A note on the preparation of sperm whale (*Physeter macrocephalus*) teeth for age determination

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ABSTRACT

We describe a modification to the most common method of preparing sperm whale teeth for age determination. The first mandibular or nearest straightest tooth was sectioned in half with a slow-rotating band saw, polished and, rather than subjecting the sectioned tooth to 10% formic acid for 30 hours, etched in 15% formic acid. The exposure time of each tooth to the acid varied depending on the size, and especially, the density of the tooth. Clear, well defined growth layer groups in sperm whale teeth suitable for age determination can be produced in substantially shorter periods of time. A method for the preparation of teeth from young sperm whales is also described. Thin sectioning and staining of teeth is used to prepare small teeth from young animals and avoids potential decalcification, which may possibly occur using acid etching methods.

KEYWORDS: AGE DETERMINATION; SPERM WHALE; AUSTRALASIA; SOUTHERN HEMISPHERE; STRANDINGS

INTRODUCTION

Determination of the age of animals is essential in understanding the ecology and physiology of populations. Knowledge of the age of a population of animals provides information on demographics, growth rates, population structure and age at sexual and physical maturity (Langvatn, 1995). Structures used to determine age (e.g. teeth) can also yield information on general health, reproductive history and the influence of environmental factors on growth, health and reproduction (Lockyer, 1995).

The teeth of sperm whales, *Physeter macrocephalus*, have been used to determine the age of individuals since the 1950s. Laws (1952) identified layers within the dentine of sperm whale teeth similar to those found in pinniped teeth and suggested they could be used for age determination. Nishiwaki *et al.* (1958) identified these dentinal layers as growth layer groups (GLGs) and suggested that two GLGs represented one year of growth for sperm whales. Limited mark-recapture studies investigating the accumulation rate of growth layers and studies calibrating seasonal changes in the thickness of the most recently formed dentine layer have been conducted (Ohsumi *et al.*, 1963; IWC, 1967; 1971; Best, 1970; Gambell, 1977). These however, suggest that the rate of deposition is more likely to be one GLG per year for this species.

Direct calibration of this assumption is difficult. Calibration of GLGs has been established for only a small number of odontocete species. For some small cetaceans, calibration has been carried out using captive 'known-age' animals (Goren *et al.*, 1987; Hohn *et al.*, 1989; Hohn, 1990) or tetracycline marking experiments (Best, 1976; Gurevich *et al.*, 1980; Brodie *et al.*, 1990; Myrick *et al.*, 1988; Myrick and Cornell, 1990).

The reading of GLGs is not a simple procedure. Counting GLGs can be confounded by poor definition in both the dentine and cementum (Klevezal', 1980), confusion with 'accessory bands' representing growth rates other than annual rates (Pierce and Kajimura, 1980; Mikhalev, 1982) and disturbances such as the mineralisation anomalies described by Lockyer (1993; 1995). With the aim of increasing the accuracy of counts from odontocete teeth, Myrick *et al.* (1983) listed four requirements for the preparation and reading of odontocete teeth: (1) a familiarity with the deposition and distribution of dental tissues; (2) a method of preparation that yields clear resolution of GLGs; (3) a definition of these GLGs in order to obtain consistency of counts; and (4) the representation of the amount of time associated with each GLG.

The most common method of tooth preparation for age determination of sperm whale teeth is to section either a mandibular or maxillary tooth (usually the first or the nearest straightest and unworn) longitudinally, followed by a period of acid etching (e.g. see review in IWC, 1980). Teeth are rinsed with water to remove any remaining acid and air-dried before reading.

Little has been published concerning the preparation of the teeth from young sperm whales. The smallest animal addressed in IWC (1980) was an 890cm female. This reflects the fact that most available material originated from whaling catches and minimum size limits were in force for the period since the International Whaling Commission was established, varying between 30-38ft or 9.2-11.6m (e.g. Beddington and Cooke, 1981). Small animals were usually the foetuses of pregnant females.

