SC/F16/JR/52

# A study on the improvement of age estimation in common minke whales using the method of gelatinized extraction of earplug

Hikari Maeda, Tadafumi Kawamoto and Hidehiro Kato

INTERNATIONAL
WHALING COMMISSION

NAMMCO
SCIENTIFIC PUBLICATIONS

*Online Early Version*

# A study on the improvement of age estimation in common minke whales using the method of gelatinized extraction of earplug

Hikari Maeda[1,*], Tadafumi Kawamoto[2], and Hidehiro Kato[1]

[1]*Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan*
[2]*Radioisotope Research Institute, Tsurumi University School of Dental Medicine, 2-1-3 Tsurumi, Tsurumi-ku, Yokohama 230-8501, Japan*

**Corresponding Author: Hikari Maeda, E-mail: hikarim@affrc.go.jp; Current address: National Research Institute of Far Seas Fisheries, 2-12-4 Fukuura, Kanazawa, Yokohama 236-8648, Japan*

## ABSTRACT

We attempted to settle the potential problems of bias caused by too soft earplugs and poor formation of the growth layers in age readings of common minke whales. Thus, we examined the feasibility of a new technique of incorporating gelatin in order to collect earplugs for age assessment. Frozen sectioning and histology of the earplug core were also used as methods to improve age estimation. Earplugs were collected by filling the space in the external auditory meatus with gelatin, hardening the gelatin, earplug and its fragments, by spraying with cooling gas, and removing the earplug embedded in gelatin. In 174 trials with common minke whales in the Western North Pacific of coastal waters of Japan in 2007–2009, it was revealed that embedding earplugs with gelatin minimized breakage and protected the neonatal line (NL). This method was particularly effective in younger animals. As a result, the readability was improved. We also examined the histological sections, which were sliced using the Kawamoto specialized frozen sectioning technique, and stained them separately with toluidine blue, haematoxylin and eosin, Sudan III, Sudan VII, and alizarin red S to display a clearer core surface image of the growth layers. The histological sections stained with alizarin red S provided the clearest images, in which we could easily identify both dark and pale laminations. This suggested a close relationship with the seasonal changes in calcium intake from feeding. Earlier age estimation methods focused on fat content in the growth layers; however, we found potential for an improvement in the readability of unclear growth layers when focusing on calcium.

## INTRODUCTION

Animal age is important basic information for population studies. Earplugs were examined as an age character for the first time in baleen whales by Purves (1955). Even today, earplugs are widely used as an age character (Gabriele *et al.* 2010, Lockyer 1984a, Nielsen *et al.* 2012). As shown in Figure 1, the earplug accumulates in the external auditory meatus (Lillie

Maeda, H., Kawamoto, T., and Kato, H. (2013) A study on the improvement of age estimation in common minke whales using the method of gelatinized extraction of earplug. *NAMMCO Scientific Publications.* doi: http://dx.doi.org/10.7557/3.2609

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). Since 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 (Best 1982, Ohsumi 1964, Gabriele *et al.* 2010, Lockyer 1972; 1984b, Roe 1967). Besides, the neonatal line (NL) which is created at birth (Ichihara 1964), is formed at the apical portion of the core, and germinal layers which are newly created, are formed at the basal portion (Fig. 2).

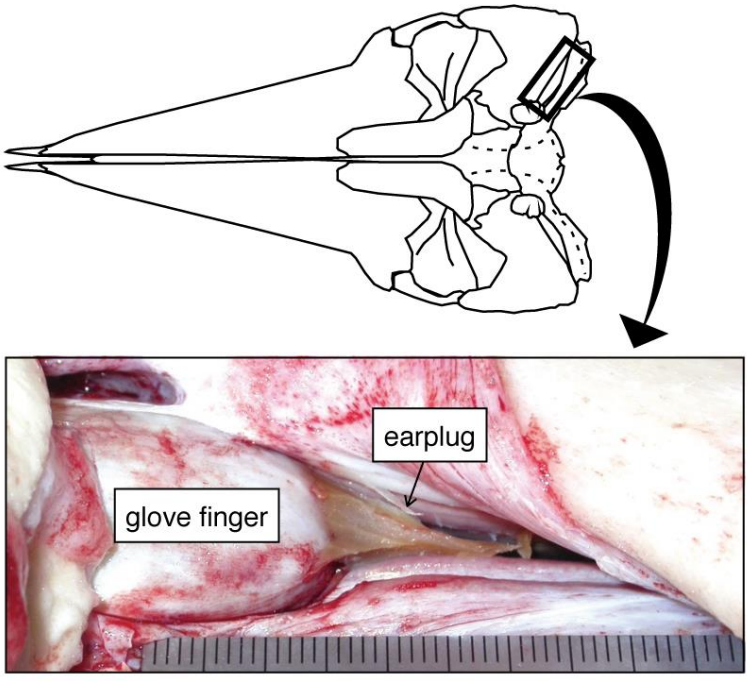


**Fig. 1.** Illustration of the ventral surface of the skull of a common minke whale, and a magnified view (below) of an exposed external auditory meatus. The ruler is graduated in millimeters.

Age estimations from earplugs have been conducted in several species of baleen whales (Lockyer 1984a). However, earplugs of the common minke whale (*Balaenoptera acutorostrata*) are generally believed to poorly reflect the age because of their softness and poor formation of growth layers (Sergeant 1963, Christensen 1981, Christensen *et al.* 1990, Kato 1992, Auðunsson *et al*. 2013). Improving the age estimation rate and acquiring more accurate age information are necessary for analyses, such as age dynamic studies. Thus, it is necessary to examine the causes for the low age estimation rate. There are two main factors that lower the age estimation rate of common minke whales using earplugs. The first factor is breakage during the collection stage. Most earplugs, particularly those of younger animals, are easily damaged during the collection because of their softness and small size. The second factor is the presence of earplugs with unclear layering patterns in which it is difficult to identify the growth layers using the existing age estimation method (Maeda and Kato 2012).

Traditionally, earplugs have been collected by exposing and cutting the external auditory meatus and then directly obtaining the internal earplug by using tweezers. However, it is very difficult to collect earplugs in their perfect condition by employing the existing method.

