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Basic information of earplugs as age character of common minke whales in western North Pacific

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ABSTRACT

Age reading from the earplugs of the common minke whale (*Balaenoptera acutorostrata*) are generally believed to be difficult and impractical because of their softness and poor formation of growth layers. However, under JARPN and JARPNII surveys, all earplugs of common minke whales were carefully collected and tried to read growth layers. We provide basic information of earplug readability and age composition of North Pacific common minke whales collected from 1994 to 2013. Age readability was 45.2% for male, 41.2% for female. Readability of mature animals (49.2% for male and 59.0% for female) was higher than immature animals (35.4% for male and 34.7% for female) in both sexes. From inter-reader calibration experiment, the age reading outcomes of two readers appeared similar. Distinct difference was observed in body length distribution and also age distribution of common minke whales between coastal and offshore areas. Whales of less than five years old were dominant in coastal areas. On the other hand, males of more than six years old were dominant in offshore areas. Result of age distribution supported segregation of common minke whales by sex and maturity status in western North Pacific. It had been believed that earplugs of common minke whales were not available for age characteristics. However, in conclusion, earplugs of common minke whales in western North Pacific were considered useful as a valid age tool.

KEYWORDS: EARPLUG, AGE DATA, COMMON MINKE WHALE, WESTERN NORTH PACIFIC

INTRODUCTION

Information on whale age is of key importance for estimating life-history parameters that can be used for stock management. Earplugs were examined as an age character for the first time in baleen whales by Purves (1955). Even today, using earplugs as an age character, age estimations have been conducted in several species of baleen whales (Lockyer, 1984a; Gabriele *et al.,* 2010; Nielsen *et al.,* 2012; Kitakado *et al.,* 2013). As shown in Figure 1, the earplug accumulates in the external auditory meatus (Lillie, 1910) and consists of a core and outer covering (Purves, 1955; Ichihara, 1959). The outer covering is secreted by epithelial cells in the external auditory meatus, whereas the core, comprising concentric light and dark laminae, is secreted by papillae on the surface of the glove finger (Purves, 1955). Fat content tends to be lower in the dark layer and higher in the light layer from histological observations of fin whale (*Balaenoptera physalus*) earplugs (Roe, 1967). The light and dark layers are formed during the feeding and breeding periods, respectively (Roe, 1967). Because many baleen whales migrate between a breeding place in low-latitude waters (winter) and a feeding place in high-latitude waters (summer), which is approximated by an annual cycle, such a living cycle is reflected as a growth layer; therefore, one growth layer represents 1 year (Roe, 1967; Lockyer, 1972; 1984b). In addition, the neonatal line (NL) which is created at birth (Ichihara, 1964), is formed at the apical portion of the core (Figure 1).

Age reading from earplugs of the common minke whale (*B. acutorostrata*) are generally believed to be difficult and impractical because of their softness and poor formation of growth layers (Sergeant, 1963; Mitchell and Kozicki, 1975; Christensen, 1981; Larsen and Kapel, 1983; Christensen *et al.,* 1990; Kato, 1992; Auðunsson *et al*., 2013). However, under JARPN and JARPNII survey, all earplugs of common minke whales were carefully

collected and tried to read growth layers. Furthermore, we tried to improve age readability especially young animals to prevent breakage and loss of neonatal line using new collection technique "gelatinized extraction" (Maeda *et al*., 2013). Here we provide basic information of earplug readability and age distribution of North Pacific common minke whales collected 1994 to 2013.

MATERIALS AND METHOD

Samples and data used

All earplugs of common minke whales were collected during the 1994 to 2013 JARPN and JARPANII surveys. Earplugs were collected from 2,572 whales (1,888 males and 684 females). Some biological datasets, including body length, sex, weight of testis, number of ovulations and genetic information were used. Each whale was assigned to the O and J stock based on the microsatellite DNA analysis presented in Pastene *et al*. (2016a: SC/F16/JR38). Only animals with membership probability of over 90% were included in the analysis. Males with a single testis weight of 290g or more, and females that had at least one *corpus luteum* or *albicans* in their ovaries were regarded as sexually mature.

