Mitochondrial DNA analysis in minke whales from Antarctic Areas V and VI

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ABSTRACT

A restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) in the ordinary form minke whale from Antarctic Areas V and VI was conducted using samples from the 1988/89-1998/99 JARPA surveys. Samples were divided following the same criteria used in the previous analyses in Areas IV and V. They were divided into three longitudinal sectors: Area V Western (130°E-160°E), Area V Eastern (160°E-170°W) and Area VI Western (170°W-140°W) and two temporal periods (Early and Late). A total of 1,814 samples were examined in six longitudinal/temporal groups as follow: VWE, n=208; VWL, n=509; VEE, n=143; VEL, n= 791, VIWE, n= 91 and VIWL, n=72. As out-group, we used the group IVWE 89/90+91/92 ('western stock', n=160). Quantification of the mtDNA differentiation among groups was carried out using the Analysis of Molecular Variance (AMOVA). Both haplotype (Fst) and sequence (PHIst) statistics were used. Most of the test conducted showed significant differences between groups in Areas V/VIW and the 'western stock'. Overall no significant mtDNA heterogeneity was found within Areas V and VIW. However, certain degree of heterogeneity was found in Group VWE, which seems to be differentiated using the Fst, from both the 'western stock' and the rest of groups in Areas V and VIW. Results of an additional analysis of the late samples in Areas V grouped into north and south (at 70°S), showed no evidence for significant latitudinal genetic heterogeneity.

KEYWORDS: ANTARCTIC ORDINARY FORM MINKE WHALE, STOCK IDENTITY, GENETICS, MANAGEMENT

INTRODUCTION

Until now the genetic analyses of JARPA minke whale samples has been based on a RFLP analysis of the mtDNA. Based on these analyses, the only significant source of mtDNA heterogeneity in Areas IV and V was attributed to whales sampled in the western part of Area IV in the 1989/90 and 1991/92 JARPA surveys (Pastene *et al.*, 1996). For practical use, such sample has been called as 'western stock'. A summary of these analyses is presented in Table 1.

These results suggested that the stock structure of the Antarctic minke whale could be more complex than it was thought initially and it could be determined not only by geographic factors (longitudinal) but also by temporal factors. For example a temporal component in the distribution of stocks in the western part of Area IV, was suggested (Pastene *et al.*, 1996).

The pattern of mtDNA differentiation shown in Table 1 can be used as a baseline on which future comparisons in mtDNA composition can be based. For example samples could be compared with a representative sample of the 'western stock' (as represented by group IVWE in Table 1). We presented here the results of a mtDNA RFLP analysis on the total samples available from Areas V and VI from 1988/89 to 1998/99. The analysis was aimed to find any additional source of mtDNA heterogeneity in these Areas. Samples in Areas V and VI are grouped and examined considering temporal (years and periods within an austral summer season) and geographical (longitude and latitude) factors. As an out-group, we used the sample of the 'western stock'.

MATERIALS AND METHODS

Samples

Samples of the ordinary form minke whale from Areas V and VI were available from the 1988/89-1998/99 JARPA surveys. For each year and for each sex, samples were grouped into: Area V Western Early (group VWE, n= 208), Area V Western Late (group VWL, n=509), Area V Eastern Early (group VEE, n=143), Area V Eastern Late (group VEL, n=791), Area VI Western Early (group VIWE, n=91) and Area VI Western Late (group VIWL,

n=72). 'Early' refers to whales sampled in December and first half of January. "Late' refers to whales sampled in the second half of January, February and March (Table 2). For comparison, we used a sample of the 'western stock' (IVWE 1989/90+1991/92, n=160) (Table 1).

Figs. 1 to 6 shows the geographical distribution of each longitudinal/temporal group for each JARPA survey, by sex.

RFLP analysis
Crude mtDNA extracted from liver tissues was digested with the same restriction enzymes of the previous study (Pastene *et al.*, 1996): *Acc*I, *Ban*I, *Eco*RV, *Hin*cII, *Hpa*I and *Ssp*I. All the procedures for DNA extraction and DNA digestion were the same as in the previous study.

Statistical analysis
The geographic/temporal differentiation of mtDNA was quantified using the Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) as implemented in the computer program AMOVA ver. 1.55. The statistics of primary interest are the haplotype (Fst) and sequence (PHIst), both of which were used. The significance of the observed variance values was tested using a modification of a matrix permutation procedure available in the computer program. All tests of statistical significance were based on 10,000 random permutation of the original data sets. The level of significance obtained by this procedure is referred in this paper as the P-value.

Samples were grouped into year/longitudinal/temporal groups. Females and male were compared first. If no significant differences were found, then these samples were pooled and each longitudinal/temporal group was tested for yearly variation. If no significant yearly differences were found, then samples from different years were pooled. Finally a hierarchical analysis using AMOVA was performed to test for among longitudinal differences, temporal differences within longitudinal sector and within longitudinal/temporal groups.

In an additional analysis, we tested our samples for latitudinal variation in Area V. Because samples from the Ross Sea are available only from the late period, we restricted the analysis to this period of the summer season. On one side we considered samples from south of 70°S involving mainly the Ross Sea. Samples were available from five JARPA surveys: 1988/89, 1990/91, 1992,93, 1994/95 and 1996/97. On the other side we considered samples from the western and eastern parts of Area V north of 70°S. We used samples for the same surveys. After checking for yearly variation in the 'south' and 'north' groups, we tested these two groups for latitudinal differences using the Fst and PHIst.

