# A Review of the Studies on Stock/Species Identity in the Minke and other Baleen Whale Species, conducted under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA)

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## ABSTRACT

We examined the studies and information on species/stock identity in the minke and other baleen whale species conducted under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA). Studies conducted on the minke whale have been focused to investigate a) the phylogenetic relationships between ordinary and dwarf forms minke whale and b) the stocks structure in the ordinary form. The dwarf form was reported for Antarctic waters by the earlier JARPA surveys. Mitochondrial DNA (mtDNA) analyses revealed that the two southern minke whale forms differ at least at the sub-species level. On this basis, the IWC Scientific Committee had recognised the existence of these forms and agreed that dwarf and ordinary forms should definitely considered separately for management purposes. Studies on stock identity in the ordinary form have been based largely in mtDNA RFLP analyses and in a lesser extension in morphometric analyses, at this moment. Results of the genetic approach has revealed considerable mtDNA heterogeneity, but little geographic concordance with IWC Areas IV and V. The results are consistent with the hypothesis that at least two genetic stocks are found in these Areas, a 'western stock' in the western part of Area IV and a 'core stock' in Area V and eastern part of Area IV, with a temporal component to their distribution in the western part of Area IV. We discuss the possibility that this putative 'core stock' can be used as a stock unit for the final estimation of biological parameters at the end of the entire program (a JARPA main research objective) and for the future implementation of the RMP's multi-stocks rules. Several additional analyses are suggested in order to investigate the utility of the 'core stock' for such management purposes. It is noted that the JARPA surveys in Areas IV and V have made possible the attainment of skin biopsy samples and photo-identification data from other baleen whale species such as the humpback, blue and right whales and that these materials are being used in studies on species/stock identity in these species.

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## 1-INTRODUCTION

The JARPA began with two feasibility surveys in management Areas IV and V, respectively (Kato *et al.* 1989; 1990). Since the austral summer season of 1989/90 Japan implemented full-scale research surveys in these areas. Until now, four have been conducted in Area IV (Fujise *et al.*, 1990; 1993a; Nishiwaki *et al.*, 1994; 1996) and three in Area V (Kasamatsu *et al.*, 1993; Fujise *et al.*, 1993b; Nishiwaki *et al.*, 1995).

The JARPA has a main lethal research component involving the random sampling of minke whale (*Balaenoptera acutorostrata)*. This research component deal with the main JARPA research objectives, e.g. "the estimation of the biological parameters required for the stock management of the Southern Hemisphere minke whale" and "elucidation of the role of whales in the Antarctic ecosystem" (Government of Japan, 1987). Although the species/stocks identity of the minke whale in Areas IV and V was not an emphasised research objective of the JARPA in the beginning, samples and data were obtained systematically from the start of the program in order to conduct studies on stock identity under a multi-approaches perspective. From each minke whale caught, skin/blubber, muscle, liver, kidney and heart tissues have been sampled for genetic analyses. Photographs of some parts of the body (dorsal side, dorsal fin and pectoral fin) and several external measurements have been obtained in order to investigate variation in external characters. With the progress of the investigations under the JARPA, the importance of the stock identity in the minke whale became evident.

Apart of the main lethal research component of the program, the JARPA also has a non-lethal component involving sighting and oceanographic surveys, skin biopsy sampling and experiments on photo-identification (photo-ID). Skin biopsy sampling and photo-ID experiments have been conducted on three baleen whale species: the humpback whale (*Megaptera novaeangliae*), the blue whale (*Balaenoptera musculus*) and the right whale (*Eubalaena australis*). These materials are being used to investigate species/stock identity in these species.

Hoelzel and Dover (1989) defined three categories of stocks: 'dynamic stock' is the fundamental unit described by a population model or assessment procedure, 'management stock' is the group of whales occurring within a specific geographical boundary which is actively or potentially exploited (individual stocks whose status is assessed by the International Whaling Commission Scientific Committee, SC) and 'genetic stock' is a genetically differentiated population within a species. Ideally the minke whale, as well as any other species, should be managed on the basis of genetically identified stocks and the studies on stock identity under the JARPA are designed to fulfill that aim.

It was pointed out the importance of the identification of stocks boundaries (geographical and temporal) for management schemes of both exploited and protected species. For exploited species this identification is necessary to properly estimate abundance, catch limits, and interpret catch statistics and life-history parameters. For protected species this identification is important for assessing population changes, establishing territorial jurisdiction and identifying critical habitats (Baker *et al.*, 1994).

This report presents a review of the studies on species/stock identity in the minke whale, conducted under the JARPA in Areas IV and V. Also we briefly summarise some information

on ongoing studies on stock/species identity conducted on the other baleen whale species mentioned above.

## 2- STOCK/SPECIES IDENTITY IN THE MINKE WHALE

### 2.1 Taxonomy and phylogenetic relationships

#### 2.1.1 Background

The first documented study that demonstrated marked morphological differences between 'diminutive' or 'dwarf' and ordinary forms minke whale was based on samples from South Africa (Best, 1985). Subsequently, Arnold *et al.* (1987) presented evidence for morphological differentiation along the Australian coast. Those authors described several morphological differences between the dwarf and ordinary forms of which the most distinctive concerned the colour of the body and baleen plates and the shape of the skull. The dwarf form has a predominantly light baleen series and a white base to the flippers, whilst the ordinary form has asymmetrically coloured baleen plates. Although the flippers of the ordinary form may have one or two tones of gray, they are never white at the base, as in the dwarf form. In the dwarf form, the dark pigmentation in the neck region extends onto the ventral grooves whereas in the ordinary form it does not occur below eye level. The dwarf form also has anteriorly convex nasal bones as opposed to the concave nasal bones in the ordinary form. In most of these morphological characteristics the dwarf form more closely resemble Northern Hemisphere minke whales.

The first genetic study involving the dwarf form was carried out by Wada (1983) who analysed a single sample from a South Africa animal that presented similar morphological characteristics to the dwarf form. His allozyme survey, however, found no significant differences between this sample and those from the Antarctic ordinary form. All the subsequent genetic analyses on the dwarf form have involved samples taken in the Antarctic by the JARPA.

The dwarf form had been identified from the Brazilian minke whale catches (da Rocha and Braga, 1982). Baker (1983) identified and illustrated this form among minke whales stranded on the New Zealand coast. Recently Zerbini *et al.* (1996) reviewed the records on dwarf minke whale in Brazil, and their results suggest that this form could be relatively common in that country. Prior to the JARPA in the Antarctic (1987/88) the southern dwarf form was only believed to be found between 7-41°S (Best, 1985). The dwarf form minke whale was reported for Antarctic waters for the first time, on the basis of catches of this form conducted during earlier JARPA surveys (Kato *et al.*, 1989; 1990; Fujise *et al.*, 1990;1993b; Kasamatsu *et al.*, 1993). Materials from those catches have been used in several genetic studies and comparative morphological and morphometric analyses between forms are underway.

#### 2.1.2 Genetics

Associated information of dwarf forms animals taken during the JARPA surveys is given in Table 1. Fig. 1 shows the distribution of their catches in the Antarctic and records of stranding and sightings of this form in lower latitudes. Each of the individuals sampled during JARPA surveys were identified as of the dwarf form by biologists on board of the research vessels (Kato *et al.*, 1989;1990; Fujise *et al.*, 1990;1993b; Kasamatsu, 1993). Most of the catches occurred between 55-62°S. One individual was caught at 65°S.

Wada *et al.* (1991) examined genetic diversity in minke whale samples from the North Pacific and the Antarctic using a restriction fragment length polymorphism (RFLP) analysis of the whole mtDNA genome. The Antarctic sample included a single individual of the dwarf form, which had been sampled during the first JARPA feasibility study in Area IV in the austral summer 1987/88 (Kato *et al.*, 1989). They digested mtDNAs with 14 six-base sequence recognition restriction enzymes and, based on the composite digestion patterns of a total 142 minke whales, 19 haplotypes were resolved. There was no shared haplotype among the three forms examined (North Pacific and Antarctic ordinary and dwarf forms). Furthermore an UPGMA-derived dendrogram based on genetic distances among these forms, showed that the dwarf form and minke whales from the North Pacific are more similar to each other than they are to the southern ordinary form.

