Age estimation of male Stejneger’s beaked whales (*Mesoplodon stejnegeri*) based on counting of growth layers in tooth cementum

Kazumi Arai¹, Tadasu K. Yamada²,* and Yoshiro Takano¹

¹Graduate School of Tokyo Medical and Dental University, Biostructural Science, Yushima 1-5-45, Tokyo 113-8549, Japan
²The National Science Museum, Tokyo, Hyakunin-cho 3-23-1, Tokyo 169-0073, Japan

Abstract. The age of six mature and one juvenile Stejneger’s beaked whales *Mesoplodon stejnegeri* were estimated by examining the growth layers appearing in ground thin sections of tooth cementum under the light microscope. In order to determine a reliable observation method for counting the growth layers of tooth cementum, serial thin slices of root cementum were cut out from one well-grown tooth and examined using various histological methods. Observation of ground thin sections under dark field illumination was shown to give the highest contrast of growth layers of various dimensions, and hence chosen as the method to examine whole ground sections of the tooth samples. Using this method, growth layer groups (GLGs), or growth layers of the first order, most probably representing yearly deposition of cementum, were clearly identified and shown to decrease in width toward the root surface. The number of GLGs thus counted in the tooth cementum of each whale ranged from 15 to 35.5 for the adults, and two for the juvenile. Furthermore, analysis of root elongation rate and its relation to GLG counts of the individual teeth indicated a wide variety of growth patterns in tooth development, and that the extent of characteristic wear on the mesial edge of the tooth represents the period after eruption, and may not reflect the actual age of the whales.

Key words: age estimation, cementum, growth layer, *Mesoplodon stejnegeri*.

Several age estimation methods for odontocetes using teeth have been proposed; these methods have varied depending on the structure of the teeth examined. Some targeted mainly dentine (Sergeant 1959; Christensen 1973; Pierce and Kajimura 1980; Hohn et al. 1989), while others focused on cementum (Kasuya 1977) or both tissues (Kasuya 1976; Lockyer 1993; Herman et al. 1994). In these studies, observations were made of: untreated ground sections; “etched” surfaces of bisected tooth; decalcified ground sections stained with hematoxylin; or frozen sections of decalcified specimens stained with hematoxylin. Either reflected light or transmitted light was used for macroscopic or microscopic observations of these specimens.

The growth layers in dentine or cementum in histological preparations can be distinguished as: alternately arranged translucent and opaque layers under transmitted light; dark and bright layers under dark field illumination; layers of different staining intensity with hematoxylin; ridges and grooves on the etched and dried surface; or layers of different X-ray translucency, dependent on the methods used. One growth layer group (GLG) (Perrin and Myrick 1980), which Klevezal (1996) defined precisely as a growth layer of the first order, is known to be produced annually in some species such as bottlenose dolphin *Tursiops truncatus* (Sergeant 1959), Baird’s beaked whale *Berardius bairdii* (Kasuya 1977), common dolphin *Delphinus delphis*, sperm whale *Physeter macrocephalus*, short-finned pilot whale *Globicephala macrorhynchus* (Kasuya and Matsui 1984) and spinner dolphin *Stenella longirostris* (Myrick et al. 1984).

Despite an abundance of reports on many cetacean species, little is known about the Genus *Mesoplodon*, which has the widest variety of species in the Family Ziphiidae or even all cetacean families. In recent years Stejneger’s beaked whale *Mesoplodon stejnegeri* has been found to strand frequently along the Japanese coasts of the Sea of Japan (Nagasawa and Yamada 1997;
Various studies of the species, including toxicology (Tanabe et al. 2003), molecular biology (Kakuda and Yamada 2003), nutrition (Komura et al. 2002), reproductive biology (Maeda et al. 2002) and pathology (Tajima 2001) have recently begun. For further analysis of this species, precise age estimation is essential.

