Genetic analysis of western North Pacific minke whales from Korea and Japan based on microsatellite DNA

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ABSTRACT

At the final stage of the *Implementation* process for the western North Pacific (WNP) common minke whales, the IWC Scientific Committee (SC) adopted four stock structure scenarios (baselines A, B, C, and D)(IWC, 2004). Previous papers by some of the current authors conducted stock identification at an individual level using a Bayesian clustering method (Structure; Pritchard *et al*., 2000), and subsequently examined the plausibility of these four stock baseline scenarios, based on the genetic variation at 16 microsatellite DNA markers analyzed from samples of minke whales collected during JARPNII as well as JARPN from 1994 to 2007. The stock identification successfully distinguished most of the sampled whales (91%) to the J and O stocks, and subsequent stock structure analysis with the identification showed that 1) whales from the J stock existed in the SA7 west (7W) with low but large enough number to cause genetic heterogeneity observed in the 7W samples as well as between the 7W and other samples, 2) except the J stock whales, the survey area was mainly occupied by O stock, and 3) the stock structure scenarios that assumed genetically distinct stock in the 7W (baselines C and D) were not supported. It was argued, however, that Structure could have overlooked weakly differentiated stocks under the genetic difference between the J and O stocks, and thus some, if not all, of the unassigned individuals from the stock identification may belong to different stocks from the J and O. Alternately, these individuals were unassigned due to the lower statistical power than we have expected. In order to examine the status of the unassigned individuals, this paper conducted some additional Structure runs and Principal Coordinate analysis. The results of these analyses failed to detect evidence of the additional stocks and suggested that these unassigned individuals were probably either J or O stocks. Similar to the previous papers, our study support that WNP is mainly occupied by the O stock.

In addition to the stock structure in WNP, examination of the stock structure in Sea of Japan (SOJ) and Yellow Sea (YS) is important for pre *Implementation* assessment for common minke whales. The SC listed seven stock structure hypotheses that proposed a single to maximum three stocks in SOJ and YS. We combined the Korean genetic data of bycaught minke whales from 1999 to 2007 to the Japanese genetic data to examine stock structure of them in the WNP, SOJ, and YS. The samples of 2005 to 2007 were not used in the previous papers. Both conventional hypothesis testing and Structure analysis were conducted to identify the number of stocks in the SOJ and YS. The Structure analysis did not show evidence of stock structure in SOJ and YS, while the conventional hypothesis testing detected very weak genetic differences within the Korean samples as well as between the Korean and Japanese samples. The results suggested there may be a sub-stock that mainly occupies YS (Y stock) and some whales in the Y stock appear to migrate north along the Korean coast that could have caused the genetic difference between the Korean and Japanese samples in SOJ. More samples from YS will be needed for further analyses.

In summary, we accept the previous view that main stocks inhabiting the Korean and Japanese waters were the J and O stock. In addition to the two stocks, the Y sub-stock may occupy YS but further analyses with more samples will be needed to reach a final conclusion.

KEY WORDS: MINKE WHALE, MICROSATELLITE, STOCK STRUCTURE, NORTH PACIFIC, SEA OF JAPAN, YELLOW SEA

INTRODUCTION

Common minke whales, *Balaenoptera acutorostrata*, are the smallest and the most abundant baleen whale species inhabiting major open oceans world-wide with spatial and temporal separations among populations (Pastene *et al*., 2007). They live up to 50 years in age and the adult size is, on average, 6-7m. They feed on various prey species, such as copepods, Euphausiids, and fish. Their age at first reproduction is five, and they are thought to reproduce every year. As typical baleen whales, minke whales undergo seasonal movement from winter breeding grounds in low latitude to summer feeding grounds in high latitude. Around the ocean off the Japanese coast, at least two different stocks of minke whales are known to exist: one stock distributes in the western North Pacific (WNP) and the other in the Sea of Japan (SOJ) (Omura and Sakiura, 1956; Ohsumi, 1977; Kato, 1992; Wada and Numachi, 1991; Goto and Pastene, 1997; Pastene *et al*., 2007).

The IWC Scientific Committee (SC) completed the RMP *Implementation* for the WNP common minke whales during the 2003 Annual Meeting. At the final stage of the *Implementation* process, the SC adopted the following stock scenarios in the WNP (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.

The SC did not examine the plausibility of each baseline scenario at all because it was afraid that any conclusions would not have been accepted by all. Consequently, the SC rated all of the scenarios the same ‘high’ plausibility.

In Kanda *et al*. (2009b; see also Kanda *et al*., 2009a and Goto *et al*., 2009), the plausibility of these four baseline stock scenarios was examined analyzing samples of minke whales collected from JARPN and JARPNII conducted from 1994 to 2007 using 16 microsatellite DNA markers. Results of the paper indicated 1) whales from the J stock existed in the 7W with low but large enough number to cause genetic heterogeneity observed in the 7W samples as well as between the 7W and other samples, 2) except the J stock whales, the survey area was mainly occupied by O stock, and 3) the baselines C and D were not supported because no other genetically distinct stock was observed in the survey area.

Prior to conducting the analyses in Kanda *et al*. (2009b), Kanda *et al*. (2009a) genetically assigned each individual in the samples to either pure O, pure J and unassigned individuals using the Bayesian clustering method implemented in the computer program Structure (Pritchard *et al*. 2000). The tests for stock differentiation were then carried out with the suspected J stock individuals (all individuals included) and without the suspected J stock individuals (individuals of unassigned and O stock included) as well as with only the suspected O stock individuals (individuals of unassigned and the J stock excluded).

