J-STAGE Advance published date: May 12, 2021

AGE ESTIMATION FROM TEETH IN LONGMAN'S BEAKED WHALES (*INDOPACETUS PACIFICUS*) STRANDED IN NEW CALEDONIA (SOUTH PACIFIC)

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Abstract

Seven Longman's beaked whales mass stranded in New Caledonia in November 2013, of which 4 ultimately died, in a first worldwide event reported for this poorly known Ziphioid species. Teeth were extracted, collected and thoroughly cleaned of gum tissue from 3 females ranging from juvenile to adult and one adult male. These were sectioned (crown-root) and prepared using two different methods and examined under microscope magnification when Growth Layer Groups (GLGs) in both dentine and cement were successfully identified. The methods employed for aging included 1) sectioning centrally at approx. 150 μm through crown and root on an Isomet circular diamond saw and examining under a microscope using both transmitted polarised light and plain light; and, 2) thick sectioning (wafering) at approx. 2.5 mm and subsequent decalcification in RDO™ (a proprietory brand, Illinois, USA) and then thin sectioning the wafer at 10–25 μm and staining with Ehrlich's acid haematoxylin. GLGs were investigated in both dentine (25 micron) and cementum (10–15 μm). Layering was evident in both tissues but higher counts were more evident in thin stained sections of cementum. Although dentinal GLGs in untreated tooth sections have been used successfully for aging in Ziphioid species *Hyperoodon ampullatus* (Christensen 1973, Feyrer *et al.*, 2020), it is believed this is the first time that teeth have been used for estimating age from GLGs in this tropical species.

Key words: Age estimation, Ziphioid whales, *Indopacetus pacificus*, teeth.

Introduction

Longman's beaked whale (*Indopacetus pacificus*), first described as a large species of the genus *Mesoplodon* (Longman, 1926) from a beachcast skull found in northern Queensland, Australia, was later assigned to its own genus, *Indopacetus*, after comprehensive morphological evaluation (Moore, 1968) and the discovery of a second skull in Somalia (Azzaroli, 1968). This species has long been considered one of the rarest and least known of all whales. Groups of *Indopacetus* are encountered with some regularity in the tropical Indian and Pacific Oceans (Anderson *et al.*, 2006; Afsal *et al.*, 2009; Yamada *et al.*, 2012), and there have been occasional reports of strandings (<20 stranded animals to date, Yamada *et al.*, 2012) most of which have been singletons, with the exception of a cow-calf pair in Taiwan (Yao *et al.*, 2012). More recently, a stranding was reported off the Phillipines (Acebes *et al.*, 2019), and two separate strandings were reported off Okinawa, Japan, including a neonate (Kobayashi *et al.*, 2021, 2021a).

This paper focuses on tooth material and relevant data from a mass stranding of Longman's beaked whales in New Caledonia in the southwest Pacific Ocean, and describes the details of the aging methods used, which may be helpful for researchers investigating more precise age in other beaked whales. Among the samples collected from three females ranging from juvenile to adult and one adult male, were the paired teeth, extracted and thoroughly cleaned of gum tissue. The details of the stranding event are described by Garrigue *et al.* (2016) when seven animals came ashore together on the south coast of the Grande Terre on 16 November 2013, four of which ultimately died. Only a brief account of the age estimation was reported at this time. This is believed to be the first worldwide mass stranding event for this poorly known species, and the first time tooth layering has been investigated for Longman's beaked whale.

Methods

Age was estimated using a single tooth from each of the four animals examined and was determined without other detailed biological information apart from sex and body length. The remaining tooth was retained untouched for other analyses. Two methods of aging were applied to investigate which might be more informative, and are detailed here. Although aging methods have been detailed for other beaked whales—Northern Bottlenose whale *Hyperoodon ampullatus* (Christensen, 1973; Feyrer *et al.*, 2020)—Longman's beaked whale is a tropical species, and in general little is known about aging in beaked whales.

