Age estimation of white whales (*Delphinapterus leucas*) from Greenland

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Various techniques of displaying growth layer groups (GLGs) in teeth from white whales, *Delphinapterus leucas*, from Greenland were tested. The dentine and cementum in unprepared longitudinal thick sections (150–200μm) displayed under transmitted polarized light microscope generally provided the simplest and clearest display of GLGs. Teeth taken from the lower jaw of white whales in West Greenland show significant wear at a much earlier age than teeth of white whales from northern Québec, the White Sea and the Kara Sea. The least worn teeth are usually positioned towards the rear of the lower jaw (tooth numbers 7–9). They give the highest number of GLGs and thus most accurately reflect the age of the whale. Teeth from one white whale that was captured when approximately three years old and maintained in captivity for 15 years showed 30–36 GLGs. This finding adds to the evidence that two GLGs are deposited annually in the teeth of white whales.

Key words:
White whale, *Delphinapterus leucas*, Greenland, age estimation.

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Introduction

In studies of vital parameters and life history it is critically important to use reliable techniques of age estimation. This also applies to studies in which the goal is to evaluate changes in age structure due to exploitation or to analyse the accumulation of contaminants by various age classes within a population.

A number of studies of white whales, *Delphinapterus leucas*, have used longitudinal sections of teeth for displaying dentine growth layer groups (GLGs) to estimate ages of the whales. One growth layer group (GLG) is assumed to consist of a dark and a translucent layer. Longitudinally bisected, polished teeth were examined under reflected light with a dissecting microscope by Sergeant (1973), Brodie (1982) and Goren *et al.* (1987). Finley *et al.* (1982) stained the bisected teeth with haematoxylin before examining them in a microscope with reflected light. Participants in a workshop on odontocete age determination found that bisected teeth were difficult to "read" and they therefore etched the teeth in 10% formic acid (Perrin & Myrick 1980). However, etching failed to improve the readability of the teeth. Instead, longitudinal sections of 25–300 μm were examined under a microscope with transmitted light or under a microfiche reader (Perrin & Myrick 1980, Burns & Seaman 1986). Doidge (1990a) also used unstained 300 μm longitudinal midsections of teeth of white whales from northern Québec. Goren *et al.* (1987) prepared 100 μm sections for scanning with a microdensitometer. These same authors also prepared 250–300 μm sections, bathed them in 5% formic acid and examined them with a scanning electron microscope. However, this method did not prove satisfactory.

The present study evaluates different tooth preparation procedures, examines differences in wear between teeth taken from the same white whale, considers sexual dimorphism in the dimensions of teeth and identifies differences in tooth wear between populations. Teeth from a "known-age" captive white whale were used for validating our age estimation techniques.

Table 1. Counts of growth layer groups (GLGs) in dentine in unstained and stained thin sections and unprepared thick sections from 13 white whales. '+' indicates that the teeth were worn and only minimum counts of GLGs could be obtained. Numbers in parentheses indicate the position of the teeth in the jaw.

Whale ID no.	Unstained thin sections	Toluidine blue thin sections	Unprepared thick sections
1104	8 (8)	8 (8)	+ 8 (1)
1114	7 (8)	7 (8)	7 (1)
1172	5 (8)	5 (8)	5 (1)
1176	6 (8)	6 (8)	6 (1)
1178	13 (8)	13 (8)	13 (1)
1181	10 (8)	10 (8)	+ 8 (1)
1182	17 (6)	17 (6)	+13 (1)
1185	11 (7)	11 (7)	11 (1)
1186	10 (8)	10 (8)	+10 (1)
1495	+16 (least worn)	+24 (least worn)	+24 (least worn)
1496	+15 (7)	+15 (7)	+20 (1)
1498	+20 (least worn)	+35 (least worn)	+30 (least worn)
1499	+24 (8)	+30 (8)	+26 (8)

