WHAT DO WE KNOW ABOUT THE STOCK STRUCTURE OF THE ANTARCTIC MINKE WHALE? A SUMMARY OF STUDIES AND HYPOTHESES

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ABSTRACT

A review of the studies on stock structure in the Antarctic minke whale was conducted with the purpose to establish a plausible hypothesis on stock structure of this species in the JARPA research area (Areas IIIE-VIW). Studies on stock structure started at the end of the decade of the 70's and results were revised by the SC during the comprehensive assessment of the species in 1990. All the analyses were conducted using samples and data from commercial pelagic whaling in the Antarctic. Genetic studies were based mainly on allozyme although studies based on mitochondrial and nuclear DNA were also conducted. Most of these analyses involved small sample sizes 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. Analysis of sighting data suggested the occurrence of five breeding Areas. Studies on stock structure under the JARPA started after the comprehensive assessment. It is considered that samples taken by JARPA are more useful for studies on stock structure given the wider geographical covering of the surveys and because minke whales were taken along track-lines in a random mode design. Initially the JARPA studies on stock structure were based on mtDNA and a considerable genetic heterogeneity in Areas IV and V was found. More recently the total minke whale samples taken by JARPA in Areas IIIE, IV, V and VIW were examined by different analytical approaches, genetics and non-genetics, using the same samples and kind of grouping. The results from the different techniques provide strong evidence to reject the hypothesis of a single stock in the JARPA research area. The most parsimonious explanation of the results is that there are at least two stocks present in the research area: an eastern Indian (I) and a western South Pacific (P) stock. These stocks would mix across a soft boundary, which would probably best be placed near 165ºE. This stock division is adopted for the objective of the estimation of biological parameters in JARPA. Although the most recent analyses by JARPA indicated that whales in Area IIIE are related to the I stock, other analyses suggested some degree of heterogeneity between Areas III and IV. Therefore the possibility of additional stock structure in Area III (e.g. the occurrence of animals from the western Indian Ocean breeding ground) should be investigated in the future. Any stock boundary (or equivalently sectors of mixing) among stocks in the Antarctic are likely to vary according to oceanographic conditions, which in turn controls the distribution of the key prey species of the Antarctic minke whale, the krill. Several future research needs on stock structure are identified. The results will inform the specifications and testing of appropriate management areas under the Revised Management Procedure.

INTRODUCTION

The Antarctic minke whale, like all the other Southern Hemisphere baleen whales species apart from the Bryde's whale, was managed by the International Whaling Commission (IWC) on the basis of six geographical 'Areas' (Figure 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 the Antarctic minke whale and determine to what extent genetic stocks and IWC management Areas coincide. These approaches included genetics, morphology, analysis of mark-recapture data, patterns of catch and sighting distribution and ecological markers, and the analyses were conducted using samples and data derived from past commercial pelagic whaling (CPW) in the Antarctic. The output of most of these studies were presented and discussed during the Comprehensive Assessment (CA) of this species conducted by the IWC Scientific Committee (SC) in 1990 (IWC, 1991). 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 (1999a).

I present here an update of the information on stock structure in the Antarctic minke whale. The review is conducted for studies based on CPW samples and JARPA samples, separately. The output of this review is used to formulate a stock structure hypothesis of this species in the feeding ground so that the estimation of biological parameters, which is the main objective of the JARPA, can be carried out on the basis of the biological stocks proposed in the hypothesis.

As a background for the review, three related matters are reviewed: abundance, catch history and seasonal movements.

Abundance

Abundance of the Antarctic minke whale was given during the 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, 6.10 (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. The IWC/SC is currently conducting an update of these abundance estimates.

Catch history

The total catch of minke whales to 1990 was summarized during the 1990 SC meeting. For Areas I to VI was 12,108, 19,739, 27,541, 34,586, 15,165 and 4,999 whales, respectively (IWC,1991). From 1987/88 to 2005/06 JARPA catches in Areas III, IV, V and VI should be added.

Seasonal movements

The Antarctic minke whale like all the other balaenopterids (except the Bryde's and pygmy blue whales) are believed to undertake seasonal migrations between feeding grounds in the Antarctic waters in summer (south of 60ºS) 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.

Kasamatsu *et al*. (1995) investigated breeding areas and southbound migrations of the Antarctic minke whales using Japanese sighting surveys data obtained during 1976 to 1987. Latitudinal occurrence by month suggested that minke whales moved southward from the breeding areas by October-November, and that most of them had migrated into Antarctic waters by January. According to the authors, whales tend to move almost directly south from the breeding areas to feeding areas, and subsequently disperse after arriving at the feeding areas.

STUDIES ON STOCK STRUCTURE BASED ON COMMERCIAL PELAGIC WHALING SAMPLES

Several techniques were used to examine stock structure of Antarctic minke whale based on CPW samples. As mentioned earlier most of these studies were presented to the CA in 1990. I summarize here only the main studies.

Genetics

Several molecular techniques were used to investigate stock structre 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) were used. Table 1 summarizes data and results of the most relevant genetic studies.

