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Age estimation and growth layer patterns in teeth of Commerson's dolphins (*Cephalorhynchus c. commersonii*) in subantarctic waters

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The Commerson's dolphin, *Cephalorhynchus c. commersonii*, one of the world's smallest cetaceans, occurs in the southwestern South Atlantic from about 41°30'S to near Cape Horn (56°S), including the central and eastern Strait of Magellan and the Falkland (Malvinas) Islands (Goodall 1978, Goodall *et al.* 1988). Along the coasts of Patagonia, northern Tierra del Fuego, Argentina, and the eastern Strait of Magellan, Chile, this is the cetacean most often seen near shore, where the animals feed and surf in the breakers. They seldom strand, but are commonly taken incidentally in shore-based gill nets set perpendicular to the coast over the wide tidal flats (Goodall 1978, 1994; Goodall *et al.* 1994, 2008; Iñíguez *et al.* 2003). Although there have been few aerial surveys, this is probably the most abundant small cetacean in these waters and is the most affected by bycatch in artisanal fisheries.

Age estimation in marine mammals by counting incremental layers in teeth has been carried out since the 1950s (Scheffer and Myrick 1980). As mentioned in Hohn (2002), the importance of age estimation goes beyond population dynamic studies, and the information on age at sexual maturation and population age structure may help to adopt adequate policies in conservation management (Read and Hohn 1995, Hohn *et al.* 1996). Additional information can be obtained from teeth, such as life history or environmental events (Klevezal and Myrick 1984, Manzanilla 1989; Lockyer 1993, 1995; Klevezal 1996, Luque *et al.* 2009). Therefore, the description

of a “basic or typical” pattern of a species gives us not only more accurate and precise age estimates, but also allows for the identification of accessory layers (ALs) and other tooth-tissue alterations, which could be correlated with intrinsic or extrinsic factors.

Early studies of the teeth of Commerson’s dolphins for ageing provided no detailed description of dentinal growth layer pattern (Lockyer *et al.* 1981, 1988; Pedraza 2008), although one of these studies (Lockyer *et al.* 1981) examined a small sample of teeth ($n = 40$). In our study we were able to significantly increase that sample size, allowing us to (1) define, identify, and characterize dentinal growth layer groups (GLGs, Perrin and Myrick 1980) using quantitative (relative size of growth layers) and qualitative (appearance of growth layers) features in good-quality sections; (2) describe the “basic” growth layer pattern and; (3) examine sexual dimorphism in GLG characteristics.

Teeth from 198 Commerson’s dolphins from the R. Natalie P. Goodall (RNP) collection, held at the Museo Acatushún de Aves y Mamíferos Marinos Australes (AMMA) at Estancia Harberton, Tierra del Fuego, were available for this study. Specimens were collected during the RNP program of beach surveys along the coast of northern Tierra del Fuego from 1974 to present (Goodall 1978, Goodall *et al.* 1994). Data on collection date, stranding location, total length and sex were available for all specimens.

Initially, one tooth was selected from the center of the lower jaw or, if the teeth were no longer in the mandible, the straightest and least worn teeth were chosen. When the initial thin section was poor, another tooth was similarly selected and processed. Teeth were prepared following the method described in Myrick *et al.* (1983) and Hohn *et al.* (1989). They were placed in 10% formalin for up to 3 h, rinsed well in water, and then decalcified in RDO.¹ Decalcification time ranged from 40 min (in neonates) to 5.5 h (in older specimens). Decalcified and well-rinsed teeth were thin sectioned to 25 μm on a freezing-stage microtome. For most specimens, tooth sections were obtained in a buccal-lingual (BL) orientation. In specimens with twisted teeth (usually older animals), the teeth were rotated, sometimes as much as 90°, to find an orientation that would allow for a mid-longitudinal section. Sections were stained in Mayer’s hematoxylin and “blued” in a weak ammonia solution. The best sections were selected and mounted on a microscope slide in 100% glycerin.