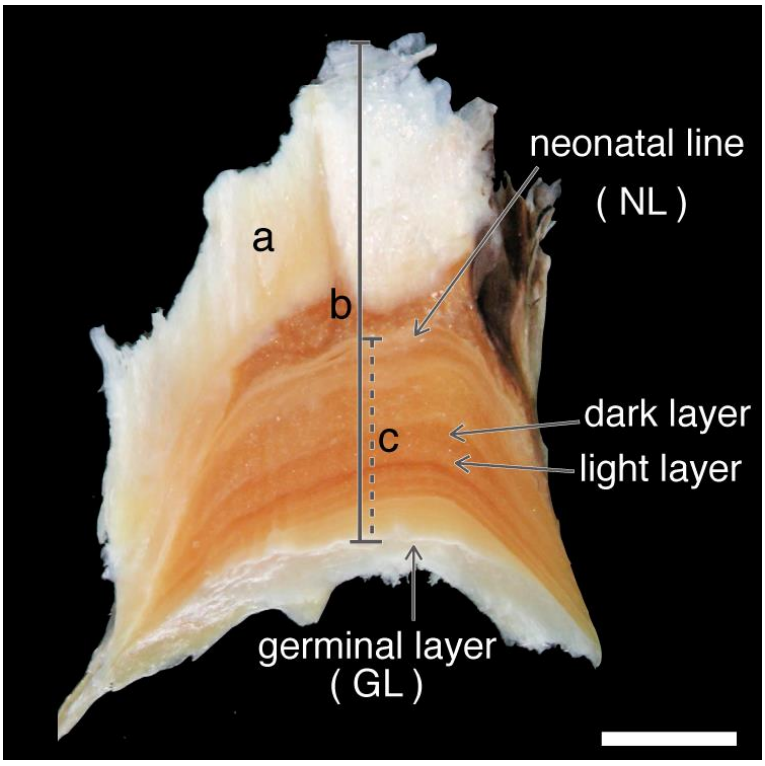


**Fig. 2.** Bisected surface of an earplug of a common minke whale. a: Outer covering, b: total earplug length, c: core length. Scale bar: 5 mm

Previously, Ichihara (1959) and Roe (1967) tested several staining solutions (Heidenhain's iron haematoxylin, haematoxylin and eosin, Azan, Dopa reagent, methyl green-pyronin (MGP), Schiff's reagent, Sudan III and Sudan Black) using frozen earplug sections. They found that Sudan III, which detects fat cells, was the most useful staining solution for earplug growth layers. Subsequently, there have been few earplug studies using these histological methods.

In the present study, we describe a method to minimize the damage done to earplugs at the collection stage by developing a new collection technique using gelatin. We also examine the possibility of using a frozen section and staining method to determine the age on the basis of histology of common minke whales.

## MATERIALS AND METHODS

### Surveys

Earplugs collected from common minke whales were sampled off Sanriku (Pacific coast of northern Japan) and Kushiro (Pacific coast of eastern Hokkaido) in Japan during 2007–2009. The samples were collected by the Japanese Whale Research Programme under a Special Permit in the Western North Pacific - Phase II (JARPN II) Coastal Components off Sanriku and Kushiro (Bando *et al*. 2008, Kishiro *et al*. 2008, 2010, Yasunaga *et al*. 2009, 2010, Yoshida *et al*. 2009). This study was approved by the Japanese government in compliance with Article VIII of the International Convention for the Regulation of Whaling. The research operations were headed by the Institute of Cetacean Research and the National Research Institute of Far Seas Fisheries (Government of Japan 2004). Common minke whales for this study were collected off Sanriku and Kushiro during spring (April–May) and autumn (September–October), when the maximum limit of 60 individuals is permitted. The survey area was within 50 nautical miles from the ports of Ayukawa and Kushiro. Four small whale catching boats (30.0–47.3 GT) were employed as sampling vessels. All captured whales were landed at the land station of each port for biological examination.

### Earplug collection using gelatin

A total of 174 trials on the common minke whale were conducted through the platform of JARPN II programmes (coastal component) during 2007–2009 (Table 1). We used some biological datasets, including body length, sex, weight of testis, and number of ovulations. Based on Kishiro *et al*. (2010) and Yoshida *et al*. (2009), a male with a testicle weighing more than 290 g and a female with at least 1 *corpus luteum* or *albicans* in the ovaries were regarded as sexually mature.

**Table 1.** Number of earplugs examined in the present survey by the programme in each year.

| Year | | Research programme | Number of individuals |
|---|---|---|---|
| 2007 | JARPN II | Coastal component off Kushiro | 38 |
| 2008 | JARPN II | Coastal component off Sanriku | 47 |
| 2008 | JARPN II | Coastal component off Kushiro | 20 |
| 2009 | JARPN II | Coastal component off Sanriku | 40 |
| 2009 | JARPN II | Coastal component off Kushiro | 29 |

Left earplugs were collected by using gelatin, and right earplugs were collected by using a standard procedure. Gelatin was selected as the embedding agent because it has good adhesiveness to the earplug, it is easy to handle in the field, and it is transparent, safe and non-toxic. The standard collection procedure was to cut open the external auditory meatus and pick out the earplug directly using tweezers. During the collection using gelatin, the external auditory meatus was impregnated with 40% liquid gelatin in prefilled syringes after opening the *corium* of the *dermis*, followed by cooling of the gelatin with coolant gas. Both earplugs were preserved in 10% neutral buffered formalin solution. In the laboratory, the earplugs were cut flat along the central axis of the core using a sharp blade according to Lockyer (1972). Then, the earplugs were ground on a wet stone to expose the NL and the growth layers and to smooth the surface. The cut surfaces of the earplugs were then examined under water using a stereomicroscope (Olympus SZX10, magnification: 3.2-31.5). Age was determined by reading the growth layers appearing on the bisected surface of the earplug, assuming annual deposition of growth layers (a pair of dark and light laminae accumulated per year; Maeda 2012). In case the age could not be determined, we categorized it according to one of three reasons, such as obscure formation, fracture of the core, and lost NL. Photographs of all earplugs were taken using a digital camera attached to the stereomicroscope (Nikon D90), and the entire core length along the central axis and earplug length were measured using an image analysis software (ImageJ, a public domain, Java-based image processing programme, National Institutes of Health, USA; Fig. 2).

