Some analyses on biological parameters of western North Pacific minke whales, from a view point of stock identification

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ABSTRACT

The present study examines some biological parameters of western North Pacific minke whales such as body length distribution, mean body length, growth curve, maximum body length and incidence of anomalous testis using biological materials obtained through the JARPN surveys in 1994 to 1999 from a view point of how biological parameters demonstrate nature of difference between genetically different stocks (so called "O stock" and "J stock"). For the present analysis, we accepted genetic identification on individual animals sampled under the JARPN research project, based on mtDNA analyses which were independently carried out. In addition, we further examined local differences in biological parameters among O stock animals so as to investigate existence of unknown stock hypothesized as "W stock". Consequently, for the present study, we used 39 J stock animals (26 males and 13 females) and 459 O stock animals (393 males and 66 females) collected through the 1994-1999 JARPN surveys. The clear differences of biological parameters, as mature body length distribution, maximum body length and asymptotic length, for female, are found between J stock and O stock. However, with regard to local difference of biological parameters among O stock samples collected from sub-areas 7, 8, 9, and 11, no clear differences indicated as it is found in the case of J stock and O stock comparison, are detected. In addition, evidence of sexual and reproductive segregation related to time and locality suggest unreality of assumption which another independent stock unit such as W stock exists within O stock region (sub-area 7, 8 and 9). And it is indicated that one and independent stock distributed widely in area with segregation depending on sex and reproductive status. As conclusions, present analysis supports genetic identification of the minke whale stock (J and O stocks), on the other hand the analysis does not reveal any aspects to suggest that hypothesized W stock actually exist.

INTRODUCTION

It has been reported that there are reproductive and sexual segregation around Japan and other associated area as in through examinations on biological materials collected by the small-type commercial whaling and pilot expeditions by the *Miwamaru* in 1973 to 1975, in which mature males and females tended to migrate further northern area, and immature animals tended to distribute in the southern coastal area from spring to early summer (Matsuura, 1936; Omura and Sakiura, 1956; Ohsumi, 1983; Wada, 1989; Kato, 1992). Furthermore, Hatanaka and

Miyashita (1997) presented possible migration pattern of the minke whales inferred from examination of local and temporal differences in biological composition based on the data obtained thorough operations by the small-type whaling, the *Miwamaru* and the 1994-95 JARPN surveys; as that, immature males migrate into the Sanriku coast (southern part of sub-area 7) in April and then disperse to the Pacific coast of Hokkaido (northern part of sub-area 7) and the Okhotsk coast of Hokkaido (sub-area 11); immature females follow similar pattern to their male counterparts, but larger immature females are also relatively abundant in the Okhotsk coast of Hokkaido (sub-area 11) in May and June; mature males widely occur from coastal areas to offshore areas on May; mature females enter the Okhotsk coast of Hokkaido (sub-area 11) in April and May and then move further to the middle and northern Okhotsk Sea (sub-area 12). On the other hand, Kato (1992) estimated several biological parameters for population analyses with Hitter/Fitter exercise such as reproductive status, length at sexual maturity and other necessary parameters. During course of his study, he also found two different foetus cohorts due to different peak of conception as in winter and autumn derived from different biological stock. And this was confirmed further analyses by Best and Kato (1992) and now it is believed that Okhotsk Sea-West Pacific stock has peak for conception in mid winter (February to March) while in October to November for Sea of Japan stock

In order to clarify stock structure of western North Pacific minke whales, Japan presented proposal of research program under scientific permit (so called JARPN survey) to the IWC (Government of Japan, 1994) and the program has conducted since then in western North Pacific region (e.g. Fujise *et al*, 1995,1996,1997; Ishikawa *et al.*, 1997; Zenitani *et al.*, 1999; Fujise *et al.*, 2000). The program mainly focused on stock identification by genetic markers such as mtDNA, other biological markers such as morphometric, pollutant burdens, parasite loads and also biological parameters have been examined from view points of stock identification (e.g. Fujise, 1995,1996; Fujise and Kato, 1996; Fujise *et al.*, 1998; Araki *et al.*, 1997; Kuramochi *et al.*, 1996).

The present study examines some biological parameters of western North Pacific minke whales using biological materials obtained through the JARPN surveys in 1994 to 1999 from a view point of how biological parameters demonstrate nature of difference between genetically different stocks (so called "O stock (the Okhotsk Sea- West Pacific stock)" and "J stock (the Sea of Japan-Yellow Sea-East China Sea stock)". For the present study, we accepted genetic identification on individual animals sampled by the JARPN surveys, based on mtDNA analyses which were independently carried out by Goto *et al.* (2000). In addition, we further examined local differences in biological parameters among O stock animals so as to investigate existence of unknown stock hypothesized as "W stock".

MATERIALS AND METHOD

The present study used biological materials collected from 498 individuals (419 males, 79 females) under JARPN surveys in 1994 to 1999.

1. Biological data collection

Number of qualified biological data and samples have been collected onboard of *Nisshin Maru*, as summarized in Appendix I and following laboratory procedure under the JARPN program. The present study used data set of reproductive status including testis weight and corpora counts, animal sex and body length, foetus data (number, length and sex) which were obtained through standard sampling procedure developed in the JARPN program. We also examined incidence of anomalous testis tissues (such as milk-white purulence and calcification of tissue) incorporating visual recording.

2. Age determination

It has been believed that age readability for North Pacific minke whales is poor (Kato, 1992). This was due to two reasons (1) poor formation of growth laminae and (2) low sampling rate of earplugs during the flensing. Under JARPN program, at least the sampling rate was largely improved because of careful treatment of carcasses, led to increasing in age readability.

Age reading was made by Kato incorporating standard manner developed in Kato (1987) by counting number of growth layers on core of the earplug with stereoscopic microscope under reflecting light. As a result, a total of 222 individuals was finally determined their ages.

3. Genetic identification of respective individuals

As explained above, we compare values of biological parameters between J and O stock animals divided by phylogenetic method (see Goto *et al.*, 2000). The J stock animals are identified by the following two criteria; (a) animals which have the same haplotype with samples collected in the Sea of Japan within the box in Fig. 2 from Goto *et al.* (2000) , or (b) animals which have RFLP haplotype "3" or "5" within the same box.

