Morphometric analysis on stock structure in the western North Pacific common minke whales (*Balaenoptera acutorostrata*)

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ABSTRACT

This study uses morphometric data obtained by JARPN II to investigated differences between “J” and “O” stocks and the plausibility of four stock structure scenarios adopted by IWC in RMP *Implementation Simulation Trial*s (*ISTs*) for western North Pacific “O” stock common minke whales (*Balaenoptera acutorostrata*), Analysis of covariance (ANCOVA) using body length as a covariate is used to test if there are significant differences in morphometric measurements among the groups compared. There are some significant differences in morphometric between “J” and “O” stocks, but no significant differences were found within the “O” stock in sub-areas 7, 8 and 9. Results of the morphometric analysis are consistent with those of the genetic analyses and therefore they provide support for the single stock structure scenario (Baseline B) used in the *ISTs*. These results provide no support for the stock structure scenarios represented by Baselines C and D.

INTRODUCTION

In the western North Pacific two stocks of the common minke whale have been recognized by the International Whaling Commission Scientific Committee (IWC SC), the Okhotsk Sea – west Pacific stock (O stock) and Sea of Japan – Yellow Sea – East China Sea stock (J stock). Previous analyses based on genetics (Goto and Pastene, 1997) and non-genetic (e.g. morphometric, Kato *et al*., 1992; Fujise and Kato, 1996) approaches revealed marked differences between these two stocks.

A study based on microsatellite analysis of minke whales sampled by the JARPN and JARPN II in sub-areas 7, 8 and 9 assigned individual minke whales to either stock (Kanda *et al*., 2009). These results provide a unique opportunity to further examine differences between the two stocks using non-genetic approaches. The first objective of this study was to compare external measurements between “O” and “J” stock whales as identified by the microsatellite analysis.

A second objective of this study was to evaluate the plausibility of the four stock structure scenarios used in the RMP *Implementation Simulation Trials* (*ISTs*) for the minke whales in 2003 (IWC, 2004), using analysis of morphometric measurements as a non-genetic marker.

The following four stock structure hypotheses were used in the *ISTs* by the IWC SC:

(1) Baseline A: 3-stock scenario (‘J’ ‘O’ and ‘W’) with W found only sporadically in subarea 9W.
(2) Baseline B: 2-stock scenario (‘J’ and ‘O’) with no W stock as a limiting case of Baseline A.
(3) Baseline C: 4-stock scenario (‘J’, ‘Ow’, ‘Oe’ and ‘W’) with fix boundaries at 147°E and 157°E.
(4) Baseline D: 3-stock scenario (‘J’, ‘O’ and ‘W’) with O and W mixing over 147°-162°E, O being dominant to the west and W to the east.

MATERIALS AND METHODS

Samples

Minke whales sampled by JARPN II surveys in sub-areas 7 (140°-150°E), 8 (150°-157°E), and 9 (157°E-170°E) between 2000 and 2007, were used in the analysis. Only mature male animals were used because body proportion could be different between mature and immature animals and because the very limited number of mature females in the research area. Males of minke whales were defined as sexually mature by testis weight (larger side) of more than 290g (Bando *et al*., unpublished data).

For the first objective of the study whales were separated into two groups, “J” and “O” stocks according to the results of the microstellite analysis (Kanda *et al*., 2009). For the second objective of the study, whales of the “O” stock were divided according to the six longitudinal sectors used during JARPA surveys: 7W (140°-147°E), 7E (147°-150°E), 8W (150°-153°E), 8E (153°-157°E), 9W (157°E-162°E), 9E (162°-170°E) (Figure 1). Table 1 shows the sample sizes by year and longitudinal sectors. This geographical division was used for testing the four stock structure scenarios used in the *ISTs*.

External measurements

The measurements that were less susceptible to differences among researchers were selected for the analysis. Selection of these measurements took into consideration the opinion of experienced researchers. We also excluded girth because they are likely to change in the feeding season, according to sampling date. The ten external measurements used are shown in Figure 2. Logarithms of the measurements were used for the analyses.

