Variability in Techniques of Counting Dentinal Growth Layer Groups in a Tooth of a Known-Age Dolphin, *Tursiops truncatus*

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ABSTRACT

A photograph of a mid-longitudinal tooth section of a known-age bottlenose dolphin was used to evaluate the techniques of six experienced tooth readers in counting the growth layer groups (GLGs).

The results point up the problem of using different criteria to define GLGs. When age is known, GLG counts may be similar even if somewhat different landmarks in the tissue are used. However, when age is unknown, GLG definitions tend to be more variable among readers using different criteria. The results of the present exercise suggest a critical need to intercalibrate the various aging techniques for given species.

INTRODUCTION

The method of age determination in odontocete or toothed whales according to the number of layers in the dentine was first developed for striped dolphins *(Stenella coeruleoalba)* by Nishiwaki and Yagi (1953). Subsequently it was applied to sperm whales *(Physeter macrocephalus)* by Nishiwaki, Hibiya and Ohsumi (1958); to white whales *(Delphinapterus leucas)*, pilot whales *(Globicephala melaena)*, and bottlenose dolphins *(Tursiops truncatus)* by Sergeant (1959); and to common dolphins *(Delphinus delphis)* by Kleinenberg and Klevezal' (1962). In recent years, the method has become standard procedure for determining age in many other odontocetes.

In the course of dental appositional growth, layers of differential mineral and optical density are accumulated in the dentine in complex patterns. Although in many cases prominent 'annual' layers may be distinguished in sectioned teeth, two or more prominent sublayers (i.e. accessory layers) within each annual layer may be apparent also. The presence of accessory layers has made difficult the delineation of annual layers; they may be counted inadvertently as annual layers.

Berzin (1964) and Klevezal' and Kleinenberg (1967) discussed the problems posed by the existence of accessory layers; the latter authors called attention to the need to calibrate dentinal layers because of the continuing disagreement among workers arising from differing interpretations of accessory layers. In 1968, the International Whaling Commission sponsored a special meeting of sperm whale biologists from several nations to resolve differences in tooth-reading methods and to agree upon a standard descriptive terminology (IWC, 1969). As a result of the meeting, the following terms were defined: *Lamina* – a translucent or opaque zone in the dentine. The *translucent zone* appears clear or light in transmitted light, dark in reflected light. The *opaque zone* appears dark in transmitted light, light in reflected light. A *growth layer* (layer) consists of two adjacent laminae, one translucent, the other opaque.

Sergeant (1959), Sergeant, Caldwell and Caldwell (1973) and Hui (1978), who independently undertook studies of teeth of a few known-age bottlenose dolphins in attempts to identify annual dental layers, obtained layer counts that correspond to the ages of the animals. In many cases however, the definition of countable layers (GLGs, terminology of the workshop report – this volume) continues to be highly judgmental and variable among workers. This may be due chiefly to the wide variety of criteria used in defining layers.

To examine this problem more thoroughly, an exercise was conducted that compared the methods of six experienced tooth readers in defining GLGs of a tooth of a bottlenose dolphin of known age. Since the true age of the animal represented was withheld from four of the six readers, one of the aims of the experiment was to compare various methods of GLG identification rather than to determine the accuracy of GLG counts in relation to age. Comparison of the interpretations between the two readers who had access to the age information was carried out to evaluate the criteria used in defining the GLGs when age is known.

MATERIALS AND METHODS

The material for the present exercise consisted of a photograph of a mid-longitudinal section of an acid-etched tooth from 'Pinger', a 3.3-year-old bottlenose dolphin, *Tursiops truncatus*, which was born on 4 November 1970 and died on 4 February 1974. The photograph was used in an earlier calibration study (Hui, 1978). Preparation of the section was described by Hui (ibid).

Copies of the photograph were distributed to six tooth readers experienced in aging delphinids. To facilitate comparisons of GLG definitions among the readers, the same part of the tooth section in each photograph was overlain with a strip of clear plastic tape. Each participant was instructed to record his readings of GLGs on the tape (Fig. 1).

RESULTS

The photograph of the thin-sectioned tooth (Fig. 1) shows three thick layers of postnatal dentine separated from each other by strongly optically opaque boundary layers. These thick layers contain two or more sublayers, each separated from the other by thin, optically opaque boundary layers of variable intensity.