Allozymes

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

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 Figure. 1. In addition they examined 195 samples (liver) from coastal Brazil. Samples in the Antarctic were from 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-I, Idh-l, 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 10º 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.

RFLP analysis of mitochondrial DNA control region

Hoelzel and Dover (1991) analysed variation in the control region of the mtDNA and compared 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 *(Ddel, MhoI 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.

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 using 14 restriction enzymes: *AccI, AvaI, BamHI, BgIII, EcoRI, EcoRJ;; RaeII, 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.

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 uncovered diagnostic differences between North Atlantic and North Pacific. Three families 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 discrete populations which only mix certain times of the year.

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.

Single locus mini- and microsatellite
van Pijlen *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 sub-clusters 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 synchronized 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.

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 l/4 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 1/2 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 10º 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 were 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 they found a statistically significant age dependent variability. Differences over three successive seasons in a same Antarctic Area was small. Comparisons among Areas were 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.

Other approaches

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 Atlantic, 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).

Kato *et al.* (1993) examined all available marks (2,864 mark release and 110 recoveries of Discovery tag) at the Antarctic. Through their analyses it was revealed that: the average distance of longitudinal movement is about 30º, 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. Marked movement of whales was observed through 130ºE, the boundary between Areas IV and V. Regarding boundaries they noted that the absence of mark crossing at 80ºE and 160º E suggests the possibility of separate feeding stocks. Following an examination of mark distribution and catching effort they concluded that discontinuity at 80ºE could be more suggestive of a boundary between stocks than that at 160ºE.

Pollutant burden

Tatsukawa *et al.* (1990) examined tissue samples from minke whales taken in Areas IV, V and VI by CPW 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 during a CPW operation in 1984/85 and during a JARPA survey in 1990/91 (Area V). They detected five organochlorine compounds (PCBs, DDTs, HCBs, CHIs 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 1990/91than in 1984/85.

Fujise *et al.* (1997) examined the level of accumulation of Hg in liver samples of Antarctic minke whales. They used a total of 534 samples obtained during CPW and JARPA operations. 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.

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 structure. 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.

Sighting 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 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.

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.

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.

STUDIES BASED ON PARTIAL JARPA SAMPLES

Partial samples and data obtained during the JARPA surveys were used in different studies on stock structure. I summarize here only the main studies.

Genetics
Both mtDNA and nDNA have been used to examine stock structure of minke whales sampled by JARPA. Table 1 summarizes data and results of the most relevant studies.

RFLP analysis of whole mtDNA
Pastene *et al.* (1993) conducted the first mtDNA study using JARPA samples. In the study a total 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 six-base restriction enzymes: *AccI, BanI, BglII, EcoRJ, 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 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). 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.

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 six 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 once 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. 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).

In response to these recommendations, Pastene *et al.* (1996) 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 (AMOV A) of Excoffier et *al.* (1992). Genetic distance between haplotypes was estimated using a maximum-likelihood method (equation 5.55 of Nei, 1987). 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.

Pastene and Goto (1999) 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. Samples in Areas IIIE and IV in 1995/96 and 1997/98 were similar in haplotype frequencies to whales of the core stock (Areas IVWL, IVE, VW). In addition an examination of the offshore component of group IVWE of several surveys was conducted considering the distance from the ice-edge as a factor. Furthermore a mtDNA analysis considering different categories of school sizes was conducted. No significant differences were found in haplotype frequencies among school sizes categories.

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.

Microsatellites

Abe *et al.* (1999) used five microsatellites (GT211, GATA098, GT023, EV1 and EV104) 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.

Morphometric analyses

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.,* 1996) the samples (n= 326) were grouped 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 among the three strata of males, but in females only the length of the dorsal fin base differed among 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 tended to support the finding of the genetic analysis (Pastene *et al.* 1996), the author could not exclude the possibility that some of the apparent differences were due to seasonal changes in body fatness.

Other approaches

Pollutant burden

Kunito *et al*. (2002) determined the concentrations of 12 trace elements in 19 liver and 161 skin tissues of minke whales from various regions in the Antarctic. Minke whales were collected by JARPA in Areas IIIE (1995/96), IV (1993/94 and 1995/96) and V (1988/89, 1990/91 and 1992/93). The data of elements in the skins were subjected to principal component analysis (PCA) and discriminant analysis to assess the ability of trace elements to discriminate among stocks of the Antarctic minke whales. Principal components were derived from standardized data via correlation matrix. For this analysis only the whales with body length over 8m were used.

Large interannual variation of the accumulation pattern of trace elements in the skin was observed in Area V. There were significant differences in the skin element concentrations among Areas III, IV and V, especially for males. To eliminate the effect of annual variation of concentration in the skin only the data for 1995/96 (Areas IIIE and IV) were analyzed. These samples were grouped into west and east by longitude 80ºE, which was proposed by mark-recapture studies (Wada, 1984; Best, 1990; Kato *et al*., 1993). The discriminant analysis allowed for a correct classification of 90% of these minke whales.

Ecological markers

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%).