It should be noted that the genetic studies of Wada (1983) and Wada *et al.* (1991) were based on only a single dwarf form individual and generated contradictory results. The allozyme survey found no significant difference between the two forms, whereas the mtDNA analysis found substantial genetic differences. Using a larger number of samples of the dwarf form, Pastene *et al.* (1994a) examined further the differentiation of mtDNA between southern ordinary and dwarf forms. These authors conducted a RFLP analysis of the whole mtDNA molecule using 11 samples of the dwarf form and 18 of the ordinary form taken during JARPA surveys. MtDNAs extracted from liver samples were digested with 11 six-base restriction enzymes revealing a total of thirteen mtDNA haplotypes in the total sample. There were no shared haplotypes between forms. Three haplotypes were found in the dwarf form while the remainder were found in the ordinary form. The larger number of samples of the dwarf form enabled the degree of genetic variation within this form to be investigated. The index of nucleotide diversity (Nei and Li, 1979) was higher in the ordinary form than in the dwarf form The degree of genetic variation within each of these forms is similar or lower to that of other cetaceans (Pastene *et al.*, 1994a). The net genetic distance between forms was estimated at 0.0524. A comparison with published information for other species revealed that the genetic distance between the dwarf and ordinary forms is larger than that for some recognised species of land and marine mammals. Using RFLP data from Wada *et al.* (1991) for the North Pacific minke whale, Pastene *et al.* (1994a) studied the genetic relationships among the three forms (North Pacific and Antarctic ordinary and dwarf forms). There was no shared haplotypes among the three forms and an UPGMA-derived dendrogram of mtDNA haplotypes based on genetic distances (Fig. 2), showed that the dwarf form and the North Pacific minke whale are more similar to each other than they are to the Antarctic ordinary form. Then the work by Pastene *et al.* (1994a), which was based on a larger number of samples of the dwarf form, supported the previous finding of Wada *et al.* (1991).

Another genetic study involving the dwarf form minke whale was that conducted by Hori *et al.* (1994), which was extended by Pastene *et al.* (1996c). These authors investigated the phylogenetic relationships of this form with minke whales from the North Pacific, North Atlantic and Antarctic ordinary form. Samples included minke whale from the North Pacific (n=9), Antarctic dwarf form (n=15) and Antarctic ordinary form (n=20). Antarctic dwarf and ordinary minke form samples were taken during JARPA surveys. In the analysis, published sequence data of the North Atlantic minke whale (n=87) and southern ordinary minke whale (n=1) were used. The method used was the amplification by the polymerase chain reaction (PCR) of the non-coding control region of mtDNA followed by sequencing of 343 base-pairs.

A neighbor-joining-based phylogenetic tree (Saitou and Nei, 1987) of 56 unique sequences (Fig. 3) showed that dwarf and ordinary forms of the Southern Hemisphere, North Pacific and North Atlantic minke whales are separated from each other, suggesting independent genetic populations. The tree also showed that dwarf minke whales are more closely related to Northern Hemisphere minke whales than to the ordinary form of the Southern Hemisphere, and are more closely related to North Atlantic minke whales than to North Pacific minke whales. The dwarf form minke whale is clearly remote from the ordinary form despite the overlap in their distribution and apparent ecological niche.

Table 2 shows the nucleotide diversity and the net genetic distances among minke whale forms based on the Kimura's two parameters method (Kimura, 1980). The larger inter forms distances are obtained in the pairwise comparisons involving the southern ordinary form. These values range from 0.082 to 0.088. The values among the other three forms range from 0.015 (comparison between Antarctic dwarf and North Atlantic minke) and 0.025 (comparison between Antarctic dwarf and North Pacific minke).

**2.1.3 Morphology and morphometry**

Until now there is no documented information on comparative morphological and morphometric analyses among dwarf and ordinary forms minke whale using material from the JARPA. For each dwarf individual caught several external measurements and visual observations have been recorded by biologists on board of the research vessels (as in the case of the ordinary forms sampled), and each individual has been photographed for external morphological analyses (Kato *et al.*, 1989; 1990; Fujise *et al.*, 1990; 1993b; Kasamatsu *et al.*, 1993). A study is underway (Kato and Fujise, in prep) involving the analyses of morphology and morphometric data collected on the dwarf form. Data being analysed are body colouration, morphometric and skeleton measurements. Results of these analyses will be available at some later stage (Fujise, pers. comm.).

**2.1.4 Conclusions and management implications**

Previous to the JARPA, genetic studies using allozyme (Wada and Numachi, 1991) and DNA (Hoelzel and Dover, 1991; Amos and Dover, 1991; vanPijlen *et al.*, 1991) had shown striking differences between Northern Hemisphere and southern ordinary form minke whales. The genetic studies under the JARPA further showed that dwarf and southern ordinary form minke whale are genetically independent entities and that they are separated from both North Pacific and North Atlantic minke whales.

The degree of mtDNA differences between both southern minke whale forms are large and similar to that found between southern ordinary form and Northern Hemisphere minke whales. Phylogenetic analyses showed that the southern dwarf form minke whale is more closely related to Northern Hemisphere minke whales than it is to the southern ordinary form minke whale. This is in agreement with the results found by Arnold *et al.* (1987) who examined morphological and morphometric characters.

Some authors (Omura, 1975; Rice, 1977) had proposed sub-specific status for the North Atlantic (*Balaenoptera acutorostrata acutorostrata*), North Pacific (*B. a. davidsoni*) and Antarctic ordinary form (*B. a. bonaerensis*). Given the large genetic distances between the southern ordinary form and each of the other geographical/morphological forms, the Antarctic ordinary form should be given full species status *Balaenoptera bonaerensis* as proposed

recently by Arnason *et al.* (1993). However, the morphological and genetic resemblance between the dwarf form and the Northern Hemisphere minke whales suggest that the taxonomic status of the dwarf form should be examined further as part of a comprehensive study of minke whale taxonomy incorporating morphological and biochemical characters of animals from all oceans. Here, the ongoing morphological and morphometric analyses using JARPA dwarf samples can make an important contribution.

On the basis on the morphological differences documented by Best (1985) and Arnold *et al.* (1987) and genetic differences (Wada *et al.*, 1991), the SC recognised the existence of two morphological forms of southern minke whales and agreed that the two forms in the Southern Hemisphere should definitely be considered separately for management purposes (IWC, 1991). In 1993, after examining the information given by Pastene *et al.* (1994a), the SC recommended the inclusion of the dwarf or diminutive form of minke whale in the Schedule, so that catch limits for Antarctic minke whales recognise the distinction between the two forms (IWC, 1994).

Recognising the striking genetic differences between both forms, by which dwarf and ordinary forms could be separated at least at the sub-species level, the Government of Japan suspended the catches of the dwarf form in the Antarctic from the JARPA survey of the 1993/94 season (Government of Japan, 1993).

As mentioned earlier, the Committee had recommended the management of the two forms separately. This would have several practical implications for population assessment studies and possible future commercial catches of minke whales in the Antarctic, due to the difficulty of distinguish both forms at sea. According to the records of the JARPA, the sampling locations of the dwarf and ordinary forms overlap between 55°S-65°S. Although dwarf form individuals are generally smaller, this alone is not a sufficient criterion as juvenile ordinary form individuals are also found in the Antarctic. However, field observations by experienced crew members involved in the JARPA surveys in the Antarctic indicate that there are distinct differences that can be detected at sea, e.g. body colouration, the white patch on the base of flipper and the swimming pattern. Some of these probably require good weather and observation conditions to be identified. Since it was determined to halt the catches of dwarf form minke whales in 1993/94, effectively no individuals of this form has been taken, showing that both forms can be recognised at the field by experienced researchers.

