Mitochondrial DNA analyses of J and O stocks common minke whale in the western North Pacific

Jung Youn Park[1], Mutsuo Goto[2], Naohisa Kanda[2], Toshiya Kishiro[3], Hideyoshi Yoshida[3], Hidehiro Kato[4] and Luis A. Pastene[2]

1 Biotechnology Research Center, National Fisheries Research and Development Institute, Busan, Korea
2 The Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo, 104-0055, Japan
3 National Research Institute of Far Seas Fisheries, 2-12-4 Fukuura, Kanazawa, Yokohama Kanazawa, 236-8648, Japan
4Tokyo University of Marine Science and Technology, 1-5-7 Konan, Minato-ku, Tokyo, 108-0075, Japan

ABSTRACT

Genetic variation at the mtDNA control region in the western North Pacific common minke whales was analyzed to examine the plausibility of proposed stock structure scenarios for both J and O stocks. Analyses were based on samples collected during JARPN and JARPNII surveys from 1994 to 2007 in the area from the Japanese coast to offshore waters (to 170°E) on the Pacific side; and from by-catches around Japan and the Korean Peninsula. The IWC SC quality control guidelines were followed as much as possible. Samples were first separated into J and O stocks according to the results of the microsatellite analysis, and the subsequent mtDNA heterogeneity tests were conducted for different categories of grouping (total samples, 'pure' O or J+unassigned samples and 'pure' J or O only). Heterogeneity tests were based on the randomized chi-square test and the *Fst* values were calculated to get an idea of the effect sizes of the groups compared. Tests were specifically focused to examine stock structure hypotheses listed by the IWC SC for both J and O stocks. In the case of the J stock none of the comparisons resulted in significant mtDNA heterogeneity when only 'pure' J category was used, except in the case of the comparison between whales of Japan and Korea. In this case a chi-square *p*-value smaller than 0.05 was found however the *Fst* was very small. Alternative explanations are given for this result. Tests for examining sub-stock structure in the O stocks followed the four stock structure hypotheses adopted at the final stage of the *ISTs* process in 2003 and was based on an updated mtDNA database. No significant heterogeneity was found when the samples were grouped and tested according to the geographical boundaries of the stock scenarios C and D and 'pure' O + unassigned animals were used. Therefore the present results provide no support for the occurrence of sub-structure within the O stock. Hypothesis A, which suggested the sporadic occurrence of a 'W' stock in sub-area 9, can not be discarded completely at this stage. In general results of these mtDNA analyses, which were based on a substantial number of samples, confirm the previous view of two stocks of common minke whale in the western North Pacific, the J and O stocks. The possibility of a W stock in offshore waters of the Pacific and a Y stock in the Yellow Sea should be further investigated in the future.

KEY WORDS: COMMON MINKE WHALE, MTDNA, STOCK STRUCTURE, NORTH PACIFIC, RMP

INTRODUCTION

The *Implementation Simulation Trials* (*ISTs*) for North Pacific common minke whale under the Revised Management Procedure (RMP) was completed during the 2003 International Whaling Commission Scientific Committee (IWC SC) meeting. However these *ISTs* were conducted in absence of the 'Requirements and Guidelines for *Implementation'* adopted by the IWC SC in 2004 (IWC, 2005). Instead of carrying out a typical *Implementation Review* after five years of completed the *ISTs*, it has been agreed to carry out a *pre-implementation assessment* and the two-year *Implementation* process as specified in the guidelines.

One of the issues on which the *pre-implementation assessment* is focused is on the establishment of plausible stock structure hypotheses consistent with the data that are inclusive enough that it is deemed unlikely that the collection of new data during the *Implementation* process would suggest a major novel hypothesis (IWC, 2005).

In the case of the North Pacific common minke whale RMP *Implementation* is conducted with the final aim to provide advice on sustainable catch limits for the O stock common minke whale. J stock common minke whales are not a target of future whaling operations however it is important to investigate the interaction of this stock with the O stock particularly in the areas where future whaling operations are likely to occur so that catches of J stock animals can be avoided or

minimized.