Using criteria above, we got resultant number of 39 J stock samples (26 males and 13 females) and 459 O stock samples (56 animals, 34 males and 22 females in the sub-11; 132 animals, 116 males and 16 females in the sub-area 7; 86 animals, 81 males and 5 females in the sub-area 8; 185 animals, 162 males and 23 females in the sub-area 9) (Table 1).

4. Statistical analyses

We used Kolmogorov-Smirnov 2-sample test (significant level of 5%) for body length distribution and t-test (significant level of 5%) for mean body length of mature animals and maximum body. And we used the logistic regression analysis for incidence of anomalous testis.

RESULTS AND DISCUSSION

1. Body length distribution

1.1. Comparison between J and O stock

1.1.1 Male

Fig. 1 indicates body length distributions of J stock and O stock samples in each sex, in which data were pooled by every 20cm. Range of body length of J stock male animals (26 individuals) is 6.2m to 7.8m and its peak exist 7.4-7.6m. In O stock males (393 individuals), their body length range is 4.4m to 8.4m with peak in 7.4m. Klomogorov-Smirnov 2-sample test revealed no statistical significant difference in the distribution between two different stocks (z=0.920, p=0.301).

If we compare only mature animals the range is similar as 7.0-7.8m for J stock vs. 6.2-8.4m for O stock and their mean body length are 7.44m (S.D.=0.25) for J stock vs. 7.42m (S.D.= 0.34) for O stock, in which no statistical difference are found (Klomogorov-Smirnov 2-sample test, z=0.675, p=0.6964; t-test, t=0.359, p=0.720).

1.1.2 Female

In female, the range of body length of two stocks are almost similar as that J stock female range (13 individuals) is 4.6m to 8.0m with peak in 7.2m and O stock range (66 individuals) is 4.4m to 8.6m with peak in 8.0m, in which Klomogorov-Smirnov 2- sample test revealed no statistically significant difference in the distribution between two different stocks (z=0.895, p=0.315).

However, if we compare the body length distributions by only mature animals significant differences were detected (Klomogorov-Smirnov 2- test, z=1.416, p=0.025). This is also true in comparison of mean body length as; their mean body length are 7.44m (S.D.=0.31 range; 7.0-8.0m) and 7.83m (S.D.=0.38, range; 7.0-8.6m) for J and O stocks respectively, in which statistical difference was detected (t-test, |t|=2.803, p=0.008).

1.2 Local variation within O stock

1.2.1 Male

Fig. 2 compares body length distributions with pooling every 20cm interval among four sub-areas within O stock in each sex. Ranges of male body length and their peak are:

Sub area 11 ; range 5.0-8.0 m, peak 7.2-7.4m .
Sub area 7 ; range 4.6-8.0m, peak 7.6m.
Sub-area 8 ; range 5.2-8.2m, peak 7.6m.
Sub-area 9 ; range 4.4-8.4m, peak 7.4m.

Klomogorov-Smirnov 2-sample test revealed statistical difference in body length distribution between sub-areas 7 and 8. In other combinations, no statistical difference was found. Any comparison of the distribution between sub-areas as summarized in Table 2.

If we comparebody only mature animals the range, peak and corresponded mean body length are:

Sub-area 11; range 6.8-8.0m (n=31), peak 7.2-7.4m, mean 7.35m (S.D.=0.29).
Sub-area 7; range 6.2-8.0m (n=94), peak 7.6m, mean 7.38m (S.D.=0.33).
Sub-are 8; range 6.4-8.2m (n=73), peak 7.6m, mean 7.48m (S.D.=0.34).
Sub-area 9; range 6.4-8.4m (n=147), peak 7.4m, mean 7.42m (S.D.=0.34).

Again no statistical differences are detected in any comparisons on mature male body length distribution between two sub-areas (Table 2). It is also true for mean body length comparison by t-test (Table 3).

1.2.2 Female

Comparison for females is rather limited than in males due to small sample size when the data is arranged by sub-area, however, we follow the same comparison for female samples. Then, range of body length and their peak in females are:

Sub-area 11; range 4.6-8.2m, peak 8.0m.
Sub-area 7; range 4.8-8.4m, no peak.
Sub-area 8; range 5.6-8.6m, no peak.
Sub-area 9; range 4.4-8.2m, peak 8.0m.

Klomogorov-Smirnov 2-sample test revealed no statistical difference in any comparison of the distribution between sub-areas as summarized in Table 2.

If we compare only mature females the range, peak and corresponded mean body length are:

Sub-area 11; range 7.0-8.2m (n=12), peak 8.0m, mean 7.85m (S.D.=0.35).
Sub-area 7; range 7.0-8.4m (n=8), no peak, mean 7.82m (S.D.=0.47).
Sub-area 8; range 7.8-8.6m (n=3), no peak, mean 8.14m (S.D.=0.45).
Sub-area 9; range 7.0-8.2m (n=13), peak 8.0m, mean 7.75m (S.D.=0.34).

Although body length is likely to be slightly larger in sub-area 8 than other sub areas, no statistical differences can be detected in any comparisons on length distribution of mature females between two sub-areas (Table 2). There are also no statistical difference in mean body length comparison by t-test (Table 3).

2. Growth curve

Kato (1992) examined local variation of growth curve and asymptotic length of females from view points of stock separation. However, he had to use corpora numbers instead of age due to lack of age information. For the present study age information were available though age readability is just as high as 44.65% (222/498). However, age information can be used for not only females but also for males, thus this has much merits than corpora number. Also age readability by earplug generally has age-specific aspect which increases with age (Kato, 1984), this does not give serious bias to analysis of asymptotic length using older animals.

Then this section examined the growth curve using earplug age reading from a view point of stock identification.

