Ranking the plausibility of stock structure hypotheses of western North Pacific common minke whale

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ABSTRACT

This paper ranks the plausibility of the components characterizing the three stock structure hypotheses agreed for the *Implementation* of western North Pacific common minke whale. First we determined the criteria for using different kinds of information to define stocks: genetics, biological, life history, distribution gaps and migration, ecology and abundance and CPUE trend. In this exercise we took into account the discussions conducted at the Standing Group on Stock Definition and at the western North Pacific Bryde's whale *Implementation* on the use of different information to define stocks. Then based on previous IWC SC work we developed a simple procedure to rank hypotheses. Results suggested the following rank of plausibility for the components characterizing the three hypotheses on stock structure in the western North Pacific common minke whale: Hypothesis I: High, Hypotheses II: Low and Hypothesis III: Low.

KEYWORDS: COMMON MINKE WHALES, *IMPLEMENTATION*, WESTERN NORTH PACIFIC, STOCK STRUCTURE HYPOTHESES, PLAUSIBILITY

INTRODUCTION

The current *Implementation* of western North Pacific common minke whales is being conducted under the new 'Requirements and Guidelines for *Implementations*' developed by the IWC SC in 2004.

The development of the requirements and guideline was a good step forward following the previous *Implementation,* which in absence of such guidelines it took an abnormal long period of time to be completed (1993-2003). However one of the most important tasks of any *Implementation* is the assignment of plausibility rank to hypotheses on stock structure. Unfortunately the IWC SC has been unable to develop a procedure that assigns plausibility to hypotheses in an objective and scientific manner.

In this paper we determined the criteria for using different kind of information to define stocks, both genetics and non-genetics. In this exercise we took into account the discussions conducted at the Standing Group on Stock Definition on the use of different information to define stocks (IWC, 2002) as well the discussions held during the *Implementation* of the western North Pacific Bryde's whale (see Table 1 of IWC, 2007). Then based on previous IWC SC work we developed a simple procedure to rank the plausibility of the components characterizing the three stock structure hypotheses of western North Pacific common minke whale.

CRITERIA FOR DIFFERENT APPROACHES TO DEFINE STOCKS

Genetics differences

Examine whether there is genetic differences between stocks, at both maternal inherited and bi-parental inherited markers, and the extent of such differences. The most used approach is hypothesis testing, in which whales from different geographic localities are compared statistically for their allele frequencies under the null hypothesis of panmixia. It is important to check whether the number of samples is large enough and if the genetic markers used are sensitive enough to detect differences among weakly differentiated stocks (sequencing of the mtDNA control region and microsatellite DNA using a sufficient number of loci e.g. at least 15 loci are the preferred techniques). If no significant differences are found check if the power of the statistical analysis has been determined before reach the conclusion of panmixia.

Biological differences
Examine whether there is morphometric and morphological (e.g. flipper color pattern, fluke color pattern) differences between stocks. Check whether the number of morphometric traits, and the number of samples examined are sufficiently large to get conclusions on panmixia with confidence.

Life history parameters differences
Examine whether there is difference in parameters such as age at sexual maturity, age and growth and conception date between stocks. Check whether the number of samples examined is sufficiently large to get conclusions with confidence.

Completeness of the stock
For some baleen whale species such as the minke whales the spatial and temporal segregation by sex and reproductive classes is well documented. This could confound the interpretation of stock structure. All reproductive classes should be represented in a biological stock. Use the available biological data to examine whether all sexual classes are represented in the proposed spatial and temporal distribution range of the stock.

Distribution gaps and migration
Examine gaps in distribution. To determine if such gaps can be attributed to stock segregation it is important to check whether the discontinuity is stable with no movement between. It is also important to examine distribution of effort as some gaps can be explained simply by no surveys in particular areas.

Check if the migration pattern proposed for a given stock is consistent with the available sighting, commercial whaling operation and biological data.

