the genetic analyses; the need for optimization of the use of morphometric data and the need to examine further some non-traditional but promising new approaches such as the use of ecological markers.

CONTENTS

1- INTRODUCTION

In response to a request from the Steering Group on Stock Definition, we presented here a review of the studies on stock identification in the Southern Hemisphere minke whale. The review of case studies is part of Task 2 defined by the *ad hoc* Working Group on Stock Definition during the 50th SC Meeting. This *ad hoc* Working Group identified five main tasks, which were specified in Annex D, Appendix 4 of the 1998 SC meeting report (IWC, 1998).

The Task 2 read: 'Review case studies of management advice for large whales, with special emphasis on the extent to which the definition of a stock used in the assessment contributed to or detracted from the success of the assessment, with particular reference to the level and nature of the available data' and 2b 'Prepare a report summarizing the results of the review'.

To write this report, in general we followed the report format offered by the Steering Group. The review emphasizes on the nature of samples and data, approaches used and on the statistical methods used to analyze these data.

2- BACKGROUND

The Southern Hemisphere minke whale, like all the other Southern Hemisphere baleen whales species apart the Bryde's whale, was managed by the IWC on the basis of six geographical 'Areas' (Fig. 1). The IWC established these Areas from the 1974/75 season, based mainly upon information from Mackintosh (1942; 1966) on distribution of catches of blue, fin and humpback whales (see review by Donovan, 1991). These areas were used by the IWC for the implementation of the New Management Procedure (NMP). However, biological evidences for the particular boundaries are weak, especially for those species such as the minke whale, whose data were not considered when the original management Areas were established.

In this regard, some related and important questions were formulated by Hoelzel and Dover (1989): 'are the whales found in two geographically distinct management Areas from two different genetic stocks? or are individuals from more than one genetic stock present in a particular management Area?. If so, what level of interchange may have occurred between different genetic stocks?'. Several approaches were used in the past to try to identify genetic stocks of minke whale in the Southern Hemisphere and determine to what extent genetic stocks and IWC management Areas coincide. They included genetics, morphology, analysis of mark-recapture data, patterns of catch and sighting distribution and ecological markers. Detailed reviews of studies on stock identity in the Southern Hemisphere minke whale have been conducted by Horwood (1990), Best (1990) and Pastene and Goto (1997). We presented here an update of the information on stock identity in the southern minke whale. This review deal only with the ordinary type minke whale.

As a background for the review, we briefly described here three related matters:

abundance, catch history and seasonal movements.

2.1 Abundance

Abundance of the southern minke whale were given during the comprehensive assessment (CA) of the species in 1990 (IWC, 1991). Estimates were based on sighting data collected during the IDCR (International Decade for Cetacean Research) surveys in Antarctic Areas I-VI south of 60°S. Estimates for Area I, which was based on a survey conducted in 1982/83, was 73,302 (CV=0.254). The estimates for Area II, based on a survey conducted in 1986/87, was 122,156 (CV=0.190), for Area III (1987/88 survey), was 88,735 (CV=0.273), for Area IV (1988/89 survey), was 74,692 (CV=0.257), for Area V (1985/86 survey) was 294,610 (CV=0.138) and the estimates for Area VI, based on a 1983/84 survey, was 106,901 (CV=0.277). All these estimates were made following Scientific Committee standard methodology.

2.2 Catch history

The total catch of minke whales to 1990 was summarized during the 1990 Committee meeting. For Areas I to VI was 12,108, 19,739, 27,541, 34,586, 15,165 and 4,999 whales, respectively (IWC, 1991).

2.3 Seasonal movements

The minke whale like all the other balaenopterids (except the Bryde's whale) are believed to undertake seasonal migrations between feeding grounds in the Antarctic waters in summer and breeding grounds in the tropical or temperate regions in winter. For this species, however, there is only a single evidence of such linkage, based on mark-recapture data. Two whales that had been marked in the Antarctic were recovered off Brazil (Buckland and Duff, 1989).

There are also a few indirect evidences on this linkage based on ecological markers (Nemoto *et al.*, 1980; Ohsumi, 1973).

Kasuya and Wada (1991) examined sighting data obtained from Japanese sighting vessels in the Indian Ocean and because of the considerable sightings in summer to the north of 55°S, they suggested that not all individuals migrate to waters south of the Antarctic Convergence in summer. On the other hand, it is possible that not all individuals, distributed in the Antarctic feeding grounds in summer, migrate to lower latitudes in winter. Aguayo (1994) made 37 sightings (211 individuals) of minke whale in Antarctic Area I in winter suggesting that some minke whales stay in the Antarctic over the winter.

Further information on movement of whales in the feeding ground is given in Section 3.3.1.

3- METHODS USED TO INVESTIGATE STOCK IDENTITY

3.1 Genetics

Several molecular techniques have been used to investigate stock identity in the Antarctic minke whale. The most extensive (geographically) genetic surveys were based on allozymes but also techniques based on restriction fragment length polymorphism (RFLP) and sequencing of mitochondrial DNA (mtDNA) as well nuclear DNA (nDNA) have been used. We summarize here only the main or the most recent studies for a particular technique (see also Table 1).

3.1.1 Allozymes

Wada and Numachi (1991) conducted an allozyme analysis involving a large sample size of the Southern Hemisphere minke whale. This analysis was part of an extensive allozyme analysis covering minke whales from other localities and other baleen whales species as well. The analysis of Antarctic samples was an extension of those of Wada and Numachi (1979) and Wada (1982).

The authors used 45 allozyme loci to examine a total of 11,414 Antarctic minke whale samples (liver and muscle) from all the Areas indicated in Fig. 1. In addition they examined 195 samples (liver) from coastal Brazil. Samples in the Antarctic were from commercial pelagic whaling (CPW) operations conducted between 1975/76 and 1983/84. Samples from Brazil were from a commercial coastal whaling (CCW) operation in 1981.

A total of 10 loci (*Adh-2, Gdh, Got-1, Idh-1, Me, Mpi, PepD, 6Pgd, Pgm-3 and Sdh*) were polymorphic in the Antarctic sample.

There was not significant departure from the Hardy-Weinberg equilibrium in the Antarctic sample. Furthermore a homogeneity test based on G-statistics was conducted using the following loci: *Adh-1, Adh-2, Gpi, Sdh and 6Pgd*. No significant spatial (IWC Areas or 10° sectors) or temporal (between years and between months within a year) heterogeneity was found in the Antarctic sample. In addition no significant differences were found between the Brazilian and Antarctic minke whales.

3.1.2 RFLP analysis of mitochondrial DNA control region

Hoelzel and Dover (1991) analysed variation in the control region of the mtDNA to study minke whales from the Antarctic, North Pacific and North Atlantic. From the Antarctic, they used 20 samples from Area IV and 21 from Area V available from the CPW. They used three kind of four-base restriction enzymes (*DdeI, MboI and HinfI*) to investigate polymorphism in the control region. A total of five haplotypes were discriminated in the total Antarctic sample. Genetic distance between the two Areas was 0.003 substantially lower than that found between Southern and Northern Hemispheres minke whales.