Fig. 1 shows the size and shape of a tooth. Each tooth was thoroughly cleaned of gum tissue and 2–3 sections, *ca.* 150 μm thick, were cut centrally through the crown and root of each tooth, using an Isomet

a) b) c) d)

Fig. 1. The series of photos shows the size and dimensions of a tooth from specimen #4: a) and b) the length of the tooth in different aspects; c) the greatest width of the tooth; d) the elliptical open pulp cavity at the root. Only the top 10 mm of the tooth crown are exposed above the gum line which is clearly visible from the darker colour.

Fig. 2. Buehler Isomet low-speed diamond saw for sectioning teeth. The chuck and wood block mount for the tooth are shown. The tooth is affixed to the wood block using a standard hobby glue gun employing glue sticks. The glue is easily removed after cutting and can be made easier by soaking the tooth sections and block in water for some minutes.

Fig. 3. A portable benchtop freezing microtome used for sectioning decalcified teeth. Note the horizontal cutting plane, the fixed freezing stage and the mobile blade. The specimen is frozen by means of a Peltier device with flowing water in counter current. The blade is not cooled and the microtome is operated at room temperature. The temperature of the freezing stage is generally around −12°C and can be adjusted by altering the water flow to the device.

slow-speed circular diamond saw (see Fig. 2). These thin sections were examined under a binocular dissecting microscope at magnification x7–x40 using alternately transmitted plain light and polarized light, focusing on Growth Layer Groups (GLGs) in both dentine and cementum (see Perrin and Myrick Jr., 1980; Lockyer *et al.*, 2016 for GLG definition). Each GLG observed was assumed to have been deposited over a 1-year period as validated in other odontocetes *e.g. Physeter macrocephalus* (Gambell, 1977), *Globicephala melas* (Lockyer, 1993), *Tursiops truncatus* (Hohn *et al.*, 1999), *Delphinapterus leucas* (Lockyer *et al.*, 2016), and various mammal species (Read *et al.*, 2018). In addition, a thicker central section (wafer) (2–2.5 mm) was removed from each tooth, again through crown-root axis adjacent to the first thin section, and decalcified in a rapid decalcifying solution RDO™ (a proprietory brand, Illinois, USA) for up to 15 h. Thin sections of (10–25 μm) of this central wafer were then made using a freezing microtome (see Fig. 3) and stained with Ehrlich's Acid Hæmatoxylin (EAH) as described for other species (Lockyer, 1993; Lockyer and Braulik 2014; Read *et al.*, 2018). The thinnest sections were destined for cemental examination and the thicker sections for dentinal study. In this instance, the thin decalcified sections were placed in large histo-cassettes in an agitated ripe solution of EAH for up to 45 min until stain uptake was deemed optimal. The sections were then transferred to water in the histo-cassettes and rinsed thoroughly of excess stain for several minutes. Subsequently, the sections were transferred into a weak ammonia solution (just a few drops of ammonia in the solution enough to turn the pH alkaline) and soaked here for about 3 min. until the sections had turned uniformly from deep red to purple-blue. After a second rinsing in water, the sections were then examined wet under a microscope and the best sections were removed for mounting. Sections used for age estimation were floated onto 5% gelatin-coated microscope slides under water and arranged with a maximum of 2 sections per slide. The slides were then carefully blotted, section downwards, on absorbent paper (a type that does not shed fibres) and then air-dried in a fume cupboard for about 30 min. and permanently mounted under glass cover slips with DPX, a fast drying mixture of distyrine (a plasticizer) and xylene. The slides were then allowed to completely dry in the fume cupboard for several days before examination. Plain transmitted light was then used for examination under a microscope as for the unstained thick sections. Age data estimated from both preparation methods were recorded in both dentine and cement.

