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Further microsatellite analysis of common minke whales in the western North Pacific

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ABSTRACT
This paper is a revised version of the microsatellite analysis presented in SC/J09/JR30 to cover the recommendations from the Expert Workshop to review the JARPNII Programme. The IWC Scientific Committee (SC) completed the RMP *Implementation* for the western North Pacific common minke whales during the 2003 Annual Meeting. At the final stage of the *Implementation* process, the SC adopted four stock scenarios (baselines A, B, C, and D) in the western North Pacific (IWC, 2004). The SC did not examine the plausibility of each scenario at all, however, 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 irrespective of available information for each hypothesis. This study examined the plausibility of these four stock baseline scenarios by analyzing samples of minke whales collected during JARPNII as well as JARPN conducted from 1994 to 2007 using 16 sets of hypervariable microsatellite DNA markers. The samples from 2003 to 2007 were not used during the previous *Implementation* process. In addition to their collection years, we further divided the samples by their sighting sites into 7W (140.01°E -147.00°E), 7E (147.01°E -150.00°E), 8W (150.01°E -153.00°E), 8E (153.01°E -157.00°E), 9W (157.01°E -162.00°E), and 9E (162.01°E -170.00°E). All of the samples were polymorphic for the 16 microsatellites analyzed, and the genetic diversity was high. We examined if there was any evidence of genetic differences between the coastal and offshore samples collected in the same year from the 7W, among the samples collected in the different years from the same sub-area, and among the samples divided and compared on the basis of proposed stock divisions from each of the four baseline scenarios with the suspected J stock individuals (all individuals included) and without the suspected J stock individuals (individuals of unknown origin and O stock included) as well as with only the suspected O stock individuals (individuals of unknown origin and the J stock excluded). We found 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. Our simulation study indicated that from genetics standpoint the statistical power for testing the baseline scenarios with our data set was quite high. Results of this revised paper confirmed the main conclusion in SC/J09/JR30.

KEY WORDS: MINKE WHALE, MICROSATELLITE, STOCK STRUCTURE, JARPN, JARPNII, NORTH PACIFIC

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 (Wada and Numachi, 1991; Bakke *et al*., 1996; Martinez and Pastene, 1999; 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, common 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 common minke whales are known to exist: one stock distributes in the western North Pacific and the other in the Sea of Japan (Omura and Sakiura, 1956; Ohsumi, 1977; Kato, 1992; Wada and Numachi, 1991; Goto and Pastene, 1997; Pastene *et al*., 2007). Contrary to the clear genetic differences detected between these two stocks, previous analyses of allozymes and mtDNA restriction fragment length polymorphism (RFLP) failed to present evidence of genetic heterogeneity among samples within the western North Pacific east of Japan even though these samples were collected from a very wide geographic area from 142°E to 170°E and from 35°N to 45°N (Wada and Numachi, 1991; Goto and Pastene, 1997). This could simply indicate a single stock of minke whales in the area. Alternate explanation is that previously used genetic markers were not sensitive enough to detect genetic differentiation among stocks of highly migratory species like minke whales because they represent very small portion of genetic differences on genome. In addition to that, large stock size and the ability to long distance migration of minke whales suggests low degree of genetic differences. Their breeding grounds have not yet been found partially because no aggregation of minke whale females has been found during the breeding season (Kasamatsu, 2000).

The IWC Scientific Committee (SC) completed the RMP *Implementation* for the western North Pacific 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 western North Pacific (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.

The primary objective of this study was to examine the plausibility of these four baseline stock scenarios by analyzing samples of minke whales collected from JARPN and JARPNII conducted from 1994 to 2007 using hypervariable microsatellite DNA markers. The samples of 2003 to 2007 were not used during the previous *Implementation* process.

This paper is the revised version of SC/J09/JR30 to cover the recommendations of the expert panel of the Expert Workshop to review the JARPNII Programme held at Tokyo from 26-30 of January. The panel recommends that the revised paper should include estimates of genetic divergence in addition to probabilities of homogeneity, report P values from the tests of homogeneity for all loci combined rather than each locus separately, and assess statistical power for the tests of homogeneity using simulated data.

