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SEASONAL CHANGES IN THE EARPLUG GERMINAL LAYERS OF NORTH PACIFIC COMMON MINKE WHALES

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Abstract

We investigated the annual accumulation rate of the most recently formed layer of earplugs, the germinal layer (GL), of the North Pacific common minke whales, which is important for age estimation. The pale GLs from Kushiro in September and October were found to be significantly thicker than those from Ayukawa in April and May. The results suggest that the pale lamina accumulated during an approximately six-month period from spring to autumn in their feeding season. This was consistent with the hypothesis that one pale and one dark lamina, comprising one growth layer group, are formed annually in this species.

Key words: earplug, common minke whale, age determination, germinal layer, accumulation rate.

The earplugs of baleen whales were first considered to show aging characteristics by Purves (1955). The external auditory meatus lumen is flattened dorso-ventrally, so that the dorsal and ventral epidermis walls are in apposition forming a "blind" section (Purves, 1955). Because of this, the earplug will never fall out throughout a whale's life. The earplug is composed of a "core" and an "outer covering" (Fig. 1). The core of the earplug (b in Fig. 2), where the growth layer group (dark lamina and pale lamina) is formed, is composed of exfoliated and keratinized epidermis of a finger-sack-like tissue called the glove finger (Fig. 1). It is surrounded by a layer of wax-like material from the external auditory canal membrane (outer covering, a in Fig. 2).

Currently, earplugs are used in age estimation for several species of baleen whales (Lockyer, 1984a; Gabriele *et al.*, 2010; Nielsen *et al.*, 2012; Kitakado *et al.*, 2013; Maeda *et al.*, 2016). Many baleen whales are known to migrate seasonally, breeding in low-latitude waters in winter and feeding in high-latitude waters in summer. It is thought that this annual cycle is reflected in earplug growth layers, with a pale lamina forming during the summer feeding season and a dark lamina forming during the winter breeding season (Fig. 2); therefore, one growth layer group represents one year (Roe, 1967;

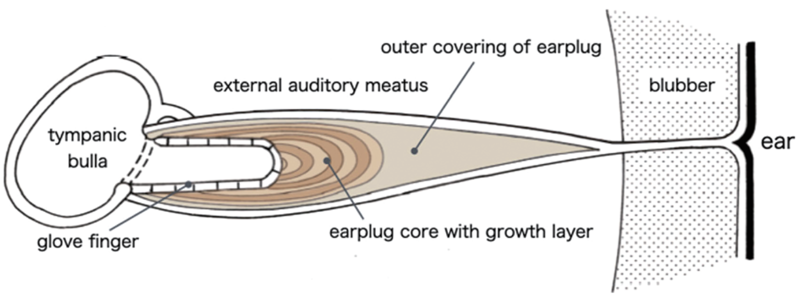

Fig. 1. Diagram showing the anatomical position of the earplug in a typical baleen whale. After Purves (1955).

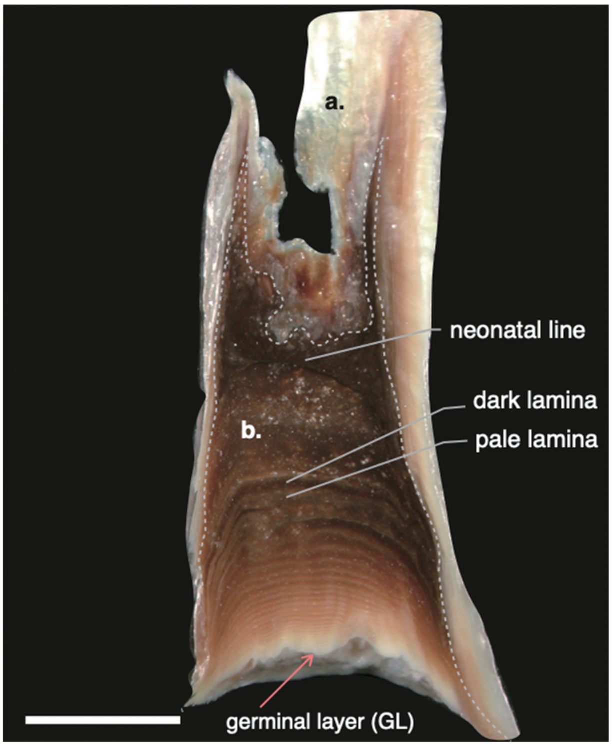

Fig. 2. Bisected surface of an earplug of a common minke whale. **a**: (outside the dotted line), outer covering; **b**: (inside the dotted line), core with growth layers. Scale bar represents: 5 mm.

Lockyer, 1972, 1984b). Fat content tends to be lower in the dark lamina and higher in the pale lamina according to histological observations of fin whale (*Balaenoptera physalus*) earplugs (Roe, 1967). Many other studies have examined whale earplugs; for example, Ohsumi (1964) investigated the annual accumulation rate of earplugs in southern-hemisphere fin whales using the mark-recapture method, and Gabriele *et al.* (2010) conducted a comparative study of the earplug growth layer and long-term individual identification in North Pacific humpback whales (*Megaptera novaeangliae*).