Pastene *et al*. (2005) examined the prevalence (percentage of infected individuals per host examined) of the gastric nematode *Anisakis simplex* in the JARPA research area in the context of stock structure. The minke whale is known to be the most important final host of *A. simplex* in the northern North Pacific. Combined female and male samples were used for the analysis. A total of 6,338 minke whales sampled by JARPA in Areas IIIE, IV, V and VIW between 1987/88 and 2003/04, were examined.

Prevalence is similar between female and males host. Results indicated that prevalence is higher in Areas V and VIW than in Areas IV and IIIE. The higher rates of *Anisakis simplex* was observed from longitude 155°E to 170°W, with a peak in sector 165°-170°E.

STUDIES BASED ON TOTAL JARPA SAMPLES (FINAL ANALYSES)

A comprehensive study on stock structure involving genetics and non-genetics markers was presented to the JARPA review meeting sponsored by the Government of Japan in 2005 (Pastene *et al*., 2005). The analyses involved sample and data collected by JARPA between 1987/88 and 2003/04. These analyses were updated to incorporate the samples collected during the last JARPA survey of 2004/05. Below a summary of these updated analyses is presented.

Genetics

RFLP analysis of whole mtDNA

Pastene *et al*. (SC/D06/J9 in this meeting) examined Antarctic minke whales sampled by JARPA in Areas IIIE, IV, V and VIW between 1987/88 and 2004/05 (whole period) using mtDNA RFLP. Samples were grouped according the longitudinal sectors used by the JARPA surveys and used in previous mtDNA analyses. A total of 6,256 samples were used in the analysis. Consistent with previous JARPA genetic studies a significant genetic heterogeneity was found. Whales in the most distant sectors (IIIE+IV and V+VIW) were differentiated genetically. The pattern of differentiation was similar between sexes. Genetic results were consistent with the hypothesis of at least two stocks occur in the JARPA research area. A fine-scale mtDNA analysis suggested a longitudinal division between stocks in the sector 150º-160ºE.

Microsatellites

Pastene *et al*. (SC/D06/J9) examined Antarctic minke whales sampled by JARPA in Areas IIIE, IV, V and VIW between 1989/90 and 2004/05 using microsatellite variation at six loci (EV1, EV104, GT211, DlrFCB14, GT195, GT23). Samples were grouped according to the longitudinal sectors used by the JARPA surveys and used in previous mtDNA analyses. A total of 6,260 samples were used in the analysis. No significant deviation from Hardy-Weinberg equilibrium was found. Consistent with previous JARPA genetic studies heterogeneity tests suggested a significant genetic heterogeneity. Heterogeneity test suggested more partitioning I females than males. Whales in the most distant sectors (IIIE+IV and V+VIW) were differentiated genetically. Genetic results were consistent with the hypothesis of at least two stocks occur in the JARPA research area.

Morphometric analyses

Hakamada (SC/D06/J10) conducted a morphometric analysis based on JARPA samples collected in Areas IIIE, IV, V and VIW between 1987/88 and 2004/05. Grouping for the analysis followed the strata used by the JARPA survey. In order to eliminate the effect of bias due to difference among researchers, the ten measurements that were less susceptible to such differences were selected for the analysis. Selection of these measurements took into consideration the opinion of experienced researchers.

Only mature animals were used in the analysis because body proportion could be different between younger and older animals. First the relationship between body length and longitudinal sectors was investigated using ANCOVA. After obtaining an estimated average length of measurements, cluster analysis was applied to examine the degree of relationships among whales in the six longitudinal sectors. Mature males were separated into two groups at the division between Areas IV and V (130ºE). Mature females were separated into two groups at the middle of Area V (165ºE).

It should be noted that the analytical approach used in SC/D06/J10 is different to that used in the 2005 analysis (Pastene *et al*., 2005). The reason is that in the previous analysis many 'significant' p values were obtained in the comparison among longitudinal sectors. This was because the sample size used is large and then the statistical approach used detected small differences in measurements as significant. Results obtained by the cluster analysis are considered more reliable.

Other approaches

Mean length of physical maturity

Bando *et al*. (SC/D06/J11) compared the mean body length of physically matured whales (MBLM) among whales sampled by JARPA in Areas IIIE, IV, V and VIW between 1987/88 and 2004/05. Grouping for the analysis followed the strata used by the JARPA survey. Physically matured individuals were defined as those with epiphysis fusion occurring in the 6th thoracic vertebrae. The t-test and ANOVA were used for testing differences in mean body length among strata. A P-value smaller than 5% was used as a criterion to reject the null hypothesis of panmixia. LSD (Least Significant Differences) tests were made in case of multiple tests. Analyses were conducted for males and females separately. A total of 2,359 minke whales were used in the analysis.

The total P-values were highly significant for both females and males. Results of pairwise comparisons were similar for females and males. No significant differences were found among IIIE, IVW, IVE and VW. Also no significant differences were found between VIW and VE. These two strata differed significantly among them. Although VEN and VES differed in the case of males, both of these strata showed a similar pattern of variation in the comparison to other strata.

Analysis of tagging data
Three 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 63º11'S, 100°5'E on 29 December 1978, then the time elapsed between marking and recapture was 13 years. This case was incorporated into the analysis by Kato *et al*. (1993).