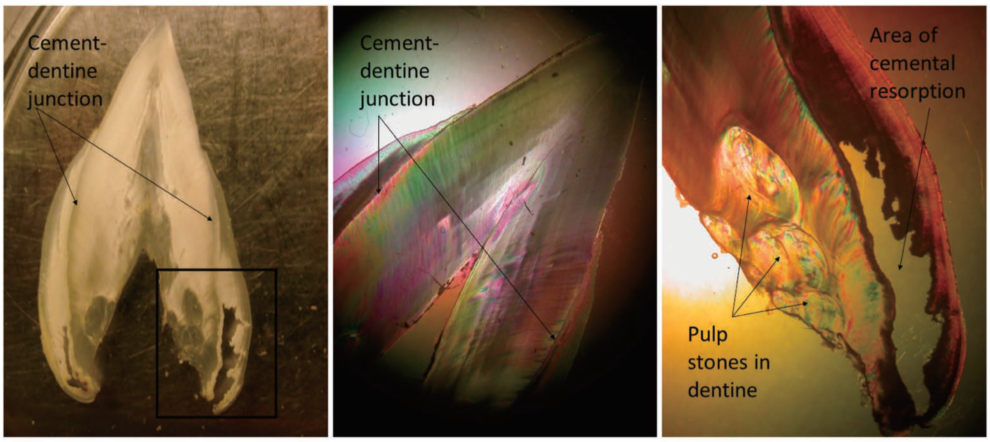
Results

The ages estimated from the different sections are presented in Table 1 where both stained and untreated sections are recorded as well as GLGs in both the dentine and cementum. The first point to note is that for all specimens, the stained sections are generally providing higher GLG counts in both dentine and cementum, with the exception of the dentine in specimen #6 where no identifiable GLGs could be seen in the stained section. The other point is that cementum is generally providing higher counts and greater clarity than the dentine. The most consistent ages for both the adult female #6 and adult male #2 were estimated as 20–22 and >20–26 GLGs respectively, which by analogy with GLG deposition rate in other cetaceans may be equivalent to years (see review in the Methods). Ages of the sub-adult females #4 and #5 were less clear, but #4 was estimated as 8–9 GLGs and #5 as 14–17 GLGs. Figs. 5–7 incl. show the untreated and stained sections of cementum for specimens #2, #5 and #6, respectively. In each case, more GLGs appear to be visible in the stained than in the untreated sections. The GLGs identified are those that fall in the range shown for cementum in Table 1. At this stage when the method of age estimation is experimental, it is unclear whether some of the identified

Table 1. Age estimates for four whales based on the examination of dentinal and cementum Growth Layer Groups (GLGs) under two different treatments (After Garrigue *et al.*, 2016).

Specimen number	Age-sex class	Treatment	No of GLGs[2] observed		Overall Age[1] Estimate (GLGs)[2]
			Dentine	Cementum	
#2[a]	Adult M, length 590 cm	unstained stained	7+++[3] 20	20+ 26	20–26 (range 7++ to 26)
#4[b]	Sub adult F, length 564 cm	unstained stained	8 9–12	5++[3] 8+[3]	8–9 (range 5++ to 12)
#5[c]	Sub adult F, length 590 cm	unstained stained	8++[3] 12+[3]	14 17[3]	14–17 (range 8++ to 17)
#6[d]	Adult F, length 618 cm	unstained stained	12++[3] no GLGs visible	20 22	20–22 (range 12++ to 22)

[1] Overall age was based on the modal age from several counts.
[2] One GLG is assumed to be equal to one year but this requires future validation.
[3] Addition symbol (+) indicates that more GLGs were present, but were too indistinct to be counted, and multiple+symbols reflect the relative amount of indistinct GLGs. In addition, the following comments relate to individual teeth.
[a] Crown worn down. Some GLGs may be missing. Dentinal resorption in root.
[b] The presence of pulp stones interfered with the GLG pattern in the dentine such that it was difficult to count at the base. Cement poorly stained and ill-defined. Dentinal resorption or decay in the root.
[c] GLGs had poor definition, including double laminae. Dentinal resorption, or decay in root.
[d] No dentinal resorption in root.



a) b) c)

Fig. 4. Untreated section 150 microns thickness, of specimen #4: a) complete section examined under plain transmitted light showing central dentine, surrounding cementum, and large areas of resorption at the dentine-cementum junction in the root; b) dentine examined under polarised filters, showing GLGs; c) close up of the resorption area from a) examined under polarised filters showing pulp stones in the dentine root.

