Yearly trend in the proportion of pregnant animals among mature female Antarctic minke whales in the JARPA and JARPAII period

SC/65b/J9Rev

Takeharu Bando and Takashi Hakamada

Institute of Cetacean Research, 4-5 Toyomi-cho, Chuo-ku, Tokyo, 104-0055, Japan
Contact e-mail: bando@cetacean.jp

ABSTRACT
This paper is a revised version of SC/F14/J9 presented to the JARPAII Review Workshop. The discussion of the paper was modified to take into consideration some recommendations from the Review Workshop. The proportion of pregnant animals among mature females (PPF) in Antarctic minke whales was examined from samples collected during 1987/88-2004/05 JARPA and 2005/06-2010/11 JARPAII surveys. Analysis was conducted for two biological stocks which were separated at 165ºE. The PPF of both stocks appeared stable at around 0.9 and when data from all years were combined, the PPF was 0.932 for I-stock and 0.904 for P-stock. Linear regression analysis showed no significant yearly trend in either stock during the JARPAII period. From logistic regression analysis, no significant trend was detected during the JARPAII period but a significant increasing trend was detected for P-stock during the JARPA and all period. This latter result was influenced by just two lower PPF values in the 1990/91 and 1994/95 seasons. Although a high PPF was observed throughout the survey period, monitoring of PPF is important to detect possible change in nutritional condition and reproduction which affect sustainable management of these whale stocks.

KEYWORDS: ANTARCTIC MINKE WHALE, PREGNANCY RATE, TRENDS

INTRODUCTION

Pregnancy rate is necessary to estimate composition of young whales which recruit into the stock, which is an important parameter to evaluate reproductive condition of stock. Pregnancy rates of large baleen whales were known to change in response to changes in abundance, food availability or competition with other species (Gambell, 1973; Kato, 1986a; 1986b; Masaki, 1979; Lockyer, 1984). Therefore, monitoring of pregnancy rate is important for sustainable management of baleen whale stocks.

Pregnancy rate in the Antarctic minke whale had been studied mainly from commercial whaling data by several authors (Best, 1982; Kato, 1982; Masaki, 1979; Ohsumi *et al*., 1970; Ohsumi and Masaki, 1975). Ohsumi *et al*. (1970) reported the proportion of pregnant animals among mature females (PPF) from 1967/68 commercial whaling data as 0.789 (n=114), and this value becomes 0.895 if individuals that had corpus luteum but no fetus were considered as pregnant. Kato (1986a) examined apparent pregnancy rate from 1971/72 to 1982/83 commercial whaling data and reported no significant yearly trend during this period and a mean value of 0.895. The first phase of JARPA was conducted from 1987/88 to 2004/05 and data on pregnancy status were continuously collected during the survey period. Results were reported to the JARPA review meeting held at Tokyo in 2006, which showed no yearly trend for two newly identified stocks. The PPF was estimated as 0.929 and 0.854 for I-stock and P-stock, respectively (Bando *et al*., 2006).

JARPA II started in 2005/06 and new data from six years surveys were accumulated. In this study, yearly trend of PPF in Antarctic minke whale was examined for the hypothesized two stocks (I-stock and P-stock) for JARPA period, JARPA II period and both periods combined.

MATERIALS AND METHODS

Biological samples and data used

All of the mature female Antarctic minke whale samples collected during JARPA (1987/88 to 2004/05, n=2,045) and JARPAII (2005/06 to 2010/11, n=1,198) surveys were used for the analysis (Table 1).

Proportion of pregnant animals among the mature females (PPF)

The proportion of pregnant animals among the mature females is defined as the proportion of pregnant females within the sample of sexually mature females. Sexual maturity for females was determined by the presence of corpora luteum or albicans in both ovaries.

Stock identification

Whales collected in Areas IIIE, IV and VW were treated as the ‘Eastern Indian Ocean Stock’ (I-stock) and those collected in Areas VE and VIW were treated as the ‘Western South Pacific Stock’ (P-stock), following 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 PPF would be necessary in future.