Ecological differences
Examine whether there is differences in accumulation level of pollutants (PCB, heavy metals) between stocks. Check whether such differences are indeed due to stock differences or simply to other ecological reasons (e.g. differences in the kind and amount of prey consumed, etc.). Check whether the source, decay rates and effect of contaminants are known. This information is important for interpreting differences in accumulation.

Examine whether there are differences in parasite load between stocks. Check whether such differences are indeed due to stock differences. Check whether the life history and movement/distribution of the parasites are known. This information is important for interpreting differences in parasite load.

Abundance and CPUE
Examine whether the abundance and CPUE trends changed accordingly with the pattern of exploitation of the proposed stock. Check whether the assumption made for abundance estimates are appropriate.

Examine whether the results of conditioning for each stock structure hypothesis agree with abundance trend.

Validity of pure stocks
Under different hypotheses of an *Implementation* some spatial and temporal delimited samples are selected as ‘pure stock’ for the purpose of estimating mixing proportion. Definition of pure stock can be better obtained if samples from breeding grounds are available. Unfortunately in the case of the common minke as well other whale species under assessment by the IWC SC such samples are not available (the exception is the humpback whale).

The selection of spatial and temporal samples for this purpose should be carefully examined to elucidate whether such groups contain really whales from a single stock rather a mixture of two stocks or hybrid whales. The spatial and temporal pattern of mixing of stocks should be examined to investigate whether such pattern is consistent with the ‘pure stock sample’.

At least Hardy-Weinberg equilibrium should be tested (including power analyses test) to examine the possibility of a mixture of more than one stock in the selected sample. Because this is not a powerful test, use alternatives methods to investigate the possibility of mixture of more than one stock

A more general criteria applying to all information above is that some weight should be given to results of analyses on stock structure recommended by the IWC SC in previous years.

DESCRIPTION OF THE HYPOTHESES ON STOCK STRUCTURE IN WESTERN NORTH PACIFIC COMMON MINKE WHALES

Three stock structure hypotheses (Hypotheses I, II and III) were proposed and specified for the *Implementation* (see Figure 1 for the new sub-areas).

Hypothesis I (Figure 2a)
Single J stock distributed in the Yellow Sea, Sea of Japan and in the Pacific side of Japan. Single O stock occurs in sub-areas 7, 8 and 9, which migrate in summer mainly to the Okhotsk Sea (sub-areas 12SW and 12NE). Both J and O stocks overlap temporally in the Pacific coast (sub-areas 7CS, 7CN and 2C) and the southern part of the Okhotsk Sea (sub-areas 11 and 12SW).

Hypothesis II (Figure 2b)
Same as Hypothesis I but a different stock (Y stock) resides in the Yellow Sea and overlaps temporarily with the J stock in the south part of sub-area 6W.

Hypothesis III (Figure 2c)
There are five stocks, referred to as Y, JW, JE, OW, and OE, two of which (Y and JW) occur in the Sea of Japan, and three of which (JE, OW, and OE) are found to the east of Japan. The JE distributes in the Pacific coast of Japan around the year and mix with the OW in sub-areas 7CS and 7CN between April and September. The JW distributes in sub-areas 6W, 6E and 10W and mix with the OW and OE in sub-areas 11 and 12SW. The OW distributes nearshore in sub-area 7W around the year and mix with the offshore OE there and with the JW and OE in sub-area 12SW. Only the OE migrates into sub-area 12NE.

Stock structure hypotheses I and II are updated versions of the previous Hypotheses A and B, with some elements of these hypotheses (e.g. Y and C stocks) mimicing some of the aspects of two of the sensitivity tests considered during the 2003 *Implementation* (IWC, 2004). A difference between Hypotheses I/II and previous Hypotheses A/B is the more extensive distribution of J stock animals in the Pacific side of Japan and the spatial and temporal mixing of J and O stock animals in sub-areas 7CS and 7CN is now more documented under Hypotheses I and II.

Stock structure hypothesis III is new.