Analytical approach

Analysis of covariance (ANCOVA) using body length as a covariate was used to test if there are significant differences in morphometric measurements among groups. In these analyses, it was assumed that lengths of the measurements depend on body length and that their relation can be described by the formula,

$$\log(x_n) = a_i \log(x_1) + b_i \quad (1)$$

where V1 is body length, x_n is length of the measurement V*n* (n=2,…,11), i is an index representing group to be compared, a_i is the slope and b_i is intercept of the formula (1) for group i. Using formula (1), ANCOVA can be explained as follows. ANCOVA consists of two steps. The first one is to test null hypothesis that

$$a_1 = a_2 = ... = a_m = a \quad (2)$$

where m is the number of the groups to be compared by ANCOVA. If the null hypothesis was rejected, the second test would be conducted. Assuming that equation (2) is true, formula (1) would be

$$\log(x_n) = a \log(x_1) + b_i \quad (1)'$$

The second one is to test null hypothesis that

$$b_1 = b_2 = ... = b_m = b \quad (3)$$

in formula (1)' when the null hypothesis (2) was not rejected at the 5% significant level. When m is more than 2, multiple comparison tests would be conducted if the null hypothesis (3) was rejected. In any ANCOVA conducted in this document, the null hypotheses (2) were not rejected. Note that derivation of b_i (i.e. average of b_i subtract from b_i) can be regarded as a correction factor to eliminate the biases to due the comparison factor. Especially, in case of comparison among researchers, they can be used to correct the bias due to difference of researchers.

The steps followed in the analysis were the following: .first, we tested if there is a temporal heterogeneity within each longitudinal sector. Second, we examined the morphometric differences between whales identified as “O” stock and “J” stock animals by the microsatellite analysis (first objective). Third, we examined morphometric differences among “O” stock whales according to geographical division underlining the stock structure scenarios of the *ISTs* (second objective).

RESULTS

Temporal heterogeneity in each longitudinal sector

Table 2 shows results of ANCOVA to test if there are any significant differences among years in each longitudinal sector. There are some significant differences in most of the longitudinal sectors. Such temporal heterogeneity could not have biological basis as genetic analyses based on mtDNA and microsatellite showed no significant yearly differences in these sectors. Rather these significant differences might reflect difference in measurement among the researchers. Assuming this, in order to eliminate possible effect of these differences among the researchers on the results of analyses, the data from a single researcher who measured samples in all longitudinal sectors in different years, were used. Assuming that the averaged measurements of all researchers are unbiased, measurements by the selected researcher are corrected in the cases that significant temporal heterogeneity was observed. Table 3 shows the numbers of samples by the single researcher. Correction factors were estimated by comparison among the researchers using ANCOVA. Table 4 shows the estimated correction factors.

The difference between "J" and "O" stocks

Two samples from "J" stock and 118 samples from "O" stocks were used for this analysis (Table 3). The two "J" stock whales were sampled in 2002, one from 7W sector and the other from 9W. Table 3 shows the number of samples "J" and "O" stock samples measured by the single researchers. There were some significant differences between "J" and "O" stocks (Table 5). This result suggests that external measurements can be used as a non-genetic marker to differentiate these stocks. For example the average marginal mean of the length of the measurements V2-V5 are longer for "J" animals than for "O" animals. Those of the measurement V6-V9 are shorter for "J" animals than "O" animals. Those of V10-V11 are longer for "J" animals than "O" animals. These results suggest that "J" animals tend to have bigger heads and shorter lower half of the body than "O" animals.

Evaluation of the plausibility of stock structure scenarios for "O" stock

Baseline A

Data from the single researcher were available for sub-area 9W only for years 2002 and 2003. Table 6 shows that there is no significant difference between 9W (data combined for the two years) and other sectors. Therefore this result provides no support for baseline A.

Baseline B

There is no significant difference in the measurements among whales in 7W, 7E, 8W, 8E, 9W and 9E as shown Table ７. This result suggests that there is no sub-stock of the O stock in sub-areas 7, 8 and 9 and agrees with the baseline B.

Baseline C

There is no significant difference among 7E, 8W and 8E as shown in Table 8. This means 7E, 8W and 8E can be combined as one group. There was no significant among whales in 7W, 7E+8W+8E, 9W and 9E as shown in Table 9. These results provide no support for the occurrence of three stocks (O_W, O_E and W) with divisions at 147° and 157°E (stock structure baseline C).

Baseline D

There is no significant difference among 7E, 8W, 8E and 9W as shown in Table 10. This means 7E, 8W, 8E and 9W can be combined. There was no significant among 7W, 7E+8W+8E+9W combined, and 9E as shown in Table 11 This result suggests that the 147-162^{o}E longitudinal sector hypothesized as a mixing region in baseline D is not significantly different from east and west of this longitudinal sector. This is inconsistent with baseline D.