Statistical method

Two statistical methods were applied to examine yearly trend of PPF. Firstly, linear regression analysis was applied to catch year of JARPAII (2005/06 to 2010/11) and PPF following Government of Japan (2005). The null hypothesis was set that the slope = 0 (H_0) to examine whether the null hypothesis can be rejected at 5% level. Secondly, logistic regression analysis was conducted to examine yearly trend of PPF during JARPA, JARPAII and during all period, respectively.

RESULTS

The PPF of I-stock whales was stable at around 0.9 throughout survey period and no significant yearly trend was detected during JARPAII by linear regression analysis (Figure 1, Tables 1, 2). No significant yearly trend was detected by logistic regression analysis during JARPA, JARPAII and all period (Table 3). When data from all years was combined, the PPF was calculated as 0.932.

For P-stock whales, the PPF was stable at around 0.9 but a slightly lower value was observed in 1990/91 (PPF=0.770) and 1994/95 (PPF=0.740) (Figure 1, Table 1). No significant yearly trend was detected during JARPAII by either linear or logistic regression analysis but a significant increasing trend was detected during the JARPA and all period by logistic regression analysis (Table 2, 3). When data from all years was combined, the PPF was calculated as 0.904.

DISCUSSION

Full scale exploitation of Antarctic minke whale started at 1971/72 and the reported apparent pregnancy rate was stable at a high value throughout the commercial whaling period (Kato, 1982; 1986a; Masaki, 1979; Ohsumi *et al.*, 1970; Ohsumi and Masaki, 1975; Zenitani, *et al.*, 2001). A high PPF was also observed during the JARPA and JARPAII periods in this study. A significant increasing trend was detected in P-stock but this is attributed to lower PPF observed in just two year in the early period of JARPA (1990/91 and 1994/95). Therefore this ‘significant’ increasing trend result should be seen with caution.

The observed high PPF during the commercial whaling period and the JARPA and JARPAII periods means that Antarctic minke whale stocks have maintained a good reproductive condition from the 1970s. Age at sexual maturity of minke whale was reported to have decreased from 10-12 in the 1940s cohorts to 7-8 in the 1970s cohorts and stabilized until the 1990s cohorts (Kato, 1983; Zenitani and Kato, 2006; Bando *et al.*, 2014). The decreasing trend from the 1940’s seems to be associated with improved nutritional conditions from the mid of the past century.

On the other hand, the increasing trend of abundance in large baleen whale species such as blue, humpback and fin whales have been reported in recent years (Branch, 2011; Hakamada and Matsuoka,, 2014; Matsuoka *et al.*, 2011; Matsuoka and Hakamada, 2014). Antarctic minke whales utilize krill as their food species and are thought to compete with other baleen whales. Therefore, an increase in the abundance of other whale species might have an adverse effect on the nutritional condition of Antarctic minke whales. Blubber thickness and stomach content weight of Antarctic minke whales have been reported to be decreasing during the JARPA/JARPAII period (Konishi *et al.*, 2008; Konishi and Walløe, 2014a; 2014b), which might indicate possible changes in the feeding environment of minke whales.

It is known that the PPF of Antarctic minke whale might be biased by segregation or date of sampling (Kato, 1986b; Ohsumi and Masaki, 1975). Whales that conceive earlier in the breeding season tend to migrate to Antarctic feeding areas earlier (Kato and Miyashita, 1991; Kato, 1995). Therefore, the observed PPF in this study might be biased upward from the true pregnancy rate, which was estimated as 0.78 from the commercial whaling data in the breeding area (Best, 1982). However, the PPF would reflect migration strategy, distribution pattern and feeding environment of Antarctic minke whales and could be an important parameter for monitoring of this stock.

Although continuation of the high PPF was observed during the JARPA II period, monitoring of the PPF is important to detect possible change in nutritional condition and reproduction which affect sustainable management of these whale stocks.

