Yearly trend in the age at sexual maturity of Antarctic minke whales examined by transition phase in earplugs collected during JARPA and JARPAII surveys

Takeharu Bando[1)], Toshiya Kishiro[2)] and Hidehiro Kato[3)]

[1)] *Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo, 104-0055, Japan*
[2)] *National Research Institute of Far Seas Fisheries, 2-12-4 Fukuura, Kanazawa-ku, Yokohama*
[3)] *Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-0075, Japan*
Contact e-mail: bando@cetacean.jp

ABSTRACT
This paper is a revised version of SC/F14/J8 presented to the JARPAII Review Workshop. The discussion of the paper was modified to take into consideration some recommendations from the Review Workshop. Yearly trend of age at sexual maturity in Antarctic minke whales was examined by age and transition phase (TP) data from earplugs collected during 1987/88-2004/05 JARPA and 2005/06-2010/11 JARPAII surveys. Analysis was conducted by sex and stocks that were separated at 165ºE. Truncation bias was corrected by standard method. The results confirmed that the age at sexual maturity of both stocks declined from the mid 1940s cohorts at around 10-12 years old to the early 1970s cohorts at around 7-8 years old, presumably as response to improved nutritional conditions at that time. Age at sexual maturity remained constant at 7-8 years old till the 1990s cohorts. Statistical analysis to investigate trend from the 1970s cohorts showed slight but statistically significant increasing trend for the I stock (both sexes) and P stock (females). Whether or not these trends respond to deteriorated nutritional conditions is not clear at this stage. If so, the level of current changes in response to deteriorated conditions is not the same as the decreasing trend level observed from the 1940's cohorts in response to improved nutritional conditions at that time. Continuous monitoring of age at sexual maturity is needed to detect possible changes in nutritional condition of Antarctic minke whales.

KEYWORDS: ANTARCTIC MINKE WHALE, AGE AT SEXUAL MATURITY, TRENDS

INTRODUCTION

It is generally known that the transition phase in earplugs of large baleen whales indicates the age at sexual maturity (Lockyer, 1972; Masaki, 1979; Kato, 1983a; Ohsumi, 1986a). Several authors have reported a decline of Antarctic minke whales age at sexual maturity from 14 years in the 1940s cohorts to 7 years in the late 1960s cohorts from the analysis of transition phase in earplugs collected by commercial whaling (Masaki, 1979; Best, 1982; Kato, 1983b; 1985; Thomson *et al*., 1999). It was considered that the decline of age at sexual maturity in the Antarctic minke whales was caused by reduction in the abundance of other large baleen whale stocks, which led to greater availability of food for Antarctic minke whales (Masaki, 1979; Best, 1982; Kato, 1983b; 1985; 1987; Kato and Sakuramoto, 1991).

At the JARPA review meeting held in Tokyo in 2006, results of long-term changes in age at sexual maturity of Antarctic minke whales from the 1940s to the 1991 cohorts were reported by newly identified Eastern Indian Ocean Stock (I-stock) and Western South Pacific Ocean Stock (P-stock) (Zenitani and Kato, 2006). The age at sexual maturity estimated using transition phase in earplugs as an indicator of sexual maturity *(tmp)* of both stocks declined from the mid-1940s cohorts or the mid-1950s cohorts at around 10-12 years to the late 1960s cohorts or the early 1970s cohorts at around 7 years. However, *tmp* for both stocks and sexes remained constant at 7-8 years or slightly increased in the early 1970s cohorts onwards (Zenitani and Kato, 2006). They interpreted this trend as a reflection of nutritional conditions for Antarctic minke whales that supported the decline of *tmp* which then shifted to conditions that did not to support a further decline.

In recent years, increasing abundance in large baleen whale species such as blue, fin and humpback whales has been reported (Branch, 2011; Hakamada and Matsuoka, 2014; Matsuoka *et al*., 2011; Matsuoka and Hakamada, 2014). Furthermore, blubber thickness and stomach contents weight of Antarctic minke whales has been reported to have decreased during the JARPA/JARPA II period (Konishi *et al*., 2008; Konishi and Walløe, 2014a; 2014b), which implies that nutritional conditions for Antarctic minke whales might be deteriorating.