One of the arguments for the results of these papers was how to interpret the unassigned individuals. Probably due to the low statistical power of the data, it was difficult to distinguish the two possibilities, that is, they were from the J or O stocks or from additional stocks. Because the two stocks are not completely distinct, there may be individuals genetically indistinguishable to the J and O stocks. Alternately, signals from the additional weakly differentiated stocks might have been overwhelmed by the relatively large O-J differences.

In addition to the stock structure of minke whales in the North Pacific, Japanese and Korean scientists have worked together to analyze the samples from Japan and Korea using mtDNA and microsatellites to investigate stock structure of common minke whales in SA5 and SA6 of the Japanese and Korean waters (Goto *et al*., 2007; Kanda *et al*., 2006; Park *et al*., 2006). On the basis of the results from these analyses, the Working Group on the in-depth assessment of WNP common minke whales, with a focus on J stock, has listed following seven stock structure hypotheses for minke whales inhabiting Yellow Sea (YS), SOJ, and Pacific coast of Japan (IWC, 2010).

(1) One stock, J: J stock migrates to YS, SOJ, and the Pacific coast of Japan.
(2) Two stocks, J and Y: J stock migrates along both coasts of Japan, and Y stock migrates along the Korean coast.
(3) Two stocks, J and Y: J stock migrates through the SOJ and Pacific coast of Japan, and Y stock migrates up to the YS.
(4) Two stocks, J and Y: J and Y stocks migrate through YS and SOJ at different times of year.
(5) Three stocks, J, K, and Y: J stock migrates along both coast of Japan, K stock along Korean coast, and Y stock up to YS.
(6) Three stocks, Je, Jw and Y: Je stock migrates along the Pacific coast of Japan, Jw stock through SOJ, and Y stock up to YS.
(7) Three stocks, Je, Jw and Y: Je stock migrates along the Pacific coast of Japan, Jw stock along the west coast of Japan, and Y stock migrates along the Korean coast including YS.

Among the seven hypotheses, only the first four were agreed by the members of the working group.

The primary objectives of this study were two-fold. First, we attempted to better identify the status of the unassigned individuals in the previous papers conducting analyses recommended from the working group on the in-depth assessment of WNP common minke whales, with a focus on J-stock in the SC61 (IWC, 2009). We did some new Structure analyses with only unassigned individuals as well as with pure O plus unassigned individuals to detect the evidence of some additional, weakly-differentiated stocks. Moreover, we also conducted Principal Coordinate analyses to better identify the status of the unassigned individuals to describe their relations in the two dimensional space.

Second, we combined the Korean genetic data of bycaught minke whales from 1999 to 2007 to the Japanese genetic data to examine stock structure of them in the WNP, SOJ, and YS. The samples of 2005 to 2007 were not used in the previous papers. We conducted both conventional hypothesis testing and Structure analysis to identify the number of stocks in the SOJ and YS.

MATERIALS AND METHODS

Samples

Table 1 shows the number of individuals used in this study for each of the sub-areas (SA) set for management purpose of the WNP common minke whale (Fig.1). Common minke whale samples from 1994-1999 JARPN and 2000-2007 JARPNII offshore component were used. These surveys were conducted mainly summer in SA7, 8, 9, and 11. Another source of the minke whale samples was the coastal component of the JARPNII survey conducted from 2002 to 2007. A total of nine surveys had been conducted as the coastal component of the JARPNII: spring surveys at Sanriku in 2003, 2005, 2006, and 2007, and fall surveys at Kushiro in 2002, 2004, 2005, 2006, and 2007. Minke whales that were bycaught on set net fishery conducted along the Japanese coast from 2001 to 2007 were also used (bycatches). As of July 1st 2001, the new regulation governed by the Japanese government has allowed the set net fishermen to harvest whales found in their set net and to sell these on to the market after DNA registration of these for individual identification. The bycatches used were obtained from the SA2, SA6, SA7, SA10, and SA11 year-round. Details about the samples can be found in Kanda *et al*. (2009a; 2009b), Kishiro *et al*. (2009), and Tamura *et al*. (2009).

Bycatches from Korea are those bycaught in coastal fishing gears along the Korean peninsula in SA6 and SA5 from 1999 to 2007 (Table 1). The Korean samples were divided into three on the basis of where they came from: K6 = eastern side of the Korean Peninsula (SA6), K5-6 = southern side of the Korean Peninsula (Tushima (Korean) Strait; SA5+SA6), and K5 = western side of the Korean Peninsula (SA5).

In order to look for evidence of seasonal variation for the bycatches, we first looked at the number of bycatches by month from Korea (K6, K5-6, and K5 samples) and Japan (J6) (Table 2; Fig. 2). In SA6, both the K6 and J6 showed a similar pattern that the number increased in spring to early summer as well as in winter and decreased from summer to fall. Similar seasonal increase in May and decrease in summer was also observed in the K5-6 and K5 although the increase in winter was not observed. It could be partially because of the small sample size. Based on these observations, we divided these samples from SA5 and 6 into three groups by their collection date to describe seasonal pattern of genetic differentiation: MJ = March to June, JO = July to October, and NF = November to February.

Microsatellite analysis

Skin tissues of minke whales taken during the JARPNII were stored in 95% ethanol until DNA extraction. Genomic DNA was then extracted from 0.05g each of the skin tissues using standard proteinase K, phenol-chloroform procedure described by Sambrook *et al*. (1989). Extracted DNA was stored in the TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Microsatellite polymorphisms were analyzed using 16 sets of primers: EV1, EV14, EV21, EV37, EV94, (Valsecchi & Amos 1996), GT23, GT195, GT211, GT310, GT509, GT575 (Bérubé *et al*., 2000), GATA28, GATA98, GATA417, TAA31 (Palsbøll *et al*., 1997), and DlrFCB14 (Buchanan *et al*., 1996). EV1, EV14, EV21 were developed from sperm whale (*Physeter macrocephalus*), EV37, EV94, GT23, GT310, GT575, GATA28, GATA98, GATA417, TAA31 were from humpback whale (*Megaptera novaeanglia*), and DlrFCB14 from beluga whale (*Delphinapterus leucas*). All GT, EV, and DlrFCB primers were dinucleotide repeat, TAA31 trinucleotide repeat, and all GATA primers tetranucleotide repeat. Primer sequences and PCR profiles follows those of the original authors with slight modifications.