Three mass strandings on the north and west coasts of Tasmania, Australia in 1998 provided teeth from sperm whales ranging in size from 417cm to 1140cm. This paper describes a modification to the most commonly used preparation method for the teeth of adult sperm whales that we believe: (1) helps in improving the resolution of GLGs and hence in a more accurate reading of the teeth and thus estimation of age; and (2) is quicker. A method for the preparation of teeth from young sperm whales is also described.

MATERIALS AND METHODS

Descriptions of growth layer groups (GLGs) particular to sperm whale teeth can be found in Nishiwaki *et al.* (1958); Ohsumi *et al.* (1963); IWC (1969); Gambell (1977); Perrin and Myrick (1980); and Donovan (1985). Definitions of terms involved in age determination were published in the

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Proceedings from the International Conference on Determining Age of Odontocete Cetaceans (and Sirenians) and a following workshop associated with that conference (IWC, 1980; Perrin and Myrick, 1980). These definitions are used here and thus a GLG is 'a repeating or semi-repeating pattern of adjacent groups of incremental growth layers within the dentine which is defined as a countable unit involving a change…. from a ridge to groove' in the case of etched teeth and 'intensely stained to lightly stained' in the case of thin-sectioned, stained teeth.

Adults/sub-adults

Near complete or smaller, partial lower jaws with teeth were collected from 94 sperm whales derived from three mass strandings on the north and west coasts of Tasmania over the months of February and March 1998. The first mandibular or the nearest straightest tooth from 52 of these animals were prepared using sectioning and acid etching (see below). In addition, ten teeth (four from the subset of 52 and six additional teeth from the original set of 94; two of these teeth were from the same individual) were prepared for a comparative study. Of these, one half of each tooth was prepared using the methods detailed below, the other half was etched for 30 hours using the methods detailed in Lockyer (1980), to compare the effects of longer-term exposure to formic acid.

Sectioning teeth

The two methods used for preparing teeth for sectioning are described below.

(1) The front mandibular or nearest straightest tooth was set in polyester resin to which a hardener, methyl ethyl ketone peroxide (MEKP), was added. The tooth was placed in an appropriate vessel and oriented such that its concave curvature was uppermost before pouring the resin into the vessel. The resin was left to set overnight.
(2) The front mandibular or nearest straightest tooth was set onto a wooden block with Thermoplastic Quartz Cement Lakeside No. 70C (Hugh Courtright and Co., Illinois, USA). Again the orientation of the tooth was such that its concave curvature was uppermost.

The tooth was sectioned in half using a circular band saw set at low-speed, with the cut orientated along the bucco-lingual plane. Cutting followed the curvature of the tooth and the resulting sections followed the midline of the tooth as closely as possible. While it is common and less time consuming to use a slow rotating diamond saw to section large teeth such as those from sperm whales, we did not have access to such equipment. More extensive polishing is required when a band saw is used and thus each tooth was sectioned slightly further from the centre than is probably necessary using a diamond saw, in order to compensate for the extra polishing required to achieve a polished, on-centre section.

Preparation of sections for acid etching

The most suitable half (that half closest to the mid-line of the tooth and exposing the most complete surface of the mid-line) or both halves of the tooth were polished with 150 grade sandpaper followed by 320 grade sandpaper until the majority of saw marks were removed from the cut surface.

Acid etching

The polished half section was placed in a bath of 15% formic acid, cut surface down, and agitated to remove any air bubbles that might disrupt etching and result in uneven etching. The bath contained a minimum of 600ml and a maximum of 800ml of formic acid, allowing for the covering of 0.5-1.0cm of the side of the tooth. Up to seven tooth sections (depending on the size of the sections) were placed in the bath at any one time. The tooth section was kept in the bath at room temperature for a period of three hours. The section was then removed and placed under running tap water for three minutes, then removed and placed in a bath of acetone for three minutes, to remove any traces of acid. It was placed again under running water for three minutes, removed and left to dry. Checks on the state of etching were made under a magnifier and the tooth section placed back into 15% formic acid for periods of 30 minutes until a clear and complete etched surface was produced. Each bath of formic acid was only used for 24 hours: the acid was discarded after this period and replaced with a fresh mixture.

