

FOR CONSIDERATION BY THE SCIENTIFIC COMMITTEE OF
THE INTERNATIONAL WHALING COMMISSION
TROMSO, NORWAY, JUNE 2011

Stock structure of western North Pacific minke whales based on mtDNA *haplotypes* from Korean ‘bycatch’ and Japanese ‘bycatch’ and scientific whaling, in relation to Hypothesis III

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Summary

We update previous results of SC/D10/NPM3 for differences in mtDNA control region sequences in western North Pacific minke whales by combining information on mtDNA haplotypes from samples collected from Japanese ‘bycatch’ and scientific whaling with information from samples from Korean ‘bycatch’. Information from Japanese ‘bycatch’ and scientific whaling was made available in three datasets, courtesy of the Institute for Cetacean Research of Tokyo (ICR) through the IWC Data Availability Group (with correction in versions 2.0 and 3.0). Genetic information on reported Korean ‘bycatch’ for three subregions was made available courtesy of the Cetacean Research Center (CRC), National Fisheries Research and Development Institute, Korea, through the IWC Data Availability Group on 15 September 2010. Minor corrections to the Korean dataset, received on 15th April 2011, were not incorporated here due to limitations of time. The combined Japanese and Korean datasets included mtDNA haplotypes from 3,021 samples, representing a total of 130 haplotypes, 118 of which were found in the Japanese dataset and 39 of which were found in the Korean dataset. Results of a quality control review and comparisons of the CRC Korean BC record to Korean market surveys are reported elsewhere (SC/63/RMP15, updated from SC/D10/NPM2_r1).

Overall results are consistent with previous analyses of haplotypes reported in SC/D10/NPM3 and haplogroup analyses reported in SC/62/NPM20, showing significant differentiation for almost all comparisons of subareas and strata. The results of the mtDNA haplotype analyses and the microsatellite loci analyses reported in SC/63/RMP17 are consistent with the predictions of Hypothesis III, showing evidence for differentiation of JE from JW, and evidence of differentiation of OW from OE. We did not find evidence for differences in haplotype frequencies between the Yellow Sea (subarea 5) and the East Sea/Sea of Japan (subarea 6W), or evidence of a seasonal effect predicted by the presumed migration of a Yellow Sea stock into subarea 6W. The absence of significant differentiation in mtDNA and the absence of significant deviation from Hardy-Weinberg for microsatellite frequencies in a simulated mixing of stocks (SC/63/RMP15), require further investigation given the results presented previously by Kanda et al. (SC/62/NPM11) and the biological evidence of a Yellow Sea stock (SC/63/RMP8).

Introduction

Here we report analyses of the population structure of western North Pacific minke whales using comprehensive (and corrected) datasets of mtDNA haplotypes made available courtesy of the Institute for Cetacean Research of Tokyo (ICR) and the Cetacean Research Center (CRC), National Fisheries Research and Development Institute, Korea. The intent of the analyses is to test plausible stock hypotheses as outlined in discussions of the ‘Working Group on the in-depth assessment of western North Pacific common minke whales, with a focus on J stock (NPM)’. Analyses rely primarily on exact tests to investigate differences in mtDNA haplotype frequencies and sex ratios for various geographic and temporal strata. Such ‘hypothesis testing’ is considered the most sensitive approach to identifying populations characterized by moderate differences in haplotype or allele frequencies (i.e., moderate levels of gene flow, Waples and Gaggiotti 2006). The report repeats and extends previous analyses reported for mtDNA haplogroups by Baker et al. (2010, SC/62/NPM20), as well as those based on uncorrected haplotypes in Goto et al (2009a; 2009b), corrected haplotypes in Park et al. (2010) and on microsatellite genotypes by Kanda et al. (2009; 2010).

Methods

Information from Japanese ‘bycatch’ and scientific whaling was made available in three datasets, courtesy of the Institute for Cetacean Research of Tokyo (ICR) through the IWC Data Availability Group: Japanese ‘bycatch’ (n=832), ‘coastal whaling’ (n=481) and ‘offshore whaling’ (n=1238). Following quality control review of mtDNA sequences and communication with ICR, a revised dataset with corrected haplotypes was received on 13 April 2010