Stejneger’s beaked whale, as in most of the Ziphiid species, has a pair of teeth in the lower jaw, which erupt only in adult males. The tooth of a mature male has a bucco-lingually flat trapeziform shape with a triangular cusp on its tip, and a thick root with parallel margins at its mesial (anterior) and distal (posterior) ends. A characteristic semilunar abrasion is formed on the mesial margin of the tooth right above the gum line. The root consists almost entirely of cementum, and ageing causes further deposition of irregular cementum on its surface. The only available description of age estimation of this species was reported by Perrin and Myrick (1980), but without any histological data. These authors strongly recommended using a combination of “etching” bisected tooth and ground thin sectioning, and their observation by obliquely reflected or transmitted light, targeting tooth cementum. The purpose of this study was therefore to establish a reliable method of tooth-based age estimation for Stejneger’s beaked whale and, using the method, to estimate the actual age of several individuals of this species.

**Materials and methods**

Six teeth (specimens A to F) each from adult males, and one (specimen G) from a young female, were sampled from different individuals, all stranded on the Japanese coasts of the Sea of Japan (37°07’–39°48’N) (Table 1). Adult whales were physically mature with fused vertebral epiphyses. The adult whale’s teeth were boiled for cleaning and kept in a dry condition until use. All the adult teeth thus obtained were classified in four categories by the extent of abrasion on the mesial margin: (−) no abrasion; (+) abrasion within cementum; (++) abrasion with exposure of dentine; (+++) abrasion with exposure of pulp cavity. Surface irregularity of root cementum was classified in three categories (−, +, ++). The pulp cavity is almost occluded in early tooth development resulting from the deposition of irregular dentine, whereas cementum deposition continues throughout the animal’s life. Therefore, the cementum was chosen as the region for age estimation as suggested by Perrin and Myrick (1980). Test samples for the evaluation of age estimation methods were taken from specimen D, which was fully erupted with little abrasion (+) and had light cementum deposition on the root surface. Specimen G was excised as a fresh specimen from a young female and kept in 10% formalin.

### Table 1. List of materials used in this study.

<table>
<thead>
<tr>
<th>Tooth Specimen</th>
<th>Size (H × W × T mm)</th>
<th>Abrasion</th>
<th>Irr. Cem.</th>
<th>GLG No.</th>
<th>Museum Number</th>
<th>Found Date</th>
<th>BL (cm)</th>
<th>Locality (Prefecture)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>L 163+ × 90 × 26</td>
<td>+++</td>
<td>++</td>
<td>30–35.5</td>
<td>NSMT M24661</td>
<td>1960.05.13</td>
<td>UN</td>
<td>Akita (Akita)</td>
<td>M</td>
</tr>
<tr>
<td>B</td>
<td>L 140+ × 94 × 17</td>
<td>+++</td>
<td>++</td>
<td>21–25</td>
<td>NSMT M32565</td>
<td>2000.03.12</td>
<td>466</td>
<td>Washizaki (Niigata)</td>
<td>M</td>
</tr>
<tr>
<td>C</td>
<td>R 137+ × 84 × 19</td>
<td>+</td>
<td>+</td>
<td>26.5</td>
<td>NSMT M32497</td>
<td>1996.02.01</td>
<td>510</td>
<td>Abraroto (Yamagata)</td>
<td>M</td>
</tr>
<tr>
<td>D*</td>
<td>R 125+ × 95 × 15</td>
<td>+</td>
<td>+</td>
<td>15.0</td>
<td>NSMT M30137</td>
<td>1996.04.08</td>
<td>519</td>
<td>Niigata (Niigata)</td>
<td>M</td>
</tr>
<tr>
<td>E</td>
<td>R 110+ × 100 × 15</td>
<td>+++</td>
<td>++</td>
<td>30.0</td>
<td>NSMT M32510</td>
<td>1999.06.20</td>
<td>498</td>
<td>Yonago (Yamagata)</td>
<td>M</td>
</tr>
<tr>
<td>F</td>
<td>R 112+ × 69 × 12</td>
<td>+</td>
<td>–</td>
<td>15.5</td>
<td>NSMT M32507</td>
<td>1998.10.21</td>
<td>446</td>
<td>Koikawaka (Yamagata)</td>
<td>M</td>
</tr>
<tr>
<td>G</td>
<td>L 42 × 46 × 8</td>
<td>–</td>
<td>–</td>
<td>2.0</td>
<td>NSMT M32691</td>
<td>2001.04.13</td>
<td>296</td>
<td>Mano (Niigata)</td>
<td>F</td>
</tr>
</tbody>
</table>

*Used for evaluation of method. Museum No.; registration number of the National Science Museum, Tokyo. Irr. Cem; irregular cementum deposition on the root surface.