Reading

Before counting GLGs, the surface of the half-section was rubbed with a soft lead pencil (No. 1 grade) to emphasise the relief of the etched surface. GLGs were counted under a magnifier. Differences in the number of GLGs estimated between halves of teeth subjected to different concentrations of acid were tested at the 95% significance level with a t-test.

Juveniles/calves

Teeth from six juvenile animals (4 females, 2 males, ranging from 417-780cm) were prepared for ageing using similar methods to those used for small delphinids (Myrick *et al.*, 1983; Lockyer, 1993).

Sectioning teeth

The straightest tooth from each jaw was mounted on a wooden block with Thermoplastic Quartz Cement Lakeside No. 70C and sectioned in half using an Isomet (Buehler Ltd., Illinois, USA) low speed rotary diamond saw machine. The cut was made longitudinally to one side of the mid-line of each tooth so that two unequal halves resulted, the thicker still containing the most central mid-line of the tooth.

Decalcification

The thicker halves were placed in histological baskets and decalcified in RDO decalcifying agent (Apex Engineering Products Assoc., Illinois, USA) for three hours, agitating the specimens every one to two hours. Checks were made on the state of decalcification and the section removed or placed back into RDO and checked every subsequent hour until decalcification was complete. Decalcification was regarded as complete when the section was flexible and slightly translucent. The teeth were then flushed in running water overnight to remove any traces of the decalcification reagent. Decalcification ranged from four hours (428cm female) to 30 hours (667cm male).

Thin sectioning

Decalcified half-sections were mounted with OCT compound (Diagnostics Division, Miles Inc. Indiana, USA) onto the CO_2 freezing stage of a sledge microtome. The cut surface of the tooth was orientated upwards and as horizontal as possible to ensure that the straightest possible sections were achieved. Serial 35μm longitudinal sections were cut until the mid-line of the tooth was passed and the central cavity began to reduce. A painters brush was used to remove the sections from the microtome, placing each into a petri

dish of distilled water for later sorting. The thin-sections were sorted, retaining only those sections closest to the central line of the tooth and most complete.

Staining
The retained sections were placed in histological baskets, rinsed again, blotted dry and stained with Haemotoxylin for 20 minutes. Specimens were agitated in the stain to ensure complete coverage by the stain and checked for the extent of staining under a dissecting microscope. Sections were 'blued' in a 2% ammonia solution for 30 seconds and rinsed under running water for a minimum of 30 minutes.

Mounting
Stained sections were sorted again, and the most central, well-stained sections retained. Sections were floated onto a slide previously coated with a 5% agarose/gelatin solution (this was carried out in a petri dish of distilled water to minimise the wrinkling of sections). The slides were dried on a slide warmer and permanently mounted under glass slide covers with DPX mountant (APS finechem, New South Wales, Australia). Slides were returned to the slide warmer for 3–5 minutes to disperse any air bubbles and then left to air dry for 24 hours.

Reading
The sections were examined using a transmitted light microscope under 2-4X power.

