

*Technical Report (not peer reviewed)*

## Earplug-based age determination and estimation of biological parameters in North Pacific Bryde's whales based on samples collected by JARPNII

Takeharu BANDO\*

*Institute of Cetacean Research, Toyomi-cho 4–5, Chuo-ku, Tokyo 104–0055, Japan*

\*Contact e-mail: bando@cetacean.jp

### ABSTRACT

This study summarizes the results of earplug-based age determination and estimation of biological parameters in the western North Pacific Bryde's whales sampled by the JARPNII surveys between 2000 and 2016. A total of 730 whales (314 males and 416 females) was sampled in the western North Pacific north of 35°N and between the Pacific coast of Japan and 170°E. Age information was obtained from earplugs of 476 (65.2%) whales. Earplug readability was higher for mature (76.4%) than immature (43.8%) whales and no difference between sexes was observed. The growth curves for males and females were  $L_t = 12.63(1 - e^{-0.208(t+4.266)})$ , and  $L_t = 13.28(1 - e^{-0.172(t+4.834)})$ , respectively. The age at sexual maturity for males and females were 10.24 and 8.56 years, respectively. The annual ovulation rate was estimated as 0.526/year. It is concluded that substantial biological information was obtained for the North Pacific Bryde's whales during the 17 years of JARPNII. In particular the readability of earplugs for age determination increased notably compared to that in the period of commercial whaling. The analyses of biological data will contribute to the management of this whale species in the North Pacific, for example by incorporating such data into the conditioning process of the RMP Implementation Simulation Trials.

### INTRODUCTION

Bryde's whales are widely distributed throughout the world, especially in waters with temperature of 20°C or more (Kato and Perrin, 2017). Two types of Bryde's whales are distributed around Japan, one in the south-western part of Japan (coastal type) and the other in offshore waters in the Pacific side of Japan (offshore type) (Kishiro, 1996; Yoshida and Kato, 1999).

Some authors (*e.g.*, Wada *et al.*, 2003) recognize these Bryde's whale types as two separate species, the smaller coastal one *Balaenoptera edeni* Anderson, 1879 (Eden's whale) and the larger offshore one *B. brydei* Olsen, 1913 (Bryde's whale). Other authors (*e.g.*, Kershaw *et al.* 2013) assign these species a sub-specific status: *B. edeni edeni* and *B. edeni brydei*, respectively. This study focused on the larger, offshore type Bryde's whale (Figure 1).

JARPNII started in 2000 with three research objectives, i) feeding ecology and ecosystem studies, ii) monitoring of environmental pollutants in cetaceans and marine ecosystems and iii) elucidation of stock structure (GOJ, 2000; 2002). Bryde's whale (offshore type) was selected as one of the target species for sampling. The estima-



Figure 1. North Pacific Bryde's whale (offshore type). Note the three distinct ridges in the head, which are characteristic of this species.

tion of biological parameters of whales was not included among the main objectives of JARPNII, however biological samples for such estimates were collected systematically during the surveys. The JARPNII was completed in 2016, and a total of 730 Bryde's whales were sampled during the 17 years survey period.

Age data is one of the most important information for

stock assessment and management of large whales. Age estimation based on earplugs is considered the most reliable tool for age determination in whales (Lockyer, 1984; Maeda *et al.*, 2016). Earplug readability of North Pacific Bryde's whales was reported as 17.4% from commercial whaling samples collected in the pelagic whaling ground of the North Pacific from 1971 to 1974 (Ohsumi, 1977). During the JARPNII, earplugs were collected carefully from each whale sampled, and efforts were made to increase the readability.

This study summarized the results of age determination in North Pacific Bryde's whales based on earplugs and some biological parameters based on age data. The analyses were based on Bryde's whale samples collected by JARPNII in the period 2000–2016 in the western North Pacific.

## MATERIALS AND METHODS

### Whale sampling

A total of 730 (314 males and 416 females) Bryde's whales were sampled by JARPNII during 2000–2016. The geographical distribution of the sampled whales is shown in Figure 2. Whales were sampled between the Japanese coast and 170°E, and between 35°N and approximately 42°N. Two biological stocks of Bryde's whales (offshore type) have been suggested for the western North Pacific, one distributed between the Japanese coast and approximately 165°E and the other, east of 180°, with a transition area between 165°E and 180° (IWC, 2017). The analyses in this study are mainly focused on the former stock.