Previously, age estimation from the earplugs of the common minke whale (*B. acutorostrata*) was generally thought to be difficult and impractical because of the softness of the earplugs and the 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). Under the Japanese Research Program and a special permit in the Western North Pacific (JARPNII), common minke whale earplugs were carefully collected using the gelatinized extraction method (Maeda *et al.*, 2013) and attempts were made to read the growth layer groups. Maeda *et al.* (2016) reported age readability and age estimation errors in common minke whales and concluded that the earplugs of this species can be used as a valid age estimation tool.

However, the annual accumulation rate of growth layers has not been analyzed for North Pacific common minke whales, and age estimation was conducted on the assumption that one growth layer group is formed per year as in other baleen species. In the case of the common minke whale, it is important to understand the periodicity of growth layer formation for age estimation. In this study, we focused on the recently formed layer, the germinal layer (GL), of the earplug and aimed to determine the periodicity of growth layer formation based on seasonal changes in the GL (Fig. 2).

Earplugs obtained from JARPNII Coastal Components from 2003 to 2016 were used. These surveys were conducted off Ayukawa (the Pacific coast of northern Honshu; within a 50-mile radius of Ayukawa Port 38°17′N, 141°30′E) in April and May and off Kushiro (the Pacific coast of eastern Hokkaido; within a 50-mile radius of Kushiro Port 42°59′N, 144°22′E) in September and October (Fig. 3).

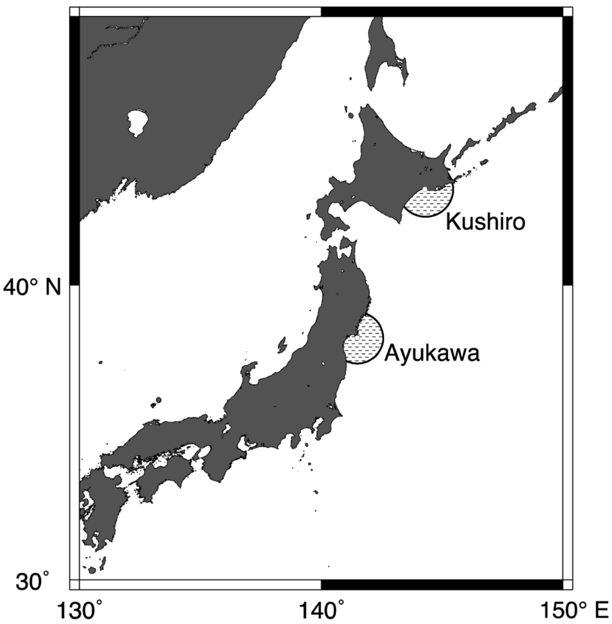

Fig. 3. Survey areas off Ayukawa (the Pacific coast of northern Honshu; within a 50-mile radius of Ayukawa Port 38°17′N, 141°30′E) in April and May, and off Kushiro (the Pacific coast of eastern Hokkaido; within a 50-mile radius of Kushiro Port 42°59′N, 144°22′E) in September and October.

Table 1. Pale and dark ratio of germinal layer (GL) of the earplug of common minke whales in Ayukawa and Kushiro.

	Number of individuals	Pale (%)	Dark (%)	Unknown (%)
Ayukawa (April to May)	175	111 (63.4)	63 (36.0)	*1 (0.6)
Kushiro (September to October)	185	185 (100)	0 (0.0)	0 (0.0)

*The GL was very thin and the type of the color could not be classified.

The earplugs were preserved in 10% neutral buffered formalin solution. In the laboratory, after cutting flat along the central axis of the earplug by using a sharp blade, the earplug was ground on a wet stone under running water to expose the neonatal line and growth layers (Fig. 2). Growth layers were counted underwater using a stereoscopic microscope (magnification: 3.2–31.5). The age in years was defined as one growth layer group comprising a pair of pale and dark laminae in the core. The earplugs of 360 individuals with clear growth layers from the neonatal line to the GL were selected and observed to determine whether the GL was pale or dark (Ayukawa: 175 individuals; Kushiro: 185 individuals; age range: 1 to 44 years). For 204 of these individuals, the thickness of the pale GL was measured using an image analysis software (ImageJ, a public domain, Java-based image processing program, National Institute of Health, USA).

Pale GLs were observed in 63.4% of the earplugs from Ayukawa in April and May and in 100% from Kushiro in September and October (Table 1). For the pale GLs, the mean thickness from Ayukawa was 0.21 mm, while that from Kushiro was 0.40 mm (Fig. 4A). The pale GLs from Kushiro were significantly thicker than those from Ayukawa (t-test; $p<0.05$).