MATERIALS AND METHODS

Samples

Common minke whales samples of the JARPNII offshore component were taken from 2000 to 2007. The JARPN samples from 1994 to 1999 were also used in this study. Eighteen sub-areas were set for management purpose of the western North Pacific common minke whale during the *Implementation* Specification conducted in 2003 (Figure 1). Although the JARPN survey was conducted at the SA11 in 1995 and 1997, we used the samples collected only from the sub-areas 7, 8, and 9. Each of the three sub-areas was further divided into western and eastern strata for analyses: 7W (140.01°E -147.00°E), 7E (147.01°E -150.00°E), 8W (150.01°E -153.00°E), 8E (153.01°E -157.00°E), 9W (157.01°E -162.00°E), and 9E (162.01°E -170.00°E). Because of other scientific purposes of the survey (e.g., feeding ecology of minke whales), the sampling locations differed from year by year. Details of offshore component of JARPNII survey can be found in Tamura *et al*. (2009). 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. Sample size was maximum 60 minke whales per survey. Details of coastal component of the JARPNII can be found in Kishiro *et al*. (2009). Table 1 shows the number of individuals used in the present microsatellite analysis by year, sub-area and the offshore/coastal components, and Figure 2 shows sighting positions of the collected individuals.

Microsatellite analysis

We followed the IWC guidelines for DNA data quality (IWC, 2009) as much as possible at the moment. 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. Most of the primers used here were already tested for amplification on minke whales by these authors. 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 and expected heterozygosity per locus was calculated using the software FSTAT 2.9.3 (Goudet, 1995). Statistical tests for deviations from the expected Hardy-Weinberg genotypic proportions were conducted using the software GENEPOP 4.0 (Rousset, 2008).

In order to detect genetic differences in the samples of minke whales, we performed conventional hypothesis testing procedure using heterogeneity test in frequencies of the microsatellite alleles among samples. 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. Markov chain method implemented in the GENEPOP was used to conduct the heterogeneity tests. Statistical significance was determined using the chi-square value obtained from summing the negative logarithm of *p*-values over the 17 microsatellite loci (Sokal & Rohlf 1995). F_{ST} was calculated using ARLEQUIN 2.0 (Schneider et al. 2000). The samples with less than 5 individuals were excluded from the genetic divergence analyses.

Assessment of statistical power for the tests of homogeneity

In order to assess statistical power for tests of homogeneity (e.g., Waples and Gaggiotti, 2006), we generated genotypic data using computer software EASYPOP (Balloux, 2001) and conducted heterogeneity tests with these generated data. We assumed two or three populations depending on the stock structure scenario we tested (baseline A and D = 2 populations, baseline C = 3 populations), each of which consists of diploid individuals with a constant size and equal sex ratio with random mating. We assumed ratio of effective population size to census population size to be 1/3 to 1/4 (Roman and Palumbi, 2003). The effective population size of the populations was thus set as it becomes 1/3 or1/4 of the census

populations size. We used census population size of approximately 20000 for the baselines A and D, and approximately 10000 for the baseline C. These numbers were used on the basis of the IWC's accepted population abundance of this species in the North Pacific. Each generation, simulation produces genotype data set for 16 independent nuclear gene loci (microsatellites) for each individual. The number of the loci simulated and maximum number of the allelic states (29) was set based on the observed data in this study. For the two population model, bidirectional migration was assumed with an equal migration rate (m), while for the three population model, stepping stone migration model was assumed. Migration rates ranged from 0.01 to 0.5, some of which (0.1-0.5) were quite high for genetic method to detect. We specified a range of genetic divergence using F_{ST} values estimated assuming island model between the two populations by changing migration rate. Mutation rate of $5x10^{-4}$ was chosen to represent microsatellite loci. For each simulation parameter set, we made 100 replicates. We ran 5000 generations for each replicate before collecting data. In the final generation of each replicate, sample of 100 individuals for the baseline A, 270 for the baseline C, and 170 for the baseline D were taken from each population for genetic analysis. The sample size of 100 for the baseline A was selected to reflect sporadic distribution of the W stock in the SA9 in some years (e.g., Goto *et al*., 2000). The sample size for the baseline C approximately equals to the sum of the samples size from SA7E to SA8E where the Oe stock was assumed. The sample size for the baseline D approximately equals to the samples size from the SA9 where the W stock was assumed. We conducted homogeneity tests for the generated data set using pairwise tests of differentiation option in the FSTAT. In this option, for each pair of samples, multi-loci genotypes are randomized between the two samples. The overall loci G-statistic is given and statistical significance was decided with a table wide level of significance at 5%.