Fig. 1. Photomicrograph of longitudinal tooth section of the 3.3-year-old specimen of *Tursiops truncatus*.

The GLGs as defined by the six participants are compared in Fig. 2. The interpretations made by Coe, Perrin, Hui and Kimura are similar. Perrin, Coe and Kimura used the strongly opaque layers to divide the postnatal dentine tissue into three and a fraction GLGs, but Hui used a thin, translucent layer adjacent to each strongly opaque layer to separate GLGs. Sergeant used the primary (strongly opaque) and some of the more intense secondary (thin opaque) boundary layers to divide the tissue into six and a fraction GLGs. Miyazaki's interpretations were similar to those of Sergeant except for the third GLG defined by Miyazaki, in which Sergeant identified two GLGs. Miyazaki's count was five and a fraction GLGs.

DISCUSSION

Although GLG counts made by Coe, Perrin, Kimura and Hui corresponded closely, the criteria used by Kimura and Hui (to whom Pinger's age was known) differ from each other somewhat. Miyazaki's and Sergeant's GLG definitions were similar, but their counts differed from each other by one GLG and from those of the other participants by two and three GLGs, respectively.

The results of the exercise point up the problem of using different criteria to define GLGs. When age is known, as in the cases of Kimura and Hui, GLG counts may be similar using somewhat different landmarks in the tissue. However, when age is unknown, as in the cases of Coe, Perrin,

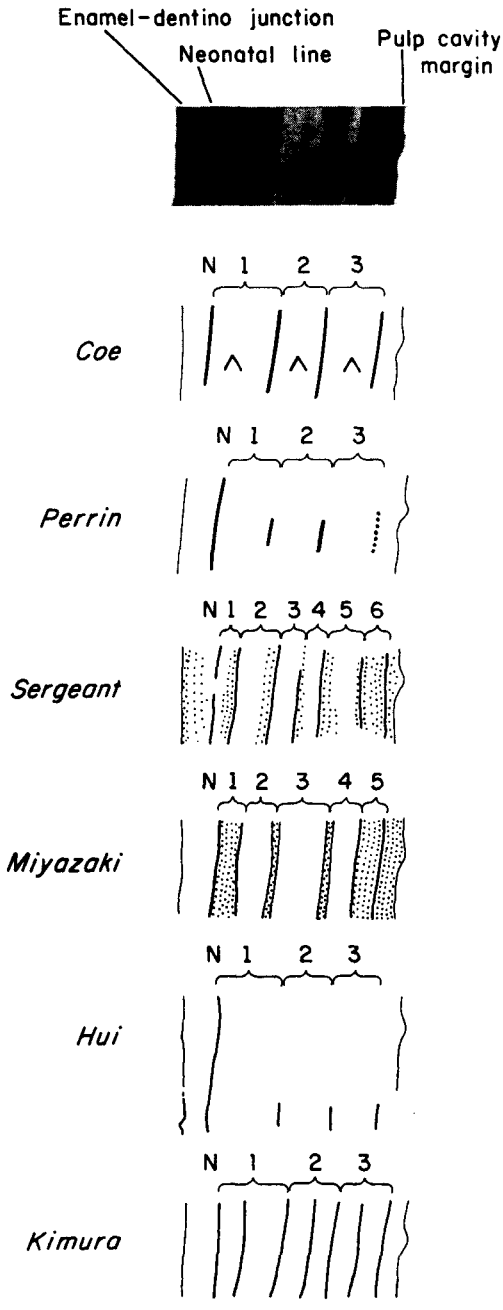

Fig. 2. GLG counts for the 3.3-year-old specimen of *Tursiops truncatus* as estimated by various readers. Inverted v's indicate accessory layers. The photograph of the field in the tooth section where the GLG counts were made is shown on the top.

Sergeant and Miyazaki, GLG definitions tend to be more variable among readers using different criteria.

Dentinal GLGs are complex in pattern and variable in optical density. Because of the wide range of preparation and counting techniques in use, there remains a critical need to intercalibrate the various techniques used for dental age determination for given species. Without precise definitions of what units are being counted, the existing confusion over growth rates, age composition, and other life history parameters of odontocete species will go unresolved.

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