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# Laboratory and analytical approaches to estimate biological parameters in the Antarctic minke whale and summary of results

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## ABSTRACT

Life history-related biological parameters in marine mammals such as age at sexual maturity and pregnancy rate are known to change in response to changes in abundance, food availability or competition among species. Therefore, monitoring of biological parameters and investigations of how these parameters change with time are important information for whale stock assessment and management. This paper presents an outline of the laboratory and analytical approaches used to estimate biological parameters in large whales and a summary of biological parameters estimated for the Antarctic minke whales based on JARPA and JARPAII biological samples.

## INTRODUCTION

Biological parameters such as age at sexual maturity and pregnancy rate are directly correlated to reproduction of whale stocks. Information on sex ratio and age at sexual maturity is necessary to assess the proportion of whales which contribute to reproduction; fetus sex ratio and litter size is information necessary to estimate the composition of young whales which recruit into the stock. Some of these parameters are known to change in response to changes in abundance, food availability or competition among whale species (Gambell, 1973; Kato, 1986a, 1986b, 1987; Kato and Sakuramoto, 1991; Masaki, 1979; Lockyer, 1972, 1979, 1984a). Therefore, monitoring of biological parameters is indispensable for assessment and sustainable management of whale stocks (Ohsumi *et al.* 1997).

The Antarctic minke whale *Balaenoptera bonaerensis* is one of the smallest Balaenopterid species with body length generally less than 10 m. Antarctic minke whales are widely distributed in the Southern Hemisphere, migrating seasonally between feeding grounds in the Antarctic in summer (south of 60°S) and breeding grounds in the tropical or temperate regions in winter (Perrin and Brownell, 2009). The Antarctic minke whale was exploited by commercial whaling in the decades of the 1970′s and 1980′s, until the moratorium on commercial whaling was implemented in the 1987/88 austral summer season. Given their large abundance, this species is a target of possible future commercial whaling operations in the Antarctic.

Previous estimates of biological parameters of Antarctic minke whale were based on samples collected during the commercial whaling period (Ohsumi *et al.*, 1970; Ohsumi and Masaki, 1975; Best, 1982; Kato, 1982, 1983, 1987; Masaki, 1979). However samples from commercial whaling are not representative of the stock as whaling operations targeted large individuals concentrated around the ice edge. Therefore biological parameters such as the age at sexual maturity estimated from those samples were thought to be biased (Kato, 1982, 1987).

The Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) was conducted between the seasons 1987/88 and 2004/05. The main objective of JARPA was the estimation of biological parameters to improve the management of Antarctic minke whales. Relevant samples and data to estimate several biological parameters were collected systematically throughout the 18-year research period. Under the JARPA, track lines were designed in each stratum established in the research area independently and random sampling was conducted to ensure representativeness of samples to accurately estimate biological parameters. Similar procedures were used in the second phase of JARPA (JARPAII).

The International Whaling Commission′s Scientific Committee (IWC SC) had recommended previously a recalculation of biological parameters of Antarctic minke whale by biological stock (IWC, 1998). Since then, stock structure analyses of Antarctic minke whale were refined, and a new stock structure hypothesis proposing an

'Eastern Indian Ocean Stock (I-stock)' (distributed mainly between 35°–130°E) and a 'Western South Pacific Stock (P-stock)' (distributed mainly between 165°E–145°W) in the Antarctic Ocean, was proposed. Pastene and Goto (2016) presented the latest genetic analyses in support of this hypothesis.

This paper presents an outline of the laboratory and analytical approaches to estimate biological parameters of large whales and a summary of biological parameters estimated for the Antarctic minke whales.

## MATERIALS AND METHODS

This section summarizes the sampling and analytical procedures used by different authors for estimating several biological parameters in the Antarctic minke whale.

### Age determination based on earplugs

One of the most important information obtained by JARPA and JARPAII was the age of the whales, which was obtained by examining the internal earplugs of whales.