RESULTS

Kanda *et al.* (2009) showed that there were the suspected J stock individuals in the samples of minke whales from the Pacific side of Japan. On the basis of the individual identifications to the stocks according to the criteria in Kanda *et al*. (2009), we conducted the tests with three different kinds of sample groups: 1) one that included all the analyzed individuals, 2) one that excluded the suspected J stock individuals (samples contained individuals of unknown origin and the O stock) and 3) one that used only the suspected O stock individuals (samples excluded individuals of unknown origin and the J stock). The number of the suspected J stock individuals in the offshore component samples was 24 in the 7W and two in the 9W, while that in the coastal component samples was 79. The number of the suspected O stock individuals in the samples was 1,365.

Genetic diversity within samples

All of the 16 microsatellites were polymorphic in the overall samples (Table 2). The number of alleles at each of the loci ranged from two at EV21 to 29 at EV1 with an average of 12.6. Expected heterozygosity at the loci ranged from 0.328 at EV21 to 0.881 at GT23 with an average of 0.698. These results indicated substantial genetic diversity in the minke whales used in this study. Evidence of deviation from the expected Hardy-Weinberg genotypic proportions was detected at two loci (GT195 and GT509) in the sample group with the suspected J stock individuals, but disappeared in the sample groups without the suspected J stock individuals as well as with only the suspected O stock individuals.

Genetic divergence between samples

Genetic differences between offshore and coastal samples in the west of SA7

We looked for evidence of genetic differences between the coastal and offshore samples collected in the same year from the 7W. None of the comparisons from 2002 to 2007 showed statistically significant differences in the sample groups with only the suspected O stock individuals (Table 3). Significant difference was detected at the 2004 sample in the sample group with and without the suspected J stock individuals. In 2004, only 12 individuals were available for the test in the offshore sample compared to 54 in the coastal one, and the heterogeneity appeared to be due to lack of some minor alleles in the former. This suggested the difference had little biological meanings. We thus combined the coastal and offshore samples collected from the same year into one, respectively, for subsequent analyses in all the sample groups.

Temporal genetic differences within sub-areas

We looked for evidence of genetic differences among the samples collected in the different years within the same sub-area. No statistically significant genetic differences were detected within the 7E, 8W, 8E,

9W, 9E and in each of the sample groups (Table 4). For the subsequent analyses, we combined the samples of the different survey years from the same sub-area into one, respectively. Contrary, significant genetic differences were detected within the 7W in the sample group with the suspected J stock individuals (Table 4). The heterogeneity found in the 7W samples, however, disappeared in the sample group without the suspected J stock individuals. We combined the samples of the different survey years into the single 7W sample, at all the sample groups for the subsequent analyses

Baseline A. Baseline A is a three-stock scenario (J, O, W stocks) with the W stock found only in part of SA9 and only sporadically. In order to test the heterogeneity within the SA9, we conducted the heterogeneity test between the 9W and 9E samples. Statistically significant difference was detected at all of the sample groups (Table 5). Considering the result from the previous test above, we decided to treat the 9W and 9E samples separately for the following tests. F_{ST} value between the samples was 0.00091 for with the suspected J stock individuals and 0.00082 with only the suspected O stock individuals, and both were significantly different from zero.

Baseline B. Baseline B is a two stock scenario (J and O) with no W stock. Statistically tests for the scenario B are same as those for the baseline A shown above.

Baseline C. Baseline C is a four-stock scenario with O_W, O_E, and W to the east of Japan in addition to the J stock in the Sea of Japan. Boundaries are fixed at 147°E and 157°E and there is no mixing between the stocks. We first conducted the heterogeneity tests among the 7E, 8W, and 8E samples that were assumed to belong to the O_E stock in the scenario. No statistically significant difference was detected (7E x 8W x 8E; Table 6), so that these samples were combined into one as 7E-8E for the following analyses in all the sample groups. We then tested for genetic differences among the 7W, 7E-8E, 9W, and 9E samples (7W x 7E-8E x 9W x 9E, 7W x 7E-8E x SA9; Table 6). In the sample groups with and without the suspected J stock individuals, statistically significant difference was detected (Table 6). Pair-wise comparisons for the sample group with the suspected J stock individuals showed statistically significant differences between the 7W and other three samples (7E-8E, 9W, and 9E; Table 7). These differences, however, disappeared in the sample group without the suspected J individuals (Table 7). Contrary, in the sample group with only the suspected O stock individuals, no evidence of genetic difference was detected among the 7W, 7E-8E, and SA9 samples. In addition to the 9W and 9E pair, statistically significant F_{ST} value was detected between the samples from 7W and 7E-8E, and 7W and 9E both for with the suspected J stock individuals (Table 8).