RANKING THE PLAUSIBILITY OF THE STOCK STRUCTURE HYPOTHESES OF COMMON MINKE WHALES

We constructed a table to evaluate the three hypotheses in the context of the available genetic and non-genetic information and the criteria determined above (see Table 1). There are common and unique components among some hypotheses. For example Hypotheses I and II share the component of panmixia of J and O stocks. The occurrence of the Y stocks in sub-area 5 is the component differentiating these hypotheses. On the other hand Hypotheses II and III share the concept of a Y stock in sub-area 5 but differ on the concept for O and J stocks (panmixia of these stocks in case of Hypothesis II and multiple J and O stocks in the case of Hypothesis III).

We evaluate the components characterizing each of those hypotheses in the context of the available genetics and non-genetic information and the criteria determined above: Hypothesis I (panmixia of J and O stocks and spatial/temporal mixing of these two stocks); Hypothesis II (occurrence of Y stock in sub-area 5) and Hypothesis III (multiple J and O stocks) (Table 1).

Following the previous work of the IWC SC during the western North Pacific Bryde's whale *Implementation* (IWC, 2007, pp 95), we used the following key: '+'= indicates evidence in favour of a hypothesis; '-'= indicates evidence against a hypothesis; '(+)'= indicate weak evidence in favour of a hypothesis; '(-)'= indicate weak evidence against of a hypothesis; '(())'= indicates very weak evidences; 'NIW'= indicates that the evidence is not inconsistent with the hypothesis, and ND= indicates the absence of particular data to evaluate a hypothesis. The designation 'NIW' often reflects the asymmetrical nature of information on stock structure (i.e. existence of differences can be viewed as positive evidence for multiple stocks, but absence of differences provides no information, and can not be viewed as positive evidence for a single stock (IWC, 2007). In using the 'NIW' key, consideration should be given to sample sizes used and/or power analysis results. The category 'ND' was not considered in the Bryde's whale *Implementation*.

During the Bryde's whale *Implementation* the key shown above was agreed and used to complete the information table. However there was no discussion on how to rank different hypotheses into 'High', 'Medium' and 'Low' based on the information from the table. In this paper we used the following general criteria.

A hypothesis should be ranked 'High' if a) data is available for at least 50% of the boxes; AND, b) the 'ND' key is not in the boxes of key evidence such as the genetics and completeness of the stock; AND, c) there are several evidences in favour (in the three degree of support indicated above) and few or none evidence against (in the three degree of support indicated above);

A hypothesis should be ranked 'Low' if a) data are not available for more than 50% of the boxes; OR, b) the 'ND' key is present in the boxes of key evidence such as the genetics and completeness of the stock; OR, c) if there are several evidences against (in the three degree of support indicated above) and few or none evidence in favour (in the three categories indicated above).

A hypothesis should be ranked 'Medium' if it represents a status intermediate between the criteria for 'High' and 'Low' e.g. similar to criteria for 'High' but where a similar number of evidences in favour and against are found.

Accordingly the result on the plausibility rank for the component characterizing stock structure hypotheses in the western North Pacific minke whale was the following:

Stock Hypothesis I: High
Stock Hypothesis II: Low
Stock Structure Hypothesis III: Low

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Table 1. General summary of the information to assess plausibility of components characterizing stock structure hypotheses. Key: : '+'= indicates evidence in favour of a hypothesis; '-'= indicates evidence against a hypothesis; '(+)'= indicate weak evidence in favour of a hypothesis; '(-)'= indicate weak evidence against of a hypothesis; '(())= indicates very weak evidences; 'NIW'= indicates that the evidence is not inconsistent with the hypothesis and ND= indicates the absence of particular data to evaluate a hypothesis. The designation 'NIW' often reflects the asymmetrical nature of information on stock structure (i.e. existence of differences can be viewed as positive evidence for multiple stocks, but absence of differences provides no information, and can not be viewed as positive evidence for a single stock (IWC, 2007). In using the 'NIW' key however consideration should be given to sample sizes used and/or power analysis results.