The second 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.

The third mark was recovered during the 2001/02 JARPA survey in Areas IIIE and IV (Ishikawa *et al.,* 2002). The mark was recovered from a whale sighted at position 66°37'S, 120°47'E on 31 January 2002. The whale was a pregnant female and the body length and body weight of this individual were 8.9m and 7.7t, respectively (Ishikawa *et al.,* 2002). The whale had been marked during the 1980/81 IWC/IDCR cruise at position 65°15'S, 134°24'E on 7 January 1981, then the time elapsed between marking and recapture was 21 years.

DISCUSSION AND HYPOTHESIS ON STOCK SRUCTURE

As indicated earlier the Antarctic minke whale was managed by the IWC on the basis of the six geographical Areas shown in Figure. 1. These Areas were established based mainly upon information on distribution or different whale species like blue, fin and humpback whales. Then, biological evidences for the particular boundaries for the minke whale are weak. In this paper I reviewed the relevant studies on stock structure for two different sample/data sets, that derived from past commercial whaling in the Antarctic and that derived from JARPA.

Studies on stock structure based on samples from commercial pelagic whaling samples

Most of the studies summarized in this paper were presented and discussed during the CA of the species in 1990 (IWC, 1991).

As reviewed in this paper the genetic analyses that used CPW samples 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. During the CA, 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 from the allozyme, were based on very small sample sizes (see Table 1). Furthermore samples available from the past commercial whaling were obtained mainly in areas near the pack-ice. Probably these samples were not representative of all genetic diversity of whales from Areas IV and V. Pastene and Goto (1999b) showed that studies based exclusively on samples obtained near the pack-ice are not informative on the stock structure. 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.

Morphological analysis provided no evidence of unambiguous genetic differences between Areas. Although some studies showed some differentiation among longitudinal sectors in the Antarctic (Doroshenko, 1979), the analyses were restricted to some few Areas and the issue of the criteria classification technique among investigators participating in different whaling expeditions was a common problem in these studies.

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.

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). However the interpretation of these results in the context of stock structure is complicated (see Balbuena *et al*., 1995).

Perhaps the most useful information on stock structure discussed at the 1990 CA was that derived from pattern of sighting distribution in low latitudes (published later by Kasamatsu *et al*., 1995), which allowed to hypothesize the occurrence of five breeding grounds in the Southern Hemisphere. The other relevant piece of information came from Discovery marks analyses. The SC noted in 1990 that it is difficult to interpret the implications of such movement for stock structure 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. Notwithstanding, several authors recognized marked movement of whales through 130ºE, which is the boundary separating Areas IV and V and a discontinuity at about 80°E, even after giving consideration to distribution of marking and catching effort.

In summary all the genetic and non-genetic approaches reviewed by the SC in 1990 failed to identify unambiguously any isolated stock in the Antarctic. Recognizing 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 recognizing 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).

Since 1987/88 austral season the JARPA surveys started in the Antarctic and new samples from Areas IV and V became available.

Analyses on stock structure based on JARPA samples

JARPA was started in 1987/88 in Areas IV and V with the main objective to estimate some biological parameters to improve the management of the Antarctic minke whale. At that time it was assumed that Areas IV and V were occupied by different genetic stocks. However the evidences supporting such assumption were weak as showed by the different studies on stock structure based on CPW samples.

The sampling procedure under the JARPA (see SC/D06/J2) allowed for a more extensive geographical and temporal covering than it was possible under CPW 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. Therefore samples taken during JARPA should be more representative of the genetic diversity of the species in the research area. Several techniques were used to examine stock structure of Antarctic minke whale using JARPA samples.

Earlier analyses based on partial samples

The genetic analyses based on both mtDNA and nDNA consistently showed significant degree of genetic heterogeneity of Antarctic minke whales, which was attributed to the occurrence of different genetic stocks in the feeding grounds. The first proposal on stocks structure was provided by Pastene *et al*. (1993). They suggested the occurrence of two stocks in Areas IV and V with a possible overlap region. However the analysis was based on samples from a few JARPA surveys and involved longitudinal sectors probably too broad.

The second proposal on stock structure was based on a larger sample size and more fine-scale grouping (Pastene *et al*., 1996). A total of eight area-time groups were designed in Areas IV and V. The results were originally presented to the mid-term JARPA review meeting in 1997 and they were summarized as follows:

a) A high degree of heterogeneity (as measured by mtDNA) exists with the ordinary form of the minke whale (Antarctic minke whale) within Areas IV and V.
b) The heterogeneity observed in Areas IV and V is attributable to both longitudinal and temporal components in distribution.
c) The observed pattern of genetic heterogeneity is not consistent with current stock boundaries (i.e. between Areas IV and V).
d) There are at least two distinct 'genetic stocks' in Areas IV and V.

The meeting recognized that the available mtDNA data were consistent with the occurrence of more than one stock in the JARPA research area. However no boundaries were proposed or discussed at that opportunity. Based mainly on these results, a working hypothesis on stock structure was presented, which suggested a main stock ('core') distributed in Areas IV and V and an additional stock ('western') distributed in western part of Area IV only early in the season. The term 'core stock' implied the concept of a large stock occupying all (or most of) Areas IV and V.