Age estimation using earplugs

The age of the whales was estimated by the counting of growth layers of earplug (Purves, 1955; Ohsumi, 1964; Lockyer, 1972; Maeda and Kato, 2012). The left and right earplugs with glove-finger were collected carefully, and immediately fixed in 10% formalin solution until age estimation. Traditionally, earplugs have been collected by exposing and cutting the external auditory meatus and then directly obtaining the internal earplugs using tweezers. According to Maeda *et al*. (2013), earplugs of small individuals were collected using the method of gelatinized extraction which was introduced in 2007.

In the laboratory, after cutting flat along the central axis of the earplug using a sharp blade, it was ground on a wet stone to expose the neonatal line and growth layers. Growth layers were counted under water using stereoscopic microscope under low magnification (3.15x – 31.5x). A year of age was defined as one pair of the light and dark laminae in the core.

Age reading was conducted in the following manner: i) earplug of the left side was read. If the earplug 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 reading of all sample was completed, age data was compared with biological data such as body length or sexual maturity, and some samples were re-read to check outlier, incomplete sample or invalid reading. All earplugs collected during the 1994 to 2013 were read by a single reader (Maeda).

Inter-reader calibration

The inter-reader calibration experiment and analysis were conducted following the method of Kitakado *et al*. (2013) which was applied for Antarctic minke whales. A total of 150 earplugs were chosen from three periods of year (1994 to 2003, 2004 to 2008, 2009 to 2013) from male common minke whales of O-stock sampled during 1994 to 2013. Fifty earplugs were randomly selected from each period and read by control reader (Bando) and Maeda.

RESULTS AND DISCUSSION

Age readability

Earplugs of immature animals had soft and light-colored core, and its outer covering was undeveloped (Figure 2, A). On the other hand, mature animals had toughened and dark-colored core with developed outer covering (Figure 2, B and C). Readability of mature animals (49.2% for male and 59.0% for female) was higher than immature animals (35.4% for male and 34.7% for female) in both sexes. Figure 3 shows readability by body length class and stocks. Readability increased with body length class in both stocks. Age readability of all animals was 45.2% for male, 41.2% for female, respectively.

Most of unreadable earplugs of mature animals had growth layers with unclear formation such as irregular lamination and partially-formed growth layers. About lower readability in immature animals, it was difficult to identify growth layers because of its solid and light-colored core and easily damaged because of its softness and small size. In the past, Kato (1992) reported that readability of common minke whales off Northern Japan collected by commercial whaling was only 8.7% (321 animals, 183 males and 133 females). However, under

JARPN and JARPANII surveys, readability of common minke whales was improved dramatically, because of careful collection and efforts in technical development of earplugs sampling.

Age reading error

Figure 4 showed the scatter plots of age estimates of common minke whales from two independent readers (Bando and Maeda) on 1st trial (read 150 samples). Figure 5 and 6 showed scatter plots of age estimates from reading data for three trials. The age reading outcomes of each reader appeared similar. Table 2 and Figure 7 showed the result of age reading error with Bando assuming control reader (unbiased). Compared to Bando, Maeda tended to estimate on the small side, however, the extent of the difference is not so large. Thus, it was considered that age data read by a single reader (Maeda) from earplugs collected during 1994 to 2013 had a constant reliability.

Age and body composition

Male and female were distributed over a wide range of age, however sample size of female was smaller than male and there were biased under ten years old. From age composition by stock (Figure 8), sample size of J-stock was smaller than O-stock and there were few old individuals. Distinct difference was observed in age distribution between coastal and offshore areas (Figure 9). Whales of less than five years old were dominant in coastal areas. On the other hand, males of more than six years old were dominant in offshore areas. Hatanaka and Miyashita (1997) reported on segregation of common minke whales in feeding season from body length composition. From body length distribution and maturity composition of JARPN/JARPANII data (1994 to 2001), Zenitani *et al*. (2002) concluded that common minke whales were distributed widely from coastal to offshore with segregation depending on sex and reproductive status. Furthermore, Kanda *et al*. (2010) analyzed length composition and sex ratio data from bycaught minke whales around Japan from 2001 to 2007 and JARPN/JARPANII data (1994 to 2007). They reported immature animals were distributed in mainly coastal areas whereas mature animals were distributed mainly in offshore areas. Results of age distribution from this study supported segregation of common minke whales by sex and maturity status in western North Pacific. From body length composition (Figure 10 and 11), it was shown that body length compositions of readable earplugs in each stock and survey are not always exactly reflected in all whales. Especially under six meter animals which considered immature animals had a large proportion of unreadable earplugs in both stock and sex. O stock male or offshore male had also a large proportion of unreadable earplugs in seven to eight meter body length ranges. The age structure could be reliably estimated by summarizing the relationship between age and length (Age length key). It was assumed that Age length key works to some extend to fill in gaps of outcomes between readable and unreadable earplug for under six meter animals.