DISCUSSIONS

Difference between "J" and "O" stocks

Marked morphometric differences found between "J" and "O" whales (Table 5) confirmed the finding of previous JARPN studies. . Hakamada and Fujise (2000) examine the difference between the "J" and "O" stocks using JARPN external measurement data obtained during 1994-1999 and suggested significant differences between the two stocks, and a tendency that "J" animals have shorter lower half of the body than "O" stock animals (Table 12).

Evaluation of the stock structure scenarios of "O" used in the RMP *ISTs*

The only source of morphometric heterogeneity was due to differences among years in sub-areas, which was assumed to reflect differences among researchers obtaining the measures in different years. It should be noted that the genetic analyses based on mtDNA (Goto *et al*., 2009) and microsatellites (Kanda *et al*., 2009) found no differences among years in the sub-areas, which provide support for the assumption of differences among researchers rather a biological event. In general results of the genetic and morphometric analyses were consistent and they provided no support for stock structure scenarios C and D used in *ISTs*. They were consistent with the view of a single "O" stock in sub-areas 7, 8 and 9 (stock structure scenario B).

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REFERENCE

Fujise, Y. and Kato, H. 1996. Some morphological aspects of the western North Pacific minke whales; Preliminary Analyses of materials from JARPN surveys in 1994-5. Paper SC/48/NP11 presented to IWC Scientific Committee, May 1996 (unpublished). [Available from IWC Secretariat]. 10pp.

Goto, M and Pastene, L. A. 1997. RFLP analysis of the mitochondrial DNA control region in minke whales sampled during the 1996 JARPN. Paper SC/49/NP10 presented to IWC Scientific Committee, September, 1997. (unpublished). [Available from IWC Secretariat]. 10pp

Goto, M., Kanda, N., Kishiro, T., Yoshida, H., Kato, H. and Pastene, L.A. 2009. Mitochondrial DNA analysis on stock structure in the western North Pacific common minke whales. Paper SC/J09/JR29 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 10pp.

Hakamada, T. and Fujise, Y. 2000. Preliminary Examination of the heterogeneity of external measurements of minke whales in the western North Pacific, using data collected during 1994-1999 JARPN surveys. Paper SC/F2K/J15 presented to the IWC Scientific Committee Workshop to Review the Japanese Whale Research Program under Special Permit for North Pacific Minke Whales (JARPN), Tokyo, 7-10 February 2000 (unpublished). 12pp.

International Whaling Commission. 2004. Report of the Scientific Committee. *J.Cetacean Res. Manege.* 6: 1-60.

Kanda, N., Goto, M., Kishiro, T., Yoshida, H., Kato, H. and Pastene, L. A. 2009. Individual identification and mixing of the J and O stocks around Japanese waters examined by microsatellite analysis. Paper SC/J09/JR26 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 9pp.

Kato, H., Kishiro, T., Fujise, Y. and Wada, S. 1992. Morphology of minke whales in the Okhotsk Sea, Sea of Japan and off the East Coast of Japan, with respect to stock identification. *Rep. Int. Whal. Commn.* 42: 437-442.

Table 1. The numbers of samples of the "O" stock used in the morphometric analysis, by longitudinal sectors for each year (see text for explanation).

year	7W				7E	8W	8E	9W	9E	total
	Kushiro	Sanriku	offshore	total						
2000	0	0	4	4	0	0	0	10	0	14
2001	0	0	21	21	4	0	13	19	0	57
2002	11	0	30	41	0	0	5	19	0	65
2003	0	7	4	11	5	12	11	18	11	68
2004	23	0	7	30	0	0	0	25	23	78
2005	24	3	18	45	0	2	0	8	19	74
2006	10	8	14	32	0	6	20	14	0	72
2007	11	8	39	58	0	1	9	2	2	72
total	79	26	137	242	9	21	58	115	55	500

Table 2. Temporal heterogeneity of the external measurement examined by ANCOVA. Bold letters indicate the differences are significant at 5% level. "n.s." is abbreviation for no significant.