Stock structure, abundance and other aspects of the J stock have been discussed within the 'Working Group on the in-depth assessment of western North Pacific common minke whales, with a focus on J stock (NPM)'. Based mainly on the analyses of Korean and Japanese DNA data some hypotheses on stock structure have been proposed (IWC, in press). These hypotheses involve the following general questions: what is the stock structure of J stock whales around the Korean Peninsula? What is the stock structure of J stock whales around the Japanese archipelago? How the J stock whales around Japan relate to those around the Korean Peninsula?

With regard the O stock at the final stage of the *ISTs* process the IWC SC adopted the following four stock structure baseline scenarios (IWC, 2004):

(1) Baseline A: three-stock scenario ('J', 'O', 'W') with the 'W' stock found only in part of sub-area 9 and only sporadically.
(2) Baseline B: two stock scenario ('J' and 'O') with no W stock as a limiting case of Baseline A.
(3) Baseline C : four-stock scenario overall, with 'O_W', 'O_E' and 'W' to the east of Japan. Boundaries are fixed at 147°E and 157°E and there is no mixing between the stocks.
(4) Baseline D : three-stock scenario ('J', 'O', 'W'), with 'O' and 'W' mixing over 147°E and 162°E, O being dominant to the west and W to the east.

Unfortunately the IWC SC was unable in 2003 to scientifically assign plausibility to these stock structure scenarios. In order to get agreement among members it gave the same 'high' plausibility to the four scenarios (IWC, 2004).

In this study analyses of mtDNA control region sequences of common minke whale are conducted with the objective to evaluate the hypotheses on stock structure listed by the IWC SC for both J and O stocks. Analyses are based on two mtDNA data sets one for common minke whales distributed around the Korean Peninsula and the other for common minke whales distributed in adjacent waters of Japan.

MATERIALS AND METHODS

Samples

Samples used in the mtDNA analysis were common minke whales by-caught in set net fisheries in Korea and Japan and whales sampled under JARPN/JARPN II in the western North Pacific by Japan (coastal and offshore whaling). Table 1 shows the number of Japanese samples by source, sub-area and month. Table 2 shows the number of Korean samples by sub-area and month. By-catch samples were for the period 1999-2007 while that JARPN/JARPN II samples were for the period 1994-2007. During the *IST* specifications conducted in 2003, eighteen sub-areas were set for management purpose of the western North Pacific common minke whale (Figure 1). These sub-areas were used for testing the hypotheses on stock structure of common minke whales.

Sequencing of the mtDNA control region

Using established protocols (Sambrook *et al.*, 1989), total-cell DNA was extracted from skin tissue samples. The first half of control region of the mitochondrial genome was amplified using the polymerase chain reaction (PCR). In order to amplify an approximately 500 bp of the mtDNA control region, primers light-strand MT4 (Árnason *et al.*, 1993) and heavy-strand Dlp5R (*5'*-CCATCGAGATGTCTTATTTAAGGGGAAC-*3'*), were used. PCR products were purified by MicroSpin S-400HR columns (Pharmacia Biotech). Cycle sequencing was performed with the same primers, using BigDye terminator cycle sequence Kit (Applied Biosystems, Inc). The cycle sequencing products were purified by AutoSeq G-50 spin Columns (Pharmacia Biotech). The labeled sequencing fragments were resolved by electrophoresis through a 5% denaturing polyacrylamide matrix on an ABI 377™ and ABI3100 Automated DNA Sequencer (Applied Biosystems, Inc), following the protocols of the manufacturer. For each sample both strands were sequenced.

In 2008 the IWC SC adopted the 'Guidelines for DNA quality control for genetic studies relevant to IWC management advice' and it recommended adherences to these guidelines in studies explicitly carried out to give stock definition advice to the Commission (IWC, 2009). In this study these guidelines were followed as much as possible. Samples used for extracting DNA came from scientific surveys (JARPN and JARPN II) where biologists are in charge to collect samples and stored it a suitable buffer. Samples from JARPN and JARPN II and those from by-catches were considered of good quality (e.g. no problem with degraded samples). Labeling of each DNA samples are crosschecked with the labeling of animals in the comprehensive data base of JARPN/JARPN II. Primer used for the amplification and sequencing of the mtDNA control regions have been used in previous studies of the common minke whales. Some of the sequences were checked through DNA surveillance routines (Ross and Shearman, 2009) and these were found of high quality.