It had been believed that earplugs of common minke whales were not available for age characteristics. However, in conclusion, earplugs of common minke whales in western North Pacific were considered useful as a valid age tool.

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TABLES

Table 1

Age readability of common minke whale collected by JARPN and JARPNII surveys from 1994 to 2013 by sex and maturity status.

	Sex	Number of whales	Number of whales with readable earplugs	Readability (%)
Total	Male	1888	853	45.2
	Female	684	282	41.2
Immature	Male	548	194	35.4
	Female	501	174	34.7
Mature	Male	1340	659	49.2
	Female	183	108	59.0
Total		2572	1135	44.1

Table 2

Result of the age reading error analysis based on Kitakado *et al*. (2013). The expected age-reading outcomes of the control and unbiased reader (Bando) was assumed to be $b_{L1} = 0, b_{H1} = 50$ for minimum and maximum ages 0 and 50 respectively, and those for the tested reader (Maeda), b_{L2}, b_{H2}, were estimated as the table below. The estimates and associated SEs for the standard deviation for the two readers were also given in the following table.

Parameters	b_{L2}	b_{H2}	σ_{L1}	σ_{H1}	σ_{L2}	σ_{H2}
Estimate	0.000045	47.533000	0.529320	3.814100	0.697990	2.876300
SE	0.000063	0.414020	0.215810	0.700810	0.200380	0.641390

FIGURES

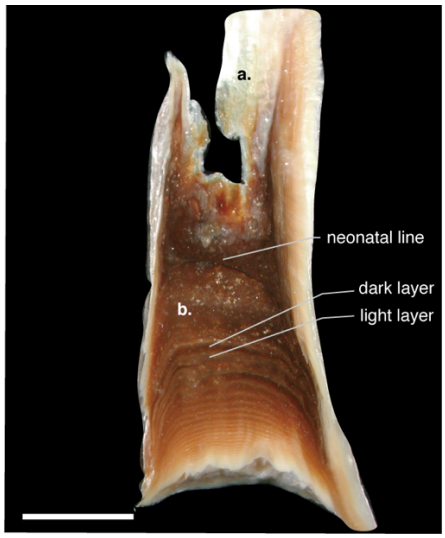

Figure 1. Bisected surface of an earplug of a common minke whale. a: Outer covering, b: Core. Scale bar: 5 mm

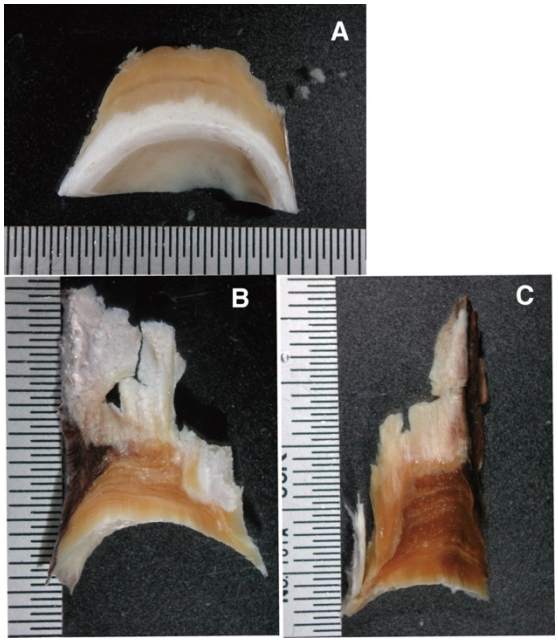

Figure 2. Bisected surface of an earplug of a common minke whale. A: 4 years old, B:18 years old, C:28 years old. A scale indicates 0.5 mm.