3.1.3 RFLP analysis of whole mtDNA

Wada *et al.* (1991) used a RFLP analysis of whole mtDNA to examine minke whales from Antarctic Areas IV and V and from the North Pacific. Samples from Areas IV (n=40) and V (n=39) were from a CPW operation in 1983/84.

Polymorphism in the mtDNA was investigated by use of 14 restriction enzymes: *AccI, AvaI, BamHI, BglII, EcoRI, EcoRV, HaeII, HincII, HindIII, HpaI, ScaI, SphI, StuI and XbaI*. All but *AvaI, BamHI, ScaI, SphI* and *XbaI* showed polymorphism. A total of 15 haplotypes were discriminated in Areas IV and V.

The G-statistics showed no significant heterogeneity between Areas IV and V. MtDNA diversity for the combined sample was 0.0016.

Pastene *et al.* (1993a) conducted the first mtDNA study using samples from the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA). Minke whales are sampled randomly under the JARPA on pre-determined survey tracks, which cover both offshore and ice-edge areas.

In the study a total sample of 318 minke whales (from seasons 1988/89 and 1990/91 in Area V and 1989/90 in Area IV), was used. Crude mtDNAs were digested with 12 six-base restriction enzymes: *AccI, BanI, BglII, EcoRI, EcoRV, HaeII, HincII, HindIII, HpaI, PvuII, SspI and StuI*. All of them showed polymorphism, apart one (*HaeII*). Restriction enzyme digestions of mtDNA from the total sample revealed a total of 71 mtDNA haplotypes.

For the analysis, samples were divided a priori into three geographical strata, western (70°-110°E), central (110°-150°E) and eastern (150°E-180°). Haplotype frequencies were employed to determine genetic relationships between the samples of the designed strata. Genetic relationships were quantified using the chi-square statistics for heterogeneity of mtDNA haplotype frequencies (Roff and Bentzen, 1989). This Monte Carlo approach estimates the significance of the chi-square test computed from the raw data. In each trial, 1,000 randomisations of the original data sets were made. Heterogeneity chi-square decomposition began by estimating the significance of the chi-squares computed from the raw data for all the three strata. This result showed that mtDNA haplotypes are not randomly distributed in these strata. Results of pair-wise comparisons indicated that mtDNA frequency distributions in the western and eastern strata were different. However, no significant differences were found between the haplotype frequencies of the western and central strata nor between central and eastern strata. The authors interpreted these preliminary results as the occurrence of different stocks in the feeding grounds of Areas IV and V and a possible mixing of them in the central stratum.

Pastene *et al.* (1993b) used JARPA samples from Area IV taken in two different austral summer seasons: 1989/90 (n= 306) and 1991/92 (165). Crude mtDNAs was digested with six of the 12 restriction enzymes used in the previous study (*AccI, BanI, EcoRV, HincII, HpaI and SspI*). These enzymes were chosen given their polymorphic character. Enzyme

digestion from the total sample (n= 471) revealed a total of 57 mtDNA haplotypes. For the analysis, samples from each season were divided a priori into three area/time strata: Area IV western early (IVWE, whales sampled in the western sector of Area IV from December to 15 January), Area IV western late (IVWL, whales sampled in the western sector of Area IV from 16 January to March) and Area IV eastern early (IVEE, whales sampled in the eastern part of Area IV in the early period). The temporal criteria was added in order to investigate the hypothesis that different stocks migrating into the Antarctic feeding ground either: (1) mix with each other as the feeding season progresses; or (2) occupy different longitudinal sectors in different periods of the feeding season. The randomized chi-square statistic for heterogeneity was used for investigating the pattern of mtDNA variation. In each area/time stratum samples did not differ significantly between seasons. Pairwise comparisons were carried out for the three area/time strata for both seasons combined. Each pairwise comparison showed significant differences in mtDNA haplotype frequencies distribution. This result was consistent with the hypothesis of different stocks migrating into the Area IV in summer with their composition changing longitudinally and with progress of time in a feeding season.

A more extensive study considering both geographical and temporal criteria was conducted by Pastene *et al.* (1994). These authors carried out a mtDNA analysis on 1,257 minke whales from Areas IV and V collected by JARPA. Samples of Area IV were from two seasons, 1989/90 (n= 307) and 1991/92 (n= 260) and those of Area V from three seasons, 1988/89 (n= 77), 1990/91 (n= 308) and 1992/93 (n= 305). Digestion with the six previously used restriction enzymes revealed a total of 123 mtDNA haplotypes. As in the previous study, samples were arbitrarily divided into four longitudinal sectors (Area IV western and eastern, Area V western and eastern), and two time periods, early (December-15 January) and late (16 January-March). Thus a total of eight area/time strata were examined. Mitochondrial DNA haplotype frequencies and the randomised chi-square statistic were used to determine relationships between the area/time strata. After pooling samples from different sexes and seasons, a marked spatial and temporal heterogeneity was found. A group of whales sampled in Area IVWE was significantly different in haplotype composition from all but one of the other spatial and temporal strata analysed. Furthermore, a group of whales sampled in Area VEL was significantly different from all but one other grouping. Of 123 haplotypes identified, 8 were dominant, but were present in all groups, so that no stock markers could be identified. Again, the results were consistent with the occurrence of different stocks in Areas IV and V and a temporal component to their distribution.

This information was revised during the 1994 SC meeting. During the discussion it was noted that the data presented were amenable to more powerful statistical analyses to examine relationships and clustering and analysis of molecular variance by permutation procedures approaches were suggested (IWC, 1995). The Committee recommended that further analysis on a temporal basis should be undertaken to examine the distinctions between the apparently different groupings early in Area IVW and late in VE (IWC, 1995).

In response to these recommendations, Pastene *et al.* (1996a) conducted a new analysis

of mtDNA variation in Areas IV and V, this time involving a total of 2,124 minke whales collected by the JARPA. Digestions with the same six restriction enzymes revealed 137 haplotypes. For the analysis samples were grouped into eight area/time strata as in the previous study. Following the recommendation from the SC, the quantification of the temporal and geographical differentiation of mtDNA was carried out using the analysis of molecular variance (AMOVA) of Excoffier *et al.* (1992). The AMOVA program calculates variance component from a distance matrix and the PHI statistic (PHIst) reflecting the correlation of haplotypic diversity at different levels of hierarchical subdivision. Information on the genetic distance among all pairs of haplotypes was used to construct the inter-haplotype file. Genetic distance between haplotypes was estimated using a maximum-likelihood method (equation 5.55 of Nei, 1987). The significance of the variance components and PHIst were tested using a random permutation procedure. For each trial, 2,000 randomisations of the original data sets were made. Samples from different sexes and seasons were pooled. There was five predominant haplotypes and these were found in all eight strata tested. The AMOVA test showed that the molecular differences were significantly less within the area/time strata than between them (PHIst=0.001; P=0.0340). Pairwise testing showed that all the PHIst values involving the Area IVWE were larger than all the other pairwise comparisons and all of them showed P values below 0.01 or 0.05. The PHIst value obtained between Area IVWE and all the other strata combined was 0.0090 (P=0.0025). The authors concluded that a significant source of mtDNA heterogeneity was attributable to the group of minke whales sampled in the western part of Area IV early in the feeding season. This group was composed by minke whales sampled during the JARPA surveys of 1989/90 and 1991/92. Then the differentiation of this stratum shown by the previous chi-square analysis was corroborated by the analysis using AMOVA. However, the apparent differentiation of the Area V eastern late stratum of the previous analysis could not be corroborated using the AMOVA.