RESULTS AND DISCUSSION

Adults/sub-adults

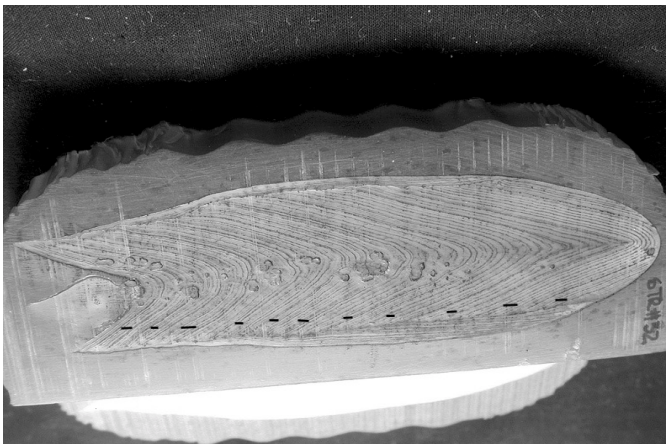
The revised and quicker method for acid etching detailed above yields clear, well defined growth layers in adult sperm whale teeth (Figs 1a and 1b). Of the 10 teeth exposed to 10% formic acid for 30 hours, two had etched surfaces in which GLGs were more pronounced and easier to discern than those on sections exposed to 15% formic acid, three had similar etching results (where GLGs were as equally defined) and five had etched surfaces in which GLGs were less pronounced and less easier to discern or had totally lost GLGs which were clearly visible on the sections exposed to 15% formic acid for a shorter period (Figs 3a and 3b, 4a and 4b).

Counts of GLGs in teeth for which the two methods produced a similar etching result were not significantly different (Table 1). Counts of GLGs in teeth for which the exposure to 15% formic acid produced more clearly discernable GLGs differed significantly from those in the half exposed to 10% formic acid. Counts of the remaining teeth in which exposure to 10% formic acid resulted in more clearly discernable GLGs were significantly different in one of two cases from those in halves exposed to 15% formic acid. Although the sample size restricts any firm conclusions being drawn from these results, as would be expected different etching results produce different age estimates. It is possible that the extended exposure of tooth sections to formic acid may result in the over-demineralisation of the cut surface of the tooth. This would result in an etched surface in which GLGs are less pronounced, difficult to discern or are totally missing. The effect would be either to underestimate the number of GLGs, or render the section more difficult to read or not readable at all. This should be investigated further with larger sample sizes.

The amount of time a tooth should be subjected to formic acid appears to be dependent on the tooth itself. Etching times in this study varied from a minimum of 3.0 hours to a maximum of 8.0 hours (Fig. 2). The time taken to produce an etched surface with clear, easily discernable GLGs was found to be significantly related to the length and width of the tooth (Multiple regression, df = 45, $P = 0.027$). Factors such as the size and the density of the tooth appear to play an important role in dictating the amount of time taken to produce a suitably etched surface (Pierce and Kajimura, 1980). It should also be noted that the age of the acid used is also an important factor in dictating the time needed to produce an adequately etched surface and re-use of acid is not recommended. Biases associated with the re-use of acid in etching were kept to a minimum by replacing the acid after 24 hours (less than the 30 hours exposure associated with the 10% formic acid).

From the results presented here, we propose that an appropriate method for preparing the teeth of adult sperm whales is to acid-etch half-sections of teeth for a baseline period of 3.0 hours in 15% formic acid. If required after checking, the sections should be etched for further periods of 30 minutes, until suitable etching is complete. These times are similar to those proposed by Pierce and Kajimura (1980).

(a) Specimen SPW3(32), 985cm adult female. Number of GLGs estimated as 35.



(b) Specimen SPW3(36), 1110cm adult female. Number of GLGs estimated as 26.

Fig. 1a and 1b. Sections of sperm whale teetch etched with 15% formic acid showing clear, well-pronounced GLGs. The last GLG in successive groups of three is marked on each figure.

[Text continues on p. 106]