JARPA II started in 2005/06 and new data from six years of surveys were accumulated. In this paper, yearly trend of age at sexual maturity in Antarctic minke whales was examined over the long-term and for recent cohorts by sex and stocks.

MATERIALS AND METHODS

Biological data used

The present study used a total of 2,801 age and transition phase (TP: age at transition phase layer) data collected in 1987/88-2004/05 JARPA and 1,374 data collected in 2005/06-2010/11 JARPA II surveys in Areas IIIE, IV, V and VIW. Samples from Areas IIIE, IV and VW were assumed as I-stock and samples from Areas VE and VIW were assumed as P-stock (Pastene, 2006). It should be noted here that the stock structure hypothesis has been refined recently (e.g. Kitakado *et al.*, 2014) and that new grouping for estimating age at sexual maturity would be necessary in future.

Age determination

Age of whales was determined by reading growth layers appearing on the bisected surface of the earplug, assuming an annual deposition of growth layers (i.e. one pair of dark and pale laminae accumulated per year) in accordance with Best (1982) and Lockyer (1984). Individual ages from 1987/88-1989/90 and 1992/93 JARPA samples were mainly read by reader-K. Individual ages of 1990/91 and 1993/94-2004/05 JARPA samples were read by reader-Z. Individual ages of 2005/06-2010/11 JARPA II samples were read by new reader (reader-B). The age at transition layer was recorded if it was present.

Cohort (= year of birth)

Cohorts (Year classes) were defined as; Cohort = (starting year of research season) – (age at capture).

Terminology for age at sexual maturity

"*Tmp*" (Kato, 1987): Mean age at sexual maturity estimated using transition phase in earplugs as an indicator of sexual maturity.
"*Tmov*" (Kato, 1987): Mean age at the first ovulation which is defined as mean age of females with a corpus luteum and no corpus albicans in both ovaries.

Correction of truncation bias

Truncation bias was corrected by the method of Kato (1985). For JARPA samples, we only used TP data for animals older than the following ages in each cohort group which was same as Zenitani and Kato (2006); 36 years (1951-55 cohort group), 29 years (1956-60), 25 years (1961-65), 19 years (1966-70), 15 years (1971-75), 12 years (1976-80), 13 years (1981-85) and 13 years (1986-95). For JARPA II samples we used TP data older than the following ages in each cohort group; 12 years (1991-1995 cohort group), 12 years (1996-2000), no data was used from the 2001-2005 cohort group because age of this group was less than 10 years.

Statistical analysis

Linear regression analysis was applied to two periods. First, regression analysis was applied to *tmp* and 1970-1997 cohorts for male of I-stock, 1968-1997 cohorts for female of I-stock, 1971-1998 cohorts for male of P-stock, 1969-1998 cohorts for female of P-stock, following Zenitani and Kato (2006), to examine long term trend from 1970s. The null hypothesis was set that the slope = 0 (H_0) and we examined whether the null hypothesis can be rejected at 5% level. Regression analysis was also applied to 1992 and younger cohorts (new data provided by JARPAII) for both sex and stocks.

RESULTS

Evaluation of new reader

Age and *tmp* data of reader-K and reader-Z were thought to almost coincide (Zenitani and Kato 2006). We examined difference of *tmp* recorded by the new reader (reader-B) and the previous reader group (reader-K and reader-Z). Figure 1 shows *tmp* against cohort based on TP data read by the two reader groups by sex and stock. The temporal trends of the two reader groups were similar and *tmp* values almost coincide with each other.

Temporal trend of age at sexual maturity by transition phase

As described above, no differences of temporal trend in *tmp* were found between the two reader groups. We estimated *tmp* using all TP data. Figure 2 and Table 1 shows *tmp* against cohort based on TP data by sex and stocks.

In both sex and stocks, *tmp* declined from the 1940s cohorts to the early 1970s cohorts from 10-12 to 7-8 years old. From early 1970s onwards, the *tmp* remained stable or slightly fluctuated at 7-8 years old. *Tmp* of the 1990s cohorts, which were newly added in this study from JARPAII surveys, were 7-8 years and no distinct difference was observed from previous cohorts (Figure 2, Table 1).

