Microsatellite analysis of minke whales in the western North Pacific

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ABSTRACT

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 and without the suspected J stock individuals as well as with only the suspected O stock individuals. 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 coastal stock was observed.

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, 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 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.

MATERIALS AND METHODS

Samples

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 (Fig. 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 Fig. 2 shows sighting positions of the collected individuals.

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. 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). When simultaneous multiple tests were conducted, Rice (1989) correction for the multiple tests was performed.

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. When multiple tests were conducted, the observed p-values from the heterogeneity tests at each of the loci were compared to the modified level of significance proposed by Rice (1989). The samples with less than 5 individuals were excluded from the genetic divergence analyses.

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 and 3) one that used only the suspected O stock individuals. 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 1365.

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 after the correction for multiple tests in the sample groups with the suspected J stock as well as only the suspected O stock individuals (Table 3). Significant difference was detected at GT195 in the 2004 sample in the sample group 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, and 9W 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 to these four sub-areas presented above, significant genetic differences were detected within the 7W in the sample group with the suspected J stock individuals and within the 9E in the sample groups with the suspected J stock and with only the O stock individuals (Table 4). In all of the cases, only one of the 16 loci (GATA28 in the 7W and DlrFCB14 in the 9E) showed a significant p-value even after the correction for multiple tests. The heterogeneity found in the 7W samples, however, disappeared in the sample group without the suspected J stock individuals. For the 9E case, none of the pair-wise comparisons was statistically significant after the corrections for the multiple tests in the both sample groups although some of the pairs with the 1994 sample showed very low p-values. We combined the samples of the different survey years into the single 7W and 9E sample, respectively 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 one of the 16 loci even after the correction for multiple tests in the sample groups with and without the suspected J stock individuals, but not in the sample group with only the suspected O stock individuals (Table 5). Considering the result from the previous test above, we decided to treat the 9W and 9E samples separately for the following tests in the sample groups with and without the J stock individuals, but combined them into one as SA9 in the sample group with only the suspected O stock individuals.

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 scenario 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 at one (GT509) of the 16 loci after the correction for multiple tests (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) even after the correction for multiple tests (Table 7). These differences, however, disappeared in the sample group without the suspected J individuals (Table 7). The comparison between the 9W and 9E samples were not significant after the correction for multiple tests in the both sample groups, but the observed p-value was the fourth lowest among the tests in the sample group with the J stock individuals and the lowest in the sample group without the J stock individuals. 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.

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 8) 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 8). Statistically significant difference was detected at one (GT509) of the 16 loci for the sample groups with the suspected J stock as well as with the only suspected O stock individuals after the correction for multiple tests (Table 8). 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) even after the correction for multiple tests (Table 9). Pair-wise comparisons for the sample group with only the suspected O stock individuals showed no statistically significant difference in any pairs, but the pairs between the 7E- 9W and 9E showed the lowest p-value (Table 9).

DISCUSSION

We believe that the results of this study substantially improve our knowledge of the stock structure of 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, allowing us to look for evidence of distribution of the individuals from the J and W stocks, if they exist, in our survey area. We conducted heterogeneity tests in the sample groups with and without the suspected J stock whales as well as with only the suspected O stock whales (Kanda *et al*., 2009). The SC has recommended that the suspected J stock individuals should be excluded from the analyses of the North Pacific 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 just 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. The analyses with only the suspected O stock individuals in the samples are relatively conservative approach compared to those with and without the suspected J stock individuals in the samples. The former sample group contains only the suspected O stock individuals, whereas the latter groups contain not only the individuals assigned to the J stock with the lower probabilities but also those assigned to neither stock (see more details in Kanda *et al*., 2009). 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 genetically distinct stocks, O_W (7W) and O_E (7E-8W), in the area westward of 157°E with a stock boundary at 147°E (IWC, 2004). The baseline D suggests western (O) and eastern (W) stocks in the JARPNII survey area with the two mixing over 147°E and 162°E. Although looked different, both baselines are actually similar to each other in terms of assuming a distinct coastal North Pacific stock along the Japanese coast. Our study, however, did not support that possibility. The results of the heterogeneity tests for the baselines C and D differed between the sample groups with and without the suspected J stock whales. 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. 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. In addition to that, although we detected the heterogeneity among the 7W, 7E-9W, and 9E samples in the sample group with only the suspected O stock individuals, the pair-wise comparisons showed that the heterogeneity was largely due to the difference between the 7E-9W and 9E. These results thus indicate that although some, but not many, numbers of the J stock individuals occur in the JARPNII survey area of the western North Pacific, it is primarily occupied by the whales from the O stock. The baselines C and D are thus rejected because no other genetically distinct coastal stock exists.