Table 1 shows the number of sampled whales by year and sexual maturity status.

### Biological data

Body length was measured in a straight line from the tip of snout to the notch of flukes using stainless steel measuring tapes. Sexual maturity of females was preliminarily determined in the field by the presence or absence of corpora lutea/albicantia in ovaries from both sides and confirmed later by counting corpora number at the laboratory. If there was at least one corpora lutea or albicantia in the ovaries, the female was determined as sexually mature. Maturity of males was determined by testis weight. Testis weight (heavier side) of more than 560g was regarded as sexually mature (Bando unpublished data).

### Earplug sampling and age determination procedure

Earplugs were collected from all sampled animals, following the method developed for baleen whales (Omura, 1963; Maeda *et al.*, 2016). The left and right earplugs

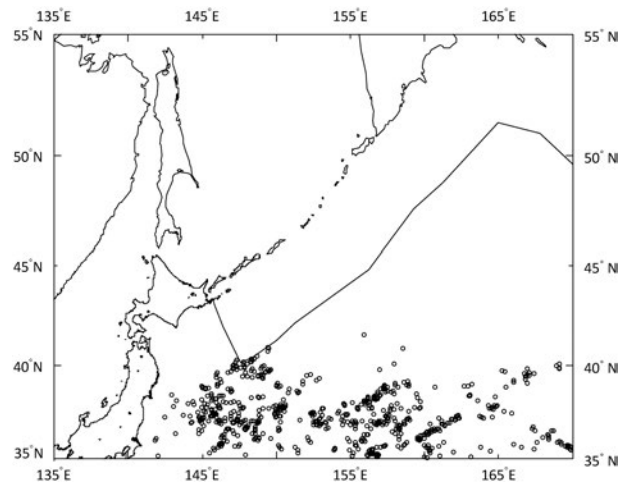


Figure 2. Research area of JARPNII and sighting positions of Bryde's whales sampled during the 2000–2016 surveys.

Table 1

Sex and sexual maturity status of Bryde's whales sampled during 2000 to 2016 JARPNII.

|       | Male     |        |       | Female   |        |       | Total |
|-------|----------|--------|-------|----------|--------|-------|-------|
|       | Immature | Mature | Total | Immature | Mature | Total |       |
| 2000  | 9        | 12     | 21    | 6        | 16     | 22    | 43    |
| 2001  | 13       | 4      | 17    | 12       | 21     | 33    | 50    |
| 2002  | 18       | 7      | 25    | 11       | 14     | 25    | 50    |
| 2003  | 10       | 9      | 19    | 9        | 22     | 31    | 50    |
| 2004  | 5        | 14     | 19    | 7        | 24     | 31    | 50    |
| 2005  | 10       | 11     | 21    | 13       | 16     | 29    | 50    |
| 2006  | 8        | 13     | 21    | 8        | 21     | 29    | 50    |
| 2007  | 13       | 10     | 23    | 5        | 22     | 27    | 50    |
| 2008  | 6        | 24     | 30    | 4        | 16     | 20    | 50    |
| 2009  | 9        | 9      | 18    | 14       | 18     | 32    | 50    |
| 2010  | 5        | 20     | 25    | 4        | 21     | 25    | 50    |
| 2011  | 10       | 10     | 20    | 11       | 19     | 30    | 50    |
| 2012  | 6        | 5      | 11    | 4        | 19     | 23    | 34    |
| 2013  | 3        | 10     | 13    | 3        | 12     | 15    | 28    |
| 2014  | 2        | 4      | 6     | 5        | 14     | 19    | 25    |
| 2015  | 3        | 11     | 14    | 2        | 9      | 11    | 25    |
| 2016  | 1        | 10     | 11    | 2        | 12     | 14    | 25    |
| Total | 131      | 183    | 314   | 120      | 296    | 416   | 730   |

were collected carefully, and immediately fixed in 10% formalin.