2.1 Comparison between J and O stocks

2.1.1 Male

Fig. 3 plots mean body length against animal age separately by genetic stock in each sex. In O stock males, mean body length rapidly increases until around 7 years, then slowly increases after this age class and cease the increase at about 7.5m around 15 years. Mean body length of J stock is smaller than of O stock in age class until 10 years, however no difference between two stock after this age class.

2.1.2 Female

On the other hand, there is no difference between the two stocks in younger age classes than 10 years, in which mean body length rapidly increase. However, it seems J stock mean body length is clearly smaller than of O stock after 10 years at which the growth rate is decreasing. Due to lack of older animals in females, it is not clear at cessation point of increase of body length, but it is likely growth ceases around 15 years at about 8.0m and 7.5m for O and J stock females respectively.

2.2 Local variation within O stock

Fig. 4 plots body length of all of animals against age with arranging by combination of local comparison in each sex. Although we spent much efforts to compare the plots between sub-areas, no specific difference can be found throughout all of combinations.

3. Maximum body length (mean body length of animals older than 14 years)

It can be interpreted that mean body length of animals older than cessation points of growth curve (i.e. physical maturity) gives asymptotic length or maximum body length of each sample group which is useful key to discriminate the biological stock. However, physical maturity data are not available at this time, thus, the asymptotic length could not be estimated. As an alternative, we used the mean body length of individuals older than 14 years as maximum body length (Table 4).

3.1 Comparison between J and O stock

3.1.1 Male

Maximum body length for J stock is calculated to be 7.47m (n=8, S.D=0.22, range; 7.01-7.70m) . And that of O stock males is 7.54m (n=57, S.D.=0.25, range; 6.96- 8.13m). Using t-test, no significant difference was found between two stocks (t-test; $|t|=0.743$, $p=0.460$).

4.1.2 Female

Maximum body length for J stock is calculated be 7.44m (n=2, S.D.= 0.18, range; 7.31-7.57m) and this value is considerably smaller than that of O stock females which is calculated to be 8.00m (n=10, S.D.=0.26, range; 7.50-8.45m) . Although sample size is rather small for comparison, t-test revealed significant difference in maximum body length between two stocks (t-test; |t|=2.900, p=0.016).

3.2 Local variation within O stock

3.2.1 Male

Maximum body length is also calculated in each sub-area within O stock in order to examine its local variation. The respective values of maximum body length are indicated Table 4. The resultant values of maximum body length (7.51-7.61m) are very close each other and no statistical difference was found by t-test (Table 5).

3.2.2 Female

Calculation of maximum body length for females is rather limited than in males due to small sample size when the data is arranged by sub-area. However, we follow the same procedure to statistically compare the values between sub-areas within O stock in order to examine its local variation. Table 4 indicates the respective value of maximum body length of females. It was difficult to compare the value in sub-area 8 with other values due to extremely small sample sizes. In the other combinations, no statistical difference was found (Table 5), however more samples will be necessary to endorse the given result.

4. Conception date

Kato (1992) found two separate foetus cohorts due to difference in conception peak as one in winter and the other in autumn derived from different biological stock and this was confirmed further analyses by Best and Kato (1992). And now it is believed that the Okhotsk Sea-West Pacific stock has peak for conception in mid winter (February to March) while in autumn (October to November) for Sea of Japan stock. Thus, now no further analyses would be necessary in terms of stock discrimination. Furthermore, Okamura *et al.* (2000) independently examined possibility of W stock existence or existence of unknown stock within O stock and they are going to submit their paper to the IWC/JARPN review meeting. Thus, this issue refer to their current study.

5. Anomaly in gonadal tissues of sampled whales

It has already been reported that anomaly in gonadal tissues occurred, as milk-white purulence and calcification of tissue were observed by the naked eyes from some male minke whales (Fujise *et al.*, 1996, 1997, 1998; Ishikawa *et al.*, 1997; Zenitani *et al.*, 1999; Fujise *et al.*, 2000). Table 6 indicates the incidence of the anomalous testis tissues with separating by sexual status (sexually immature or mature).

Such anomalous occurred from 72 mature males of 370 total male samples, whereas only one case occurred from immature males of a total 49 sexually immature male samples.

5.1 Comparison between J and O stocks

Among J stock mature males (25 individuals), anomalous tissue is confirmed from four individuals, and corresponded incidence of anomalous testis is 16.0%. And among 345 O stock mature males, anomalous tissue occurred to be observed from 68 individuals (19.7%). The incidence of anomalous testis of O stock was slightly higher than that of J stock.

5.2 Local variation within O stock

Resultant values of incidence of anomalous testis among O stock mature males are indicated in Table 6. Anomalous testis are 16.0% (5/31) and 13.8% (13/94) in sub-areas 11 and 7, respectively. The values are 27.4% (20/73) in sub-area 8 and 20.4% (30/147) in sub-area 9. Incidences of anomalous testis are likely to be higher in sub-areas 8 and 9 than other sub areas.

In order to extract real pattern of incidence of the anomalous testis, a logistic regression model is applied incorporating a variable selection method. We examined what variables among year of sampling, body length, sexual maturity and stock (or sub-area) differentiation significantly affect the incidence of the anomalous testis. Through several trials, it was revealed the incidence of anomalous testis is strongly related to animal growth (body length) rather than other factors, i.e. the incidence is increasing with growth of animals. Thus the apparent local variation of this parameter does not mean indication due to stock differentiation.

6. Sexual and reproductive segregation related to time and locality

Many authors have already reported there is sexual and reproductive segregation among minke whales distributed in waters around Japan (Matsuura, 1936; Omura and Sakiura, 1956; Ohsumi, 1983; Wada, 1989; Kato, 1992; Hatanaka and Miyashita, 1997). In addition Fujise et al. (1998) and Zenitani *et al.* (1999) reported more detailed based on JARPN data such aspects as; (1) mature males are dominant in sub-areas 7, 8 and 9 in May to September. (2) Proportion of immature animals is relatively higher in Spring than in Summer in sub-areas 7, 8 and 9. (3) Mature females were present in a relatively high proportion in summer (July and August) in sub-area 11 (southern part of Okhotsk Sea).