(version 2.0). Following a second data availability request for access to microsatellite genotypes, additional information on position of bycatch and distance from shore of coastal and offshore whaling was received on 23 October 2010 (version 3.0). Genetic information on reported Korean ‘bycatch’ for three subregions was made available courtesy of the Cetacean Research Center (CRC), National Fisheries Research and Development Institute, Korea, through the IWC Data Availability Group on 15 September 2010: Yellow Sea (n=366), Korean Strait (n=47) and the East Sea (n=64). The Yellow Sea subregion represents subarea 5, and the East Sea represented subarea 6W. The Korean Strait includes both subregions. The two datasets (ICR and CRC) were combined and sorted into various strata for the purposes of investigating differentiation in mtDNA and heterogeneity of sex ratios. The binomial test of frequencies, available in Excel, was used for sex ratios. The program GENEPOP (Rousset 2008) was used for calculation of indices of differentiation (F_{ST}) and test of differentiation (i.e., modified exact test) for mtDNA haplotypes.

Results

Haplotype sequence review and revised datasets

The revised ICR datasets (Japanese Coastal, Offshore and Bycatch) totalled 2,551 samples, of which 2,544 included mtDNA control region sequences and haplotype codes. The Korea Bycatch dataset included 477 records of bycatch samples, of which 477 included mtDNA control region sequences and haplotype codes. Initial review indicated that haplotype codes had been reconciled (i.e., were in agreement), allowing a combined analysis of the Japanese and Korean datasets. Results of a quality control review and comparisons of the CRC Korean BC record to Korean market surveys are reported elsewhere (SC/63/RMP15, updated from SC/D10/NPM2_r1). Minor corrections to the Korean dataset, received on 15th April 2011, were not incorporated here due to limitations of time. In total, the 3,021 samples represented 130 haplotypes, 118 of which were found in Japanese samples and 39 of which were found in Korea (with 27 in both datasets). Frequency histograms of the haplotypes in the two datasets are shown in Figure 1.

Differentiation of haplotypes by revised subareas and source

Preliminary investigations of pairwise tests of differentiation from all subareas and sources allowed us to reduce the complexity of the overall comparisons to 9 sample strata (Table 1). For this, we deleted subareas with small sample sizes, e.g., the Korean Strait, subareas 1, 10 and 11, and combined some adjacent subregions that showed no significant differences, e.g., 7W>60 and 7E, 8 and 9. The final 9 strata include the represented subareas 5(bc) (i.e., Yellow Sea), 6W(bc) (i.e., East Sea), 6E(bc), 2(bc), 7CS, 7CN, 7W>60nm+7E and 8+9. Given the strong signal of differentiation by 7(bc) within the other subarea strata, we did not pool these samples. Further details of comparisons with subarea 7 are discussed below.

Overall, the pattern of differentiation was similar to that reported for the haplogroup analyses (Table 2). Of the 36 pairwise comparisons all but 7 were significant: 3 of these non-significant tests involved subarea 5, which had the smallest sample size, 3 involved comparisons with subareas 7W>60+7E and 1 was between 6E and 6W. The comparison between 7CS and 7CN, which include the coastal Sanriku and Kushiro catches and the ‘offshore’ catches within 60 nm of shore, showed a small but significant difference. The largest effect size was between the most distant samples, e.g., 5(bc) and 8+9 (F_{ST} =0.0967; p <0.0001). The smallest significant effect was between 7CS and 7CN (F_{ST} =0.0004; p =0.042).

Sex ratios by revised subarea and source

Significant male bias was found in 5 of the 9 sample strata: subareas 5(bc), 6W(bc), 7CN, 7W>60+7E and 8+9. A significant female bias was found in subarea 2(bc) (Table 3)

Differentiation of haplotypes by sex - for revised subarea and source

There were no significant differences in haplotypes frequencies by sex within any of the 9 sample strata (Table 3).

Differentiation of haplotypes by season – for bycatch in subarea 2(bc) and 7(bc)

In an effort to understand seasonal differences in distributions of stocks along the two coasts, we stratified haplotype frequencies by 2 six-month seasons (spring/summer and autumn/winter) for bycatch in subareas 2(bc) and 7(bc) (Table 4). There was no significant difference between seasons for subarea 2(bc) but there was a significant difference for subarea 7(bc) and an absence of difference between subarea 2(bc) summer and subarea 7(bc) winter strata.