There was however an unusual large number of singleton haplotypes in the Japanese mtDNA data base, which were defined by an unusual large number of transversion, which is uncommon in intra-specific surveys of cetacean control region sequences (SC/62/NPM20). Therefore all the singletons in the database (70) were re-sequenced two or three times to check for inconsistencies. It was concluded that there was no problem with 28 of the original singletons, but that there were some sequence errors in the other 42. Once corrected the sequences for these 42 cases the sequences were allocated to haplotypes: 39 were allocated to existing haplotypes while three remained as singleton haplotypes. Although no inconsistencies were found for the non-singleton haplotypes re-sequencing was carried out for randomly selected samples (non-singleton haplotypes) in the database. No inconsistencies have been found so far for the non-singleton haplotypes. Most of the sequence errors occurred in more recently collected samples in the data base. This coincides with the date in which a new, less experienced technician started working at the laboratory. Then a combination of a less experienced person at the laboratory and a less strict checking of the new haplotypes by the researcher in charge (due to excess of work), was the main cause for these inconsistencies.

Considering the problems found with the singletons in the Japanese database it was decided to check the Korean database for similar problems. This exercise resulted in a number of corrections in the Korean database as follow: 13 singleton haplotypes in the original database were corrected allocated to other common haplotypes; 16 samples had wrong assigned haplotypes ID.

Measures are being taken so that these errors do not occur again in the future. The present mtDNA analysis is based on the corrected databases (ver 2.0 in the case of the Japanese database).

Data analysis

Assignment of whale individual to stock by an independent genetic marker

O and J stock common minke whales are highly differentiated for both genetic and non genetics markers. (Kanda *et al*., 2009a) used a set of 16 microsatellites and the program STRUCTURE to assign individual common minke whales to J and O stocks. In their study individual whales with membership probability of over 90% for either stock were defined as 'pure' J or O while those with probability less than 90% were defined as individual whales of unknown origin (unassigned individuals).

For the present mtDNA analysis samples were first separated for these two differentiated J and O stocks based on the work by Kanda *et al*. (2009a). Then hypothesis testing (which is more powerful tool to detect differences in more weakly differentiated stocks) was conducted for the samples considered to be composed largely by individuals from one of these stocks. Hypothesis testing was carried out at three levels depending of the classification of the microsatellite analysis: total samples (including 'pure J', 'pure O' and unassigned individuals); 'pure J' <u>or</u> 'pure O' plus unassigned individuals; and 'pure J' <u>or</u> 'pure O' (without the unassigned individuals).

The randomized chi-square Test of Independence (Roff and Bentzen, 1989) was used to investigate the temporal/spatial differentiation of mtDNA. F_{ST} was also calculated using the software of Analysis of Molecular Variance (AMOVA) (Excoffier *et al*. 1992), to get an idea of the effect size between the groups of samples tested. For each chi-square test a total of 10,000 permutations of the original data were performed. A *p*-value smaller than 0.05 was used as a criterion to reject the null hypothesis of panmixia.

Samples were grouped under temporal (months) and geographical criteria following the specifications of the hypotheses listed by the IWC SC for both J and O stocks (IWC, 2004; in press).

RESULTS

mtDNA haplotypes

The Japanese data set included mtDNA sequences from a total of 2,544 individual whales, 1,232 from JARPN/JARPN II offshore, 480 from JARPN/JARPN II coastal and 832 from by-catches. The total number of 2,551 individuals in Table 1 includes some individual for which sequences were not available. This data set contained 118 unique sequences (haplotypes) of which 31 corresponded to singletons.

The Korean data set included mtDNA sequences from a total of 478 individual whales. This data set contained 31 haplotypes, 28 of which were shared with the Japanese samples. Three haplotypes were specific to the Korean sample: '2' (n=3), '180' (n=1) and '181' (n=1).

