Morphometric analysis on stock structure of the O stock common minke whale in the western North Pacific

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ABSTRACT
Morphometric analyses were conducted to examine stock structure of western North Pacific common minke whales (*Balaenoptera acutorostrata*) by using external measurement data collected during 1994 and 2014 JARPN and JARPNII surveys. Most of the analyses conducted followed recommendations from the 2009 JARPNII review workshop. External measurements of mature males were first compared between O and J stock animals assigned by the microsatellite DNA analysis. Then only O stock animals were compared among sub-areas. The analytical procedures used were the Analysis of Covariance (ANCOVA) and Discriminant Analysis (DA). Significant differences were detected between O and J stock whales. J stock animals had longer head region compared to O stock animals. No significant differences were detected in O stock animals among sub-areas. The results of the present morphometric analyses provided no evidences for sub-structuring of the O stock into Ow and Oe as proposed in one of the hypotheses used in the RMP *Implementation*, as common minke whales from coastal and offshore sub-areas did not differ in morphometric characters.

INTRODUCTION

In the western North Pacific, two stocks of common minke whales, the Okhotsk Sea – western North Pacific stock (O stock) and Sea of Japan – Yellow Sea – East China Sea stock (J stock) has been recognized based on genetic (Goto and Pastene, 1997; Goto *et al*., 2009; Kanda *et al*., 2009) and non-genetic (Fujise *et al*., 2000; Fujise and Kato, 1996; Goto *et al*., 2009; Kanda, *et al*., 2010; Kato, 1992; Mitani *et al*., 2000) studies. Morphometric analysis has been conducted to clarify stock structure of common minke whales and the results supported the view of only two stocks distribute in the western North Pacific (Fujise and Kato, 1995; 1996; Hakamada and Bando, 2009a; 2009b; Hakamada and Fujise, 2000; Kato, 1992).

During the *Implementation Review* for western North Pacific common minke whales conducted by the IWC SC from 2010 to 2013, three stock structure hypothesis were proposed (IWC, 2014). Among the three hypothesis, Hypothesis C claimed three stocks (Je, Ow and Oe) distributed in coastal and offshore sub-areas of the Pacific side of Japan. In contrast, Hypotheses A and B proposed only O and J stocks distributed in the Pacific side of Japan, and mixing of both stocks in some sub-areas and periods.

Elucidation of stock structure in large whales is one of the objectives of JARPNII and several kinds of samples and data were collected for this purpose from the start of the program. In this study, the stock structure of common minke whales in western North Pacific was investigated based on morphometric analysis by using external measurement data collected during 1994 and 2014 by JARPN and JARPNII surveys. The analyses followed most of the recommendations from the 2009 JARPNII review workshop (IWC, 2010).

Firstly external measurements between O and J stocks were compared. Second the external measurements of only O stock animals were compared among sub-areas to examine the plausibility of sub-structuring of the O stock into Ow and Oe as proposed under Hypothesis C. Animals were assigned to O and J stocks based on microsatellite DNA analysis (Pastene *et al*., 2016a (SC/F16/JR38)).

MATEIALS AND METHODS

Samples

Common minke whales sampled in sub-areas 7CN, 7CS, 7WR, 7E, 8 and 9 (Figure 1) during 1994 to 2014 JARPN and JARPNII offshore surveys were used in the analysis. Only mature males were used because body proportion could differ between mature and immature animals and the very limited number of mature females in the research area. Males of minke whales were defined as sexually mature based on testis weight (heavier side) of more than 290g (Bando *et al*., unpublished data). All mature males were used to compare O and J animals (Table 1). Only morphometric data obtained by the same researchers who measured whales from all sub-areas (three persons) were used to compare O stock animals among sub-areas (Table 2). This was done in order to reduce possible bias derived from measurement differences among researchers.

External measurements
External measurements were conducted on the research vessel. 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 with progress of the feeding season. The ten external measurements used are shown in Figure 2. Logarithms of the measurements were used for the analyses.

Assignment of O and J stocks
Each whale was assigned to the O and J stock based on the microsatellite DNA analysis presented in Pastene *et al*. (2016a: SC/F16/JR38). Only animals with membership probability of over 90% were included in the analysis (see discussion of Pastene *et al*. (2016a: SC/F16/JR38) on the validity of this approach).