The present study examined this aspect with combining all of data set obtained through the studies above. As in Table 7, proportion of males in the sample set (male sex ratio) are higher in sub-areas 7, 8 and 9 in May to September being 74.1-100.0% while the value (56.0-63.3%) is relatively lower in sub-area 11 in July to August. Sexual maturity rate is also different related to time and locality as; (1) mature males generally dominate in sub-areas 7, 8 and 9 (71.9-85.7%). (2) Immature animals (combined both sexes) in coastal sub-area 7 is slightly higher as being (22.1%) than other sub-areas 8 and 9 (11.0-13.9%). (3) Proportion of mature females and immature females are higher in sub-area 11 in July to August than other sub-areas in the same season. (4) Mature males dominate than other components in sub-area 7, 8 and 9 in May to September. (5) Proportion of immature males in sub-area 7, 8 and 9 are slightly increasing in spring (May to June) than in summer (July to September).

The JARPN surveys clarified mature males are dominated from coastal sub-area 7 to offshore sub-areas 8 and 9 in May to September. The main distribution area of mature females and immature animals already reported by many authors; as that, mature females are dominated in southern part of Okhotsk Sea (sub-area 11) in April to May and them move further to the middle and northern Okhotsk Sea (sub-area 12); immature animals are dominated in the Sanriku coast (western and southern part of sub-area 7) in April to May (Matsuura, 1936; Omura and Sakiura, 1956; Ohsumi, 1983; Wada, 1989; Kato, 1992; Hatanaka and Miyashita, 1997).

Thus, as indicated above, incomplete components of all of sex and sexual status in every sub-area and season indicate unreal existence of independent stock unit in each sub-area.

CONCLUSION

As already indicated by Kato (1992) and Best and Kato (1992), difference in conception dates due to different mating timing, which is one of biological parameters provides clear key to discriminate two different stock of minke whales. Thus, the present study again examine available biological parameters which have potential to indicate some kind of difference if the stock would be separate with incorporating independent genetic marker given for respective animals.

Through the analyses, significant differences have been detected in several parameters especially in females (Table 8) as: (1) The body length distribution of mature females between two stocks are significant difference and mean body length for J stock mature females is significantly smaller than for O stock mature females. (2) Growth curve figure shows asymptotic length for J stock females (7.5m) is smaller than for O stock females (8.0m). (3) Maximum body length of J stock females (7.44m) significantly smaller than of O stock females (8.00m). Among them, because both asymptotic length and maximum body length are free from reproductive segregation, they give much more stable solution on the difference due to different genetic stocks, in another words, such differences support stock identification by genetic markers.

On the other hand, no such difference has been detected among local areas within O stock while some differences exist in body length of males but due to sexual and reproductive segregation which have been confirmed by many previous authors (Omura and Sakiura, 1956; Ohsumi, 1983; Wada, 1989; Kato, 1992; Hatanaka *et al.*, 1997) (Table 9). If the different genetic stock supposed as "W stock" actually exists, some difference such as conception dates or maximum body length/asymptotic can be also expected. However, none of difference has been detected within O stock animals, thus existence of W stock is not supported by the present analysis . This also agree conclusion of conception date analysis by Okamura *et al.* (2000), in which they denied plausibility of existence of W stock through mathematical modeling test.

The present study also clarified sexual and reproductive segregation related to time and spatial localities (sub-area), which indicates there is no case complete all of animal components in specific sub-area. And it is indicated that a independent stock unit distributed widely in area with segregation depending on sex and reproductive status. This seems to be also another evidence not to suggest existence of independent stock unit (W stock) within O stock.

In conclusion, present study has found that some biological parameters showed significantly different value between J and O stock, which supports stock identification by genetic marker, while the present study does not indicate that independent stock such as W stock within O stock region (sub-area 7, 8, 9).

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Table 1. Number of samples by genetic stocks collected under the JARPN surveys in 1994 to 1999.

Stock*	Male	Female	Total
J	26	13	39
O-11	34	22	56
O-7	116	16	132
O-8	81	5	86
O-9	162	23	185
O-combined	393	66	459
Total	419	79	498

*: classified on the basis of mtDNA analyses (Goto *et al.*, 2000)

Table 2. Comparison of body length distribution between two sub-areas within O stock using Klomogorov-Smirnov 2-sample test. Above diagonal line: z value, below diagonal line: probability.

Male

Sub-area	O-11	O-7	O-8	O-9
O-11		0.873	1.230	0.738
O-7	0.360		1.304	1.288
O-8	0.077	**0.050**		1.146
O-9	0.554	0.053	0.111	

(Mature male)

Sub-area	O-11	O-7	O-8	O-9
O-11		0.860	1.266	0.769
O-7	0.357		1.270	0.911
O-8	0.057	0.057		1.160
O-9	0.487	0.306	0.097	

Female

Sub-area	O-11	O-7	O-8	O-9
O-11		1.063	0.682	0.480
O-7	0.155		0.647	0.750
O-8	0.622	0.705		0.644
O-9	0.940	0.536	0.675	

(Mature female)

Sub-area	O-11	O-7	O-8	O-9
O-11		0.639	0.516	0.673
O-7	0.744		0.554	0.835
O-8	0.941	0.836		0.801
O-9	0.625	0.392	0.455	

Table 3. Comparison of mean body length of mature animals in each sub-area within O stock animals using t-test. Above diagonal line: t value, below diagonal line: probability.

Male

Sub-area	O-11	O-7	O-8	O-9
O-11		-0.511	1.948	1.170
O-7	0.610		1.967	0.975
O-8	0.054	0.051		-1.204
O-9	0.244	0.330	0.230	

Female

Sub-area	O-11	O-7	O-8	O-9
O-11		0.200	1.197	-0.771
O-7	0.843		1.025	-0.397
O-8	0.253	0.332		-1.719
O-9	0.449	0.696	0.108	

Table 4. Maximum body length of minke whales older than 14 years by genetic stock.