**Preparation of test specimen**

To determine the most reliable and practical method for tooth-based age estimation for Stejneger’s beaked whale, serial sections of cementum were made and different observation techniques were compared. Specimen D was cut bucco-lingually through the plane slightly distal from the cusp (Fig. 1a) using a diamond saw E300 (EXAKT, Norderstedt, Germany). Subsequently the distal half of the tooth was cut horizontally at the middle level of the tooth followed by a further cut 1 cm below the first horizontal cut, taking out a 1 cm thick block (Fig. 1b, c). This block of tooth consisted mainly of cementum, in which thin irregular dentine and a narrow pulp cavity were sandwiched in between thick cemen-
Arai et al., Age estimation of Mesoplodon stejnegeri

The block was then sliced into 1.5 mm thick serial test pieces parallel to the first vertical cut (Fig. 1d). All test pieces showed similar arrangement of incremental layers of various dimensions on its surface under the microscope, the thick incremental layers near the pulp area were produced when young and the thin layers near the surface area were deposited when the animal was older.

Thin ground sections. The distal sides of some test pieces were polished with waterproof abrasive papers (#800–2000), further with lapping papers (grading; 3.0–9.0 μm), and glued on to acrylic plates (76 × 26 × 2 mm) with cyanoacrylate adhesives. The glued test pieces were then ground into 50–100 μm thick sections, and fixed in 4% formalin in 0.1 M sodium phosphate buffer (pH 7.4) for 2–3 days. After a thorough examination under the microscope as described below, the surface of one of the ground sections was lightly etched in 1% HCl for 2 min, rinsed in running water, stained with Bömer’s hematoxylin at a dilution of 1 : 10 for 15 min, and mounted in 50% glycerine (light etching). Another ground section was totally decalcified in 5% EDTA for 3–4 hr, stained with Bömer’s hematoxylin at a dilution of 1 : 10 for 1 hr, rinsed and mounted in 50% glycerine according to Kasuya (1977).

For contact microradiography, a 50 μm thick ground section attached to the acrylic plate was put into acetone to dissolve the acrylic plate. The freed ground section was placed on a PELICULA FILM (Kodak, NY., U.S.A.) and a contact microradiogram was taken by irradiating soft X-rays at 20 kV, 4 mA for 24 min in a cabinet type X-ray apparatus SRO-M50 (SOFRON, Fig. 1. Orientation of Stejneger’s beaked whale tooth for preparation of test pieces. (a) Lingual view of a fully erupted tooth (specimen D) of a male Stejneger’s beaked whale showing slight abrasion (arrow) right above the tooth gum (dotted curve) and mild deposition of irregular cementum on the root surface. The straight line indicates the direction of the first bucco-lingual cut plane (1st. CP) through the cusp region whereby the tooth was bisected. (b) A diagram showing the structure appearing on the first bucco-lingual cut surface of the distal half of the tooth. The second horizontal cut at the middle level and the third one 1 cm below the second cut are drawn with solid lines. (c) One cm thick tooth block and 1.5 mm thick test pieces cut from the block. (d) Coronal surface of the one cm thick tooth block (upper panel) and serial test pieces cut from the block (lower panel). Note some incremental lines on the coronal surface of the block as well as the cut surfaces of the serial slices. M, mesial (anterior); L, lingual; C, cementum; D, dentine; P, pulp cavity.

Table 2. Summary of evaluation of preparation and observation methods on visibility of GLGs.