To investigate this hypothesis JARPA surveys covered the eastern part of Area III and western part of Area VI since the austral summer season 1995/96. MtDNA analysis on the new samples obtained from Area IIIE failed to detect any boundary of the 'core stock' to the west as the pattern of mtDNA heterogeneity found in the western part of Area IV early in the 1989/90 survey (attributed to a hypothetical 'western stock') (Pastene *et al*, 1996) was not found in samples from Area IIIE (Pastene and Goto, 1997). Furthermore the same analysis conducted by Pastene *et al*. (1996) was repeated in the present study for the total JARPA samples (1987/88-2004/05). This time no significant genetic heterogeneity was found for the samples of group IVW taken early in the season (data not shown). Two possible interpretations are possible for the results of group IVWE in 1989/90, a) heterogeneity observed in that group reflects a biological event e.g. the sporadic (only in some years) intrusion of a different stock or the mixing of two stocks in Area IVW early in the season, b) the heterogeneity observed for that group do not reflect a biological event. Rather it is a product of the low effect size and high genetic diversity of Antarctic minke whales, which produce misleading results for small sample sizes.

In reviewing the results on stock structure derived from JARPA, the SC has noted that only preliminary conclusions can be drawn at this stage and that more concrete conclusions will be able to be made following the completion of different analyses. It further supported the suggestion that additional analyses using alternative groupings and analytical methods should be conducted (IWC, 2004). On this regard the 1997 JARPA review meeting recommended that other analyses be conducted (in addition to mtDNA) and the possibility of further structure in the 'core stock' was not discarded. These suggestions are related to the widely accepted concept that the most effective way to address questions on stock identity is to consider results from several techniques, genetics and non-genetics (Donovan, 1991; Pastene *et al*., 2000; Perrin, 2001; Rugh *et al*., 2003). In the case of the Antarctic minke whale the application of multi-approaches to investigate stock identity was particularly desirable because previous genetic analysis suggested that the effect size in this species is low and then, results obtained by a single genetic marker should be checked using independent markers. Consequently the study on stock structure under the JARPA was extended by the use of several biological markers, both genetics and non-genetics and more detailed grouping of samples. These approaches were used for examining samples of the JARPA from 1987/88 to 2004/05 as reviewed above.

Analyses based on total samples (final analyses) and hypothesis on stock structure

It is reasonable to conclude that the most plausible hypothesis on stock structure in the Antarctic minke whales is that derived from the comprehensive analysis of total JARPA samples and data. The same samples and the same grouping were used by different analytical approaches.

The results of different analytical approaches used by JARPA were presented to this meeting in Documents SC/D06/J9, J10 and J11, and summarized in this review paper. Table 2 shows a summary of the results of these approaches. Based on this information it is reasonable to conclude that the single stock scenario can not be applied to Antarctic minke whales in the Antarctic feeding grounds of Areas IIIE-VIW. Results of the different approaches are consistent with the occurrence of at least two stocks in the research area. Probably these

stocks are related to the breeding areas in the eastern Indian Ocean and western South Pacific suggested by Kasamatsu *et al*. (1995) (Figures 2 and 3). The following names are proposed for these stocks: Eastern Indian Ocean Stock (I-Stock) and Western South Pacific Ocean Stock (P-Stock).

Because the different analytical approaches were able to differentiate whales from different longitudinal sector, the extreme scenario of full mixing of stocks in the Antarctic can be discarded because different stocks can not be discriminated under such level of mixing. The remaining question is whether these stocks are fully segregated in the feeding ground or if they partially mix. The possibility of mixing can not be discarded but such possibility can be better explored with analysis of samples from the breeding grounds.

For practical reason (to define a biological unit on which the estimation of biological parameters can be estimated) stock boundaries among stocks should be identified. While the microsatellites and morphometrics analyses were unable to identify any boundary between I and P stocks, the analysis of mtDNA suggested a boundary in the sector 150º-160ºE. The mean body length of physically matured whales suggested a possible stock separation around longitude 165°E, this is between sectors VW and VE. Although there is not a clear longitudinal 'cut', it is reasonable to conclude that the western part of Area V is more related to I-stock than P-stock. Therefore for the objective of the estimation of biological parameters a boundary between I and P stocks was adopted at the division between Areas VW and VE (165°E). This is consistent with the results of mark-recapture that showed marked movements of whales through 130ºE (division between Areas IV and V) suggesting that whales in Area IV are related to Area VW.