RESULTS

mtDNA haplotypes
Characterization of the mtDNA RFLP haplotypes was documented in a previous paper (Pastene *et al.*, 1996). By using a set of six polymorphic restriction enzymes, a total of 153 haplotypes have been discriminated in the Antarctic minke whale.

mtDNA of the sexes
No significant differences were found between male and female samples in each of the year/longitudinal/temporal groups. Subsequent analyses considered male and female samples pooled.

Yearly variation in the longitudinal/temporal groups
Results of the analysis of yearly variation in the longitudinal/temporal groups are shown in Table 3. No significant yearly variation was observed in these groups. In the case of Group VWE, however, the Fst showed a P value below 5% level and the PHIst was also near to significant. Pairwise comparison showed that the sample from 1990/91 (n=23) was somewhat different from the 1992/93 (n=83) and 1994/95 (n=102) samples. No significant difference was found between these two latter samples. Subsequent analyses ignored the sample of 23 individuals of the 1990/91 survey.

Hierarchical analysis by AMOVA
Table 4 shows the results of the hierarchical analyses by AMOVA for Fst and PHIst statistics. Neither of these statistics showed significant genetic spatial/temporal heterogeneity in Areas V and VI, although the Fst showed lower P values for each of the tests conducted than those obtained for the PHIst.

Comparison with the 'western stock'
Although the overall test by Fst and PHIst showed no evidence for significant mtDNA heterogeneity, we conducted pairwise comparison among the longitudinal/temporal groups and included into this analysis the sample from the 'western stock'. Table 5 shows these results for the Fst and PHIst.

Comparison involving the 'western stock' showed higher Fst or PHIst values and most of them presented P values below 5%. It should be noted here that the sample of Group VWE (n=185) was differentiated from both, the 'western stock' and the rest of the groups by the Fst.

Latitudinal differences
No significant yearly differences were found in the 'south' (Fst=0.0030, P=0.1426; PHIst=0.0050, P=0.0822) and 'north' (Fst=-0.0010, P=0.5644; PHIst=-0.0000, P=0.5479) groups in Area V. Both, Fst and PHIst statistics showed no significant differences between samples to the south and north of 70°S in Area V (Fst=-0.0010, P=0.7410; PHIst=-0.0010, P=0.6693).

DISCUSSION

In the study by Pastene *et al.* (1996) the mtDNA analysis was focused to examine samples from Areas IV and V from 1987/88 to 1994/95. A summary of the results obtained in such study is given in Table 1. Subsequently Pastene and Goto (1998) examined samples from Area V and western part of Area VI, which were obtained during the 1996/97 JARPA survey. This latter study also included a set of historical samples from Area VIW, taken in a commercial operation in 1985/86. Results of these previous studies in Areas V and western part of Area VI showed no evidence for significant mtDNA heterogeneity.

In this study we examined the pattern of mtDNA variation in minke whales from Area V and western part of Area VI using all the samples available from JARPA surveys in these Areas. We concentrated in the analysis of JARPA samples because commercial whaling concentrated its operations in areas of high density around the ice-edge. Recent studies have shown that the distance from the ice-edge to the sampling position could be an important factor determining the stock structure in the Antarctic minke whale (Goto *et al.*, 1998; Pastene and Goto, 1999). Samples in the JARPA surveys are taken on pre-determined transects designed near and far from the ice-edge position and only one whale is taken from a school. Samples from six surveys were available for Area V and from two surveys for Area VIW. Thus we were able to study the pattern of yearly variation in these Areas, in addition to the longitudinal and temporal factors within a given survey.

In general no substantial yearly variation was observed in the longitudinal/temporal groups. The only exception was the Group VWE where some degree of mtDNA heterogeneity was observed among years. In this group, samples from three different surveys were available (1990/91, 1992/93 and 1994/95). Pairwise comparisons showed that the sample of the 1990/91 survey (n=23) was somewhat different from the other two. An examination of Group VWE 1990/91 in Fig. 2 suggest that such a sample was taken from a restricted area and then the genetic differences observed could be reflecting the restricted geographical range from where the sample was taken. Samples from the other two surveys in this group showed no significant differences in mtDNA composition and then the sample combined from these two surveys was used in the subsequent analysis.

The nested AMOVA analysis showed no significant differences in mtDNA composition. Neither samples from different longitudinal sectors nor from different temporal groups within longitudinal sectors were significantly different. These results are the same as those found by Pastene *et al.* (1996) and Pastene and Goto (1998). Notwithstanding the overall tests showed no significant differences, we conducted pairwise comparisons among the longitudinal/temporal groups in Areas V and VIW and compared these groups with a sample of the 'western stock'.

From such exercise, two main results were obtained. Firstly both statistics showed the same trend regarding the differentiation of the 'western stock', as most of the tests involving this sample showed P values below 0.05. This result was more evident by using the PHIst. It should be noted that in both cases (Fst and PHIst), the sample from Area VIWL showed negative values and the P values were abnormally high (0.8563 and 0.7662, respectively) when compared with the 'western stock'. Again, if we look Fig. 6 the samples of the 1998/99 JARPA survey, which compose mainly this group, were particularly restricted regarding the geographical covering and this fact could be reflected in the results of the statistical test. Then, for the genetic analysis on stock structure it is particularly important that the samples be taken from wide areas and distributed evenly in time as it has been the cases of most of the JARPA surveys. The second result from the exercise was that for one of the statistics used (Fst) we found some preliminary evidence for genetic differentiation of Group VWE. This group was differentiated

from both the 'western stock' and from the rest of the groups examined in Areas V and VIW. This result could provide evidence for additional stock structure in these Areas. This result should be considered as preliminary as the overall test for heterogeneity showed no significant differences in these Areas.