*Sampling at the field*

The left and right earplugs were collected carefully by researchers at the field, which were immediately fixed in 10% formalin.

*Laboratory work*

At the laboratory, individual age was determined by counting growth layers appearing on the bisected surface of the earplug using a stereoscopic microscope, assuming an annual deposition of growth layers (i.e. one pair of dark and pale laminae accumulated per year) (Figure 1) (Lockyer, 1984b).

*Calibration work*

The inter-reader variability has been evaluated under suggestions and guidance from the IWC SC. After such evaluation, the estimated ages with this variability was incorporated into the Statistical Catch-At-Age (SCAA) analysis (IWC, 2012; Kitakado *et al.*, 2013; Punt *et al.*, 2014), which was conducted to estimate the historical and current population trajectories of the stocks as well the natural mortality rates by age classes in the Antarctic minke whale (see below).

The ages of whales with unreadable earplugs have been estimated by applying an age-length-key for the purposes of the SCAA. In more recent years the racemization technique was developed for the Antarctic minke whale (Yasunaga *et al.*, 2017) and therefore this technique can provide age estimation for unreadable earplugs, which in turn will improve the SCAA performance.

### Sexual maturity determination

*Sampling at the field*

Testes samples of about 1.5 cm square were collected from testes from the right side and immediately fixed in 10% formalin. Ovaries from both sides were collected and preserved at −20°C for later analyses.

*Laboratory work*

Sexual maturity of males was determined by examination of histological samples of testes stained by hematoxylin-eosin. Males with seminiferous tubules over 100 µm diameter, spermatid or open lumen in the tubules were determined as sexually mature (Figure 2) (Kato, 1986a; Kato *et al.*, 1990, 1991). Sexual maturity for females was


Figure 1. Earplug of Antarctic minke whale.

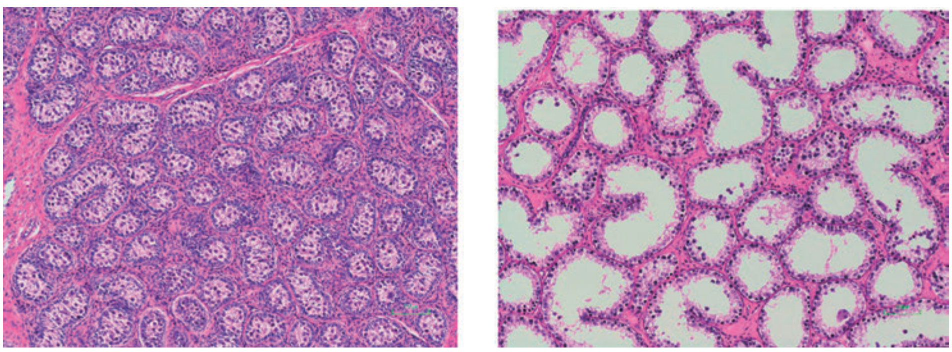

Figure 2. Histological testis sample of immature (left) and mature (right) Antarctic minke whales.

determined by the presence of corpora luteum (CL) or albicans (CA) in both ovaries (Lockyer, 1984a) (Figure 3).

**Physical maturity**

*Sampling at the field*

Physical maturity status was identified by examination of vertebrae. The fusion of the vertebral epiphysis to the centrum was known to start at the anterior cervical, then at the posterior caudal vertebrae, and is completed on the middle or posterior dorsal vertebrae (Kato, 1988). A part of the 6th dorsal vertebra which contains cartilage between centrum and anterior epiphysis was collected and fixed in the 10% formalin solution.

*Laboratory work*

Physical maturity was determined by examination of the vertebrae stained by 0.25% toluidine blue-O solution. Cartilage between epiphyses and centrum was observed by naked eye or stereoscopic microscope. Whales with the epiphyses fused to the centrum were defined as physically mature (Figure 4) (Kato, 1988).

