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## Progress in the research on earplug-based age determination and biological parameters of North Pacific sei whales at the Institute of Cetacean Research

Takeharu BANDO<sup>1,\*</sup> and Hikari MAEDA<sup>2</sup>

<sup>1</sup>Institute of Cetacean Research, 4–5 Toyomi-cho, Chuo-ku, Tokyo 104–0055, Japan

<sup>2</sup>Japan Fisheries Research and Education Agency, 2–12–4 Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa 236–8648, Japan

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

### ABSTRACT

This study summarizes the progress in the research on earplug-based age determination and estimation of biological parameters of the western North Pacific sei whales sampled by the JARPNII/NEWREP-NP surveys between 2002 and 2018. A total of 1,622 whales (744 males and 878 females) were sampled in the western North Pacific north of 35°N and between the Pacific coast of Japan and 170°E. Earplugs were read for whales collected up to 2016 ( $n=1,354$ ). Age information was obtained for 887 whales (65.5%). Earplug readability was higher for mature (69.7%) than immature (52.1%) whales, and no difference between sexes was observed. The growth curves for males and females were  $L_t=14.14 (1-e^{-0.174(t+6.650)})$  and  $L_t=15.17 (1-e^{-0.150(t+7.407)})$ , respectively. The age at sexual maturity for males and females were 6.7 (SE=0.29) and 6.9 (SE=0.27) years, respectively. The body length at sexual maturity was estimated as 12.72 m (SE=0.12) for males and 13.31 m (SE=0.12) for females. The annual ovulation rate was estimated as 0.744/year. Substantial biological information was obtained for the North Pacific sei whale during the 17 year of JARPNII and NEWREP-NP. The analyses of biological data will contribute to the management of this whale species in the North Pacific.

### INTRODUCTION

Sei whale (*Balaenoptera borealis*) is widely distributed throughout the world. As with other baleen whale species, they undertake seasonal migration spending the summer feeding season in subpolar waters and returning to low latitude waters to calve in winter (Horwood, 2017). In the North Pacific, sei whales (Figure 1) distribute widely in the offshore area associated with the productive polar and subarctic fronts during the summer feeding season (Murase *et al.*, 2014).

The second phase of the Japanese Whale Research Program under Special Permit in the western North Pacific (JARPNII) started in 2000 (GOJ, 2000; 2002), and was completed in 2016. The sei whale was added as one of the target species for sampling in 2002. The New Scientific Whale Research Program in the western North Pacific (NEWREP-NP) started in 2017 (GOJ, 2017) and also had the sei whale as one of the target species. During the annual surveys, biological data and samples such as body length measurements and reproductive organs, were obtained systematically. A total of 1,622 sei whales were

sampled during a 17 year survey period. The NEWREP-NP was completed in 2018.

Age data is one of the most important information for stock assessment and management of large whales. Age estimation based on counting of growth layers accumulated in the earplugs is considered the most reliable tool for age determination in baleen whales (Lockyer, 1984a;



Figure 1. A sei whale feeding on plankton near the sea surface in the western North Pacific.

Maeda *et al.*, 2016). During JARPNII and NEWREP-NP, earplugs were collected from each whale sampled by experienced biologists in order to increase the readability. Some age-related biological parameters are known to change in response to changes in abundance, food availability or competition among whale species (Gambell, 1973; Kato and Sakuramoto, 1991; Lockyer, 1984b). Therefore, monitoring of biological parameters is indispensable for the assessment and management of whale stocks.

This study summarized the progress in the research on earplug-based age determination and estimation of the biological parameters of the western North Pacific sei whales sampled by the JARPNII and NEWREP-NP surveys between 2002 and 2018.

## MATERIALS AND METHODS

### Whale sampling

A total of 1,622 (744 males and 878 females) sei whales were sampled by JARPNII and NEWREP-NP during 2002–2018. The geographical distribution of the sampled whales is shown in Figure 2. Table 1 shows the number of sampled whales by year and sexual maturity status.