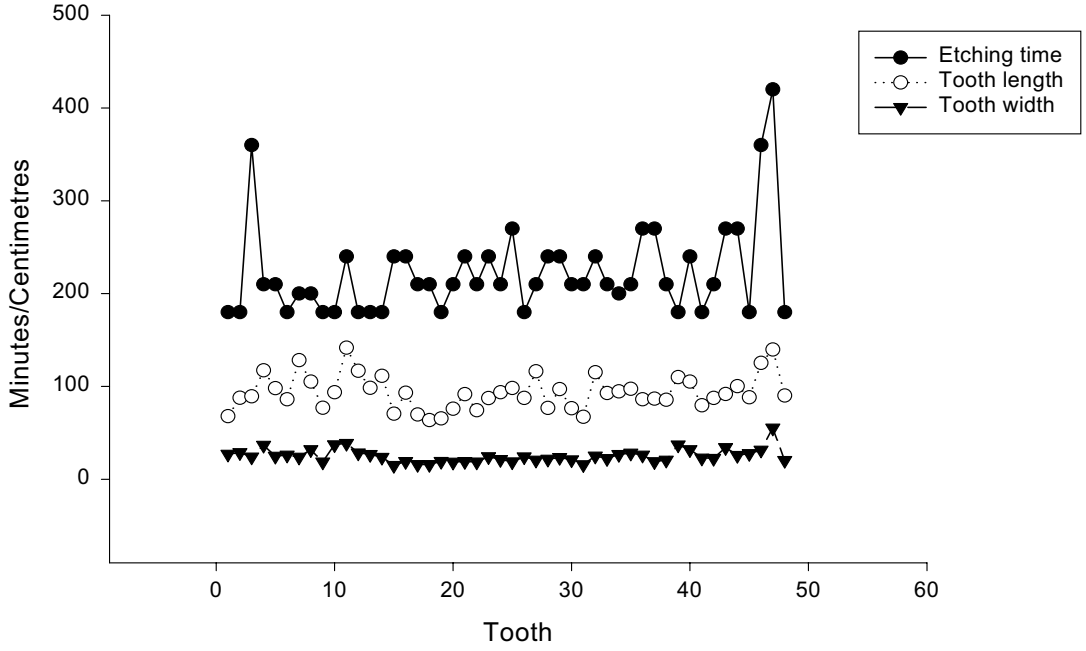
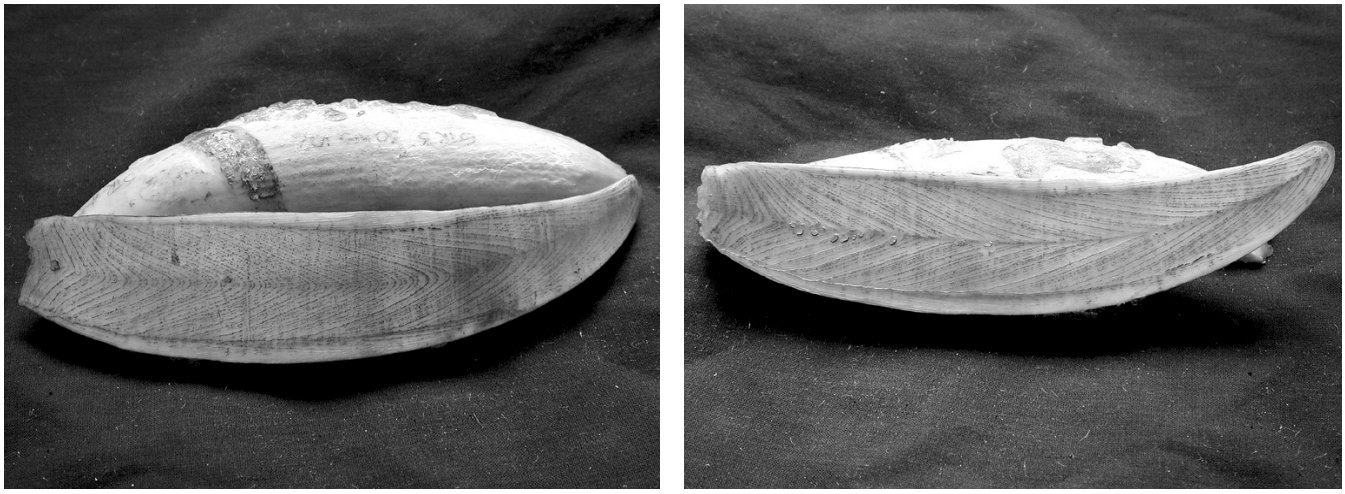


Fig. 2. Length and width (cm) of sperm whale teeth in relation to etching times (min).