Significant increasing trend was detected by linear regression analysis between *tmp* and cohorts for male of I-stock (p=0.044, 1970-1997 cohorts), female of I-stock (p=0.022, 1968-1997 cohorts), and female of P-stock (p=0.004, 1969-1998 cohorts) (Table 2). Furthermore, significant decreasing trend was detected between *tmp* and 1992 and younger cohorts in female of P-stock (p=0.042, Table 3).

DISCUSSION

In the previous study *tmp* was estimated until the 1991 cohort based on JARPA samples (Zenitani and Kato, 2006), and this study examined a further seven cohorts since then. *Tmp* in recent cohorts was in general stable, at about 7-8 years.

The present analysis confirmed a temporal trend of age at sexual maturity in I and P stock of Antarctic minke whales over the long term as suggested in previous studies. The results confirmed that the age at sexual maturity of both stocks declined from the mid 1940s cohorts at around 10-12 years old to the early

1970s cohorts at around 7-8 years old.

Statistical analyses for the cohorts after 1970's suggested slight but statistically significant increasing trend of the *tmp* for the I stock (both sexes) and P stock (female). Whether or not these trends respond to deteriorated nutritional conditions is not clear at this stage. If so, the level of current changes in response to deteriorated conditions is not the same as the decreasing trend level observed from the 1940's cohorts in response to improved nutritional conditions at that time. Zenitani and Kato (2006) reported slightly increasing trend of *tmp* from the 1970s to the early 1990s cohorts and suggested that nutritional condition for Antarctic minke whale might have negatively changed. Similar trend was also detected in the present study from the 1970s to the late 1990s cohorts. Supporting evidence for the deteriorated conditions hypothesis is the increasing abundance of other krill-eater baleen whale stocks (Branch, 2011; Hakamada and Matsuoka, 2014; Matsuoka *et al*., 2011; Matsuoka and Hakamada, 2014).

Regarding the statistical analysis of the cohorts after 1992 a significant decreasing trend was detected in P-stock for females. However *tmov*, which was considered to be free from the segregation and catch selectivity (Kato, 1987), did not show a decreasing trend in the 1990s cohorts for this stock, although the analysis involved a small sample size (Figure 3). This means that reliability of the decreasing trend detected in P-stock for females after 1992 was uncertain and further analysis and continuous monitoring is needed.

Sexual maturity of baleen whales depends on body size rather than age, and growth speed of body size is largely affected by nutritional condition of each individual (Kato, 1987; Lockyer, 1981: Ohsumi, 1986b). Therefore, age at sexual maturity can be a useful indicator for monitoring the status of Antarctic minke whale stocks. Continuous monitoring of age at sexual maturity is a valuable tool to detect possible changes in nutritional conditions for Antarctic minke whales.

The JARPAII Review Workshop made some recommendations regarding the analytical methods which will improve reliability of yearly trend in age at sexual maturity presented in this paper. For example the Workshop recommended:

(1) Use the approach of Thomson et al. (1999) to better account for truncation bias (which is considered in SC/F14/J8) as well as the fringe effect, which relates to the low proportion of animals with ages slightly higher than the age-at-maturity, an issue likely related to the need for contrast to detect a transition phase in an earplug;

The authors examined long time trend of mean age at sexual maturity derived from transition phase observations based on data sets where truncation effects were solved. While the resultant trends were mostly agreed with those given by Thomson *et al.* (1999) in the cohorts concerned, it was suggested that the analyses needed to take account of fringe effects (as in Thomson *et al*., 1999), as well as truncation effects and ageing errors. The authors consider the method of Thompson *et al.* (1999) is effective in reducing bias on *tmp* and consequently they will start to address this recommendation with high priority.

(2) Incorporate the effects of age reading errors presented in SC/F14/J11;

The authors agree that reading error is an important factor when combining age data from multiple readers. The authors will start discussion on methodology how to incorporate results of SC/F14/J11 into present analysis.