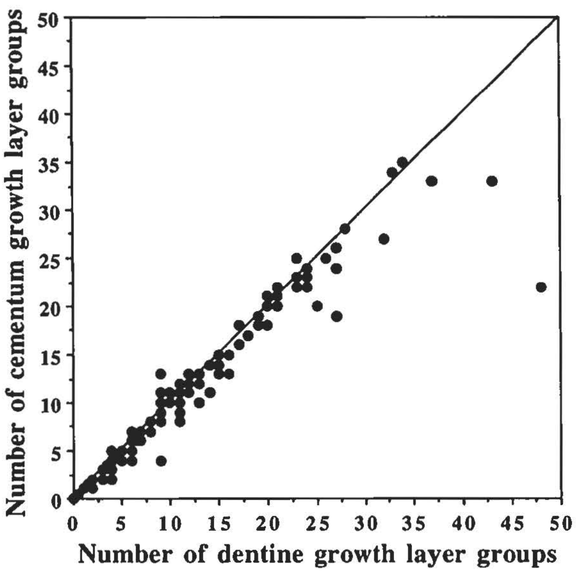


Fig. 2. Correlation between the number of dentine and cementum growth layer groups in white whale teeth.

Materials and methods

The entire lower jaws were collected from 44 white whales taken in the harvest by Inuit in West Greenland. It proved to be too time-consuming under field circumstances to collect the entire jaws from a larger sample of the catch. Instead, the lower jaws of 282 additional white whales were cut off with a saw. The jaws were kept frozen until extraction of teeth, which was done after boiling in water for 5 min or maceration in water at 40°C for a few days. As a standard, we numbered the teeth in each side of the lower jaw from 1 to 10, starting anteriorly. The effect of boiling versus maceration was tested on two whales in which the left side of the lower jaw was boiled and the right side was macerated.

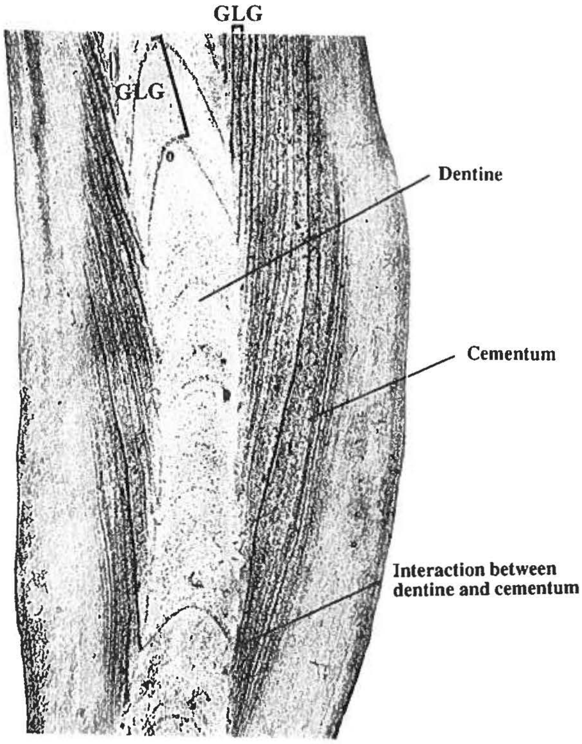


Fig. 1. Detail of white whale tooth showing the interaction between cementum and dentine.

Two types of section were prepared:

1) *Thin sections:* Teeth were decalcified for 2–20 days (depending on the size of the tooth) in 5% HNO_3. Central longitudinal thin sections (14 µm, 30 µm and 40 µm) were then cut with a freezing microtome at −22°C (Reichert Jung type 1206 and Frigomobil OM) and stained with toluidine blue following methods described by Dietz *et al.* (1991). The sections were mounted on glass slides. The thin sections were read both before and after staining with toluidine blue.