Stock*	Male					Female				
	Mean	S.D.	Max.	Min.	n	Mean	S.D.	Max.	Min.	n
J	7.47	0.22	7.01	7.70	8	7.44	0.18	7.31	7.57	2
O-11	7.61	0.22	7.38	7.95	7	8.05	0.10	7.95	8.18	4
O-7	7.51	0.29	7.05	8.05	16	8.19	0.37	7.93	8.45	2
O-8	7.51	0.29	6.96	8.10	17	7.75	-	-	-	1
O-9	7.56	0.20	7.31	8.13	17	7.89	0.35	7.5	8.18	3
O-combined	7.54	0.25	6.96	8.13	57	8.00	0.26	7.50	8.45	10

*: classified on the basis of mtDNA analyses (Goto *et al*., 2000)

Table 5. Comparison of maximum body length between two sub-areas within O stock using t-test.
Above diagonal line: t value, below diagonal line: probability.

Male

Sub-area	O-11	O-7	O-8	O-9
O-11		0.820	-0.877	-0.584
O-7	0.421		-0.047	0.544
O-8	0.390	0.963		0.612
O-9	0.565	0.590	0.545	

Female

Sub-area	O-11	O-7	O-8	O-9
O-11		-0.529	-	-0.873
O-7	0.685		-	-0.909
O-8	-	-		-
O-9	0.423	0.430	-	

-: not calculate

Table 6. Incidence of anomalous testis tissues of minke whales by genetic stock.

Stock*	Sexual status	n	Testis tissues		Incidence
			Normal	Anomalous	%
J	Imm.	1	1	0	0
	Mat.	25	21	4	16.0
O-11	Imm.	3	3	0	0.0
	Mat.	31	26	5	16.1
O-7	Imm.	22	22	0	0.0
	Mat.	94	81	13	13.8
O-8	Imm.	8	7	1	12.5
	Mat.	73	53	20	27.4
O-9	Imm.	15	15	0	0.0
	Mat.	147	117	30	20.4
O-combined	Imm.	48	47	1	2.1
	Mat.	345	277	68	19.7

*: classified on the basis of mtDNA analyses (Goto *et al*., 2000)

Table 7. Sex ratio and reproductive status of minke whales collected under the surveys in 1994 to 1999 by sub-area and month.

Sub-area	Month	Male Imm.	Male Mat.	Male Total	Female Imm.	Female Mat.	Female Preg.	Female Total	Total	Sex ratio
11	Jul.	2	26	28	8	14	[12]	22	50	56.0
		(4.0)	(52.0)		(16.0)	(28.0)				
	Aug.	2	17	19	4	7	[5]	11	30	63.3
		(6.7)	(56.7)		(13.3)	(23.3)				
	Total	4	43	47	12	21	[17]	33	80	58.8
		(5.0)	(53.8)		(15.0)	(26.3)				
7	May	8	41	49	4	3	[2]	7	56	87.5
		(14.3)	(73.2)		(7.1)	(5.4)				
	Jun.	9	36	45	5	2	[2]	7	52	86.5
		(17.3)	(69.2)		(9.6)	(3.8)				
	Jul.	0	0	0	0	1	[1]	1	1	0.0
		(0.0)	(0.0)		(0.0)	(100.0)				
	Aug.	1	14	15	0	0		0	15	100.0
		(6.7)	(93.3)		(0.0)	(0.0)				
	Sep.	4	9	13	0	2	[1]	2	15	86.7
		(26.7)	(60.0)		(0.0)	(13.3)				
	Total	22	100	122	9	8	[6]	17	139	87.8
		(15.8)	(71.9)		(6.5)	(5.8)				
8	May	0	7	7	1	0		1	8	87.5
		(0.0)	(87.5)		(12.5)	(0.0)				
	Jun.	5	28	33	1	2	[2]	3	36	91.7
		(13.9)	(77.8)		(2.8)	(5.6)				
	Jul.	3	38	41	0	1	[1]	1	42	97.6
		(7.1)	(90.5)		(0.0)	(2.4)				
	Aug.	0	5	5	0	0		0	5	100.0
		(0.0)	(100.0)		(0.0)	(0.0)				
	Total	8	78	86	2	3	[3]	5	91	94.5
		(8.8)	(85.7)		(2.2)	(3.3)				
9	May	3	17	20	5	2	[2]	7	27	74.1
		(11.1)	(63.0)		(18.5)	(7.4)				
	Jun.	10	39	49	2	3	[3]	5	54	90.7
		(18.5)	(72.2)		(3.7)	(5.6)				
	Jul.	0	62	62	2	5	[5]	7	69	89.9
		(0.0)	(89.9)		(2.9)	(7.2)				
	Aug.	2	27	29	2	3	[3]	5	34	85.3
		(5.9)	(79.4)		(5.9)	(8.8)				
	Sep.	0	4	4	0	0		0	4	100.0
		(0.0)	(100.0)		(0.0)	(0.0)				
	Total	15	149	164	11	13	[13]	24	188	87.2
		(8.0)	(79.3)		(5.9)	(6.9)				

Table 8. Summery of differences in biological parameters between O and J stocks[1), with note on statistical significant.

Biological parameters	Male	Female	Difference
Body length distribution for mature animals	NS	S*	J stock is smaller than O stock
Mean body length of mature animals	NS	S*	J stock is smaller than O stock
Maximum body length	NS	S*	J stock is smaller than O stock
Growth curve	ND	D	J stock is smaller than O stock
Anomalous testis, incidence	NS	-	

[1): classified on the basis of mtDNA analyses (Goto *et al*., 2000)

NS: not significant (significant level of 5%), **S***: significant (significant level of 5%)

D: different by visual examination, **ND**: not different by visual examination

Table 9. Summery of differences in biological parameters between sub-areas within O stock, with note on statistical significant.