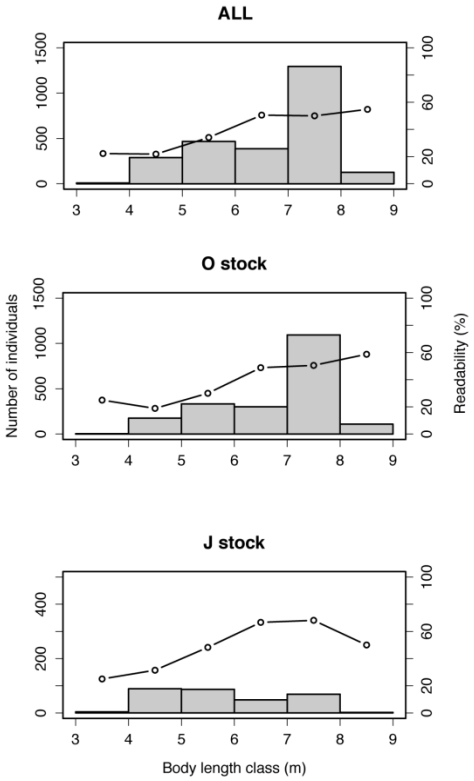

Figure 3. Age readability of common minke whales collected by JARPN and JARPNII (1994 to 2013) surveys by body length class (upper; all combined, middle; O stock, bottom; J stock).

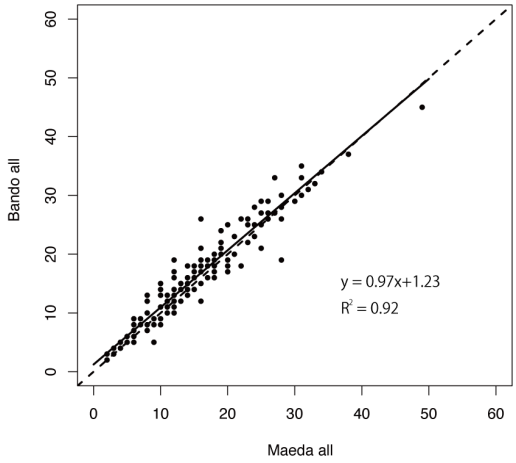

Figure 4. Scatter plots of age estimates of common minke whales from Bando against Maeda on 1st trial (read 150 samples). The dashed line shows a 45 degree line. Solid line shows regression line.

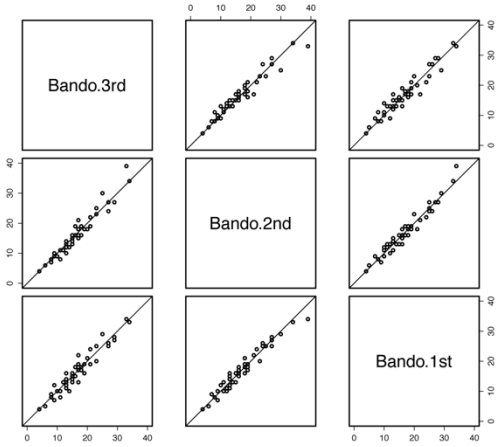

Figure 5. Scatter plots of age estimates of common minke whales from Bando's age-reading data for three trials. The solid line shows a 45 degree line.

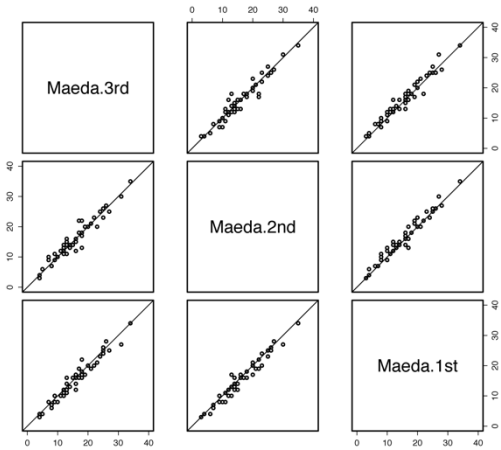

Figure 6. Scatter plots of age estimates of common minke whales from Maeda's age-reading data for three trials. The solid line shows a 45 degree line.