### 2.2 Stock identity in the southern ordinary form minke whale

#### 2.2.1 Background

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).

Only one breeding ground has been identified in Brazil based on Discovery mark recovery analysis (Buckland and Duff, 1989). Of interest for the stock identity studies of JARPA in

Antarctic Areas IV and V, however, is the information on location of possible breeding grounds in the Indian Ocean and in the western South Pacific. 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 suggesting that not all individuals migrate to waters south of the Antarctic Convergence in summer (Kasuya and Wada, 1991). 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.

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. 4). 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). The JARPA surveys have been conducted in two of these Areas, Area IV (70°E-130°E) and Area V (130°E-170°W), under the assumption that whales found in these two distinct Areas are from two different genetic stocks. 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. Detailed reviews of studies on stock identity in the Southern Hemisphere minke whale have been conducted by Horwood (1990) and Best (1990).

Before going into a review of the studies on stock identity in the minke whale conducted under the JARPA, we summarise the previous information on this topic, based mainly in the review conducted by the SC during the comprehensive assessment of this species in 1990 (IWC, 1991). During the comprehensive assessment, information on genetic, morphology, Discovery-marks, ecological markers, sighting pattern distribution and catches distribution was reviewed in order to formulate a range of feasible hypotheses about the organization of the minke whale population in the Southern Hemisphere.

*Genetics*

An allozyme study conducted by Wada and Numachi (1991) examined a total of 11,414 samples from the six Antarctic management Areas and they found no significant differences among them. Whales from Areas IV and V were compared using several other biochemical genetic techniques. Hoelzel and Dover (1991) analysed variation in the control region of the mtDNA, vanPijlen *et al.* (1991) used multilocus DNA fingerprinting and Amos and Dover (1991) used satellite DNA sequences. The only significant differences between these two Areas were found by the latter authors. They found significant differences in frequencies

although no characteristic variant of an Area was found. They concluded that there is only small differences between Areas and that the observed variability was spread between them.

In 1992 the SC examined a genetic study by vanPijlen *et al.* (1992) who used single locus mini and microsatellites to compare minke whales from Areas IV and V, using samples from past commercial whaling operations. These 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.

None of the molecular genetic techniques reviewed in 1990 provided any evidence of unambiguous genetic differences between minke whales in Areas IV and V and 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 most of the genetic works were based on very small sample sizes. 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. Finally it should be mentioned 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 analyses*

The SC then examined the information on morphology based in analyses conducted by Bushuev (1990) and reviewed by Best (1990). The author had examined 34 non-meristic characteristics, ten meristic and 2 linear features of minke whales. A total of 6,646 whales were examined from Areas I, II, III and IV. These analyses failed to identify any isolated populations on the Antarctic.

*Discovery mark analysis*

Information on the recovery of 94 Discovery marks from minke whales in the Southern Hemisphere was reviewed on the basis of a paper presented 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 finally 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.

In 1992 the SC examined a study by Kato *et al.* (1993) who examined all available marks (2,864 mark release and 110 recoveries of Discovery tag) at the Antarctic and recognised 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.

*Ecological markers*

On this topic, the study by Bushuev (1990) was reviewed. This author studied the ectoparasite occurrence in minke whales from Antarctic Areas I, III and IV and found significant differences in infestation rate of the warm-water parasite *Xenobalanus globicipites* among these three areas. These differences suggested that whales in Antarctic Area I, III and IV come from different wintering grounds.

*Pattern of distribution from sightings*

An original version of the study by Kasamatsu *et al.* (1995) was reviewed by the SC in 1990. These authors 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 south of 35°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. 5). Also reviewed in 1990 was a document by Kasamatsu *et al.* (1990) in which data from the IWC/IDCR minke whale cruises made from 1978/79, were summarised. 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.

*Patterns of catches distribution*

The SC examined information on this topic analysed by van Beek (1983) and Best (1990). 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.

In summary all the genetic and non-genetic approaches reviewed by the SC in 1990 failed to identify 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. 5).

Under the JARPA different approaches, genetic and non-genetics, are being used to investigate stock identity in the minke whale. In the next paragraphs we reviewed the information and results of analyses obtained using these different approaches.

**2.2.2 Genetics**

The genetic studies on stock identity under the JARPA have been based on analyses of the maternal-inherited mtDNA. This small, closed circular molecule has an evolutionary rate as much as ten times faster than nuclear DNA (Brown *et al.*, 1979). This should allow greater resolution of genetic differences between conspecifics and closely related species using mtDNA analysis than was possible with protein electrophoresis. Mitochondrial DNA analyses based on JARPA samples have been presented in different documents (Pastene *et al.*,1993a;1993b;1994b;1996b;1996d), which have been examined at SC meetings. Here we summarise those analyses conducted on minke whales from Areas IV and V.

The first genetic study using JARPA samples was conducted by Pastene *et al.* (1993a). In this study priority was given to develop a rapid and inexpensive method for analysing mtDNA with restriction enzymes, from a large number of samples obtained during the JARPA surveys. A 'mini-prep' procedure was reported by those authors. In the same study, an RFLP analysis of mtDNA using a total sample of 318 minke whales (from seasons 1988/89 and 1990/91 in Area V and 1989/90 in Area IV), was carried out. Crude mtDNAs were digested with 13 six-base restriction enzymes. All of them showed polymorphism, apart one. 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 clearly 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 mixing of them in the central stratum.

The genetic information given by Pastene *et al.* (1993a) and van Pijlen *et al.* (1992) and that on movement of minke whales in the feeding ground based on mark-recovery analysis given by Kato *et al.* (1993), was examined during the 1992 SC meeting. The SC noted that 'none of these works contradicted the broad conclusion reached in the 1990 comprehensive assessment based on an examination of feeding concentrations, movement indicated by mark recoveries

and knowledge of likely breeding grounds, which suggested that biological stocks typically spanned 60° (IWC, 1993). The SC recommended that biopsy sampling be carried out on breeding grounds, to allow further investigation of the discreteness of Southern Hemisphere minke whale stocks and also suggested that in addition to mtDNA analyses, isozyme analyses should be undertaken to provide more information on stock separation (IWC, 1993).

Pastene *et al.* (1993b) used JARPA samples from Area IV taken in two different austral summer seasons (= season): 1989/90 (n= 306) and 1991/92 (165), in a new RFLP analysis of mtDNA. Crude mtDNAs was digested with six of the 13 restriction enzymes used in the previous study. 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 (whales sampled in the western sector of Area IV from December to 15 January), Area IV western late (whales sampled in the western sector of Area IV from 16 January to March) and Area IV eastern early (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.* (1994b). These authors carried out a mtDNA analysis on 1,257 minke whales from Areas IV and V. 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 IVW in the early part of the season 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 VE late in the season 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.* (1996b) conducted a new analysis of mtDNA variation in Areas IV and V, this time involving a total of 2,124 minke whales. 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. The number of samples used in that analysis, by stratum and season is shown in Table 3 and the geographical distribution of the area/time strata for all seasons combined is shown in Fig. 6. 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 IV western early stratum were larger than all the other pairwise comparisons and all of them showed P values below 0.01 or 0.05 (Table 4). The PHIst value obtained between stratum IV western early 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. 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.

These results suggested a small but real distinction between at least two stocks and a temporal component to their distribution. It should be noted that the analysis by Pastene *et al.* (1996b) was conducted by pooling the samples from several seasons under the assumptions that the pattern of seasonal movement of a given breeding stock is the same in different years and that lateral movement in the feeding grounds and patterns of mixing are similar between years. The authors discussed these assumptions and on the basis on some preliminary analyses, suggested that yearly variation may occur in Area IV.

The SC revised these information during the 1995 meeting and 'noted that genetic sampling was needed from the putative breeding grounds to assist interpretation of mtDNA variation in the Antarctic'. The Committee recommended that 'special efforts should be made to obtain biopsies (or tissues from stranded animals) in other low latitudes areas, including investigation of material in museum and elsewhere' (IWC, 1996).