2) *Thick sections:* Unprepared longitudinal sections of 150–200 µm were cut with diamond wafering blades mounted on a Buehler Isomet lowspeed saw. When necessary, sections were polished by hand using wet 600-grit silicon carbide paper disks (3M) before examination under a microscope. Sections were kept in a mixture of alcohol and glycerin.

Counting of GLGs in both dentine and cementum was facilitated by the use of a transmitted light microscope (10 ×, Carl Zeiss Jenaval) with polarized light. Two or three trained readers examined all teeth independently. The number of GLGs in both dentine and cementum was

Table 2. Discrepancies in counts of growth layer groups (GLGs) in dentine and cementum of white whale teeth. Two or three independent observers counted the GLGs and the difference between the minimum and maximum counts was used as a qualifier (Q). In some cases a final age could not be determined (ND). The number of teeth (N(Q)) is shown for each qualifier.

	Dentine		Cementum	
Q	N(Q)	%	N(Q)	%
0	189	56	146	43
1	82	24	104	31
2	28	8	27	8
3	8	2	13	4
4	3	1	4	1
5	3	1	2	1
ND	27	8	44	13
		N = 340		

determined to be the mean of the readings, rounded upwards to the next whole number. Disagreement among the various readers was quantified as the difference between minimum and maximum values. This figure was used as a "qualifier" for the age estimation. Teeth in which a neonatal line could be detected were assumed to reveal a "complete age", whereas those where the neonatal line had disappeared due to apical wear were considered to provide only a "minimum age".

Teeth from a 372 cm female white whale that died on 26 July 1984 after 15 years in captivity at Duisburg Zoo, Germany, were used for validating the age estimation technique. The whale was estimated by her captor to have been 3 years old (length 294 cm) when captured in the Seal River near Churchill, western Hudson Bay, 5 August 1969 (Gewalt 1970). During maceration the teeth were mixed and no position numbers could be assigned to them when the cranium was deposited at the Institut für Haustierkunde in Kiel, Germany. Thus teeth from upper and lower jaws could not be distinguished, nor could the anterior and posterior teeth be identified. All teeth from this specimen are now being kept at the Greenland Fisheries Research Institute (ID no. 74). For age estimation four teeth, judged to be the least worn, were selected for sectioning (150–200 μm).

To test whether there was any difference in the number of GLGs in various teeth from the same animal, we prepared sections (150–200 μm) of 5 teeth from the same side of the jaw (usually nos 1, 3, 5, 7, 8) from 6 individuals with unworn teeth and 12 individuals with worn teeth (Figs 3 & 4).

The proportion of worn teeth in both males (N = 142) and females (N = 184) was calculated for the teeth from West Greenland. This proportion was compared with that for a sample from northern Québec (Doidge 1990b) and a large sample from the White and Kara seas. Ages for the latter sample had already been estimated and were made available to us by G.N. Ognetov (SEVPinro, Arkhangelsk). These ages were estimated by methods described in Ognetov (1985a, 1985b), which are consistent with methods described by Sergeant (1973). For both samples, ages were estimated on the assumption that 2 GLGs are deposited annually.

Mean age at tooth wear (MATW) was calculated by the technique described in DeMaster (1978).

Teeth from 206 individuals were measured to assess sexual dimorphism in tooth development. Weight, length, width and thickness were measured with a Sartorius labweight (1/10 g) and a digital micrometer (1/100 mm).