Hypothesis testing for J stock

Seasonal structure

Hypothesis 4 for J stock (IWC, 2009) proposes two J stocks which migrate through the Sea of Japan at different times of the

year. To test this hypothesis samples were grouped into temporal group (three monthly groups, Table 3) both in the Sea of Japan and Pacific side of Japan. The rational for this seasonal division followed that used in the microsatellite analysis (SC/62/NPM11). Previous observations had found that the number of bycatches differed by month. The only mtDNA significant difference was found for sub-area 7 for total samples (Table 3).

Geographical structure
Hypothesis 2 proposes two stocks, one distributed around the Korean Peninsula and the other in the west and east coast of Japan while hypothesis 3 proposes two J stocks, one distributed in the Yellow Sea and the other in the Sea of Japan and Pacific coast of Japan.

Table 4 shows the results of the tests focused to evaluate these hypotheses. The *p*-value in the comparison of samples obtained around Japan increases (and the *Fst* values decrease) from the situation of using total samples to the situation of using 'pure J' (Table 4). No significant differences were found between J stock animals obtained in different sub-areas around Japan. No significant differences were found in the comparison between sub-area 5 (Yellow Sea) and sub-area 6 around the Korean Peninsula (Table 4). A *p*-value smaller than 5% level was found when Japanese and Korean J animals ('Pure J') were compared (Table 4). The *p*-value of this comparison becomes larger than 5% when only whales in subarea 6 in Korea are compared to whales in Japan. The *Fst* value was very small in both cases.

Hypothesis testing for O stock
Geographical structure
Samples were grouped according the specifications of hypotheses A (Table 5), C (Table 6) and D (Table 7). Samples involved the category 'pure O' + unassigned individuals. This means that 'pure J' individuals were excluded from sub-area 7W. As no significant differences were found at this level there was no need to test for the category 'Pure O'.

As shown in Tables 5, 6 and 7 no significant differences were found in these comparisons and the *Fst* values were quite small. These results suggest no sub-structure within the O stock.

DISCUSSION

In this study hypothesis tests were conducted to examine different stock structure hypotheses listed for both J and O stocks. Analyses were based on updated mtDNA data bases for both Korea and Japan. Revision of these two data sets derived in the corrections of several singleton haplotypes. The reasons for the errors in the previous versions of the data bases were identified and measures taken so that these errors do not occur again in the future.

In this study the samples were assigned to J or O stocks according to the results of microsatellite analyses and three broad categories were used: total samples, 'pure' stock plus unassigned samples and 'pure' stock. This was done in an attempt to avoid bias derived from the presence O and J stock animals in the hypothesis testing analysis of the J and O stocks, respectively. Although there is room for additional discussion of the categories by the microsatellite analysis, the grouping categories used in this study are considered reasonable. It is important to note here that O types and J types defined by haplogroups showed good concordance with the O and J clusters in the STRUCTURE analysis of microsatellite loci by Kanda *et al*. (2009a) (SC/62/NPM20). The occurrence of two differentiated stocks in the western North Pacific have been recognized for a long time, and marked differences in genetic and non-genetics characters have been reported. It is not reasonable to conduct analysis on stock structure without attempting to separate individuals from these two stocks at first. Conclusions on sub-stock structure within the J and O stocks based on samples containing individuals from both stocks are misleading. Here the mtDNA analysis was conducted on samples grouped into J and O stocks by an independent marker.

Hypothesis testing was the main analytical tool to examine genetic differentiation between samples grouped under temporal and geographical criteria. Limitations of the hypothesis testing for delineating stocks have been already identified. There are difficulties when the level of dispersal is low and panmixia can not be rejected owing to low statistical power, or when the degree of statistical power is sufficient to reject panmixia even if the dispersal rate is such that populations are demographically correlated (Palsboll *et al*., 2006). Then careful considerations should be given to interpret *p*-values from the hypothesis testing particularly when the groups tested are set under arbitrary geographical and temporal criteria.

This study deliberately avoided excessive sub-divisions of the samples. Additional sub-divisions derive in groups with smaller sample size decreasing the power of the analysis and making difficult the interpretation of results. Instead the analyses were focused to test samples grouped under broad hypotheses. Furthermore hypothesis testing was carried out only by the chi-square test and the *Fst* was calculated only with the purpose to get an idea of the effect sizes between two groups tested. This avoided the difficulty to interpret different p-values from different analytical tests.