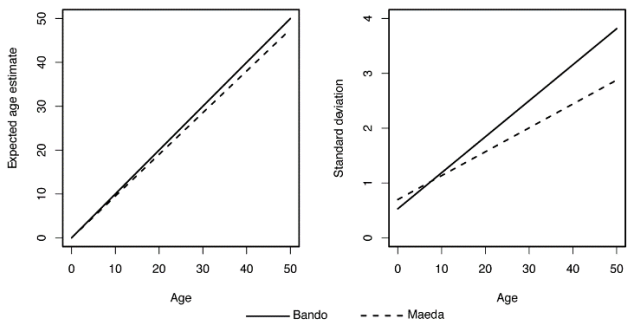

Figure 7. Expected age estimates (left) for the control reader (Bando: solid line) and Maeda (dotted line) if the control reader is assumed to be unbiased. Right: standard deviations of both readers.

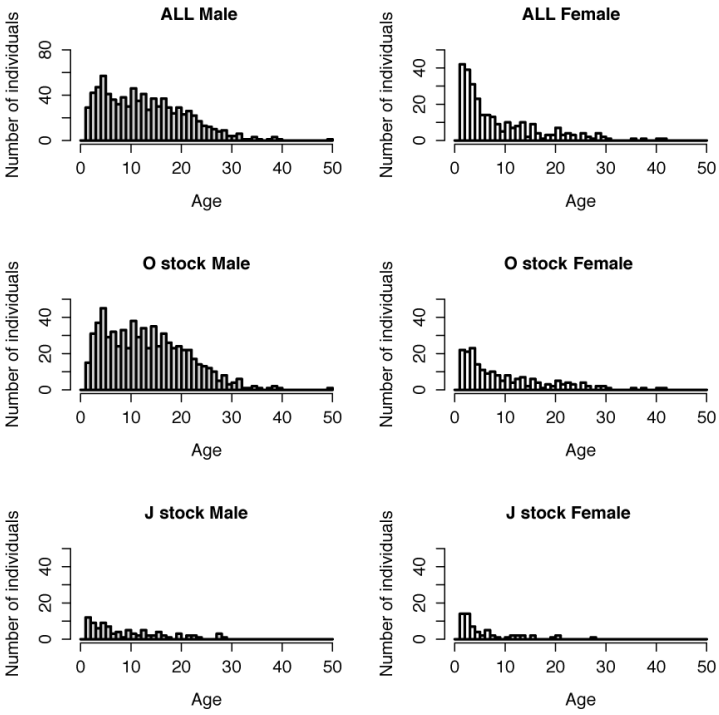

Figure 8. Age distributions of common minke whales collected during JARPN and JARPNII (1994-2013) surveys (upper; all combined, middle; O stock, bottom; J stock).

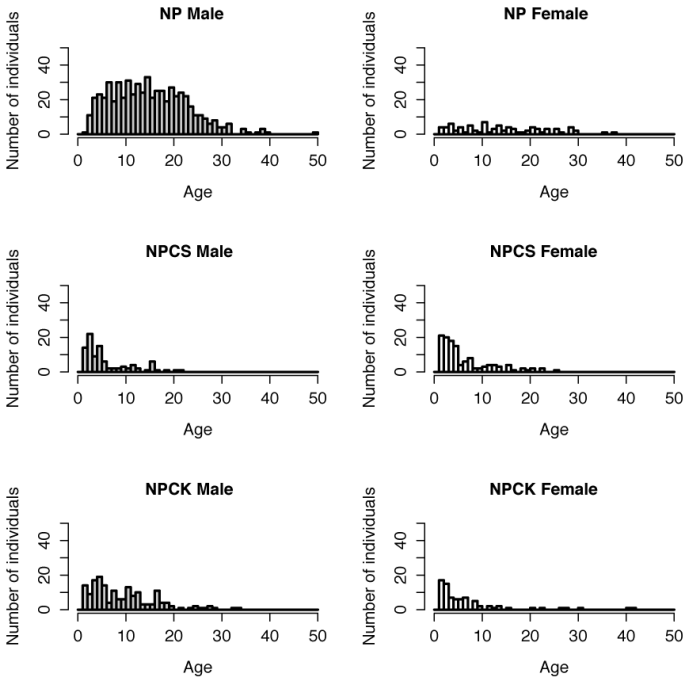

Figure 9. Age distributions of JARPN and JARPNII (1994-2013) samples (NP; offshore, NPCS; coastal area off Sanriku, NPCK; coastal area off Kushiro, by sex).