In addition to longitudinal and temporal factors we conducted an analysis considering the latitudinal factor. In most of the JARPA surveys, samples in the late period in the eastern part of Area V are taken in the Ross Sea. Then we tested differences between two groups of samples in the late period: north of 70°S including western and eastern part of Area V and south of 70°S including mainly the Ross Sea. We found no evidence for genetic heterogeneity among years in these groups. The samples of north and south of 70°S showed no significant differences in mtDNA composition. The analysis of mtDNA in Area IV has incorporated information on the distance between the sampling place and the ice-edge and such factor seems to play an important role in determining stock structure in the Antarctic minke whale (Goto *et al.* 1998; Pastene and Goto, 1999). Future analyses in Areas V and VI should consider information on the distance from the ice-edge as well.

Regarding the statistics used, our results are consistent with the view that the haplotype statistics is more sensitive to detect differences between stocks than the sequence statistics. For example, stronger evidence for yearly differentiation was found in Group VWE using the Fst (Table 3). Possible additional heterogeneity in Area V was also detected using the Fst (Table 5A). However, the PHIst seems to be more sensitive to detect differences with the 'western stock' (Table 5B).

The genetic analysis of JARPA samples has been based on RFLP analysis of the mtDNA. Recently Abe *et al.* (1999) started the analysis of microsatellite and now we are conducting analysis of sequencing of the mtDNA control region. Future studies will be based, in addition to RFLP data, on sequencing and microsatellite data.

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Samples from Areas V and VI used in this study were obtained during different JARPA surveys. We would like to acknowledge the researchers and crewmembers that participated in those surveys. We thank H. Oikawa and S. Azumi for their help in the process of DNA extraction.

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Table 1: Haplotypic correlation (PHIst, below diagonal) and their probabilities (P, above diagonal) among eight area/time groups of minke whales from Areas IV and V. In parenthesis is the sample size. Note that all pairwise comparisons involving group IVWE showed higher PHIst values, all of them with P values below 0.01 or 0.05 (After Pastene *et al.*, 1996). P values below 5% are shown in **bold**.

Area/time groups	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	-

Table 2: Samples of minke whales used for the mtDNA analysis in this study. They were grouped by Area, longitudinal sector, period within an austral summer season, sex and JARPA survey. See text for details of the groupings.

JARPA Survey	Area V								Area VI			
	Western				Eastern				Western			
	Early		Late		Early		Late		Early		Late	
	M	F	M	F	M	F	M	F	M	F	M	F
1988/89					5	5	39	57				
1990/91	7	16	91	47	20	7	39	82				
1992/93	42	41	64	39	17	9	38	56				
1994/95	82	20	18	5	6	7	75	93				
1996/97			43	55	41	26	45	105	60	31	8	5
1998/99			73	74			121	41			39	20
Total	131	77	289	220	89	54	357	434	60	31	47	25

Table 3: Results of the analysis of yearly variation in the longitudinal/temporal groups. See Table 1 for surveys involved and sample size used in each test. No test was conducted for Group VIWE as JARPA data are available only from a single year. P values below 5% are shown in **bold**. P values below 10% are shown underlined.

Long./temporal group	Fst	P	PHIst	P
VWE	0.0120	**0.0447**	0.0080	0.0735
VWL	-0.0030	0.9399	-0.0040	0.9683
VEE	-0.0080	0.8475	0.0050	0.2553
VEL	0.0000	0.3573	0.0000	0.4679
VIWL	-0.0180	0.8611	-0.0230	0.8463

Table 4: Results of the nested analysis of molecular variance of minke whale mtDNA haplotypes in Areas V and VI. The P-value is the probability of a more extreme variance component or PHIst than that observed, in comparison to a null distribution of these values on 10,000 random permutations of the data matrix. PHIct and the among longitudinal sectors variance component involves the permutation of whole longitudinal/temporal groups among sectors; the PHIsc and the among temporal groups within sectors involves the random permutation of individuals among temporal groups within sectors; the PHIst and the within longitudinal/temporal groups components involves the random permutation of individuals among the six longitudinal/temporal groups. P-values below 10% are underlined. A sample of 23 individuals from Group VWE 1990/91 is excluded from the analysis (see text).