In the laboratory, the flat along the central axis of the earplug was cut using a sharp blade, then it was placed on a wet stone to expose the neonatal line and growth layers (Figure 3). Growth layers were counted under water using a stereoscopic microscope. A growth layer group (GLG) was defined as one pair of light and dark laminae in the core and considered as one year of age. Age reading was conducted in the following manner: i) earplug of the

left side was read. If the growth layers were ambiguous, earplug from the right side was also read. Reading from the less ambiguous side was adopted; ii) age reading was conducted only once without any knowledge of biological information such as body length or sex; iii) when the reading of all sample was completed, age data was compared with biological data such as body length or sexual maturity status, and some samples were re-read to check outlier, incomplete sample or invalid reading. All earplugs were read by a single reader (TB).

**Estimation of biological parameters**

*Growth curve*

To estimate growth curve, the von Bertalanffy growth model was fitted to the body length and age as:

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

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.

*Age at sexual maturity*

Age at sexual maturity ( $tm$ ) was estimated by the following equation (Cooke, 1984):

$$tm = l - 0.5 + \sum_l^k \left( \frac{I_a}{N_a} \right)$$

$$\text{var}(tm) = \sum_l^k \frac{M_a I_a}{N_a^2 (N_a - 1)}$$

where

- $M_a$  is number of mature animals in age  $a$
- $I_a$  is number of immature animals in age  $a$
- $N_a$  is total number of animals in age  $a$
- $l$  is age of youngest mature animal in the sample
- $k$  is age of oldest immature animal in the sample.

*Ovulation rate*

Annual ovulation rate was estimated by applying linear regression analysis between age and total number of corpora (corpora lutea and albicantia). The regression line was fitted to age 10 and older because almost all animals mature at the age of 10.

**RESULTS AND DISCUSSIONS**

**Age readability**

The readability of earplugs varied depending on the maturity status. Readability of sexually immature individuals was 46.6% for males and 40.8% for females (Table 2). Readability of earplugs in sexually mature animal was higher, 76.5% and 76.4% for males and females, respectively (Table 2). Readability of all samples was 65.2%, which was higher than that of western North Pacific common minke whales (44.1%) (Maeda *et al.*, 2016), and

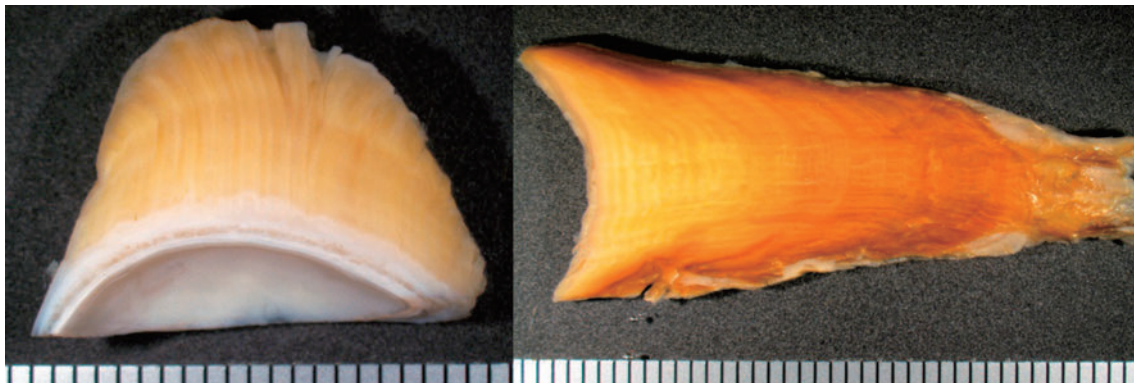


Figure 3. Bisected earplug surfaces of Bryde's whales (left: 5 GLGs, right: 20 GLGs). The scale shows 1 mm interval.

Table 2  
Earplug-age readability of Bryde's whales by sex and sexual maturity status.

|          | Male             |                   |                 | Female           |                   |                 | Total            |                   |                 |
|----------|------------------|-------------------|-----------------|------------------|-------------------|-----------------|------------------|-------------------|-----------------|
|          | Number of whales | Readable earplugs | Readability (%) | Number of whales | Readable earplugs | Readability (%) | Number of whales | Readable earplugs | Readability (%) |
| Immature | 131              | 61                | 46.6            | 120              | 49                | 40.8            | 251              | 110               | 43.8            |
| Mature   | 183              | 140               | 76.5            | 296              | 226               | 76.4            | 479              | 366               | 76.4            |
| Total    | 314              | 201               | 64.0            | 416              | 275               | 66.1            | 730              | 476               | 65.2            |