Regarding the western boundary of the I-stock the JARPA analyses based on mtDNA, morphometric and mean body length of physical maturity suggested no significant differences between whales in Areas IV and IIIE. For this reason it is assumed that whales in Area IIIE belong to the I-stock. It should be noted, however, that the microsatellite analysis showed some degree of genetic heterogeneity between these two Areas (SC/D06/J9). In addition one of the explanations for the heterogeneity observed for group IVWE in 1989/90 in a previous mtDNA analysis was the sporadic intrusion or mixing with a third stock presumably distributed in Area III. Also mark-recapture analysis suggested a boundary at 80ºE, near the boundary between Areas III and IV, which was consistent with the results of a pollutant study (Kunito *et al*., 2002) and parasite occurrence (Bushuev, 1990) suggesting differences between Areas III and IV (see review above). For the purpose of the estimation of biological parameters whales in Area IIIE are considered as part of the I stock given the results of JARPA shown in Table 2. However the possibility of additional stock structure in Area III, or the presence of a mixing sector around the division between Areas III and IV can not be discarded at this stage given the results of some analyses. For the estimation of the biological parameters it is suggested to consider longitude 145ºW as the eastern boundary of P stock. The extension of the P stock to the east should be investigated in the future.

Analyses based on pollutant accumulation (Kunito *et al*., 2002) and parasites load (Bushuev, load suggested differences between Areas III and IV. Interpretation of results from pollutant accumulation and occurrence of parasites is, however, difficult in the context of stock structure. With regard organochlorines Aguilar (1987) reviewed the methods of using these pollutants to discriminate marine mammal populations. He suggested that among other factors, nutritional state, sex, age, trophic level, distance of habitat from mainland and pollution source, excretion, metabolism and tissue composition should be identified and their effect ascertained before attempting any comparison between populations. Regarding the use of differences in the incidence of parasites for stock structure in whales Balbuena *et al*. (1995) noted that the application of the technique to marine mammals is hampered by the lack of control over sampling conditions and the paucity of information about the biology of their parasites.

Distribution of Antarctic minke whales is related to distribution of their main prey species in the Antarctic, the krill (*Euphausia superba*). The distribution of Antarctic krill concentrations has been related to bottom topography, sea-ice and hydrographic features (Ichii, 1990). Yearly variability in these features in the sector around 165°E could have some effect on annual fluctuation on the distribution of krill concentrations and this, in turn, could affect the distribution of minke whale around that longitudinal sector. Therefore a boundary between stocks or equivalently a longitudinal sector of mixing between two stocks is expected to change according changes in the distribution of krill (see discussion of Document SC/D06/J9).

Results of the different analyses are not consistent with the current boundaries of the IWC Areas. These results allows for a response to the questions formulated by Hoelzel and Dover (1989): Area IV is occupied

mainly by a single stock (I) while Area V is occupied by two different stocks (I and P). Only a partial portion of Area III has been investigated by JARPA. While results of JARPA suggested that Area III is occupied mainly by the I stock, we can not discard the possibility of additional stock structure in that Area (e.g. the intrusion and mixing with animals from the western Indian Ocean breeding ground).

The results on stock structure will inform the specifications and testing of appropriate management areas under the Revised Management Procedure.

Further research needs
The following topics are recommended for future studies on stock structure:

a) Yearly changes in stocks distribution and boundaries (or changes in the proportion of stocks in areas of mixing) in the region 150ºE-180ºshould be investigated in the future in relation to bottom topography, currents and krill distribution.
b) Western boundary (or areas of mixing) of the stock originating in the eastern Indian Ocean and the eastern boundary (or areas of mixing) of the stock originating in the western Pacific Ocean should be investigated in the future.
c) To elucidate the possibility of mixing in the feeding ground at a better resolution, samples and genetic analysis of whales from the breeding grounds are desirable. Experiments of satellite tracking would be very useful to examine lateral movement and possible mixing in the feeding grounds as well movement between feeding grounds and lower latitudes wintering grounds.
d) 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.

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Table 1: Summary of the main genetic analyses on stock structure in the Antarctic minke whale. CPW=commercial pelagic whaling; CCW=commercial coastal whaling; HW=Hardy-Weinberg; RE= restriction enzyme; SE= length of sequence segment. A sign '=' means that the statistical test found no significant heterogeneity.

Marker/ Source	Locality	Period of Sampling	n	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 CCW	45 loci (10 polymorp.)	H-W test Homogeneity test (G-test)	Areas I=II=III=IV=V=VI Areas I-VI=BRAZIL
RFLP mtDNA Control Region							
Hoelzel and Dover (1991)	Area IV Area V		20 21	CPW CPW	3 restriction enzymes	Genetic distance	Area IV=Area V
RFLP Whole mtDNA							
Wada *et al.* (1991)	Area IV Area V	1983/84 1983/84	40 39	CPW CPW	14 restriction enzymes (9 polym)	Homogeneity test (G-test)	Area IV=Area V
Pastene *et al.* (1993)	70˚-110˚E (W) 110˚-150E (C) 150˚E-180˚ (E)	1989/90 1989/90,90/91 1990/91,88/89	118 93 107	JARPA	12 restriction enzymes (11polym)	Homogeneity test (Randomized chi-square test)	W=C C=E W≠E