**Frozen sectioning and staining**

The normally prepared earplug sections that had clear growth layers were selected for frozen sectioning. Frozen sections were prepared according to the method described by Kawamoto (2003) using a Multi-purpose Cryosection preparation kit (SECTION-LAB Co. Ltd., Japan). First, the earplugs were rapidly frozen in cooled hexane coolant. Then, the frozen earplugs were placed in a container filled with embedding gel and frozen completely in cooled hexane. The frozen block was then removed from the container, fixed to the sample stage, and attached to a cryomicrotome (Leica, CM3050S). The blocks were sectioned at 4–10 µm. The cutting surface was rotated by 90° from the normal surface which is the normally prepared earplug section. Then, the sections were stained in separate trials with toluidine blue (10 s), hematoxylin (5 min) and eosin (15 s), Sudan III (30–60 min), Sudan VII (30–60 min), and alizarin red S (5–10 min), each staining method being used separately and not sequentially on each section. Stained sections were mounted with mounting medium (SCMM-R2; SECTION-LAB Co. Ltd., Japan). Photographs of the stained sections were taken by a digital camera attached to a light microscope. We also analysed the gray value (8 bits / pixel) from the photographs by using image analysis software Image J. Elements in the section were analysed with an energy dispersive X-ray spectrometer (EDS).

**RESULTS**

**Earplug collection using gelatin**

Readability was improved in the samples collected using gelatin for each body length class of whale sampled (Fig. 3). Particularly, in animals with less than 7 m body length class, NL loss was reduced in the earplugs collected using gelatin. This clearly indicated that embedding earplugs in gelatin prevented breakage and loss of the NL (*Fisher's exact* test, $P < 0.05$). Figure 4 illustrates an example of a pair of earplugs. An earplug from the right external auditory meatus collected by the standard procedure had lost the NL; whereas, the gelatinized earplug from the other side held the NL. One year difference was found in age counting. As just described, age readability was improved in the earplugs collected using gelatin.

Figure 5. shows the change in the proportion of animals by age class for which age readability was improved by earplug collection using gelatin. The results showed that earplug collection using gelatin was effective in the < 12-year-old whales and particularly in the 1–3 year age class (*Cochran-Armitage* test, $P < 0.05$).

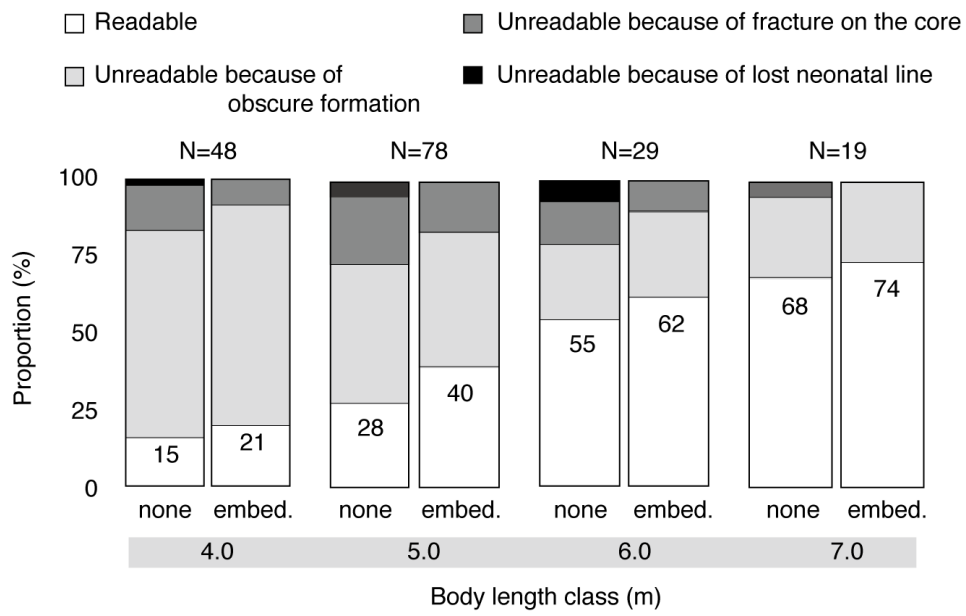


**Fig. 3.** Comparison between proportions (%) of readable earplugs by the state of preservation. none: without gelatin embedding, embed.: with gelatin embedding

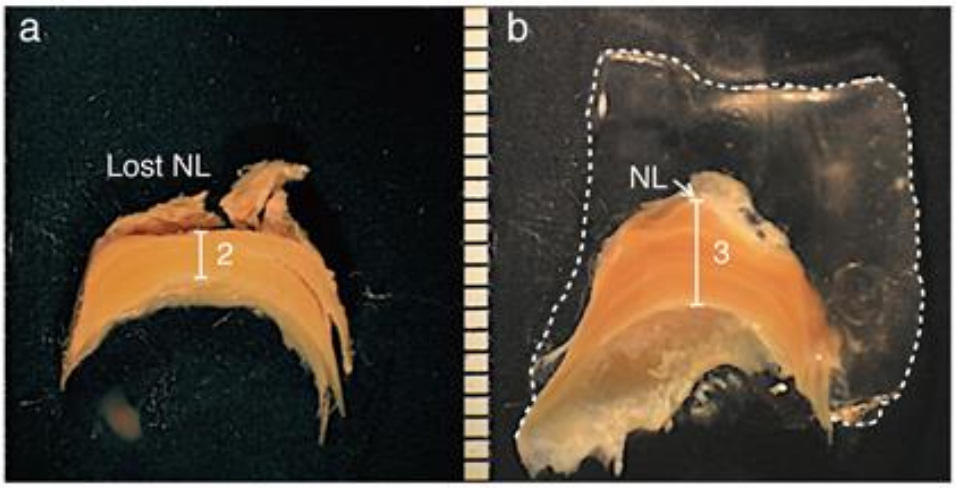


**Fig. 4.** Comparison of age readings from earplugs of a single animal (a) without gelatin embedding and (b) with gelatin embedding. Dotted line delimits the gelatinised area. The ruler at the center is graduated in millimeters. NL: neonatal line.

