Efficiency of wear and decalcification technique for estimating the age of estuarine dolphin *Sotalia guianensis*

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Most techniques used for estimating the age of *Sotalia guianensis* (van Bénéden, 1864) (Cetacea; Delphinidae) are very expensive, and require sophisticated equipment for preparing histological sections of teeth. The objective of this study was to test a more affordable and much simpler method, involving of the manual wear of teeth followed by decalcification and observation under a stereomicroscope. This technique has been employed successfully with larger species of Odontoceti. Twenty-six specimens were selected, and one tooth of each specimen was worn and demineralized for growth layers reading. Growth layers were evidenced in all specimens; however, in 4 of the 26 teeth, not all the layers could be clearly observed. In these teeth, there was a significant decrease of growth layer group thickness, thus hindering the layers count. The juxtaposition of layers hindered the reading of larger numbers of layers by the wear and decalcification technique. Analysis of more than 17 layers in a single tooth proved inconclusive. The method applied here proved to be efficient in estimating the age of *Sotalia guianensis* individuals younger than 18 years. This method could simplify the study of the age structure of the overall population, and allows the use of the more expensive methodologies to be confined to more specific studies of older specimens. It also enables the classification of the calf, young and adult classes, which is important for general population studies.

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1. Introduction

The estuarine dolphin, *Sotalia guianensis* (van Bénéden, 1864) (Cetacea; Delphinidae), is a small cetacean common along the Brazilian coast, whose range stretches from Honduras in Central America to the southern Brazilian state of Santa Catarina (Geise and Borobia 1987, Simões-Lopes 1988; Borobia *et al*. 1991). The maximum body length and absolute age recorded for *Sotalia guianensis* were 220 cm length (Flores 2002) and 31 years (Ramos *et al*. 2008). Most animals captured by fishing nets or found dead on the beach were up to 6 years old and comprised most of the available specimens in scientific collections (Ramos 1997; Rosas 2000; Ramos *et al*. 2008). Among them it was observed that the females were frequently older than the males (Ramos *et al*. 2008). The definition of different age classes in the population is of extreme importance in analyses pertaining to the reproductive stock, causes of mortality and demographic patterns (Ricklefs 2003; Rosas *et al*. 2003). Ascertaining an individual's age is of fundamental importance to ontogenetic studies (Hohn 2009), and according to Hohn (1990), estimating the age of an individual is considered a priority in studies of the life cycle and the biology of populations of odontocetes.

Age estimations of Odontoceti have been carried out since 1953 (Nishiwaki and Yagi 1953) by observing and counting the growth layer group (GLG) found in teeth. GLG

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is formed by the cyclical deposition of dentine and cementum tissue and is a record of an individual life. As toothed whales have non-deciduous dentition, all layers formed throughout their lives can be found within their teeth. The pulp cavity is reduced over time as dentine deposition takes place, due to the fact that deposition occurs from the outer to the inner portion of the tooth (Slooten 1991). The layers can be complete (annual layers) – meaning they start at the base of the tooth, reach the top and return to base – or incomplete (subannual layers) – meaning they are not present along the length of the tooth. The latter, also known as accessory layers, should not be considered when estimating ages, as they do not constitute annual deposition (Hohn 1990). In the estuarine dolphin, each growth layer is considered to represent 1 year of life (Rosas *et al*. 2003). Some examples of the techniques that have previously been employed to prepare *S. guianensis* teeth for GLG counting are the following: wear without decalcification; thin stained section of decalcified tooth; and thin stained section of decalcified tooth, treated with alum (described in Ramos *et al*. 2008). In spite of the variety of methods that enable age estimation for this species, many of the available osteological samples from this dolphin do not have age estimates, probably because of the high cost and complexity of the techniques traditionally used. Many of these techniques are costly because they require sophisticated equipment for histologicals sections and an optical microscope for reading GLG, which are not available to most scientific collections and institutions.

One of the simplest and most affordable techniques, successfully used for larger odontocetes such as *Tursiops truncatus* (Montagu, 1821) and *Orcinus orca* (Linnaeus, 1758), is the manual wear of teeth followed by decalcification and observation under a stereomicroscope (Pierce and Kajimura 1980). However, as *Sotalia guianensis* teeth are smaller, the layers would not be as apparent as in the larger odontocetes. The purpose of this study is to apply this technique to the estuarine dolphin in order to ascertain whether GLG can be observed and whether the layers can be counted. This technique, if efficient, will simplify the process estimating the age of these animals, enabling an increase in studies of age structures. At the same time, it will remedy the lack of information regarding material currently deposited in scientific collections.