Some explanations are given here for the low *p*-value (0.035) found in the comparison between Korean and Japanese 'pure

J' : a) despite the criteria used to exclude O stock animals from the sample from Japan some of them could have remained causing some effect in the statistical test. This is supported by the result of a comparison of samples from sub-area 6 Korea vs sub-area 6 Japan in which a *p*-value of 0.092 was found (*Fst*=-0.000). This suggests that the effect of unrecognized O stock animals could be larger in the sample from the Pacific side of Japan; b) whales from sub-area 5 (Yellow Sea) could have an effect on the statistical test. There are some previous biological and genetic evidences (SC/62/NPM11) suggesting possible differentiation of whales in the Yellow Sea. This is supported by the result of the comparison between Korean and Japan, which excluded sub-area 5. The *p*-value in this case was 0.071 (*Fst*=-0.001); c) there are two different J stocks. It should be noted here that the small *Fst* values obtained in the mtDNA comparison suggest substantial dispersal between Korean and Japanese J stock whales and therefore explanations a) and b) above are more plausible.

When no significant differences are found then we should ask whether or not the power of the analysis has been high enough to detect differences between stocks. On this regard the power of the genetic analyses on western North Pacific common minke whales has been evaluated for microsatellites (Kanda *et al*., 2009b) and these authors concluded that the power of their analysis was high. Although the power of the mtDNA analysis has not been evaluated yet the powers can be considered high given the sensitivity of this marker to detect differences and the large sample size used (Tables 1 and 2).

In general the updated mtDNA provided no support for additional sub-division in the J and O stocks, although the low *p*-value obtained in the comparison between Korean and Japanese 'pure J' should be further investigated under the two explanations provided above. Results of the mtDNA analysis in general are consistent with those of the microsatellites (Kanda *et al*., 2009b; SC/62/NPM11). The microsatellite analysis detected small differences between Korean and Japanese whales, which were attributed to a different stock in the Yellow Sea. One of the explanations for the low p-value found in the mtDNA comparison between Japan and Korean whales was also attributed to whales in the Yellow Sea.

The results of the analyses focused on the stock structure hypotheses of the O stock in the Pacific side of Japan provide little support for sub-division of the O stock. No support was found for hypotheses C and D. Regarding hypothesis A the microsatellite analysis detected some differences in sub-areas 9 (Kanda *et al*., 2009b) which is similar to some differences found by the mtDNA analysis in sub-area 9 in a previous analysis (Goto *et al*., 2009). This result requires further consideration in the context of hypothesis A.

ACKNOWLEDGEMENTS

We thank researchers and crew of the JARPN and JARPN II surveys conducted between 1994 and 2007 for the collection of samples used in this study. We also thank H. Oikawa for his laboratory assistance.

REFERENCES

Árnason, Ú., Gullberg, A. and Widegten, B. 1993. Cetacean mitochondrial DNA control region: Sequences of all extant baleen whales and two sperm whale species. *Mol. Biol. Evol.*, 10, 960-970.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-91.

Goto, M., Kanda, N., Kishiro, T., Yoshida, H., Kato, H. and Pastene, L.A. 2009. Further mitochondrial DNA analysis on stock structure in the western North Pacific common minke whales. Paper SC/61/JR7 presented to the IWC Scientific Committee, May 2009, Madeira, Portugal (unpublished), 10pp.

International Whaling Commission. 2004. Report of the Sub-Committee on the Revised Management Procedure. *J. Cetacean Res. Manage.* 6 *(Suppl)*: 57-184.

International Whaling Commission. 2005. Report of the Sub-Committee on the Revised Management Procedure. *J. Cetacean Res. Manage.* 7 (Suppl.): 77-113.

International Whaling Commission. 2009. Report of the Working Group on Stock Definition. *J. Cetacean Res. Manage*. 11 (Suppl.): 248-257.

International Whaling Commission. (*in press*). Report of the Working Group on the In-Depth Assessment of Western North Pacific Common Minke Whales, with a focus on J-stock (Annex G1).