### Biological data

Body length was measured in a straight line to the near-

est 1 cm, from the tip of the snout to the notch of the flukes using a stainless measure. Sexual maturity in males was determined by examining the histological samples from the testis. Males with seminiferous tubules over 100  $\mu\text{m}$  diameter, spermatid or open lumen in the tubules were determined to be sexually mature (Masaki, 1976; Lockyer, 1984b). Maturity of females was preliminarily determined in the field by the presence or absence of at

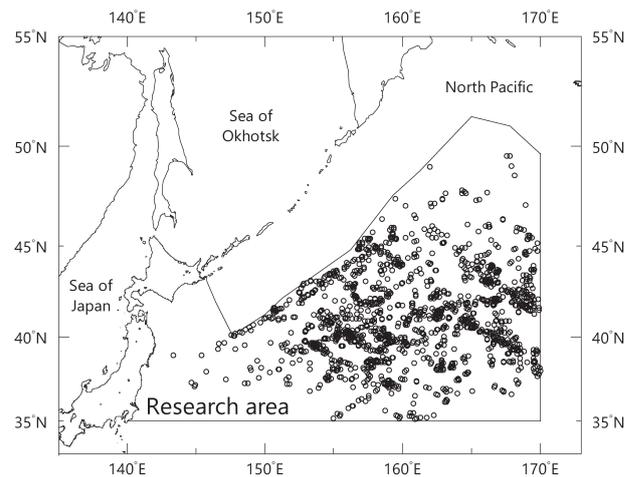


Figure 2. Research area of JARPNII and NEWREP-NP and sighting positions of sei whales sampled during the 2002–2018 surveys.

Table 1

Number of sei whales sampled by JARPNII and NEWREP-NP in the western North Pacific in the period 2002–2018, by year, sex and sexual maturity status.

Year	Male				Female			Total
	Immature	Mature	Unknown	Total	Immature	Mature	Total	
2002	3	12	0	15	4	20	24	39
2003	4	19	0	23	4	23	27	50
2004	11	36	0	47	14	39	53	100
2005	6	45	0	51	14	35	49	100
2006	18	30	0	48	14	38	52	100
2007	21	33	0	54	16	30	46	100
2008	6	38	0	44	17	39	56	100
2009	10	36	0	46	9	45	54	100
2010	12	31	0	43	10	47	57	100
2011	8	46	0	54	13	28	41	95
2012	12	32	0	44	11	45	56	100
2013	15	29	0	44	6	50	56	100
2014	7	31	0	38	11	41	52	90
2015	9	20	0	29	15	46	61	90
2016	10	28	0	38	16	36	52	90
2017	13	50	0	63	15	56	71	134
2018	12	50	1	63	17	54	71	134
Total	177	566	1	744	206	672	878	1622

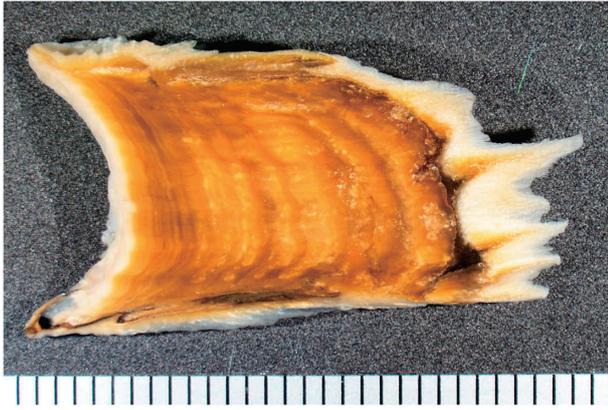


Figure 3. Bisected earplug surface of sei whale with light and dark laminae of growth layer groups (GLGs). The scale shows a 1 mm interval.

least one corpus luteum or corpus albicans in both sides of the ovaries, and confirmed later in the laboratory by counting number of corpora.

#### Earplug sampling and age determination

Earplugs were collected from all sampled animals, following the method developed for baleen whale (Omura, 1963; Maeda *et al.*, 2016). The left and right earplugs were collected and immediately fixed in 10% formalin until age determination. In the laboratory, the surface 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 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.