Pastene *et al* .(1996)	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 Early (VWL) 160˚E-160˚W Early (VEE) 160˚E-160˚W Late (VEL)	1987/88-1994/95	160 383 233 321 208 264 76 479	JARPA	6 polymorphic restriction enzymes	Homogeneity test (AMOVA / PHI-st)	Significant heterogeneityin Group IVWE
Pastene *et al*. (this meeting)	35˚-70˚E (IIIE) 70˚-100˚E (IVWN) 70˚-100˚E (IVWS) 100˚-130˚E (IVE) 130˚-165˚E (VW) 165˚E-170˚W (VEN) 165˚E-170˚W (VES) 170˚W-145˚W (VIW)	1987/88-2004/05	529 462 959 1234 1094 712 790 476	JARPA	6 polymorphic restriction enzymes	Homogeneity test (AMOVA / PHI-st, Randomized chi-Square test)	IIE=IVW=IVE VIW=VE IIIE,IVW,IVE ≠VE,VIW Possible boundary in the sector 150˚-160˚E

Sequencing mtDNA Control Region							
Bakke *et al.* (1996)	Area IV Area V	1991/92 1990/91	13 10	JARPA JARPA	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 CPW	10 gene families		Area IV≠ Area V using clone cl.5M1-19
Multilocus minisatellite							
van Pijlen *et al.* (1991)	Area IV Area V			CPW CPW	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 CPW	3 loci	H-W test Homogeneity test clustering analysis	Area IV=Area V
Microsatell							
van Pijlen *et al.* (1992)	Area IV Area V	1982/83 1982/83	19 61	CPW CPW	4 loci	H-W test Homogeneity test clustering analysis	Departure from H-W equilibrium in one locus Area IV=Area V
Pastene *et al*. (this meeting)	35°-70°E (IIIE) 70°-100°E (IVWN) 70°-100°E (IVWS) 100°-130°E (IVE) 130°-165°E (VW) 165°E-170°W (VEN) 165°E-170°W (VES) 170°W-145°W (VIW)	1989/90-2004/05	549 484 1022 1084 1164 675 784 498	JARPA	6 loci	H-W test Homogeneity test	IVWN,IVWS ≠VE,VIW Some degree of heterogeneity between Areas IVW and IIIE (females)

Table 2: Summary of the results on stock structure in JARPA. MBLM= Mean body length of physically matured whales. A sign '=' means that the statistical test found no significant heterogeneity.

Method	Sex	JARPA samples	Pattern of Geographical variation
mtDNA	F+M	1987/88-2004/05	IIIE=IVW=IVE VIW=VE IIIE,IVW,IVE≠VE,VIW Possible boundary in the sector 150°-160°E
Microsatellites	F	1989/90-2003/04	IVWN,IVWS ≠VE,VIW Some degree of heterogeneity between Areas IVW and IIIE
	M	1989/90-2004/05	No significant heterogeneity
	F+M	1989/90-2004/05	IVW≠ VE
MBLM	F	1987/88-2004/05	IIIE=IVW=IVE=VW VIW=VE IIIE,IVW,IVE,VW≠VE,VIW
	M	1987/88-2004/05	VEN≠VES IIIE=IVW=IVE=VW VIW=VEN,VES IIIE,IVW,IVE,VW≠VEN,VES,VIW
Morphometrics	F	1987/88-2004/05	IIIE=IVW=IVE=VW VIW=VE IIIE,IVW, IVE, VW≠VE,VIW
	M	1987/88-2004/05	IIIE=IVW=IVE VIW=VE IIIE,IVW, IVE≠VW,VE,VIW

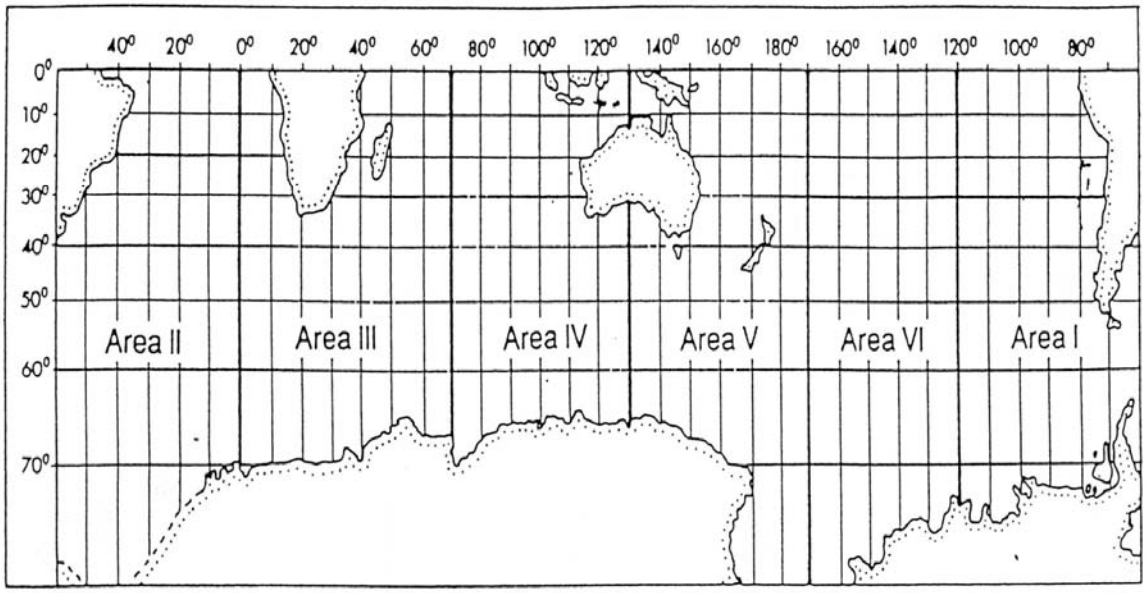

Figure 1: IWC Antarctic Areas for the management of baleen whale species (except Bryde's whale).