In Commerson’s dolphins, cement is not well developed and its layers are inconspicuous (Lockyer *et al.* 1981), so only dentinal layers were counted in this study. Using a stereo microscope with transmitted light at 40 \times and 100 \times magnifications, the dentine of each specimen was read, without access to biological data, three times by NAD. Estimated age was taken as the last reading, under the assumption that the accuracy of counting increases with time (Pinedo and Hohn 2000). A subsample of teeth was read independently by AAH and the readings were compared. If different, a consensus was achieved. Additionally, consultation on growth-layer patterns continued between the authors during the readings in order to begin describing and defining the growth-layer pattern. We assumed, as in Lockyer *et al.* (1981, 1988), that in this species one GLG represents one year’s growth.

¹Reference to trade names does not imply endorsement by the authors’ institutions.

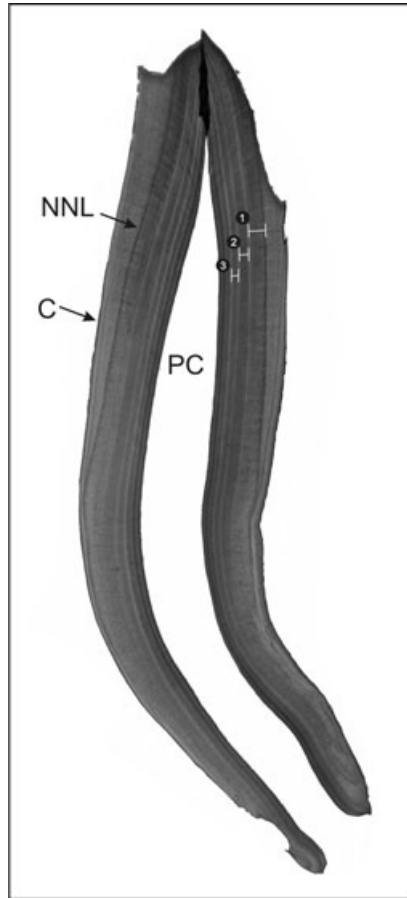


Figure 1. Location of GLG thickness measurements taken on a good quality (on-center) section of Commerson's dolphin *C. commersonii* tooth at 40 \times magnification. NNL = neonatal line, C = cement, PC = pulp cavity, numbers = GLG number.

In order to characterize the growth layer pattern, the GLG thickness was measured at specific locations on good quality BL sections (Fig. 1). Sections were considered as "good quality" following the criteria used by Hohn *et al.* (1989) and Pinedo and Hohn (2000). Due to the marked lateral asymmetry of Commerson's dolphins' teeth, measurements were always taken on the convex side. All measurements were obtained at 100 \times using Image-Pro Plus processing software.

We tested for sexual dimorphism in the mean GLG width using the Student's *t*-test at a 5% significance level. In addition, dentinal anomalies occurred in 50.5% ($n = 100$) of the specimens, usually within the four first GLGs. In those animals it was more challenging to delimit annual layers, so in order to help identify GLG boundary, the thickness of "typical" and anomalous GLGs were compared (Student's *t*-test at 0.05). In all cases the measurements compared were only from the first three