A- Fst

	df	% total variance	Fst	P
Among long. Sectors	2	-0.04	CT: -0.0000	0.4724
Among temp. in sect.	3	0.14	SC: 0.0010	0.1394
Within long/temp. g.	1,785	99.90	ST: 0.0010	0.0991

B- PHIst

	df	% total variance	Fst	P
Among long. Sectors	2	-0.05	CT: -0.0010	0.6600
Among temp. in sect.	3	0.01	SC: 0.0000	0.4336
Within long/temp. g.	1,785	100.04	ST: -0.0000	0.5993

Table 5: Pairwise tests among six longitudinal/temporal groups in Areas V and VI. The sample from the 'western stock' (IVWE) is included for comparison as an out-group. Note that the overall test for the samples of Areas V and VI was not significant, for either Fst and PHIst (see Table 4). In parenthesis is the sample size. Fst or PHIst values below diagonal; P-values above diagonal

A- Fst

Groups	VWE (185)	VWL (509)	VEE (143)	VEL (791)	VIWE (91)	VIWL (72)	IVWE (160)
VWE	-	**0.0280**	0.0647	**0.0493**	0.0757	**0.0166**	**0.0007**
VWL	0.0042	-	0.5195	0.2722	0.6742	0.4403	**0.0323**
VEE	0.0049	-0.0005	-	0.4440	0.8174	0.5594	0.2075
VEL	0.0029	0.0003	-0.0002	-	0.4874	0.1719	**0.0180**
VIWE	0.0057	-0.0015	-0.0030	-0.0006	-	0.7480	0.1551
VIWL	0.0136	-0.0004	-0.0013	0.0025	-0.0033	-	0.8563
IVWE	0.0168	0.0041	0.0016	0.0046	0.0027	-0.0034	-

B- PHIst

Groups	VWE (185)	VWL (509)	VEE (143)	VEL (791)	VIWE (91)	VIWL (72)	IVWE (160)
VWE	-	0.5222	0.6344	0.5902	0.6341	0.2315	**0.0032**
VWL	-0.0005	-	0.6414	0.2972	0.5092	0.4702	**0.0033**
VEE	-0.0014	-0.0010	-	0.4305	0.6387	0.1991	**0.0093**
VEL	-0.0006	0.0002	-0.0001	-	0.7143	0.4033	**0.0033**
VIWE	-0.0016	-0.0007	-0.0019	-0.0018	-	0.3076	**0.0145**
VIWL	0.0026	-0.0005	0.0031	0.0001	0.0019	-	0.7662
IVWE	0.0125	0.0085	0.0126	0.0095	0.0129	-0.0039	-

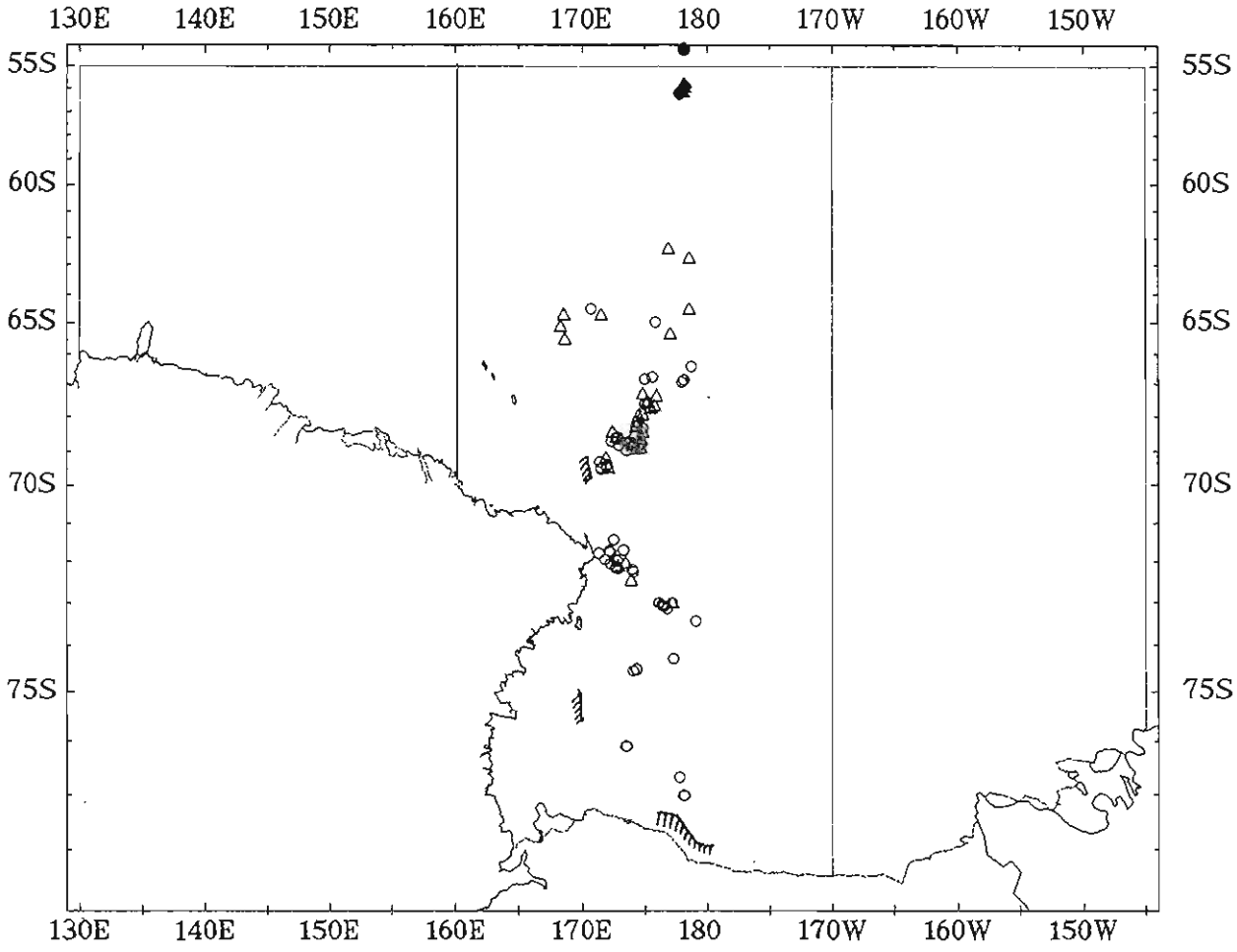

Fig. 1: Geographical distribution of longitudinal/temporal groups of minke whales in Area V in 1988/89, by sex. Area V was divided into west and east at 160°E. Explanation of symbols is as follow: ● =early female; ▲ =early male; O =late female; △ =late male. The position of the ice-edge is also shown.