The JARPAII Review Workshop suggested the investigation of the limitations of the criteria used to identify a pregnant female:

> *(1) Further discussion of the limitations of the criteria used to identify a pregnant female − in particular, better account should be taken of the fact that small foetuses may be missed by scientists and that foetuses may be lost due to abortion during the hunt or loss during towing leading to an underestimate of true pregnancy rate;*
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> *(2) To assist with (1), the authors should consider examination of other criteria for pregnancy (in some cases this may be possible from existing data or tissue samples) including diameter of the largest uterine horn, histology of the uterine mucosa, examination of the mammary glands and examination of hormone levels in blubber.*

JARPAII researchers on board the research base ship examine ovaries and uterus of all females to identify sexual maturity and pregnancy status. Table 4 shows the number of females in each reproductive category collected during JARPAII surveys. The possibility that abortion could occur during hunt or towing cannot be denied. However, only six individuals among 1,701 females (0.35%) were classified as “Unknown mature” which means corpora lutea or albicantia exist in the ovary (only a part of ovary was collected in some case) but presence of fetus could not be confirmed due to damage by harpoon. There was no case in unbroken uterus that a part of umbilical code or placenta existed but no fetus was found, which could indicate abortion. These results suggests that the case of abortion or loss during towing is rare. ‘Unknown mature’ were excluded from the calculation of PPF.

Among 1,205 matured females collected during JARPAII, 19 individuals (1.6%) were classified as “ovulating”, which means corpora lutea exists in ovary but no fetus was found in uterus (Table 4). Although the proportion is small, among the ‘ovulating’ females there is the possibility of occurrence of fetus that was not detected, which leads to an underestimation of true pregnancy rate. The Review Workshop recommended the examination of four other criteria for identification of pregnancy. Examination of uterus would be useful to identify pregnancy status (Lockyer and Smellie, 1985). Diameter of both uterine horn were measured for all females during 1987/88 to 2004/05 JARPA survey and collection of uterine mucosa was conducted during the same periods. Therefore, a feasibility study to identify the usefulness of these data and samples for identification of pregnancy can start within this year. Examination of the mammary glands will be only applicable to late pregnancy because development of mammary gland will start at late stage of pregnancy (Laws, 1961; Lockyer, 1984). There is a possibility that early pregnant and ovulation without fertilization could not be differentiated from the examination of hormone in blubber (Mansour *et al*., 2002; Kellar *et al*., 2013).

Although recommendations (1) and (2) will increase the accuracy of PPF, the authors note that the degree of changes will be small (may be less than 1.6%) and therefore the conclusion of this paper (high PPF throughout JARPA and JARPA II periods) will not change.

The Review Workshop noted that ‘in terms of effects at the population level the ultimate parameters of interest are successful births and calf survival rather than pregnancy rates *per se*’. In this context the

Workshop provided some useful comments. For example it recommended that:

> *(3) The Panel recommends that the feasibility of using histological examination of mammary glands to assess recent lactation is examined. If this is possible, the proportions of recently lactated to [apparently] pregnant females and to mature females would be a more appropriate index of reproductive success that would likely be more sensitive to environmental fluctuations than crude pregnancy rates because perinatal mortality or abandonments of newborns by females in low nutritive condition is known to occur in many mammal species.*

The authors agree that the proportion of recently lactated to (apparently) pregnant females and to mature females would be more appropriate index of reproduction. Although Antarctic is feeding grounds for Antarctic minke whales, a trace of the secretion of milk might remain in the mammary gland (Chittleborough, 1958; van Utrecht, 1968). Measurement and collection of mammary gland was conducted for all females during 1987/88 to 2010/11 JARPA/JARPAII surveys. The authors will start a feasibility study to examine the possibility of recent lactation by observation of histological sample of mammary gland within 2014.