Baseline D. Baseline D is another three-stock scenario (J, O, W stocks), with the O and W stocks mixing over 147°E and 162°E, the O being dominant to the west and W to the east. If this scenario is true, we should detect genetic differences not only between the 7W and 9E but also among the 7E, 8W, 8E and 9W samples. No statistically significant difference was detected among the 7E, 8W, 8E and 9W samples (7E x 8W x 8E x 9W; Table 9) in all the sample groups. These samples were combined into one as 7E-9W for the following analyses. We then conducted the heterogeneity tests among 7W, 7E-9W, and 9E (7W x 7E-9W x 9E; Table 9). Statistically significant difference was detected at the sample groups with as well as without the suspected J stock, but not with only the O stock individuals (Table 9). Pair-wise comparisons for the sample group with the suspected J individuals showed statistically significant differences in the two pairs between the 7W and other two samples (7E- 9W, and 9E; Table 10). F_{ST} was statistically significant at the pairs between the 7W and 7E-9W, and 7W and 9E both for with the suspected J stock individuals, but not with only the O stock individuals (Table 11).

Assessment of statistical power for the tests of homogeneity

Table 12 shows the input parameters used and the results of simulation analysis to assess the statistical power for the tests homogeneity conducted in each of the baselines. From genetics perspective, our simulation attempted to test the statistical power for very small genetic divergence between two samples. For instance, estimated F_{ST} values were all smaller than 0.01 for the two stocks baseline scenario.

Because both the baselines A and D assumed existence of the two stocks, O and W, the input parameters for simulating them were all same except the sample size for the homogeneity tests. The sample size differed between the baselines because we looked for the power to detect the W stock sporadically appeared in the SA9W for the baseline A while we looked for the power to detect the W stock always existing in the SA9 for the baseline D. The difference between the results for the two baselines thus

reflected the difference in the samples size. High power was detected with m=0.01 for the baseline A and with m=0.01 and 0.02 for the baseline D.

Stepping stone model was assumed for the baseline C because three populations, O_W, O_E, and W from west to east were assumed to distribute with fixed boundaries between them, suggesting lower migration rate between the O_W and W than between O_W and O_E, and between O_E, and W. Census population size was also assumed to be 10000 compared to 20000 in the baselines A and D. Estimated F_{ST} values in the table 12 represented for between the neighbor populations (i.e., between O_W and O_E, and between O_E and W). High statistical power was observed at the migration rate of 0.1 for the comparisons between the neighbor populations (i.e., between O_W and O_E, and between O_E and W) and even at 0.2 for between the first and third populations (i.e., O_W and W).

DISCUSSION

We believe that the results of this study substantially improve our knowledge of the stock structure of common minke whales in the western North Pacific and are quite informative for effective management of this species. Additional 923 minke whales were collected after 2003 *Implementation* process and used for the current study. Approximately 90% of these additional minke whales were collected from the 7W and SA9. As shown in the Figure 2, now our samples spatially covered the survey areas quite well. These facts allowed us to look for evidence of distribution of the individuals from the J and W stocks, if they exist, in our survey area. In addition to that, in this study we conducted simulation analysis to assess statistical power to see if the number of minke whales we sampled and the number of the microsatellite loci we analyzed was sufficient to test adequately the alternate stock structure hypothesis.

We conducted the heterogeneity tests with three different kinds of sample groups: 1) one that included all the analyzed individuals, 2) one that excluded the suspected J stock individuals (samples contained individuals of unknown origin and the O stock) and 3) one that used only the suspected O stock individuals (samples excluded individuals of unknown origin and the J stock). Identification of the stock origins for the individual whales was according to Kanda *et al*., (2009). The SC has recommended that the suspected J stock individuals should be excluded from the analyses of the North Pacific common minke whales because they could have large effects on the analyses. In fact, evidence of deviations from the expected Hardy-Weinberg genotypic proportions was detected in the sample group with the suspected J stock individuals, but disappeared in the sample group without them. Similarly, the temporal genetic heterogeneity detected in the 7W samples should have reflected the difference in the number of the J stock individuals between the earlier and later samples. The J stock individuals were fewer in the offshore than in the coastal 7W samples (Kanda *et al.* 2009) and the earlier samples from 1994 to 2001 consisted of only the offshore ones. Since no diagnostic marker has been found between the O and J stock individuals, we think the genetic identification used is the best available so far.