Pastene *et al.* (1996b) used the same polymorphic restriction enzymes of the previous study to examine minke whale samples from Areas IIIE and VIW available from CPW operations. Samples from Area IIIE (n=154) were obtained in December 1978 while samples from Area VIW (n=134) were obtained in December/January 1985. Given the fact that these samples had been storage for a long time, a different procedure for extracting and analyzing DNA was used. The method used (Southern-hybridization) gave comparable RFLP profiles to those obtained for the JARPA samples. Statistical procedures were the same as in the previous study. Samples from Area VIW were similar to the core samples of Areas IV and V while that samples from Area IIIE were more similar to the group of whales sampled by JARPA in Area IVWE in 1989/90 and 1991/92. A simulation exercise suggested that at least a sample size of 150-200 individuals is needed to detect significant mtDNA differences among putative stocks within the Southern Hemisphere minke whales.

Goto *et al.* (1998) examined a total of 563 minke whales from two JARPA surveys in Area IV (1989/90 and 1991/92). In addition to the longitudinal and temporal factors examined in the previous studies, samples were divided into 'offshore' and 'ice-edge' by a line on 60 n.miles from the ice-edge. Homogeneity test was conducted using the same

procedure of the previous studies. The only significant source of heterogeneity was attributable to the 'offshore' component of Area IVWE.

Pastene and Goto (this meeting) examined minke whales sampled by the JARPA in Areas IIIE and IV in two summer seasons, 1995/96 and 1997/98. Criteria for grouping the samples and method used for the statistical analysis were the same as in the previous studies. A total of 812 whales were examined into ten area/time/year groups. The pattern of mtDNA variation in these two surveys was similar. The significant mtDNA heterogeneity detected in the group Area IVWE in 1989/90 and 1991/92 was not detected in 1995/96 and 1997/98. An additional analysis of this group in several surveys, which incorporated information on the distance from the ice-edge, suggested the possibility of yearly variation in the distribution of stocks.

3.1.4 Sequencing of the mtDNA control region

Bakke *et al.* (1996) using sequencing analysis examined 13 and 10 minke whale samples from Areas IV and V, respectively. The analysis also included samples from the North Atlantic. Samples in Areas IV and V were from the 1991/92 and 1990/91 JARPA surveys, respectively.

They sequenced a 345bp-segment of the mtDNA control region. The nucleotide diversity in the Antarctic total sample was 0.0159 higher than in the North Atlantic (0.0064). They used a randomization test to estimate the statistical significance of the genetic differences. Areas IV and V were different at the 10% significance level.

3.1.5 Repeated DNA sequences

Amos and Dover (1991) used repeated DNA sequences (gene families) to examine minke whales from the North Atlantic, North Pacific and Antarctic Areas IV and V. Approximately 50 samples were available from each of these Areas. These were available from CPW operations.

A total of 10 gene families were identified and six of these uncovered diagnostic differences between North Atlantic and North Pacific. Of these three also revealed differences between either or both the Northern Hemisphere and the Antarctic. However no consistent differences were found between Areas IV and V although clone cl.5M1-19 revealed a polymorphism which had a strongly skewed distribution with respect to these two Areas. The authors offered three possible explanations for such apparent differences: i) biased sampling due to related individuals ii) a genuine difference in allele frequencies between the populations and iii) an indication of two discreet populations which only mix certain times of the year.

3.1.6 Multilocus minisatellite (DNA fingerprinting)

van Pijlen *et al.* (1991) used this molecular technique to examine minke whales from the North Atlantic, North Pacific and Antarctic Areas IV and V. Samples from the Antarctic

were from CPW operations.

When Jeffreys' probe 33.15 was used, striking differences were observed between North Atlantic and North Pacific and Antarctic minke whales. Such differences disappeared when probe 33.6 was used. No significant differences between Areas IV and V were found.

3.1.7 Single locus mini- and microsatellite

vanPijlen *et al.* (1992) used single locus mini and microsatellites to compare minke whales from the North Pacific, North Atlantic and Antarctic Areas IV and V. Samples from the Antarctic were from the CPW operation of 1982/83.

Three minisatellite loci (cBac02, cBac10 and cBac34) were screened in 24 and 21 whales from Areas IV and V, respectively. Four microsatellite loci (199/200, 417/418, 464/465 and 415/416) were screened in 19 and 61 whales from Areas IV and V, respectively.

Regarding minisatellite, no significant differences in allele frequencies were found for any of the loci between Areas IV and V and then these two samples were pooled for further analyses. Striking differences were observed among Antarctic, North Pacific and North Atlantic minke whales. No deviations from the Hardy-Weinberg equilibrium were detected in any population for each of the three loci.

Regarding microsatellite, allele frequencies were homogeneous in Areas IV and V. These two Areas pooled were substantially different from North Pacific and North Atlantic. For one locus (415/416) a significant deviation from the Hardy-Weinberg equilibrium was observed for the Antarctic sample.

Clustering analysis of microsatellite showed two subclusters in the Antarctic sample in 84% of the trees. One of them was further divided into two clusters in 86% of the trees. However these clusters were not linked with catch position, time, date or sex.

The authors noted that the interpretation of their negative results (no significant differences between Areas) is difficult without further information on movement of whales between Areas during the feeding season. Furthermore they added that more extensive and synchronised sampling is required before it will be possible to distinguish unequivocally between a truly panmictic population and a subdivided population, which may display temporal and spatial mixing on the feeding ground.

Abe *et al.* (this meeting) used five microsatellites (GT211, GATA098, GT023, EV1PM and EV104Mn) to examine 914 minke whales sampled by the JARPA in Areas IIIE, IV, V and VIW. The mean observed heterozygosity in these Areas varied from 0.8206 to 0.8906. Allele frequencies of the five loci were very similar among Areas. Using three different statistical methods, a significant deviation from the Hardy-Weinberg equilibrium was found in the total sample and Area IIIE suggesting some degree of stock

structure. A preliminary heterogeneity test suggested nuclear DNA heterogeneity in Area V. Although this analysis was considered preliminary and grouping of samples was not exactly the same as in the mtDNA analyses, the nuclear DNA pattern of variation seems to differ from that of mtDNA in these Areas.