<table>
<thead>
<tr>
<th></th>
<th>Transmitted light</th>
<th>Dark field illumination</th>
<th>Angled reflected light</th>
<th>Contact microradiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin ground section</td>
<td>-untreated</td>
<td>○</td>
<td>NA</td>
<td>●</td>
</tr>
<tr>
<td></td>
<td>-lightly etched and stained</td>
<td>○</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>-totally decalcified and stained</td>
<td>○</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Thick ground section</td>
<td>-deeply etched</td>
<td>NA</td>
<td>NA</td>
<td>●</td>
</tr>
<tr>
<td>Cryosection</td>
<td>-stained</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

○, low contrasted; ◎, highly contrasted; ●, not distinct; NA, not applicable.

127
Tokyo, Japan) (Table 2).

Thick ground sections. Several other test pieces glued on to acrylic plates were ground to 500 μm and fixed in buffered 4% formalin as already mentioned. For observations of surface topography, the surfaces of the specimens were then deeply etched with 10% formic acid for

Fig. 2
2 hr or with 0.5% HCl for 1 hr, rinsed in running water, and air dried after staining with Mayer’s haematoxylin for 1 hr or without staining. This method is equivalent to the “etching” procedure of Perrin and Myrick (1980). We use the term “deep etching” to discriminate this method from the former “light etching”.

Cryosections of decalcified specimen. Several 1.5 mm thick test pieces cut out from the block of cementum were immediately fixed in 4% buffered formalin, decalcified in 5% EDTA for three weeks, placed in 30% sucrose over night, and processed for cryosectioning to make 40 μm thick sections. The free-floating cryosections were stained either with Mayer’s haematoxylin for 1–1.5 hr or with Bömer’s haematoxylin at a dilution of 1 : 10 for 1 hr, and mounted in 50% glycerine.

All samples thus prepared were examined using a standard light microscope or a stereomicroscope M420 (LEICA, Wetzlar, Germany) under transmitted light, or under angled reflected light (Fig. 1a) using the diamond saw. To observe all growth lingually through the plane slightly distal from the cusp (Fig. 1a) using the diamond saw. To observe all growth layers in the cementum, the cut surface of the mesial half of each tooth specimen was ground until the plane through the cusp or the putative cusp tip was obtained, and polished. The polished surface was then glued on to a large acrylic plate (150 × 50 × 3 mm) with cyanoacrylate adhesives. Subsequently, the tooth specimen was cut out using the diamond saw along the acrylic plate, leaving a 1 mm thick section adhering to the acrylic plate. The adhering section was then ground to 50–120 μm thick, polished and mounted in 50% glycerine. As the best contrast of the growth layers was obtained under dark field illumination from these ground sections (see results), counting was done under this illumination several times at an interval of several weeks with the understanding that a GLG can follow parallel to the root surface and is more distinct than growth layers of the second order (Perrin and Myrick 1980).

Tracing GLGs

The ground specimens were placed in a photographic enlarger 45MXT (BESELER, NJ, U.S.A.), and the enlarged image was printed on Fuji projection paper Type C (Fuji Photo Film, Tokyo, Japan). The outline of the individual GLG was then traced and pencilled on the printed image while examining the sections under dark field illumination. The image was then erased using Weiger’s variation of Lugol’s solution (Bock and Shear 1972), followed by treatment with photographic fixative to leave only pencil outlines of GLGs.

Vertical growth rates of the teeth

In order to evaluate the rate of root elongation and its relation to the timing of GLG formation in the individual teeth, the vertical distance of every GLG was measured along the dentinocementum junction, and plotted in order of the length of time (in order of the GLG number).

Results

Evaluation of tissue preparation and observation methods

Different preparations and observation methods affected the visibility of GLGs in test specimens (see Table 2 for a summary).