(a) Half-section of tooth after 8 hours in 15% formic acid.

(b) Half-section of tooth after 30 hours in 10% formic acid.



(c) Detail of the basal portion of each tooth section.
Left: section etched in 15% formic acid; right: section etched in 10% formic acid.

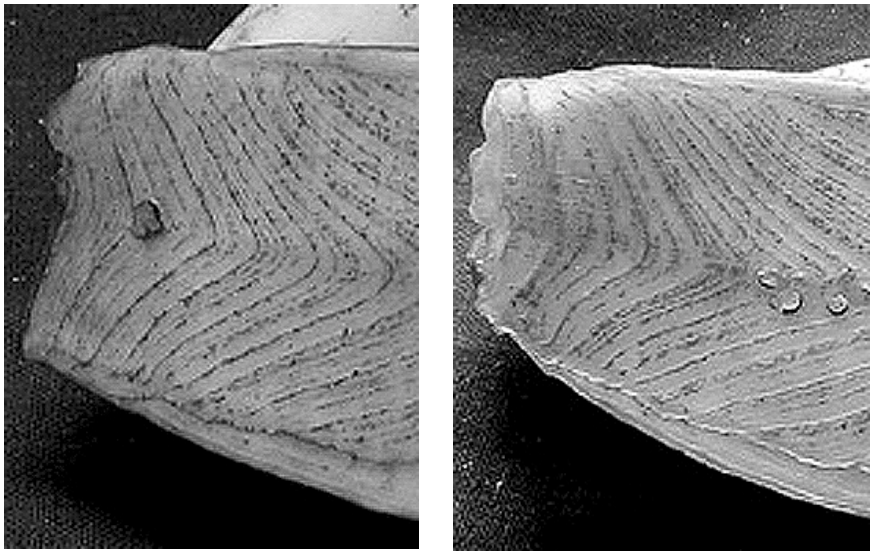


Fig. 3a, 3b and 3c. Tooth sections from SPW3(3), 1026cm adult female sperm whale. Number of GLGs estimated as 40.

(a) Half-section of tooth after 8 hours in 15% formic acid.

(b) Half-section of tooth after 30 hours in 10% formic acid.

(c) Detail of the area just anterior to the basal portion of each section (from the third GLG).
Left: section etched in 15% formic acid; right: section etched in 10% formic acid.

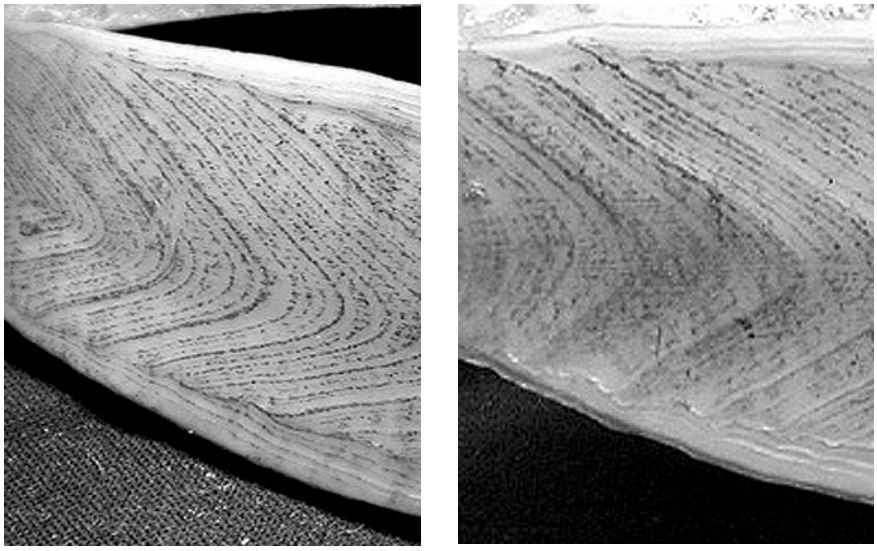


Fig. 4a, 4b and 4c. Tooth sections from SPW3(54), 1119cm adult female sperm whale. Number of GLGs estimated as 36.