2. Materials and methods

A total of 26 *S. guianensis* specimens, from the collection of the Museu de Ciências Naturais (MCN), Universidade Federal do Paraná, Curitiba, Brazil, were selected. All specimens were collected in the Paraná State, Brazil. The teeth were dry-preserved and one tooth of each specimen was used.

The selection process of teeth to be used in this study involved choosing the larger and less curved teeth with no dental abrasions, which are generally located in the median portion of the mandible and maxilla (Ramos *et al*. 2008). The average size of a tooth located in this region is 2 cm in length and 0.5 cm in width. According to Rosas *et al*. (2003), the number of layers counted in any single tooth located in the median area is the same for every tooth in that area; therefore a single tooth was selected from each specimen. The method used to estimate age in this study was manual wear followed by tooth decalcification, based on the method proposed by Pierce and Kajimura (1980), described below. Each tooth should be embedded in an individual block of polymerized acrylic resin in order to favour manual wear.

1. A mixture of resin and catalyst was prepared and placed into a cardboard mold lined with contact paper, forming a layer approximately 1 cm thick.
2. Two minutes after the tooth was placed on the surface of the resin, with its lingual surface facing down, an additional resin and catalyst layer was added to cover the tooth.
3. After 24 h, the block was easily removed from the mold.
4. Accurate on-centre sections were cut along the labial–lingual plane (Rosas *et al*. 2003). This factor was crucial, because in off-centre sections the accessory layers cannot be identified, which can lead to erroneous estimations of age. Thus, on-centre or very close-to-centre sections are fundamental to this technique (Pinedo and Hohn 2000).
5. Each resin block containing a tooth was manually worn, with circular movements, until the medial portion of the tooth was attained. Wearing was executed with successive sheets of sandpaper with decreasing grit sizes (220, 400, 600 and 1200) in order to remove any flaws and grooves (Ramos *et al*. 2008).
6. The worn blocks were immersed in a 25% formic acid solution for about an hour, with their worn sides facing upwards, in order to decalcify the teeth.
7. Each block was rinsed in water and dried at room temperature for 24 h.

With this procedure, the dentine layers were highlighted according to differences in their rates of demineralization (Ramos *et al*. 2008). Growth layers were counted under a stereomicroscope of 10× to 20× magnification, without reference to specimen information (body length or previous age estimates). Reflected illumination was used, as it is considered the most appropriate illumination for this technique because it improves visualization of the layers (Ramos and Di Beneditto 2005). For an accurate GLG reading, the resin blocks containing the tooth were placed on a Petri dish positioned above a mirror. A spotlight was

directed onto the tooth and the mirror, thus highlighting the dentine layers. Distinction between the layers was enhanced by changing the direction of the light source, in order to obtain the ideal degree of shading and reflection (Ramos and Di Beneditto 2005).

The neonatal line, which appears just below the prenatal dentine, and which forms before birth, was not counted. Only complete layers were counted, whereas the accessory layers, which do not represent annual deposition, were ignored. The first growth layer formed (which is found after the neonatal layer) is represented by a depression followed by a crest and so on. When a depression followed by a crest was detected as the innermost tooth layer, i.e. the latest growth layer formed, it was counted (Hohn 2009). Before the final analysis, a series of independent readings were carried out by each author and the criterion for the counting of layers was established by consensus. Following the recommendations of Pinedo and Hohn (2000), the last reading of the teeth was used to estimate the age, on the assumption that the accuracy of reading improves with practice.

3. Results

The wear procedure took on average 30 min for each tooth. In all cases, growth layers were evidenced; nevertheless, in 4 of the 26 teeth (15.38%) not all the layers could be clearly observed. In these teeth, there was significant decrease in GLG thickness, which hindered the counting of layers. After differential demineralization, which occurs through the reaction of formic acid with dentine minerals, it was possible to observe that the growth layers are formed by a depression followed by a crest. Figure 1 shows a tooth after the wear and the decalcification procedure, as well as the counting of its layers, as observed under a stereomicroscope. For this individual, 11 layers were counted.