Information	**HYPOTHESIS I**	**HYPOTHESIS II**	**HYPOTHESIS III**
	J/O and mixing	**Y stock**	**JW/JE and OW/OE**
Genetics markers			
Mitochondria DNA[1]	+	((+))	((+))
Microsatellite[2]	+	(+)	((+))
Biological markers			
Morphometric[3]	(+)	ND	NIW
Flipper color pattern[4]	(+)	ND	(-)
Fluke color pattern[5]	(+)	ND	(-)
Life history markers			
Conception date[6]	+	(+)	-
Completeness of the stock[7]	+	ND	-
Distribution gaps and migration			
Distribution gaps[8]	NIW	ND	NIW
Migration[9]	+	ND	-
Ecology			
PCB accumulation[10]	NIW	NIW	NIW
Heavy metal accumulation[11]	NIW	NIW	NIW
Cookie cutter shark scars[12]	NIW	ND	NIW
Abundance and CPUE			
Trend[13]	(+)	ND	(-)
Conditioning[14]			
'Pure' stock[15]	+	(+)	(-)

[1,2]Some mtDNA and microsatellite analyses in the Pacific side of Japan were conducted separately for J and O stocks as recommended by the IWC SC since 2002. This was possible after the microsatellite work to assign individuals to O and J stocks (Kanda *et al*., 2009a), which was updated following recommendations from the IWC SC (Kanda *et al*. 2010a).

No significant mtDNA differences were found among O stock animals in the Pacific side of Japan providing no support for sub-structuring in this stock; weak mtDNA differences between Japanese and Korean J stock animals disappeared when whales from the Yellow Sea were excluded from the analysis. No significant mtDNA differences were found when samples from sub-area 5 were compared with those from sub-area 6 (Park *et al*., 2010; Baker *et al*., 2010).

No significant microsatellite DNA differences were found among O stock animals in the Pacific side of Japan providing no support for sub-structuring in this stock; weak differences were found within the J stock animals, which were attributed to a temporal mix of two stocks in sub-area 6W (Kanda *et al*., 2010a). Additional microsatellite analyses conducted on the basis of the new sub-areas (Figure 1) showed no significant heterogeneity among J stock animals from sub-areas 1E, 6E, 10E, 2C, 7CS and 7CN; and no significant heterogeneity among O stock animals from sub-areas 2C, 7CS, 7CN, 7W, 7E, 8 and 9) (Kanda *et al*., 2010b). Slikas and Baker (2010) found significant deviation from Hardy-Weinberg equilibrium along the Korean coast of the Sea of Japan in summer. However such deviation was very strong compared to slight or not differences (based on *Fst*) found between sub-areas 5 and 6 for both mtDNA and microsatellite DNA.

Mixing of two stocks (O and J) in sub-area 7 was suggested based on STRUCTURE analyses (Kanda *et al*., 2009a) with the proportion of the J stock decreasing from the coast to offshore waters (higher proportion within the 10n miles from the coast but also occurring in smaller proportion until 60n miles from the coast (Kanda *et al*., 2010c).

The number of samples used in those analyses is large and the genetic markers sensitive (more than 400bp of the control region and 17 microsatellite loci). Furthermore the power of the microsatellite analyses was estimated to be high (Kanda *et al*., 2009b).

Principal Component Analysis revealed some sub-structuring within the O stock of sub-areas 7, 8 and 9 but such suggested structuring was not related with body length and geographical position and the authors noted that such result does not have clear biological interpretation (Gaggiotti and Gascuel, 2011). The same analysis showed not genetic structuring among J stock animals east and west of Japan.

Other analyses in the Pacific side of Japan (e.g. mtDNA analysis by Baker *et al*., 2010 and microsatellite analysis by Slikas and Baker, 2010) were conducted for all samples combined (J and O stock animals). Several 'significant' differences among sub-areas were found but it is difficult to interpret such differences in the context of stock structure. No effort was made to differentiate between the two possibilities of O/J mixing and independent stock with intermediate characteristics.