Table 1

Results of t-tests on sections of teeth exposed to the two acid-etching techniques. Number of GLGs given are those values with the highest repeatability after four sessions in which the number of GLGs in each section were counted three times. The mean given is based on the counts from each four sessions (n=3) pooled. The method that produced sections in which GLGs were more pronounced and more clearly defined is given in **bold**, where there is no discernible difference no bold type is given.

	10% formic acid, 30 hours			15% formic acid, (exposure in minutes)				
Reg. No.	No. GLGs	Mean	SE	No. GLGs	Mean	SE	df	*P*
SPW3(3)	32	32.67	0.51	**42 (180)**	40.58	0.63	11	<0.001
SPW3(34)	29	28.92	0.43	**22 (180)**	22.08	1.06	11	<0.001
SPW3(37)	35	34.08	0.62	**35 (480)**	36.42	0.45	11	0.012
SPW3(54)	35	32.75	0.85	**36 (345)**	35.92	0.26	11	0.004
SPW4(1)	33	29.33	0.94	**33 (300)**	35.75	0.84	11	<0.001
SPW4(4)	**18**	19.83	0.62	21 (405)	21	0.30	11	0.036
SPW4(8)	**33**	39.58	2.22	39 (300)	37.83	0.83	11	0.37
SPW4(16)	19	19.17	0.44	18 (420)	19.67	0.48	11	0.37
SPW4(16)	20	21.92	0.93	18 (300)	22.33	0.89	11	0.68
SPW4(21)	17	19.17	0.96	17 (390)	19.33	0.66	11	0.87

Even with the small sample size given here we believe that it is sufficient to show that this method produces consistently reliable results and reduces the potential problems associated with over-etching. However, the sample size is small and the possibility of a formal internationally-organised trial with larger sample sizes should be considered, perhaps under the auspices of the IWC Scientific Committee.

Juveniles/calves

Teeth from small sperm whales (animals < 800cm) were found to be shaped like small, hollow cones, similar to those described by Berzin (1971). As we considered acid-etching to be a potentially unsuitable (too harsh) method of preparation for age determination on such small teeth, we did not test that method here. Four of the six animals examined contained fewer than two GLGs (the number of GLGs estimated ranged from 0.75-7 GLGs), and we also considered acid etching too coarse a method to discern GLGs with precision. Pierce and Kajimura (1980) found that in small teeth of phocoenids and delphinids, the cementum tended to decalcify rather than etch when exposed to acid. While these are different taxonomic groupings of cetaceans, it is possible that acid has a similar effect on small sperm whale teeth. The preparation of small sperm whale teeth using thin sectioning and staining yielded clearly discernable GLGs within the dentine (Figs 5 and 6), and provided suitable specimens for age determination.