One of the objectives of the JARPNII was to look for any evidence of existence of the W stock. Past mtDNA studies (e.g., Goto *et al*., 1997) found the genetic heterogeneity in the 9W in some years. In this study, we did not find the genetic heterogeneity concordant to the past studies. The heterogeneities found within the 9E samples as well as between the 9W and 9E samples were not strong or clear enough to convince an additional stock. It is important to note that the Bayesian clustering analysis we conducted for these samples failed to detect a signal of the third stocks in the area (Kanda *et al*., 2009). We await results from other independent studies conducted on the same samples as well as from continued monitoring of minke whales migrating to the SA9 to make a final decision.

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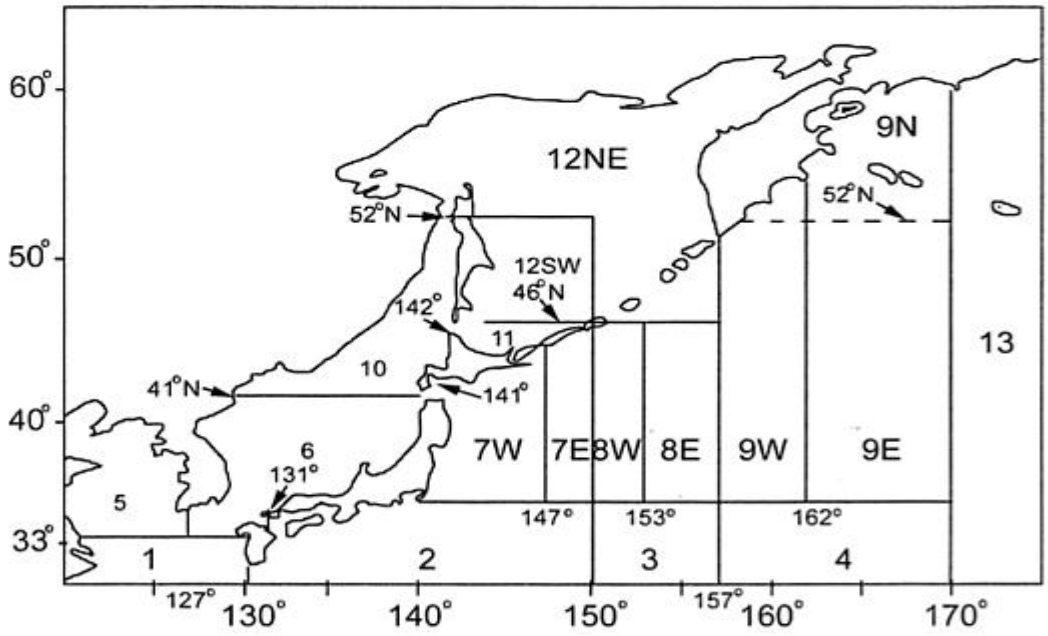

Fig. 1. Eighteen sub-areas used for the *Implementation Simulation Trials* for the North Pacific minke whales.