Kanda, N., Goto, M., Kishiro, T., Yoshida, H., Kato, H., and Pastene, L.A. 2009a. Individual identification and mixing of the J and O stocks around Japanese waters examined by microsatellite analysis. Paper SC/61/JR5 presented to the IWC

Scientific Committee, May 2009, Madeira, Portugal (unpublished), 14pp.

Kanda, N., Goto, M., Kishiro, T., Yoshida, H., Kato, H., and Pastene, L.A. 2009b. Further microsatellite analysis of minke whales in the western North Pacific. Paper SC/61/JR8 presented to the IWC Scientific Committee, May 2009, Madeira, Portugal (unpublished), 14pp.

Palsboll, P.J., Berube, M. and Allendorf, F.W. 2006. Identification of management units using population genetic data. *Trends in Ecology and Evolution* 22 (1):11-16.

Roff, D.A., and Bentzen, P. 1989. The statistical analysis of mtDNA polymorphisms: χ^2 and the problem of small samples. *Mol. Biol. Evol.*, **6**, 539-545

Ross, H.A. and Shearman, H. 2009. GenBank sequence assessment for species assignment-control region sequences published for baleen whales in 2007. Paper SC/61/SD3 presented to the IWC Scientific Committee. May 2009 (unpublished). 10pp.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. *Molecular cloning: A laboratory manual. 2nd Ed*., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Table 1. Number of samples in the Japanese mtDNA data base, by month, sub-area and source (by-catch（JBC) and JARPN/JARPNII (coastal and offshore)).

Source	Sub-area	Month												Total
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
JBC	2	32	26	14	8	15	6	13	5		5	18	42	184
	6	61	35	38	60	48	26	16	24	14	14	31	44	411
	7	10	5	8	29	40	40	19	9	6	7	19	20	212
	10										1	6	2	9
	11						1			1	6	8		16
	Sum	103	66	60	97	103	73	48	38	21	33	82	108	832
Coastal	7				110	117				150	104			481
Offshore	7					122	178	32	27	103				462
	8					38	94	79	12	1				224
	9					38	83	155	186	10				472
	11							50	30					80
	Sum					198	355	316	255	114				1238
Total		103	66	60	207	418	428	364	293	285	137	82	108	2551

Table 2. Number of samples in the Korean mtDNA database, by month and sub-area.

	Sub-area	Month													Total
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	?	
KBC	6	39	20	31	36	38	43	17	18	26	22	36	53	1	380
	5/6	4	1	2	5	15	13	6	3			5	2		56
	5	4	1	2		4	12	4	4	3	2	3	3		42
	Total	47	22	35	41	57	68	27	25	29	24	44	58	1	478

Table 3. Seasonal (March to June, July to October and November to February) mtDNA differentiation of common minke whales (J stock) in the Sea of Japan and Pacific side of Japan based on by-catch samples.

		All samples		'Pure J' + unassigned		'Pure J'	
		chi-sq p-value	*Fst*	chi-square p-value	*Fst*	chi-square p-value	*Fst*
JCB	SA2	0.688	-0.006	0.902	-0.008	0.731	-0.009
	SA6	0.472	-.0.000	0.409	-0.000	0.313	0.000
	SA7	0.018	0.021	0.194	0.017	0.718	0.002
KBC	SA5	0.335	0.015				
	SA5/6	0.637	0.048				
	SA6	0.584	-0.000				

Table 4. Geographical (oceans, sub-areas) mtDNA differentiation of common minke whales (J stock), based on by-catch and JARPN/JARPN II samples

	Chi-square p-value	*Fst*
JP SA2, 6, 7, 10, 11(All samples)	<0.0001	0.036
JP SA2, 6, 7, 10, 11 ('Pure J'+unassigned)	0.072	0.007
JP SA2, 6, 7, 10, 11 ('Pure J')	0.115	0.005
KBC SA5 vs SA6	0.324	-0.0013
KBC SA5, SA5/6, SA6	0.736	0.001
KBC vs Japan (all samples)	<0.0001	0.026
KBC vs Japan ('Pure J'+unassigned)	0.0007	0.001
KBC vs Japan ('Pure J')	0.035	-0.001

Table 5. mtDNA differentiation between 9W and 9E by year and total samples of O stock (Hypothesis A).