The baseline C suggests the existence of the three genetically distinct stocks, O_W (7W), O_E (7E-8W), and W (9W-9E) with no mixing between the stocks. The baseline D suggests two stocks, O (7W) and W (9E), in the JARPNII survey area with the two mixing over 147°E and 162°E (7E-9W) with a cline. Both baselines are similar in terms of assuming a distinct coastal North Pacific stock in the 7W. Our study, however, did not support that possibility. The results of the heterogeneity tests for the coastal stock of the baselines C and D differed among the sample groups. The statistical significance in the heterogeneity tests between the 7W and other offshore (east of 7E) samples was disappeared when the suspected J stock individuals were excluded from the samples, that is, with only the O stock individuals, no statistical significance was detected under the baselines C and D. The number of the suspected J stock individuals excluded was 103, and there were still 789 individuals in the 7W samples for the test without the J stock individuals. The disappearance of the statistical significance is highly likely due to exclusion of the J stock individuals from the samples but not due to the reduced sample size for the tests. The genetic heterogeneity we have seen in the samples from the 7W thus indicated the existence of some individuals from the J stock, but not from the other genetically distinct coastal stock. We did not also detect any heterogeneity among the samples from the middle sub-areas (7E to 8E or 9W) after exclusion of the J stock from the analysis, which did not support the O_E and W stocks under the baselines C and the W under the baseline D. Unless the population sizes of the stocks are much larger than we have anticipated, our simulation study indicated that from genetics standpoint the statistical power for testing the baselines C and D with our data set was quite high. We believe from these results that the JARPNII survey area of

the western North Pacific is primarily occupied by the whales from the O stock. The baselines C and D are highly unlikely.

It was a little difficult to evaluate the baseline A, however. The baseline A assume sporadic migration of the W stock into the SA9 because past mtDNA studies (e.g., Goto *et al*., 1997) found the genetic heterogeneity in the area in some years. We did not find such temporal genetic differences within the samples from the SA9, but the genetic heterogeneity between the 9W and 9E samples. The simulation study indicated that from genetics viewpoint the statistical power for testing the baseline A with our data set was reasonably high. The heterogeneity we observed could be due to sporadic migration of the W stock or a group of genetically related individuals from the O stock. We should await results from more detailed genetic analysis (e.g., look for the pair of individuals that are related), from other independent studies conducted on the same samples (e.g., morphometric study) as well as from continued monitoring of minke whales migrating to the SA9 in order to better understand migration pattern of the W stock under the baseline A.

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Table 1. Samples used for the microsatellite analyses.

	Survey area							
	Coastal	Offshore						
Year	7W	7W	7E	8W	8E	9W	9E	Total
1994						7	14	21
1995						78	22	100
1996		31		1	15			47
1997		2		1	30	19	48	100
1998		25	31	44				100
1999		50						50
2000		24				16		40
2001		43	7		21	29		100
2002	50	60			8	32		150
2003	50	17	7	21	17	24	14	150
2004	58	15				42	41	156
2005	120	32		7	7	19	30	215
2006	95	36	2	10	28	23	1	195
2007	107	79		2	13	2	4	207
Total	480	414	47	86	139	291	174	1631

Table 2. The number of alleles (A), expected heterozygosity (He), and test results for deviation from the expected Hardy-Weinberg genotypic proportions (HW) at 16 microsatellite loci analyzed in the samples of minke whales used in this study. n.s. = not significant

Microsatellites	A	He	HW	HW*	HW**
DlrFCB14	5	0.379	n.s.	n.s.	n.s.
EV1	29	0.814	n.s.	n.s.	n.s.
EV14	6	0.565	n.s.	n.s.	n.s.
EV21	2	0.328	n.s.	n.s.	n.s.
EV37	12	0.726	n.s.	n.s.	n.s.
EV94	8	0.655	n.s.	n.s.	n.s.
GATA28	22	0.841	n.s.	n.s.	n.s.
GATA98	6	0.621	n.s.	n.s.	n.s.
GATA417	13	0.751	n.s.	n.s.	n.s.
GT23	16	0.881	n.s.	n.s.	n.s.
GT195	13	0.835	<0.01	n.s.	n.s.
GT211	16	0.879	n.s.	n.s.	n.s.
GT310	14	0.825	n.s.	n.s.	n.s.
GT509	23	0.861	<0.001	n.s.	n.s.
GT575	12	0.820	n.s.	n.s.	n.s.
TAA31	4	0.381	n.s.	n.s.	n.s.
all loci	12.6	0.698	0.000	0.217	0.157

*Tested without the suspected J stock individuals.
** Tested with only the suspected O stock individuals.