In summary analyses conducted separately for J and O stocks followed previous recommendations from the IWC SC. These showed no significant differences within J and within O stocks animals. Analyses were based on large sample size and the genetic markers are considered sensitive. The power of the microsatellite analyses was estimated high. For these reasons we considered that mtDNA and microsatellite provide evidence in favour of Hypothesis I. There are some signals of heterogeneity that can be interpreted as the occurrence of a Y stock in sub-area 5. However such signals are weak, particularly for mtDNA where no significant differences were found between sub-areas 5 and 6. The Fst obtained in the comparison between these sub-areas are very small for both mtDNA and microsatellite DNA. For these reasons we considered that the evidence provides weak support in case of microsatellite and very weak support in the case of mtDNA in case of Hypothesis II. No effort was made to differentiate between the two possibilities of O/J mixing and independent stock with intermediate characteristics in the mtDNA (Baker et al., 2010) and microsatellite (Slikas and Baker, 2010) and then several interpretations are possible for such results. Consequently we considered that the genetic evidence provide very weak support in case of Hypothesis III.

[3]Morphometric analyses in sub-areas 7, 8 and 9 were conducted separately for J and O stocks based on genetics. A total of 10 measurements were used and the sample size was two J stock animals and 118 O stock animals. Despite the small J stock sample size, significant differences in morphometric

measurements were found between J and O stocks animals, and no significant differences were found among O stock animals from sub-areas 7, 8 and 9 in the Pacific side of Japan (Hakamada and Bando, 2009).

We considered that morphometric data provide weak support in case of Hypothesis I due to the limited sample size of J stock. No data is available to evaluate Hypothesis II and the data is not inconsistent with Hypothesis III.

[4]Flipper color pattern analyses in sub-areas 7, 8 and 9 were conducted separately for J and O stocks. The sample size was 23 J stock animals and 166 O stock animals. Differences were found between J and O stock animals. No differences between 7CN, 7CS, 7WR, 8, and 9 O stock animals (Kanda *et al*., 2010b).

We considered that flipper color pattern data provide weak support in case of Hypothesis I. No data is available to evaluate Hypothesis II and the data are weak evidence against Hypothesis III, at least for the OW/OE component of this hypothesis (sample sizes are larger than in the case of morphometric).

[5]Fluke color pattern analyses in sub-areas 7, 8 and 9 were conducted separately for J and O stocks. The sample size was 14 J stock animals and 155 O stock animals. Differences were found between J and O stock animals. No differences were found between 7CN, 7CS, 7WR, 8, and 9 O stock animals (Kanda *et al*., 2010b).

We considered that fluke color pattern data provide weak support in case of Hypothesis I. No data is available to evaluate Hypothesis II and the data are weak evidence against Hypothesis III, at least for the OW/OE component of this hypothesis (sample sizes are larger than in the case of morphometric).

[6]Conception date analyses in sub-areas 7, 8, 9 and 11 conducted separately for J and O stocks. Sample size for J stock animals was eleven while that for the O stock was 96. Differences found between J and O stock animals. No differences among O stock animals (Bando *et al*., 2010a). Furthermore these authors found that the sample of J stock from the Pacific side of Japan have two conception dates, a pattern as in the sample of eight individuals examined by Kato (1992). Then the pattern of conception date of J stock animals on both sides of Japan is similar. Some differences have been found in conception date between the Yellow Sea animals and those in other areas (Wang, 1985). However data are limited in quantity and quality to reach a sound interpretation of those differences (Bando, personal communication).

We considered that conception date data provide support in case of Hypothesis I; weakly support Hypothesis II (due to the nature of the data examined by Wang, 1985) and the data indicate evidence against against Hypothesis III (sample sizes are larger than in the case of morphometric).

[7]Length composition and sex ratio in the Pacific side of Japan and Sea of Japan analyses was conducted separately for J and O stocks. Large sample sizes were used. At the Pacific side of Japan O stock immature animals distributed mainly in coastal areas whereas O stock mature animals distribute mainly in offshore areas, which is consistent with a single O stock with spatial segregation by sex and maturity classes. J stock animals from the Sea of Japan and Pacific coast showed quite similar characteristics in the data (Kanda *et al*., 2010d). The analyses were based on large sample size.