**Age/length at sexual maturity**

Body length and age at sexual maturity was estimated by two methods. The first is the body length and age at first ovulation (*Lmov*, *tmov*) (Kato, 1987). Under this method the mean body length and age were calculated for the whales with one corpus luteum and no corpus albicans in both ovaries.

The second method is the body length and age at 50% sexual maturity (*Lm50%*, *tm50%*) (Kato, 1987). Under this method the body length and age at 50% maturity was estimated by logistic regression analysis based on maximum likelihood. The logistic model was fitted to age/body length and sexual maturity rate as:

$$Y=\left[1+e^{(aX+b)}\right]^{-1}$$

where $Y$ is the proportion mature at age/body length $X$, and $a$ and $b$ are empirical parameters. Age/body length at 50% maturity was calculated as $-ba^{-1}$.

**Age at physical maturity**

Age at 50% physical maturity was estimated by logistic regression analysis based on maximum likelihood. This is the same as the method for estimation of the age at 50%

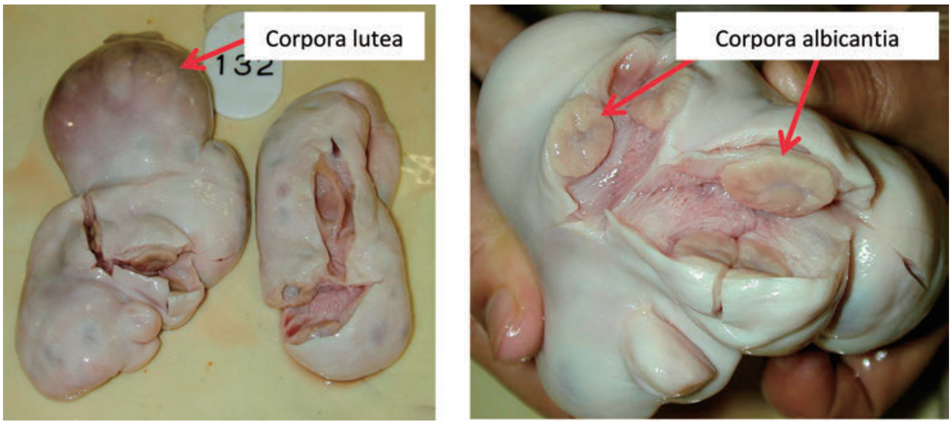


Figure 3. Ovary of Antarctic minke whales with corpora lutea (left) and corpora albicantia (right).

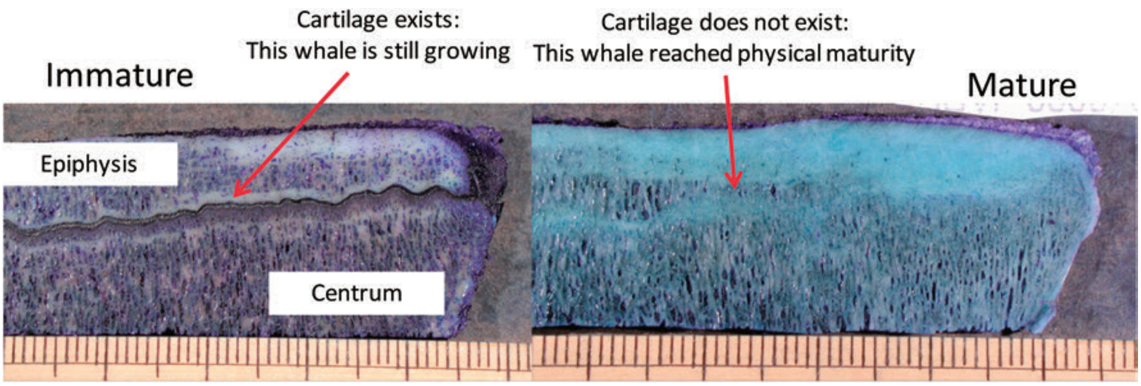


Figure 4. Vertebra of physically immature (left) and mature (right) Antarctic minke whale.

sexual maturity (*tm50%*).