measurement	*p* -value					
	7W	7E	8W	8E	9W	9E
V2	n.s.	n.s.	n.s.	n.s.	**0.043**	n.s.
V3	***p*** **<0.001**	n.s.	n.s.	n.s.	n.s.	n.s.
V4	***p*** **<0.001**	n.s.	n.s.	n.s.	n.s.	n.s.
V5	**0.012**	n.s.	n.s.	n.s.	n.s.	n.s.
V6	n.s.	n.s.	n.s.	n.s.	**0.001**	n.s.
V7	**0.034**	n.s.	n.s.	n.s.	***p*** **<0.001**	**0.021**
V8	**0.001**	**0.015**	n.s.	n.s.	**0.032**	**0.001**
V9	***p*** **<0.001**	n.s.	n.s.	n.s.	**0.010**	**0.007**
V10	**0.047**	n.s.	n.s.	n.s.	n.s.	n.s.
V11	n.s.	**0.021**	n.s.	**0.005**	***p*** **<0.001**	n.s.

Table 3. The number of samples of the "J" and "O" stocks by the researcher who measured samples all the longitudinal sectors.

year	7W		7E		8W		8E		9W		9E		total	
	O	J	O	J	O	J	O	J	O	J	O	J	O	J
2002	30	1	0	0	0	0	5	0	19	1	0	0	54	2
2003	4	0	5	0	12	0	11	0	18	0	11	0	61	0
2006	0	0	0	0	1	0	2	0	0	0	0	0	3	0
total	34	1	5	0	13	0	18	0	37	1	11	0	118	2

Table 4. Estimated biases for the researcher who measured samples all the longitudinal sectors using ANCOVA. Positive number indicates the measurement was overestimated. Negative number indicates the measurement was underestimated. “-“ indicates there were no significant differences in Table 2 and therefore no correction was made.

measurement	7W	7E	8W	8E	9W	9E
log(V2)	-	-	-	-	0.004	-
log(V3)	-0.004	-	-	-	-	-
log(V4)	-0.001	-	-	-	-	-
log(V5)	-0.002	-	-	-	-	-
log(V6)	-	-	-	-	-0.001	-
log(V7)	0.007	-	-	-	-0.004	-0.012
log(V8)	0.001	-0.011	-	-	0.000	-0.028
log(V9)	0.007	-	-	-	0.004	-0.027
log(V10)	-0.004	-	-	-	-	-
log(V11)	0.002	-0.021	-	-0.001	0.022	-

Table 5. Comparison between “O” and “J” stocks identified by microsatellite and estimated marginal mean of length of measurements for average body length (750.4cm). Bold letters indicate the differences are significant at 5% level. “n.s.” is abbreviation for no significant.

measurement	*p*- value	"J stock" (*n* =2)	"O stock" (*n* =118)
V2	n.s.	100.1	94.2
V3	**0.041**	119.9	112.8
V4	**0.032**	159.4	151.6
V5	n.s.	319.7	310.2
V6	n.s.	408.7	410.8
V7	**0.036**	345.1	355.2
V8	n.s.	236.5	243.7
V9	n.s.	191.8	195.7
V10	**0.017**	162.0	152.1
V11	**0.035**	88.3	84.0

Table 6. Results of test if there is any significant difference between 9W and other longitudinal sector in 2002 and 2003.

year	2002	2003
measurement	*p*- value	*p*- value
V2	n.s.	n.s.
V3	n.s.	n.s.
V4	n.s.	n.s.
V5	n.s.	n.s.
V6	n.s.	n.s.
V7	n.s.	n.s.
V8	n.s.	n.s.
V9	n.s.	n.s.
V10	n.s.	n.s.
V11	n.s.	n.s.

Table 7. Results of test if there is significant difference among 7W, 7E, 8W, 8E, 9W and 9E in morphometric measurements by ANCOVA. “n.s.” is abbreviation for no significant at 5% level.

measurement	*p*- value
V2	n.s.
V3	n.s.
V4	n.s.
V5	n.s.
V6	n.s.
V7	n.s.
V8	n.s.
V9	n.s.
V10	n.s.
V11	n.s.

Table 8 Results of test if there is significant difference among 7E, 8W and 8E in morphometric measurements by ANCOVA. "n.s." is abbreviation for no significant at 5% level.

measurement	*p*- value
V2	n.s.
V3	n.s.
V4	n.s.
V5	n.s.
V6	n.s.
V7	n.s.
V8	n.s.
V9	n.s.
V10	n.s.
V11	n.s.