#### 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 the 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 ( $t_m$ ) was estimated by the following equation (Cooke, 1984).

$$T_m = g - 0.5 + \sum_{a=g}^h \left( \frac{I_a}{N_a} \right)$$

$$\text{var}(t_m) = \sum_{a=g}^h \frac{M_a I_a}{N_a^2 (N_a - 1)}$$

where

$M_a$  is the number of mature animals in age  $a$ ,

$I_a$  is the number of immature animals in age  $a$ ,

$N_a$  is the total number of animals in age  $a$ ,

$g$  is the age of the youngest mature animal in the sample, and

$h$  is the age of the oldest immature animal in the sample.

##### Body length at sexual maturity

Body length at sexual maturity ( $L_m$ ) was estimated by the following equation (Cooke, 1984; Kato, 1992). When calculating, body length data was rounded to the nearest 0.1 m.

$$L_m = j - 0.05 + 0.1 \sum_{b=j}^k \left( \frac{I_b}{N_b} \right)$$

$$\text{var}(l_m) = q \sum_{b=j}^k \frac{M_b I_b}{N_b^2 (N_b - 1)}$$

where

$M_b$  is the number of mature animals in body length  $b$ ,

$I_b$  is the number of immature animals in body length  $b$ ,

$N_b$  is the total number of animals in body length  $b$ ,

$j$  is the body length of the smallest mature animal in the sample, and

$k$  is the body length of the largest immature animal in the sample.

##### Annual ovulation rate

The annual ovulation rate was estimated by applying linear regression analysis between age and the total number of corpora (corpora lutea and albicantia). The regression line was fitted to ages 14 years and older because all females were sexually mature at the age of 14 years in this study.

## RESULTS AND DISCUSSIONS

### Age readability

Earplugs were read for whales collected up to 2016 ( $n=1,354$ ). Age information was obtained for 887 whales (65.5%). The readability of earplugs varied depending on the maturity status. Readability of sexually immature individuals was 55.9% for males and 48.9% for females (Table 2). Readability of earplugs in sexually mature animal was higher, 72.5% and 67.4% for males and females,

Table 2  
Earplug-age readability of sei whales sampled during 2002–2016 surveys, by sex and sexual maturity status.

Maturity status	Male			Female			Total		
	Number of whales	Readable earplugs	Readability (%)	Number of whales	Readable earplugs	Readability (%)	Number of whales	Readable earplugs	Readability (%)
Immature	152	85	55.9	174	85	48.9	326	170	52.1
Mature	466	338	72.5	562	379	67.4	1028	717	69.7
Total	618	423	68.4	736	464	63.0	1354	887	65.5

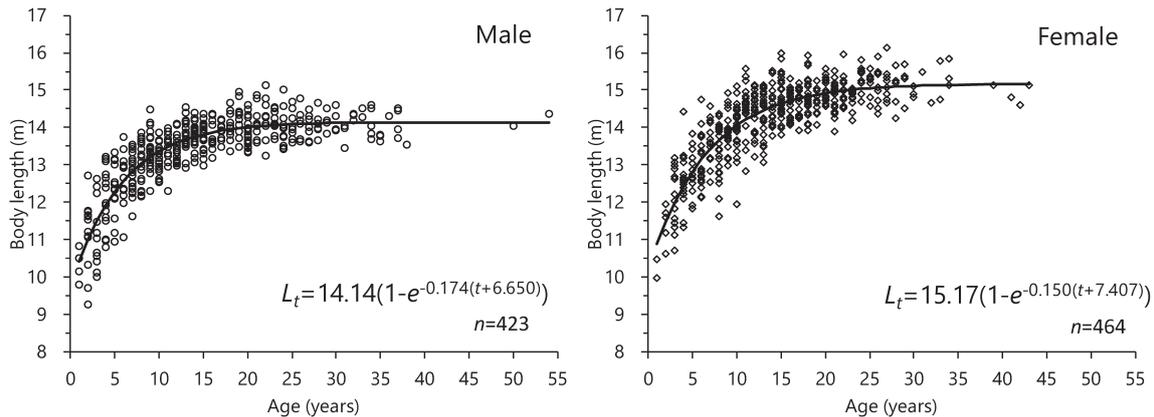


Figure 4. Relationship between body length and age in sei whales. The solid lines show the von Bertalanffy growth curves.

respectively (Table 2). Readability of all samples was 65.5%, which was higher than for western North Pacific common minke whales (44.1%) (Maeda *et al.*, 2016), and comparable to Bryde's whales (65.2%) (Bando, 2018), which were based on samples collected by JARPNI and JARPNI.