The mean total earplug length increased with age (6.19 mm in 1–3-year-old whales, 24.19 mm in more than 20-year-old whales; Fig. 6). The mean length of the outer covering (difference between total earplug length and core length) increased with age in each age class (*Jonckheere-Terpstratrent* test, $P < 0.05$).

**Frozen sectioning and staining**

Although fat cells were stained, growth layers could not be identified in frozen sections (4 μm) stained with Sudan III and Sudan VII. Hematoxylin and eosin, and toluidine blue also provided very poor results. However, alizarin red S was the most useful stain in identifying the growth layers (Table 2). Thick stained layers were observed in the core stained with alizarin red S (Fig. 7). After analyzing the gray value from the photographs of earplugs stained with alizarin red S, thick stained layers were observed periodically in the core (Fig. 8). It is known that while interlayer space between growth layers is wide and irregular in the immature stage, it becomes narrower in the mature stage (Lockyer, 1984b). Since it was close to the NL in the photographs as well, any sites considered to be in the above-mentioned characteristic of growth layers. As a result, thick stained peaks were periodically observed, the number of which was determined to be fourteen that corresponded to the numbers counted under the stereomicroscope. Most probably, these alizarin red S-stained areas reflected the growth layers. Alizarin red S solution is known to detect calcium. We analysed a part of the well-stained area by EDS in order to determine the presence of calcium. Except for the gold used for pre-treatment, calcium was the only dominant component (Fig. 9), revealing that the stained area had calcium.

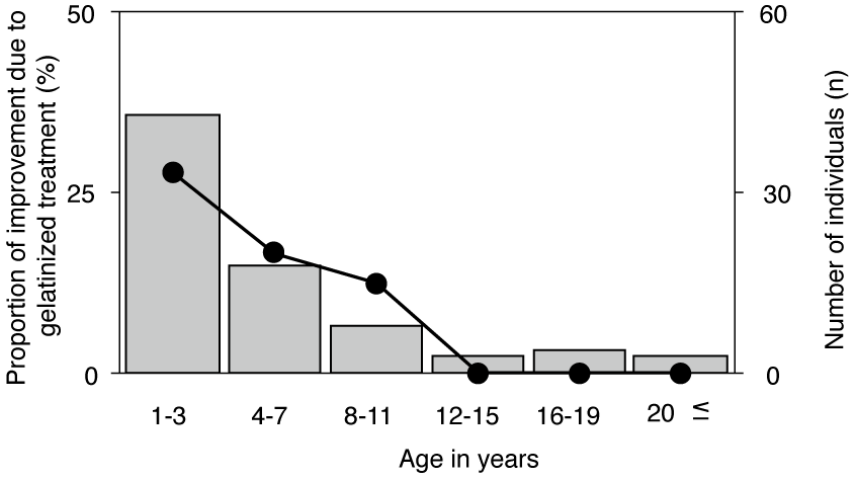


**Fig. 5.** Changes in the proportion of animals whose age readability was successfully improved with the use of gelatin embedding with age class. The bar represents the number of individuals in which growth layers existed in both earplugs in each age class.