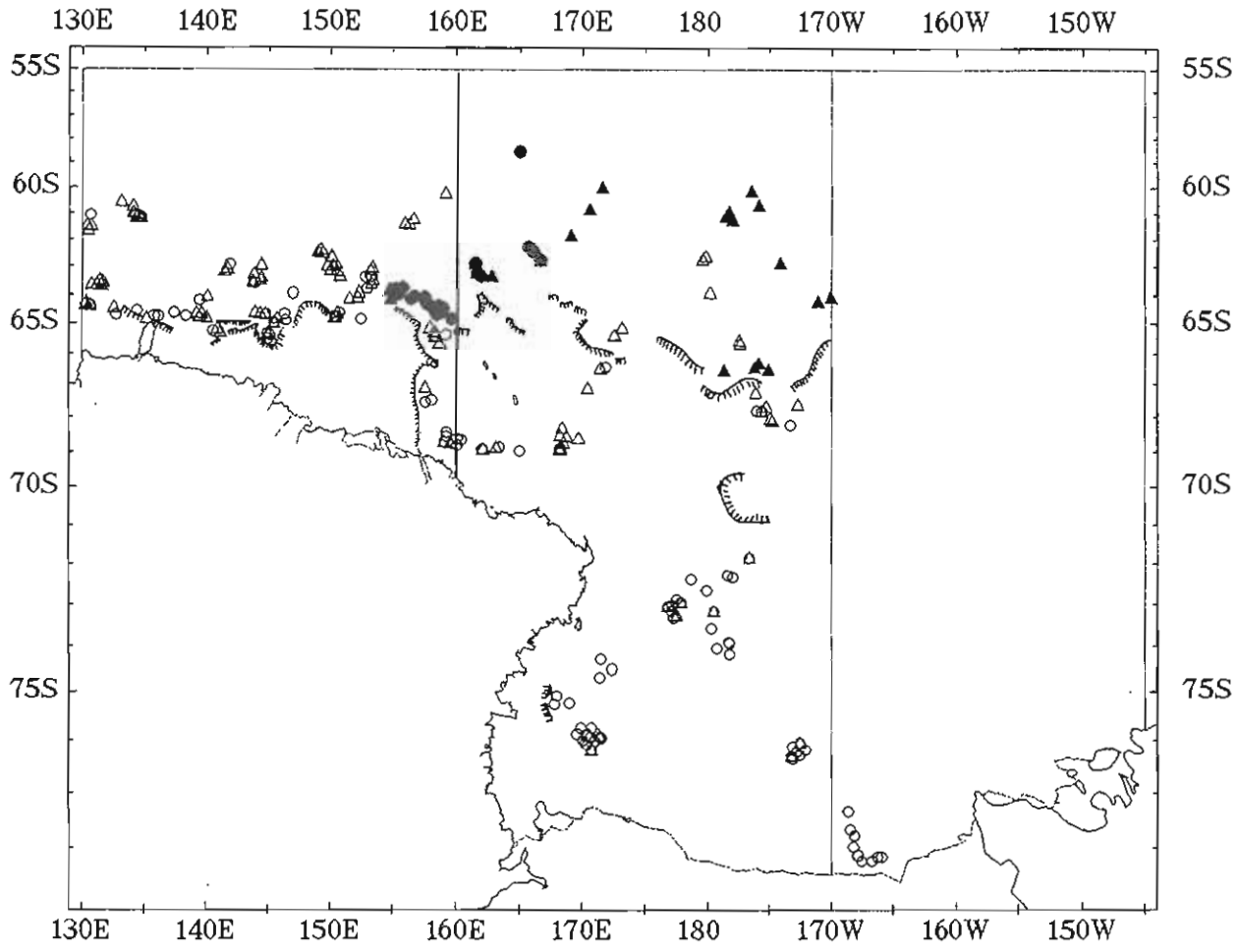

Fig. 2: Geographical distribution of longitudinal/temporal groups of minke whales in Area V in 1990/91, by sex. Area V was divided into west and east at 160°E. Explanation of symbols is as follow: ● =early female; ▲ =early male; O =late female; △ =late male. The position of the ice-edge is also shown.