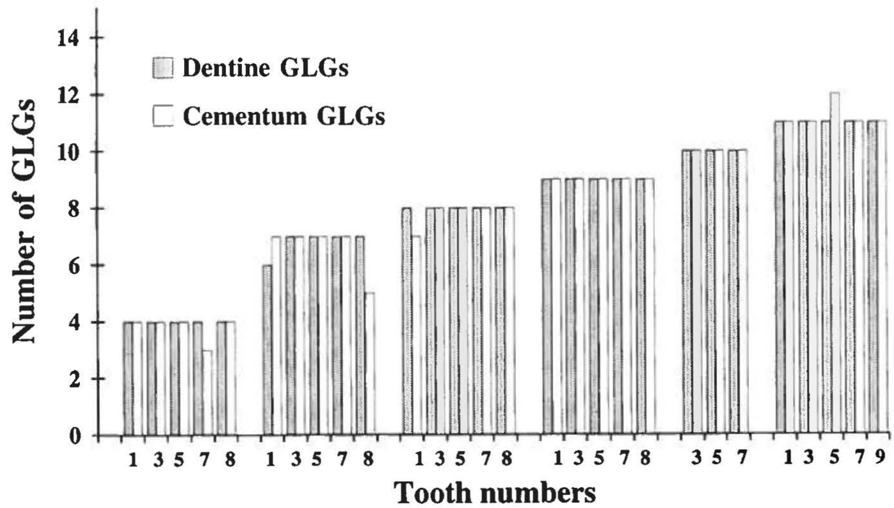


Fig. 3. Number of GLGs counted in unworn teeth from the lower jaws of six white whales from West Greenland.

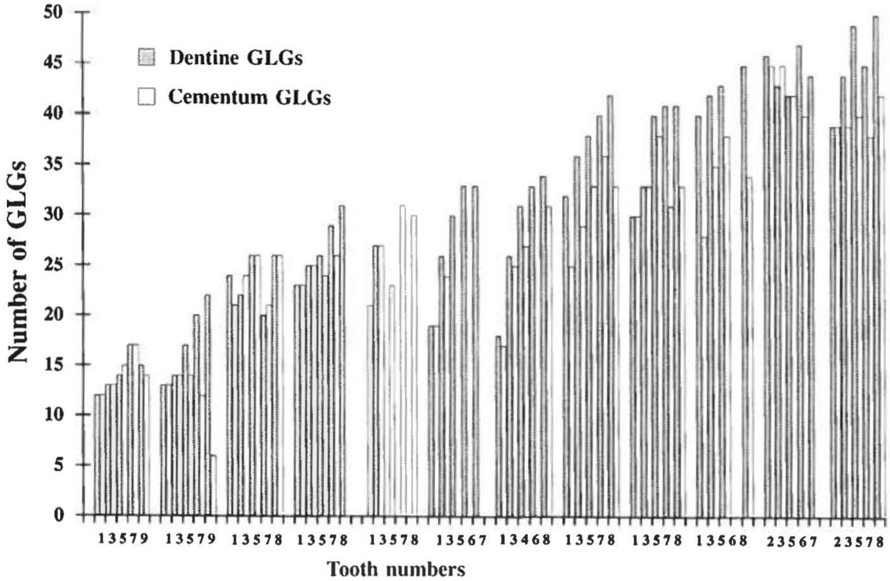


Fig. 4. Number of GLGs counted in worn teeth from the lower jaws of 12 white whales from West Greenland. The neonatal line could not be detected in any of these teeth.

Results and discussion

Preparation of teeth

There was no discernible difference in the readability of the unstained thick sections (150–200 μm) that had been boiled or macerated. Furthermore there was no obvious difference in using 14 μm, 30 μm or 40 μm thin sections, although it seemed that 14 μm sections were slightly easier to read. For younger teeth, unstained thin sections did not differ appreciably from those stained with toluidine. However, for older teeth, staining improved the readability. This effect can be seen with ID numbers 1495, 1498 and 1499 (Table 1), for which larger numbers of GLGs were counted in the stained sections.