Analytical procedures
Following recommendations from the 2009 JARPNII review workshop, the following procedures were used:

ANCOVA
Analysis of covariance (ANCOVA) using body length as a covariate was used to test if there are significant differences in external 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,

$$\log(x_n) = \alpha_i \log(x_1) + \beta_i \qquad (1)$$

where x_1 is body length, x_n is length of the measurement V_n, i is an index representing group to be compared, α_i is the slope and β_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

$$\alpha_1 = \alpha_2 = \cdots = \alpha_m = \alpha \qquad (2)$$

where m is the number of the groups to be compared by ANCOVA. If the null hypothesis was not rejected, the second test would be conducted. Assuming that equation (2) is true, formula (1) would be

$$\log(x_n) = \alpha \log(x_1) + \beta_i \qquad (1)'.$$

The second one is to test null hypothesis that

$$\beta_1 = \beta_2 = \cdots \beta_m = \beta \qquad (3)$$

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 were conducted if the null hypothesis (3) was rejected.

Discriminant analysis
Discriminant analysis using principle components were conducted in two steps. First, PCA was used to extract principal components (PC) from the 11 measurements. A total of 11 PCs was obtained from 11 measurement. If a sample has missing value in some measurements, it was excluded from the dataset. Second, 11 PCs were used to construct discriminant function to predict groups. To determine the discriminant function, stepwise variable selection was conducted. If F-statistics is more than 3.84, the PC was included in the discriminant function. If F-statistics was less than 2.71, the PC was removed from the function. This procedure is a combination of forward selection and backward selection (Landau and Everitt, 2001).

RESULTS
Difference between O and J stock animals
ANCOVA
There was no significant interaction between logarithm of body length and stocks (i.e. the null hypothesis (2) was not rejected) for all measurements and therefore ANCOVA can be applied. Table 3 shows the results of the ANCOVA test for the null hypothesis (3) for each measurement. There were significant differences between the two stocks for measurements V2, V3, V4, V5 and V10. This result suggested there are

significant difference in measurements between the two stocks.

Discriminant analysis
Table 4 shows coefficient of PCs to construct discriminant functions to discriminate between O and J stocks. PC1 was positively correlated to all measurements, which can be regarded as an index of growth. PC2 was positively correlated to V2-V5 and V10-V11, which can be regarded as an index of length of head region. Table 5 shows prediction of the stock from the discriminant functions which have the PCs as covariates. PC1, PC2, PC3, PC8 and PC9 were selected as variables of discriminant function. The table shows the prediction is correct for about 80% of the samples. Figure 3 shows a histogram of score of discriminant functions for O and J stocks. When the score is more than 0, the sample was predicted as J stock. This figure shows that discriminant function predicts the stock almost correctly for each stock. These results suggest that two stocks are different in measurements.

Difference of O stock animals among sub-areas
ANCOVA
Table 6 shows test for the null hypothesis (3) for each measurement to examine if there are significant difference in 'O' stock animal's measurement among sub-areas 7CS, 7CN, 7WR, 7E, 8 and 9. There was no significant difference for all the measurements examined.

Discriminant analysis
Table 7 shows the coefficient of PCs to construct discriminant functions to discriminate O stock animals among sub-areas 7CS, 7CN, 7WR, 7E, 8 and 9. PC1 is positively correlated to all measurements, which can be regarded as an index of growth. PC2 is negatively correlated to V2-V5 and V10-V11, which can be regarded as an index of length of head region. Effort was made to construct discriminant function by the stepwise variable selection, however no variable was selected.

DISCUSSIONS

Significant difference was detected in morphometry between O and J stock common minke whales, confirming the findings of previous studies. J stock animals have longer head region compared to O stock animals, which confirmed the results of previous studies (Hakamada and Bando, 2009a; Hakamada and Fujise, 2000). More than 80% of morphometrically discriminated animals matched with DNA-identified stock, which supported the separation of two stocks. Significant difference was also reported in colorations of flipper between O and J animals (Nakamura *et al*., 2016 (SC/F16/JR39)). These results revealed that morphology and morphometry can be used to identify O and J stocks of North Pacific common minke whales.

