SC/53/RMP13

Comments on the estimations of the J and O stocks mixing proportion and level of by-catches of common minke whale using mitochondrial DNA data from the retail market surveys

Luis A. Pastene, Mutsuo Goto and Naohisa Kanda

The Institute of Cetacean Research, 4-18 Toyomi-cho, Chuo-ku, Tokyo 104-0055, Japan

ABSTRACT

This paper reviews and discusses several technical aspects related with the identification of North Pacific common minke whale and O and J stocks products from surveys in the retail market. It is not the objective of this paper dispute or to propose any figure on the contribution of the O and J stocks to the market. This paper deals with technical aspects of the surveys identifying several factors that need more discussion if data derived from such surveys are going to be used for management purpose. The degree of genetic differentiation among different forms of minke whale allows the unambiguous identification of North Pacific common minke whale products in the market. Moreover, the near-to-fix mtDNA differences between J and O stocks allow the assignment of individuals to either of these stocks with a high degree of confidence. There are, however, several aspects of the surveys that need further considerations. Among them are the non-random nature of the surveys, the geographical variation in the proportion of J and O stock products in Japan, the 'dynamic' of the minke whale products in Japan and the representativeness of the sample used as the baseline for the J stock in the estimations of mixing proportion. Without further considerations on these aspects, the use of market data for management purpose of common minke whale is premature.

KEYWORDS: NORTH PACIFIC MINKE WHALE, J STOCK, O STOCK, MIXING PROPORTION, BY-CATCH, GENETICS

INTRODUCTION

During the last years molecular surveys of whale products have been conducted in the retail market of Japan and Korea. The original purpose of these surveys was the investigation of the species identity of different whale products to get an idea on the diversity and availability of cetacean species in the market. A workshop was carried out in 1999 to discuss several technical matters related to such surveys (Dizon *et al.*, 1999).

While the identification of species was the original objective of these surveys, more recently the investigation of the stock of origin and individual identification of whale products have been also important objectives. Of special interest has been the identification of products in the market derived from the J and O stocks common minke whale. As recently as the 2000 Scientific Committee (SC) meeting in Adelaide, the Committee received and discussed some papers, which presented estimations of the contribution of these stocks to the retail market. Furthermore extrapolations to the possible level of by-catch minke whales in Japan and Korea was made based in such figures (IWC, 2001).

Accurate information of the level of by-catches of minke whale around Japan is important for the IST of this species in the region. Then techniques conducting to obtain such information should be investigated and discussed. The use of- and extrapolation from- the number of common minke whales identified in molecular surveys in the retail market is one of those techniques.

The objective of this paper is to identify and discuss factors, which could affect the estimation of contribution of J and O stocks to the market and the estimation of by-catches based on these data. The objective of this paper is not to dispute any of the previous figures of mixing proportion and by-catch offered in previous SC meetings. Also we do not attempt to propose any new figures. This paper deals only with methodological aspects of the molecular surveys in the retail market.

DIFFERENTIATION BETWEEN J AND O STOCKS

North Pacific common minke whale can be separated unambiguously from any other form of minke whale from any other ocean basin (Hori *et al.*, 1994; Pastene *et al.*, 2001).

At a smaller scale, it has been known for a long time the existence of two different stocks on either side of the Japanese archipelago (Omura and Sakiura, 1956; Ohsumi, 1983; Best and Kato, 1992). The first genetic study showing substantial differences between these two stocks was based on allozyme (Wada, 1984). More recently these stocks have been investigated using DNA-based techniques. Goto and Pastene (1997) used RFLP analysis of the mtDNA control region and confirmed the significant differences between these two stocks. These authors expanded the RFLP analysis to sequencing and they found near-to fix genetic differences between these two stocks (Goto and Pastene, 1998; undocumented information, 1998).

Because differences between J and O stocks are striking but not absolute, maximum likelihood method can be also used to estimate the mixing proportion between J and O stocks in a given sample, provided the haplotype frequencies of the 'baseline' stocks (e.g. Pastene *et al.*, 1998).

EXAMPLE OF ESTIMATIONS OF MIXING PROPORTION OF J AND O STOCKS

Pastene *et al.* (1998) used mtDNA haplotype frequencies and a maximum likelihood method to calculate the mixing proportion of J and O stocks in sub-area 11. It was estimated that the proportion of J stock female animals in the April sample was 0.4075 (SE: 0.0806) and that of male animals in August in 0.3147 (SE: 0.1160). Goto *et al.* (2000) used three different genetic markers, mtDNA control region RFLP, control region sequencing and microsatellite to estimate the J stock proportion in sub-area 11. The estimates based on RFLP, sequencing and microsatellite were in general similar to each other.

It should be noted that the baseline population of the J stock in these three studies was the same and it was based on a sample from a 1982 whaling operation in Korea (n=28), which was assumed to be representative of the genetic diversity of the J stock.