Fig. 2. A comparison of microscopic views of incremental lines appearing in the test pieces (a–e) and a panoramic view of a whole sized ground section (f) of specimen D. The black dot in each figure (a–e) indicates identical growth layer. (a) Higher magnification of the boxed area in (f) viewed under dark field illumination. More than 7 GLGs (7th–13th GLGs), each consisting of a pair of bright and dark growth layers, are counted. The GLGs in superficial regions become progressively narrower and have fewer growth layers of the second order. In order to evaluate the rate of root elongation and its relation to the timing of GLG formation in the individual teeth, the vertical distance of every GLG was measured along the dentinocementum junction, and plotted in order of the length of time (in order of the GLG number).

Results
**Thin ground sections.** When the undecalcified ground section (50 μm thick) of the test piece was examined under dark field illumination, highly contrasting GLGs, each consisting of a pair of distinct dark and bright layers, were observed (Figs. 2a and 2f). In each GLG, a variable number of finely contrasting growth layers of the second order (Klevezal 1996) could be distinguished at a higher magnification (Fig. 2c). The GLGs near the pulp area were thick and easily distinguishable in all test pieces, however under standard transmitted light, fine GLGs in the surface layers became indistinct, and smaller number of GLGs of translucent and opaque layers relative to those observed under dark field illumination could be distinguished within the same section (cf. Figs. 2c and 2e). When the same ground section was further examined under standard transmitted bright light after light etching and hematoxylin staining, GLGs of hematoxylin stained and unstained layers were distinguished. These GLGs of hematoxylin stained and unstained layers corresponded to the bright and dark layers shown under dark field illumination, and the opaque and translucent layers under standard transmitted illumination, respectively. The GLG number and clarity was the same as that observed under standard transmitted light without treatment (cf. Figs. 2d and 2e), but less than that observed under dark field illumination.

When the ground section was examined after total decalcification and hematoxylin staining, the images of moderately stained and less stained layers similar to those observed in the lightly etched and stained specimen was obtained.

Contact microradiograph of other ground sections did not show clear images of GLGs comparable to those shown in Figs. 2a and 2c.

**Thick ground sections.** In deeply etched sections (500 μm thick), only three wide GLGs of ridges and grooves

---

**Fig. 3.** Diagrams of cemental GLGs traced on the printed images of undecalcified ground sections. Dark and bright layers appearing under dark field illumination are drawn as black and white lines, respectively. Arrows indicate the level of the apical end of the first dark layer in the cementum.
Arai et al., Age estimation of Mesoplodon stejnegeri

were recognized near pulp regions under angled reflected light. No growth layers could be seen in the outer regions of the specimen toward the cementum surface, where the cementum matrix was evenly stained with hematoxylin and did not show any layering patterns.

Cryosections of decalcified specimen. After hematoxylin staining, 40 μm thick cryosections showed the same number of GLGs as observed in lightly etched and stained sections, each comprising a pair of moderately stained and less stained layers under standard transmitted light (Fig. 2b). The overall optical contrast of growth layers of the second order in these sections was lower than that in the ground sections observed under dark field illumination. Correlation between the hematoxylin stained and less stained layers with the bright and dark layers was rather clearly visible (cf. Figs. 2b and 2c).

Taken together, these data clearly indicate that dark field illumination of ground sections reveals the most numerous GLGs and also the finer structure as growth layers of the second order. Accordingly, all the teeth to be examined in this study were then made into whole ground sections and examined mainly under dark field illumination (Fig. 2f).

GLG counting in the cementum of whole tooth ground sections

The GLG number was counted in the whole ground section under dark field illumination, with the aid of enlarged traces of growth layers as shown in Fig. 3, starting from the first dark layer exterior to the dentinocementum junction up to the root surface, where the maximum number was recorded. Since the innermost cemental layer along the dentinocementum junction always appeared bright under dark field illumination, a score of 0.5 GLG was added in case the most superficial layer of the cementum ended as a bright layer.

