

A METHOD FOR AGE DETERMINATION OF DOLPHINS

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The use of growth layers in teeth as an indicator of age in odontocetes and pinnipeds was suggested by Laws (1954) and since then the method has been used extensively in both marine and non-marine mammals. Dentinal growth layers are groups (growth layer groups) of repetitive alternating bands which in cross-section are similar to growth rings in trees. The most commonly used methods for counting growth layer groups (GLGs) are by undecalcified longitudinal thin sections (150 μm) or decalcified and stained thin sections (10-30 μm). In longitudinal sections viewed with light microscopy, GLGs appear as opaque and translucent cones nestled one inside another, with the oldest dentine lying adjacent to the enamel, and the newest layer bordering the pulp cavity.

Each GLG consists of two layers differentiated by optical or mineral densities, and, in at least dolphins (family Delphinidae), evidence from captive and marked animals has shown that one GLG is deposited each year. During one part of this annual cycle a layer of dentine is relatively hypomineralized;

during the remainder of the cycle the dentine is relatively hypermineralized. Conventional methods of counting GLGs have used this mineral pattern to differentiate GLGs by corresponding differences in optical densities visible in thin sections or by differential stainability of the layers.

Although most of the methods currently employed in defining and counting GLGs yield adequate results, the clarity of layering is often poor with repeated counts on sections of teeth giving different results because of fine "accessory layers" interfere with boundaries GLGs. An alternative method for resolving GLGs is by etching the tooth, i.e. removing superficial calcium, which produces a pattern of alternating ridges and grooves, which are easily seen in large sperm whale teeth but the small size of some dolphin teeth (e.g. Stenella sp., about 0.3 cm diameter, and Turiops, about 0.6 cm diameter) prohibits the use of the method with more conventional microscopic techniques. The use of SEM on etched sections of small dolphin teeth greatly facilitates the counting of GLGs over these conventional methods.

Cross-sections (0.25 cm) or longitudinal half-sections of teeth were cut on a Buehler Isomet low speed saw. Each section was etched in 5% formic acid for three hours, rinsed in water, cleaned in an ultrasonic cleaner in acetone, then dipped in 70% ethanol. Each prepared section was then air dried, mounted on an SEM stub, coated with gold-palladium, and viewed with a Cambridge Stereoscan MK11A or S4-10 SEM. Micrographs were taken at a 45-55 tilt angle.

Micrographs show the clear resolution of GLGs as ridges and grooves (figure 1 and 2), in many cases without the interferences of accessory layers. In addition, a finer pattern of layering can be seen, at least in young animals where the pulp is not yet occluded, which seems to indicate a rhythmic pattern of dentine deposition as incremental lines within each GLG (figure 3). This method seems to offer the clearest way to resolve and count GLGs in the teeth of small dolphins and is useful to identify additional structures in the dentine.

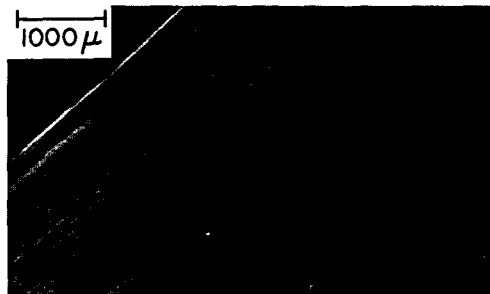


Figure 1: An etch longitudinal section of tooth from an old bottlenose dolphin, Tursiops truncatus, with 18 GLGs, where each GLG consists of one ridge and one groove. The occluded pulp cavity indicates that this is only a minimum age.



Figure 2: Longitudinal section of a tooth from Tursiops with at least 20 GLGs and an open pulp cavity. Cementum, which surrounds the dentine, is visible at the bottom of the picture.

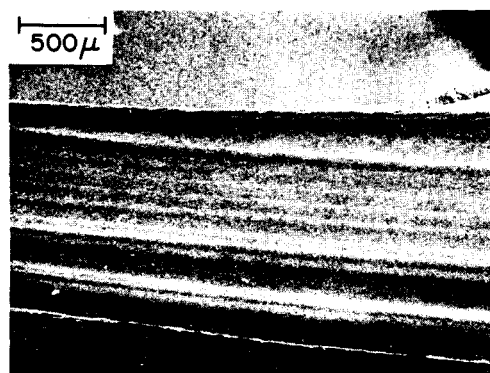


Figure 3: Incremental lines which compose the two GLGs in this tooth are visible in this section from a young Tursiops.