Regarding estimates in the retail market, Goto and Pastene (2000) summarized the information obtained from two DNA surveys in the Japanese retail market, which were sponsored by the Government of Japan (GOJ), one conducted in 1996 and the other in 1999/2000. Out of 341 whale product samples identified in the 1996 survey, 65 (19.1%) were North Pacific common minke whale products. Out of 294 samples identified in the 1999/2000 survey, 31 (10.5%) were North Pacific common minke whale products. Under the criteria that a G base in position 298 identify most of the individual of the J stock (Goto and Pastene, undocumented information, 1998) the number of J stock products in the North Pacific minke whale market sample was calculated. In the 1996 and 1999/00 surveys 9 (13.9%) and 13 (41.9%) products were from the J stock, respectively.

Baker *et al.* (2000) used unpublished and unauthorized mtDNA information mainly from Goto and Pastene (1998) and from personal communications (e.g. e-mail communication 10 November 1998) to characterize genetically J and O stocks. These authors used a maximum likelihood procedure to estimate the mixing proportion of J and O stock products in the Japanese retail market. They used the JARPN sample (1994-1998) as the 'baseline' sample of the O stock and the Korean market sample as the 'baseline' sample of the J stock. Based on the relative haplotype frequencies of these two source stocks, the maximum likelihood estimate of the J stock contribution to the Japanese retail market was found to be 31% with

confidence interval of 19%-43%. For the 31% proportion of J stock products, the required total incidental take was 100 whales per year, of which 60 would be of J stock origin (assuming that 60% of the incidental catches in Japan are from the J stock).

FACTORS AFFECTING THE ESTIMATIONS OF MIXING PROPORTION AND LEVEL OF BY-CATCHES

To illustrate the discussion of the factors listed below we conducted several calculations based on data of minke whale products obtained during the Japanese surveys in the retail market. A summary of these surveys is as follow:

- 1995 survey by the GOJ and Traffic (March-Apr.), 210 products sequenced, 16 North Pacific minke whale products.
- 1996 survey by the GOJ (March-June), 341 products sequenced, 65 North Pacific minke whale products.
- 1999/2000 survey by GOJ
  a) First set (Nov., Dec., Jan.), 294 products sequenced, 31 North Pacific minke whale products.
  b) Second set (Nov., Dec., Jan., Feb.), 291 products sequenced, 32 North Pacific minke whale products.

- 2000/01 survey by the GOJ
  a) First set (Nov., Dec.), 247 products sequenced, 16 North Pacific minke whale products.

The 1995 surveys were already reported (Government of Japan, 1997a; 1997b). The 1996 and 1999/00 (first set) surveys were reported in Goto and Pastene (2000). The 1999/00 (second set) and the 2000/01 (first set) have been recently completed and they will be reported in the future. For the purpose of this paper we make use of the number of North Pacific common minke whale products identified in these surveys. It should be noted that the figures on common minke whales refer to number of products, not individuals.

Nuclear DNA (nDNA) (microsatellite) analysis on products identified as North Pacific common minke whale have been conducted only for the survey 1999/2000 (first set). The analysis was made using the same eight loci as used by Abe *et al.* (1998).

In the sections below we identified the J stock individuals by the criteria of a G base in position 298 (Goto and Pastene, undocumented information 1998). It is noted here that other sites in the control region can be used to identify J and O stock individuals, but none of them is absolute. Test sequences in the Japanese surveys were obtained in different laboratories, which used different primers for PCR amplification. As a result we obtained a set of sequences differing in length. The only informative site common for all samples was site 298. However, this is not a problem for the objective of this paper. The idea here is not to provide absolute values on the number of J stock products. The important point here is to keep consistency in all comparisons by using the same criteria.

1- Randomness of the surveys

This is an important aspect to consider. It is a basic principle that if the surveys do not cover adequately the study area (both geographical and temporal), the samples obtained will not be representative of that area and the information obtained will be of little utility for management purposes. Furthermore sampling should be weighted according to the availability of whale products in each area.

The DNA surveys conducted in the retail market, both those sponsored by the GOJ and those conducted by foreign researchers, are not random surveys. A considerable discussion has been carried out within the Scientific Committee (SC) on this aspect. The main critical comments from several SC members were: a) sampling design are inadequately described and hence it is not possible to evaluate the resultant estimates, b) the non-random nature of the sampling scheme meant that any inferences from the data collected during the market surveys were flawed (IWC, 2001).

To illustrate this problem we examined the consistency among the results of different Japanese surveys. Table 1 shows the results of the number of North Pacific common minke whale products and that of J stock products found in different surveys in the Japanese retail market. The proportion of North Pacific common minke whale products and that of J stock products change among surveys, even between surveys conducted in similar periods of the year. The inconsistency of results among surveys is suggestive of the non-random nature of the surveys.