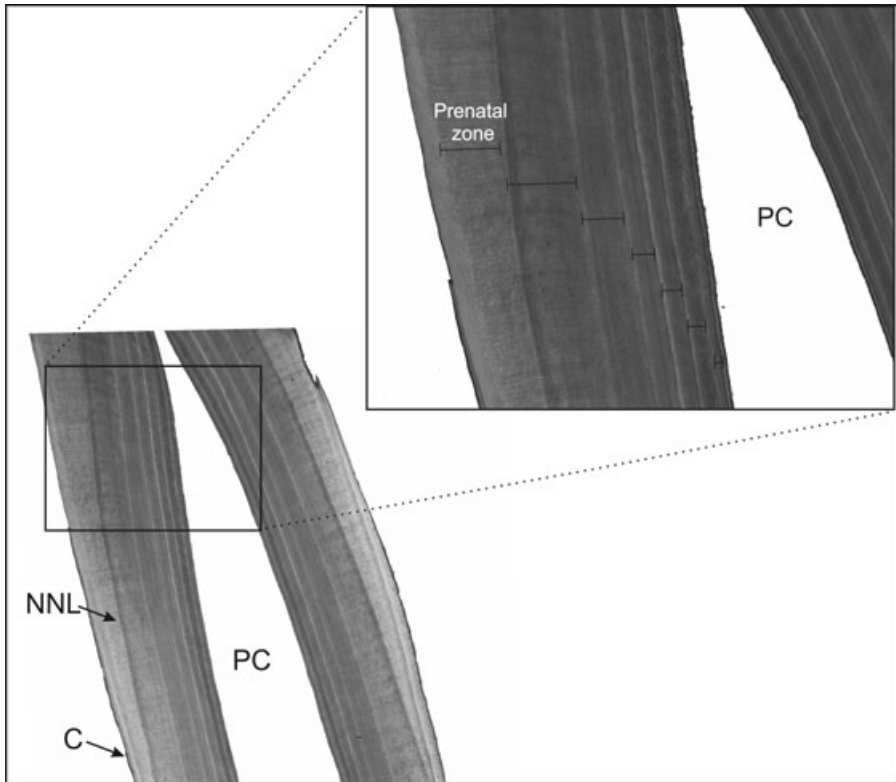


Figure 2. Dental growth-layer pattern in *C. commersonii* teeth. A 6 yr old male (RNP 1351) at 40 \times and 100 \times magnification (top right corner). NNL = neonatal line, C = cement, PC = pulp cavity.

GLGs, due to the small sample size of GLGs four and older for statistical analysis. We also examined the last layer deposited in order to determine the season of layer deposition for those specimens for which we had an exact date of death ($n = 72$).

As in other dolphin species, the dentinal GLG pattern in Commerson's dolphins consists of a thin, weakly stained layer (unstainable layer) and a thick stainable layer (Perrin and Myrick 1980, Hohn *et al.* 1989). The prenatal zone was homogeneous, without layering and considerably thinner than the first GLG at the base of crown in the convex side, where the cement layer begins. In contrast to what has been described for many species, appearance of the neonatal line was complicated. Rather than appearing primarily as a distinct, unstained layer, it consists of a very thin dark line adjacent to prenatal dentine, more evident at the apical zone, a narrow unstainable layer (the beginning of the first GLG) and an intensely stained broad layer. As previously described for other species (Myrick *et al.* 1983, Hohn *et al.* 1989, Pinedo and Hohn 2000), the boundary between two adjacent GLGs was delimited by a thin, dark stained layer, which was more conspicuous between GLGs 2–3 and subsequent GLGs than between GLGs 1–2 (Fig. 2). Moreover, the boundary between

Table 1. Measurements (μm) of GLG thickness obtained for males (M) and females (F) of Commerson's dolphin. NS = not significant.

GLG	Sex	Mean	<i>n</i>	SD	Minimum/maximum	<i>t</i> -value ($P \leq 0.05$)
1	M	237.19	27	25.12	173.11/283.29	NS
	F	225.04	14	31.85	171.72/272.97	
2	M	131.79	22	24.83	94.03/176.73	NS
	F	131.51	7	26.32	103.34/169.53	
3	M	79.32	16	13.35	58.41/97.35	NS
	F	76.15	4	5.7	71.41/82.84	

the first and second GLGs was quite distinct in most specimens. The GLG pattern changed at the fifth or sixth GLG, at which time they became better defined with the unstainable becoming relatively wider and the stainable layer becoming relatively narrower, as described for bottlenose dolphins, *Tursiops truncatus* (Hohn 1980). When more than one tooth from a dolphin was used, the layering pattern was the same.

The relative thickness of the GLGs follows the same pattern found in bottlenose dolphins (Hohn *et al.* 1989), except that the second GLG is smaller than the first in Commerson's dolphins. No significant differences were found in the corresponding GLG width between male and female specimens (Table 1). Additionally, the mean width of anomalous and typical GLGs was not different in the first and second GLG, suggesting that thickness measurements may be a useful tool to identify these GLGs if the pattern is confusing. However, the thickness of the third GLG was significantly different (Table 2).