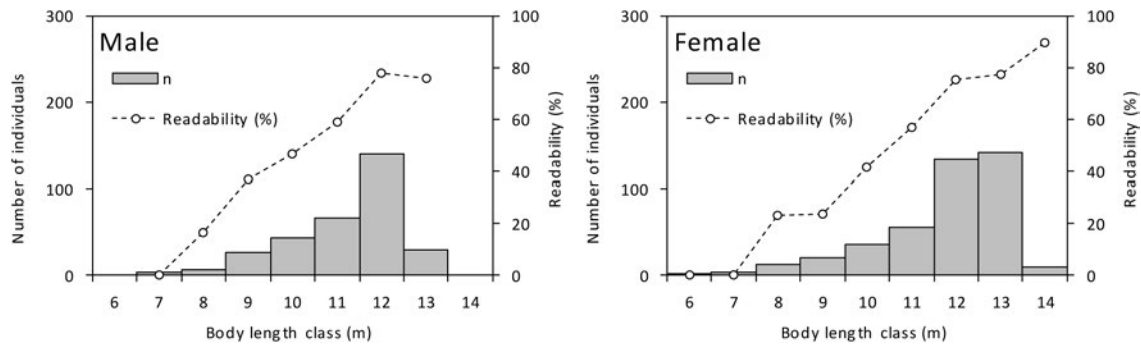


Figure 4. Earplug age readability of Bryde's whales by body length class and sex.

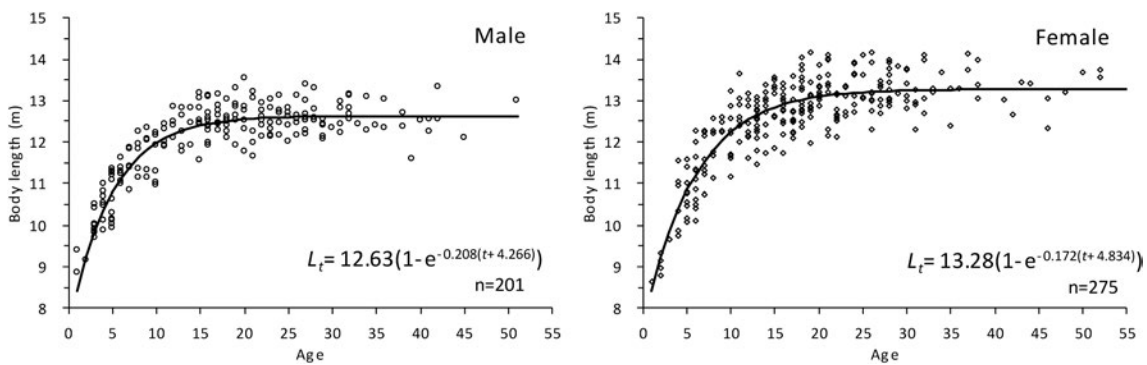


Figure 5. Relationship between body length and age of Bryde's whales. The solid line shows the von Bertalanffy growth curve.

was comparable to that of sei whales (63.0%) (Bando *et al.*, 2016), which were estimated based on samples collected by JARPN/JARPNII.

Readability increased with body length for both sexes (Figure 4). An inter-reader calibration experiment, following the method of Kitakado *et al.* (2013), will be conducted in the near future.

### Growth curve

For both sex, the growth rate was high at younger ages and stabilized after 20 years old (Figure 5). The following von Bertalanffy growth curves were estimated:

$$\text{Male: } L_t = 12.63(1 - e^{-0.208(t+4.266)})$$

$$\text{Female: } L_t = 13.28(1 - e^{-0.172(t+4.834)})$$

Ohsumi (1977) estimated growth curves of Bryde's whales based on samples collected by the commercial whaling in the North Pacific, and estimated maximum length as 12.8 m (42 ft) and 13.4 m (43.8 ft) for males and females, respectively. Similar values were obtained for Bryde's whales sampled by JARPNII.

### Age at sexual maturity

Sexually mature males first appeared at the age of 8, and from 16 years old all animals were sexually mature (Figure 6). Age at sexual maturity (*tm*) for males was esti-

mated as 10.24 years (SE=0.60).