Variations in the thickness of dark layers of GLGs in the middle area of cementum

Among the growth layers of the first order of the teeth from adult whales, there were two variations in the type of dark layers; one that increased in thickness toward the root apex (thick layer); and another that retained its thickness throughout the length (thin layer). There was no tendency in the arrangement of these two types of dark layers in each specimen. Dark layers of the first order in specimens D and F mainly consisted of thick
Fig. 5. Ground section of specimen G. (a) Whole ground section of specimen G viewed under dark field illumination. Three boxed areas in (a) respectively coincide with (b), (c) and (d), in which a standard transmitted light image (upper tile) and a dark field image (lower tile) are combined. Pulp-dentine border, dentinocementum junction, and cementum surface were indicated by the solid vertical or oblique bars from left to right respectively. White arrowheads in dentine indicate the first distinct opaque layer in standard transmitted light. The same layer is indicated by the arrowhead in dark field image (d). This layer is not clearly visible under dark field illumination for unknown reasons (b, c). The dotted bars in the cementum indicate the first dark layer bordering the first and the second GLG. Numbered horizontal bars indicate GLGs in cementum. E, enamel; D, dentine; C, cementum; P, pulp; PL, periodontal ligament.
layers, specimens C and E had alternate repeating patterns of one thick and one thin layer, while specimens A and B had alternately arranged groups of several thick layers and several thin layers (Fig. 3).

**Distinction of GLGs in the surface layers**

In all adult teeth, the width of GLG gradually became narrower and irregular in profile toward the root surface accompanied by a decrease in optical contrast of the growth layers of the second order in the individual GLGs (Fig. 4a). The narrow GLGs were observable from the coronal forming end to the root apex, but some outermost GLGs appeared to be discontinuous (Fig. 4a). In both specimens A and B, some outermost GLGs were shown to merge into a single layer or to branch out (Fig. 4b). Accordingly, the GLG counts in these specimens differed depending on the regions chosen for counting. Although the true age is not verifiable, we assume the largest number represents the actual age (Table 1).

**The first formed growth layer in dentine and cementum**

The bucco-lingual view of specimen G (un-erupted tooth from a young female) was triangular in profile, having a thin enamel covering at the tip of the cusp (Fig. 5a). When we observed dentine in the ground section of this specimen (G) by regular transmitted light, there was a distinct opaque growth layer located at the boundary between the area of obscure growth layers and the interiorly located area of sharp growth layers (white arrowheads in Fig. 5b, 5c and 5d). Under dark field illumination, the first formed cementum along the dentinocementum junction appeared as a thick bright layer followed exteriorly by a thin distinct dark layer, whose apical end was located coronally from that of the distinct opaque layer in the dentine described above (Fig. 5d). Exterior to these thick bright and thin distinct dark layers was a pair of a bright layer and a very thin dark layer. The former extended only one-third of the length from the root apex toward the cusp, but contained some vague growth layers of the second order. The latter extended from the cementoenamel junction to the root apex. Accordingly, the two pairs of bright and dark layers in the cementum of specimen G were considered to be GLG 1 and GLG 2. The pattern of initial deposition of the first thick bright layer followed by a thin distinct dark layer of cementum along the dentinocementum junction was also confirmed in all adult tooth specimens.

**Vertical growth rate of the teeth**

The vertical height of every GLG was plotted against GLG number (Fig. 6). Data indicated that the roots of all teeth undergo considerable growth in early development (growth phase), but the speed of root elongation apparently slowed down at some point of development, reaching a plateau phase. Specimens A, B and E reached the plateau phase when the 20th, 14.5th and 16.5th GLG was formed, respectively. Specimens C and D were in the beginning of plateau phase at the 22.5th and 10.5th GLG.
sections of decalcified test pieces show more stainability clear enough to distinguish fine GLGs. Although cryo-ability of the surface layers of the root cementum is not (Kasuya 1977). With this method, however, the stainability of growth layers. Total decalcification of thin ground sections is a method commonly applied to cetacean teeth of growth layers. The light etching treatment of the ground surface of hard tissues is generally contained several finely contrasting growth layers of the second order. This method is not only simple and reliable compared to the other regular decalcification and staining processes, but represents a new trial for age estimation of whales. We therefore propose that, for age estimation of male Stejneger’s beaked whale, observation of the ground section of tooth cementum under dark field illumination is the most reliable and recommendable method for the present. Further observations of teeth at different age stages in both sexes are strongly recommended to support the viability of this method for age estimation of this species.