Apart from mtDNA analyses no other genetic approach has been used for investigating stock identity using samples from the JARPA. Different internal tissues has been collected

systematically from 1987/88 to 1995/96 for isozyme analysis and studies using this approach are being planned.

### 2.2.3 Morphology and morphometry

As mentioned earlier, from each whale sampled during the JARPA surveys, photographs of some parts of the body (dorsal side, dorsal fin and pectoral fin) and several external measurements have been obtained in order to investigate variation in external characters. Fujise (1995) conducted a preliminary study on morphometry of minke whales from Area IV sampled in the 1989/90 season. 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.*, 1994b) 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.*, 1994b: 1996b), the author could not exclude the possibility that some of the apparent differences were due to seasonal changes in body fatness. Further studies in this area are expected.

### 2.2.4 Recovery of Discovery marks

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°57S, 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).

### 2.2.5 Other approaches

Genetic and morphology/morphometric analyses have been the traditional and most used approaches to study stock identity in the past, as well for analysing samples from JARPA. As noted by Donovan (1991), apart these traditional approaches, there are also another promising

approaches which could be used in stock identity studies. Among them pollutant burden and ecological markers are identified.

**2.2.5.1 Pollutant burden**

Fujise *et al.* (1997) examined the level of accumulation of Hg in liver samples of southern minke whales. They used samples 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 the age groups examined.

**2.2.5.2 Ecological markers (parasites)**

In the only report on parasites using JARPA data, Sedlak-Weinstein (1990) summarised 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.

**2.2.5.3 Conception date**

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.

**2.2.6 Conclusions and management implications**

The current status on the studies on stock identity in the southern ordinary form minke whale carried out under the JARPA, has been based largely in genetic and in a lesser extension in morphometric analyses. Other promising approaches are being investigated and their application is being considered.

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 genetic survey has revealed considerable mtDNA heterogeneity, but little geographic concordance with management Areas IV and V. The most extensive and recent mtDNA analysis (Pastene *et al.*, 1996b) showed considerable mtDNA heterogeneity in minke whales distributed in Area IV, particularly in the western sector of this area. A significant source of mtDNA heterogeneity is attributable to a group of whales in the western part of Area IV migrating early in the season. This result is supported by that derived from morphometric analysis that used samples of Area IV of the 1989/90 season.

On the basis of these results, Pastene *et al.* (1996b) proposed the occurrence of at least two

genetic stocks in Antarctic Areas IV and V: a 'core stock' in Area V, eastern part of Area IV and western part of Area IV late in the season (January-March), and a 'western stock' in the western part of Area IV early in the season (December-January). Then these authors suggested a temporal component to the distribution of stocks in the western part of Area IV. Of importance for management purposes is to investigate whether the putative 'core stock' could be used as a stock unit for both a) the estimation of biological parameters, which is a main objective of the JARPA and b) the application of the multi-stocks rules of the RMP.

*Estimation of biological parameters*

One of the main objectives of the JARPA is 'the estimation of biological parameters required for the stock management of the Southern Hemisphere minke whale' (Government of Japan, 1987). Such estimations are being conducted on the basis of the IWC management Areas IV and V on the assumption that each Area is occupied by different genetic stocks. The accuracy of the estimation of parameters such as the natural mortality, however, has faced a challenging issue to solve. In the discussion conducted during the 1994 SC meeting on the preliminary results of the estimations of this parameter, it was noted that the accuracy in the estimation stem largely from stock identity questions and seasonal variations in the migration patterns for different age groups (IWC, 1995). In other words it is possible that the survey is not covering all the range of distribution (geographical or temporal) of a genetic stock. According to the observed pattern of variation of mtDNA, it is possible that some whales from a genetic stock occurring in Area V ('core stock') distribute in Area IV displaying geographical and temporal interactions with other stock occurring in that Area ('western stock'). Thus, it is important to define the areas and periods (within a season) in which the 'core stock' is present. Then on the basis of the geographical and temporal distribution of the 'core stock', the estimation of biological parameters could be carried out.

*Application of the multi-stocks rules of the RMP*

Under the RMP, accepted by the IWC in 1994, catch limits are set on a 'small area' basis to allow for the risk associated with uncertainty about stock identity. In the case of the Southern Hemisphere minke whale the SC agreed that 10° longitude sector would the 'small areas' specified in the RMP's multi-stock rules. Catch limits are calculated by applying the Catch Limit Algorithm (CLA) at the 'small area' level, but these limits may, where appropriate, be modified by the processes of 'catch-cascading' or 'catch-capping', which involve the consideration of these 'small areas' grouped together as well separately. Obviously the implementation of these rules will depend on the availability of new biological evidence on stock identity. For example if the hypothesised 'core stock' is confirmed to be a isolated genetic stock, then in that case, it would be appropriate to combine 10-degree longitude 'small areas' into the actual range of distribution of this 'stock'.

Then, it is important to determine whether the 'core stock' can be used as a stock unit for such purposes. We consider that, at this stage, this is premature. According to the observed temporal pattern of mtDNA variation, it seems that the stocks involved in Areas IV and V could either a) mix with each other as the feeding season progresses or b) occupy different longitudinal sectors in different periods of the feeding season. It is necessary to determine which of those possibilities is the correct. In case of the mixing, areas, periods within a season and mix proportions should be investigated. In case of the second possibility, areas and periods of distribution (within a season) of the 'core stock' should be investigated. The possibility of mixing can not be excluded but it can not be checked with the actual data since

no genetic markers had ever been identified for the different strata examined in the feeding ground. With this regard the SC had recommended the genetic sampling in lower latitudes of the Southern Hemisphere in order to assist the interpretation of the mtDNA variation observed in the Antarctic.

One question to be addressed is whether the intra-seasonal patterns of spatial and temporal mtDNA variation observed in Areas IV and V is the same every season. Pastene *et al.* (1996b) discussed this topic and on the basis of some preliminary analyses they suggested that yearly variation may occur in Area IV. If this is corroborated, then the pattern of distribution of the stocks in this Area may change both intra and interseasonally. This require further investigation.

The hypothesis established by Pastene *et al.* (1996b) assume that the 'core' stock is composed of a single stock. This assumption is based on the fact that, apart from group IVWE, no other significant source of temporal and geographical mtDNA heterogeneity had been found. The lack of significant differences between samples, however, could mean either that there is no population segregation or that there was inadequate power in the analysis to detect segregation. Low power can be the result of low resolution due to the small portion of the genome examined or of small sample size. A simulation exercise conducted by Pastene *et al.* (1996d) 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. In the genetic analysis by Pastene *et al.* (1996b) most of the statistical test were based in sample sizes over 160 individuals. The only exception was the group Area VE early where only 76 whales were used and thus genetic differentiation might be missed here due to the small sample size. This sample size become a critical factor when the samples are grouped by season (year). The application of other more sensitive genetic markers in combination with morphometric and other non-genetic analyses are suggested to investigate further whether the 'core stock' is a single genetic entity or a complex of more than one stock.

## 3- INFORMATION ON STUDIES ON OTHER BALEEN WHALE SPECIES

As pointed out earlier, the JARPA has a non-lethal research component involving, among other activities, the attainment of biopsies and photographs of natural markings on the humpback, blue and right whales. Both activities are conducted along the sighting surveys. Skin biopsy samples have been collected using a biopsy dart shooting gun described by Kasamatsu *et al.* (1991).