Apart to make effort to design random sampling in the future, the results from each sample should be weighted according to the availability of whale products in each prefecture. This weighting, however, is a challenging issue as the dynamics of the whale products in Japan is very complex. The availability of minke whale products could change by location and period of the year. For example it could depend on the period and localities where the JARPN by-products are distributed. Even more JARPN by-products could be transferred among Japanese prefectures after the original split of the by-products. It will depend also on the dynamics in the market of the by-products derived from stranding or by-catches. The way as these products enter into the market is unknown. It is possible that products of a by-catch or stranded whale in a given prefecture be sold at different prefectures.

2- Geographical variation of the distribution of J/O stocks products in Japan

The proportion of J/O stocks in the retail market change with geographical locality in Japan as shown in IWC (2001, Annex S). Using additional information from the Japanese surveys we expanded the analysis of geographical variation in the proportion of J/O stock products. Figs. 1 , 2 ,3 and 4 shows the geographical distribution of J and O stocks products in Japan according the surveys of 1995, 1996, 1999/2000 and 2000/01 (first set), respectively. Larger proportion of the J stock is found in western Japan. This means that the proportion J and O stocks products will be different according to the region sampled.

Table 2 shows the results on the number of North Pacific minke whale products and that of J stock products found in different surveys in the Japanese retail market, for both western and eastern Japan. The division between western and eastern was made at approximately at the location of the Kanto area (Kanto area is included in the eastern part). The proportion of North Pacific minke whale products and that of J stock products is larger in western Japan than in eastern Japan in each of the survey, except 1996.

The contribution of J stock by-products by the JARPN is very small. According to the criteria of a G in position 298, the average annual contribution is about five individuals. Then the contribution of J stock products to the market could be mainly from stranded or by-catches of J stock animals along the Japanese coast.

It has been assumed for long time that J stock animals distribute only in the Sea of Japan and East China Sea. However, a DNA analysis of the stranding and by-catches in sub-area 2 (southeast coast of Japan) suggest a larger proportion of J stock than O stock animals. Genetic samples were available for 13 by-catch whales between 1996 and 2000. Of these 10 were characterized by a G in position 298 (76.9%). Supply of J stock products to the market from prefectures around this sub-area and others to the south east of Japan, could explain in part the larger proportion of J stock products in the market in western Japan.

These results for sub-area 2 also suggest that the distribution of the J stock could be wider than originally thought.

3- Which sample is representative of the J stock?

This is an important issue to determine. Depending on what sample is designed as the 'baseline' sample of the J stock the DNA characterization of this stock will change and obviously this will have an effect on the estimations of the mixing proportion in a mixed assemblage of O and J stocks. This issue is discussed in more details in Goto *et al.* (2001). According to their results larger haplotype diversity has been found in recent by-catch samples obtained in the Sea of Japan than in a commercial sample obtained in 1982 around the Korean Peninsula. Recent by-catch samples are from both sides of the Sea of Japan (Korean coast and Japanese coast). Several explanations are given in Goto *et al.* (2001) including a) the possibility of additional stock structure within the Sea of Japan and b) possible migration of O stock whales into the Sea of Japan. These and other explanations should be further investigated. An adequate identification of a

representative sample of the J stock is fundamental to get accurate estimation of the mixing proportion of J and O stocks in both the wild and in the retail market.

It has been assumed that all whales distributed in the Sea of Japan belong to the J stock, however, there is the possibility that some O stock animals migrates into the Sea of Japan, at least in some part of the year. If a sample containing animals from the two stocks is used as the 'baseline' sample of the J stock, then the estimates of the proportion of the J stock in a mixed assemblage will be overestimated.

4- Duplicate sampling

As noted in Goto and Pastene (2000) results of the molecular surveys in the retail market are presented as number of samples or 'test sequences' rather than number of individuals. Microsatellite analysis were conducted on samples identified as North Pacific common minke whales by the mtDNA analysis in the surveys in Korean and Japan (Dalebout *et al.*, 2000). Analysis of six microsatellite profiles indicated that the 101 minke whale products from the Japanese market originated from 87 individuals and that the 42 products from the Korean market originated from 34 unique individuals. Of the 87 individuals identified in the Japanese market, 33.3% were of likely J-stock origin, based on the characteristic polymorphisms of the mtDNA control region (Dalebout *et al.*, 2000). Furthermore these authors considered that these results provided evidence that previous estimates of J stock proportions on the Japanese market were not biased by duplicate sampling.

As established earlier microsatellite analysis has been conducted only on the whales identified as North Pacific common minke whales in the 1999/2000 Japanese survey. According the profiles of eight loci, the 31 animals identified as North Pacific common minke whale in that survey originated from 26 unique individuals and the number of individuals J stock in that sample was 11 (39.3%), which is similar than the proportion calculated on the basis of the number of products (41.9%).

Microsatellite analysis should be conducted as a routine in future market surveys and analysis of duplicate, similar to that conducted by Dalebut *et al.* (2000), should be repeated using a larger sample set.

5- How long whale products from a same individual remain in the Japanese retail market?