We confirmed that the dark layer, appearing under dark field illumination, and the translucent layer under standard transmitted light, appearing in the ground section, corresponds to the chromophobic layer in lightly etched and hematoxylin stained sections. In the case of the spotted dolphin, however, the dentinal hematoxylin stainable layer was reported to correspond to the translucent layer shown in the ground section (Kasuya 1976). The same relationship between the hematoxylin stainable layer and the translucent layer in the cementum has also been reported in the teeth of the bottlenose dolphin, the northern fur seal Callorhinus ursinus, the Caspian seal Pusa caspica (Klevezal 1996) and the Japanese macaque Macaca fuscata (Hachiya and Ohtaishi 1994). Our observations apparently disagree with these previous

Discussion

Evaluation of methods for age estimation

The thick GLGs in the inner regions of the root cementum of this species are readily distinguished by most of the methods used in this study under low magnifications, but the GLGs drastically decrease in their thickness and become difficult to discern near the root surface. Indeed precise counting of narrow indistinct GLGs in surface layers is critical for the accuracy of age estimation. Accordingly, suitability of the preparation and observation methods for age estimation should be evaluated by the findings at the cementum layers near the root surface.

All the methods were compared considering the read-ability of the structure near the root surface. The light etching treatment of the ground surface of hard tissues is a common method for the observation of microstructures of human teeth. It exposes organic substances that have been masked by dense deposits of mineral crystals, and makes some of the growth layers stainable with dyes and detectable by light microscopy. On the ground sections of the cementum of Stejneger’s beaked whale’s teeth, however, light etching does not improve the stainability of growth layers. Total decalcification of thin ground sections is a method commonly applied to cetacean teeth including the large tooth of Baird’s beaked whale (Kasuya 1977). With this method, however, the stainability of the surface layers of the root cementum is not clear enough to distinguish fine GLGs. Although cryo-sections of decalcified test pieces show more stainability of GLGs to hematoxylin than the totally decalcified thin ground sections, the cyclic repetition of growth layers of the second order is not clearly observable.

The deep etching treatment of thick ground sections, which is followed by air-drying of the decalcified surface, enhances surface topography reflecting the different matrix structures and/or matrix constituents between the growth layers in the cementum. Perrin and Myrick (1980) suggested the necessity of combined usage of this method viewed by obliquely reflected light and observation of ground sections by transmitted light for large sized specimens. In our specimens, however, this treatment rather damaged the matrix structures of the cementum in the pulpal side, and showed only little effect near the root surface. Since the cementum near the pulp cavity is porous and more easily etched than near the surface, there appear to be a great difference in the rates of decalcification within the part of the cementum.

In this experiment, we found that observation of thin ground sections under dark field illumination provides the best optical images of growth layers. The narrow GLGs near the root surface observed in dark field illumination generally contained several finely contrasting growth layers of the second order. This method is not only simple and reliable compared to the other regular decalcification and staining processes, but represents a new trial for age estimation of whales. We therefore propose that, for age estimation of male Stejneger’s beaked whale, observation of the ground section of tooth cementum under dark field illumination is the most reliable and recommendable method for the present. Further observations of teeth at different age stages in both sexes are strongly recommended to support the viability of this method for age estimation of this species.
Arai et al., Age estimation of Mesoplodon stejnegeri

reports. Although Hachiya and Ohtaishi (1994) suggested that the rapid cementum deposition in the teeth of the Japanese macaque is caused by heavy occlusion pressure during the winter time, there may be differences in the formation of the tooth cementum between the above mentioned species that have functional teeth and Stejneger’s beaked whale, whose teeth do not occlude. Analyses of chemical and physical properties of growth layers may explain the causes of this discrepancy, and furthermore, the mechanism of formation of growth layers and GLGs, related to the biology of individual whales.