In executing the wear and decalcification technique, it was observed that, as the number of layers increased, their thickness decreased, thus making the reading of a large number of layers difficult. Figure 2 shows a tooth with three layers, which were easily counted. In this tooth the layers are thicker than in those observed on a tooth from an older individual, as represented in figure 1.

Table 1 shows the age of the individuals studied. Analysis of more than 17 layers could sometimes prove inconclusive, because many of these teeth presented significant juxtaposition of layers. The 4 teeth that could not be clearly read had at least 17 layers.

4. Discussion

Although many studies use GLG count to estimate odontocetes age, the process of GLG formation is not yet fully understood (Hohn *et al*. 1989; Rosas *et al*. 2003; Hohn 2009). It might be hypothesized that although dentine deposition is continuous throughout the life of an animal, it is not homogeneous. Otherwise, demineralization would be equal in every dentine region and would not present the irregularity observed, with depressions and crests forming along the layers of growth. One hypothesis is that when acid comes into contact with the tissue, the region with lower mineral density becomes a depression while that with greater mineral density becomes a crest, thus resulting in the establishment of GLG.

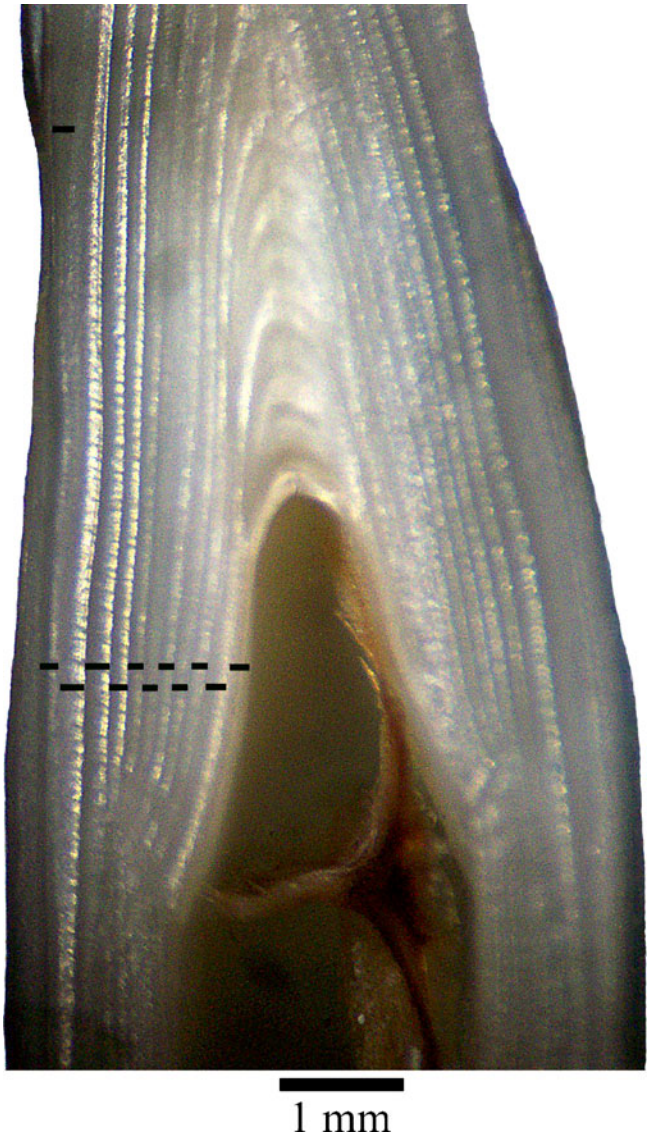

Figure 1. Partial view of a *Sotalia guianensis* tooth after the wear and decalcification procedure (MCN-026). The *dashes* indicate the dentine layers, the first one (the outer one in the upper portion of the tooth) being the neonatal layer.