With regard the humpback whale, there are a total of 47 biopsy samples collected in Areas III, IV and V between the 1993/94 and 1995/96 JARPA surveys (Nishiwaki *et al.*, 1994; 1995; 1996). Some of these samples were used in a genetic study based on mtDNA that compared whales from Antarctic Areas IV and V (Pastene *et al.*, 1996a). With regard the blue whales there are a total of five biopsy samples collected in Areas III and V during the 1994/95 and 1995/96 surveys (Nishiwaki *et al.*, 1995;1996). Given the geographical distribution of these whales, they are believed to be of the 'true' blue whale *Balaenoptera musculus intermedia*. With regard the right whale there are a total of five biopsy samples collected in Area IV during the 1993/94 and 1995/96 surveys (Nishiwaki *et al.*, 1994; 1996). Biopsy samples from these species are being used in a genetic study involving mtDNA control region sequences (Pastene, Goto, Kimura and Nishiwaki, in prep).

An outline of the photo-ID experiments on southern baleen whales conducted under the JARPA was given by Pastene and Fujise (1994). The number of whales identified for natural markings, by species and JARPA survey was summarized in ICR (1996). Copy of photographs of whale identified are sent to the Western Australian Museum every year as part of a co-operative study to investigate the linkage of these species between feeding grounds in the Antarctic Areas IV and V and migratory corridors along the Australian coast.

## 4- GENERAL CONCLUSIONS

In this report we have reviewed the studies and information on species/stock identity on the minke whale and other baleen whale species conducted under the JARPA. After this review our general impression is that some new and important information on this research item has been obtained.

Particularly important has been the new information on taxonomy and phylogenetic relationships of the minke whale. The past sampling of the dwarf form minke whale in the Antarctic, overlapping distribution with the ordinary form, gave the possibility to conduct genetic analysis on this form and then to investigate its phylogenetic relationships with the ordinary form. It was concluded that the differences were such that both southern forms should be considered separated at least at the sub-species level. On the basis of this information the SC had recommended that the two forms in the Southern Hemisphere (dwarf and ordinary) should definitely be considered separately for management purposes.

With regard the stock identity within the ordinary form minke whale, a large-scale mtDNA survey in Areas IV and V has been carried out. The use of JARPA samples for stock identity purposes has several advantages: 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.

The genetic survey has revealed considerable heterogeneity, but little geographic concordance with management Areas IV and V. The results of the studies on stock identity are consistent with the hypothesis of a 'core stock' distributed in Area V, eastern part of Area IV and western part of Area IV late in the season. This 'stock' potentially could be used as a stock unit for management purposes. We identified several areas of further research which should be addressed before considering the 'core stock' as a stock unit useful for such purposes.

Donovan (1991) suggested that several approaches, both genetic and non-genetic should be used to study stock identity, and this has been the policy under the JARPA. However, analyses on stock identity in the ordinary form minke whale have been based largely on genetics. The genetic approach, so far has involved only the maternal-inherited mtDNA. Other genetic

approaches including bi-parental inherited genetic markers should be used in future and the morphometric analysis should be expanded for analysing samples from other seasons and Areas. Non-genetics approaches such as the use of pollutant burden, ecological markers and morphometry should be further studied and developed in order to allow a multi-factors approach in the stock identity studies.

Studies on pollutant burden, organochlorine and heavy metals, have been mentioned as potentially useful for studies on stock identity and samples for such analyses have been collected by JARPA. 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. During the JARPA surveys in Areas IV and V, from each whale sampled different tissues have been collected for pollutant analyses and, because many of the factors indicated above, such as sex and age of the individuals, are identified, those samples are potentially useful for studies of stock identity. During the SC comprehensive assessment of the species in 1990, differences in the incidence of parasites in whales were identified as potentially useful in studies on stock identity (IWC, 1991). Balbuena *et al.* (1995), however, 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. With regard the first, the JARPA surveys offers a good chance for control of the sampling conditions. By using such samples, this approach could be also potentially useful for stock identity studies.

In the mtDNA analyses, geographical and temporal factors have been taken into consideration. Since the pattern of distribution in the feeding ground of animals of different sexes and ages could be different, we consider that in the future, DNA analyses should take into consideration these biological parameters, in addition to geographical and temporal factors.

Finally it should be mentioned that the JARPA surveys have made possible the attainment of biopsy samples in the Antarctic feeding ground from other baleen whale species, such as the humpback, blue and right whales. Genetic analyses using that material are underway. The same species targeted for biopsy sampling have been targeted for photo-ID experiments and a catalog with photographs of whale identified for their natural markings has been established and an international project using these materials is underway.

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**Table 1**: Biological and sampling information on the dwarf form minke whales taken by the JARPA (Kato *et al.*, 1989;1990; Fujise *et al.*, 1990;1993b; Kasamatsu *et al.*, 1993).

| Sample number | Sex | Body length | Sampling date | Sampling location |
|---|---|---|---|---|
| 87/88-273 | Male | 7.0m | 23 Mar. 1988 | 58°22'S, 111°30'E |
| 88/89-005 | Female | 4.5m | 13 Jan. 1989 | 55°22'S, 178°10'E |
| 88/89-013 | Female | 7.0m | 17 Jan. 1989 | 62°04'S, 177°28'E |
| 88/89-014 | Male | 6.0m | 17 Jan. 1989 | 62°07'S, 177°02'E |
| 88/89-070 | Female | 5.9m | 04 Feb. 1989 | 60°38 S, 175°07'E |
| 88/89-227 | Female | 4.0m | 19 Mar. 1989 | 61°54'S, 177°55'E |
| 89/90-002 | Female | 4.3m | 06 Dec. 1989 | 55°20'S, 97°00'E |
| 89/90-199 | Male | 5.4m | 12 Jan. 1990 | 61°30'S, 128°06'E |
| 89/90-215 | Female | 7.1m | 15 Jan. 1990 | 60°59'S, 116°06'E |
| 90/91-002 | Female | 3.8m | 29 Dec. 1990 | 65°04'S, 178°12'E |
| 90/91-012 | Female | 7.5m | 03 Jan. 1991 | 61°09'S, 175°21'W |
| 90/91-014 | Female | 6.6m | 03 Jan. 1991 | 60°40'S, 176°34'W |
| 90/91-118 | Female | 6.8m | 26 Jan. 1991 | 60°34'S, 146°49'E |
| 92/93-107 | Female | 7.0m | 10 Jan. 1993 | 60°51'S, 167°42'E |
| 92/93-108 | Female | 3.5m | 11 Jan. 1993 | 60°31'S, 166°05'E |
| 92/93-330 | Female | 7.2m | 22 Mar. 1993 | 61°49'S, 143°16'E |

**Table 2**: Nucleotide diversity (on the diagonal) and net inter-forms minke whale genetic distances, derived from mtDNA control region sequence data. AO= Antarctic ordinary form minke whale; AD= Antarctic dwarf form minke whale; NP= North Pacific minke whale; NA= North Atlantic minke whale (after Pastene *et al.*,1996c).

| | AO | AD | NP | NA |
|---|---|---|---|---|
| AO | 0.025 | 0.086 | 0.082 | 0.088 |
| AD | | 0.012 | 0.025 | 0.015 |
| NP | | | 0.014 | 0.020 |
| NA | | | | 0.007 |

**Table 3**: Number of samples examined in eight area/time strata, by season. Codes for strata as in Fig. 6 (after Pastene *et al.*, 1996b).

| Area/time group | 87/88 | 88/89 | 89/90 | 90/91 | 91/92 | 92/93 | 93/94 | 94/95 | Total |
|---|---|---|---|---|---|---|---|---|---|
| IVWE | | | 118 | | 42 | | | | 160 |
| IVWL | | | 92 | | 147 | | 144 | | 383 |
| IVEE | | | 82 | | 12 | | 139 | | 233 |
| IVEL | 229 | | 15 | | 55 | | 22 | | 321 |
| VWE | | | | 23 | | 83 | | 102 | 208 |
| VWL | | | | 138 | | 103 | | 23 | 264 |
| VEE | | 10 | | 27 | | 26 | | 13 | 76 |
| VEL | | 96 | | 121 | | 94 | | 168 | 479 |

**Table 4**: Haplotypic correlation (PHIst, below diagonal) and their probabilities (P, above diagonal) among eight area/time strata of minke whales in Areas IV and V. Codes for strata as in Fig. 6 (after Pastene *et al.*, 1996b).