We considered that these data provide support in case of Hypothesis I; no data to evaluate Hypothesis II and the data indicate evidence against Hypothesis III because under this hypothesis a stock would be composed exclusively by a single reproductive class (e.g OW by immature animals, OE by mature animals and JE by immature animals), which is not biologically plausible.

[8]The trend surface of the transformed density predicted by each month revealed no clear gaps in the Pacific side of Japan (sub-areas 7, 8 and 9) (Okamura *et al*., 2001).

Evidence of spatial (permanent) discontinuity would be viewed as positive evidence for multiple stocks, but absence of discontinuity is viewed as neutral information, as there is no expectation that their prey would have a discontinous distribution (IWC, 2007). Given this we considered that the information is not inconsistent with the data in case of Hypotheses I and III (at least for the OW/OE component of this hypothesis). No data is available to evaluate Hypothesis II.

[9]The only reports documenting the consistency of different kind of information (sighting, pattern of whaling operation, body length and sex ratio distribution) are those by Goto *et al*. (2010) and Hatanaka and Miyashita (1997). The information examined was consistent with the migration and mixing of two stocks along the coast of Japan.

We considered that migration pattern provides support in case of Hypothesis I, no data is available to evaluate Hypothesis II and provide evidence against Hypothesis III (see also footnote 7 above).

[10,11]There is limited information on the source, decay rate and accumulation pattern of PCB and heavy metals. This makes difficult the interpretation of different level of accumulation among groups of whales.

We considered that these data are not inconsistent with the three hypotheses.

[12]Cookie cutter shark scars analyses in 7CN+7CS and 7E+8+9 were conducted separately for J and O stocks. Differences were found between J and O stock animals. No differences were found among O stock animals (Bando *et al*., 2010b). However there is limited information on the life history and movement of the cookie cutter shark, which make difficult the interpretation of differences among groups of whales.

We considered that these data are not inconsistent with the three hypotheses.

[13]CPUE time series data were not consistent with existence of small coastal O stock, similar to the case proposed in Hypothesis III (Okamura *et al* 2004).

We considered that these data provide weak support in case of Hypothesis I, no data is available to evaluate Hypothesis II and provide weak evidence against Hypothesis III. We assigned these as weak because there some pending question on the operational data for the CPUE.

[14]Result of conditioning will be available ate the SC63.

[15]Under Hypothesis III the pure stock sample for the JE are the by-catch samples of sub-area 2C; for OW the JARPNII samples from sub-areas 7CS (April and May) and 7CN (September and October), excluding samples within 10n miles of the coast. In the first case there are genetic evidences of the occurrence of O stock animals in sub-area 2C (Kanda *et al*., 2009a). Results of the Hardy-Weinberg equilibrium test for those ‘pure stock’ samples resulted in no significant departure, however the power of this test has not been determined. Furthermore those ‘pure stock’ samples only include a single component of the assumed stocks (see completeness of the stock mentioned above).

The composition of J and O type animals in 7CN+CS changes with distance from the coast, with the proportion of the J stock decreasing from the coast to offshore waters (higher proportion within the 10n miles from the coast but also occurring in smaller proportion until 60n miles from the coast (limit for 7CS and 7CN)) (Kanda *et al*., 2010c).

Under Hypotheses I and II genetic and biological data support the selection of sub-area 6E (all months) as the pure sample of the J stock and 7WR+7E+8 as the pure sample of the O stock. J stock animals occur until the limit of sub-areas 7CS and 7CN (Kanda *et al*., 2010c). Sub-area 9 was excluded to avoid any confounding effect of the sporadic C stock in that area.

We considered that the definition of pure stock sample migration pattern provides support in case of Hypothesis I, weak support in the case of Hypothesis II and provide evidence against Hypothesis III (see also footnote 7 above).