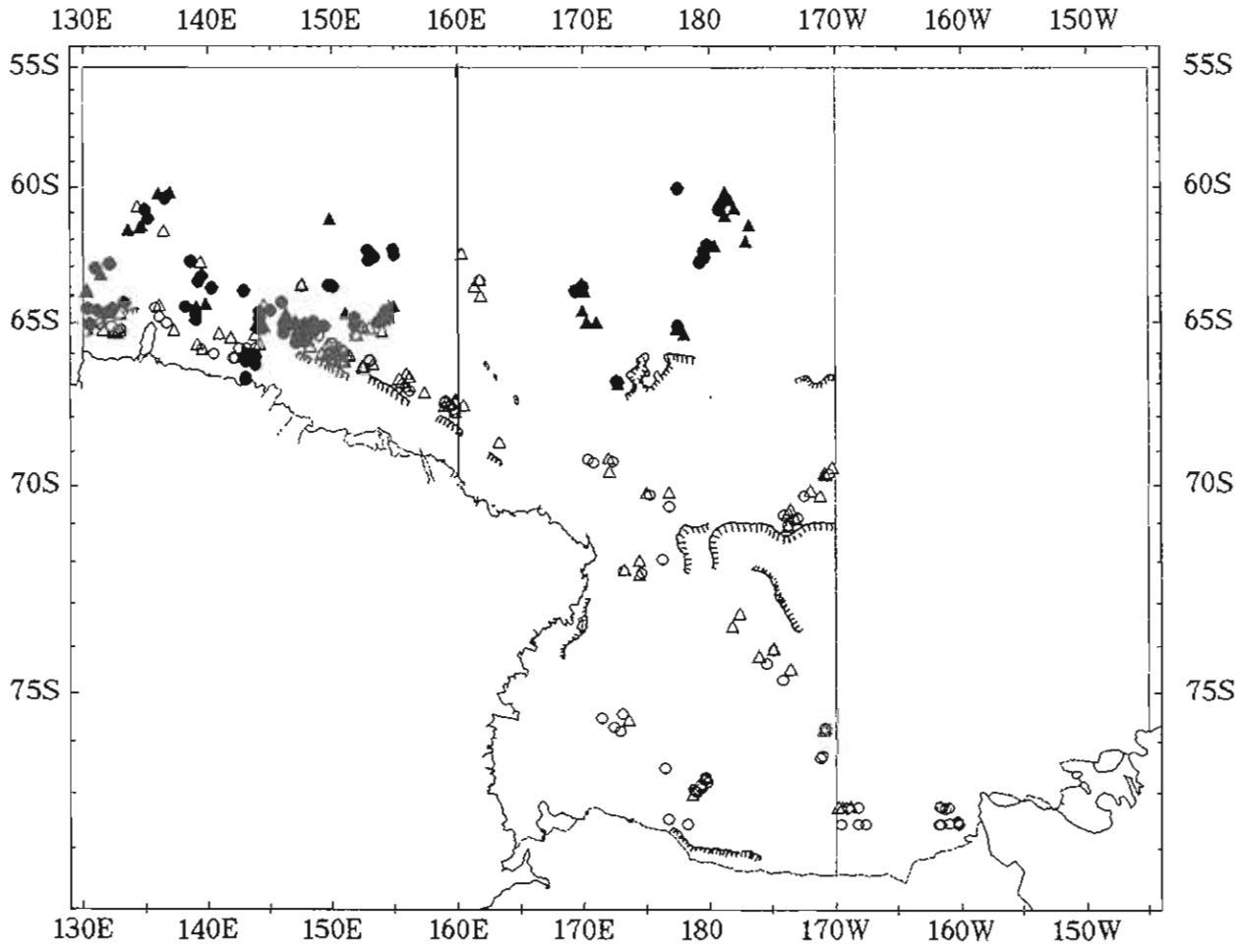

Fig. 3: Geographical distribution of longitudinal/temporal groups of minke whales in Area V in 1992/93, by sex. Area V was divided into west and east at 160°E. Explanation of symbols is as follow: ● =early female; ▲ =early male; O =late female; △ =late male. The position of the ice-edge is also shown.