Most of the specimens analyzed showed a conspicuous pale AL in the first GLG, more evident in yearling specimens (Fig. 3). In addition, 22.6% of the specimens older than 5 yr ($n = 115$) had a thin dark layer in GLG 5, 6, or 7. Both of these features were considered ALs or marker lines and were not included in GLG counts. Anomalous dentinal GLGs were present in 100 specimens, mostly among the first four GLGs. They often presented two or three conspicuous wavy dark ALs and a relative "hypomineralized" appearance (Fig. 4). In 23 specimens (>7 yr old) pulp stones were observed, which consisted of nodules with concentric rings, as described in Lockyer (1993) (Fig. 5).

Table 2. Measurements (μm) obtained for "typical" (T) and anomalous (A) GLGs, with results of significance test between GLG type. * = significantly different at $P \leq 0.05$ and NS = not significant.

GLG	GLG type	Mean	<i>n</i>	SD	Minimum/maximum	<i>t</i> -value ($P \leq 0.05$)
1	T	231.56	22	33.15	171.72/283.29	NS
	A	238.08	17	19.17	215.65/269.59	
2	T	133.55	12	27.05	94.03/169.53	NS
	A	130.65	17	24.05	95.85/176.73	
3	T	72.74	13	9.37	58.41/88.36	2.95*
	A	90.64	6	8.94	78.96/97.35	

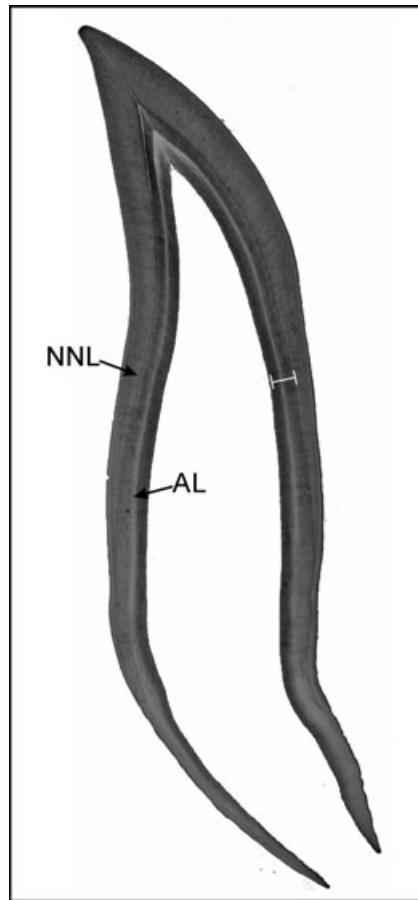


Figure 3. Prominent AL in dentine of a 1 yr old female *C. commersonii* (RNP 1478). NNL = neonatal line.

In specimens stranded during summer the last layer deposited (adjacent to pulp cavity) was the unstainable one in 67.4% of the cases ($n = 43$), whereas in specimens stranded during autumn the last layer was stainable (79.3%, $n = 29$).

In our study, each GLG, which consisted of a thin unstainable layer and a thicker stainable layer, was considered as an annual layer when estimating age. Even though the Commerson's dolphins pattern should be validated with tetracycline or other marks to set a GLG as an annual pattern, its similarity to other delphinids growth patterns where GLGs were calibrated (*i.e.*, bottlenose dolphins in Hohn *et al.* 1989) may allow this assumption. In addition, our results on the last layer deposited seem to support this assumption. Most animals, captured in the austral summer, showed the unstainable layer as the most recently formed. Births occur from November through March (Goodall *et al.* 1988). Assuming the neonatal line is deposited near the time of birth, a new annual GLG should begin to form at this time each year.