Dalebout *et al.* (2000) using microsatellite analysis identified 34 individuals among 42 North Pacific common minke whale products in the Korean market in 1999. They suggested that such minimum 'census' of the 1999 by-catch could represent an overestimate of the by-catch in that year if products from previous years are held in long-term storage and distributed over a period of time. Furthermore they indicated that the absence of a match between two sampling periods (March and October), argues against this as a common practice in the Korean market.

We examined preliminarily this issue for the case of the Japanese market. For such purpose we used the microsatellite profiles of the 31 minke whales identified in the 1999/2000 Japanese survey (first set) and compared them with the profiles of minke whales taken during JARPN surveys and documented by-catches (Table 3). As explained above, the 31 samples derived from the 26 individuals. Of these 11 matched with JARPN samples and all of them were O stock animals. By looking at the date of the JARPN samples, market products sampled from November 1999 and January 2000 contained minke whale JARPN by-products distributed in the market in 1997 (1), 1998 (4) and 1999 (6). This preliminary analysis suggests that the whale products from a same whale could be available in the market for more than one year.

It should be noted that products of a same whale can be distributed in different and distant prefectures. Then the period for which products of a same individual remain in the market, should be investigated using surveys covering wide areas and different years. It should be also noted that such period could also depend of the kind of whale products e.g. processed products could be available at the market for longer period than non-processed products.

CONCLUSIONS

North Pacific common minke whales can be separated unambiguously from any other form minke whale. Then any unknown whale products in the market having originated from North Pacific minke whale can be identified unambiguously. At a smaller scale, in the western North Pacific J and O stocks present near-to-fix mtDNA differences, then individuals can be assigned to each of these stocks with a high degree of confidence. Because the genetic differences are striking but not absolute, maximum likelihood method can be used to estimate mixing proportion between these stocks given the haplotype frequencies of 'baseline' stocks.

Although the genetic methods used to identify North Pacific common minke whale and J and O stocks products in the market are robust, there are others more general aspects that need consideration if the data obtained in molecular surveys in the retail market are going to be used for management purpose. Among the aspects that need more consideration are the sampling scheme, which has not been a random procedure; considerations on the geographical variation of O/J stocks proportion in Japan and representativeness of the samples representing the J stock.

In addition to conduct the sampling under a random scheme, consideration should be given to weight the samples according the availability of whale products across the different prefectures. This issue could prove to be a difficult one due to the complex nature of the dynamics of the whale products in the market.

It will be also important to determine the period that the products of a same individual remain in the market. To investigate this it will be important that the sampling cover wide areas and several years, as products from a same animal can be sold in very distant places from the original one.

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Table 1: Number of North Pacific common minke whale products identified in the Japanese retail market, by survey. J stock products were identified according the criteria of a G base in position 298 of the control region. In parenthesis is the proportion of the North Pacific common minke whale products in the total number of sequences examined or the proportion of J stock products in the total North Pacific common minke whale products.

Survey	Period	Test sequences	North Pacific common minke whale products	Number of J stock products
1995	March-April	210	16 (0.08)	8 (0.50)
1996	March-June	341	65 (0.19)	9 (0.14)
1999/2000	Nov.-Feb	585	63 (0.11)	19 (0.30)
2000/2001 (first)	Nov.-Dec.	247	16 (0.07)	4 (0.25)
Total		1,383	160 (0.12)	40 (0.25)

Table 2: Number of North Pacific common minke whale products identified in the Japanese retail market, by survey and geographical region in Japan (western and eastern Japan). J stock products were identified according the criteria of a G base in position 298 of the control region. In parenthesis is the proportion of J stock products in the total North Pacific common minke whale products.

Survey	Eastern Japan		Western Japan	
	North Pacific common minke whale products	J stock products	North Pacific common minke whale products	J stock products
1995 survey	0	0	16	8 (0.50)
1996 survey	12	2 (0.17)	53	7 (0.13)
1999/00 survey	12	0 (0.00)	51	19 (0.37)
2000/01	13	2 (0.15)	3	2 (0.67)
Total	37	4 (0.11)	123	36 (0.29)

Table 3: Comparison of profiles of eight microsatellite loci between North Pacific common minke whale products purchased from the Japanese retail markets between November 1999 and January 2000 and JARPN and documented by-catch samples. Samples within a box correspond to products from a same individual identified by the microsatellite analysis. Assignation of O or J stocks is according the criteria of a G base in position 298 of the mtDNA control region. Question marks correspond to cases of undocumented by-catch or documented by-catch for which no genetic sample was available.