Male

Biological parameters	Difference between sub-areas					
	9 vs.8	9 vs. 7	9 vs.11	8 vs.7	8 vs.11	7 vs.11
Body length distribution	NS	NS	NS	S*	NS	NS
Body length distribution of mature animals	NS	NS	NS	NS	NS	NS
Mean body length of mature animals	NS	NS	NS	NS	NS	NS
Maximum body length	NS	NS	NS	NS	NS	NS
Growth curve	ND	ND	ND	ND	ND	ND
Anomalous testis, incidence	NS	NS	NS	NS	NS	NS

Female

Biological parameters	Difference between sub-areas					
	9 vs.8	9 vs. 7	9 vs.11	8 vs.7	8 vs.11	7 vs.11
Body length distribution	NS	NS	NS	NS	NS	NS
Body length distribution of mature animals	NS	NS	NS	NS	NS	NS
Mean body length of mature animals	NS	NS	NS	NS	NS	NS
Maximum body length	-	NS	NS	-	-	NS
Growth curve	ND	ND	ND	ND	ND	ND

NS: not significant (significant level of 5%), **S***: significant (significant level of 5%)

ND: not different by visual examination

-: can be not calculated

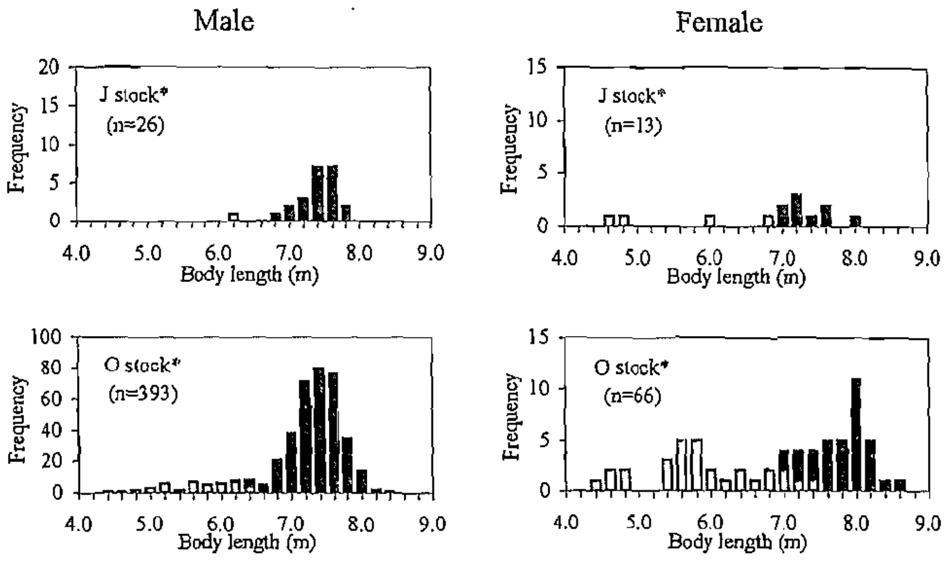


Fig.1. Body length distribution of J stock and O stock animals each sex, in which data were pooled by every 20cm
White indicate immature animals and black indicates mature animals.
*: classified on the basis of mtDAN analyses (Goto *et al*., 2000)

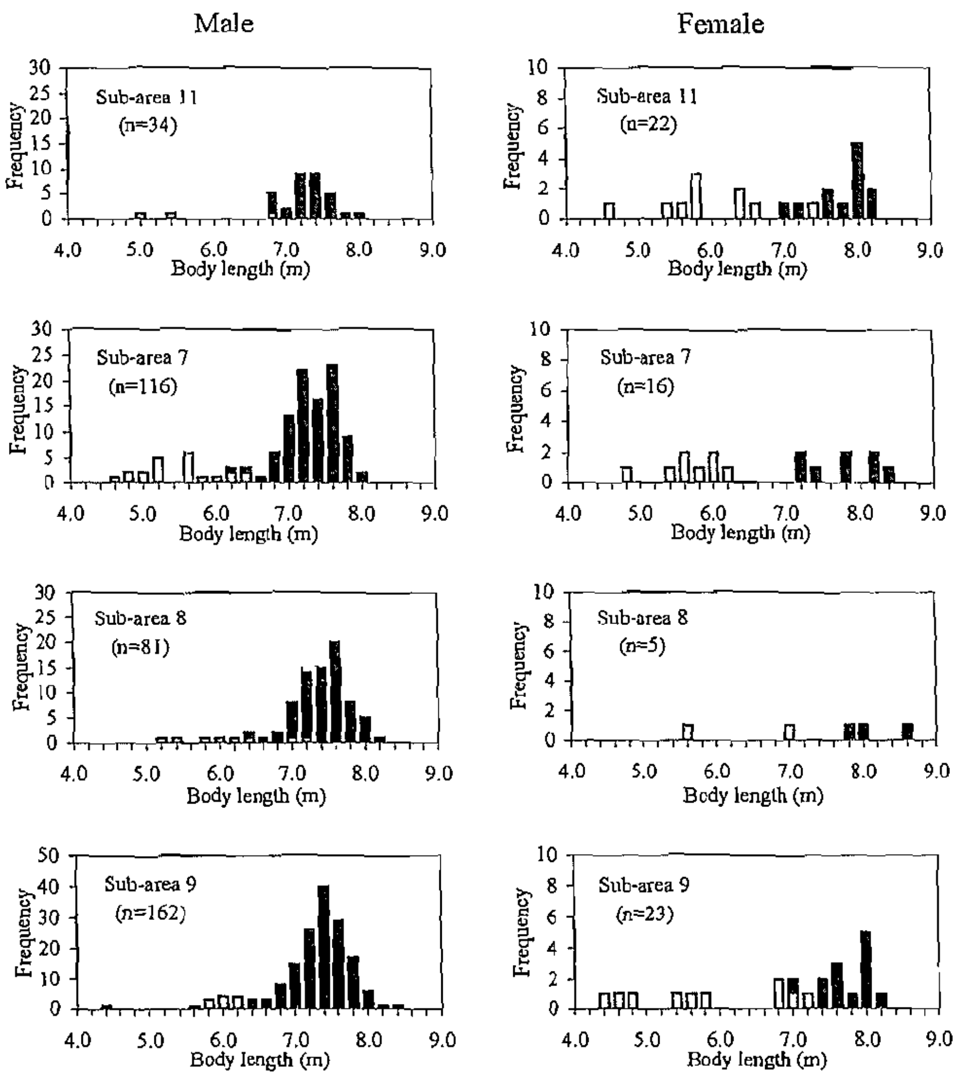
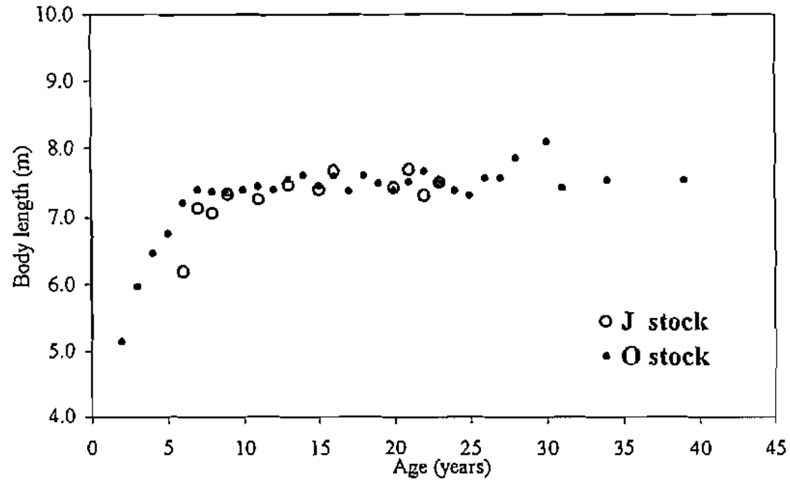