On the other hand, no significant differences were detected in O stock animals among sub-areas. This result was consistent with those of previous studies (Hakamada and Bando, 2009a; 2009b; Hakamada and Fujise, 2000). Putative Ow and Oe stocks were proposed for coastal sub-areas (7CS and 7CN) under Hypothesis C. However the results of the present morphometric analyses provided no evidences for such sub-structuring as common minke whales from coastal and offshore sub-areas did not differ in morphometric characters. These morphometric results are consistent with those from microsatellite and mtDNA analyses presented to this workshop (Pastene *et al*., 2016a (SC/F16/JR38); 2016b (SC/F16/JR40)).

Only mature males were analysed in this study because sample size of mature female was not large enough, due to sexual segregation of common minke whales in the research area. Migration strategy of O stock common minke whale differs by sex and maturity status, and majority of mature females are thought to migrate to Okhotsk Sea (Hatanaka and Miyashita, 1997). Further analysis by using mature female samples will contribute to elucidate further the stock structure and migration strategy of common minke whales in the western North Pacific.

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REFERENCES

Fujise, Y. and Kato, H. 1995. Preliminary report of morphological differences of mine whales between coastal Japan (sub-area 7) and offshore area (sub-area 9) of western North Pacific. Paper SC/47/NP6 presented to IWC Scientific Committee, May 1995 (unpublished). 9pp.

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). 10pp.

Fujise, Y., Hakamada, T., Aoki M., Niimi, S., Nakata, H., Honda, K. and Tanabe, S. 2000. An attempt to identify stocks in the western North Pacific minke whale (Balaenoptera acutorostrata) using accumulation levels of heavy metals and organochlorines as ecological tracers. Paper SC/F2K/J18 presented to the IWC Scientific Committee Workshop to Review the Japanese Whale Research Programme under Special Permit for North Pacific Minke Whales (JARPN), Tokyo, 7-10 February 2000 (unpublished). 18pp.

Goto, M. and Pastene, L.A. 1997. Population structure of the western North Pacific minke whale based on an RFLP analysis of the mtDNA control region. *Rep. int. Whal. Commn* 47:531-537.

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). 10 pp.

Hakamada, T. and Bando, T. 2009a. Morphometric analysis on stock structure in the western North Pacific common minke whales (*Balaenoptera acutorostrata*). Paper SC/J09/JR27 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 13 pp.

Hakamada, T. and Bando, T. 2009b. Further morphometric analysis on stock structure in the western North Pacific common minke whales (*Balaenoptera acutorostrata*). Paper SC/61/JR6 presented to the IWC Scientific Committee, May 2009 (unpublished). 4 pp.

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.

Hatanaka, H. and Miyashita, T. 1997. On the feeding migration of the Okhotsk Sea – West Pacific stock of minke whales, estimates based on length composition data. *Rep. Int. Whal. Commn* 47: 557-564.

International Whaling Commission. 2010. The report of the Expert Workshop to review the ongoing JARPN II Programme. *J. Cetacean Res. Manage.* 11 (Supple 2): 405-449.

International Whaling Commission. 2014. Report of the working group on the *Implementation Review* for western North Pacific common minke whales. *J. Cetacean Res. Manage*. 15 (Suppl.): 112-188.

Kanda, N., Goto, M., Kishiro, T., Yoshida, H., Kato, H. and Pastene, L.A. 2009. Microsatellite analysis of minke whales in the western North Pacific. Paper SC/J09/JR30 presented to the JARPN II Review Workshop, Tokyo, January 2009 (unpublished). 14 pp.

Kanda, N., Goto, M., Nagatsuka, S., Kato, H., Pastene, L.A. and Hatanaka, H. (2010). Analysis of genetic and non-genetic data do not support the hypothesis of an intermediate stock in sub-area 7. Paper SC/S10/NPM9 presented to the First Intersessional Workshop for western North Pacific common minke whales, Tokyo, September 2010 (unpublished).

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.

Landau, S. and Everritt, B. S. 2001 *A handbook of statistical analyses using SPSS*. Chapman & Hall/CRC. 354pp.

Mitani, Y., Bando, T., Takai, T. and Sakamoto, W. 2000. Diet record and stock structure of minke whales *Balaenoptera acutorostrata* around Japan examined by δ^{13}C and δ^{15}N analyses. Paper SC/F2K/J20 presented to the IWC Scientific Committee Workshop to Review the Japanese Whale Research Programme under Special Permit for North Pacific Minke Whales (JARPN), Tokyo, 7-10 February 2000 (unpublished). 12pp.