Sample	mtDNA ID	Microsatellite ID	Location
o-001	O	98NP023	Hokkaido (Abashiri)
o-037	O	?	Hokkaido (Kushiro)
o-064	O	97NP024	Akita (Akita)
o-086	O	98NP021	Aomori (Aomori)
o-105	O	99NP017	Miyagi (Sendai)
o-117	O	98NP029	Miyagi (Shiogama)
o-118	O	98NP038	Miyagi (Shiogama)
o-165	O	99NP025	Aichi (Nagoya)
o-175	J	?	Wakayama (Nachikatsuura)
o-189	J		Wakayama (Taiji)
o-191	J	M172	Wakayama (Taiji)
o-192	J		Wakayama (Taiji)
o-213	O	?	Nara (Nara)
o-225	J	?	Kyoto (Kyoto)
o-230	J	JBM189	Kyoto (Kyoto)
o-243	O	?	Kochi (Kochi)
o-244	J	?	Kochi (Kochi)
o-245	J	?	Kochi (Kochi)
o-247	J	JBM173	Kochi (Kochi)
o-249	J		Kochi (Kochi)
o-248	J	?	Kochi (Kochi)
o-250	J		Kochi (Kochi)
o-252	O	?	Kochi (Kochi)
o-253	O	?	Kochi (Kochi)
o-255	O		Kochi (Kochi)
o-258	O	99NP089	Ehime (Matsuyama)
o-270	O	99NP084	Hiroshima (Hiroshima)
o-279	J	?	Hiroshima (Hiroshima)
o-282	O	99NP021	Hiroshima (Hiroshima)
o-293	O	?	Hyogo (Kobe)
o-298	O	99NP010	Hyogo (Kobe)

1995 survey

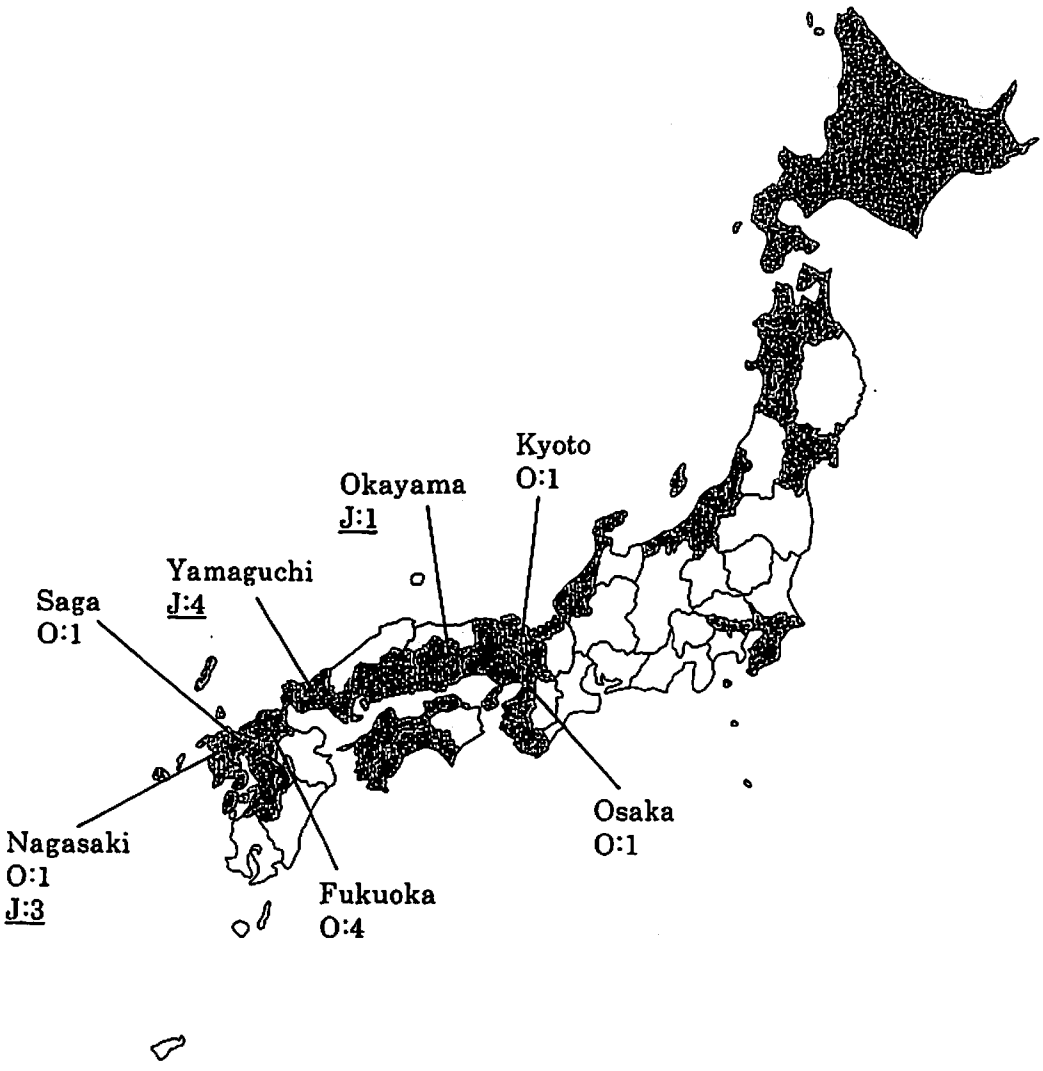

Figure 1: Geographical distribution of North Pacific common minke whale products identified in a DNA survey in the Japanese retail market conducted in 1995. J stock products were identified according to the criteria of a G base in position 298. In black are those areas covered by the survey.