**Growth curve**

The von Bertalanffy growth model was fitted to the body length and age as:

$$L_t = L_\infty\left(1-e^{-K(t-t_0)}\right)$$

where $L_t$ is the body length at age $t$, $L_\infty$ is asymptotic length, $K$ is the growth rate coefficient and $t_0$ is the theoretical time at zero length.

**Proportion of pregnant whales in matured females (PPF)**

PPF is defined as the proportion of pregnant females within the sexually matured females.

**Annual ovulation rate**

Annual ovulation rate was estimated by applying linear regression analysis between age and total number of corpora (CL and CA). Only individuals after the 1970 cohort were used because of the reported decline of age at sexual maturity (determined from transition phase in ear plug) from the 1940s to the 1960s cohorts (Kato and Sakuramoto, 1991; Thompson *et al.*, 1999). The regression line was fitted to age 10 and 30 because almost all animals are mature at the age of 10 and sample size was not large enough for ages over 30.

**Fetal sex ratio and litter size**

Presence or absence of fetus was identified by cutting both sides of the uterus horn. Fetus sex was identified by the appearance of the genital organ. Small fetuses of which sex could not be identified were excluded from the analysis.

**Natural mortality via SCAA**

Natural mortality was estimated by the Statistical Catch-at-Age (SCAA) model (Punt *et al.*, 2014). Individual age, abundance and catch history information were incorporated in the SCAA model and age dependent natural mortality was estimated (Punt *et al.*, 2014).

## SUMMARY OF RESULTS

Biological parameters of Antarctic minke whales on stock basis (Pastene and Goto, 2016) are presented in Table 1. Both stocks presented similar values for age at sexual and physical maturity. In both stocks the values were smaller in males than females.

Remarkable differences between stocks were found in body length at sexual maturity (*Lmov*, *Lm50%*) and in the asymptotic length calculated from growth curves, which was about 20 cm larger in I-stock than P-stock for both sexes. The PPF of both stocks was as high as about 0.9 and annual ovulation rate supported the high pregnancy rate in both stocks. The fetus sex ratio is almost parity for both stocks and multiplets are rare in this species.

Age dependent natural mortality was estimated from SCAA. The pattern was similar between the two stocks with natural mortality being higher in young and old animals. It was calculated as 0.048 (for age = 15) to 0.107 (for age = 35) for the I-stock and 0.046 (for age = 15) to 0.103

Table 1

Summary of biological parameters of Antarctic minke whales estimated from 1987/88 to 2004/05 JARPA samples.

| | | I-stock (35°E–130°E) | | P-stock (165°E–145°W) | |
|---|---|---|---|---|---|
| | | Male | Female | Male | Female |
| ***Growth*** | | | | | |
| Growth curve | | $L_t$=8.60(1−e$^{-0.272(t+1.97)}$) | $L_t$=9.16(1−e$^{-0.228(t+2.19)}$) | $L_t$=8.44(1−e$^{-0.269(t+2.08)}$) | $L_t$=8.97(1−e$^{-0.203(t+2.90)}$) |
| Age at physical maturity | 50%mature | 16.9 | 20.8 | 16.9 | 20.6 |
| ***Sexual maturity*** | | | | | |
| Age at sexual maturity | *tmov* | | 7.6 | | 8.5 |
| | *tm50%* | 5.3 | 7.6 | 5.4 | 8.1 |
| Body length at sexual maturity (m) | *Lmov* | | 8.39 m | | 8.32 m |
| | *Lm50%* | 7.28 m | 8.20 m | 7.14 m | 7.99 m |
| ***Reproductive characteristics*** | | | | | |
| Proportion of pregnant in matured female (%) | | | 92.1% | | 87.7% |
| Annual ovulation rate | | | 0.989/year | | 1.005/year |
| Foetal sex ratio | | 52.1% | 47.9% | 46.8% | 53.2% |
| Litter size | | | 1.002 | | 1.013 |