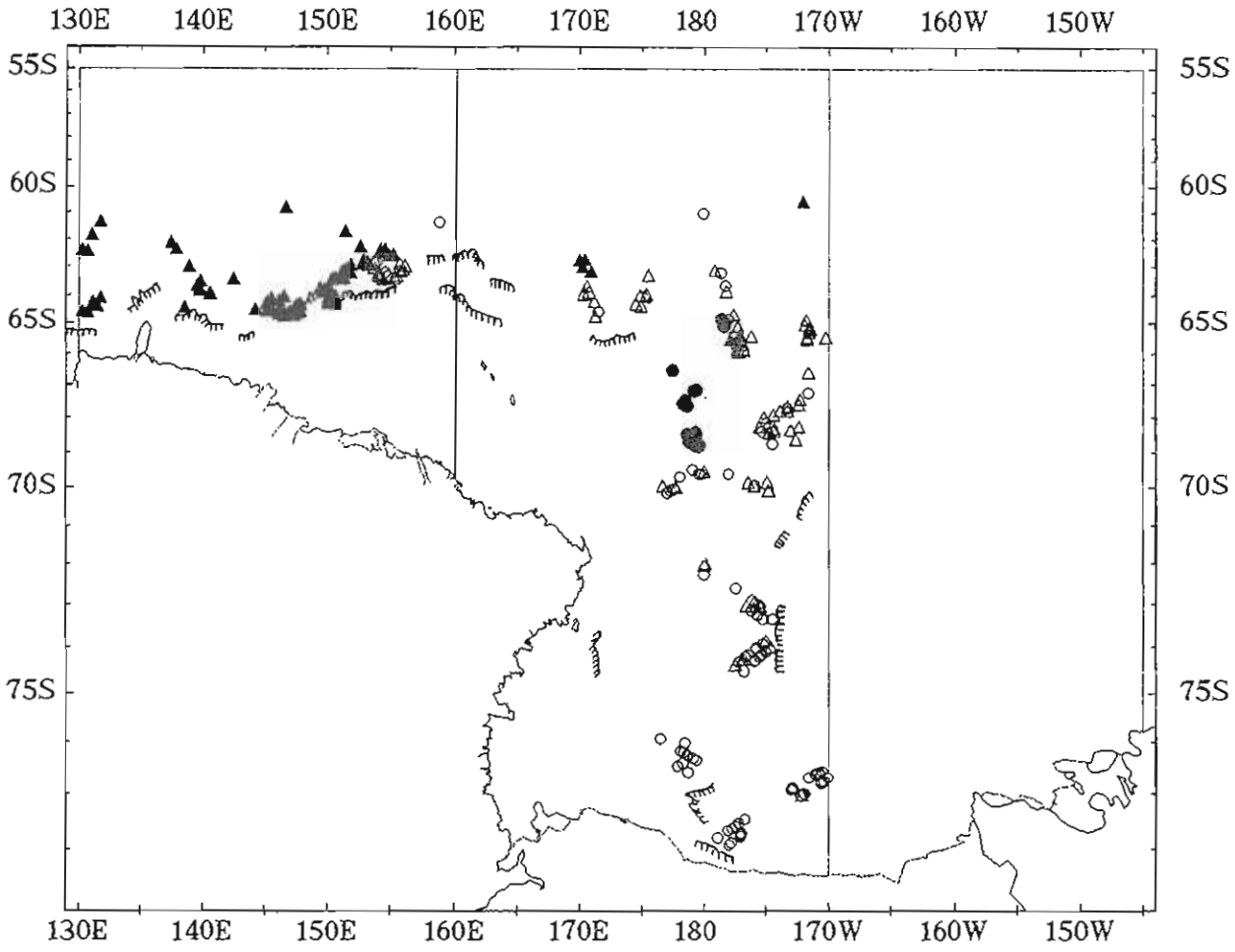

Fig. 4: Geographical distribution of longitudinal/temporal groups of minke whales in Area V in 1994/95, by sex. Area V was divided into west and east at 160°E. Explanation of symbols is as follow: ● =early female; ▲ =early male; O =late female; △ =late male. The position of the ice-edge is also shown.