PCR amplifications were performed in 15μl reaction mixtures containing 10-100ng of DNA, 5 pmole of each primer, 0.625 units of Ex *Taq* DNA polymerase (Takara Shuzo), and 2mM of each dNTP, and 10x reaction buffer containing 20mM $MgCl_2$ (Takara Shuzo). PCR amplifications followed the manufacture's instructions for the use of Ex *Taq* DNA polymerase (Takara Shuzo). Amplified products with internal size standard (GENESCAN400HD, Applied Biosystems Japan) were run on a 6% polyacrylamide denaturating gel (Long Ranger™) using an BaseStationTM 100 DNA fragment analyzer (Bio-Rad). Although alleles were visualized using Cartographer™ software specifically designed for the BaseStation, allelic sizes were determined manually in relation to the internal size standard and minke whale DNA of known size that were rerun on each gel.

Data analysis

The number of alleles per locus, allelic richness, and heterozygosity were computed using FSTAT 2.9.3 (Goudet 1995). Conventional hypothesis testing procedure using heterogeneity test in frequencies of the microsatellite alleles among samples was performed in the GENEPOP 4.0 (Rousset, 2008). Null hypothesis to be tested is if the samples came from a genetically same group of minke whales. If genetic differences exist, then it could indicate these samples came from genetically different stocks of minke whales. Statistical significance was determined using the chi-square value obtained from summing the negative logarithm of *p*-values over the 16 microsatellite loci (Sokal and Rohlf, 1995). F_{ST} was also calculated using GENEPOP 4.0. The samples with less than 5 individuals were excluded from the genetic divergence analyses.

The Bayesian clustering approach was implemented with the microsatellite data in the Structure 2.0 (Pritchard *et al*., 2000) to determine the most likely number of genetically distinct stocks present in our samples. Posterior probabilities for K were estimating from three independent runs for each value of K from one to five with only genetic information. These data were calculated based on burn-in period of 10,000 iterations and runs of 100,000 iterations. Individual assignment was then conducted for the most plausible K using estimated individual proportion of membership probability. The ancestry model we used for the simulation was the admixture model, which assumes individuals may have mixed ancestry. The allele frequency model used was the correlated allele frequencies model, which assumes frequencies in the different stocks are likely to be similar due to migration or shared ancestry.

Principal coordinate analysis (PCA) implemented in GenAlEx (Peakal *et al*., 2006) was used to describe relationships among individuals with ordination. PCA is a multivariate technique that allows us to find and plot the major patterns within a multivariate data set (e.g., multiple loci and multiple samples). GenAlEx is a computer program for population genetic analysis running within Microsoft Excel™. For PCA, we needed to convert genetic data into a pairwise individual by individual genetic distance matrix. The genetic distance matrices generated then form the input of PCA. Within the GenAlEx program, a pairwise individual-by-individual (N x N) genotypic distance matrix is calculated for codominant data using the following rules: For a single-locus, with i-th, j-th, k-th and l-th different alleles, a set of squared distances is defined as d2(ii, ii) = 0, d2(ij, ij) = 0, d2(ii, ij) = 1, d2(ij, ik) = 1, d2(ij, kl) = 2, d2(ii, jk) = 3, and d2(ii, jj) = 4. Genetic distances are summed across loci under the assumption of independence. The matrix generated is then used for subsequent PCA analysis.

DNA data quality control under the IWC guidelines

In order to minimize errors in genetic data, we were recommended to follow the IWC approved guidelines for DNA data quality control for genetic studies (IWC, 2009). Since JARPN started, we have established standard sampling, sample handling, and laboratory practice procedures to ensure consistent data generation. These procedures are quite well consistent to the IWC guidelines except we did not score microsatellite alleles by two persons for double-check. During the data collection, some individuals in the samples were genotyped more than twice for double check. For data consistency and plausibility, we used the computer program MICRO-CHECKER (van Oosterhout *et al*., 2004) to check for null alleles and reading/typing errors. We also used the GENEPOP to test deviation from Hardy-Weinberg equilibrium expectations and linkage disequilibrium in our samples. We conducted these tests for several different sample groups (by each of sub-areas, SA7 to 9, by stocks (O, J, and unassigned individuals), all Korea, etc.) respectively, and found significant deviations within some groups even after corrections for multiple tests (Rice, 1998) (data not shown). Because the loci involved in the deviations and linkage disequilibrium were not consistent over the sample groups, however, we did not remove any ones from the 16 microsatellite loci for the further analyses. Finally, the guidelines recommended to sequence the microsatellite loci for marker validation. Although we have not had the opportunity yet to do so by ourselves, we have used the published primers that were designed from the cetacean species and were checked for the reliability of amplification and scoring prior to use.

RESULTS

For genetic analyses of only the Japanese samples, we examined genetic variation at the 16 microsatellite loci as described above. For genetic analyses of the Korean and Japanese samples combined, we examined genetic variation at 11 of the 16 microsatellite loci because of difficulty of data standardization between the Korean and Japanese labs.