Nakamura, G. et al. 2016. Investigation of the spatial and temporal mixing of 'O' and 'J' stocks based on morphology and coloration of the flippers. Paper SC/F16/JR39 presented to the JARPNII special permit expert panel review workshop, Tokyo, February 2016 (unpublished).

Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016a. Temporal and spatial monitoring of the 'J' and 'O' stocks of common minke whale in waters around Japan based on microsatellite DNA. Paper SC/F16/JR38 presented to the JARPNII special permit expert panel review workshop, Tokyo,

February 2016 (unpublished).
Pastene, L.A., Goto, M., Taguchi, M. and Kitakado, T. 2016b. Updated genetic analyses based on mitochondrial and microsatellite DNA indicated no sub-structure of the 'O' stock common minke whale in the western North Pacific. Paper SC/F16/JR40 presented to the JARPNII special permit expert panel review workshop, Tokyo, February 2016 (unpublished).

Table 1. Sample size of mature male common minke whales used for comparison between O and J stock animals.

Year	Stock		Total
	J	O	
1994		15	15
1995		63	63
1996	3	30	33
1997		64	64
1998		60	60
1999	4	38	42
2000	2	16	18
2001		56	56
2002	3	54	57
2003		64	64
2004	3	54	57
2005	1	42	43
2006	1	52	53
2007	2	49	51
2008		35	35
2009		20	20
2010		7	7
2011		21	21
2012	5	17	22
2013		3	3
Total	24	760	784

Table 2. Sample size of mature males common minke whales used for comparison of O stock animals among sub-areas (only morphometric data obtained by three researchers who measured whales from all sub-areas, were used)

Year	Sub-area						Total
	7CS	7CN	7WR	7E	8	9	
1998	0	0	0	6	30	0	36
2000	1	4	0	0	0	11	15
2001	3	7	10	5	12	19	53
2002	0	28	1	0	5	20	54
2003	2	0	3	5	26	28	62
2006	7	2	6	0	23	14	45
2009	0	2	6	1	7	4	20
Total	13	43	26	17	103	96	285

Table 3. Results of ANCOVA to test morphometric differences between O and J stock animals.

Measurement	*p* -value	J stock *n* =24	O stock *n* =760
V2	**$p<0.05$**	97.0	93.1
V3	**$p<0.05$**	117.3	111.8
V4	**$p<0.05$**	155.6	150.4
V5	**$p<0.05$**	314.8	307.7
V6	n.s.	405.9	409.5
V7	n.s.	350.7	353.5
V8	n.s.	241.3	242.7
V9	n.s.	193.4	194.8
V10	**$p<0.05$**	157.3	151.7
V11	n.s.	83.6	84.1

Table 4. Component matrix extracted by PCA to test morphometric differences between O and J stock animals. Bold letter indicates the PCs selected for discriminant function. PC*x* indicates the *x*th PC.

	PC1	**PC2**	**PC3**	PC4	PC5	PC6	PC7	**PC8**	**PC9**	PC10	PC11
logV1	0.951	-0.181	0.016	-0.035	-0.140	0.022	-0.034	-0.129	-0.136	-0.036	-0.059
logV2	0.748	0.505	-0.114	0.387	0.037	0.047	-0.135	0.016	-0.022	0.011	0.015
logV3	0.808	0.517	-0.152	-0.030	0.068	0.098	0.156	0.030	0.058	-0.043	-0.102
logV4	0.859	0.430	-0.111	-0.125	0.021	0.104	0.143	-0.040	-0.047	0.050	0.106
logV5	0.897	0.225	-0.029	-0.252	-0.104	0.070	-0.238	0.069	0.049	0.005	0.003
logV6	0.805	-0.482	0.010	0.112	-0.268	0.010	0.116	0.147	0.001	-0.004	0.016
logV7	0.860	-0.454	-0.053	0.075	-0.042	0.011	0.002	-0.158	0.140	0.002	0.022
logV8	0.825	-0.501	-0.096	-0.020	0.176	-0.038	-0.010	0.038	-0.023	0.146	-0.047
logV9	0.818	-0.480	-0.141	-0.031	0.238	-0.035	-0.025	0.052	-0.026	-0.125	0.041
logV10	0.816	0.447	0.070	-0.030	-0.032	-0.357	0.027	-0.004	0.012	-0.001	0.004
logV11	0.670	0.037	0.732	0.024	0.095	0.069	0.014	0.010	0.008	-0.003	0.002

Table 5. Comparison between DNA and morphometrically discriminated stocks of common minke whales.