1996 survey

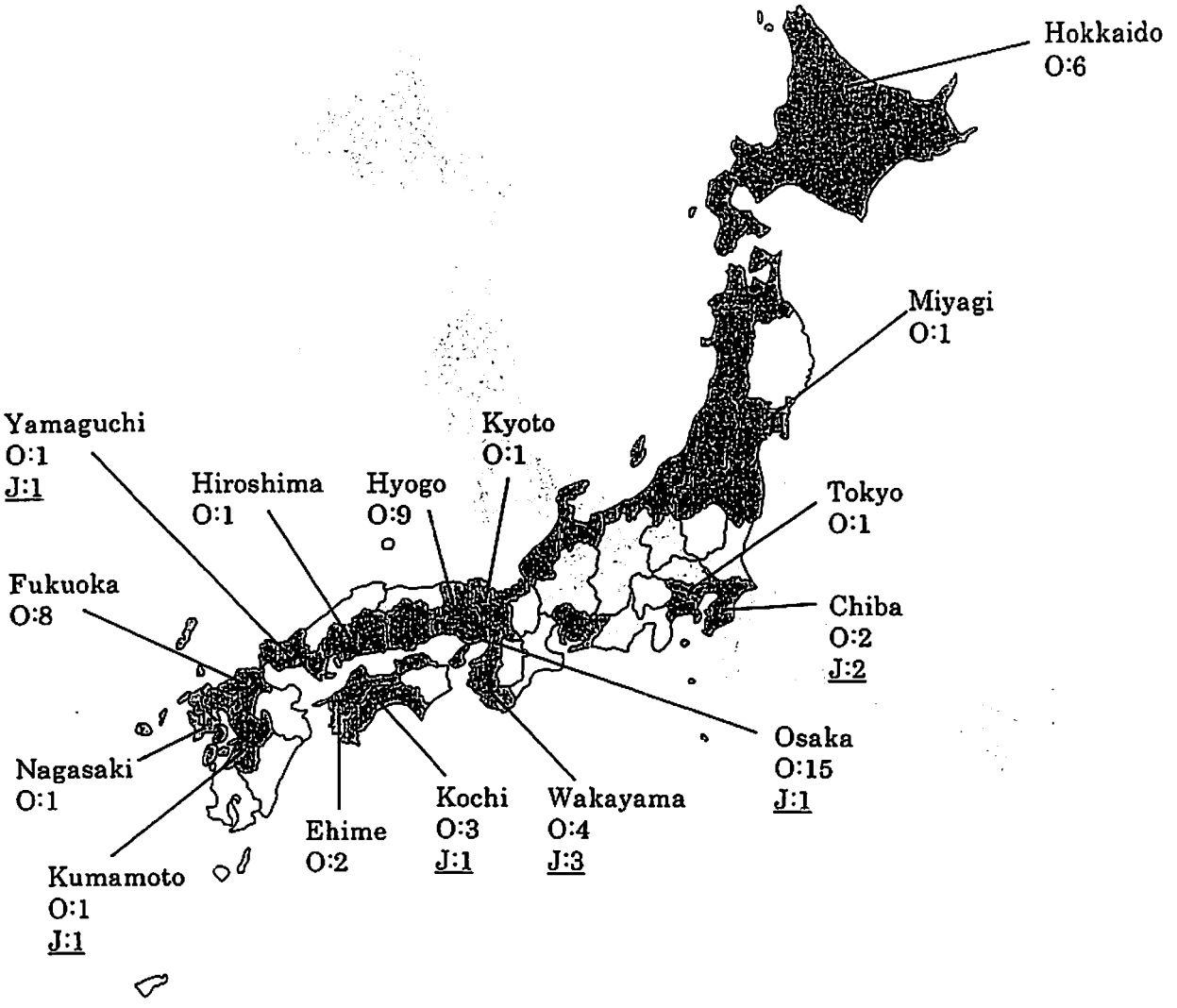

Figure 2: Geographical distribution of North Pacific common minke whale products identified in a DNA survey in the Japanese retail market conducted in 1996. J stock products were identified according to the criteria of a G base in position 298. In black are those areas covered by the survey.