| Area/time group | IVWE (160) | IVWL (383) | IVEE (233) | IVEL (321) | VWE (208) | VWL (264) | VEE (76) | VEL (479) |
|---|---|---|---|---|---|---|---|---|
| IVWE | - | 0.0345 | 0.0295 | 0.0015 | 0.0060 | 0.0103 | 0.0493 | 0.0040 |
| IVWL | 0.0052 | - | 0.4198 | 0.3628 | 0.2104 | 0.8676 | 0.5787 | 0.3058 |
| IVEE | 0.0072 | -0.0000 | - | 0.2644 | 0.1674 | 0.2489 | 0.1724 | 0.3688 |
| IVEL | 0.0136 | 0.0001 | 0.0007 | - | 0.6327 | 0.6892 | 0.4218 | 0.5297 |
| VWE | 0.0105 | 0.0010 | 0.0017 | -0.0009 | - | 0.4733 | 0.4673 | 0.7621 |
| VWL | 0.0076 | -0.0015 | 0.0009 | -0.0009 | -0.0004 | - | 0.8361 | 0.3403 |
| VEE | 0.0087 | -0.0015 | 0.0032 | -0.0004 | -0.0005 | -0.0034 | - | 0.3093 |
| VEL | 0.0083 | 0.0003 | 0.0002 | -0.0003 | -0.0011 | 0.0003 | 0.0009 | - |

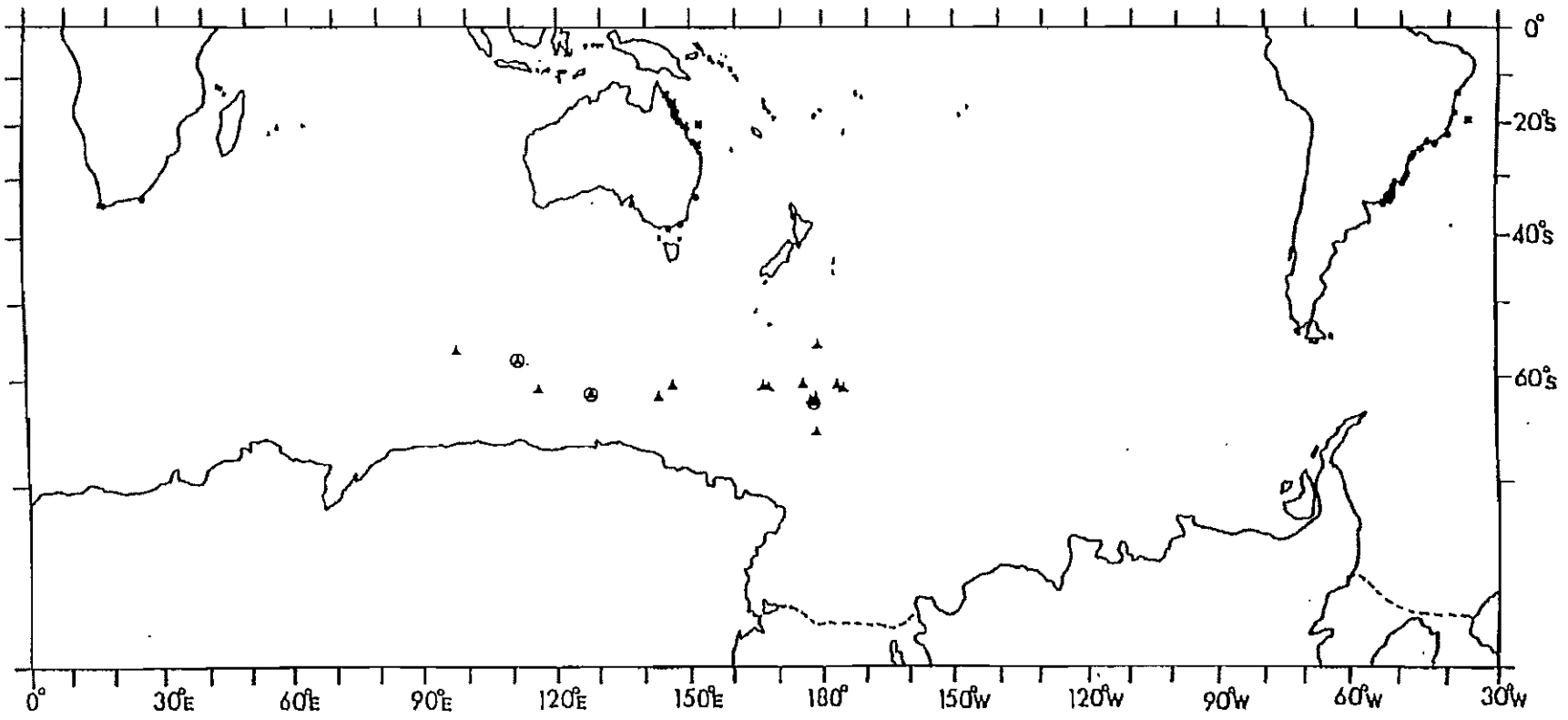


Fig. 1 Geographical distribution of the catches of the dwarf form minke whales made by the JARPA surveys in Areas IV and V (see also Table 1) and records of stranding and sightings in lower latitudes of the Southern Hemisphere. ▲=JARPA catch, female individual; ◭=JARPA catch, male individual; ● =stranding in low latitude; ■ =sighting in low latitude.