#### Growth curve

For both sexes, the growth rate was high at younger ages and stabilized after 15 years (Figure 4). The following von Bertalanffy growth curves were estimated:

$$\text{Male: } L_t = 14.14(1 - e^{-0.174(t+6.650)})$$

$$\text{Female: } L_t = 15.17(1 - e^{-0.150(t+7.407)})$$

Masaki (1976) estimated growth curves of sei whales based on samples collected from the commercial whaling in the North Pacific in the 1970s. However, the estimated curve was incomplete due to the lack of young individuals. During JARPNI and NEWREP-NP, samples from a wide range of ages, including young individuals, were collected, and for the first time a reasonable growth curve could be obtained for western North Pacific sei whales.

#### Age at sexual maturity

Sexually mature males first appeared at the age of 4 years,

and from 13 years old onwards all animals were sexually mature (Figure 5).  $T_m$  for males was estimated as 6.7 years (SE=0.29). For females, sexually mature whales first appeared at the age of 3 years, and from 14 years old onwards all animals were sexually mature (Figure 5).  $T_m$  was estimated as 6.9 years (SE=0.27). Masaki (1976) reported the  $T_m$  of sei whales from the commercial whaling in the North Pacific in the 1970s as 2.5 and 6.5 years for males and females, respectively. However, these values were underestimated as the ages of younger animals are often estimated as less than their actual ages. This is because the softer and smaller earplug of young animals, together with a lack of neonatal layers, in some cases, make it difficult to recognize growth layers (Masaki, 1976).

#### Body length at sexual maturity

The relationship between sexual maturity and body length is shown in Figure 6. Sexually mature male first appeared at body length of 12.2 m, and from 13.6 m onwards all animals were sexually mature.  $L_m$  was estimated as 12.72 m (SE=0.12). For females, sexually mature whales first appeared at the body length of 12.6 m, and from 14.0 m all animals were sexually mature (Figure 6).  $L_m$  was estimated as 13.31 m (SE=0.12). Masaki (1976) estimated  $L_m$  of male sei whales as 12.9 m based on the

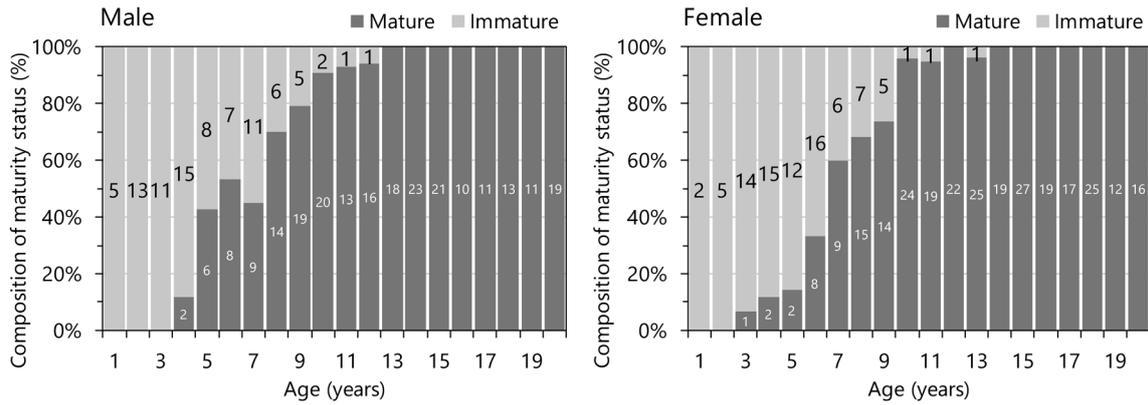


Figure 5. Sexual maturity status by age and sex in sei whales. Numbers in the bars show the numbers of samples examined.