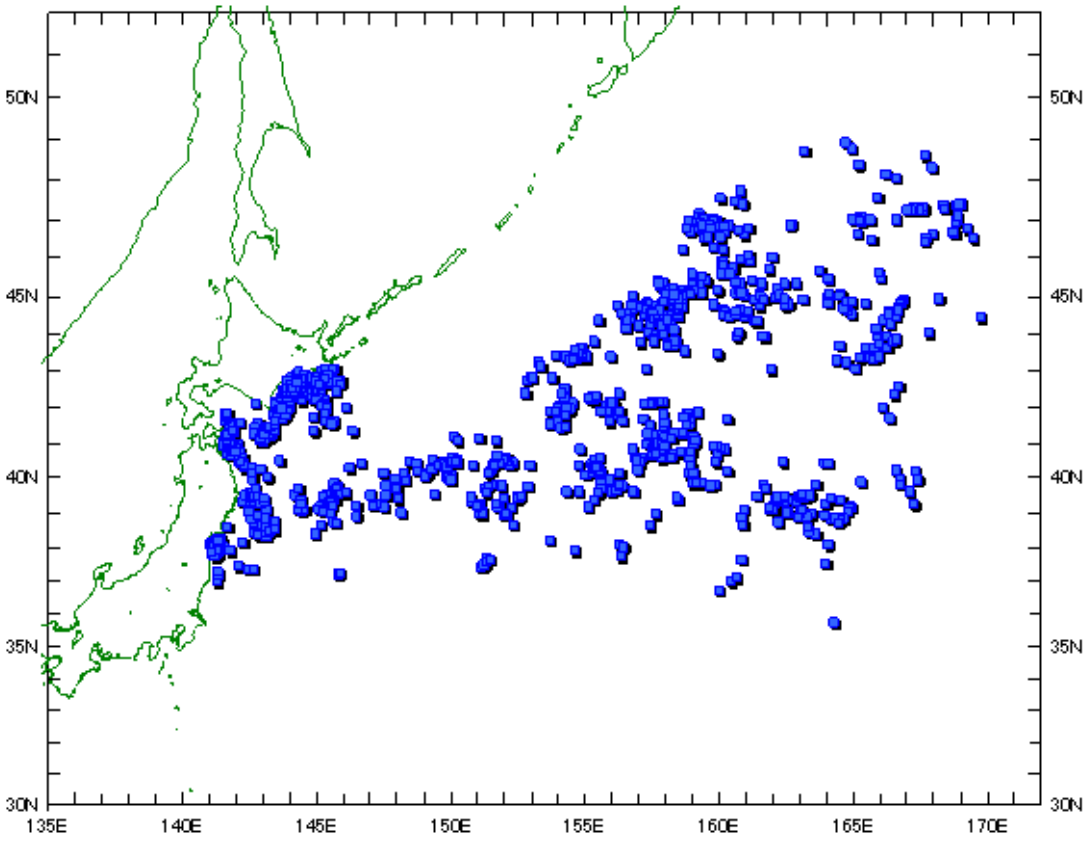

Fig. 2. Sighting positions of the collected minke whales during the JARPN and JARPNII surveys. Both the offshore and coastal component samples are included.

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.

*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 in the sample groups with and without the suspected J stock individuals as well as with only the suspected O stock individuals.