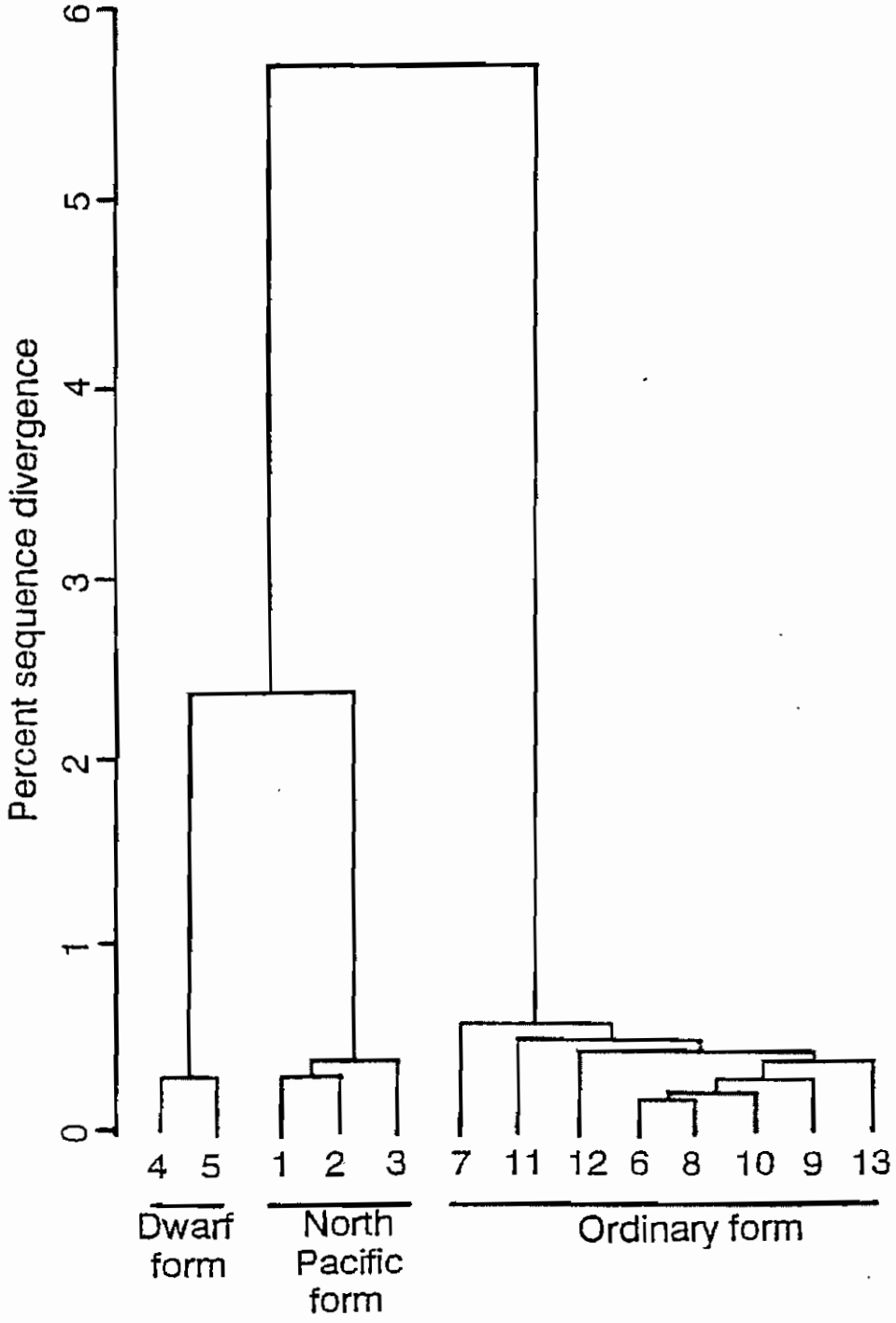


Fig. 2 UPGMA-derived dendrogram of thirteen RFLP-derived mtDNA haplotypes in the minke whale (after Pastene *et al.*, 1994a).

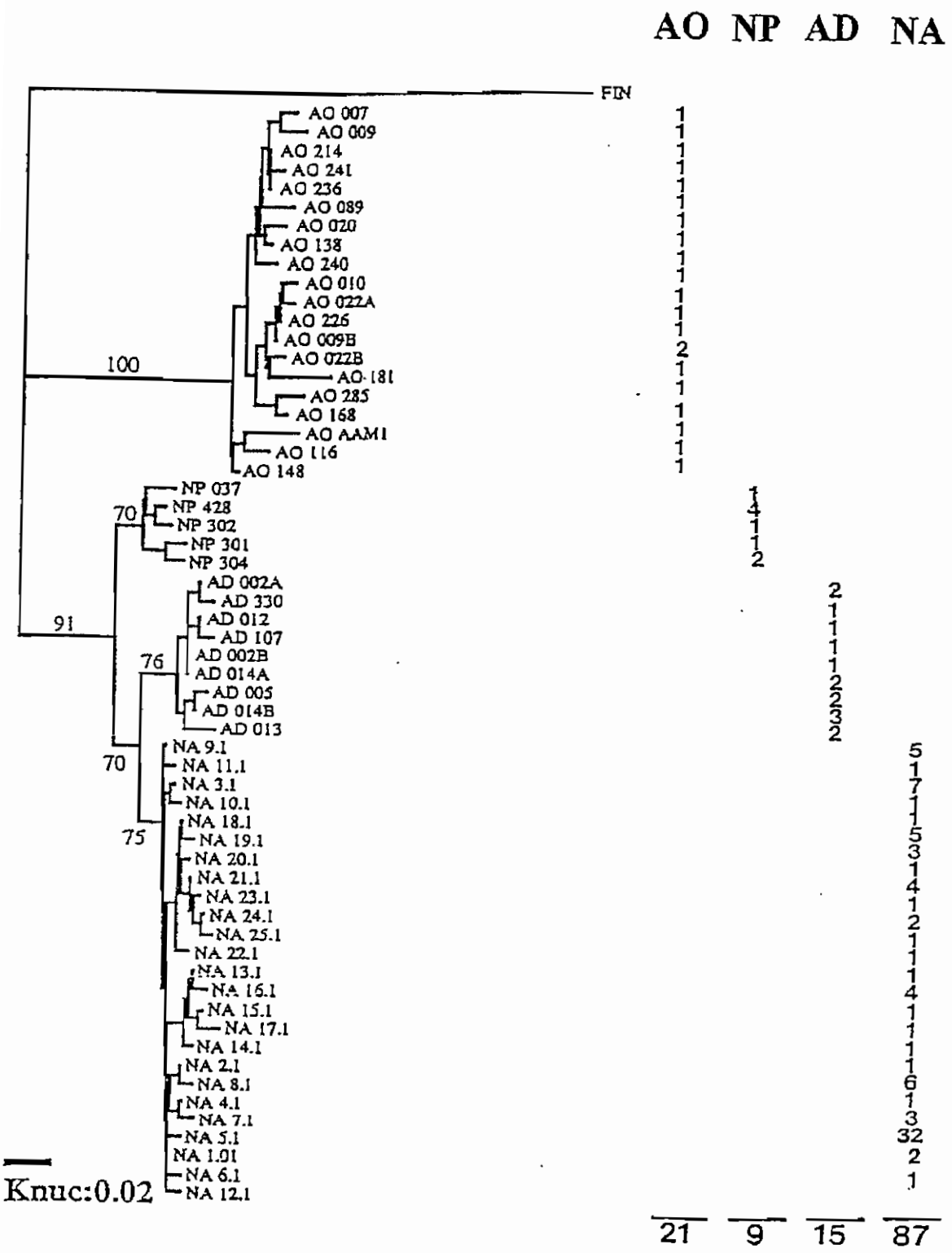


Fig. 3 Neighbor-joining-derived tree of 56 unique mtDNA control region sequences in the minke whale and haplotype frequencies in four forms of the minke whale (after Pastene *et al.*, 1996c). Number at nodes show agreement in a consensus of 100 bootstrap simulations. AO= Antarctic ordinary form; AD= Antarctic dwarf form; NP= North Pacific minke whale; NA= North Atlantic minke whale.

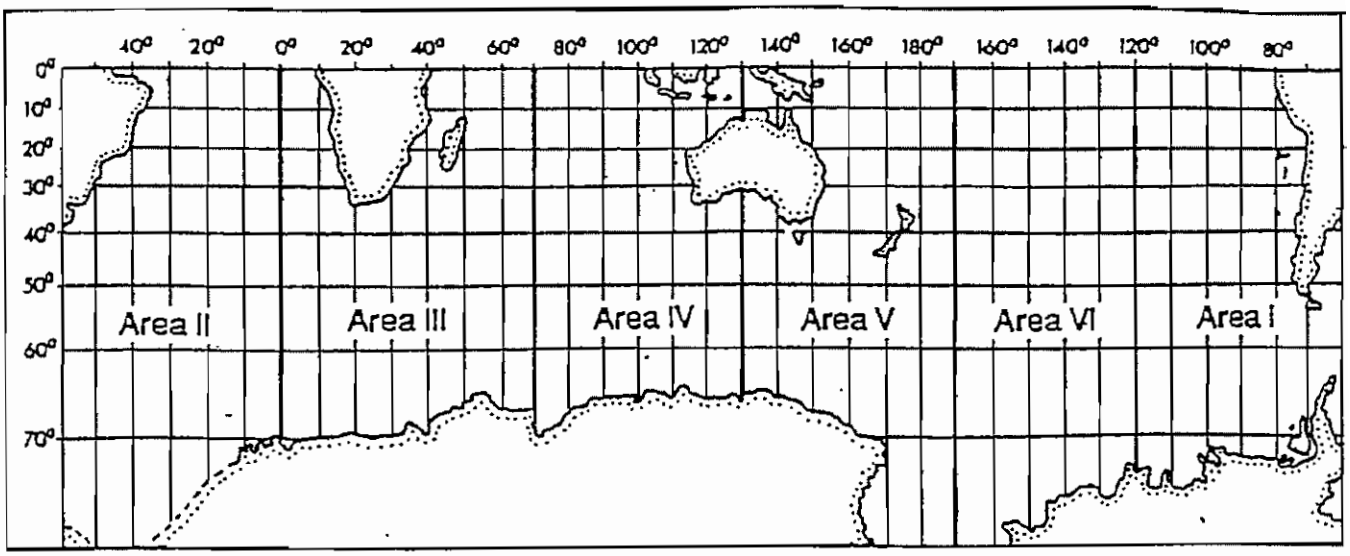


Fig. 4 Southern Hemisphere 'Areas' used by the IWC for the management of baleen whales (except Bryde's whales).