Fig.2. Body length distribution with pooling every 20cm interval among four sub-areas within O stock in each sex.of hypothetical O stock animals, by sex and sub-area.
White indicate immature animals and black indicates mature animals.

Male



Female

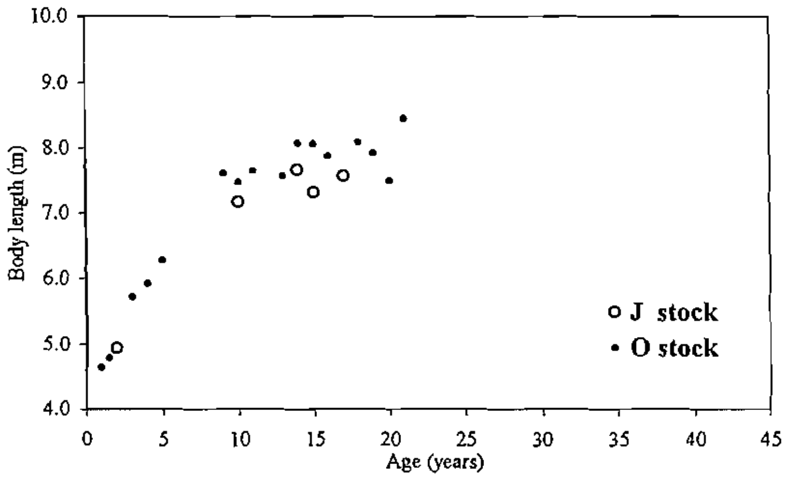


Fig. 3. Mean body length against animal age separated by genetic stock in each sex.
Open circles: J stock*, closed circles: O stock*.
*: classified on the basis of mtDAN analyses (Goto *et al*., 2000)