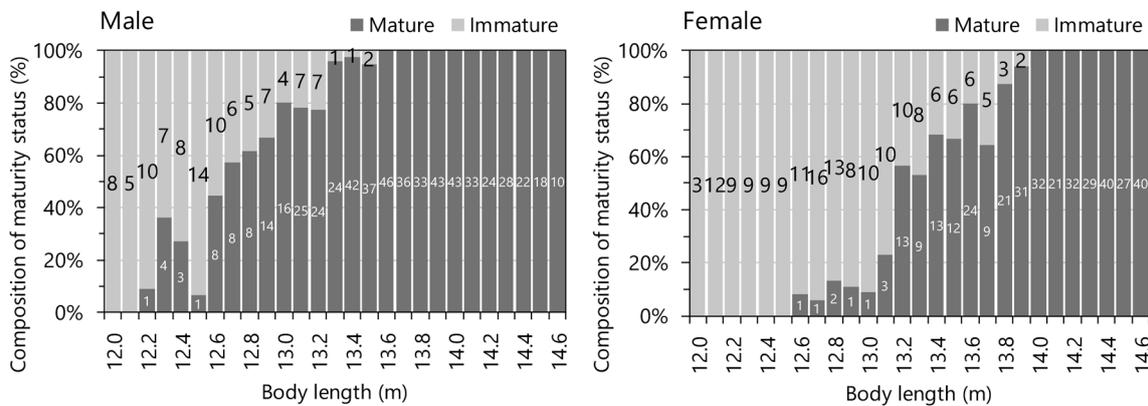


Figure 6. Sexual maturity status by body length and sex for sei whales. Numbers in the bars show the numbers of samples examined.

commercial whaling samples from the North Pacific, which was slightly higher than the value estimated in this study. However, Masaki (1976) determined the sexual maturity of males by testis weight rather than histological observation, which might have influenced the results. For females, a similar value was estimated from commercial whaling samples (13.4 m) (Masaki, 1976).

**Annual ovulation rate**

The corpus luteum and albicans first appeared at the age of 3 years and the number of corpora increased linearly after the age of 14 years (Figure 7). Annual ovulation rate was estimated as 0.744. The estimated value means that the majority of sei whales give birth (or ovulation occurs) three times in four years. This value was higher than 0.604 which was estimated from the 1970s commercial whaling samples (Masaki, 1976).

**CONCLUDING REMARKS**

Substantial biological information was obtained for the North Pacific sei whale during the 17 years of JARPNII and NEWREP-NP. In particular, more age data from young in-

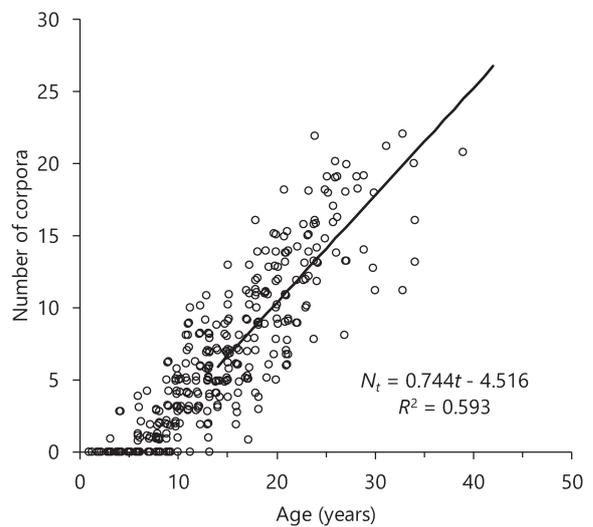


Figure 7. Jitter plot showing the relationship between age and number of corpora in sei whales. The linear regression line was fitted to ages 14 years and over.

dividuals were obtained, resulting in improved estimates of biological parameters than those obtained in the period of commercial whaling. The analyses of biological data

will contribute to the management of this whale species in the North Pacific.

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