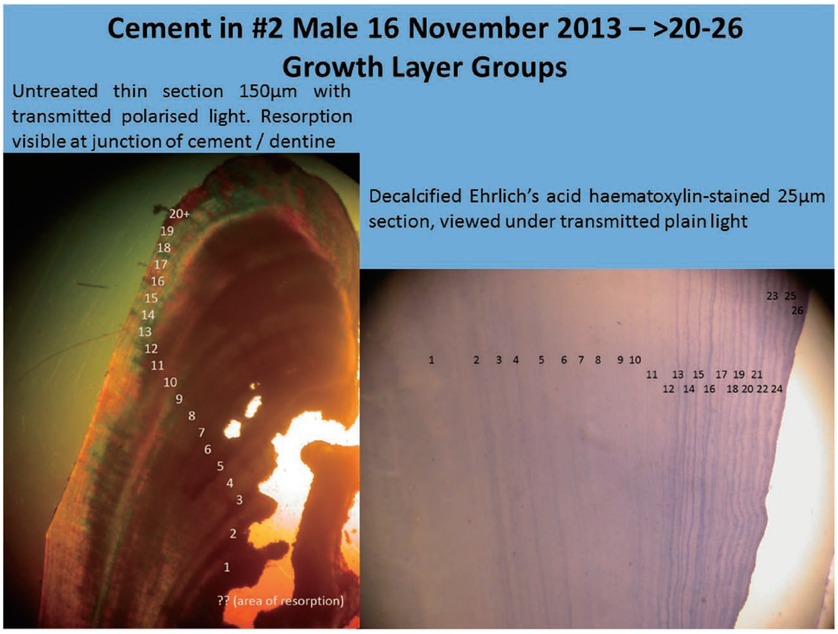


Fig. 5. Comparison of cementum in untreated and decalcified stained sections of specimen #2.

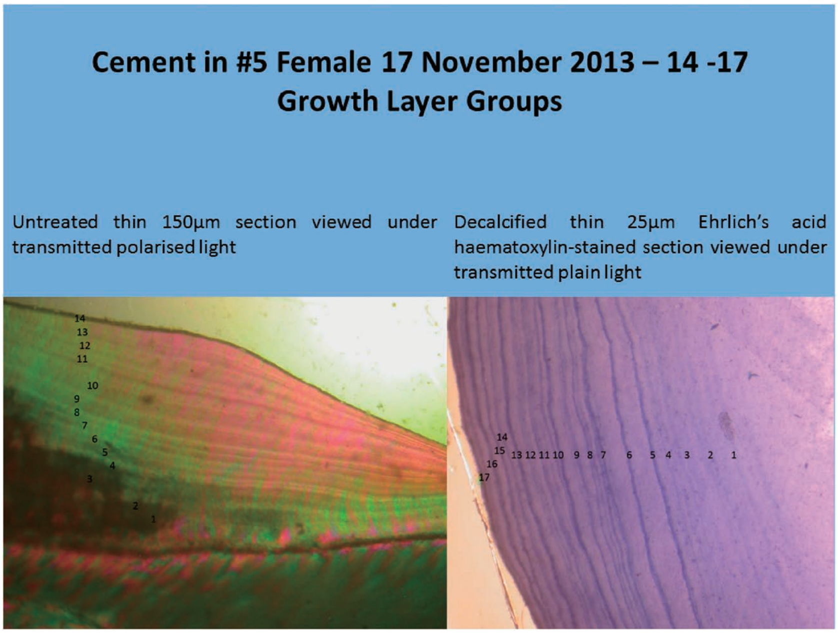


Fig. 6. Comparison of cementum in untreated and decalcified stained sections of specimen #5.