Stock structure at the Pacific side of Japan (SA7-SA9)

Structure analyses with selected individuals in the samples

On the basis of the results from the individual identifications to stocks according to the criteria in Kanda *et al*. (2009a), we conducted some new Structure runs focusing on the pure O and unassigned individuals to detect some additional, weakly-differentiated stocks. We first conducted Structure with the samples of the pure O and unassigned individuals (N=1772) combined (i.e., pure J excluded) and then with that of only the unassigned individuals (N=240). Both cases presented the highest likelihood probability at K=1 (Table 3). We were unable to detect signals of population differentiation.

Principal coordinate analysis on all samples

PCA was used to describe the relationships among the individuals from all the Japanese samples (N=2542) in a two dimensional space (Fig. 3). We plotted each individuals with the marks based on the individual identification to the stocks (Kanda *et al*., 2009a) to see if there were any concordance between the two independent analyses. The individuals assigned to the pure O and J stocks tended to occupy different spaces, while most of the unassigned individuals tended to occupy the middle space of the pure O and J. PC1 and PC2 explain 25.9 and 15.7 of variance, respectively.

Genetic difference between SOJ side and Pacific side of Japan (SA6 and SA2)

Genetic difference between the bycatch samples from SA6 and SA2 was examined using the genetic variation at 16 microsatellite loci (Table 4). With all the individuals from the samples, the genetic difference was observed between the two areas. However, after removing the pure O stock individuals, that difference was disappeared. Even for the former case, F_{ST} was quite low.

The observed genetic difference should have been resulted from the different proportion of the J and O stock existed in these areas. Approximately 13% of the individuals in the SA2 sample (24/183) were assigned to the O stock compared to only 1% in the SA6 sample (4/411).

Stock structure in SOJ and YS (SA5 and SA6)

Table 5 shows some genetic indices observed from the Korean amd Japanese samples at the 11 microsatellite loci. For the Japanese sample, although we demonstrated no structuring between SA2 and SA6, we used only the bycathes from SA6 to avoid the effect of the pure O individuals in the SA2 sample that was difficult to remove completely. The numbers of alleles per locus and allelic richness were

slightly higher in the Korean samples than in the Japanese ones (Table 5). The expected heterogeneities were similar to each other.

Genetic differentiation between seasonal groups within same areas
We first looked for evidence of genetic difference at the 11 microsatellite loci among the seasonal groups within the same area (Table 6). In the Korean samples, although no significant difference was detected in the K5-6 and K5, significant genetic difference was observed among the groups in the K6. Pair-wise comparisons indicated that the observed difference was due to the JO group. We thus combined the MJ and NF groups into one as K6-MF and separated from K6-JO in the K6. No such difference was observed in the K5-6 and K5 although it could be due to the small sample size. In the J6, no such seasonal difference was detected even though the samples size was large. In these three samples, three seasonal groups in the K5-6, K5, and J6 were combined into one, respectively, as K5-6, K5, and J6.

Genetic differentiation among the areas
We looked for evidence of genetic difference among the K6-MF, K6-JO, K5-6, K5, and J6 (Table 7). Within the Korean samples, the K6-MF showed significant difference to the K6-JO and K5, but not to the K5-6. The K6-JO did not show significant difference to the K5-6 and K5. The K5-6 did not show significant difference to the K5. F_{ST} values among these comparisons ranged from 0.0004 to 0.0044. The J6 showed significant differences to all of the Korean samples, and F_{ST} values ranged from 0.0004 to 0.0042. The lowest F_{ST} value was observed at the comparison between the J6 and K6-MF, which was much lower than that between the K6-MF and K6-JO.

Although statistically significant differences were detected among the Korean and Japanese samples from the heterogeneity tests, Structure analysis did not support the existence of multiple stocks in the area (Table 8). PCA was also used to describe the relationships among the individuals from the SA5 and SA6 (Fig. 4). All of the individuals gathered in the same space. PC1 and PC2 explain 19.4 and 19.0 of variance, respectively.

Stock structure in WNP, SOJ, and YS
Structure analysis for all of the available samples was conducted with the genetic data from the 11 microsatellites. Structure with the total samples (3019 individuals) without information on their geographic origins presented the highest likelihood probability at K=2 (Table 9). Similar to Kanda *et al*. (2009a), we assigned the individuals into the different groups on the basis of their membership probabilities over 0.900. All other individuals with the membership probability less than 0.900 to the either groups were assigned as individuals of unknown origin (unassigned individual). With this criterion, 2441 individuals (81%) were assigned to the either stocks (1085 to stock 1 and 1356 to stock 2). The proportion of the individuals with the membership probabilities over 0.900 was less than that observed from the analysis conducted in Kanda *et al*. (2009) using only the Japanese samples with the genetic data at the 16 microsatellites. This lower proportion could have been due to lower number of microsatellite loci used. Again, Structure was not able to detect the genetic difference within the Korean and Japanese samples we detected using the hypothesis testing.

Spatial distribution of the assigned individuals
Both of the assigned and unassigned individuals were grouped based on their sampling origins (offshore, coastal, bycatch, Korea, and Japan) as well as locations (IWC sub-areas) (Table 10; Fig. 5). Similar to the previous study (Kanda *et al*., 2009), distribution of the pure individuals that were genetically assigned to the different stock was clearly separated geographically. Most of the individuals collected from the SOJ and YS belonged to the Stock 1, whereas almost all of the individuals from the offshore NP (east of the SA7E) belonged to the stock 2. Intermediate areas (SA7W and SA11) contained the individuals from the both stocks. The results again indicated that the Stock 1 and Stock 2 were the J and O stock. Unassigned individuals also distributed all areas with similar proportions.