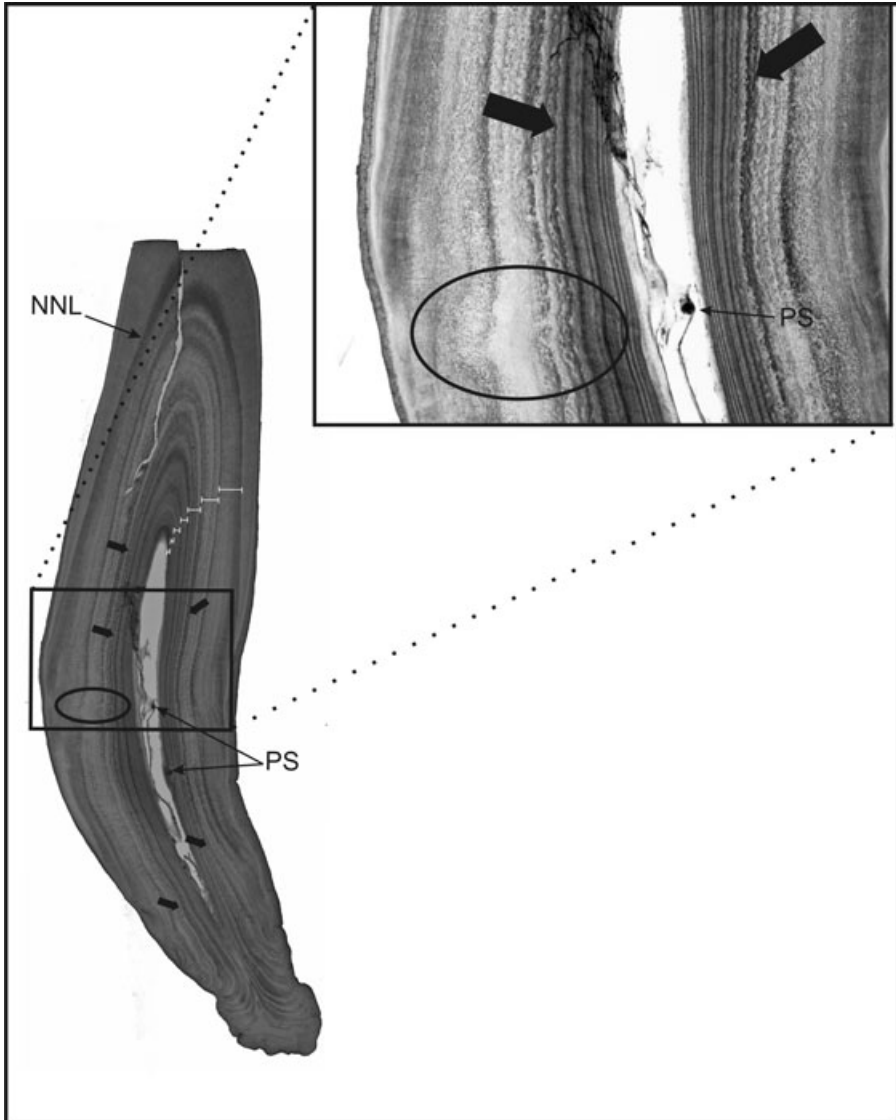


Figure 4. Wavy accessory layers (short arrows) and "hypomineralized" appearance (detailed in the circle) in an off-center section from a 7 yr old male *C. commersonii* (RNP 620) at 40 \times and 60 \times magnification (top right corner). PS = pulp stone, NNL = neonatal line.

Despite possible among-individual variation, layering was clear, GLGs distinct, and the ALs after the first GLG were not prominent in most specimens. Inconspicuous ALs make age estimation less problematic; reducing the probability of misinterpretation of the annual layering pattern (Hohn 1990). In addition, the boundary layer between the first and second GLG was conspicuous in most animals. This distinct