GLGs might actually be accessory lines. Figs. 5–7 all show identified GLGs in the decalcified and stained cementum, but it is clear that some are darkly stained and others paler; in addition, sometimes the spacing between layers is not regular but very close appearing double (for example, see GLGs 19 and 21, Fig. 7) suggesting possible accessory lines. The untreated cementum in Fig. 7 shows identifies only 20 GLGs, indicating that staining may highlight other ultrastructures in addition to GLGs. Read *et al.* (2018) discuss the significance and possible causes of accessory lines in marine mammalian teeth under the topic of tooth anomalies, including periodic changes in nutrition, hormonal levels and environmental factors. Presently it is difficult to identify likely factors for this tropical species, not knowing much about the life history.

The specimens #2, #4 and #5 all had evidence of extensive mineral resorption at the dentine-cemen-

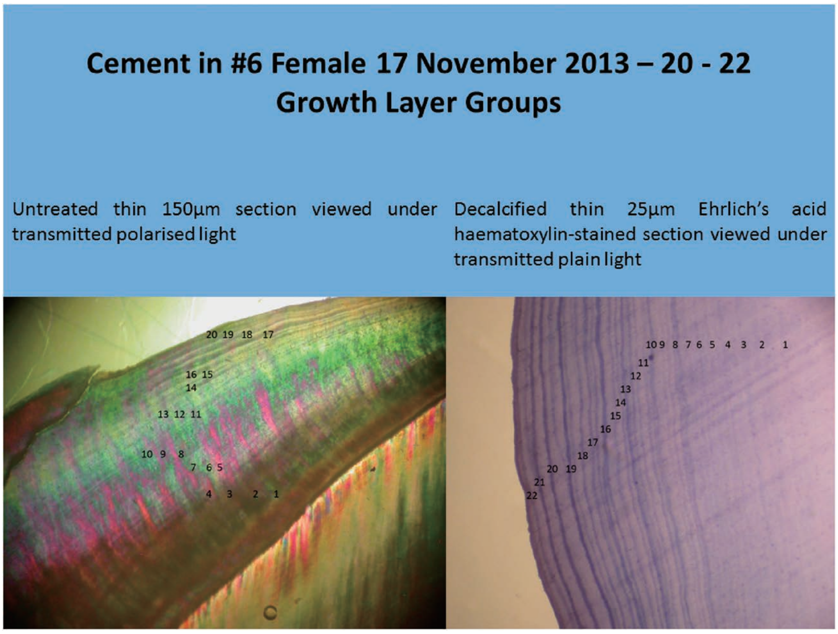


Fig. 7. Comparison of cementum in untreated and decalcified stained sections of specimen #6.