	Sample size		Chi-square	
	9W	9E	p-value	*Fst*
1994	7	14	0.543	-0.029
1995	78	22	0.103	0.029
1997	19	48	0.858	-0.007
2003	25	14	0.063	0.031
2004	42	42	0.347	0.002
2005	19	30	0.456	-0.002
Total	291*	176*	0.555	0.003

*: including 2000, 2001, 2002, 2006 and 2007 samples

Table 6. mtDNA differentiation for whales grouped according geographical boundaries of Hypothesis C of O stock.

Combination of samples	Chi-square P value	*Fst*
7E x 8W x 8E	0.234	0.003
9W x 9E	0.555	0.003
7W x 7E-8E x 9W-9E	0.031	0.001
7W* x 7E-8E x 9W-9E	0.852	-0.000

*: 'Pure J' samples were excluded from the 7W sample.

Table 7. mtDNA differentiation for whales grouped according geographical boundaries of Hypothesis D of O stock.

Combination of samples	Chi-square P value	*Fst*
7E x 8W x 8E x 9W	0.174	0.004
7W x 9E	0.249	0.001
7W* x 9E	0.629	0.000
7W x 7E-9W x 9E	0.055	0.001
7W* x 7E-9W x 9E	0.861	-0.000

*: 'Pure J' samples were excluded from the 7W sample.

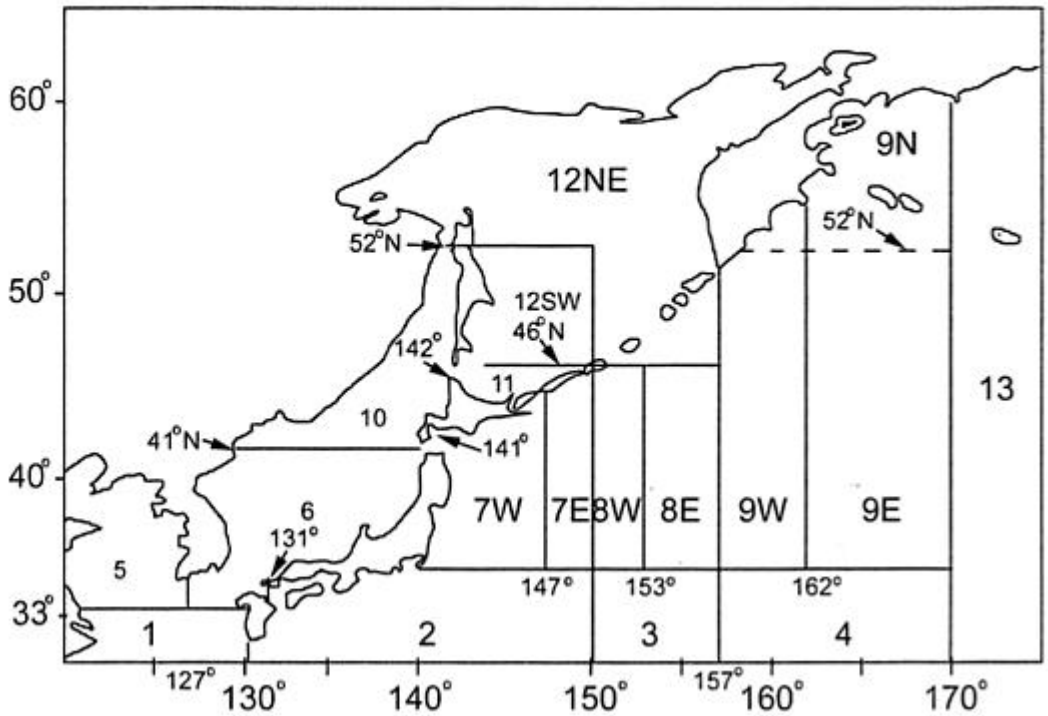

Figure 1. The 18 sub-areas used for the *Implementation Simulation Trials* for North Pacific minke whales in 2003.