3.2 Morphological and morphometric analyses

Wada and Numachi (1979) examined the morphology in minke whales collected by the CPW in Antarctic Areas I-VI in the austral summers of the period 1971/72 and 1976/77. The characters used were the following: i) the relationship between the end of the ventral grooves and umbilicus, ii) flipper coloration and iii) the proportion of the black band in the largest baleen plate. On these characters two (ventral grooves reaching umbilicus and ventral grooves not reaching the umbilicus), four (uniformly black, with dark line, with faint greyish band and with clear white band) and six (all faint amber, black band occupies ¼ area of baleen plate, black band occupies 1/3 area of baleen plate, black band occupies 2/3 area of baleen plate and all black) items were defined and each whale was classified according them.

The number of whales examined for each of the characters and items were, for character i) 532 and 18,692, respectively; for character ii) 8,934, 4,003, 6,147 and 180, respectively and for character iii) 23, 1,459, 7,103, 7,831, 2,703 and 49, respectively.

The following characters were dominant: ventral grooves reaching umbilicus, flipper coloration uniformly black and baleen plate coloration with black occupying ½ area of baleen plate. No relationship between external characters and sex was observed but considerable variation in the observed frequency patterns between CPW operations was found. Apart from this, remarkable variation even within the same 10° square by expedition or by season was found. Finally the authors concluded that the data set was not useful to identify stock units and that criterion classification technique of each observer should be unified.

Doroshenko (1979) examined the morphology of minke whales collected by CPW operations in Antarctic waters of the Indian Ocean and in the southwest Pacific. He called these as Indian and New Zealand populations, respectively. A total of 800 whales collected in the austral season 1975/76 were examined for ten morphological characters as follow: i) shape of the fluke notch, ii) color of flipper on dorsal side, iii) color of the palate, iv) location of the Jacobson's organ, v) configuration of the left liver segment, vi) sternum shape, vii) number of vibrissae, viii) number of ventral grooves on a line with the base of the flippers, ix) number of baleen plates on one side of the baleen series and x) number of phalanges on the first and fourth digits of the flippers. For some of these characters items or types were defined and the frequencies of them was compared among the two populations.

According to the author the most marked differences between both populations were the frequency of occurrence of shapes of fluke notches (character i), color of flippers (character ii), number of baleen plates and number of phalanges on the first and fourth

digits of the pectoral flipper. He suggested that the Indian population is thought to inhabit the area between 55°E and 110°E and the New Zealand population between 135°E and 165°W. He further suggested that the border line is limited by latitudes 58°S to 60°S and the southern one coincides with the ice-edge.

Bushuev (1990) examined minke whales collected by CPW operations in Areas I, II, III and IV between 1983/84 and 1985/86. A total of 6,646 whales was examined for different morphological markers.

A total of 34 nonmetric characters were used. These characters were on coloration, derivatives of cutaneous covering, skeleton, digestive system and circulatory system. In addition the author examined 10 meristic and two linear morphological features. Comparison between the samples by Area and by sex was made using the index of similarity *r* and the significance differences between *r* and 1 was calculated by *J-statistics*, which has a chi-squared distribution. Differences in the mean values of meristic and linear morphological characters between samples were evaluated using Student's test.

In relation to the analysis of nonmetric characters the author found specific pattern of variation by sex in all samples, stability of differences between the sexes over season (years) and significant differences between sexes for combined data of several seasons in nine characters. For one character only was a statistically significant age dependent variability found. Differences over three successive seasons in a same Antarctic Area was small. Comparisons among Areas was made for female and male independently. The results of several combinations and tests suggested no reason to consider the groupings of minke whales examined to be from isolated populations.

Regarding meristic and linear characters, statistical significant differences between males and females were found for four characters. No significant correlation of characters with age was found. Limited variation in these characters among seasons (years) was found. No significant differences were found among Areas.

Fujise (1995) conducted a preliminary study on morphometry of minke whales from Area IV sampled in the 1989/90 JARPA survey. All the morphological observations and external measurements had been made by the author on the field. Following the sampling design used in the genetic analysis (Pastene *et al.*, 1996a) the samples (n= 326) were grouped a priori into three strata: Area IV western early, Area IV western late and Area IV eastern early. A principal component analysis of the log transformed data revealed that most of the morphometric variation resulted from measurements of overall length, dorsal fin shape, skull size and shape of flukes. An analysis of covariance indicated that the length of the dorsal fin base and the width of the flipper significantly differed between the three strata of males, but in females only the length of the dorsal fin base differed between area/time strata. Canonical discriminant analysis revealed that the three area/time strata were not separated exactly. It was concluded that whales from the Area IV western early stratum have different external body proportions. Although this result tend to support the finding of the genetic analysis (Pastene *et al.* 1996a), the author could

not exclude the possibility that some of the apparent differences were due to seasonal changes in body fatness.

3.3 Other approaches

3.3.1 Analysis of tagging data

Information on the recovery of 94 Discovery marks from minke whales in the Southern Hemisphere was reviewed by Best (1990). The main features were the recovery of two marks from whales on the winter breeding grounds off Brazil. These whales had been marked at locations 54° of longitude apart in the Antarctic. This was a direct evidence of linkage of breeding areas with feeding areas in the Antarctic. Noting the long longitudinal distance between marking locations at the Antarctic and recovery locations at the breeding ground off Brazil, Best (1990) suggested that whales from different breeding grounds may intermingle on the Antarctic feeding grounds. Recoveries of the other 92 marked minke whales in the Antarctic indicated a substantial but limited range of longitudinal movement (up to 40°) for 90% of whales within eight years of marking. Patterns of dispersal of marked whales suggested a discontinuity around 80°E (western part of Area IV) (see also Wada, 1984). The SC had noted that it is difficult to interpret the implications of such movement for stock identity due to the small sample size and due to the fact that movements indicated by mark recoveries will be influenced by the distribution of marking and catching effort (IWC, 1991).

Kato *et al.* (1993) examined all available marks (2,864 mark release and 110 recoveries of Discovery tag) at the Antarctic and recognized a similar discontinuity at around 80°E. Through their analyses it was revealed that: the average distance of longitudinal movement is about 30 degrees, recaptured animals showed no preferential east or west movement in the Antarctic and no significant difference were found between sexes in terms of distance moved.

