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DNA profiling of North Pacific minke whales from Korean markets shows true takes exceed officially reported bycatch

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ABSTRACT
Individual identification of 'whalemeat' products by DNA profiling provides direct information on the minimum number of whales entering into commercial trade. A total of n=177 samples of North Pacific minke whales (*Balaenoptera acutorostrata* subsp.) were purchased in markets of Busan, Ulsan and Pohang during 7 market surveys from February 2004 to February 2005. In an attempt to improve on methods of previous estimates (Baker et al. 2007), these market surveys were conducted at regular intervals and from a standardised number of outlets. Following quality control review of information from mtDNA control region sequences, sex identification and genotyping of up to 6 microsatellite loci, 169 of the products provided sufficient information for individual identification. Matching of DNA profiles resolved 90 individuals, 69 of which were sampled in only one of the 7 surveys. This 'minimum census' exceeds the reported bycatch of 61 minke whales from official records in 2004 and the average of 85/year reported for the three-year period, 2003-2005. As the sampling was far from exhaustive, the true take of Korea minke whale is likely to be substantially larger than documented by the current reporting. Capture-recapture estimates based on these replicate samples could provide an improved estimate of the true takes over time and inform population dynamic models of the J stock.

INTRODUCTION

Genetic sampling of whale products on domestic markets has proven a useful tool both for identifying the species (Baker and Palumbi 1994, Baker *et al*. 1996), stock origin (Baker *et al*. 2000) and minimum numbers (Dalebout *et al*. 2002) of individuals on the market. Use of capture-recapture techniques provides a means of moving beyond the minimum number of identified individuals to provide inference on the throughput of whales on the market (Baker et al. 2007). This information can complement statistics based on reporting of whales taken as 'bycatch' or in regulated hunts, to provide an improved estimate of the 'true' takes over time (Baker 2008).

Here we present a 'minimum census' of North Pacific minke whales distributed in retail markets of the Republic of (South) Korea from February 2004 to February 2005. This minimum census is compared to the official records in the Progress Report of the government of Korea to the International Whaling Commission.

MATERIALS AND METHODS

Sample collection and genotyping. A total of 7 surveys of Korean markets were conducted from February 2004 to February 2005. On the basis of experience with Korean whalemeat markets since 1994, we attempted to sample systematically the majority of major retail outlets in the three cities during each survey (Baker et al. 2006). Each survey required 2–3 days and included a standard number of shops in each of the major fisheries markets as well as whalemeat specialty restaurants. More than 40 outlets were visited during the 7 surveys, although variation in whalemeat availability resulted in purchases from a subset of 15–20 outlets on any given survey. In an effort to improve on previous methods, market surveys were conducted at intervals of approximately 2 months, sampling from a standardised number of shops during each survey (Leaper & Cooke 2006).

The sampling of minke whales on the Korean markets is described by (Baker et al. 2006) and field methods for DNA extraction, sex identification, mitochondrial (mt) DNA control region sequencing and amplification of microsatellite loci are described in detail by Lavery et al. (2004). For microsatellite genotyping, we used a set of 8 published loci (GATA417, GATA28, GATA98, Palsbøll et al.,1997; GT23, GT211, GT310, GT509 and GT575, Bérubé et al., 2000). Following binding to streptavidin-coated plates, and washing to remove native

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DNA, amplified DNA was returned to the laboratory. Microsatellite loci were re-amplified with fluorescently labelled primers from biotin-bound PCR products collected in the field prior to genotyping. Amplified product was co-loaded in 3 sets for automated sizing (size standard 500LIZ™) on an ABI3730xl (Applied Biosystems, Table 1). Allele sizes were called automatically with GeneMapper (Applied Biosystems) and visually inspected for inconsistencies. Peaks that could not be resolved were removed from the dataset. Data organization, analyses of microsatellite allele frequency and analysis of probability of identity for each microsatellite locus and mtDNA were conducted with the program GenAlEx (Peakall & Smouse 2006). One locus, failed to amplify reliably in the field (GT23) and a second was found to have detectable rates of allelic dropout (GT575). These loci were excluded from the DNA profiles.

Genotype matching. Samples with fewer than 5 microsatellite loci successfully genotyped were considered of insufficient quality for individual identity and were removed from the dataset. Unique genotypes were resolved with the program CERVUS (Marshall *et al.* 1998), requiring an exact match of 4 loci, supported by control region haplotype and sex, where available. Given issues associated with poor quality tissue samples (e.g. allelic dropout), the initial comparison allowed for mismatches at up to 2 loci. For this minimum criterion, the probability of identity ranged from 2.74×10^{-4} to 1.68×10^{-5}. We considered this to be sufficient to establish 'likely replicates'. All mismatching loci were visually inspected and resolvable errors (e.g., a low intensity peak that had been missed in the primary analysis resulting in a false homozygote) were corrected. An initial per-allele error rate of 1.8% was calculated from samples consider to be 'likely replicates'. This initial error rate was substantial reduced through the review process.