Differentiation of haplotypes by season – for bycatch in subarea 5(bc) and 6W(bc)

We investigated seasonal differences in 6W(bc) compared to 5(bc) to test the prediction of mixing, based on previous results for microsatellites reported by Kanda et al. (SC/62/NPM11, see Table 6). This previous analysis reported a difference between samples from June-October (JO) and March-June (MJ) in subarea 6W(bc) but not in subarea 5(bc). This was interpreted as evidence of seasonal migration of the Yellow Sea stock (from subarea 5) into subarea 6W during summer (June-October), resulting in a Wahlund effect in the overall sample from 6W. Using mtDNA haplotypes, we tested for differentiation of the JO and MJ seasonal strata in 6W(bc) and compared each of these to subarea 5(bc). There was no evidence of significant differentiation in these comparisons (Table 5), confirming the findings that haplotype frequencies in the Yellow Sea do not differ from the East Sea/Sea of Japan (Table 2).

Comparison of haplotypes frequencies in subareas 6E and 2

To investigate the hypothesis of a JE stock in subarea 2, we compared the frequency of haplotypes for this subarea and those of subarea 6E (Figure 2). The frequency histograms showed that the significant differences between these two subareas is due largely to a strong frequency based difference in two shared haplotypes, #1 and #64. A frequency-based difference in shared haplotypes is consistent with the hypothesis of two related stocks (i.e., the JW and JE), rather than the mixing of 2 stocks distinguished by many unshared haplotypes.

Differentiation of haplotypes by strata in subareas 7CS and 7CN

To investigate the complexity of stock structure in subareas 7CS and 7CN, we considered differences in haplotype frequencies from 6 strata. These consisted of the north and south division of bycatch, the north and south divisions of coastal hunting (Sanriku and Kushiro) and the north and south divisions of the ‘offshore’ whaling within 60nm of shore (7oCS and 7oCN). The significant differences were entirely attributed to comparisons with the bycatch samples rather than to the north/south divisions within the bycatch, coastal whaling or offshore whaling (Table 6). Only when coastal whaling and offshore whaling <60nm are combined, is there a small but significant difference attributed to 7CS and 7CN divisions (see Table 1).

Conclusions (including SC/D10/NPM4)

Overall results of mtDNA analyses presented here and previously for haplogroups (SC/62/NPM20), and analyses of microsatellites (SC/63/RMP17) are consistent with Hypothesis III in the following, except where noted:

- 1) Subarea 5(bc) represents the best available proxy for a Yellow Sea stock that is resident year-round, based on biological information, but genetic evidence is conflicting. **Genetic Evidence:** Results of mtDNA analyses presented here and analyses of Hardy-Weinberg deviation from simulated mixing of stocks based on microsatellites (SC/63/RMP15) are not consistent with predictions of Hypotheses II or III in regards to a Yellow Sea stock and mixing of this stock in subarea 6W. Further investigation of this discrepancy is required given the results presented previously by Kanda et al. (SC/62/NPM11) and the biological evidence of a Yellow Sea stock.
- 2) Subarea 6E(bc) represents the best proxy for the ‘JW’ stock, a year-round stock in the Sea of Japan. **Genetic Evidence:** 6E(bc) differs significantly from most other subareas in haplotype frequencies and from all other subareas in microsatellite loci. 6E(bc) shows no evidence of heterogeneity in haplogroup frequencies by sex or season (SC/62/NPM20). These differences, and absence of differences, are consistent with a single non-migratory stock.
- 3) Subarea 2(bc) represents the best proxy for the ‘JE’ stock, a year-round stock along the eastern coast of Japan. **Genetic Evidence:** 2(bc) differs for mtDNA and microsatellite loci from all other subareas. Comparison of 2(bc) and 6W(bc) shows that the primary differences are in frequencies of shared common haplotypes. This is indicative of differences between 2 related stocks (e.g., JE and JW), rather than a mixing of two stocks with many unshared haplotypes (e.g., J and O). No significant differences in haplotype frequencies were found between sexes or between seasons in subarea 2(bc). The absence of these differences is also consistent with a single year-round stock, rather than a mixing of two different stocks.
- 4) Coastal Sanriku and coastal Kushiro represent the best proxy for the ‘OW’ stock. **Genetic Evidence:** The individual and combined samples from these two seasonal hunts show significant differences in both haplotypes and microsatellite loci with almost all other subareas and strata, including comparisons to 7(bc) and subareas 8 and 9. However, samples from these two hunts show no differences with each other, at either mtDNA or microsatellite loci (see SC/D10/NPM4). The combined sample of Sanriku and Kushiro shows no difference between sexes for mtDNA (microsatellite analyses are in progress). These differences,

and absence of differences, are consistent with seasonal hunting (Spring for Sanriku and Fall for Kushiro) from a single migratory stock that is differentiated from the more coastal JE stock and the more offshore OE stock.