Two .410 Discovery marks have been recovered during JARPA surveys in the Antarctic. The first occurred during the 1991/92 JARPA survey in Area IV (Fujise *et al.*, 1993a). The mark was recovered from a whale sighted at position 65°36'S, 79°E on 3 February 1992. The whale was a pregnant female and the body length and body weight of this individual were 9.0m and 10.4t, respectively (Fujise *et al.*, 1993a). The whale had been marked during the 1978/79 IWC/IDCR cruise at position 63° 11'S, 100° 5'E on 29 December 1978, then the time elapsed between marking and recapture was 13 years and 36 days. The other mark was recovered during the 1992/93 JARPA survey in Area V (Fujise *et al.*, 1993b). The mark was recovered from a whale sighted at position 66° 20'S, 153° 16'E on 10 February 1993. The whale was a male and the body length and body weight of this individual were 8.4m and 7.5t, respectively (Fujise *et al.*, 1993b). The whale had been marked during the 1980/81 IWC/IDCR cruise at position 70° 57'S, 174° 58'W on 1 February 1981, then the time elapsed between marking and recapture was 12 years and 36 days. It should be noted that both Discovery marks were recovered in the same Area in which the whales were marked. In the first case in the western part of Area IV (recovered 21° longitude apart) and in the second case in Area V (recovered 32°

longitude apart).

3.3.2 Pollutant burden

Tatsukawa *et al.* (1990) examined tissue samples from minke whales taken in Areas IV, V and VI during commercial whaling operations in 1984/85 and 1985/86. They compared the level of concentration of DDE and PCB among these Areas using male samples aged over 15 years old. No significant differences among Areas were observed in the concentration of PCB. However, significant higher values of DDE were observed in Area IV than in Areas V and VI.

Tanabe *et al.* (1995) conducted an analysis of organochlorines in the blubber of minke whales taken in a commercial operation in 1984/85 and during a JARPA survey in 1990/91 (Area V). They detected five organochlorine compounds (PCBs, DDTs, HCBs, CHLs and HCHs). No yearly variation was detected in the level of concentration of DDTs. However, even considering the age-dependent accumulation characteristics, they concluded that PCB concentration was higher in 1990/91 than in 1984/85.

Fujise *et al.* (1997) examined the level of accumulation of Hg in liver samples of southern minke whales. They used a total of 534 samples obtained from both past commercial whaling and JARPA. In their analysis of geographical variation they compared level of accumulation among Areas III-VI. No significant differences were observed between female and male samples. Also no significant differences were observed among Areas for different age groups examined.

3.3.3 Ecological markers

Nemoto *et al.* (1980) reported the presence of diatoms (*Cocconeis ceticola*) on the skin of minke whales killed off Durban, South Africa. On the assumption that typical *C. ceticola* is only contracted in high latitudes, the authors speculated that its occurrence on whales from Durban shows that these whales may recently have migrated from higher latitudes.

Ohsumi (1973) reported a broken-off bill of a marlin (*Makaira mazara* or *M. indica*) embedded in the rostrum of a minke whale killed in the Antarctic at 64°06'S, 87°14'E (Area IV). This finding also provides indirect evidence of a probable linkage between Antarctic and tropical or sub-tropical waters of the Indian Ocean.

Bushuev (1990) studied several ecological markers on minke whales from Areas I, III and IV. He used samples collected by CPW operations in these Areas between 1983/84 and 1985/86. The ecological markers investigated were: presence (absence) of *Xenobalanus globicipitis*, presence (absence) and the degree of occurrence of *Cyamus balaenoptera*, degree of occurrence of 'white' scars and degree of occurrence (number) of fresh 'brightly white' areas.

The presence/absence of *X. globicipitis* was the most useful marker for stock identity. Differences in infestation by this parasite among feeding concentrations appeared to be

largely unrelated to differences in the time of whaling. On the other hand, infestation by this parasite was found to be practically the same in different age groups. In general, females appeared more often infested by this parasite than males and this difference varied by Area.

Significant differences by Area were found in the frequency of occurrence of *X. globicipitis* with the level of geographical differences far exceeding that of the time differences (seasonal and inter-seasonal). The author explained such differences saying that the whales feeding in Area III, in the western and central parts of Area IV and in the eastern part of Area I spend the winter in different areas of the Southern Hemisphere where the probability of being infested by *X. globicipitis* varies considerably.

In the only report on parasites using JARPA data, Sedlak-Weinstein (1990) summarized the incidence of parasites in 241 minke whales sampled during the 1988/89 JARPA survey in Area V. Of the 241 whales captured 102 (42.3%) were infested with parasites as follows: *Cyamid balaenoptera* ectoparasite amphipods found on the ventral grooves (36% infestation rate), *Pennella balanae* crustacean siphonostomatid ectoparasite (2.1%), *Bolbosoma balaenae* intestinal acanthocephalan (1.3%), *Tetrabothria* sp. intestinal cestode possibly T. *affinis*, *Phyllobothrium delphini* larval cestode found in the blubber (1.7%), *Anisakis* sp stomach nematodes (7.5%). Further analyses involving another areas of the feeding ground are necessary to investigate whether the infestation rate of these parasites change with locality.

3.3.4 Conception dates

The timing of conception has been used to investigate stock identity in the North Pacific minke whale (Kato, 1992). The basic data used in this approach are the foetal lengths, which have been recorded during the JARPA surveys. The identification of different foetal cohorts in different areas of the Antarctic may suggest the occurrence of different breeding stocks. Studies on stock identity using this approach are being considered using JARPA materials.

3.3.5 Photo-identification

To our knowledge, there are no studies on photo-id to investigate stock identity in the southern minke whale.

3.3.6 Survey distribution

Kasuya and Wada (1991) examined sighting data obtained from Japanese sighting vessels in the Indian Ocean. They suggested that density of minke whale is high in the eastern and western sides of the Indian Ocean and low in the central sectors. The highest minke whale densities are found south of 60°S from November to March with considerable sightings to the north of 55°S.

Kasamatsu *et al.* (1995) analysed sightings of minke whales collected by Japanese scouting boats and research vessels operating in the Southern Hemisphere since 1976. On

the basis of this information the authors identified five areas of higher density north of 20°S in October-November, which were believed to be breeding grounds: 110°W-120°W and 130°W-170°W in the South Pacific; 40°E-50°E and 80°E-100°E in the Indian Ocean. In the South Atlantic the Brazil breeding ground had previously been identified between 20°W and 40°W. They also proposed hypothetical feeding areas used by the animals from these breeding grounds (Fig. 2).

Kasamatsu *et al.* (1990) summarize data from the IWC/IDCR minke whale cruises made from 1978/79. The document showed regions of high and low density in the Antarctic Ocean but some of these appeared to have shifted in the interval between the surveys. There was consistent discontinuity at 30°E-70°E and around 100°E.

3.3.7 Catch distribution

van Beek (1983) and Best (1990) examined CPUE series for the Antarctic minke whale. It was noted that although, there was discontinuity in the distribution of catches, it was not clear to what extend this reflected the distribution of whales rather than the distribution of catching effort. van Beek (1983) noted that although the interpretation of these plots was complicated, he suggested that only at around 100°E is there a very minor indication for a stock boundary.