Table 3. Results (p-values) of the heterogeneity tests between the offshore and coastal samples collected from the survey years in the 7W. Tests were conducted respectively for the sample groupings with and without the suspected J stock individuals as well as with only the suspected O stock individuals.

With the suspected J stock individuals						Without the suspected J stock individuals						Only the suspected O stock individuals					
2002	2003	2004	2005	2006	2007	2002	2003	2004	2005	2006	2007	2002	2003	2004	2005	2006	2007
0.380	0.653	**0.033**	0.288	0.063	0.614	0.531	0.748	**0.005**	0.252	0.150	0.711	0.363	0.700	0.056	0.117	0.335	0.551

Table 4. Results (p-values) of the heterogeneity tests among the samples collected in the different survey years from the same sub-area. Tests were conducted respectively for the sample groupings with and without the suspected J stock individuals as well as with only the suspected O stock individuals.

With J						Without J*		Only O					
7W	7E	8W	8E	9W	9E	7W	9W	7W	7E	8W	8E	9W	9E
0.014	0.422	0.190	0.785	0.949	0.061	0.162	0.926	0.073	0.356	0.225	0.795	0.924	0.157

* The suspected J stock individuals were detected only at the 7W and 9W.

Table 5. Results (p-values) of the heterogeneity tests and F_{ST} valuses between the 9E and 9W samples. Tests were conducted respectively for the sample groupings with and without the suspected J stock individuals as well as with only the suspected O stock ones.

	9W x 9E		
	With J	Without J	Only O
Heterogeneity test	**0.007**	**0.005**	**0.027**
F_{ST}	with J: 0.00091*		only O: 0.00082*

*P<0.05

Table 6. Results (p-values) of the heterogenety tests for the baseline C. Tests were conducted respectively for the sample groupings with and without the suspected J stock individuals as well as with only the suspected O stock individuals.

7E x 8W x 8E		7W x 7E-8E x 9W x 9E		
With / without J	Only O	With J	Without J	Only O
0.815	0.885	h.s.	**0.030**	0.207

h.s.: highly significant.

Table 7. Results (p-values) of the pair-wise heterogenety tests between the samples of minke whales from different areas for the baseline C. Tests were conducted respectively for the sample groups with and without the suspected J stock individuals.

	With J	Without J
7W x 7E-8E	**0.001**	0.448
7W x 9W	h.s	0.363
7W x 9E	**0.000**	0.060
7E-8E x 9W	0.758	0.740
7E-8E x 9E	0.667	0.647
9E x 9W	**0.007**	**0.006**

h.s.: highly significant.

Table 8. F_{ST} values between the samples of minke whales from different areas for the baseline C. The values were estimated respectively for the sample groupings all individuals (below diagonal) and of only O (above diagonal).

	7W	7E-8E	9W	9E
7W	-----	0.00027	-0.00001	0.00016
7E-8E	0.00067**	-----	-0.00014	-0.00021
9W	0.00019	-0.00013	-----	0.00082*
9E	0.00097**	-0.00021	0.00091*	-----

*: P<0.05, **: P<0.01

Table 9. Results (p-values) of the heterogenety tests for the baseline D. Tests were conducted respectively for the sample groupings with and without the suspected J stock individuals as well as with only the suspected O stock ones.

7E x 8W x 8E x 9W			7W x 7E-9W x 9E		
With J	Without J	Only O	With J	Without J	Only O
0.819	0.799	0.922	h.s	**0.044**	0.101

h.s.: highly significant.

Table 10. Results (p-values) of the pair-wise heterogenety tests between the samples of minke whales from different areas for the baseline D. Tests were conducted respectively for the sample groupings with and without the suspected J stock individuals.

	With J	Without J
7W x 7E-9W	**h.s**	0.255
7W x 9E	**0.000**	0.056
7E-9W x9E	0.094	0.095

h.s.: highly significant.