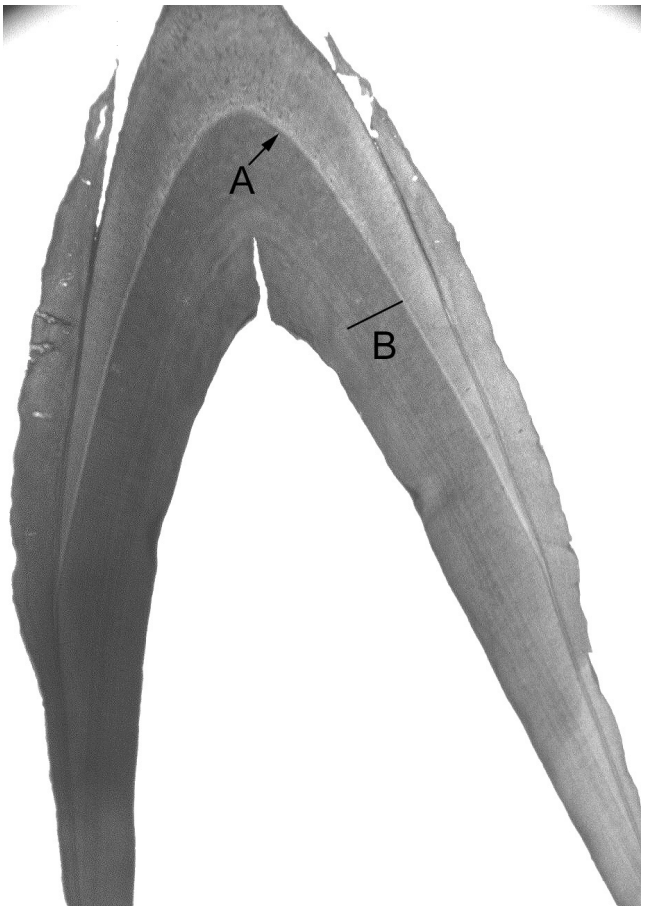


Fig. 6. Decalcified stained section of tooth from SPW4(18), 575cm immature female sperm whale. Number of GLGs estimated as 1.5.

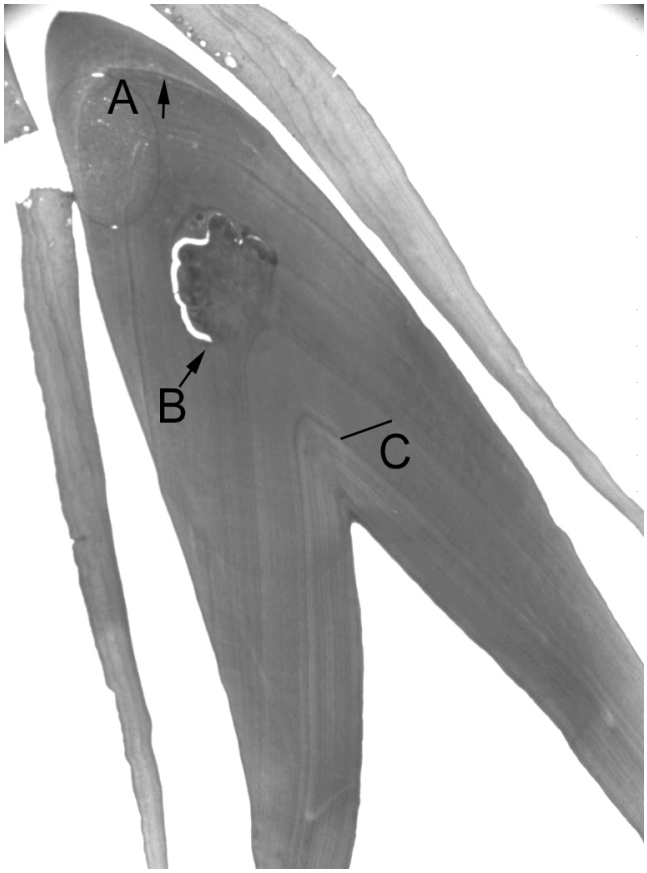


Fig. 5. Decalcified stained section of tooth from SPW4(2), 780cm immature sperm whale. Number of GLGs estimated as 5.

CONCLUSION

While we do not consider the methods described in this paper to be the only methods available that are suitable for use on sperm whale teeth, we consider them to yield consistently clear, well defined specimens suitable for age determination.

However, several authors (e.g. Perrin and Myrick, 1980; Stewart *et al.*, 1996 and Hohn and Fernandez, 1999, have found that the different preparation of teeth from the same individual can result in different age estimates. Standardisation of techniques is to be recommended to ensure accurate and consistent age estimates. A suitable international study to determine the most appropriate method should be carried out. This is essential if the results are to be used to determine biological parameter values in order to assess the status of cetaceans and develop appropriate management strategies.

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