	Survey year																	
	With the suspected J stock individuals						Without the suspected J stock individuals						Only the suspected O stock individuals					
Microsatellites	2002	2003	2004	2005	2006	2007	2002	2003	2004	2005	2006	2007	2002	2003	2004	2005	2006	2007
DlrFCB14	0.468	0.168	0.138	0.876	0.022	0.204	0.522	0.134	0.184	0.561	0.043	0.260	0.432	0.055	0.274	0.593	0.054	0.380
EV1	0.702	0.896	0.104	0.016	0.771	0.578	0.645	0.893	0.186	0.018	0.832	0.591	0.447	0.695	0.322	0.015	0.940	0.476
EV14	0.838	0.233	0.602	0.639	0.040	0.261	0.939	0.378	0.135	0.418	0.064	0.640	0.952	0.365	0.223	0.425	0.101	0.497
EV21	1.000	1.000	1.000	0.278	0.082	0.526	1.000	1.000	0.786	0.186	0.022	0.807	0.853	0.729	0.557	0.107	0.036	0.742
EV37	0.654	0.155	0.650	0.637	0.500	0.213	0.751	0.361	0.668	0.796	0.625	0.197	0.492	0.686	0.724	0.938	0.603	0.177
EV94	0.838	0.572	0.841	0.795	0.814	0.442	0.871	0.570	0.869	0.614	0.822	0.453	0.711	0.613	0.851	0.347	0.710	0.670
GATA28	0.118	0.962	0.550	0.642	0.201	0.176	0.144	0.887	0.658	0.607	0.101	0.197	0.282	0.759	0.331	0.790	0.191	0.103
GATA98	0.372	0.520	0.577	0.345	0.789	0.569	0.376	0.560	0.555	0.040	0.879	0.826	0.606	0.502	0.651	0.030	0.933	0.961
GATA417	0.469	0.958	0.289	0.905	0.282	0.465	0.346	0.969	0.333	0.933	0.423	0.592	0.281	0.889	0.272	0.991	0.403	0.573
GT23	0.288	0.109	0.133	0.123	0.074	0.242	0.365	0.082	0.214	0.428	0.030	0.194	0.074	0.225	0.381	0.367	0.080	0.106
GT195	0.112	0.327	0.004	0.085	0.172	0.518	0.106	0.454	**0.001**	0.133	0.616	0.310	0.123	0.590	0.009	0.110	0.677	0.194
GT211	0.088	0.804	0.067	0.343	0.692	0.692	0.302	0.646	0.053	0.655	0.585	0.420	0.247	0.426	0.094	0.557	0.373	0.415
GT310	0.659	0.251	0.803	0.576	0.342	0.821	0.598	0.363	0.876	0.509	0.649	0.639	0.712	0.371	0.860	0.630	0.704	0.867
GT509	0.431	0.223	0.277	0.431	0.514	0.230	0.308	0.150	0.103	0.709	0.690	0.276	0.432	0.197	0.099	0.449	0.837	0.188
GT575	0.042	0.462	0.056	0.165	0.239	0.628	0.098	0.399	0.079	0.110	0.549	0.425	0.115	0.242	0.091	0.054	0.463	0.467
TAA31	0.435	0.640	0.175	0.430	0.449	0.656	0.260	0.811	0.045	0.758	0.706	0.985	0.180	0.782	0.117	0.823	0.881	0.884

Bold indicates statistical significance after the correction for multiple tests (Rice, 1998).

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

	With J						Without J*		Only O					
Microsatellites	7W	7E	8W	8E	9W	9E	7W	9W	7W	7E	8W	8E	9W	9E
DlrFCB14	0.170	0.498	0.392	0.606	0.599	**0.001**	0.123	0.617	0.138	0.471	0.329	0.461	0.331	**0.001**
EV1	0.430	0.743	0.208	0.802	0.585	0.819	0.760	0.582	0.786	0.754	0.402	0.718	0.672	0.847
EV14	0.061	0.777	0.147	0.600	0.720	0.977	0.171	0.718	0.056	0.848	0.177	0.769	0.640	0.942
EV21	0.286	0.067	0.748	0.829	0.884	0.303	0.495	0.886	0.351	0.057	0.550	0.640	0.796	0.361
EV37	0.888	0.730	0.173	0.108	0.469	0.733	0.810	0.534	0.864	0.743	0.141	0.114	0.377	0.585
EV94	0.898	0.912	0.036	0.910	0.762	0.988	0.937	0.729	0.798	0.888	0.046	0.747	0.757	0.988
GATA28	**0.001**	0.848	0.634	0.481	0.774	0.790	0.006	0.829	0.010	0.857	0.493	0.544	0.926	0.776
GATA98	0.743	0.956	0.849	0.036	0.905	0.140	0.849	0.900	0.889	0.948	0.647	0.065	0.973	0.214
GATA417	0.550	0.811	0.622	0.562	0.343	0.454	0.710	0.357	0.740	0.829	0.530	0.516	0.242	0.739
GT23	0.248	0.266	0.277	0.252	0.139	0.109	0.249	0.139	0.465	0.140	0.249	0.390	0.415	0.238
GT195	0.318	0.074	0.014	0.966	0.344	0.401	0.402	0.251	0.271	0.071	0.063	0.991	0.359	0.511
GT211	0.682	0.173	0.961	0.697	0.531	0.512	0.840	0.517	0.575	0.170	0.844	0.649	0.485	0.704
GT310	0.040	0.901	0.764	0.257	0.290	0.684	0.045	0.202	0.016	0.916	0.675	0.221	0.102	0.639
GT509	0.153	0.352	0.690	0.750	0.891	0.010	0.500	0.863	0.325	0.371	0.431	0.736	0.937	0.027
GT575	0.274	0.034	0.796	0.431	0.439	0.653	0.351	0.420	0.567	0.036	0.641	0.335	0.585	0.717
TAA31	0.114	0.228	0.135	0.889	0.764	0.204	0.164	0.774	0.112	0.222	0.186	0.943	0.811	0.116

Bold indicates statistical significance after the correction for multiple tests (Rice, 1998).
* The suspected J stock individuals were detected only at the 7W and 9W.