Table 11. F_{ST} values between the samples of minke whales from different areas for the baseline D. The values were estimated respectively for the sample groupings with all individuals (below diagonal) and with only O (above diagonal).

	7W	7E-9W	9E
7W	-----	0.00016	0.00016
7E-9W	0.00039***	-----	0.00035
9E	0.00097**	0.00040	-----

**: P<0.01, ***: P<0.001

Table 12. Input parameter sets used for generating simulated data set using EASYPOP to asess statistical power in our samples and results of the homogenetiy tests with the simulated data. The following were fixed in all sets other than shown in the table: diploid, random mating, equal sex ratio, all subpopulations of constant Ne, mutation rate of 0.005, 16 nuclear gene loci, 29 maximum allelic states, and 100 replicates each with 5000 generations.

	Input parameters								
	n	N	Ne	m	Nem	F_{ST}	S	% rejecting panmixia	
Baseline A								O x W	
N=3Ne	2	19980	6660	0.01	67	0.0037	100	97	
	2	19980	6660	0.02	133	0.0019	100	57	
	2	19980	6660	0.05	333	0.0008	100	25	
	2	19980	6660	0.1	666	0.0004	100	11	
	2	19980	6660	0.2	1332	0.0002	100	7	
	2	19980	6660	0.5	3330	0.0001	100	6	
N=4Ne	2	20000	5000	0.01	50	0.0050	100	100	
	2	20000	5000	0.02	100	0.0025	100	80	
	2	20000	5000	0.05	250	0.0010	100	27	
	2	20000	5000	0.1	500	0.0005	100	11	
	2	20000	5000	0.2	1000	0.0002	100	10	
	2	20000	5000	0.5	2500	0.0001	100	6	
Baseline C								O_W x O_E or O_E x W	O_W x W
N=3Ne	3	10050	3350	0.01	34	0.0074	270	100, 100	100
	3	10050	3350	0.05	168	0.0015	270	100, 100	100
	3	10050	3350	0.1	335	0.0007	270	97, 97	100
	3	10050	3350	0.2	670	0.0004	270	29, 31	93
	3	10050	3350	0.67	2245	0.0001	270	1, 2	13
N=4Ne	3	10000	2500	0.01	25	0.0099	270	100, 100	100
	3	10000	2500	0.05	125	0.0020	270	100, 100	100
	3	10000	2500	0.1	250	0.0010	270	100, 98	100
	3	10000	2500	0.2	500	0.0005	270	67, 52	99
	3	10000	2500	0.67	1675	0.0001	270	2, 2	12
Baselin D								O x W	
N=3Ne	2	19980	6660	0.01	67	0.0037	170	100	
	2	19980	6660	0.02	133	0.0019	170	94	
	2	19980	6660	0.05	333	0.0008	170	44	
	2	19980	6660	0.1	666	0.0004	170	11	
	2	19980	6660	0.2	1332	0.0002	170	7	
	2	19980	6660	0.5	3330	0.0001	170	6	
N=4Ne	2	20000	5000	0.01	50	0.0050	170	100	
	2	20000	5000	0.02	100	0.0025	170	99	
	2	20000	5000	0.05	250	0.0010	170	44	
	2	20000	5000	0.1	500	0.0005	170	18	
	2	20000	5000	0.2	1000	0.0002	170	9	
	2	20000	5000	0.5	2500	0.0001	170	2	