			Morphologically discriminated stock		
			J stock	O stock	Total
DNA based stock	Frequency	J stock	20	4	24
		O stock	146	614	760
	%	J stock	83.3	16.7	100.0
		O stock	19.2	80.8	100.0

Table 6. Results of ANCOVA to test morphometric differences of O stock animals among sub-areas.

Measurement	*p* -value	7CS *n* =13	7CN *n* =43	7WR *n* =26	7E *n* =17	8 *n* =103	9 *n* =96
V2	n.s.	93.5	93.5	92.9	93.4	93.8	94.6
V3	n.s.	113.3	111.9	112.2	112.8	112.2	113.1
V4	n.s.	152.0	151.1	150.8	152.2	151.1	152.2
V5	n.s.	312.6	309.2	307.0	310.8	309.8	310.4
V6	n.s.	410.8	414.1	412.8	414.9	413.6	412.4
V7	n.s.	358.5	359.6	357.5	358.2	356.7	356.0
V8	n.s.	246.4	246.7	246.2	244.9	244.9	243.7
V9	n.s.	198.1	197.9	198.1	196.8	197.0	195.8
V10	n.s.	150.7	151.7	150.2	152.5	151.9	153.1
V11	n.s.	82.8	84.4	84.2	85.6	84.4	85.0

Table 7. Component matrix extracted by PCA to test morphometric differences of O stock animals among sub-areas.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
logV1	0.954	0.199	0.026	0.018	-0.128	-0.079	-0.016	-0.043	-0.029	-0.127	-0.089
logV2	0.771	-0.493	-0.130	0.351	0.094	-0.097	0.051	0.022	-0.022	0.004	0.017
logV3	0.817	-0.504	-0.175	-0.073	-0.007	0.106	0.107	-0.001	0.111	0.043	-0.075
logV4	0.867	-0.413	-0.116	-0.152	-0.036	0.093	0.110	-0.032	-0.104	-0.051	0.072
logV5	0.916	-0.221	-0.022	-0.167	-0.132	-0.233	-0.051	0.065	0.020	0.058	0.028
logV6	0.798	0.490	0.074	0.139	-0.259	0.135	0.013	0.108	0.007	0.030	0.025
logV7	0.850	0.470	-0.042	0.045	-0.020	-0.017	-0.006	-0.213	0.049	0.046	0.041
logV8	0.809	0.530	-0.094	-0.051	0.165	0.008	0.010	0.030	-0.117	0.088	-0.058
logV9	0.797	0.516	-0.164	-0.055	0.211	0.001	-0.014	0.089	0.088	-0.079	0.045
logV10	0.833	-0.466	0.055	0.007	0.051	0.119	-0.263	-0.012	-0.010	0.002	-0.001
logV11	0.754	-0.105	0.633	-0.019	0.115	-0.004	0.078	0.004	0.017	0.002	0.003