RESULTS AND DISCUSSION

Of the 177 samples previously identified as North Pacific minke whale, based on sequencing of mtDNA control region or cytochrome *b* sequences (Baker et al. 2006), 169 were successfully genotyped for 5 or more loci. Matching genotypes in CERVUS, confirmed that the 169 products represented 90 unique genotypes. Based on the relatively small probability of identity and the relatively small number of pairwise comparison, we inferred that the 90 unique genotypes represent 90 individual whales and that the matching genotypes represented multiple products of the same whale. This inference was further supported by mtDNA haplotype and sex available for most products.

The observed capture-recapture histories of all products are listed in Table 2. A sampling event was defined as a purchase from a sales outlet (e.g., shop, restaurant or market stall) on a given day. Products from the same whale purchased in different shops or on different days were considered replicate samples. Products from the same individual purchased in the same shop/day are shown as pseudo-replicates. Multiple occurrences of the same sampling history are listed once, and the number of individuals sharing this capture history is given. Of the 90 individuals, 69 were identified in only 1 of the seven surveys, 15 were identified in two surveys, 5 in three surveys and 1 in four surveys.

Progress reports of the Government of Korea show 87 minke whales killed as bycatch in 2003, 61 in 2004 and 107 in 2005, for an average of 85/year over these 3 years (IWC 2005; IWC 2006; IWC 2007).

CONCLUSION

The systematic sampling of Korean markets during 7 surveys revealed that a minimum of 90 individual North Pacific minke whales were killed and sold commercially across this 12-month period. This number exceeds the total reported for the year 2004 and the average reported across the three years, 2003-2005. As the sampling was far from exhaustive, our minimum census strongly suggests that the true take is substantially larger than documented by the current reporting. Capture-recapture analyses similar to those reported previously (Baker et al. 2007) would provide an improved estimate of this total. Assuming that these whales were taken with the EEZ of South Korea, they must represent the 'J' stock, providing further evidence for concern of over exploitation of this depleted coastal stock.

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Table 1: Locus information for the 6 loci used to individually identify North Pacific minke whale products sold on Korean markets

Locus	Label	Co-load group	Allele size range	N alleles
GATA 28	NED	2	196 – 216	6
GATA 417	FAM	1	202 – 222	7
GATA98	NED	1	101 – 113	4
GT 211	FAM	2	96 – 120	11
GT 310	TET	3	115 – 137	9
GT 509	HEX	3	195 – 215	11

Table 2: Capture-recapture histories of products purchased from individual North Pacific minke whale purchased during market surveys of Busan, Ulsan and Pohang for February 2004 to February 2005. Individual identification is based on DNA profiling from mtDNA control region sequences, sex identification and up to 6 microsatellite loci. Note inclusion of replicate samples and pseudo-replicate samples (as superscript) within each survey. Each line shows a specific capture history, e.g., in the Feb 04 survey, 4 individuals were found once, 2 individuals were found in 2 shops, 1 individual was found in 3 shops (and twice in the same shop), 1 individual was found twice in 1 shop and recaptured in the April 04 survey, 1 individual was recapture in April 04 (twice in the same shop).

Survey date	Feb 04			April 04				June 04	Aug 04		Oct 04		Dec 04			Feb 05			
Histories	4																		
	2	2																	
	1^2	1	1																
	1^2			1															
	1			1^2															
				5															
				1		1													
				1				1											
				1		1		1	1^2										
				1	1	1	1^2	1			1^2								
								1^2											
								10											
								1	1										
								1			1^2								
								1^2					1						
								1								1			
								1^2					1			1			
									1^2										
									11										
									1	1									
									2				2						
									1		1								
									1	1	1								
									1^2	1	1	1							
									1		1^2					1			
									1				1^2		1	1		1	
									1		1^2		1			1			
											1^2								
											5								
											1	1							
											1^2	1							
											1^2		1						
											1		1						
													1^2						
													9						
													1		1				
													1^3	1	1^3				
													1^2			1			
																2^2			
																6			
																3		3	
																1	1	1	
Individuals	9			9				16	21		10		13			12			90
+ Replicates	13			16				19	26		20		25			24			143
Samples	15			18				22	29		27		32			26			169