DISCUSSION
We believe that the results of this study substantially improve our knowledge of the stock structure of minke whales in the WNP, SOJ, and YS.

In the first part of this study, we focused on the unassigned individuals that were not assigned to either the J or O stocks from the previous Structure analysis (Kanda *et al.*, 2009a). The unassigned individuals

could be the whales form some other additional weakly differentiated stocks or could be the whales that were not assigned to the J or O stock due to the low statistical power of the current genetic data. Both additional Structure runs and PCA analysis failed to detect the evidence of additional stocks in the area. In the PCA analysis, although the J and O stock individuals tended to occupy the different spaces, the unassigned individuals tended to occupy the inbetween space, suggesting the unassigned individuals were either the J or O stocks but not the additional stock. Considering the level of the genetic differences between the J and O stocks (Kanda *et al*., 2009a), it is unlikely that all of these individuals were the hybrids between the two stocks. Addition of more number of microsatellite loci may better identify the status of the unassigned individuals.

We found the seasonal differences in the bycatch numbers in the Korean and Japanese samples from SA6. The number of bycatches increased in winter and spring but decreased in summer. Similar, but not as clear, pattern was observed in the Korean samples from SA5. Although the similar seasonal pattern was seen in both the Korean and Japanese samples, evidence of genetic difference between the seasonal groups was only detected in the Korean samples (roughly, winter/spring group x summer/fall group). Furthermore, only one of the two groups (the spring/winter group) from SA6 differed from the samples from SA5. Considering the resident Y sub-stock in YS described in the stock structure hypotheses (IWC, 2010), the evidence of genetic differences we found in the Korean samples might have been due to the stock difference between the J stock and Y sub-stock. We also found the yearly heterogeneity in the winter group ($p<0.05$). This yearly heterogeneity and the very weak heterogeneity between the Japanese and Korean samples from SA6 could be because individuals in the Y sub-stock might migrate to north at some extent along the Korean coast. Each of the SA6 Korean year samples might contain the individuals from the Y stock in different proportions. However, it is important to note that the recent mitochondrial DNA analysis indicated no evidence of sub-stocks in the Korean minke whales (Park *et al*., 2009). Park *et al.* (2009) analyzed the Korean bycatch samples from 1998 to 2005, so that most of the individuals were the same ones to this study.

The most likely explanation for the results of this study is that the main stocks inhabiting WNP, SOJ, and YS are the J and O stock, and that there maybe the Y sub-stock in YS. Further analyses with more samples and more markers will be needed to reach a final conclusion especially for the Y sub-stock.

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Table 1. Number of individuals from the Korean and Japanese samples used in this study.

	Total	SA2	SA5	SA5+6	SA6	SA7W	SA7E	SA8W	SA8E	SA9W	SA9E	SA10	SA11
Japan													
JARPN & JARPNII													
Offshore (94-07)	**1231**					**414**	**47**	**86**	**139**	**291**	**174**		**80**
Coastal (02-07)	**480**					**480**							
Bycatch (01-07)	**831**	**183**			**411**	**212**						**9**	**16**
Korea (99-07)	**477**		**42**	**56**	**379**								

Table 2. Number of bycatches per month in SA5 and SA6 in Korea (99-07) and Japan(01-07).

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Korea													
SA6	39	20	31	36	39	43	17	18	26	22	36	52	379
SA5+6	4	1	2	5	15	13	6	3	0	0	5	2	56
SA5	4	1	2	0	4	12	4	4	3	2	3	3	42
Total	47	22	35	41	58	68	27	25	29	24	44	57	477
Japan													
SA6	61	35	38	60	49	26	16	24	13	14	31	44	411

Table3. Results of Bayesian clustering method analyzed for the Japanese samples from SA7-SA9.

K	Log P(k/x)*	variance*	Pr(k/x)**
O and unassigned			
1	-90909.8	88.7	~1.0
2	-91939.7	2527.1	~0.0
3	-93413.2	5532.1	~0.0
4	-101177.4	21271.3	~0.0
5	-100444.1	19969.6	~0.0
Only unassigned			
1	-11677.1	68.1	~1.0
2	-11713.5	212.2	~0.0
3	-11882.3	564.8	~0.0
4	-12162.2	1131.5	~0.0
5	-11937.4	633.2	~0.0

* log likelihood of the data for different values of K and the variance. ** Probability for each of K.

Table 4. Results of the heterogeneity tests between the Japanese bycatches from SA2 and SA6. Tests were conducted with all individuals and without the O stock individuals.

SA2 x SA6	chi-square	F_{ST}
All individuals	*	0.0008
Without O	ns	-0.0003

* = statistically significant, ns = no significant

Table 5. The number of alleles (A), allelic richness (AR), and expected heterozygosity (He) at 11 microsatellite loci analyzed in the samples of minke whales used in this study.

	Korea all (N=477)			Japan SA6 (411)		
	A	AR	He	A	AR	He
EV37	14	13.6	0.673	7	7.0	0.652
EV1	21	20.4	0.697	18	18.0	0.671
GT575	13	12.6	0.750	10	10.0	0.735
EV94	7	7.0	0.581	4	4.0	0.568
GT23	14	13.6	0.873	12	12.0	0.875
GATA98	8	7.7	0.590	5	5.0	0.568
GATA417	9	8.7	0.670	8	8.0	0.673
GT211	13	12.6	0.775	12	12.0	0.803
EV21	4	4.0	0.334	2	2.0	0.344
DlrFCB14	5	4.9	0.413	3	3.0	0.452
GT195	15	14.8	0.812	8	8.0	0.802
Average	11.2	10.9	0.652	8.1	8.1	0.649

Table 6. Results of the heterogeneity tests between the seasonal groups in the Korean and Japanese samples from SA5 and SA6.