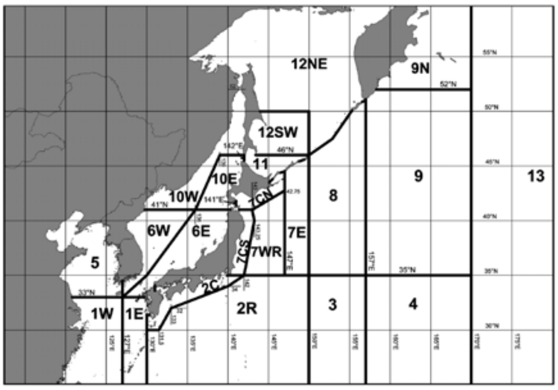
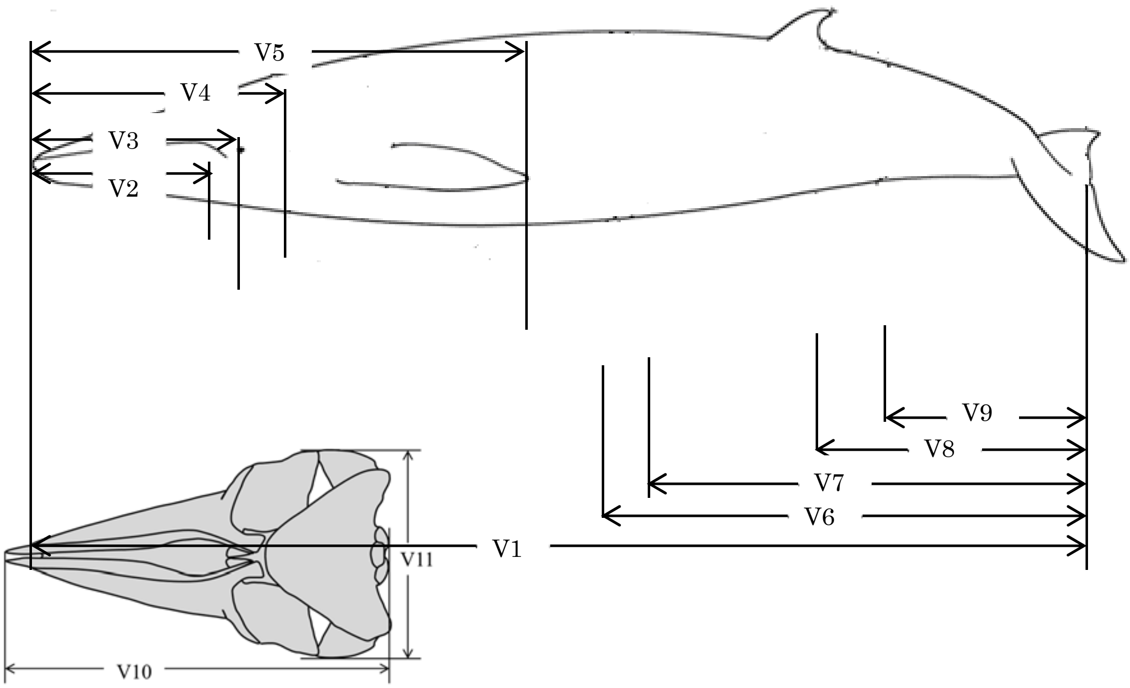


Figure 1. The sub-areas used for the *Implementation Review* for western North Pacific common minke whales (IWC, 2014).



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

Figure 2. External measurement points of common minke whale used in this study.

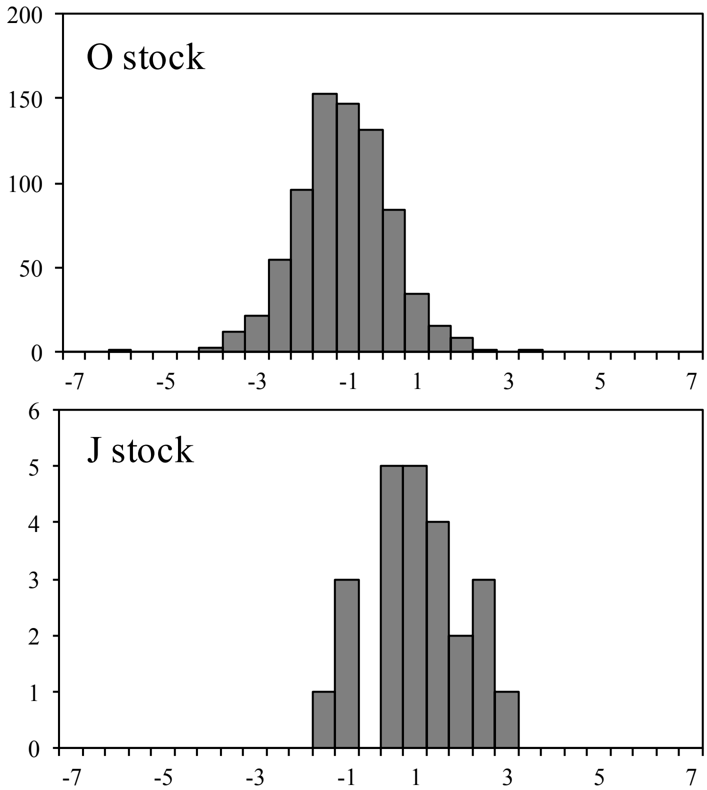


Figure 3. Results of discriminant analysis between O and J stock animals.