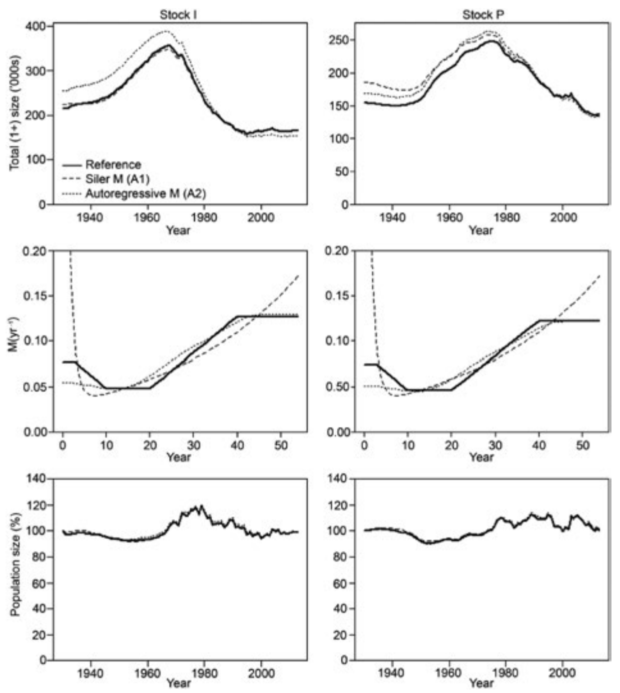


Figure 5. Time-trajectories of total (1+) population size (upper panels), age-specific natural mortality (center panels), and total (1+) population size relative to carrying capacity (lower panels) for three ways to model natural mortality (the Siler model, autoregressive and piecewise linear) of two stocks of Antarctic minke whale (from Punt *et al.*, 2014). Biological data obtained from both JARPA and JARPAII were used in the SCAA.

(for age=35) for P-stock (Figure 5) (Punt *et al.*, 2014). Time-trajectories derived from SCAA showed that total population size of Antarctic minke whale increased until 1970's and then declined until 2000's for both stocks (Figure 5) (Punt *et al.*, 2014).

## DISCUSSION

At present examination of earplugs is the only practicable means to obtain age data at the annual scale. The earplug has proved to be a valid and useful tool for age determination (Lockyer, 1984b), and is the only method providing age data accurate enough for population-level analyses such as the SCAA of Antarctic minke whales. Because there are a number of whales sampled with unreadable earplugs, the feasibility of other ageing techniques is being investigated by ICR scientists such as that based on enantiomers of aspartic acid in eye lens, which are measured using high performance liquid chromatography (Yasunaga *et al.*, 2017) and the DNA methylation approach which was applied to humpback whale (Polanowski *et al.*, 2014). Potentially these techniques could complement the age information obtained from earplugs.

Age and other reproductive data obtained by JARPA and JARPAII enabled the estimation of important biological parameters in the Antarctic minke whale, which are significant for assessment and management of this species in the Antarctic. Of particular importance was the estimate of age-specific natural mortality rate (Punt *et al.*, 2014) in this species, which was one of the main research objectives of JARPA.

Another important aspect was that the estimation of biological parameters was made on the basis of biological stocks and not on the basis of politically-based geographical boundaries. It was found that some biological parameters differed between stocks.

Some of the biological parameters are known to change in response to changes in abundance, food availability or competition with other species (Gambell, 1973; Lockyer, 1979; Kato, 1986b, 1987). Monitoring of biological parameters is useful for understanding of the present status of stocks and to predict future trends, which are essential for sustainable management of whale stocks. Moreover, information on biological parameters is useful to improve the performance of the Revised Management Procedure (RMP).


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