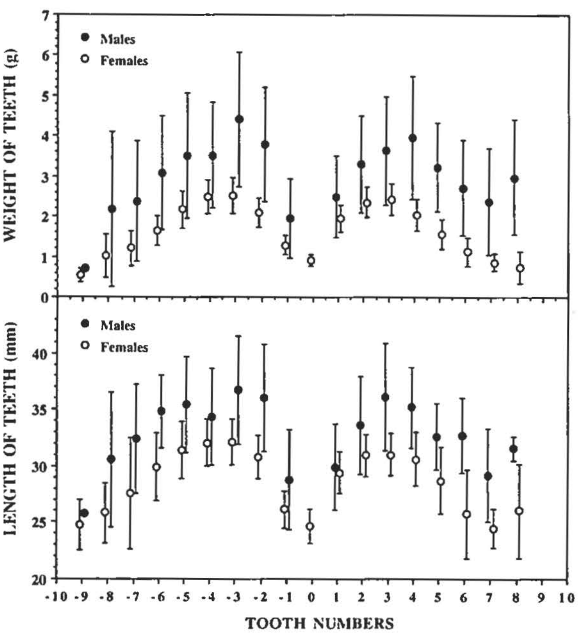


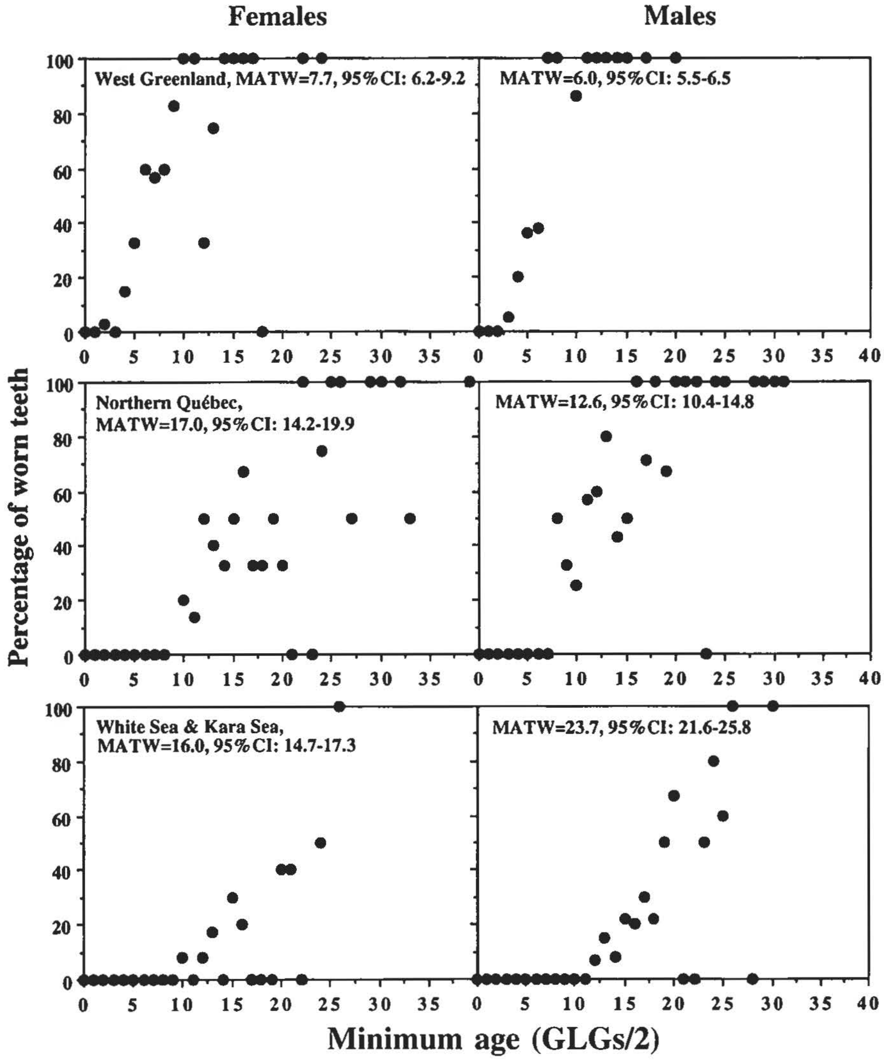
Fig. 5. Comparison of weight and length of white whale teeth from males (dots) and females (open circles) at different positions in the lower jaw. Only whales with more than 9 GLGs are shown and the vertical lines indicate 95% confidence limits. The teeth are shown from left (indicated by a minus) to right (plus).