Sexually mature females first appeared at the age of 7, and from 11 years old all animals were sexually mature (Figure 6). *Tm* was estimated as 8.56 years (SE=0.39).

Ohsumi (1977) reported age at 50% sexual maturity of Bryde's whales collected by the commercial whaling in the North Pacific in the 1970's as 10 and 8 years for males and females, respectively. Consideration should be given to the possibility of bias arising from legal size limit and the selectivity of large animals during commercial whaling. However similar estimates were obtained for Bryde's whales collected during past commercial whaling and JARPNII.

### Annual ovulation rate

The corpora lutea/albicantia first appeared at the age of 7 and the number of corpora increased linearly after the age of 10 (Figure 7). Annual ovulation rate was calculated as 0.526. The estimated annual ovulation rate means that the majority of Bryde's whale in the North Pacific give birth (or ovulation occurs) every two years. The estimated value was higher than the 0.455 estimated from the 1970's commercial whaling (Ohsumi, 1977).

### CONCLUDING REMARKS

It is concluded that substantial biological information was



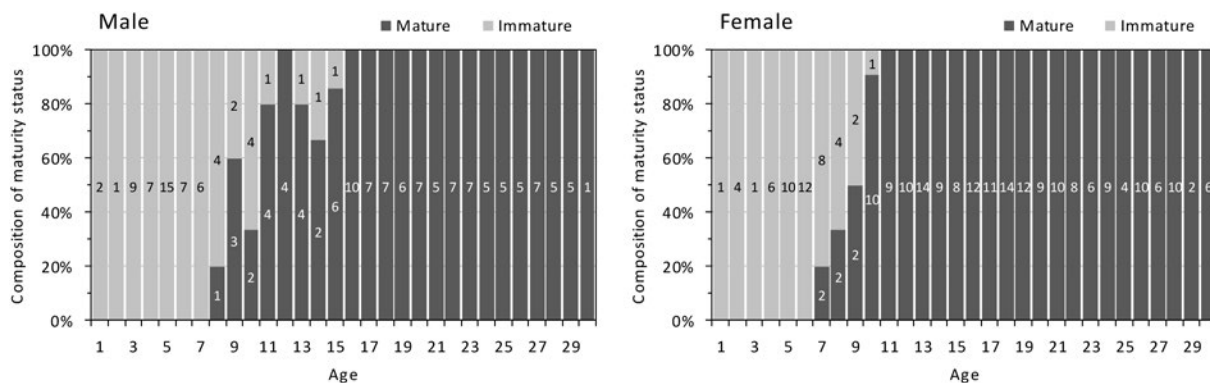


Figure 6. Sexual maturity status by age and sex in the Bryde's whales. Numbers in the bar shows the number of samples examined.

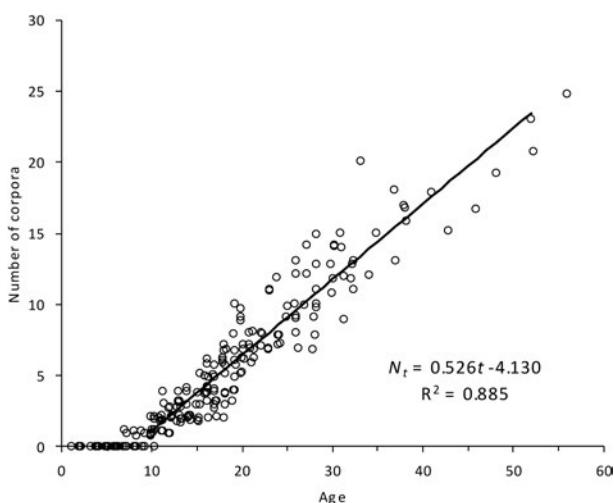


Figure 7. Relationship between age and number of corpora in Bryde's whales. Linear regression line was fitted to ages 10 and more.

obtained for the North Pacific Bryde's whales during the 17 years of JARPNII. In particular the readability of earplugs for age determination increased notably compared to that in the period of commercial whaling. The analyses of biological data will contribute to the management of this whale species in the North Pacific for example by incorporating such data into the conditioning process of the RMP *Implementation Simulation Trials* (Bando and Kato, 2017).

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