(3) Take into account the problems of the representativeness of samples in recent years due to disruption of the programme discussed under Item 12.1.2;

The transition phase had been formed in young age before the whale was caught, and no difference among survey areas is expected. Therefore, estimated *tmp* would not be affected by the representativeness of samples. This recommendation however will be addressed again when other high priority tasks have been completed

(4) Compare analyses of tm from the transition phase with those using corpora counts and, if available, histological studies of testes;

The authors agree with this recommendation. However they note that most of the JARPA/JARPAII ovary samples were lost after the 2011 tsunami. Presence/absence of corpora was determined on board the research base vessel so that information on female maturity is available for all animals. This recommendation can be addressed for JARPA samples only because corpora counting was made before the loss of samples, unlike the case of JARPAII samples, which were lost before the corpora counting could be made. Histological testis samples were collected from all males and comparison with *tmp* is possible when preparation of stained section and identification of sexual maturity status has been completed. However, priority for males is lower than for females because underestimation of age at 50% maturity due to segregation was reported for males (Kato, 1982; Bando *et al*., 2006).

(5) Compare the results of the revised study with those (re-analysed to the extent possible following Thomson et al., 1999) for fin and sei whales from the Antarctic to examine whether any observed trends are synchronous, which may provide information on whether there is a cross-specific (e.g. outside environmental) cause to the trends;

The authors agree that comparison with other whale stock is useful to understand inter-species relationship within Antarctic ecosystem. This recommendation will be addressed when application of Thomson *et al* (1999) method has been completed.

(6) Evaluate alternative models (e.g. where trends are shared across sex, stock or both);

The authors agree that model-based approach is useful to understand detail of yearly trend in *tmp*. Multiple regression analysis considering sex, stock, year class and other parameters as dependent variable, will be candidate for the model. The authors will start discussion on methodology in collaboration with statisticians.

(7) Include a more detailed description of the models evaluated and a more detailed discussion of the results that considers all plausible explanations for observed trends.

This recommendation will be considered once progress in the works under the other recommendation has been made.

Work plan and timetable for the work under each recommendation

Recommendation	Priority	Workplan and timetable
1	High	Start analysis within 2014
2	High	Start discussing methodology within 2014.
3	Medium	Start analysis when other high priority tasks are completed.
4	Medium	Methods have been already established. Start analysis within 2014.
5	High	Start analysis within 2014 (if application of Thomson *et al*. (1999) has been completed)
6	Medium	Start discussing methodology within 2014. The analysis will be conducted within a few years
7	Medium	To be addressed when the work under the recommendations above have progressed

Work progress will be reported to the Scientific Committee.