Since the thickness of the GL is expected to compress as the number of growth layers increases, the thicknesses of the pale GLs from Ayukawa and Kushiro were compared with the number of growth layers. The GL thickness from both Ayukawa and Kushiro decreased as the number of growth layers increased, but the Kushiro GLs were thicker than the Ayukawa GLs in all growth layer number classes (Fig. 4B). In growth-layer classes 1–5, 6–10 and 11–15, the pale GLs from Kushiro were significantly thicker than those from Ayukawa (t-test; $p<0.05$).

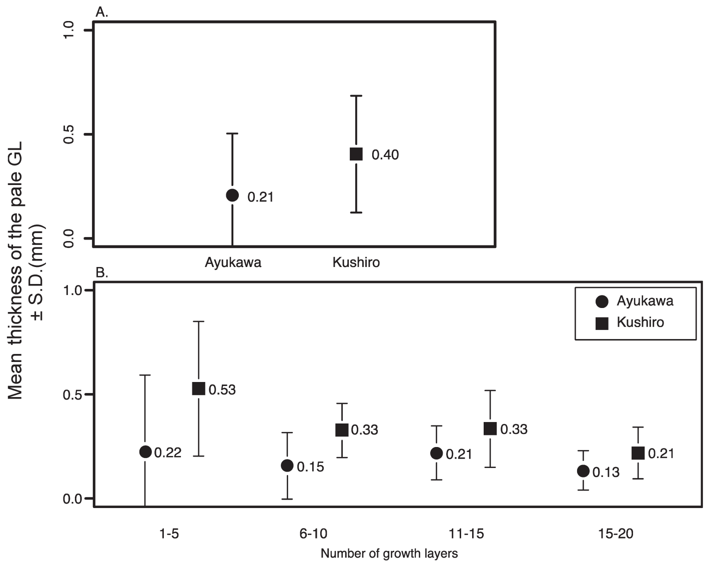

Fig. 4. **A**: Comparison of mean thickness of the pale GL (mm) between Ayukawa in April to May and Kushiro in September to October. **B**: Comparison of mean pale GL thickness (mm) between Ayukawa in April to May and Kushiro in September to October by type of growth layers.

Maeda *et al.* (2013) measured the length of earplugs in the North Pacific common minke whale and examined the growth of the earplug (the core and the outer covering) with age, and found that the outer covering that covers the core was not present in younger earplugs, but it became thicker with age. In younger individuals (with fewer layers), the outer covering has not yet formed and there is more space in the ear canal; the wax pushed out of the glove finger is not compressed and forms a thicker layer (although younger individuals have a better metabolism). However, as the number of layers increases, it was considered that the outer covering developed and the core of the earplug becomes larger; there is less space in the ear canal and the thickness of the GL formed by extrusion from the glove finger becomes thinner.

Lockyer (1972) examined fin whales from the southern hemisphere and observed a distinctly spaced growth layer that diminished suddenly, which was called the transition phase. The transition phase in the earplug, where widely spaced growth layers abruptly become much closer together, indicates the age at sexual maturity. Maeda *et al.* (2017) reported the transition phase could be identified in the North Pacific common minke whale and the mean age at transition phase was about 7 years old for both males and females. While some individuals showed the transition phase (rapid narrowing of the layer interval) as early as 4 years of age (Maeda *et al.*, 2017), the large number of young individuals with relatively wide stratum widths suggests that the SD were greater in the 1–5 years old group.

Hatanaka and Miyashita (1997) reported that immature minke whale individuals are distributed along the northern Pacific coast in early summer; mature females are abundant in high-latitude waters (Sea of Okhotsk) in summer; and mature males are distributed south of mature females in summer, mainly east of the Kuril Islands, and are abundant off eastern Hokkaido at the end of summer. To examine seasonal changes in the accumulation of the growth layers, the feeding season migration route of minke whales was assumed to be as follows. In early summer, minke whales migrate northward along the northern Pacific coast to feed, then migrate to Sanriku off Ayukawa in April and May, which is the early feeding season, and then further northward to their main feeding grounds. They then migrate southward to the waters off Kushiro in September and October, which is the latter half of the feeding season.

While individuals with dark GLs were observed in the early feeding season in Ayukawa, no individuals with dark GLs were observed in the late feeding season in Kushiro. The pale GL was significantly thicker in Kushiro than in Ayukawa, suggesting that a pale layer accumulated during the feeding season from spring to autumn, which lasts approximately six months. This was consistent with the hypothesis that the growth layer (one pair of pale and dark laminae) is accumulated once a year in common minke whales, as in fin whales and other species.

In this study, earplugs with clear growth layers were used. However, Maeda *et al.* (2016) reported that age readability of North Pacific common minke whale was 45.2% for male, 41.2% for female, and most of unreadable earplugs of mature animals had growth layers with unclear formation such as irregular lamination and partially-formed growth layers. Individuals with irregular growth layer groups may not have a clear seasonal migration, and this point requires further investigation.

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