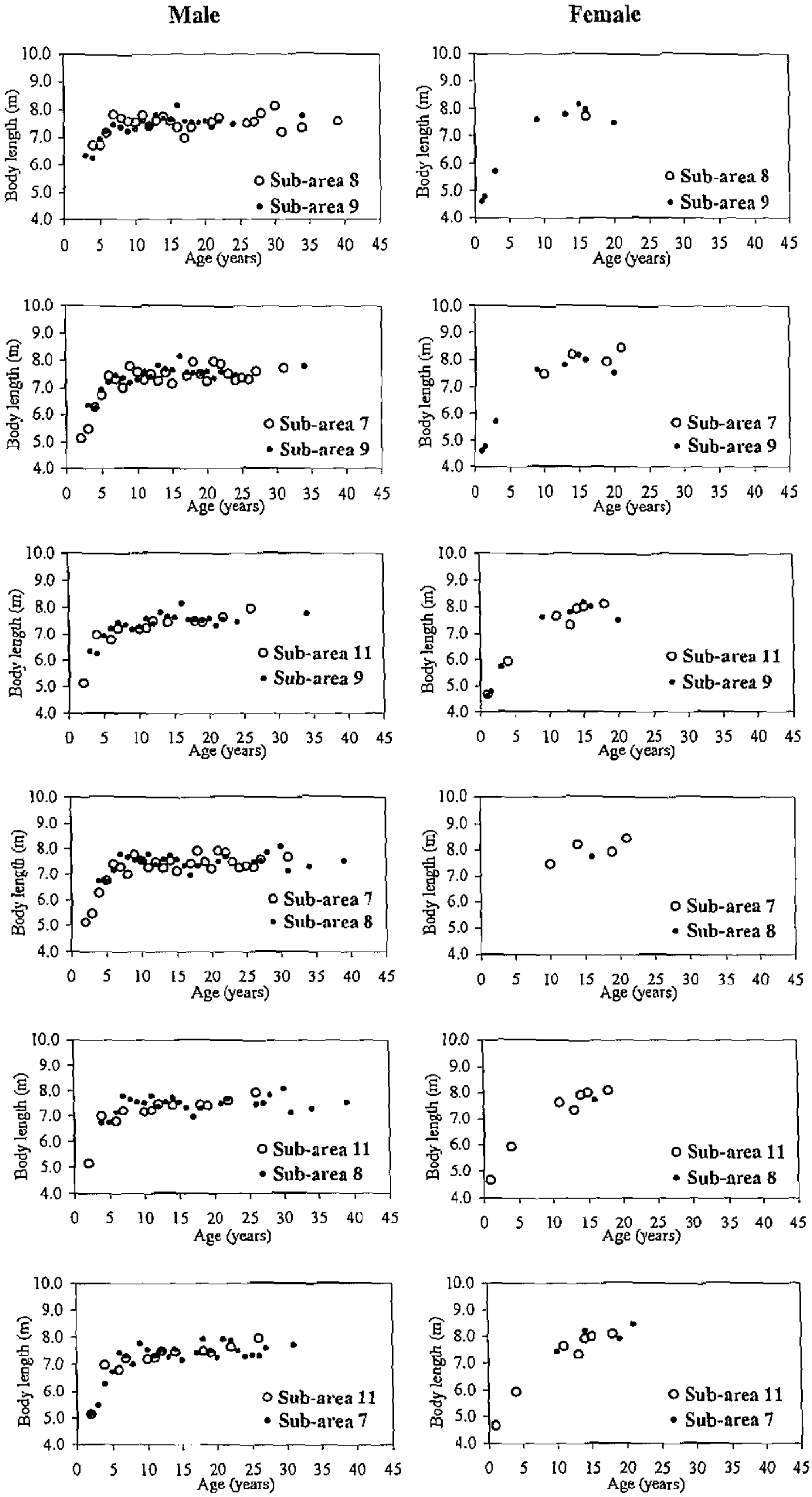


Fig.4. Body length of all of animals against age with arranging by combination of local comparison in each sex.

Appendix 1. Summary of biological data and samples.

Biological data and Samples	1994		1995		1996		1997		1998		1999	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Body length and sex	18	3	91	9	63	14	87	13	89	11	71	29
External body proportion	18	3	91	9	63	14	87	13	89	11	71	29
Photographic record of external character	18	3	91	9	63	14	87	13	89	11	71	29
Diatom film record and sampling	18	3	91	9	63	14	87	13	89	11	71	29
Blubber thickness	18	3	91	9	63	14	87	13	89	11	71	29
Body weight	18	3	91	9	63	14	87	13	89	11	71	29
Body weight by parts	12	3	25	3	18	3	25	6	19	0	14	6
Blubber, muscle, liver and kidney tissues for DNA study	18	3	91	9	63	14	87	13	89	11	71	29
Muscle, liver and heart tissues for isozyme analysis	18	3	91	9	63	14	87	13	89	11	71	29
Muscle, liver and kidney tissues for heavy metal analysis	18	3	91	9	63	14	87	13	89	11	71	29
Blubber, liver, kidney, muscle tissues for organochlorine analysis	18	3	91	9	63	14	87	13	89	11	71	29
Tissues for lipid analysis	12	3	25	3	18	3	25	6	19	0	14	6
Tissues for endocrine disrupters analysis	-	-	-	-	-	-	-	-	89	11	71	29
Muscle, liver tissues and baleen plate for stable isotopes	-	-	-	-	-	-	-	-	89	11	28	22
Mammary grand; lactation status, measurements and histological sample	-	3	-	9	-	14	-	13	-	11	-	29
Collection of ovary	-	3	-	9			-	13	-	11	-	29
Uterine horn; measurement and endometrium sample	-	3	-	9	-	14	-	13	-	11	-	29
Uterine mucus for sperm detection	-	3	-	9	-	14	-	13	-	11	-	28
Foetus number	(1)		(7)		(7)		(6)*		(4)*		14	
Photographic record of fetus	-	1	2	5	1	6	4	1	1	2	8	6
Fetal sex (identified by visual observation)	-	1	2	5	1	6	4	1	1	2	8	6
Fetal length and weight	-	1	2	5	1	6	4	1	1	2	8	6
External measurement of fetus	-	1	2	4	1	6	4	1	1	1	7	2
Collection of fetus	-	1	2	5	0	1	-	-	-	2	8	6
Testis and epididymis; weight and histological sample	18	-	91	-	63	-	87	-	89	-	71	-
Smear samples from testis and epididymis tissues	18	-	91	-	63	-	87	-	89	-	71	-
Urine sample for sperm detection	12	-	77	-	51	-	73	-	59	-	46	-
Collection of serum sample	18	3	91	9	63	14	87	13	89	11	71	29
Stomach content, conventional record	18	3	91	9	63	14	87	13	89	11	71	29
Weight of stomach content in each compartment	18	3	91	9	62	14	87	13	89	11	-	-
Volume and weight of stomach content in each compartment	-	-	-	-	63	14	87	13	89	11	71	29
Collection of stomach contents for organochlorine study	9	1	-	-	-	-	36	2	-	-	-	-
Collection of stomach contents for heavy metals study	9	1	15	2	23	1	36	2	16	1	-	-
Collection of stomach contents for lipid analysis	6	1	10	1	5	0	6	0	2	0	-	-
Record and collection of external parasites	18	3	91	9	63	14	87	13	89	11	71	29
Record and collection of parasites in stomach	18	3	91	9	63	14	87	13	89	11	71	29
Record and collection of parasites from small intestine	18	3	91	9	63	14	87	13	89	11	71	29
Record and collection of parasites from liver	18	3	91	9	63	14	87	13	89	11	71	29
Earplug for age determination	18	3	91	9	63	14	87	13	89	11	71	29
Tympanic bulla for age determination	12	1	91	9	62	14	85	12	89	11	71	29
Largest baleen plate for morphologic study and age determination	18	3	91	9	63	14	87	13	89	11	70	29
Vertebral epiphyses sample	18	3	91	9	63	14	87	13	88	11	71	28
Baleen plate set mouth cavity (length and breadth)	18	3	91	9	63	14	87	13	88	11	71	29
Length of each baleen plate series	18	3	91	9	63	14	87	13	88	11	71	29
Number of vertebrae	18	3	91	9	63	14	87	13	88	11	71	29
Number of ribs	18	3	91	9	63	14	87	13	88	11	71	29
Skull measurement (length and breadth)	18	3	91	9	61	14	84	12	88	10	71	28
Detailed measurements of skull	12	2	2	3	5	2	-	-	0	1	0	1
Collection of skull	2	0	0	1	-		2	1	-	-	0	3
Collection of whole skeleton	4	2	-	-	-		-	-	-	-	0	1

*: including fetuses of sex unidentified.