- 5) The revised subareas 7CS and 7CN require further consideration. **Genetic Evidence:** The largest effect within these subareas is due to differences between bycatch and all other strata. The north/south division at latitude 41 provides little evidence of differentiation within the other three strata, e.g., bycatch, coastal hunting and 'offshore' hunting within 60 nm of shore.
- 6) Subareas 8 and 9 represent the best proxy for the 'OE' stock. **Genetic Evidence:** These two subareas did not differ from each other at either mtDNA haplotypes or microsatellite loci, but do differ from all other subareas or strata, except for the relatively small sample from the adjacent 7W>60nm and 7E.

Acknowledgements

This report was prepared in accordance with the conditions established in the SC data access protocol for Procedure A and the agreement to use the data only for the investigation of stock structure for the Implementation Assessment of NP minke whales. For access to the Japanese datasets, we thank the members of ICR who collected these samples and those that conducted the laboratory work, particularly L. Pastene, N. Kanda and M. Goto. For access to the Korean dataset, we thank members of the CRC who collected these samples and those that conducted the laboratory work, particularly An, Y-R and Park, J-Y. Thanks also to members of the IWC Data Availability Group, T. Kitakado, G. Donovan, and D. Palka.

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Table 1. Sample strata used in the '9-population' analyses reported in Table 2, including corresponding subareas and sources, and Data Availability Agreement (DAA).

Sample Strata	Subarea	Source	DAA
5(bc)	5	Korean Yellow Sea bycatch	CRC
6W(bc)	6W	Korean East Sea bycatch	CRC
6E(bc)	6E	Japanese Sea of Japan bycatch	ICR
2J(bc)	2	Japanese Pacific Coast bycatch	ICR
7J(bc)	7CS+7CN	Japanese Pacific coastal bycatch	ICR
7CS	7CS	Japanese Pacific coastal and offshore whaling	ICR
7CN	7CN	Japanese Pacific coastal and offshore whaling	ICR
7W>60+7E	7W+7E	Japanese Pacific offshore whaling	ICR
9+8	9+8	Japanese Pacific offshore whaling	ICR

Table 2. Pairwise tests of differentiation for mtDNA haplotypes from western North Pacific minke whales showing F_{ST} values, calculated using Genepop for 9 sample strata. **Bold** values are significant at $p < 0.001$. Starred values* are significant at $p < 0.05$ but > 0.01 .

Strata		5 (bc)	6W (bc)	6E (bc)	2 (bc)	7 (bc)	7CS	7CN	7W>60+7E
5 (bc)	n=47								
6W (bc)	n=366	0.0015							
6E (bc)	n=411	0.0006	-0.0002						
2J (bc)	n=184	0.0159	0.0091	0.0090					
7J (bc)	n=212	0.0291	0.0189	0.0187	0.0086				
7CS	n=321	0.0754	0.0635	0.0622	0.0389	0.0116			
7CN	n=503	0.0742	0.0624	0.0612	0.0386	0.0118	0.0004*		
7W>60+7E	n=111	0.0844	0.0729	0.0711	0.0463	0.0162	-0.0002	-0.0011	
9+8	n=690	0.0967	0.0834	0.0819	0.0555	0.0232	0.0011	0.0016	-0.0009
Total	2845								

Table 3. Sex bias in sample of North Pacific minke whale by 9 sample strata (Excel Binomial Test). Pairwise F_{ST} and differentiation calculated in GenePop.

Strata	n	Female	Male	Binomial, p	F_{ST}	p
5(bc)	47	0.13	0.87*	<0.0001	n.a.	n.a
6W(bc)	366*	0.14	0.86*	<0.0001	0	0.499
6E(bc)	410	0.52	0.48	0.3480	0.0017	0.129
2(bc)	184	0.64*	0.36	0.0002	0	0.908
7(bc)	212	0.57	0.43	0.0630	0.0083	0.393
7CS	319	0.46	0.54	0.1450	0.0002	0.725
7CN	503	0.21	0.79*	<0.0001	0	0.996
7W>60+7E	111	0.12	0.88*	<0.0001	n.a.	n.a
8+9	696	0.09	0.91*	<0.0001	0	0.417

Table 4: Pairwise differentiation (F_{ST}) in mtDNA haplotype frequencies by seasons for subareas 2(bc) and 7(bc). **Bold** values are significant at $p < 0.001$.