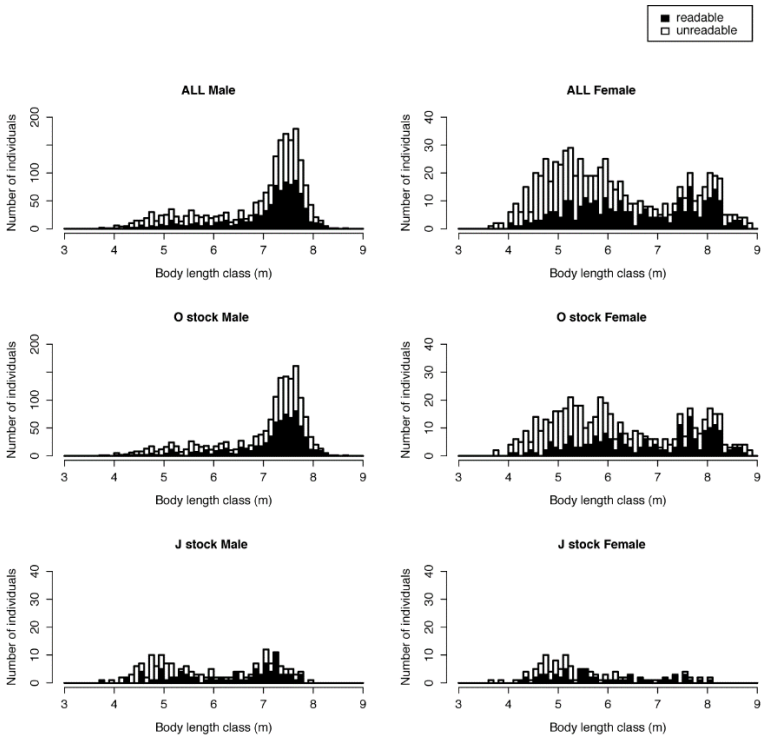

Figure 10. Body length distributions of common minke whales collected during JARPN and JARPNII (1994-2013) surveys (upper; all combined, middle; O stock, bottom; J stock).

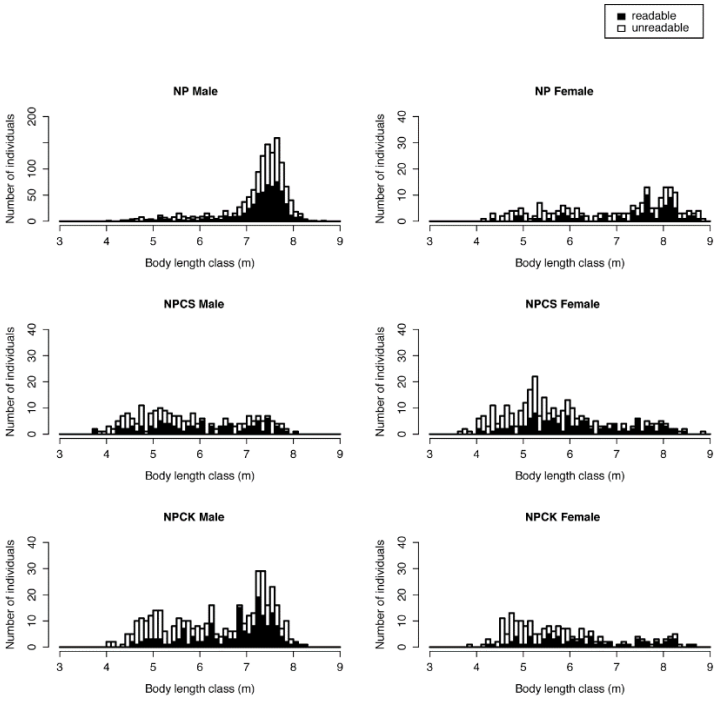

Figure 11. Body length distributions of JARPN and JARPNII (1994-2013) surveys (NP; offshore, NPCS; coastal area off Sanriku, NPCK; coastal area off Kushiro).