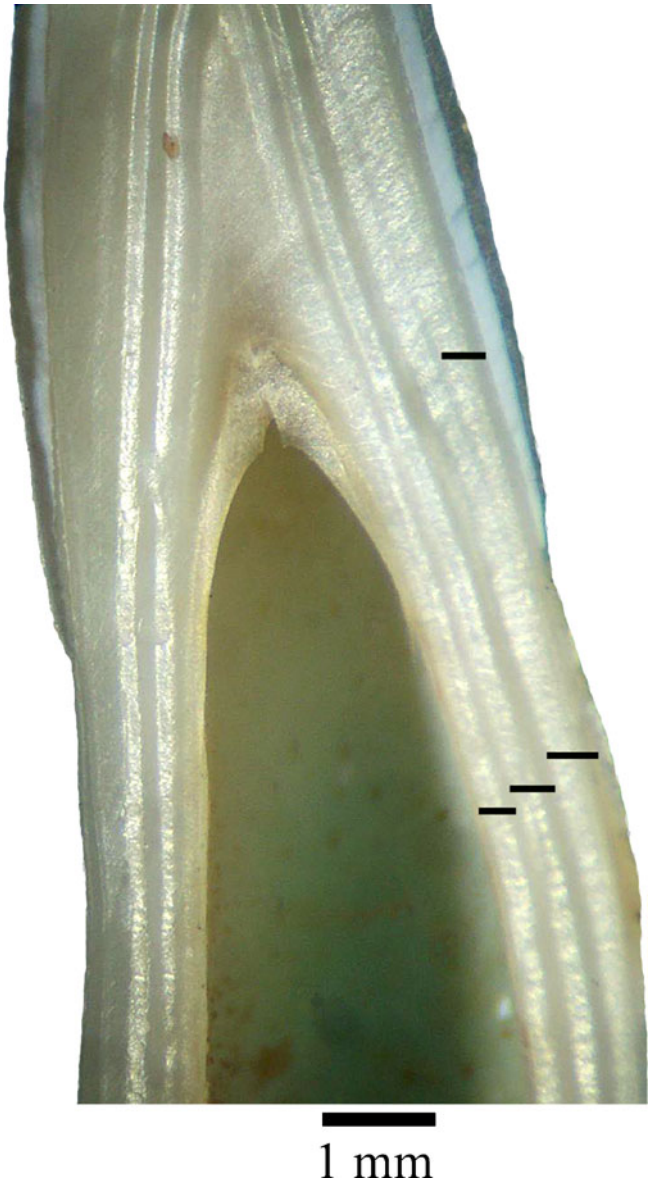

Figure 2. Partial view of a *Sotalia guianensis* tooth after the wear and decalcification procedure (MCN-045). The *dashes* indicate the dentine layers, the first one (the outer one in the upper portion of the tooth) being the neonatal layer.

However, the reason for this differential mineral deposition occuring as a regular 1-year cycle is unknown, although Boyde (1980), Klevezal (1980) and Scheffer and Myrick (1980) have suggested that seasonal variations in growth patterns, genetic and physiological cycles, diet and hormonal changes, as well as intrinsic factors involving general metabolism, may be the causes for the formation of growth layers.

According to Hohn (2009), the annual layering pattern is possibly endogenously controlled, whereas subannual layers are influenced by environment effects. Estuarine dolphins do not make great migrations, nor has large variations in their habitat or diet throughout the year, but present annual layering pattern. This fact is consistent with Hohn's (2009) hypothesis of the endogenous control of the annual layers, which appear even in species not subject to large environmental variations. Despite the gap in our understanding of the formation processes of growth layers, the technique applied here enabled an effective count of up to 17 growth layers. The recognition and reliable counting of these layers enable the use of the wear and decalcification method for small cetaceans, and similarly for the larger ones, as performed by Pierce and Kajimura (1980). This method will simplify the study of age structures of populations and confine the application of costlier methodologies to specific studies of individuals of advanced age. This technique enables classification of the calf, young and adult classes, which is important for many population studies. The adoption of this technique will enable greater use of specimens already deposited in research collections, enabling the age of individuals to be established, thus providing data on the structure of population samples.

Table 1. Estimated age of each *Sotalia guianensis* specimen using the wear and decalcification method

Museum number	Age (years)
MCN-003	9
MCN-004	12
MCN-010	11
MCN-011	14
MCN-014	7
MCN-017	11
MCN-019	4
MCN-026	11
MCN-027	15
MCN-033	9
MCN-038	7
MCN-042	23
MCN-043	7
MCN-044	4
MCN-045	3
MCN-055	5
MCN-060	17
MCN-061	15
MCN-063	4
MCN-071	5
MCN-072	19
MCN-083	4
MCN-085	18
MCN-091	8
MCN-092	6
MCN-093	2

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References

Borobia M, Siciliano S, Lodi L and Hoek W 1991 Distribution of the South American dolphin *Sotalia fluviatilis. Can. J. Zool.* **69** 1025–1039