4- HISTORICAL CONCLUSIONS ON STOCK IDENTITY OF THE SPECIES IN THE SOUTHERN HEMISPHERE

As indicated in Section 2, the Southern Hemisphere minke whale was managed by the IWC on the basis of the six geographical Areas shown in Fig. 1. These Areas were established based mainly upon information on distribution of different whale species like blue, fin and humpback whales. Then, biological evidences for the particular boundaries for the minke whale are weak.

Studies on stock identity in the Southern Hemisphere minke whale (ordinary form) started at the end of the decade of the 70's. Since then, different methods were used for studying stock identity in this species.

Results of the application of these methods, which used samples and data provided by the CPW, were presented and discussed during the CA of the species in 1990 (IWC, 1991). Until 1990 the genetic analyses involved methods based on both allozymes and DNA (RFLP analysis of mtDNA control region, repeated DNA sequences, multilocus minisatellite), which were used to investigate genetic differences mainly between Areas IV and V. None of these genetic techniques provided any evidence of unambiguous genetic differences between these Areas. The SC concluded that 'there must be sufficient interchange between the currently recognised stocks in the Southern Hemisphere to counteract the effects of genetic drift (which builds up genetic differences between populations through the random loss of variation). However, this could be achieved by the movement of one reproductively successful individual per generation between neighbouring stocks' (IWC, 1991). It should be noted, however, that all of the genetic

works apart the allozyme, were based on very small sample sizes (see Table 1). Furthermore all of those studies used samples available from the past commercial whaling in the Antarctic, which operated mainly in areas near the pack-ice. Probably these samples were not representative of all genetic variability of whales from Areas IV and V. It should be mentioned also that, apart the allozyme survey of Wada and Numachi (1991), all the other genetic studies were concentrated to Areas IV and V with no DNA information from the other Areas.

Non-genetic approaches discussed in 1990 involved morphology, pattern of distribution from sightings, analysis of Discovery marks, pattern of catch distribution and ecological markers.

Morphological analysis provided no evidence of unambiguous genetic differences between Areas (but see Doroshenko, 1979). These analyses were restricted to some few Areas and the issue of the criteria classification technique among investigators participating in different expedition, was a common problem in some of these studies. Analysis of sighting distribution allow to hypothesize the occurrence of five breeding grounds in the Southern Hemisphere. The hypothetical feeding areas for whales from these breeding grounds were also suggested (see Fig. 2). Regarding the analysis of Discovery marks, the SC noted that it is difficult to interpret the implications of such movement for stock identity due to the small sample size and due to the fact that movements indicated by mark recoveries will be influenced by the distribution of marking and catching effort. However, several authors recognized a discontinuity at about 80°E. Regarding catch distribution, it was noted by the SC in 1990 that although, there was discontinuity in the distribution of catches, it was not clear to what extend this reflected the distribution of whales rather than the distribution of catching effort. van Beek (1983) noted that although the interpretation of these plots was complicated, he suggested that only at around 100°E is there a very minor indication for a stock boundary. The use of ecological markers were more promising as significant differences in the infestation of one parasite were found among Areas I, III and IV (Bushuev, 1990).

In summary all the genetic and non-genetic approaches reviewed by the SC in 1990 failed to identify unambiguously any isolated population in the Antarctic. Recognising that most of the genetic analyses had been concentrated in Areas IV and V, the SC recommended that further work on the mitochondrial DNA genome of minke whales from stock Areas other than IV and V should be conducted to examine stock identity, if suitable samples are available (IWC, 1991). Also recognising the ecological markers (infestation rate of ectoparasites) as a promising approach to study stock identity, the SC recommended that Soviet data on the distribution of ecological markers should be analysed in more detail to provide some measure of the reliability of the conclusions presented (IWC, 1991).

Based on the information reviewed in the 1990 meeting on sighting distribution patterns and marks-recovery, the SC formulated some hypotheses on stock structure (IWC, 1991, pp. 125-126). These hypotheses were based on the assumptions that there are five breeding grounds for Southern Hemisphere minke whales (see Fig. 2).

All the analyses on stock identity conducted until 1990 were based on samples and data provided by the CPW operations. Since 1987/88 austral season the JARPA surveys began in the Antarctic and new samples from Areas IV and V became available. As mentioned earlier, the sampling procedure under the JARPA allowed for a more extensive geographical and temporal covering than it was possible under commercial operations.

The use of JARPA samples for stock identity purposes has several advantages in comparison to CPW samples: a) whales are sampled using a random design described by Kato *et al.* (1989). Between 1987/88 and 1991/92, the number of whales to be taken from a school varied with school size: if a solitary whale was found it was sampled; if a pair was encountered, both whales were planned to be taken, with the first whale to be sampled being chosen randomly; for schools of three or more, two whales were taken using the random method. From 1992/93, only one whale was randomly taken from a school, regardless of school size; b) whales are sampled on pre-determined track lines, which cover both offshore and areas near the pack ice and c) detailed sampling and biological information is available for each specimen, and this information has been collected by biologists.

Studies on stock identity under JARPA were reviewed by Pastene and Goto (1997). These studies have been based largely in mtDNA RFLP analyses and in a lesser extension in morphometric analyses. Results of the genetic approach has revealed considerable mtDNA heterogeneity, but little geographic concordance with IWC Areas IV and V

According to the results of the studies under the JARPA, it seems that the stocks structure of minke whales migrating into the Antarctic feeding grounds of Areas IV and V could be more complex than it was thought initially, and it could be determined not only by geographical factors but also by temporal factors. The most extensive and recent mtDNA analysis showed that the only significant source of mtDNA heterogeneity is attributable to a group of whales in the western part of Area IV migrating early in the season 1989/90. This result is supported by that derived from morphometric analysis that used samples of Area IV of the 1989/90 season.

Although several genetic (Amos and Dover, 1991; Pastene *et al.* 1996a; Abe *et al.*, this meeting) and non-genetic (Doroshenko, 1979; van Veek, 1983, Wada, 1984; Bushuev, 1990, Tatsukawa *et al.*, 1990, Fujise, 1995) studies suggest some degree of structuring, we can say that no definitive conclusion can be reached on the stocks structure of this species in the Antarctic feeding ground. It should be noted in particular that different studies suggest either a stock boundary or an interaction of stocks in the western part of Area IV and eastern part of Area III. For example the analysis of mark recapture data suggest a discontinuity at around 80°E (Wada, 1984; Best, 1990; Kato *et al.*, 1993); CPUE series suggested that only at around 100°E is there a very minor indication of stock boundary (van Beek, 1983) and a RFLP analysis of the mtDNA analyses under the JARPA suggest an interaction of stocks in the western part of Area IV (Pastene *et al.*, 1996a). In addition a morphological study suggested that an 'Indian population' inhabit

the area between 55°E and 110°E and a 'New Zealand population' inhabit the area between 135°E and 165°W (Doroshenko, 1979).