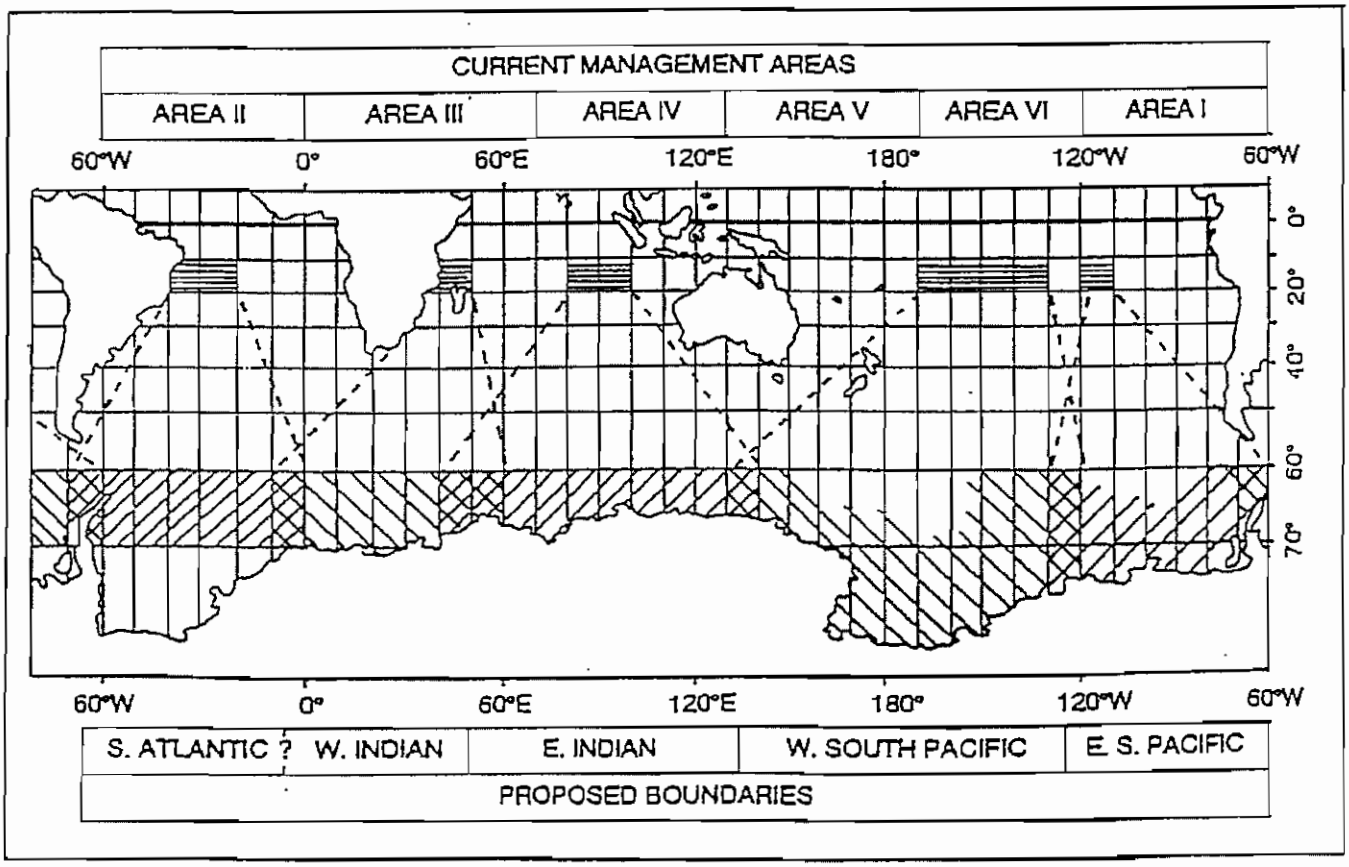


Fig. 5 Possible location of breeding grounds and Antarctic feeding areas based on sighting distribution data; the location of known breeding ground off Brazil is shown. The hypothetical feeding areas used by the animals from these breeding grounds are also shown (after IWC, 1991).

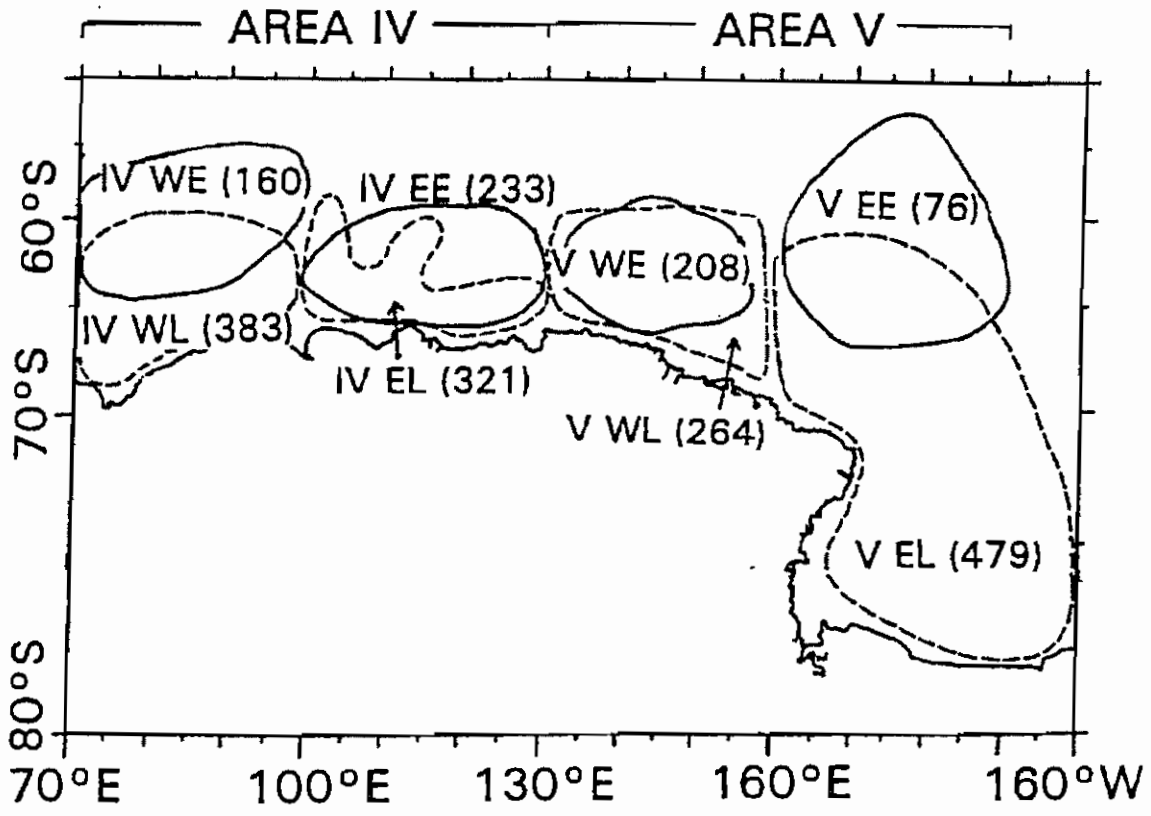


**Fig. 6**: Range of eight area/time strata of minke whales sampled by JARPA examined for mtDNA variation. In parenthesis are the sample sizes. Solid lines: early strata (sampled between December and 15 January); broken lines: late strata (sampled between 16 January and March) (after Pastene *et al.*, 1996b).

## APPENDIX

Scientific works on stock/species identity in the Southern Hemisphere minke and other baleen whale species, based on data and material obtained by the JARPA.


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