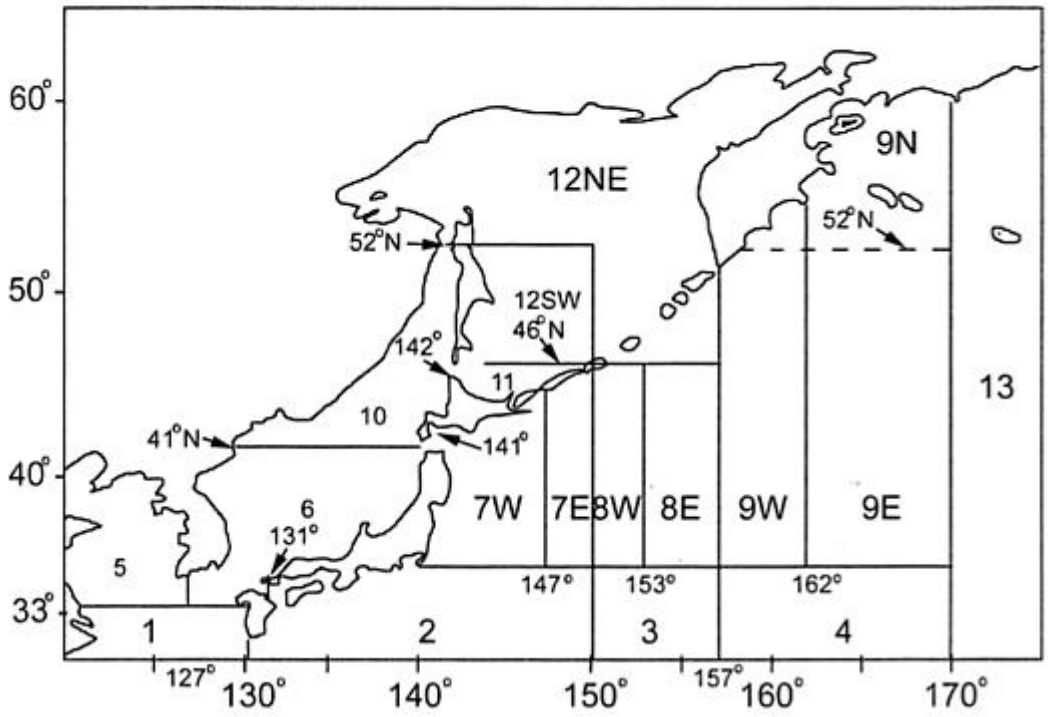

Figure 1. Eighteen sub-areas used for the *Implementation Simulation Trials* for the North Pacific minke whales.

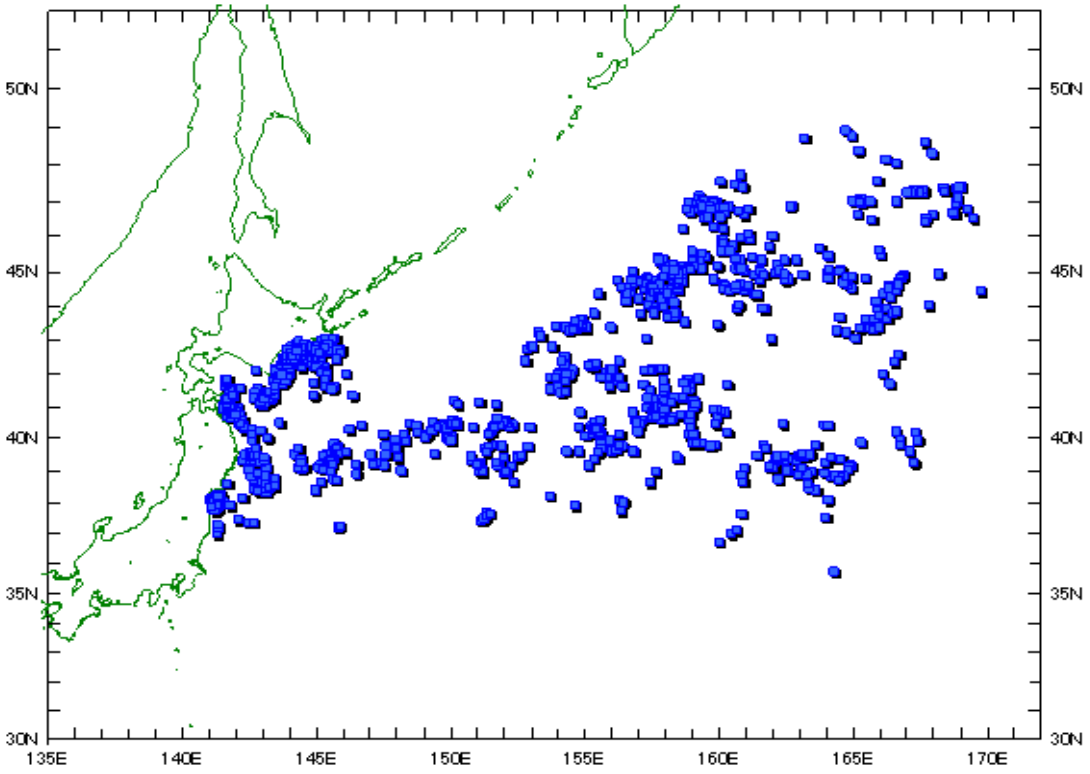

Figure 2. Sighting positions of the collected minke whales during the JARPN and JARPNII surveys. Both the offshore and coastal component samples are included.