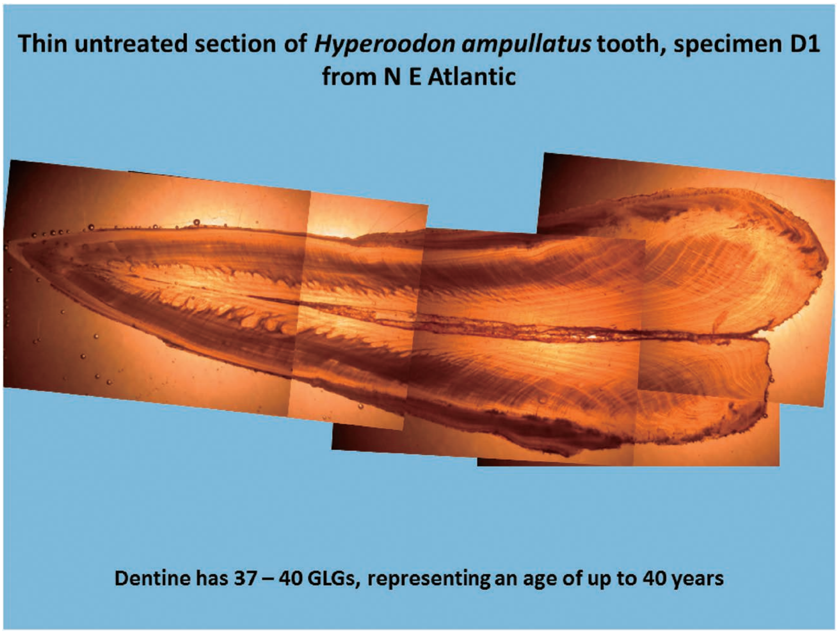


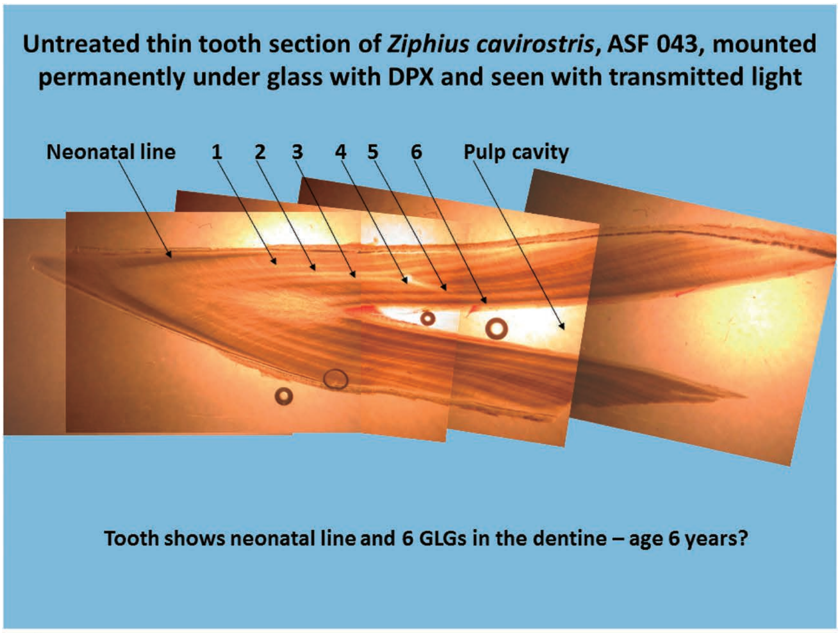
Fig. 8. Dentinal GLGs in an untreated section of a tooth of *Hyperoodon ampullatus*.

tum junction in the root region (Fig. 4a, c), while #6 had no such condition.