	all	MJ x JO	MJ x NF	JO x NF
Korea SA6	*	*	ns	*
Korea SA5-6	ns	---	---	---
Korea SA5	ns	---	---	---
Japan SA6	ns	---	---	---

MJ = March to June, JO = July to October, NF = November to February

Table 7. Resutls of the pairwise heterogeneity tests and FST among the Korean and Japanese samples from SA5 and SA6.

			chi-square	F_{ST}
Korea SA6 NJ	x	Korea SA6 JO	*	0.0029
Korea SA6 NJ	x	Korea SA5-6	ns	0.0008
Korea SA6 NJ	x	Korea SA5	*	0.0044
Korea SA6 NJ	x	Japan SA6	*	0.0004
Korea SA6 JO	x	Korea SA5-6	ns	0.0015
Korea SA6 JO	x	Korea SA5	ns	0.0044
Korea SA6 JO	x	Japan SA6	*	0.0042
Korea SA5-6	x	Korea SA5	ns	0.0004
Korea SA5-6	x	Japan SA6	*	0.0015
Korea SA5	x	Japan SA6	*	0.0039

NJ = November to June; JO = July to October; * = statistically significant; ns = no significant

Table 8. Results of Structure runs for the Korean and Japanese samples from SA5 and SA6.

K	Log P(k/x)*	variance*	Pr(k/x)**
1	-26839.4	57.2	~1.0
2	-27136.4	1043.4	~0.0
3	-27544.5	2176.0	~0.0
4	-27679.5	2787.1	~0.0
5	-28101.3	3886.6	~0.0

* log likelihood of the data for different values of K and the variance. ** Probability for each of K.

Table 9. Results of Structure runs for all of the Korean and Japanese samples (11 loci).

K	Log P(k/x)*	variance*	Pr(k/x)**
1	-104341.4	72.6	~0.0
2	-100408.9	843.1	~1.0
3	-100977.6	2652.5	~0.0
4	-100723.1	3063.1	~0.0
5	-101459.8	4968.2	~0.0

* log likelihood of the data for different values of K and the variance. ** Probability for each of K.

Table 10. Structure analysis (11 loci) for all the samples from Japana and Korea samples

	J stock	unassigned	O stock	Sum
Korea SA6	303	72	4	379
Korea SA5-6	44	11	1	56
Korea SA5	34	5	3	42
Japan SA10	7	2	0	9
Japan SA6	345	63	3	411
Japan SA2	130	33	20	183
Japan SA11	19	18	43	80
Japan SA11-BC	12	4	0	16
Japan SA7W-BC	92	45	75	212
Japan SA7W-K	34	51	168	253
Japan SA7W-S	33	55	139	227
Japan SA7W	25	76	312	413
Japan SA7E	0	6	42	48
Japan SA8W	1	18	67	86
Japan SA8E	0	31	108	139
Japan SA9W	6	55	230	291
Japan SA9E	0	33	141	174
Sum	1085	578	1356	3019
proportion	0.359	0.191	0.449	

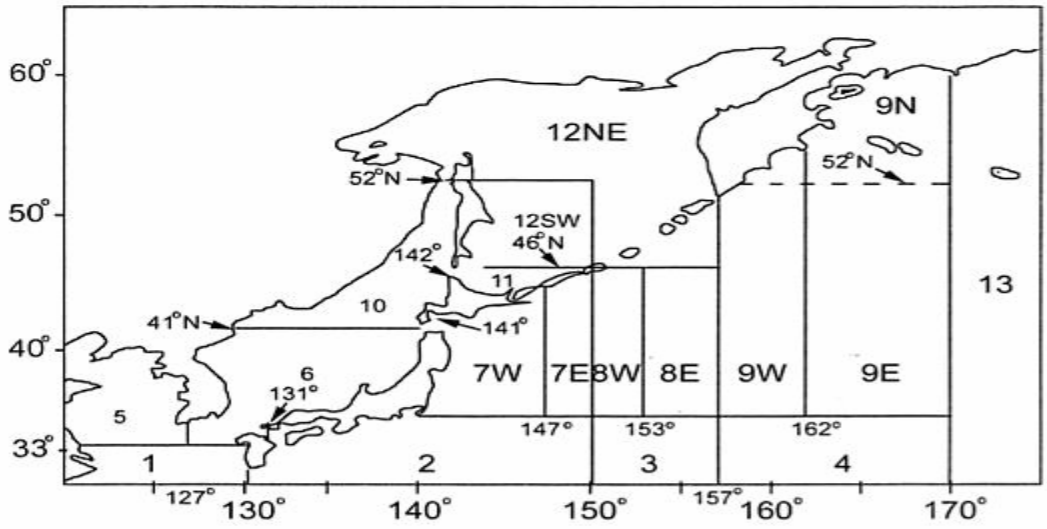

Fig. 1. Eighteen sub-areas used for the *Implementation Simulation Trials* for the North Pacific minke whales.