1999/2000 survey

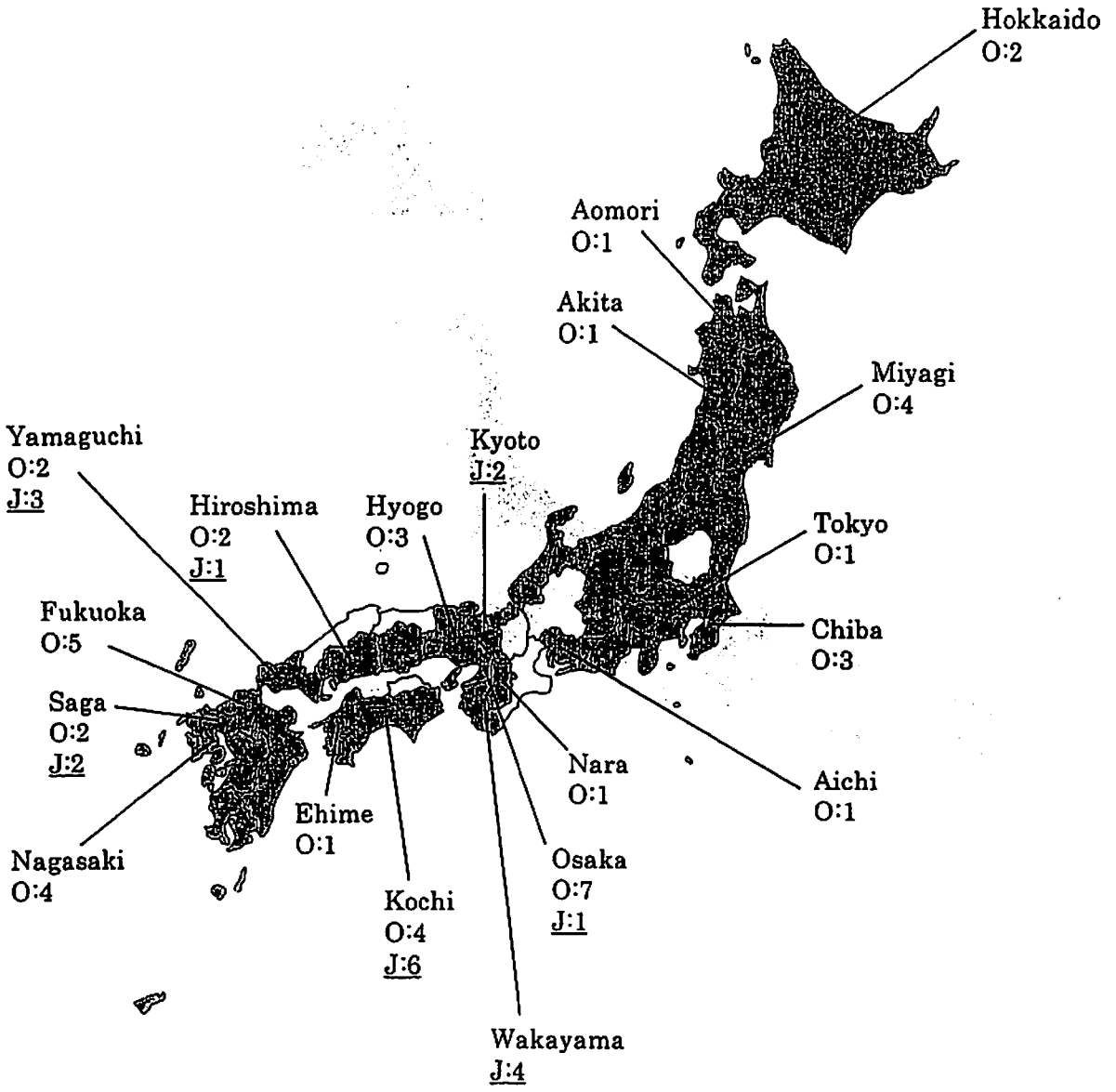

Figure 3: Geographical distribution of North Pacific common minke whale products identified in a DNA survey in the Japanese retail market conducted in 1999/2000. J stock products were identified according to the criteria of a G base in position 298. In black are those areas covered by the survey.

2000/2001 survey (First set)

