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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)).

MATERIALS 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,

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

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

$$\alpha_1 = \alpha_2 = \dots = \alpha_m = \alpha \quad (2)$$

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

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

The second one is to test null hypothesis that

$$\beta_1 = \beta_2 = \dots = \beta_m = \beta \quad (3)$$

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 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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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 | <i>p</i> -value | J stock <i>n</i> =24 | O stock <i>n</i> =760 |
|-------------|-------------------------|-------------------------|--------------------------|
| V2 | <i>p</i><0.05 | 97.0 | 93.1 |
| V3 | <i>p</i><0.05 | 117.3 | 111.8 |
| V4 | <i>p</i><0.05 | 155.6 | 150.4 |
| V5 | <i>p</i><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 | <i>p</i><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 | 7CN | 7WR | 7E | 8 | 9 |
|-------------|------------|--------|--------|--------|--------|---------|--------|
| | | $n=13$ | $n=43$ | $n=26$ | $n=17$ | $n=103$ | $n=96$ |
| 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 |

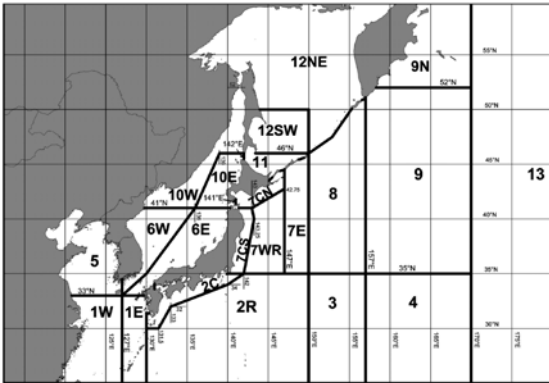
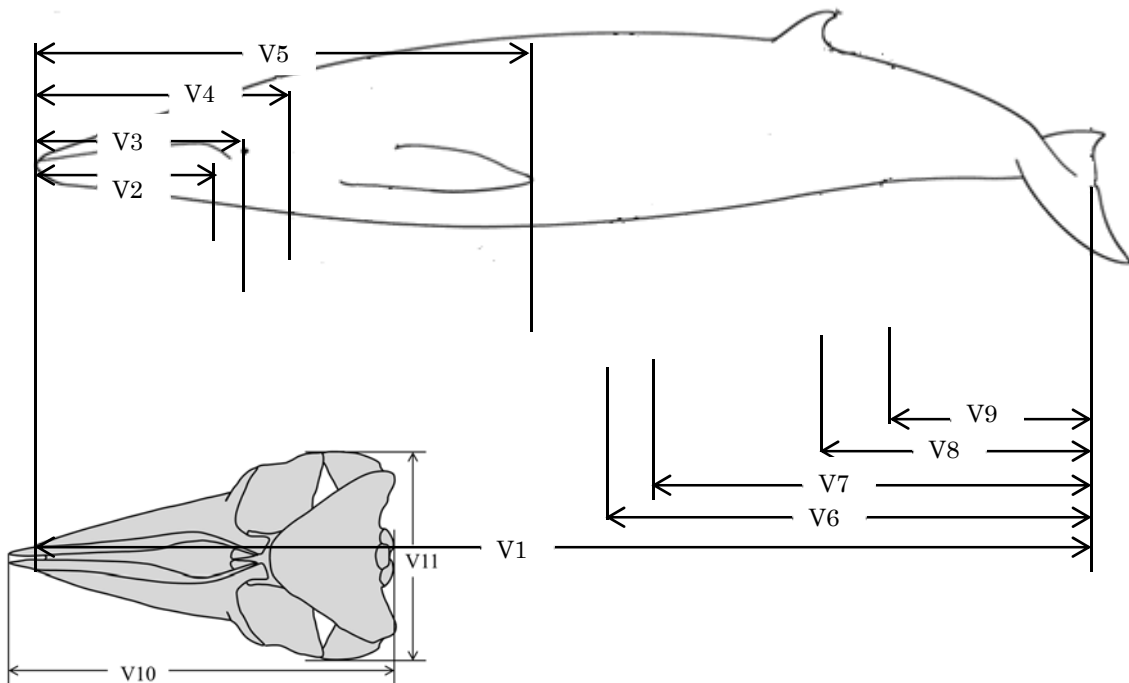


Figure 1. The sub-areas used for the *Implementation Review* for western North Pacific common minke whales (IWC, 2014).



- 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.

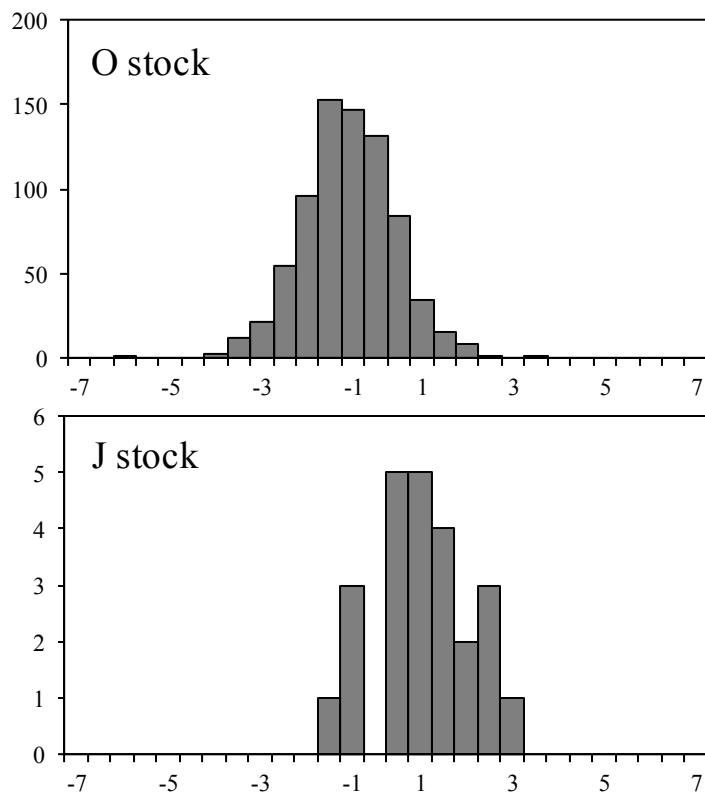


Figure 3. Results of discriminant analysis between O and J stock animals.