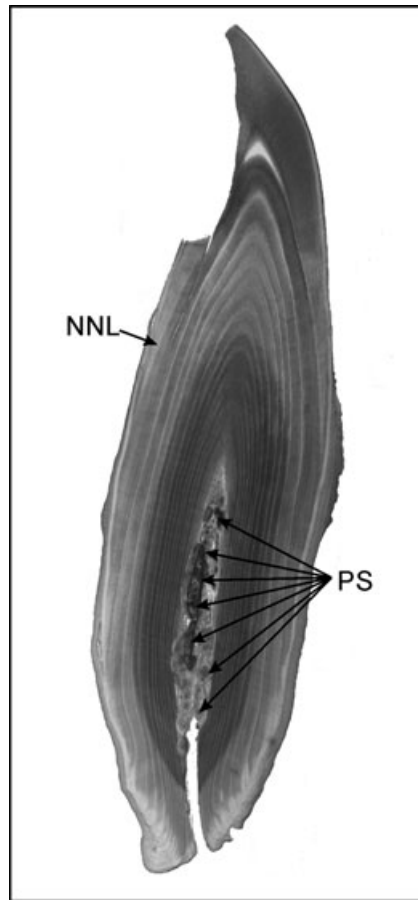


Figure 5. Many pulp stones (PS) in a 14 yr old male *C. commersonii* (RNP 1073). NNL = neonatal line.

1–2 GLG boundary was observed in franciscana dolphins, *Pontoporia blainvillei*, and related to an early calcium intake by Pinedo and Hohn (2000). The same explanation might be applied here as well, since in Commerson's dolphins the nursing period also seems to be short. According to direct observation in captivity, calves stopped nursing before one year of age and ingested amounts of food similar to those of adults at age one (Joseph *et al.* 1987, Kastelein *et al.* 1993).

Usually, populations that have individuals with distinct growth layers inhabit areas with marked seasonal variability (Klevezel 1996). This could explain the distinctness of growth layers found in Commerson's dolphins' teeth from Tierra del Fuego. It might be informative to compare the GLGs of this population to those from other areas with less seasonal change, as well as to other odontocetes in southern Argentina or Chile.

The relative width of the GLGs was consistent in all specimens indicating that this feature would be helpful to identify annual layer boundaries. However, the variability in absolute width, due to individual variation, orientation of the tooth when sectioned, or other factors, is too large to be reliable. In animals with an anomalous pattern, mean widths of the two first GLGs were similar to those of “typical” ones, reinforcing the utility of measurements. The differences observed in the third GLG could be an artifact of the small sample size, thus, a larger sample size may be necessary to elucidate it. The lack of detectable differences in the means and ranges of GLG width between males and females allows for pooling measurements for both sexes in future studies. However, given the small female sample size in this study, comparisons with a greater number of specimens are recommended.

The presence of a prominent AL midway between the neonatal line and the first GLG in most of the 198 animals sampled suggests this could be related to life events, possibly with the beginning of solid food intake. Joseph *et al.* (1987) found that a calf born in captivity began mouthing whole fish at approximately 2 mo of age and Kastelein *et al.* (1993) observed that the milk-dependent period continued for between two and eight months for six calves in captivity. The mark could also be due to a common environmental event or is regulated intrinsically since it is common in many delphinids.

Although the age at sexual maturation is not known for Commerson’s dolphins, Lockyer *et al.* (1988) suggested it to be at about 5–6 yr. That age corresponds with the appearance of a thin dark AL in GLGs 5, 6, or 7 in 26 of 115 specimens sampled. A connection between ALs (as marker lines) and sexual maturation has been suggested in other cetaceans (Klevezal and Myrick 1984, Klevezal 1996). Additionally, at GLG 5 there is a transition in the pattern; GLGs become more distinct, suggesting a possibility that this represents the age of maturation, as has been suggested for other species (Klevezal 1996). Other tooth anomalies seem to be associated with particular years and environmental factors (Dellabianca *et al.*, unpublished data), but this possible link needs further research.

The uniformity and consistency in GLG width and structure allow us to set guidelines that might be helpful to improve the precision of age estimation. Using these guidelines might be particularly important in this species because of the prominent AL found in approximately the middle of the first GLG in many of the animals. Counting this AL as a GLG would introduce a positive bias upward in age estimates, leading to underestimation of total length and age of sexual maturation (Hohn 1980, 1990). Given that Commerson’s dolphins are, within the small cetaceans, the most affected by bycatch in artisanal fisheries in Tierra del Fuego and surrounding areas (Goodall *et al.* 1994, Iñíguez *et al.* 2003), knowing the age structure of bycaught specimens is necessary in order to implement adequate conservation policies.

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