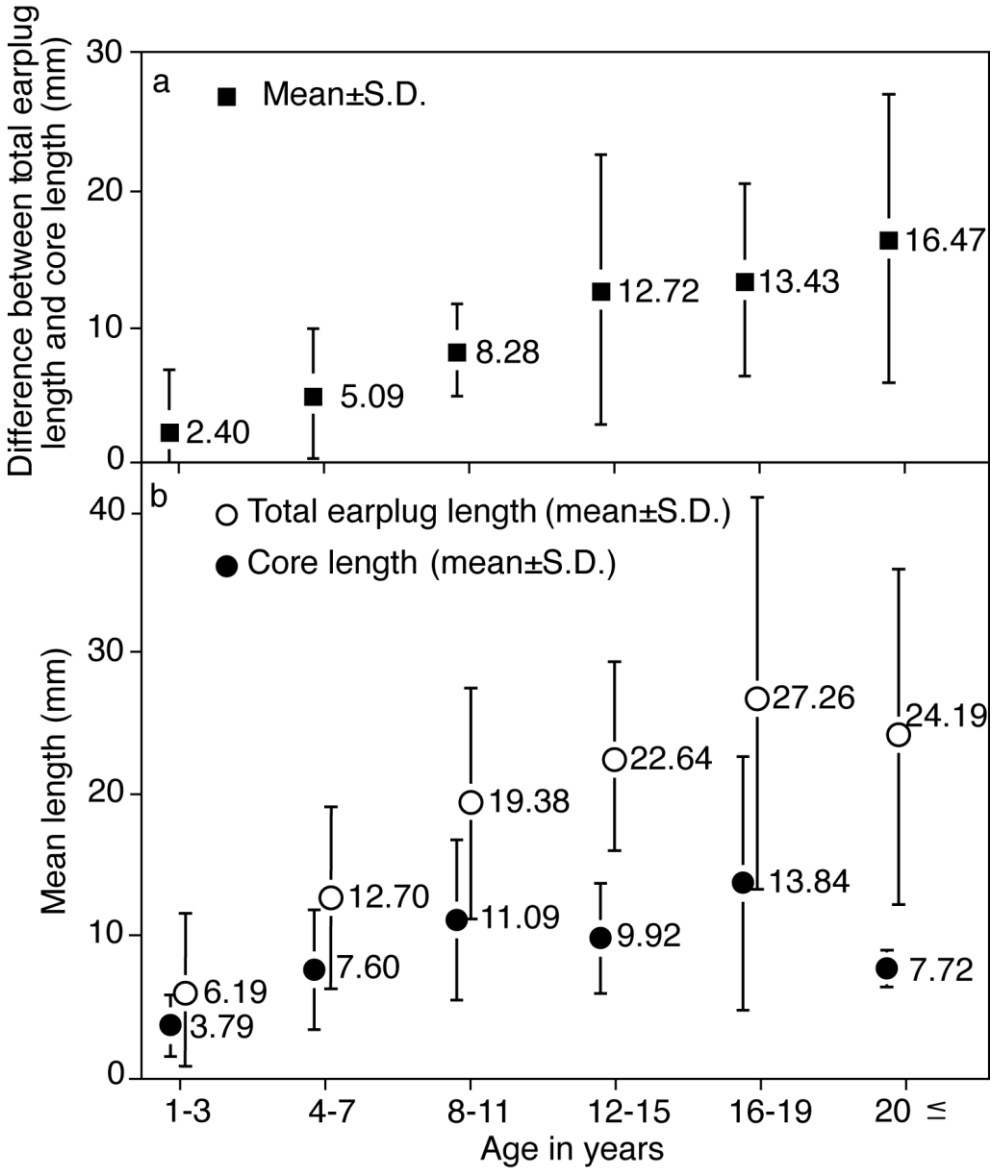


**Fig. 6.** Growth of the earplug in relation to age. a) Changes in the gaps of length between the core length and total length of the earplug and its standard deviation in relation to age class. b) Changes in the length of earplugs and the earplug core in relation to age class. Bars represent standard deviation in each age class.

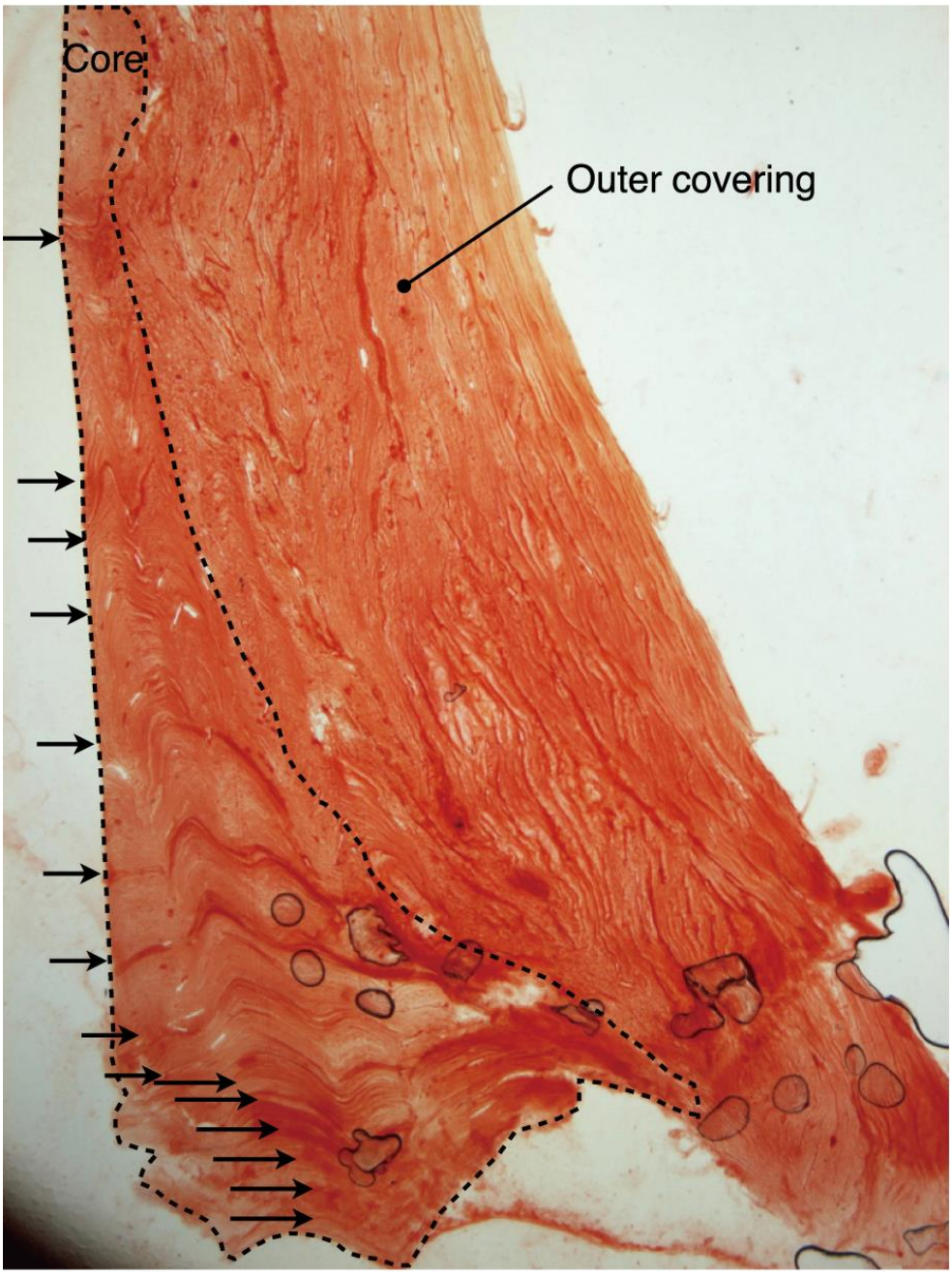


**Fig. 7.** The core surface of the growth layers is inside the dotted line. Arrowheads show the thickly stained laminae.