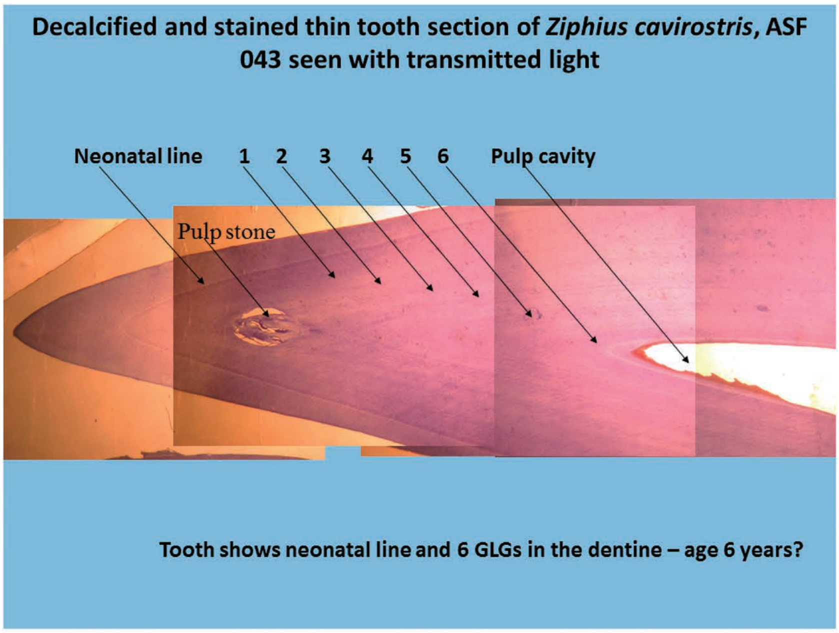
Discussion and conclusions

Both methods of preparation detailed here were adequate for identifying GLGs in cementum, with slightly higher counts in stained sections. Generally, GLGs were difficult to identify in the dentine which presented a lower age count than cementum. Thus cemental GLGs would appear to be the preferred tissue for age estimation in Longman's beaked whale. Stained sections tended to produce higher GLG counts, but at the moment it is unclear whether accessory lines may be highlighted and what time period a GLG represents. However, as suggested above, the norm in most mammals is an annual deposition of GLGs (Gambell, 1977; Lockyer 1993; Hohn *et al.*, 1999; Lockyer *et al.*, 2016; Read *et al.*, 2018). In this study, the dentine did not provide clear differentiation of GLGs so that this tissue must be regarded as unreliable for total age estimation in this species, based on this limited investigation.

Generally, dentinal GLGs are preferred for aging in cetacean teeth (Stewart and Stewart 2014; Read *et al.*, 2018). In *Hyperoodon ampullatus*, the dentinal GLGs in untreated tooth sections are the preferred aging tool, as noted earlier (Christensen, 1972; Feyrer *et al.*, 2020). This is demonstrated in Fig. 8 where an untreated section (prepared in the same manner as *Indopacetus* teeth) shows up to 40 dentinal GLGs. There is no wear at the crown so that all GLGs are represented. There is limited information about aging in Ziphioid whales, and the authors note that teeth of *Ziphius cavirostris* (prepared



a)



b)

Fig. 9. Comparison of dentinal GLGs in a) untreated and b) decalcified stained sections of teeth of *Ziphius cavirostris*.

in a similar manner as *Indopacetus pacificus* teeth) presented good GLG resolution in both untreated and decalcified stained sections of dentine (Fig. 9). It should be noted however, that the cementum in both *Ziphius* and *Hyperoodon* is thinner and less conspicuous than in *Indopacetus* (compare Fig. 4). Additionally, unlike *Ziphius* and *Hyperoodon*, for which there have been past fisheries (Japan, Norway) when some life history information was learned, there is almost nothing known about *Indopacetus*, including the extent of its distribution outside tropical waters, if at all. All that can be drawn from the methods tried presently is that cementum appears to be the best tissue to use for aging, and decalcifiying and staining the tissue tends to reveal more detail and thus higher counts than the untreated sections.

With respect to the presence of mineral resorption in the roots of the teeth of *Indopacetus*, the cause is unknown (Fig. 4a, c). It is reported that high levels of cadmium (Cd) in the body can lead to interference in bone mineral structure, specifically calcium (Kazantsis, 2004; Sughis *et al.*, 2011). Garrigue *et al.* (2016) reported high concentrations of Cd in the kidneys and liver of #4, #5 and #6, but coincidentally noted that the lowest concentration was observed in #6, the only animal with no resorption. In addition #4 had many pulp stones present in the dentine (Fig. 4b, c), the cause of which is again unknown. However, the presence of these structures made reading the dentinal GLGs very difficult. Such inclusions do not occur in cementum.

It is believed this is the first time that teeth have been used for estimating age from GLGs in this species, *Indopacetus*. These initial results are very promising and may be helpful to others who may encounter similar specimens. The method may also be helpful in investigating some other beaked whale species.

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Received: November 1, 2020
Accepted: March 5, 2021
Published online: May 12, 2021