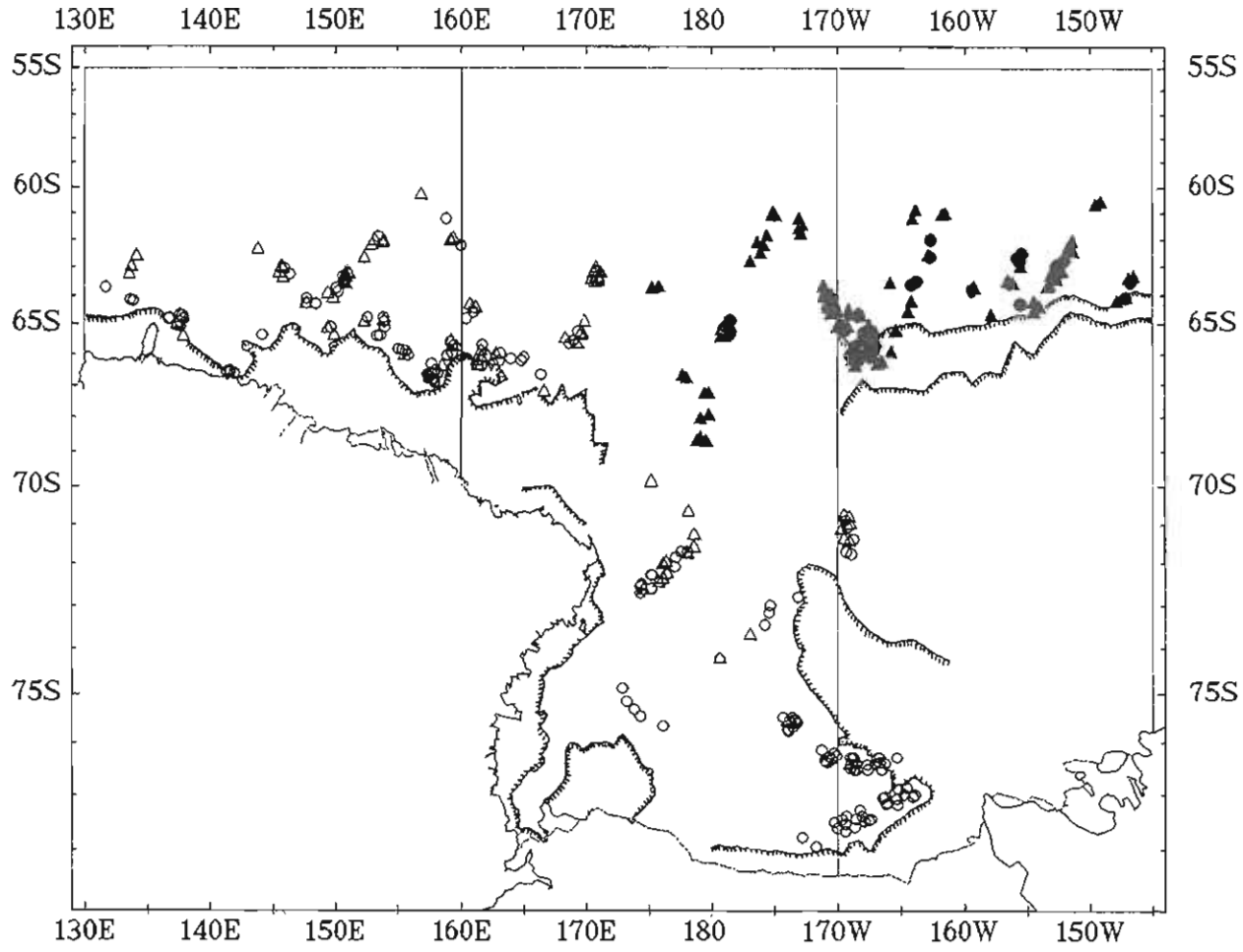

Fig. 5: Geographical distribution of longitudinal/temporal groups of minke whales in Areas V and VI in 1996/97, by sex. Area V was divided into west and east at 160°E. Explanation of symbols is as follow: ● =early female; ▲ =early male; O =late female; △ =late male. The position of the ice-edge is also shown.

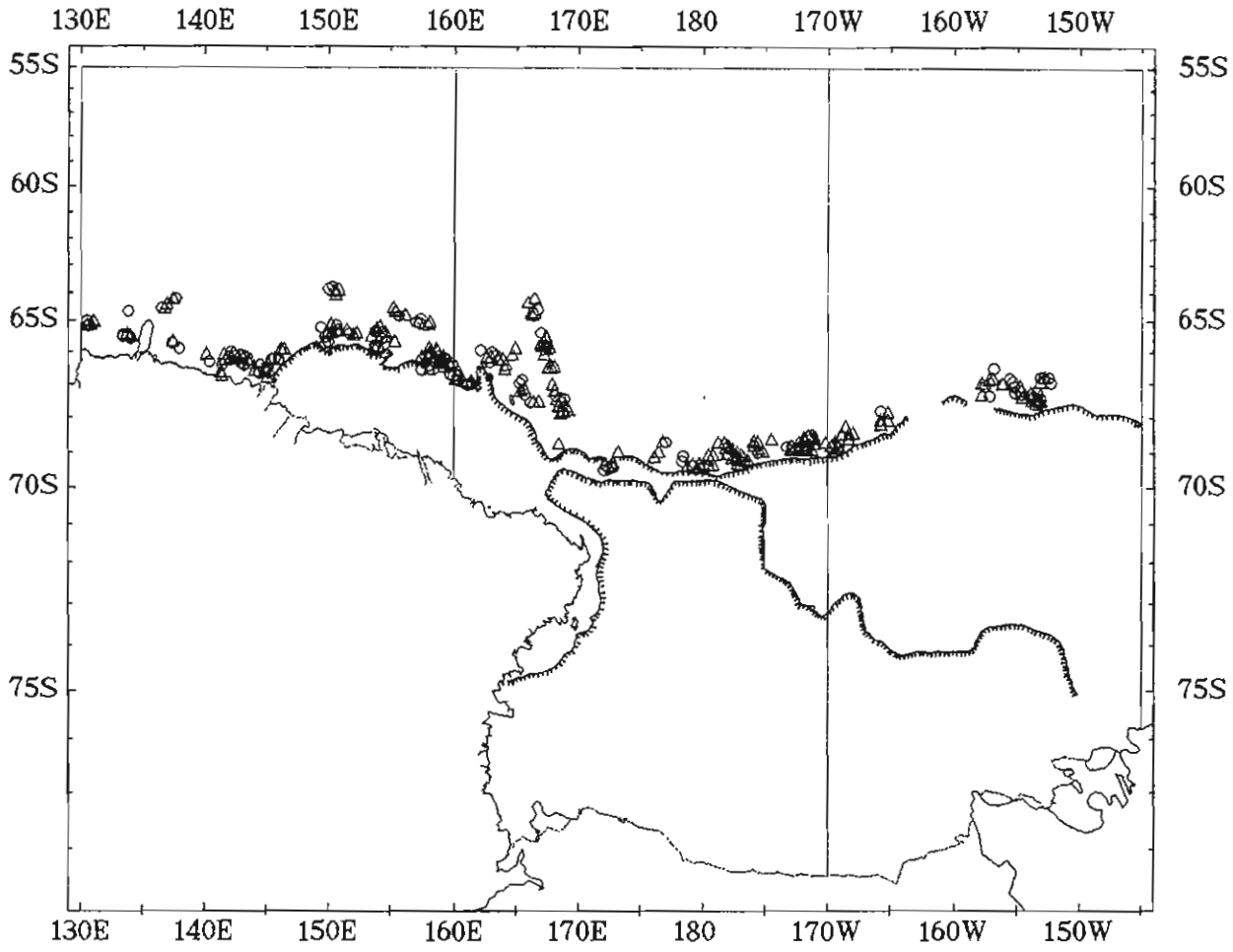

Fig. 6: Geographical distribution of longitudinal/temporal groups of minke whales in Areas V and VI in 1998/99, by sex. Area V was divided into west and east at 160°E. Explanation of symbols is as follow: ● =early female; ▲ =early male; O =late female; △ =late male. The position of the ice-edge is also shown.