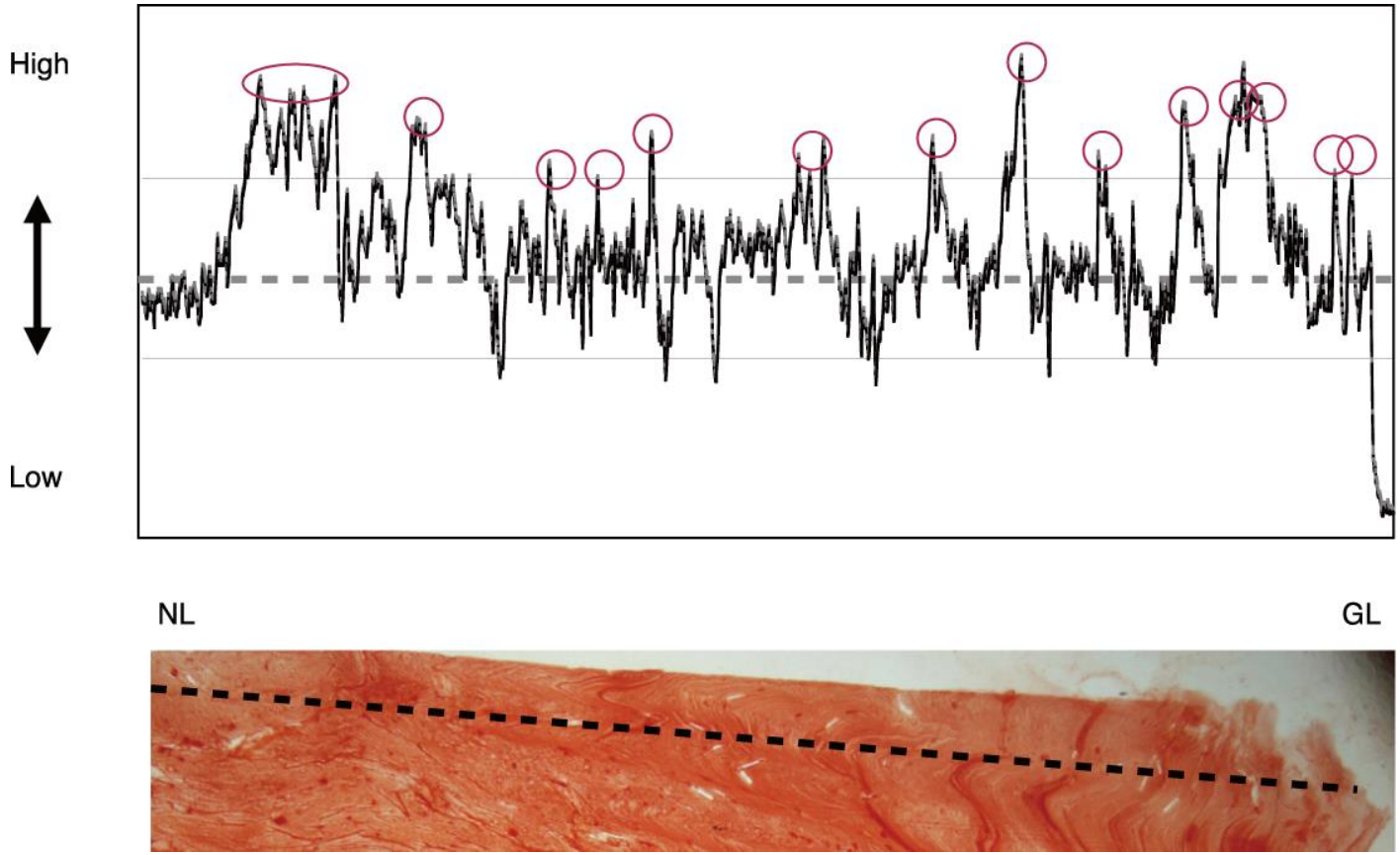


**Fig. 8.** The graph shows dark and light colouring on the dotted line in the lower photograph.

**Table 2.** Assessment of staining conditions for each staining solution.

| Staining solutions | Assessment |
|---|---|
| Toluidine blue | Poor |
| Hematoxylin & eosin | Poor |
| Sudan III | Moderate |
| Sudan VII | Moderate |
| Alizarin red S | Excellent |

## DISCUSSION

### Earplug collection using gelatin

Total earplug length and the difference between earplug and core length also increased with age. It was considered that the outer covering that covers the core was not present in younger earplugs, but it became thicker with age, which would stabilise the earplug. Since the NL, which is important for age estimation, is formed at the apical portion of the core or the border between the outer covering and the core, an earplug with an immature outer covering could break and the NL could be lost. Such cases tended to occur in individuals of less than 7 m body length during the standard collection procedure. No NLs were lost, and the fracture on the core was reduced when the earplugs were collected using gelatin. These results suggested that collecting earplugs using gelatin could be effective for individuals of less than 7 m body length and an immature outer core covering. Among individuals that had the entire suite of growth layers in both right and left earplugs, the proportion in which age could only be determined using the earplugs embedded in gelatin was highest in whales aged 1–3 years, which decreased until it was zero after the age of 12 years. Therefore, the collection method in which gelatin was used as the embedding agent was the most effective for the 1–3-year age class and less effective after the age of 12 years. As a result, collecting earplugs using gelatin would enable the age estimation rate to improve much more in young and small individuals that have soft earplugs with underdeveloped outer coverings. In contrast, we did not recognise much improvement in the age estimation rate for those individuals in the greater than 7 m body length class and those aged above 12 years. The outer covering was well developed, and the earplug itself was stabilised in these individuals; thus, the collection could be performed by the existing method without any loss or breakage of the NL. The decision to use gelatin for collecting earplugs should depend on the body length (< 7 m) and the earplug condition at the time of dissection of the external auditory meatus. The collection technique using gelatin can be employed in

accordance with the shape and hardness of earplugs for other species. However, each species has variously shaped and sized earplugs; therefore, it will be necessary to examine the specific features of other species earplugs in order to apply the collection method using gelatin.

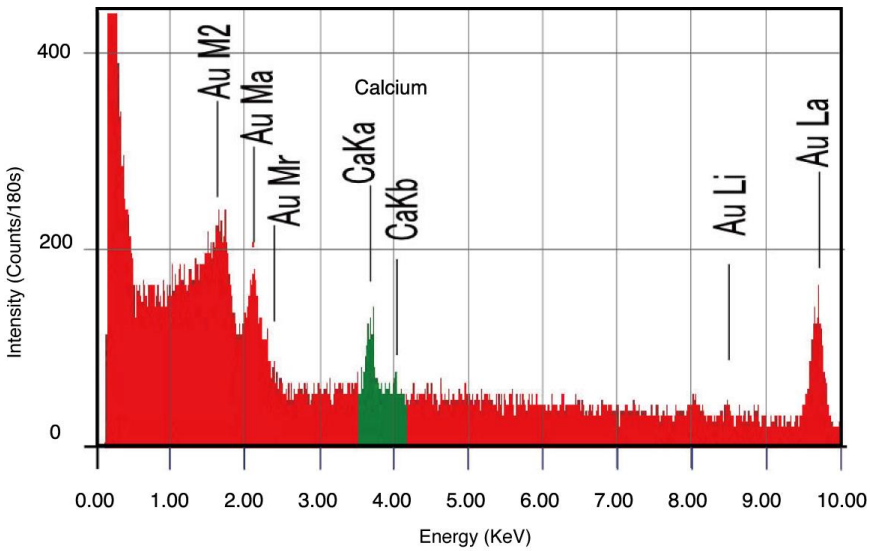


**Fig. 9.** The graph shows the analysis of a part of a well-stained area using energy dispersive X-ray spectrometry. Except for the gold used for pre-treatment, calcium was the only dominant component.