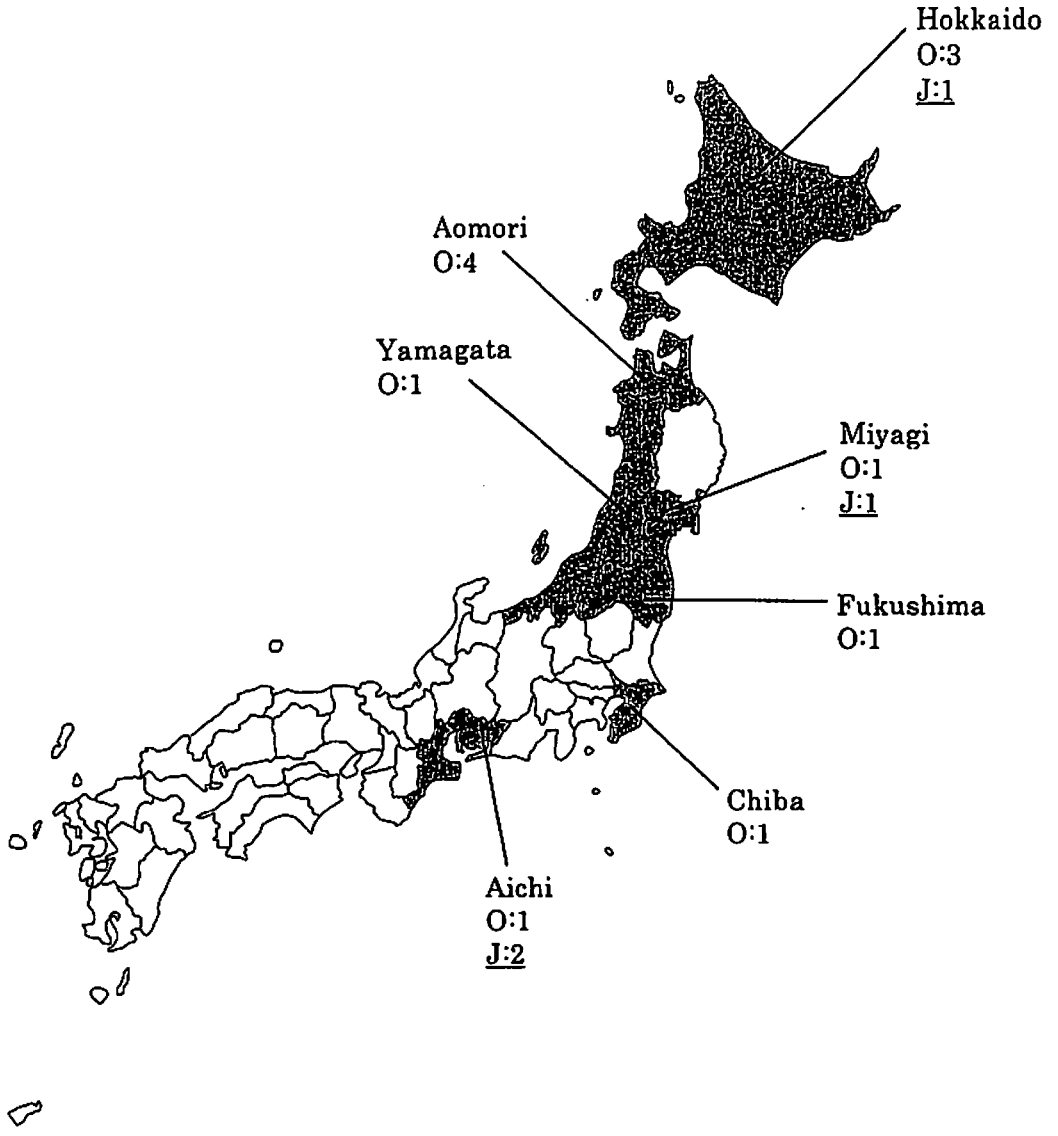

Figure 4: Geographical distribution of North Pacific common minke whale products identified in a DNA survey in the Japanese retail market conducted in 2000/2001 (first set). J stock products were identified according to the criteria of a G base in position 298. In black are those areas covered by the survey.

SC/53/RMP13 Table 3
Revised Version

Table 3: Comparison of profiles of eight microsatellite loci between North Pacific common minke whale products purchased from the Japanese retail markets between November 1999 and January 2000 and JARPN and documented by-catch samples. Samples within a box correspond to products from a same individual identified by the microsatellite analysis. Assignation of O or J stocks is according the criteria of a G base in position 298 of the mtDNA control region. Question marks correspond to cases of undocumented by-catch or documented by-catch for which no genetic sample was available.

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o-086	O	98NP021	Aomori (Aomori)
o-105	O	99NP017	Miyagi (Sendai)
o-117	O	98NP029	Miyagi (Shiogama)
o-118	O	98NP038	Miyagi (Shiogama)
o-165	O	99NP025	Aichi (Nagoya)
o-175	J	?	Wakayama (Nachikatsuura)
o-189	J		Wakayama (Taiji)
o-191	J	M172	Wakayama (Taiji)
o-192	J	Wakayama, Nov. 1999	Wakayama (Taiji)
o-213	O	?	Nara (Nara)
o-225	J	?	Kyoto (Kyoto)
o-230	J	?	Kyoto (Kyoto)
o-243	O	?	Kochi (Kochi)
o-244	J	?	Kochi (Kochi)
o-245	J	?	Kochi (Kochi)
o-247	J	JBM173	Kochi (Kochi)
o-249	J	Niigata, Dec. 1999	Kochi (Kochi)
o-248	J	?	Kochi (Kochi)
o-250	J		Kochi (Kochi)
o-252	O	?	Kochi (Kochi)
o-253	O	?	Kochi (Kochi)
o-255	O		Kochi (Kochi)
o-258	O	99NP089	Ehime (Matsuyama)
o-270	O	99NP084	Hiroshima (Hiroshima)
o-279	J	?	Hiroshima (Hiroshima)
o-282	O	99NP021	Hiroshima (Hiroshima)
o-293	O	?	Hyogo (Kobe)
o-298	O	99NP010	Hyogo (Kobe)