Table 9. Results of test if there is significant difference among 7W, 7E+8W+8E, 9W and 9E in morphometric measurements by ANCOVA. "n.s." is abbreviation for no significant at 5% level.

measurement	*p*- value
V2	n.s.
V3	n.s.
V4	n.s.
V5	n.s.
V6	n.s.
V7	n.s.
V8	n.s.
V9	n.s.
V10	n.s.
V11	n.s.

Table 10. Results of test if there is significant difference among 7E, 8W, 8E and 9W in morphometric measurements by ANCOVA.

measurement	*p*- value
V2	n.s.
V3	n.s.
V4	n.s.
V5	n.s.
V6	n.s.
V7	n.s.
V8	n.s.
V9	n.s.
V10	n.s.
V11	n.s.

Table 11. Results of test if there is significant difference among 7W, 7E+8W+8E+9W and 9E in morphometric measurements by ANCOVA.

measurement	*p*- value
V2	n.s.
V3	n.s.
V4	n.s.
V5	n.s.
V6	n.s.
V7	n.s.
V8	n.s.
V9	n.s.
V10	n.s.
V11	n.s.

Table 12. Morphometric comparison between “J” and “O” stock using JARPN data (Hakamada and Fujise, 2000). Measurements that were not used in this study were omitted to facilitate comparison.

measurement	*p*- value	"J stock"	"O stock"
V2	$\boldsymbol{p<0.05}$	95.0	91.0
V3	$\boldsymbol{p<0.05}$	114.1	109.8
V4	$\boldsymbol{p<0.05}$	151.0	146.8
V5	n.s.	305.0	299.8
V6	n.s.	398.1	399.6
V7	n.s.	344.9	345.5
V8	n.s.	236.5	238.0
V9	n.s.	190.6	191.0