> *(4) Existing sampling protocols be examined to ensure that uterine horn and mammary gland measurements are recorded and mammary gland tissue and uterine mucosa are collected for histological analysis*

Data and samples of uterine and mammary gland collected during JARPA/JARPA II were as follows:

Measurement of uterine horn:	1987/88-2004/05	n=3,151
Collection of uterine mucosa:	1987/88-2004/05	n=3,151
Measurement of mammary gland:	1987/88-2010/11	n=4,852
Collection of mammary gland:	1987/88-2010/11	n=4,852

Data and samples were collected from all females irrespective of sexual maturity status. Measurement of uterine horn and collection of uterine mucosa was not conducted after 2005/06 but these research items will be resumed if the feasibility study showed effectiveness of these sample for identification of pregnancy.

> *(5) The programme takes the opportunity to examine the efficacy of approaches to examine pregnancy using blubber analyses and faecal samples found for some other mysticetes (see Mansour et al., 2002; and Kellar et al., 2013)*

The authors agree that these approaches could be useful in other species, although PPF could not be estimated by the blubber or faecal analysis because immature and resting (mature but not pregnant) could not be differentiated by the hormone level analysis (Mansour *et al*., 2002; Kjeld, *et al*., 2004; Rolland, *et al*., 2005; Kellar *et al*., 2013). In addition, the authors have the opinion that this approach is of limited practical utility for the case of the Antarctic minke whales because the collection of biopsies (for blubber samples) (particularly in offshore areas where JARPAII operates) and faeces in the field is not feasible (Ohsumi *et al*., 2007).

> *(6) Given the proponents' primary objective of studying biological parameters in order to monitor changes in the ecosystem, the Panel recommends that the relationship between reproductive parameters and the information available on body condition, krill consumption and environmental covariates such sea surface temperature (SST), location, and other factors be examined on an annual basis. This may help to explain anomalous years like the 1990-91 or the 1994-95 seasons, when low apparent pregnancy rate values were observed.*

The authors note that this recommendation is in the context of the integration which JARPAII researchers have identified as a priority for the second part of JARPAII, and the authors will consider implementing this recommendation with high priority.

ACKNOWLEDGEMENTS
Our sincere thanks are due to all researchers and crews who participated in the JARPA and JARPA II surveys.

We thank Hiroshi Hatanaka and Luis A. Pastene, of the Institute of Cetacean Research, for useful comment on this manuscript.

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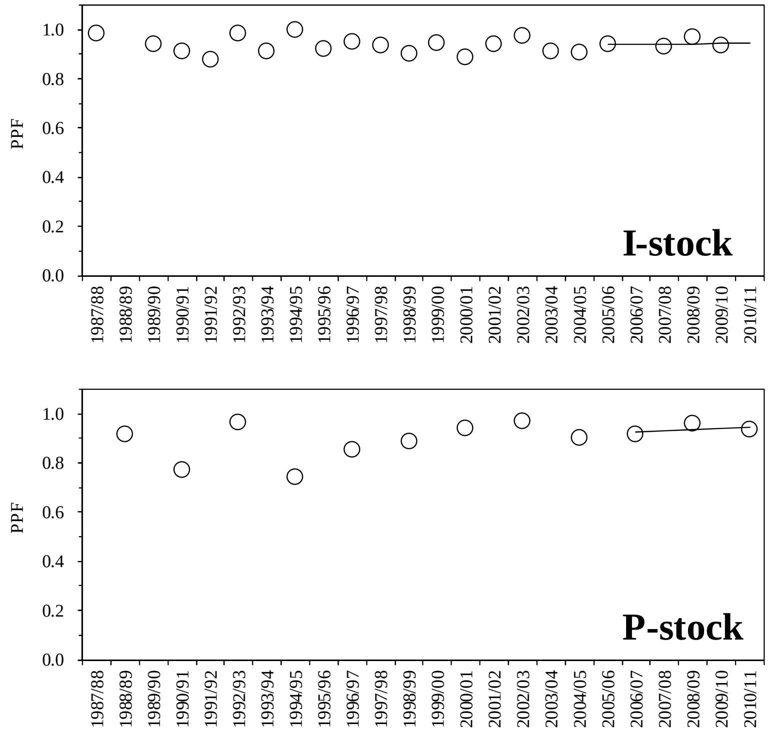

Figure 1. Yearly trend of PPF in I-stock and P-stock Antarctic minke whales collected during JARPA and JARPA II surveys. Linear regression line was applied for both stocks during JARPAII (2005/06 to 2010/11) periods.