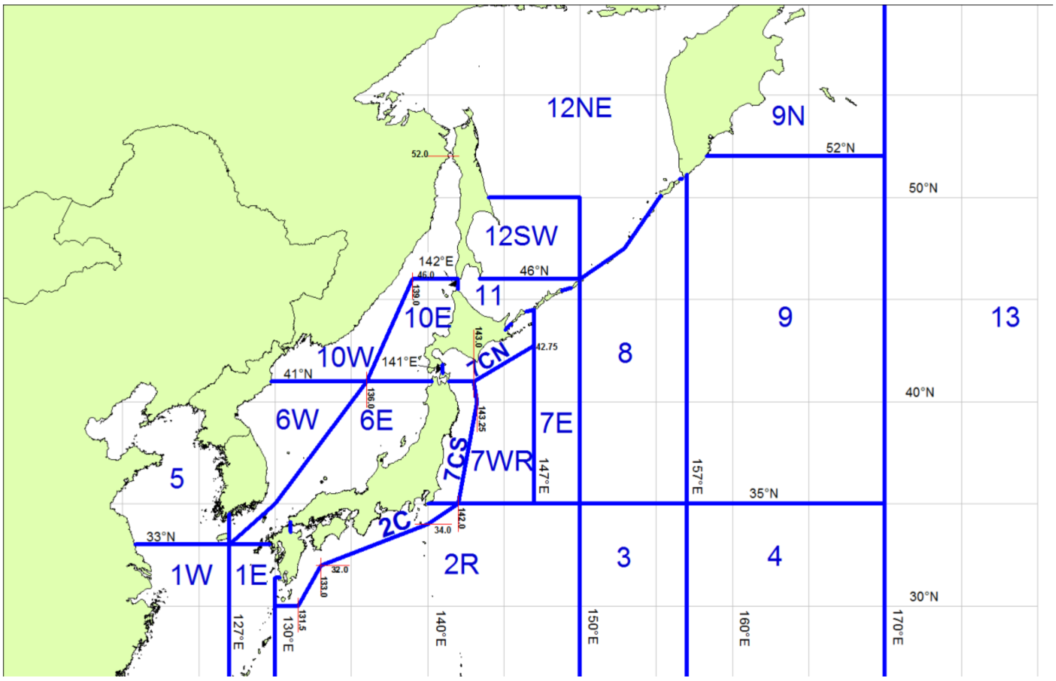

Figure 1. New sub-areas defined in the current RMP *Implementation* of western North Pacific common minke whales.

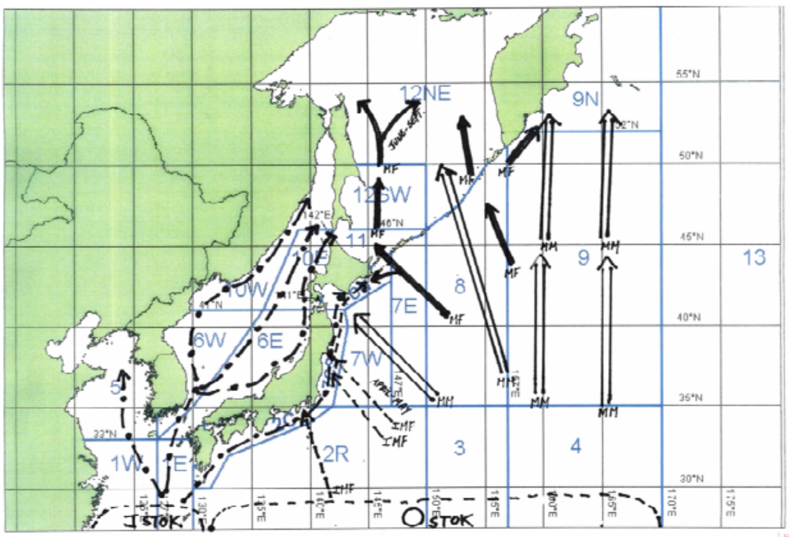

Figure 2a. Schematic representation of Hypothesis I of the current RMP *Implementation* (modified from Hatanaka and Miyashita, 1997).
MM=mature males, MF=mature females, IMF=immature males and females.