The same tooth could not be used to make both thin sections and thick sections, so the comparison between these two methods cannot be conclusive. However, stained thin sections did provide higher GLG counts than unstained thick sections in 4 of 13 cases (Table 1). This could have been due to the fact that the teeth used for staining in 10 of the 13 cases were generally from positions farther back in the jaw than those used for thick sections. However, thick sections gave a higher number of GLGs in one case (ID no. 1496, Table 1).

The same numbers of dentine and cementum GLGs were counted in most teeth where the dentine and cementum "interacted" (Fig. 1). However, there were generally more readable GLGs in the dentine than in the cementum in both decalcified (Wilcoxon $P < 0.01$, $N = 20$) and unprepared sections (Wilcoxon $P < 0.001$, $N = 212$). This was especially pronounced in older animals (Fig. 2). For age estimation the tissue with the most readable GLGs was used. This was the dentine in most cases.

Eighty percent of the dentine GLG counts and 74% of the cementum counts deviated by less than a year (0–1 GLG, Table 2). Twelve percent of the dentine counts and 14% of the cementum counts deviated by one year or more (2–5 GLGs). In 8% of the dentine counts and 13% of the cementum counts the number of GLGs could not be determined exactly either because of a twist in the

Fig. 6. Age at tooth wear in female and male white whales from West Greenland (this study), northern Québec (Doidge 1990b) and the White Sea and Kara Sea (Ognetov unpublished data). MATW indicates the mean age at tooth wear calculated according to DeMaster (1978).



tooth that made proper sectioning impossible, or because it was difficult to discern the GLGs (Table 2). In most cases it was possible to read at least one set of GLGs – either those in the dentine or those in the cementum – and therefore an age could be estimated for most whales.

Effects of tooth wear

Unworn teeth taken from different positions in the jaw showed no significant difference in the number of dentine or cementum layers (Fig. 3). However, in worn teeth there was a trend towards more dentine GLGs being present in teeth taken from the more posterior positions in the jaw (Fig. 4). The front teeth are usually worn, especially in older whales, but they often have easily distinguished GLGs in the dentine and cementum. The teeth in the middle of the jaw are usually the largest and they are less worn than the anterior teeth. The most posterior teeth tend to be the least worn, but because of their smaller size and vestigial form they are often difficult to read. In particular, the cementum GLGs are generally more difficult to discern in these rear teeth due to their smaller size (Figs 4 & 5). The closer spacing makes it difficult to distinguish the GLGs in these teeth. More dentine GLGs are present in the posterior teeth and thus they provide a better estimate of age than do the cementum GLGs for these teeth (Fig. 4).

Tooth wear is common in both sexes, but it appears and becomes more severe in males at slightly younger ages than in females (Fig. 6). However, male white whales from the White and Kara seas have a greater mean age at tooth wear (MATW) than females. The onset of tooth wear occurs at a much greater age in white whales from