**Frozen sectioning and staining**

We found that alizarin red S was the best staining solution for identifying growth layers. Even with the use of image analysis software, it produced the same results as those of the standard method of age estimation. EDS analysis showed that calcium was contained in the dyed portion. It was also found that calcium accumulation was recorded as layers in the earplug and these layers corresponded to the growth layers. Thus, it could be possible that we can get further information from calcium content in earplugs. A past chemical analysis study using fin whale earplugs indicated that calcium tended to collect less in the dark layer and more in the light layer (Hamada *et al.* 1989). Since a relationship between formation of the growth layer and change in the calcium density has been suggested, the layers that dyed darker with alizarin red S reflected the lighter layer or a layer formed during the feeding period. Earlier age estimation studies focused on fat content in light layers, such as direct counting by the naked eye or Sudan staining; however, there is potential for improving the readability of unclear growth layers while using calcium. As this was a preliminary study, it will be necessary to further clarify the relationship between formation of the growth layer and change in the calcium density while increasing the number of

samples and directly assessing the calcium content along the growth layers using wavelength dispersive X-ray spectrometry.

In past studies, the techniques of X-ray photography (Masaki 1968, Lockyer 1974), bleaching the earplug with hydrogen peroxide (Lockyer 1974) and using an image processing system (Kato *et al.* 1988) were developed to improve the readability of earplugs in fin whales, sei whales (*Balaenoptera borealis*), and Antarctic minke whales (*Balaenoptera bonaerensis*). Unfortunately, these methods have not led to any practical use. However, the earplugs of common minke whales remain a valid age tool. Particularly, the methods presented in this paper to improve the earplug readings could make a significant contribution for calibrating the racemisation method for common minke whales. Therefore, it will be necessary to continue to improve the readability as much as possible from the collection stage to the age estimation stage.

## ACKNOWLEDGMENTS

We thank all the survey leaders of JARPN II; Mutsuo Goto, Takeharu Bando, Genta Yasunaga (Institute of Cetacean Research), Toshiya Kishiro, and Hideyoshi Yoshida (National Research Institute of Far Seas Fisheries) for supporting this study. We also thank the crew of the research vessels and staff of the research station. Finally, we are indebted to Yoshihiro Fujise (Director of the Institute of Cetacean Research) for his valuable support.

## REFERENCES

Auðunsson GA, Nielsen NH, Elvarsson BT, Víkingsson GA, Kato H, Halldórsson SD, Gunnlaugsson T and Hansen SH (2013) Age estimation of common minke whales (*Balaenoptera acutorostrata*) in Icelandic waters by aspartic acid racemization (AAR),-AAR and earplug readings of Antarctic minke whales (*B. bonaerensis*) used as a reference. IWC SC/65/SP15_Rev, 23 pp.

Bando T, Kato H, Kishiro T, Goto M, Yasunaga G, Saito T, Tabata S, Morita Y, Okamoto R, Maeda H, Inagaki M, Nagatsuka S, Ebisui T, Nakai K, Matsumoto A, Gokita A and Kawahara S (2008) Cruise report of the second phase of the Japanese whale research program under special permit in the western North Pacific (JARPN II) in 2007 – coastal component off Sanriku. Paper SC/60/O6 presented to the 60th IWC Scientific Committee meeting (available at IWC office), 25 pp.

Best PB (1982) Seasonal abundance, feeding, reproduction, age and growth in minke whales off Durban (with incidental observation from Antarctic). *Rep. Int. Whal. Commn.* 32:759-786.

Christensen I (1981) Age determination of minke whales, Balaenoptera acutrostorata, from laminated structures in the tympanic bullae. *Rep. Int. Whal. Commn.* 31:245-253.

Christensen I, Haug T and Wiig Ø (1990) Morphometric comparison of minke whales Balaenoptera acutorostrata from different areas of the North Atlantic. *Mar. Mamm. Sci.* 6(4):327-338. doi: http://dx.doi.org/10.1111/j.1748-7692.1990.tb00362.x

Gabriele C, Lockyer C, Straley MJ, Jurasz MC and Kato H (2010) Sighting history of a naturally marked humpback whale (*Megaptera novaeangliae*) suggests ear plug growth layer groups are deposited annually. *Mar. Mamm. Sci.* 26(2):443-450. doi: http://dx.doi.org/10.1111/j.1748-7692.2009.00341.x

Government of Japan. (2004) Fujise Y, Tamura T, Kato H, and Kawahara S, eds. Some considerations of the feasibility components of the JARPN II research plan. Paper SC/56/O2 submitted to the 56th IWC Scientific Committee (available at IWC office), 36 pp.

Hamada N, Yamazaki S, Toda S, Fujise Y and Zenitani Y (1989) X-ray analysis of earplugs from Antarctic minke whale. 38th The Japan society for Analytical chemistry, 25-26. In Japanese.

Ichihara T (1959) Formation mechanism of ear plug in baleen whales in relation to glove-finger. *Sci. Rep. Whales Res. Inst.* 14:107-135.

Ichihara T (1964) Prenatal development of ear plug in baleen whales. *Scient. Rep. Inst.* 18:29-48.

Kato H (1992) Body length, reproduction and stock separation of minke whales off northern Japan. *Rep. Int. Whal. Commn.* 42:443-453.