Strata	2(bc)_sum	2(bc)_win	7(bc)_sum
2(bc)_summer n=61			
2(bc)_winter n=123	-0.0005		
7(bc)_summer n=145	0.0142	0.0210	
7(bc)_winter n=67	-0.0001	0.0030	0.0233

Table 5: Pairwise differentiation (F_{ST}) and exact test of mtDNA haplotype frequencies by seasonal strata (July to October and November to June) in subarea 6W(bc) compared to overall sample for subarea 5(bc). F_{ST} shown below and p values for exact test shown above the diagonal.

Strata	5(bc)	6W(bc)_NJ	6W(bc)_JO
5(bc) n=47	--	0.4936	0.4979
6W(bc)_NovJun n=283	0.0016	--	0.9456
6W(bc)_JulOct n=83	-0.0000	-0.0023	--

Table 6: Pairwise differentiation (F_{ST}) in mtDNA haplotype frequencies for 6 strata within subarea 7. **Bold** values are significant at $p < 0.001$. The 6 strata are the 7CS and 7CN divisions for bycatch (7(bc)CS and 7(bc)CN), coastal hunting at Sanriku and Kushir0 (CSan and CKush) and 'offshore' hunting within 60 nm of shore (7oCS and 7oCN).

Strata	7(bc)CS	7(bc)CN	CSan	CKush	7oCS
7(bc)CS n=116					
7(bc)CN n=96	0.0031				
CSan n=227	0.0041	0.0151			
CKus n=253	0.0043	0.0175	0.0000		
7oCS n=94	0.0124	0.0337	0.0028	0.0045	
7oCN n=250	0.0085	0.0222	0.0001	-0.0002	0.0014

Figure 1. Haplotype frequencies for all Japanese reported bycatch and scientific whaling (top) and Korean reported bycatch, sorted according to haplotype code#.

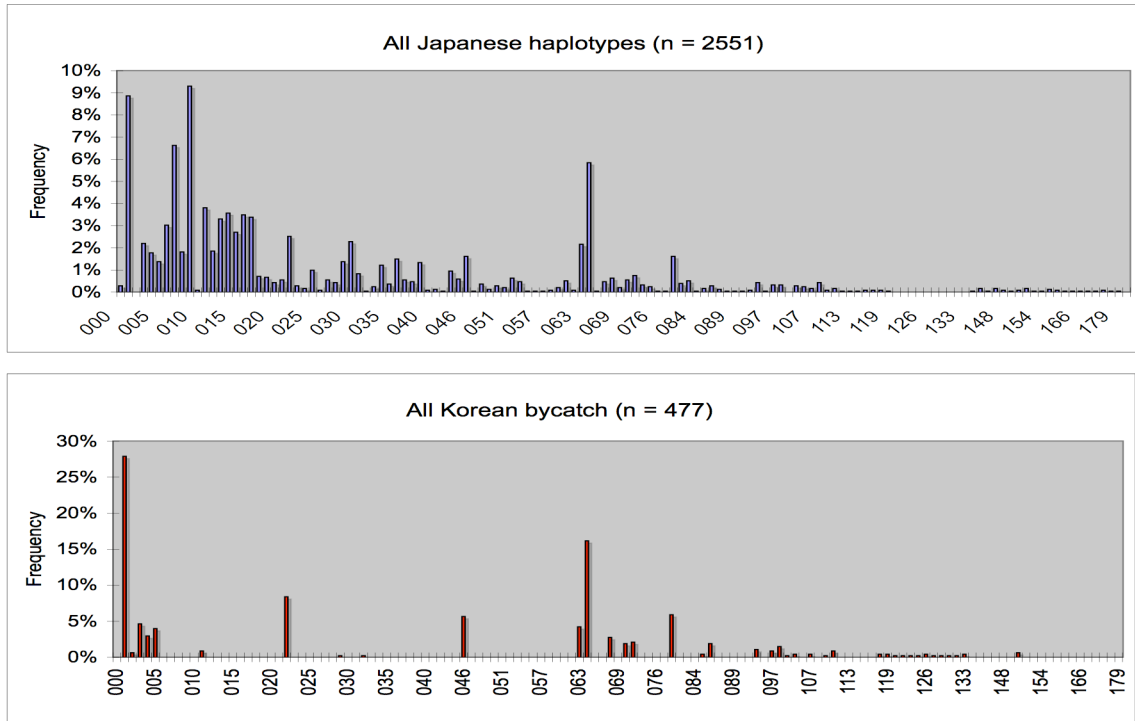


Figure 2: Haplotype frequencies for subareas 6E(bc) (top) and 2(bc) (bottom), sorted according to haplotype code#.