Table 5. Results (p-values) of the heterogeneity tests between the 9E and 9W samples in the sample groups with and without the suspected J stock individuals as well as with only the suspected O stock individuals.

	9W x 9E		
Microsatellites	With J	Without J	Only O
DlrFCB14	0.436	0.436	0.477
EV1	0.873	0.856	0.890
EV14	0.991	0.987	0.999
EV21	0.445	0.407	0.332
EV37	0.344	0.338	0.499
EV94	0.097	0.089	0.106
GATA28	0.457	0.495	0.562
GATA98	0.089	0.072	0.050
GATA417	0.080	0.098	0.126
GT23	0.917	0.913	0.860
GT195	0.071	0.062	0.264
GT211	0.384	0.368	0.415
GT310	0.130	0.129	0.053
GT509	**0.001**	**0.001**	0.003
GT575	0.039	0.033	0.064
TAA31	0.542	0.599	0.749

Bold indicates statistical significance after the correction for multiple tests (Rice, 1998).

Table 6. Results (p-values) of the heterogenety tests for the baseline C in the sample groups 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		7W x 7E-8E x SA9
Microsatellites	With / without J	Only O	With J	Without J	Only O
DlrFCB14	0.236	0.219	0.560	0.466	0.555
EV1	0.516	0.465	0.320	0.757	0.772
EV14	0.921	0.820	0.527	0.938	0.832
EV21	0.371	0.383	0.588	0.543	0.353
EV37	0.142	0.214	0.528	0.679	0.639
EV94	0.957	0.952	0.157	0.101	0.105
GATA28	0.934	0.929	0.154	0.459	0.450
GATA98	0.087	0.086	0.286	0.235	0.686
GATA417	0.549	0.573	0.186	0.123	0.411
GT23	0.568	0.816	0.410	0.815	0.472
GT195	0.310	0.302	0.026	0.487	0.554
GT211	0.993	0.895	0.469	0.820	0.967
GT310	0.330	0.479	0.023	0.053	0.315
GT509	0.439	0.584	**0.000**	**0.000**	0.066
GT575	0.775	0.895	0.121	0.242	0.807
TAA31	0.839	0.693	0.538	0.797	0.690

Bold indicates statistical significance after the correction for multiple tests (Rice, 1998).

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

	Microsatellites															
	DlrFCB14	EV1	EV14	EV21	EV37	EV94	GATA28	GATA98	GATA417	GT23	GT195	GT211	GT310	GT509	GT575	TAA31
With J																
7W x																
7E-8E	0.311	0.144	0.061	0.421	0.569	0.447	0.229	0.876	0.072	0.136	0.195	0.704	0.044	**0.000**	0.153	0.454
9W	0.456	0.598	0.671	0.243	0.616	0.325	0.108	0.267	0.855	0.248	0.012	0.231	0.258	**0.000**	0.682	0.465
9E	0.952	0.249	0.648	1.000	0.128	0.041	0.148	0.126	0.210	0.659	0.172	0.257	0.017	**0.000**	0.091	0.276
7E-8E x																
9W	0.155	0.626	0.741	0.775	0.941	0.423	0.569	0.284	0.225	0.786	0.322	0.810	0.633	0.037	0.555	1.000
9E	0.636	0.377	0.712	0.603	0.468	0.489	0.754	0.202	0.252	0.701	0.639	0.751	0.196	0.131	0.197	0.529
9E x 9W	0.423	0.867	0.991	0.443	0.343	0.100	0.436	0.083	0.086	0.913	0.063	0.376	0.122	0.001	0.031	0.534
Without J																
7W x																
7E-8E	0.277	0.408	0.462	0.414	0.652	0.276	0.391	0.716	0.153	0.409	0.810	0.998	0.096	0.069	0.396	0.643
9W	0.290	0.850	0.961	0.202	0.674	0.298	0.330	0.480	0.679	0.413	0.192	0.380	0.284	0.011	0.675	0.586
9E	0.967	0.723	0.731	1.000	0.240	0.016	0.362	0.104	0.110	0.930	0.794	0.424	0.040	0.029	0.135	0.377
7E-8E x																
9W	0.169	0.540	0.756	0.768	0.933	0.406	0.603	0.282	0.228	0.827	0.304	0.805	0.674	0.027	0.602	1.000
9E	0.630	0.348	0.728	0.603	0.455	0.469	0.761	0.197	0.244	0.694	0.629	0.742	0.210	0.116	0.211	0.531
9E x 9W	0.434	0.859	0.987	0.399	0.329	0.079	0.493	0.082	0.094	0.906	0.063	0.375	0.115	0.001	0.036	0.595