Age estimation

The pattern of the first distinct opaque layer in dentine occurring along the boundary between the areas of obscure and distinct growth layers, agrees with the description of the dentinal neonatal line by Perrin and Myrick (1980). As this putative dentinal neonatal line was confirmed in all specimens examined, the cutting plane of our specimens was worth GLG counting even though the cusp of the tooth crown had been worn out in specimens A, B, D, E and F, and that of specimen C had been cut off-center (Fig. 3).

For precise age estimation of the Stejneger’s beaked whale, an interpretation of the significance of the presence of two GLGs in the cementum of specimen G appears crucial. Based on observations of newborn whales, Yamada (1998) estimated that the season for parturition of this species is from April to May, and that the average body length at birth is about 210 cm. The body length of whale G (stranded in April) was 296 cm, some 1.4 times larger than the estimated length of this species at birth. The correlation between the body length and the number of cemental GLGs in specimen G (two GLGs) appears in line with that of Baird’s beaked whale reported by Kasuya (1977) that a 6.4 m long whale had two GLGs in its tooth cementum. The estimated body length at birth of Baird’s beaked whale is reported to be 4.6 m (Omura et al. 1955). Kasuya (1977) concluded that the cementum in the teeth of this species begins to be laid down soon after birth because almost the same number of growth layers had formed in postnatal dentine and cementum. In the case of Stejneger’s beaked whale, therefore, on the assumption that the cementum also starts to form soon after birth and that cemental GLG is produced annually, whale G is estimated to be almost two years old. In fact, formation of single cemental GLG per year has been reported in several cetaceans (Kasuya 1976, 1977; Perrin and Myrick 1980; Kasuya and Matsui 1984; Lockyer 1993) and some terrestrial mammals (Klevezal 1996). In this context, the GLG counts in the cementum of whole ground sections of the seven teeth examined, directly indicate the actual age of the individual whales (Table 1). To verify the reliability of the above assumptions and consequent age estimation, further analyses of larger numbers of specimens at different ages including those at fetal, perinatal, and postnatal stages of development are awaited.

Variation in the rate and timing of growth in individual teeth

Specimens A, B and E are characterized by heavy abrasion along the mesial margin of the erupted portions and irregular deposition of thick cementum on the root surface (Table 1). It is notable that each of these three individuals had a long plateau phase lasting 10.5–15.5 GLGs (Fig. 6). It appears reasonable to state that the degree of abrasion of the erupted part of the tooth and the deposition of cementum on the root surface depends on the length of the period after cessation of vertical growth, i.e. the length of the plateau phase. Since the beginning of the plateau phase varies widely among the individuals (Fig. 6), deep abrasion and heavy cementum deposition on the root surface do not necessarily represent the actual age.

There is no correlation between the body length and the growth rate (root elongation speed), or size of the tooth. In other words, male Stejneger’s beaked whale appears to exhibit a wide range of size, growth rate and other phenotypes in their teeth, irrespective of their actual age. Therefore for precise age estimation of Stejneger’s beaked whale, GLG counts in the tooth cementum are essential.

Acknowledgments: We are grateful to Atsumi town office, Dr. M. A. Brazil, Johetsu Municipal Aquarium, Mr. S. Kimura, Mr. T. Koike, Dr. T. Kuramochi, Mano town office, Mr. T. Mizuno, Mr. K. Nagasawa, Mr. Y. Nakamura, Niigata City Aquarium, the late Mr. E. Noda, Ms. M. Ogino, Ryotsu city office, The Sea of Japan Cetology Research Group, Mr. J. Shindo, Mr. M. Suzuki, Ms. Y. Tajima, Tsuruoka city office, and Yamagata Prefectural Museum for their help in collecting samples and Dr. M. Amano, Dr. A. A. Hohn, Prof. T. Kasuya, Dr. T. Kishiro and Mr. K. Nagasawa for their helpful comments and suggestions.
References


Received 15 December 2003. Accepted 16 August 2004.