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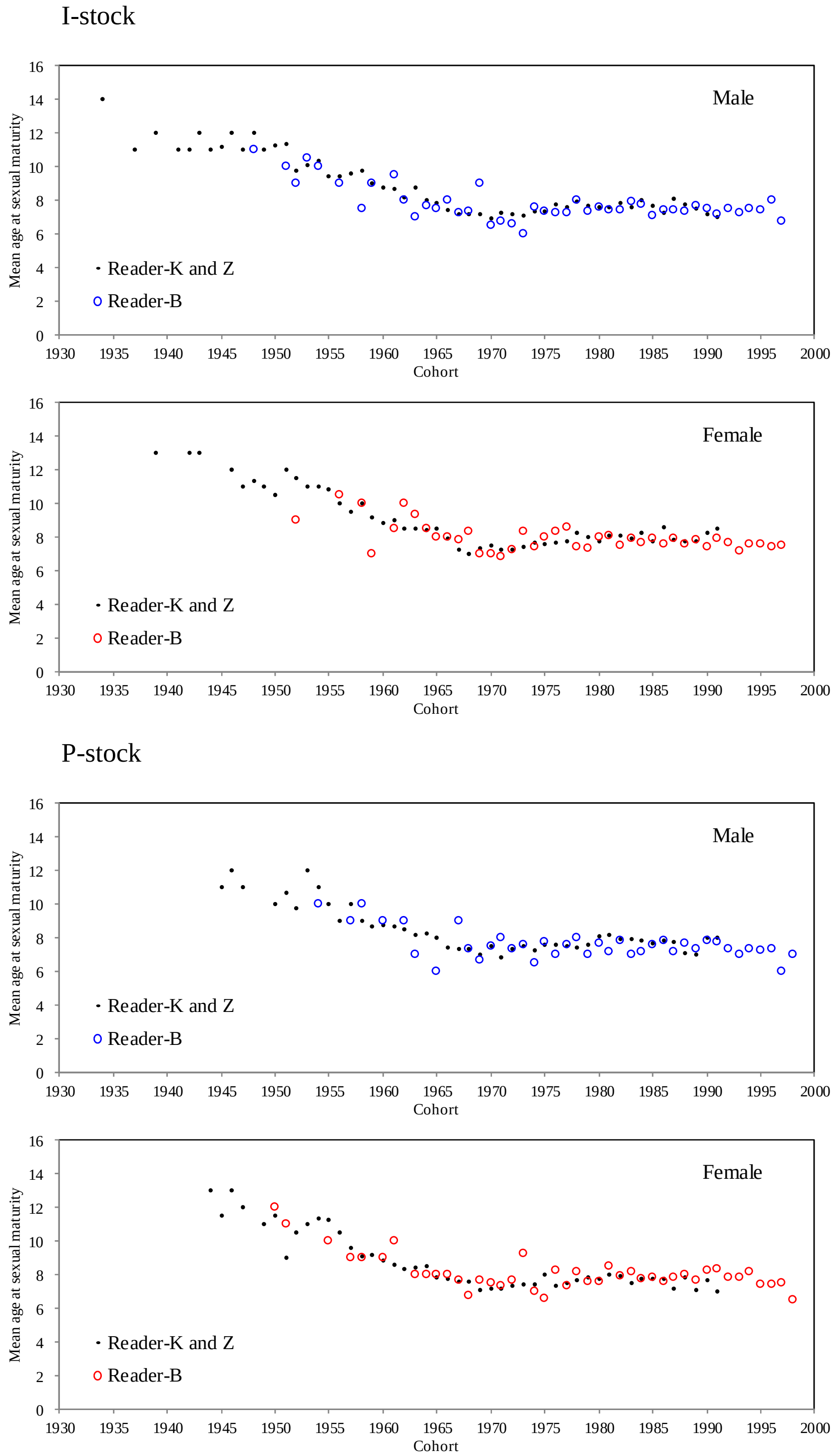


Figure 1. A plot of *tmp* against cohorts based on TP data of I and P stocks separated by two age reader groups. Reader-K and reader-Z read JARPA (1987/88-2004/05) samples and reader-B read JARPA II (2005/06-2010/11) samples.