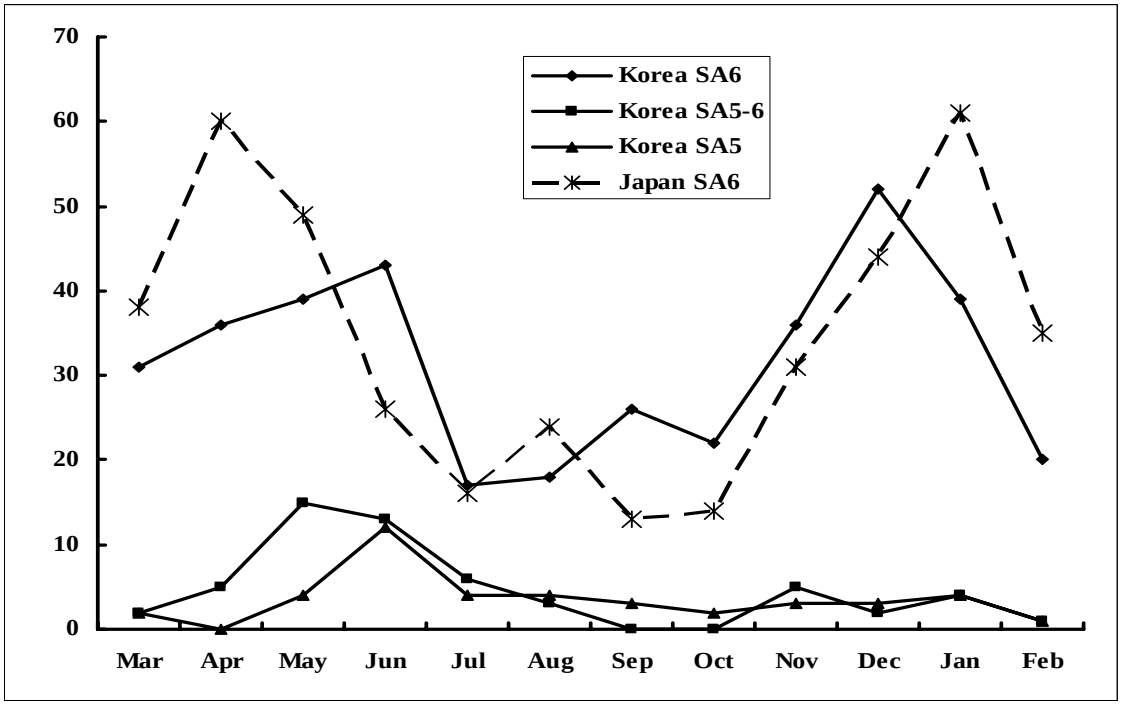

Fig. 2. Number of bycatch individuals from SA5 and SA6 in Korea and Japan.

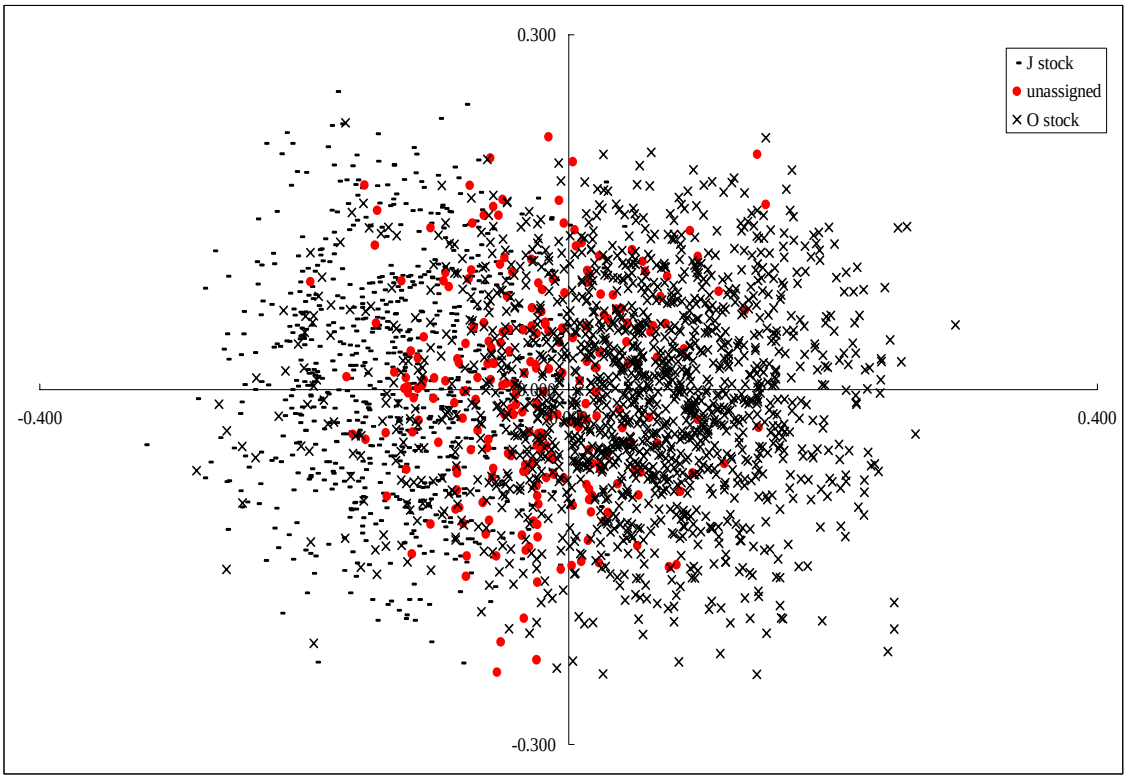

Fig. 3. Results of the principal coordinate analysis (first two axes) among the individuals from all of the Japanese samples in the WNP. Assignment of the individuals was based on the Structure analyses conducted with these individuals. See details in the text.