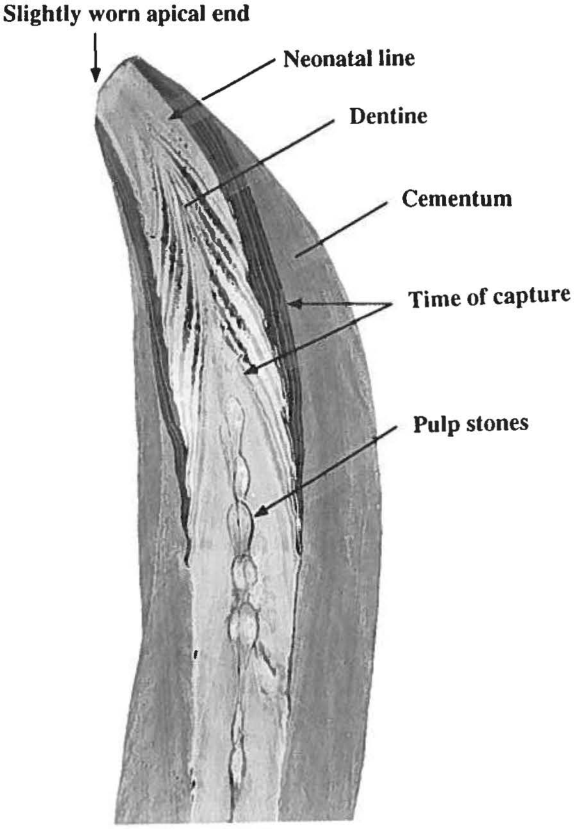


Fig. 7. Section of distal end of a tooth from a known-age white whale from western Hudson Bay. A distinct change in deposition of GLGs is evident after 6 GLGs.

the White and Kara seas (both males and females) than in white whales from West Greenland and northern Québec (Fig. 6). MATW is significantly lower for white whales from West Greenland compared with the other areas. Females from northern Québec have a MATW similar to that of females from the White and Kara seas but that of males differs greatly between the two areas (Fig. 6). These differences in tooth wear (which affects readability) must be taken into account when comparing age-related parameters between populations.

Pulp stones

Pulp stones appear occasionally in the dentine, but never in the cementum, of both males and females. Pulp stones are deposited in the pulp cavity (Fig. 7). None or several pulp stones could be found in teeth from whales of the same age and different sex. No meaningful pattern could be detected in the deposition of pulp stones.

Sexual dimorphism

Perrin & Myrick (1980) suggested that there were sexually dependent morphological differences in tooth development. Thus tooth measurements from 206 white whales were tested by discriminant analysis for evidence of sexual dimorphism, but no significant differences between males and females could be detected. However, teeth from male white whales with more than 9 GLGs are clearly larger than teeth from similar-aged females (Fig. 5).

Calibration of age

All four teeth from the captive whale had characteristics that clearly distinguished them from the teeth collected from white whales in West Greenland. In the four teeth a clear difference was evident in the distinctness of GLGs in both cementum and dentine after deposition of the sixth one (Fig. 7). The deposition pattern changed from a distinct one with well-defined dark and translucent zones through GLG 6 to a less pronounced layering pattern from GLG 7 to the death of the whale. A similar change was observed in the teeth of two other captive white whales (Brodie 1982, Goren *et al.* 1987). Brodie (1982) was unable to count the GLGs deposited after the time of capture in one specimen.

The neonatal line was present in three of the four teeth, and this made it possible to estimate the whale's "complete age". Two of the four teeth were difficult to read, but the estimated number of GLGs in the dentine of the two most readable teeth varied between 30 and 36. The GLGs in the cementum were even more difficult to read but also gave counts of between 30 and 36.

Despite the uncertainty in our GLG counts for this whale, they allow us to reinforce the conclusion of Sergeant (1973) and Brodie (1982) that 2 GLGs are deposited annually in the teeth of white whales. The only previous validation of annual deposition of 2 GLGs in the white whale was that by Goren *et al.* (1987). These last authors tested various techniques for estimating age from the teeth of a male white whale from Alaska whose known age at death was approximately 24 years. Approximately 40 GLGs could be counted consistently in this whale's teeth.

Recommended procedure for age estimation

We recommend that the less worn of tooth numbers 7 and 8 from either side of the lower jaw be selected for age estimation (Fig. 4), that unprepared thick longitudinal sections (150–200 μm) be used and that a transmitted-light microscope with polarized light be used for counting GLGs. Counts in the dentine seem to result in higher values and more reliable (*i.e.* more nearly complete) estimates of age than counts in the cementum (Fig. 2 & Table 2), although counts in the cementum can provide a valuable supplement. For whales with many GLGs, thin sections stained with toluidine could be used to supplement the thick sections (Table 1).

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