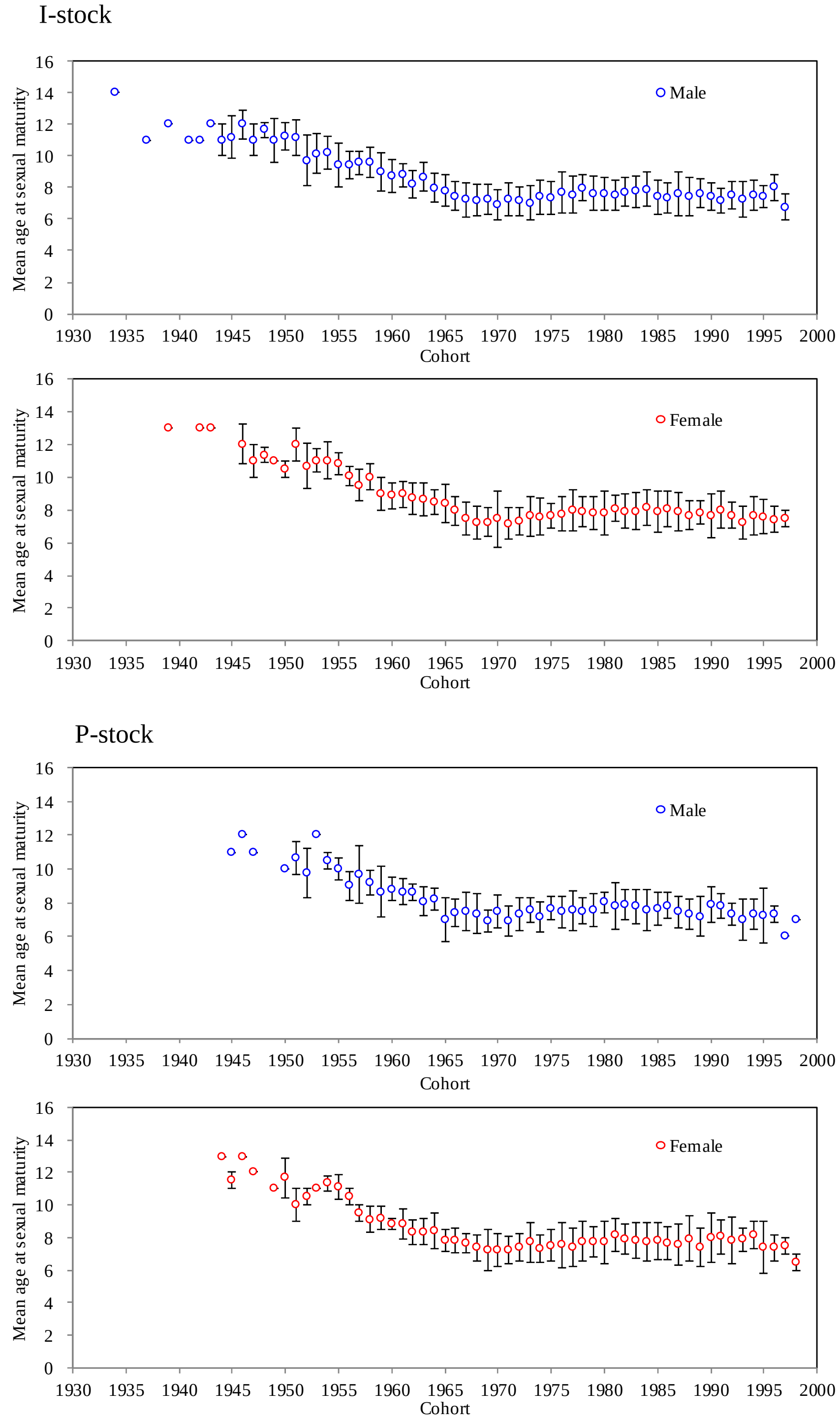
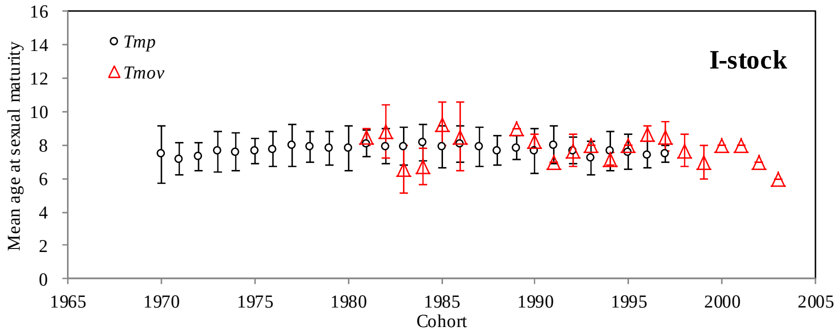


Figure 2. Temporal trend of *tmp* by cohort for each sex and stocks. Open circle is mean age and solid bar is range of standard deviation. All JARPA and JARPA II samples were combined.



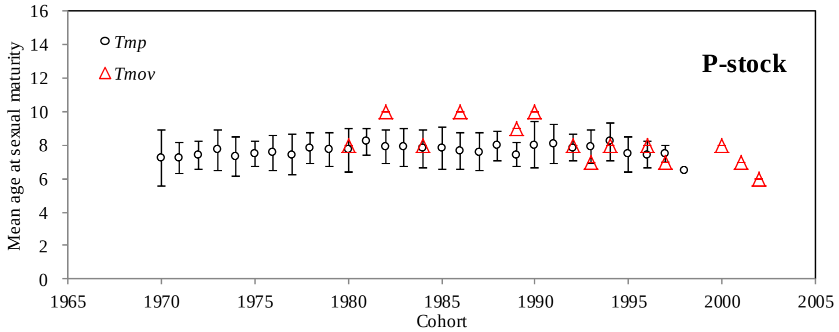


Figure 3. Comparison of two kinds of estimates of age at sexual maturity for females in each stock. *Tmp* is mean age at sexual maturity estimated using transition phase in earplug and *tmov* is mean age at first ovulation. Open circle and triangle shows mean age and solid bar shows range of standard deviation.