Boyde A 1980 Histological studies of dental tissues of Odontocetes. *Rep. Int. Whaling Comm. Special Issue* **3** 65–87

Flores PAC 2002 Tucuxi – *Sotalia guianensis*; in *Encyclopedia of marine mammals* (eds) Perrin WF, Würsig B and Thewissen JGM (San Diego: Academic Press) p. 1414

Geise L and Borobia M 1987 New Bazilian records for *Kogia, Pontoporia, Grampus,* and *Sotalia* (Cetacea, Physeteridae, Platanistidae, and Delphinidae). *J. Mamm.* **68** 873–875

Hohn, AA 1990 Reading between the lines: analysis of age determination in dolphins; in *The bottlenose dolphin* (eds) S Leatherwood and RR Reeves (San Diego: Academic Press) p. 653

Hohn AA 2009 Age estimation; in *Encyclopedia of marine mammals* 2nd edition (eds) WF Perrin, B Würsig and JGM Thewissen (London: Academic Press) p. 1316

Hohn AA, Scott MD, Wells RS, Sweeney JC and Irvine AB 1989 Growth layers in teeth from known-age, free-ranging bottlenose dolphins**.** *Mar. Mamm. Sci*. **5** 315–342

Klevezal GA 1980 Layers in the hard tissues of mammals as a record of growth rhythms of individuals. *Rep. Int. Whaling Comm. Special Issue* **3** 89–94

Nishiwaki M and Yagi T 1953 On the age and growth of teeth in a dolphin, *Prodelphinus coeruleo-albus*. *Sci*. *Rep*. *Whales Res*. *Inst.* **8** 133–146

Pierce VK and Kajimura H 1980 Acid etching and highlighting for defining growth layers in cetacean teeth. *Rep. Int. Whaling Comm. Special Issue* **3** 99–101

Pinedo MC and Hohn AA 2000 Growth layer patterns in teeth from the franciscana, *Pontoporia blainvillei*: developing a model for precision in age estimation. *Mar. Mamm. Sci.* **16** 1–27

Ramos RMA 1997 *Determinação de idade e biologia reprodutiva de* Pontoporia blainvillei *e da forma marinha de* Sotalia fluviatilis *(Cetacea: Pontoporiidae e Delphinidae) no Norte do Rio de Janeiro*. Dissertação de mestrado. UENF, Campo dos Goytacazes-RJ

Ramos RMA and Di Beneditto APM 2005 *Guia para estudo de cetáceos. Volume 3: Métodos para determinação de idade em cetáceos odontocetos* (Campos dos Goytacazes: Editora da Universidade Estadual do Norte Fluminense Darcy Ribeiro) p. 38

Ramos RMA, Rosas FCW, Simões-Lopes PC, Zanelatto RC, Dans S and Crespo EA 2008 Estimativa de idade; in *Biologia ecologia e conservação do boto-cinza* (eds) ELA Monteiro-Filho and KDKA Monteiro (São Paulo: Páginas & Letras Editora e Gráfica) pp 193–209

Ricklefs REA 2003 *Economia da natureza* (Rio de Janeiro: Guanabara Koogan) p. 503

Rosas FCW 2000 *Interações com a pesca, mortalidade, idade, reprodução e crescimento de* Sotalia guianensis *e* Pontoporia blainvillei *(Cetacea: Delphinidae e Pontoporidae) no litoral sul do Estado de São Paulo e do Estado do Paraná, Brasil*. Tese de doutorado. UFPR, Curitiba-PR

Rosas FCW, Barreto AS and Monteiro-Filho ELA 2003 Age and growth of the estuarine dolphin (*Sotalia guianensis)* (Cetacea, Delphinidae) on the Paraná coast, Southern Brazil. *Fish. Bull*. **101** 377–383

Scheffer VB and Myrick AC 1980. A review of studies to 1970 of growth layers in the teeth of marine mammals. *Rep. Int. Whaling Comm. Special Issue* **3** 51–63

Simões-Lopes PC 1988 Ocorrêcia de uma população de *Sotalia fluviatilis* Gervais 1853, (Cetacea, Delphinidae) no limite sul de sua distribuição, Santa Catarina, Brasil. *Biotemas* **1** 57–62

Slooten E 1991 Age, growth, and reproduction in Hector's dolphins. *Can. J. Zool.* **69** 1689–1700

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