Kato H, Sone M, Kashiwa M and Tokiwa H (1988) Summary of preliminary experiments using an image processing system to enhance the age determination of the southern minke whale from earplug. *Rep. Int. Whal. Commn.* 38:269-272.

Kawamoto T (2003) Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. *Arch. Histol. Cytol.* 66(2):123-143. doi: http://dx.doi.org/10.1679/aohc.66.123

Kishiro T, Kato H, Yoshida H, Miyashita T, Iwasaki T, Kanaji Y, Ryono T, Tabata S, Morita Y, Okamoto R, Maeda H, Nagatsuka S, Ogawa N, Nakai K, Ebisui T, Matsumoto A, Gokita A, Kiwada H and Kawahara S (2008) Cruise report of the second phase of the Japanese whale research program under special permit in the western North Pacific (JARPN II ) in 2007 – coastal component off Kushiro. Paper SC/60/O7 presented to the 60th IWC Scientific Committee meeting (available at IWC office), 33 pp.

Kishiro T, Kato H, Yoshida H, Yasunaga G, Tabata S, Nakamura G, Maeda H, Inoue S, Miyakawa N, Okamoto R, Oikawa H, Watanabe H, Iwasaki T, Kanaji Y, Minamikawa S, Otani S, Ishikawa H, Kanda N,

Gokita A, Yamazaki K and Yonehara Y (2010) Cruise report of the second phase of the Japanese whale research program under special permit in the western North Pacific (JARPN II ) in 2009 (part II) – coastal component off Kushiro. Paper SC/62/ O6 presented to the 62nd IWC Scientific Committee meeting (available at IWC office), 13 pp.

Lillie DG (1910) Observation on the anatomy and general biology of some of members of the larger Cetacea. *Proc. Zool. Soc. Lond.* 80(3):769-792. doi: http:dx.doi.org/10.1111/j.1096-3642.1910.tb01916.x

Lockyer C (1972) The age at sexual maturity of the southern fin whale (*Balaenoptera physalus*) using annual layer counts in the ear plug. *J. Cons. Int. Explor. Mer.* 34(2):276-294. doi: http://dx.doi.org/10.1093/icesjms/34.2.276

Lockyer C (1974) Investigation of the earplug of the southern sei whale, *Balaenoptera borealis*, as a valid means of determining age. *J. Cons. Int. Explor. Mer.* 36(1):71-81. doi: http://dx.doi.org/10.1093/icesjms/36.1.71

Lockyer C (1984a) Age determination by means of the earplug in baleen whales. *Rep. Int. Whal. Commn.* 34:692-696.

Lockyer C (1984b) Review of baleen whale (Mysticeti) reproduction and implications for management. *Rep. Int. Whal. Commn.* (Special Issue 6):27-50.

Maeda H (2012) Investigation of the earplug in common minke whales as age characters and their age dynamics [Dr. Scient thesis]. Tokyo University of Marine Science and Technology, 122 pp. (In Japanese)

Maeda H and Kato H (2012) Technical improvements of the age character (earplug) collection in common minke whales. *Bull. Jpn. Soc. Fish. Oceanogr.* 76:59-65. (In Japanese)

Masaki Y (1968) A trial for reading of earplug lamination of the sei whale by means of soft X-ray. Paper presented to the International Whaling Commission meeting on age determination in whales, Oslo.

Nielsen NH, Garde E, Heide-Jørgensen MP, Lockyer CH, Ditlevsen S, Ólafsdóttir D and Hansen SH (2012) Application of a novel method for age estimation of a baleen whale and a porpoise. *Mar. Mammal Sci.* 29(2):E1-E23. doi: http://dx.doi.org/10.1111/j.1748-7692.2012.00588.x

Ohsumi S (1964) Examination on age determination of the fin whales. *Sci. Rep. Whales Res. Inst.* 18:49-88.

Purves PE (1955) The wax plug in the external auditory meatus of the Mysticeti. *Discovery Rep.* 27:293-302.

Roe HSJ (1967) Seasonal formation of laminae in the earplug of the fin whale. *Discovery Rep.* 35:1-30.

Sergeant D (1963) Minke whale, *Balaenoptera acutorostrata lacepede*, of the Western North Atlantic. *J. Fish. Res. Board Can.* 20:1489-1504. doi: http://dx.doi.org/10.1139/f63-101

Yasunaga G, Kato H, Kishiro T, Yoshida H, Ishikawa H, Goto M, Tabata S, Maeda H, Nakamura G, Inoue S, Miyakawa N, Tamura T, Kumagai S, Iwasaki T, Kanaji Y, Watanabe H, Hiruta H, Yamazaki K, Yonehara Y and Oikawa H (2010) Cruise report of the second phase of the Japanese whale research program under special permit in the western North Pacific (JARPN II ) in 2009 (part II) – coastal component off Sanriku. Paper SC/62/O5 presented to the 62nd IWC Scientific Committee meeting (available at IWC office), 20 pp.

Yasunaga G, Kato H, Kishiro T, Yoshida H, Nishiwaki S, Saito T, Tabata S, Okamoto R, Maeda H, Nakamura G, Inoue S, Otani S, Iwasaki T, Kanaji Y, Mogoe T, Murase H, Wada A, Nakai K, Matsumoto A, Gokita A, Yamazaki K, Oikawa H, Onodera K, Shiraishi K and Nagashima H (2009) Cruise report of the second phase of the Japanese whale research program under special permit in the western North Pacific (JARPN II ) in 2008 (part II) – coastal component off Sanriku. Paper SC/61/O6 presented to the 61st IWC Scientific Committee meeting (available at IWC office), 24 pp.

Yoshida H, Kato H, Kishiro T, Miyashita T, Iwasaki T, Kanaji Y, Yasunaga G, Oikawa H, Ryono T, Tabata S, Okamoto R, Maeda H, Mashumoto A, Ogawa N, Nagatsuka S, Inoue S, Hiruta H, Gokita A and Matunaga H (2009) Cruise report of the second phase of the Japanese whale research program under special permit in the western North Pacific (JARPN II ) in 2008 (part II) – coastal component off Kushiro. Paper SC/61/O5 presented to the 61st IWC Scientific Committee meeting (available at IWC office), 10 pp.