Table 1. Mean age at sexual maturity of Antarctic minke whales derived from TP. All JARPA and JARPA II samples were combined.

Cohort	I-stock						P-stock					
	Female			Male			Female			Male		
	mean	n	S.D.	mean	n	S.D.	mean	n	S.D.	mean	n	S.D.
1934				14.00	1	0.00						
1935												
1936												
1937				11.00	1	0.00						
1938												
1939	13.00	1	0.00	12.00	1	0.00						
1940												
1941				11.00	1	0.00						
1942	13.00	1	0.00	11.00	1	0.00						
1943	13.00	1	0.00	12.00	3	0.00						
1944				11.00	2	1.00	13.00	1	0.00			
1945				11.20	5	1.33	11.50	2	0.50	11.00	1	0.00
1946	12.00	4	1.22	12.00	5	0.89	13.00	1	0.00	12.00	1	0.00
1947	11.00	4	1.00	11.00	2	1.00	12.00	1	0.00	11.00	1	0.00
1948	11.33	3	0.47	11.67	3	0.47						
1949	11.00	1	0.00	11.00	3	1.41	11.00	1	0.00			
1950	10.50	2	0.50	11.25	4	0.83	11.67	3	1.25	10.00	1	0.00
1951	12.00	2	1.00	11.14	7	1.12	10.00	2	1.00	10.67	3	0.94
1952	10.67	6	1.37	9.70	10	1.62	10.50	2	0.50	9.75	4	1.48
1953	11.00	4	0.71	10.13	15	1.26	11.00	1	0.00	12.00	1	0.00
1954	11.00	8	1.12	10.22	9	1.03	11.33	3	0.47	10.50	2	0.50
1955	10.83	6	0.69	9.43	7	1.40	11.13	8	0.78	10.00	5	0.63
1956	10.06	16	0.56	9.42	12	0.86	10.50	4	0.50	9.00	3	0.82
1957	9.50	6	0.96	9.57	14	0.73	9.50	6	0.50	9.67	3	1.70
1958	10.00	12	0.82	9.60	25	0.94	9.13	8	0.78	9.20	5	0.75
1959	9.00	10	1.00	9.00	18	1.20	9.18	11	0.72	8.67	6	1.49
1960	8.88	16	0.78	8.77	22	1.04	8.86	7	0.35	8.83	6	0.69
1961	8.94	17	0.80	8.77	31	0.71	8.83	6	0.90	8.67	6	0.75
1962	8.68	22	0.92	8.17	23	0.87	8.33	6	0.75	8.60	5	0.49
1963	8.64	22	0.98	8.68	28	0.93	8.36	11	0.77	8.08	12	0.86
1964	8.44	18	0.76	7.96	25	0.92	8.40	10	1.11	8.25	8	0.66
1965	8.41	22	1.15	7.81	32	1.01	7.85	13	0.66	7.00	6	1.29
1966	7.95	20	0.86	7.47	47	0.92	7.80	15	0.75	7.43	14	0.82
1967	7.48	21	1.01	7.22	27	1.10	7.64	14	0.61	7.50	14	1.12
1968	7.22	18	0.97	7.19	37	0.98	7.40	20	0.80	7.36	11	1.15
1969	7.25	20	0.89	7.24	29	0.93	7.21	14	1.26	6.94	16	0.66
1970	7.44	16	1.69	6.91	33	0.93	7.22	18	1.03	7.50	16	1.00
1971	7.15	26	0.95	7.22	50	1.04	7.23	22	0.85	6.94	18	0.91
1972	7.29	28	0.84	7.13	48	0.95	7.41	29	0.81	7.35	17	0.97
1973	7.61	18	1.21	7.03	33	1.06	7.71	24	1.24	7.57	28	0.73
1974	7.59	22	1.15	7.41	37	1.08	7.32	22	0.87	7.17	24	0.90
1975	7.65	17	0.76	7.35	43	1.03	7.50	20	0.97	7.67	24	0.69
1976	7.75	20	1.04	7.72	36	1.30	7.55	22	1.37	7.48	23	0.93
1977	7.95	20	1.24	7.54	41	1.15	7.42	26	1.18	7.56	16	1.17
1978	7.86	22	0.92	7.97	35	0.81	7.77	30	1.20	7.54	13	0.75
1979	7.81	27	0.98	7.63	32	1.08	7.74	23	0.94	7.58	24	0.95
1980	7.81	27	1.31	7.62	39	1.03	7.71	17	1.27	8.05	19	0.60
1981	8.07	27	0.81	7.53	38	0.94	8.19	16	1.01	7.82	17	1.34
1982	7.93	40	1.01	7.70	43	0.90	7.92	24	0.91	7.92	25	0.89
1983	7.93	42	1.12	7.74	43	1.01	7.86	28	1.09	7.80	25	1.02
1984	8.11	27	1.10	7.89	37	1.06	7.77	26	1.19	7.60	20	1.20
1985	7.89	36	1.24	7.39	28	1.11	7.79	28	1.18	7.65	20	0.96
1986	8.08	25	1.09	7.32	37	0.96	7.66	29	0.99	7.86	14	0.74
1987	7.89	27	1.13	7.62	42	1.40	7.57	14	1.29	7.46	28	0.94
1988	7.67	36	0.88	7.44	27	1.20	7.95	20	1.40	7.35	17	0.90
1989	7.82	17	0.71	7.63	19	0.93	7.40	15	1.20	7.19	16	1.18
1990	7.63	16	1.36	7.42	24	0.86	8.00	14	1.51	7.88	8	1.05
1991	8.00	23	1.14	7.18	22	0.78	8.07	15	1.06	7.82	11	0.72
1992	7.67	18	0.82	7.52	23	0.88	7.84	19	1.42	7.33	9	0.67
1993	7.21	24	1.00	7.24	29	1.10	7.89	18	0.74	7.00	11	1.21
1994	7.61	18	1.16	7.50	14	0.98	8.18	11	0.83	7.36	14	0.89
1995	7.57	14	1.05	7.44	16	0.70	7.43	7	1.59	7.25	4	1.64
1996	7.40	5	0.80	8.00	3	0.82	7.40	5	0.80	7.38	8	0.48
1997	7.50	4	0.50	6.75	4	0.83	7.50	2	0.50	6.00	1	0.00
1998							6.50	4	0.50	7.00	1	0.00