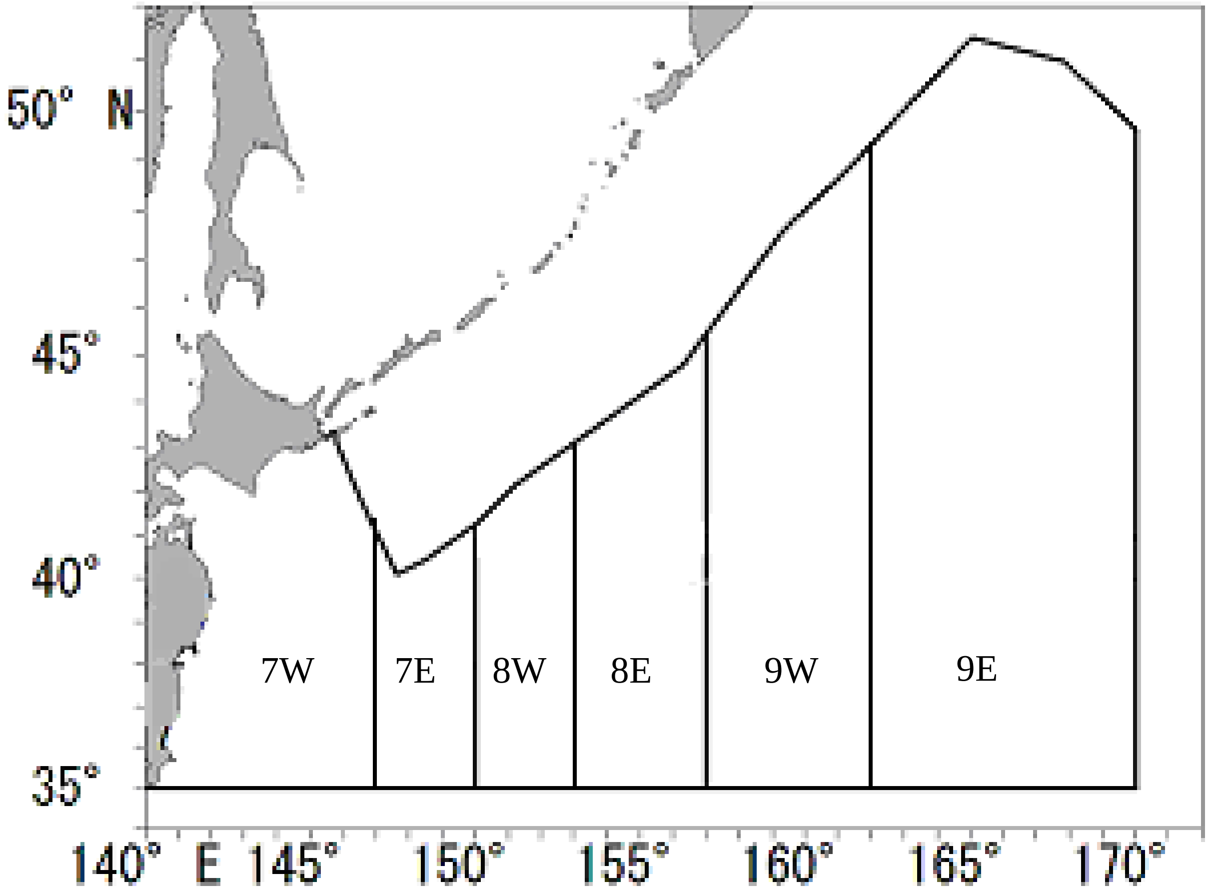
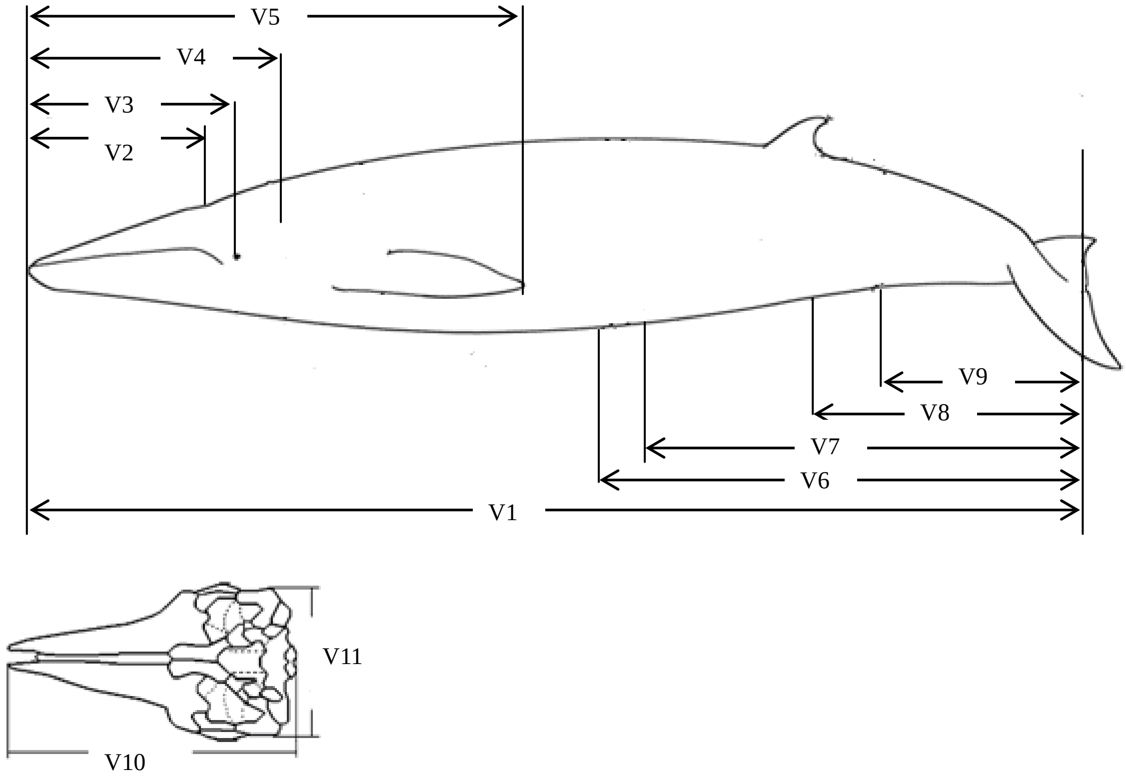


Fig. 1. Geography of the six longitudinal sectors.



V1: Body length

V2: From the tip of snout to blow hole

V3: From the tip of snout to center of eye

V4: From the tip of snout to ear

V5: From the tip of snout to tip of flipper

V6: From notch of flukes to end of ventral gloves

V7: From notch of flukes to center of umbilicus

V8: From notch of flukes to sexual apparatus

V9: From notch of flukes to anus

V10: Length of skull

V11: Maximum width of skull

Fig. 2. External measurements of western north Pacific common minke whale used in this study.