If we consider that the behavior of minke whale stocks in the feeding ground could change within and between seasons, according to changes in oceanographical conditions and dynamic of prey species. Then the results indicated above for Areas III and IV could suggest a 'dynamic boundary' rather than a fixed geographical boundary in these Areas.

There are several tasks, which should be addressed in order to clarify the stock structure in the Southern Hemisphere minke whale. Among them we can mention the following:

i) No genetic analysis has been conducted for minke whales in lower latitudes of the Southern Hemisphere. Ways on how to obtain genetic samples from suspected breeding areas (as suggested by pattern of sighting distribution) should be investigated. Information on the genetic composition of minke whales in lower latitude is important as such information could be used to interpret the pattern of variation observed in the Antarctic.

ii) It seems that the effect size (the expected degree of genetic differentiation between putative populations) in the southern minke whale is very small. Although powerful DNA techniques were used in the past (reviewed at the SC 1990 meeting), these were applied on a small number of commercial samples from Areas IV and V. These techniques should be applied on a larger number of samples from these and other Areas and the analyses should consider both geographical and temporal criteria. Available samples from JARPA could be used for this purpose.

iii) Methods to estimate the power of the statistical analysis of genetic data should be developed and applied in future.

iv) An optimization in the use of morphological data to investigate stock structure is needed. A study using commercial data concluded that a standardization of procedure to obtain morphometric data was needed among surveys. Although detailed and large amount of data has been obtained during the JARPA surveys, only data from one survey (1989/90) have been examined. Standardizing procedure among researchers participating in different surveys should optimize the use of morphological data.

v) Promising new techniques to investigate stock identity such as the use of ecological markers and pollutant burden should be further investigated and applied in the future. Because some of these technique are age or sex dependent, samples available from the JARPA could be used.

vi) Results of the different approaches to investigate stock identity should be interpreted in the light of oceanographic process and dynamic of prey species because the behavior of stocks in the feeding ground could change inter and/or

intra-seasonal depending of changes in the dynamic of prey species.

At this stage is difficult to suggest the 'best' approach for studying stock identity in the southern minke whale. As mentioned above, the optimal use of several approaches have not been reached yet i.e. use of morphometric and ecological marker data. Regarding to genetics, the only techniques used more extensively have been allozyme and RFLP of the mtDNA. The allozyme survey covered all the IWC Areas but proved to be uninformative of the stock identity. The mtDNA RFLP analysis has proved to be more informative but it has been used only on Areas IV and V. Other DNA-based techniques has been based on limited sample sizes and restricted to Areas IV and V.

On the other hand further discussion should be carried out on the meaning of 'significant' geographical or temporal differences found by the different approaches. Are the significant differences found in the level of DDE concentration between Area IV and Areas V-VI (Tatsukawa *et al.*, 1990) indicative of the occurrence of different stocks? What about different results being obtained using different approaches or even different genetic techniques?

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Table1: Summary of the main genetic analyses conducted on the Southern Hemisphere minke whale to investigate intra-specific structure.
Key: CPW=Commercial Pelagic Whaling; CCW=Commercial Coastal Whaling; JARPA=Japanese Whale Research Program under Special Permit in the Antarctic; it=internal tissue; H-W=Hardy-Weinberg; RE=restriction enzymes; SE=length of sequence segment

Gen.marker / Authors	Geographic Locality	Period of Sampling	n	Sample Source	Number of Loci/RE/SE	Statistical Analysis	Main Results
Allozyme							
Wada and Numachi(1991)	Areas I-VI BRAZIL	1975/76 - 1983/84 1981	11,414 195	CPW(it) CCW(it)	45 loci (10 polymorphic)	H-W test Homogeneity test (G-test)	Areas I=II=III=IV=V=VI Areas I-VI = BRAZIL
RFLP Whole mtDNA							
Wada *et al.*(1991)	Area IV Area V	1983/84 1983/84	40 39	CPW(it) CPW(it)	14 restriction enzymes (9 polymorphic)	Homogeneity test (G-test)	Area IV = Area V
RFLP mtDNA Control Region							
Hoelzel and Dover(1991)	Area IV Area V		20 21	CPW(it) CPW(it)	3 restriction enzymes	Genetic distance	Area IV = Area V
Pastene *et al.*(1993a)	70°-110°E (W) 110°-150°E (C) 150°E-180° (E)	1989/90 1989/90, 90/91 1990/91, 88/89	118 93 107	JARPA(it) JARPA(it) JARPA(it)	12 restriction enzymes (11 polymorphic)	Homogeneity test (Randomized chi-squane test)	W = C C = E W ≠ E
Pastene *et al.*(1996a)	70°-100°E Early* (IVWE) 70°-100°E Late (IVWL) 100°-130°E Early (IVEE) 100°-130°E Late (IVEL) 130°-160°E Early (VWE) 130°-160°E Late (VWL) 160°E-160°W Early (VEE) 160°E-160°W Late (VEL)	1989/90, 91/92 1989/90, 91/92, 93/94 1989/90, 91/92, 93/94 1987/88, 89/90, 91/92, 93/94 1990/91, 92/93, 94/95 1990/91, 92/93, 94/95 1988/98, 90/91, 92/93, 94/95 1988/89, 90/91, 92/93, 94/95	160 383 233 321 208 264 76 479	JARPA(it) JARPA(it) JARPA(it) JARPA(it) JARPA(it) JARPA(it) JARPA(it) JARPA(it)	6 polymorphic restriction enzymes	Homogeneity test (AMOVA / PHI-st)	Significant heterogeneity in Group IV WE

Table1: cont.

Gen.marker / Authors	Geographic Locality	Period of Sampling	n	Sample Source	Number of Loci/RE/SE	Statistical Analysis	Main Results
Pastene *et al.*(1996b)	66°-70°E (IIIEE) 145°-165°W (VIWE)	Dec.1978 Dec./Jan. 1986	154 134	CPW(it) CPW(it)	6 polymorphic restriction enzymes	Homogeneity test (AMOVA / PHI-st)	IIIEE = IVWE VIWE ≠ IVWE
Sequencing mtDNA Control Region							
Bakke *et al.*(1996)	Area IV Area V	1991/92 1990/91	13 10	JARPA(it) JARPA(it)	345bp-segment	Homogeneity test (Randomized Yst test)	Area IV≠Area V at 10% significant level
Repeated DNA sequences							
Amos and Dover(1991)	Area IV Area V		50 50	CPW(it) CPW(it)	10 gene families		Area IV ≠Area V using clone cl.5M1-19
Multilocus minisatellite							
van Pijlen *et al.*(1991)	Area IV Area V			CPW(it) CPW(it)	2 Jeffreys' probe (33·15; 33·6)		Area IV = Area V
Single locus minisatellite							
van Pijlen *et al.*(1992)	Area IV Area V	1982/83 1982/83	24 21	CPW(it) CPW(it)	3 loci	H-W test Homogeneity test clustering analysis	Area IV = Area V

Table1: cont.