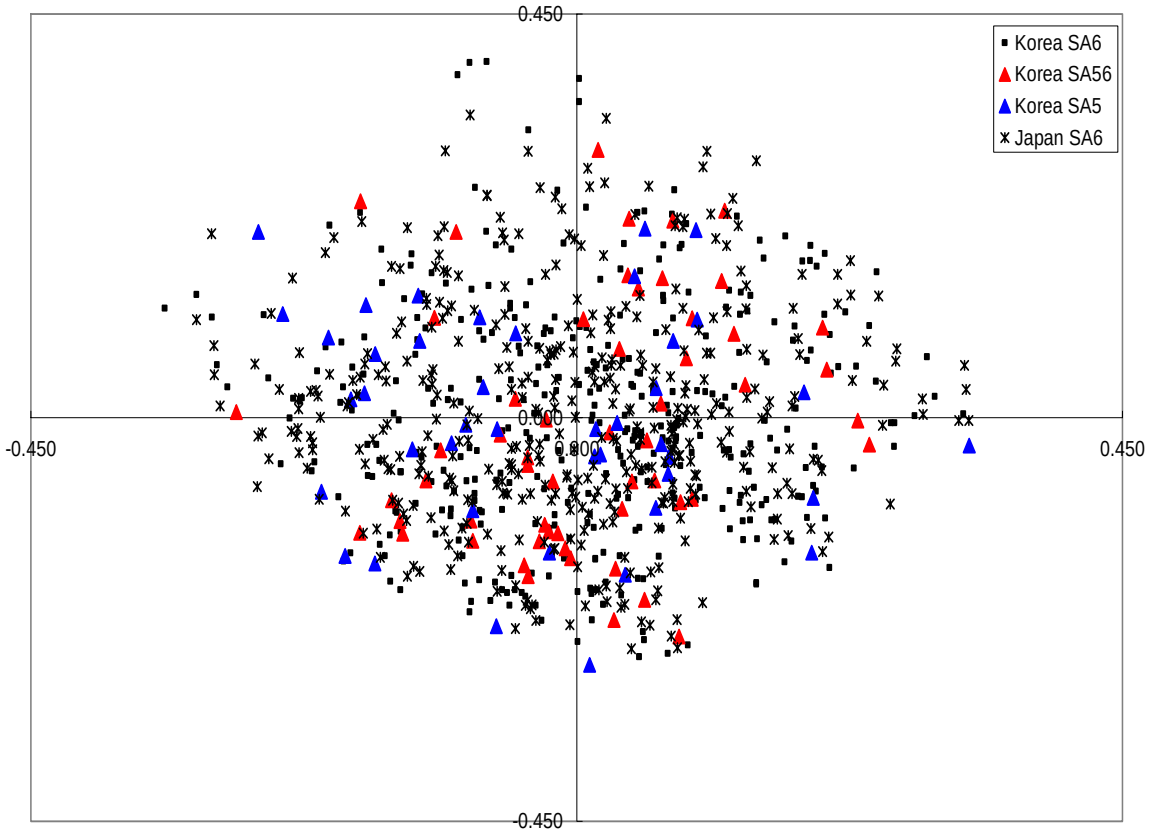

Fig. 4. Results of the principal coordinate analysis (first two axis) among the individuals from SA5 and SA6 in Korea and Japan.

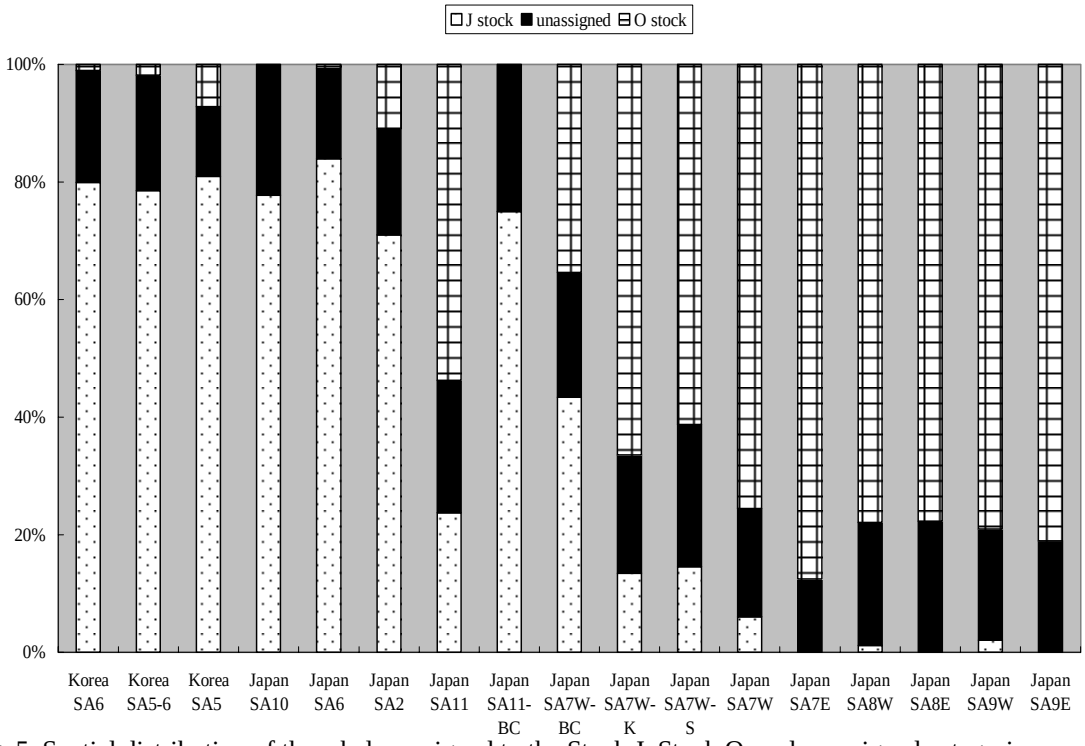

Fig. 5. Spatial distribution of the whales assigned to the Stock J, Stock O, and unassigned categories. BC = bycatches, K = coastal survey of JARPNII at Kushiro, S = coastal survey of JARPNII at Sanriku.