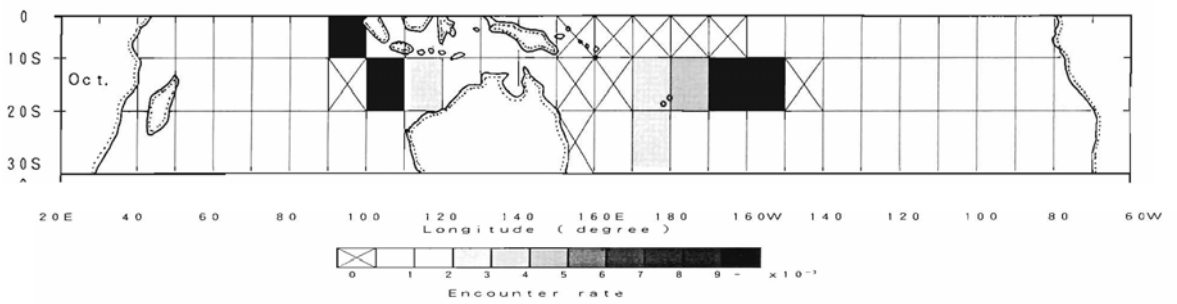

Figure 2: Encounter rates of Antarctic minke whales in 10º squares of latitude and longitude in waters 0º-30ºS in October (from Kasamatsu *et al*., 1995).

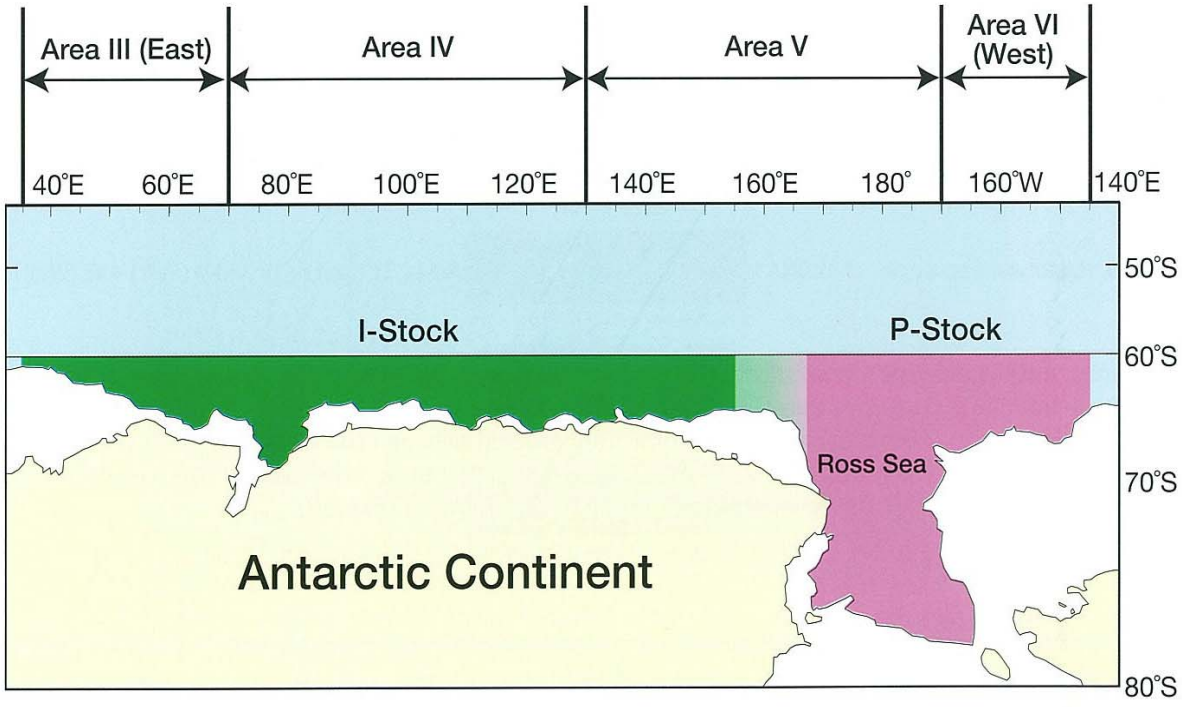

Figure 3: Hypothesis on stock structure in the Antarctic minke whale in the JARPA research area. The most parsimonious explanation of the results is that there are at least two stocks present in the research area: an eastern Indian (I) and a western South Pacific (P) stock. These stocks would mix across a soft boundary, which would probably best be placed near 165ºE. These stocks could be related to the breeding areas proposed for the eastern Indian Ocean and western South Pacific (Figure 2) (see text for details).