Table 1. Number of pregnant, not pregnant matured females and PPF in I-stock and P-stock Antarctic minke whales.

Period	Season	I-stock				P-stock			
		Pregnant	Not pregnant	Number of matured female	PPF	Pregnant	Not pregnant	Number of matured female	PPF
JARPA	1987/88	57	1	58	0.983				
	1988/89					96	9	105	0.914
	1989/90	80	5	85	0.941				
	1990/91	51	5	56	0.911	57	17	74	0.770
	1991/92	72	10	82	0.878				
	1992/93	64	1	65	0.985	54	2	56	0.964
	1993/94	62	6	68	0.912				
	1994/95	14	0	14	1.000	54	19	73	0.740
	1995/96	96	8	104	0.923				
	1996/97	54	3	57	0.947	112	19	131	0.855
	1997/98	43	3	46	0.935				
	1998/99	56	6	62	0.903	16	2	18	0.889
	1999/00	106	6	112	0.946				
	2000/01	23	3	26	0.885	94	6	100	0.940
	2001/02	141	9	150	0.940				
	2002/03	35	1	36	0.972	92	3	95	0.968
	2003/04	154	15	169	0.911				
	2004/05	39	4	43	0.907	144	16	160	0.900
JARPA II	2005/06	226	15	241	0.938				
	2006/07					262	24	286	0.916
	2007/08	168	13	181	0.928				
	2008/09	30	1	31	0.968	162	7	169	0.959
	2009/10	184	13	197	0.934				
	2010/11					87	6	93	0.935
	Total	1755	128	1883	0.932	1230	130	1360	0.904

Table 2. Result of linear regression analysis for both stocks of Antarctic minke whales during JARPA II period.

I-stock

	value	SE	t	p-value
Intercept	0.916	0.162	5.647	0.030
Coefficient	0.00115	0.008	0.151	0.894

P-stock

	value	SE	t	p-value
Intercept	0.830	0.209	3.974	0.157
Coefficient	0.00485	0.009	0.512	0.699

Table 3. Result of logistic regression analysis for both stocks of Antarctic minke whales during JARPA, JARPA II and all period.

I-stock: JARPA

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	35.486	44.153	0.804	0.422
Year	-0.016	0.022	-0.745	0.456

P-stock: JARPA

	Estimate	Std. Error	z value	Pr(>|z|)	
Intercept	-89.238	39.380	-2.266	0.023	*
Year	0.046	0.020	2.317	0.021	*

I-stock: JARPA II

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	15.427	193.561	0.080	0.936
Year	-0.006	0.096	-0.066	0.947

P-stock: JARPA II

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-264.655	243.264	-1.088	0.277
Year	0.133	0.121	1.099	0.272

I-stock: JARPA+JARPA II

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	7.396	28.183	0.262	0.793
Year	-0.002	0.014	-0.170	0.865

P-stock: JARPA+JARPA II

	Estimate	Std. Error	z value	Pr(>|z|)	
Intercept	-98.810	25.960	-3.806	0.000	*
Year	0.051	0.013	3.891	0.000	*

Table 4. Number of female Antarctic minke whales classified by sexual maturity and pregnancy status. PPF was calculated as {(C)+(E)}/{(A)+(B)+(C)+(D)+(E)}

Year	Female							Total
	Immature	Ovulating	Resting	Pregnant	Lactating	Pregnant & Lactating	Unknown mature	
		(A)	(B)	(C)	(D)	(E)	(F)	
2005/06	149	4	11	224		3		391
2006/07	63	4	20	262			2	351
2007/08	96		12	164	1	4	1	278
2008/09	104	3	5	188		4		304
2009/10	70	7	6	174		10	2	269
2010/11	14	1	5	87			1	108
Total	496	19	59	1099	1	21	6	1701