Gen.marker / Authors	Geographic Locality	Period of Sampling	n	Sample Source	Number of Loci/RE/SE	Statistical Analysis	Main Results
Microsatellite							
van Pijlen *et al*.(1992)	Area IV Area V	1982/83 1982/83	19 61	CPW(it) CPW(it)	4 loci	H-W test Homogeneity test clustering analysis	Departure from H-W equilibrium in one locus Area IV = Area V

* ' Early ' refers to whales sampled between December and 15 January; ' Late ' refers to whales sampled between 16 January and March.

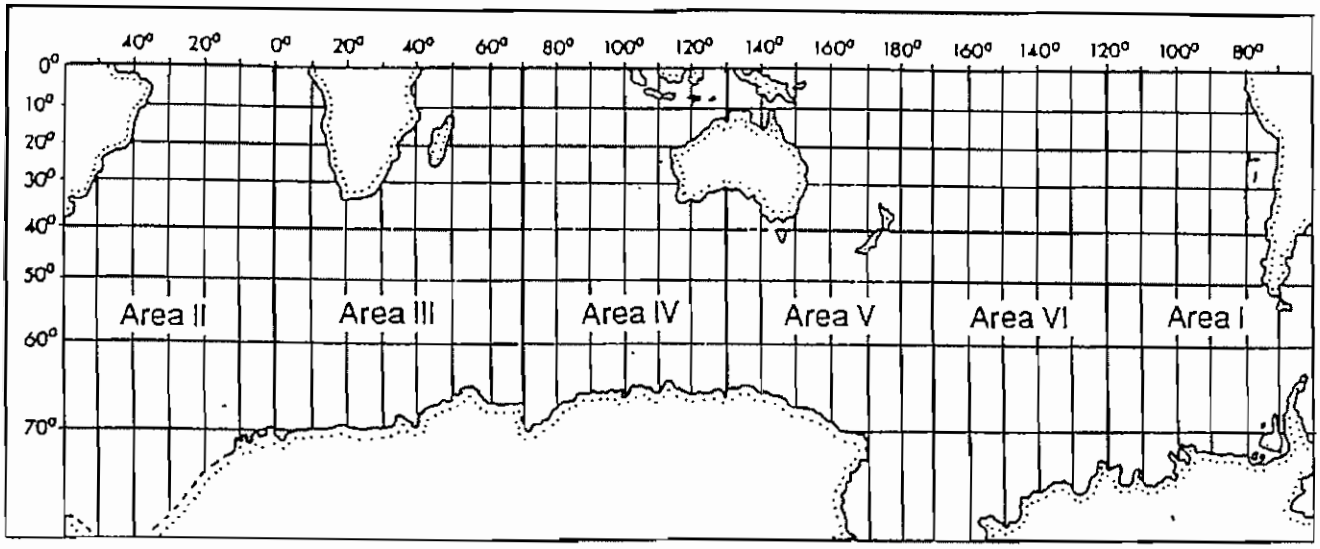

Figure 1: IWC Antarctic Areas for the management of baleen whale species (except Bryde's whale).

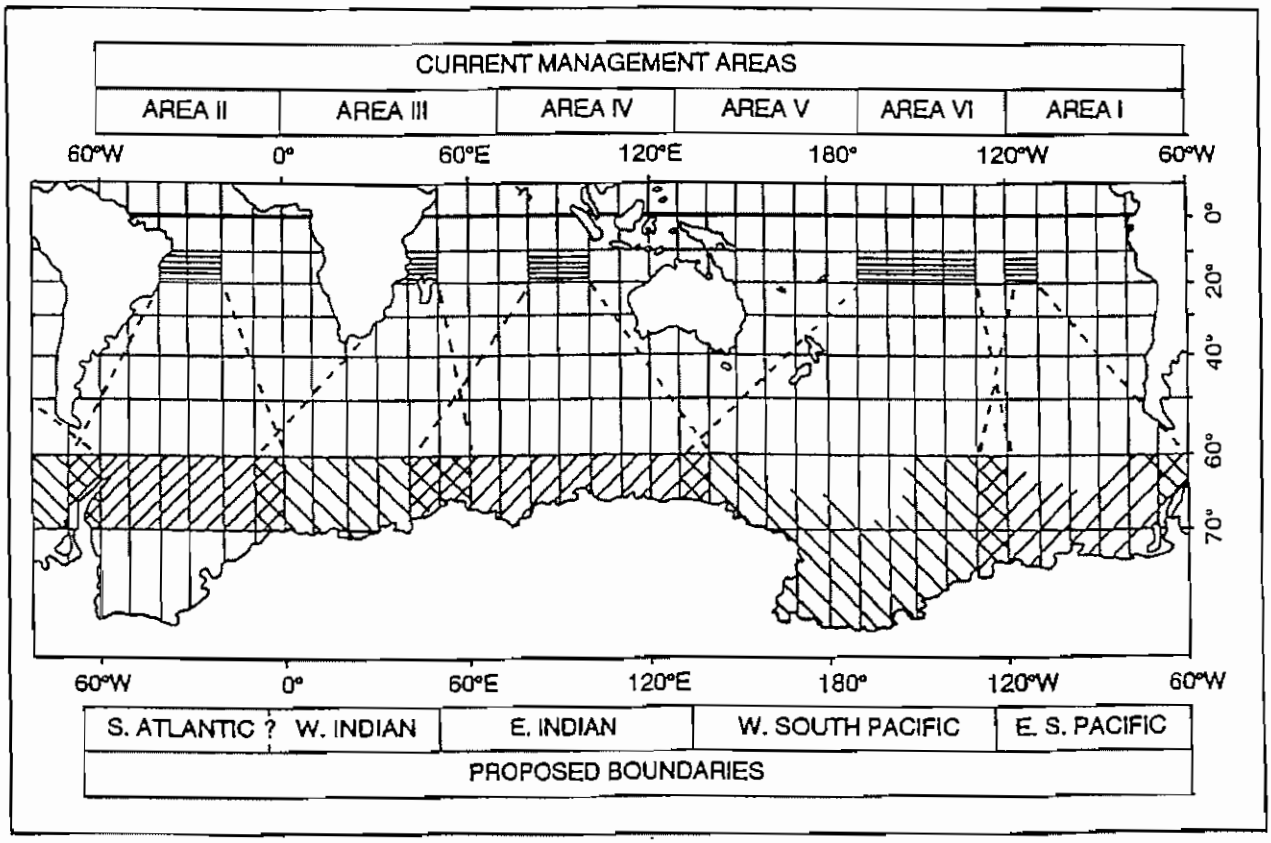

Figure 2: Possible location of breeding grounds and Antarctic feeding areas as proposed from sighting data distribution (after IWC, 1991).