Bold indicates statistical significance after the correction for multiple tests (Rice, 1998).

Table 8. Results (p-values) of the heterogenety tests for the baseline D in the sample groups with and without the suspected J stock individuals as well as with only the suspected O stock individuals.

	7E x 8W x 8E x 9W			7W x 7E-9W x 9E		
Microsatellites	With J	Without J	Only O	With J	Without J	Only O
DlrFCB14	0.161	0.161	0.204	0.863	0.745	0.742
EV1	0.648	0.552	0.688	0.207	0.823	0.920
EV14	0.969	0.972	0.932	0.366	0.886	0.979
EV21	0.551	0.541	0.604	0.443	0.377	0.230
EV37	0.405	0.407	0.483	0.153	0.315	0.334
EV94	0.859	0.844	0.828	0.067	0.052	0.043
GATA28	0.914	0.918	0.874	0.079	0.357	0.533
GATA98	0.089	0.082	0.075	0.309	0.271	0.266
GATA417	0.414	0.446	0.367	0.151	0.107	0.368
GT23	0.739	0.765	0.858	0.245	0.710	0.574
GT195	0.325	0.321	0.317	0.020	0.576	0.496
GT211	0.995	0.993	0.965	0.244	0.706	0.763
GT310	0.524	0.540	0.516	0.006	0.019	0.049
GT509	0.081	0.068	0.312	**0.000**	0.005	**0.002**
GT575	0.704	0.739	0.838	0.081	0.138	0.336
TAA31	0.943	0.950	0.819	0.260	0.552	0.696

Table 9. Results (p-values) of the pair-wise heterogenety tests between the samples of minke whales from different areas for the baseline D in the sample groups with the suspected J stock individuals and with only the suspected O stock individuals.

	Microsatellites															
	DlrFCB14	EV1	EV14	EV21	EV37	EV94	GATA28	GATA98	GATA417	GT23	GT195	GT211	GT310	GT509	GT575	TAA31
With J																
7W x																
7E-9W	0.601	0.221	0.102	0.196	0.235	0.355	0.077	0.764	0.234	0.044	0.010	0.256	0.024	**0.000**	0.293	0.262
9E	0.953	0.213	0.655	1.000	0.127	0.038	0.120	0.131	0.192	0.659	0.144	0.259	0.019	**0.000**	0.089	0.267
7E-9W x																
9E	0.691	0.694	0.974	0.456	0.296	0.212	0.570	0.125	0.113	0.862	0.238	0.515	0.102	0.013	0.068	0.487
Only O																
7W x																
7E-9W	0.472	0.894	0.885	0.098	0.463	0.107	0.315	0.832	0.391	0.145	0.231	0.886	0.179	0.029	0.746	0.486
9E	0.759	0.835	0.938	1.000	0.252	0.028	0.533	0.081	0.361	0.973	0.823	0.407	0.050	0.023	0.229	0.779
7E-9W x																
9E	0.796	0.726	0.983	0.313	0.316	0.249	0.750	0.108	0.290	0.824	0.457	0.595	0.066	0.002	0.112	0.885

Bold indicates statistical significance after the correction for multiple tests (Rice, 1998).