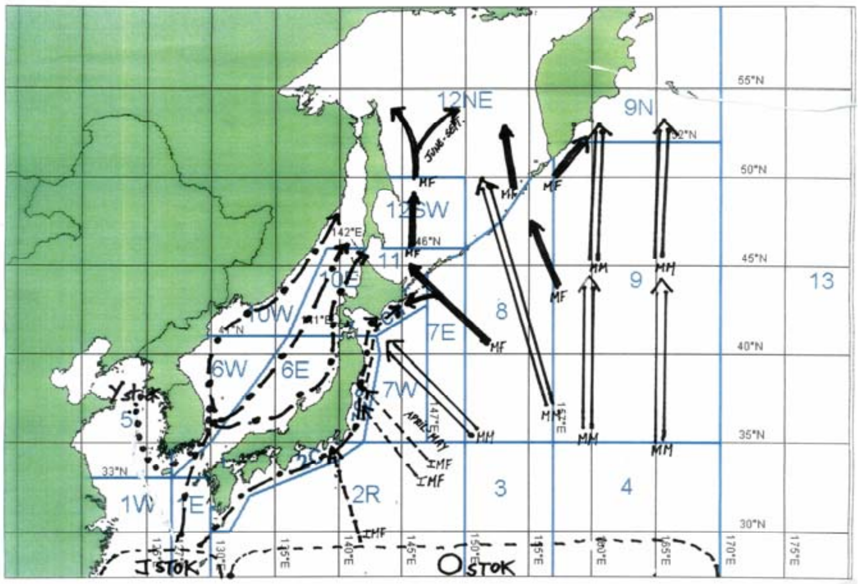

Figure 2b. Schematic representation of Hypothesis II of the current RMP *Implementation* (modified from Hatanaka and Miyashita, 1997).
MM=mature males, MF=mature females, IMF=immature males and females. Y stock residing in the Yellow Sea and mixing with the J stock in the southern part of sub-area 6W in summer.

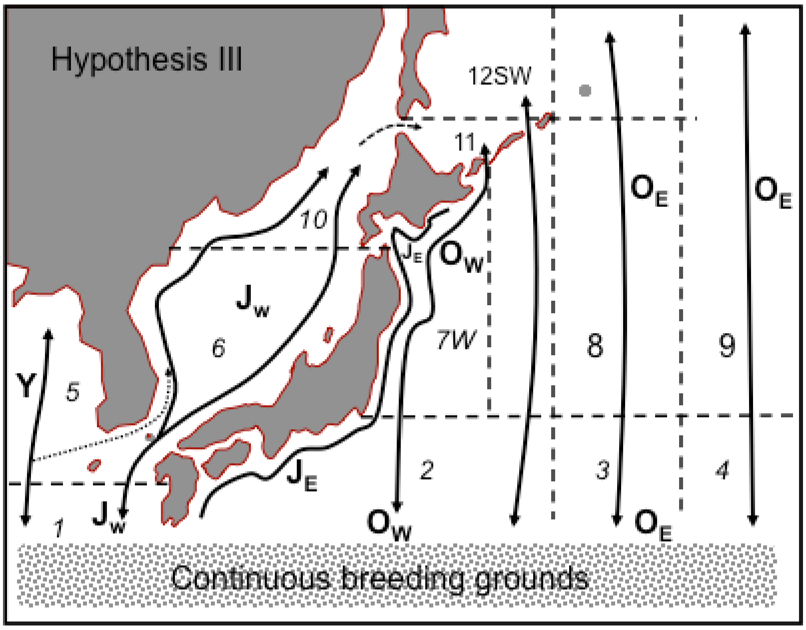

Figure 2c. Schematic representation of Hypothesis III of the current RMP *Implementation* (taken from SC/63/RMP8).