Table 2. Results of linear regression analysis applied to cohort and age at sexual maturity by sexes and stocks. Regression analysis was applied to 1970-1997 cohorts for male of I-stock, 1968-1997 cohorts for female of I-stock, 1971-1998 cohorts for male of P-stock, 1969-1998 cohorts for female of P-stock, respectively.

Male: I-stock (1970-1997 cohorts)

	value	SE	t	p-value	
Intercept	-12.664	10.004	-1.266	0.206	
Coefficient	0.01016	0.005	2.012	0.044	*

Female: I-stock (1968-1997 cohorts)

	value	SE	t	p-value	
Intercept	-17.525	11.031	-1.589	0.113	
Coefficient	0.01274	0.006	2.288	0.022	*

Male: P-stock (1971-1998 cohorts)

	value	SE	t	p-value
Intercept	7.121	13.318	0.535	0.593
Coefficient	0.00021	0.007	0.031	0.975

Female: P-stock (1969-1998 cohorts)

	value	SE	t	p-value	
Intercept	-30.031	12.915	-2.325	0.020	*
Coefficient	0.01902	0.007	2.919	0.004	*

Table 3. Results of linear regression analysis applied to cohort and age at sexual maturity by sexes and stocks. Cohorts of 1992 and younger were used for analysis.

Male: I-stock

	value	SE	t	p-value
Intercept	65.916	151.163	0.436	0.664
Coefficient	-0.02936	0.076	-0.387	0.700

Female: I-stock

	value	SE	t	p-value
Intercept	0.384	159.752	0.002	0.998
Coefficient	0.00356	0.080	0.044	0.965

Male: P-stock

	value	SE	t	p-value
Intercept	35.714	192.894	0.185	0.854
Coefficient	-0.01429	0.097	-0.148	0.883

Female: P-stock

	value	SE	t	p-value	
Intercept	342.796	161.115	2.128	0.037	*
Coefficient	-0.16805	0.081	-2.080	0.042	*