AGE DETERMINATION AND AGE RELATED FACTORS IN THE TEETH OF WESTERN NORTH ATLANTIC BOTTLENOSE DOLPHINS

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ABSTRACT

Teeth were taken from 120 bottlenose dolphins, *Tursiops truncatus*, which had stranded on the mid-Atlantic coast of the United States. The number of annual growth layer groups (GLGs) for each animal was used to construct a growth curve. The growth rate of coastal North Atlantic Ocean *Tursiops* is similar to other cetaceans in having a high initial rate of growth, with no differences in growth between females and males. In females, the first dentinal GLG is thickest and is followed by GLGs which become progressively narrower. In males, the second GLG is thicker than the first; GLGs beyond number two become progressively smaller but at a slower rate than in females. In males and females, the translucent layer makes up proportionally larger parts of the GLG as the animal ages, but in males the percent translucent layer remains constant at about 50% while in females it continues to increase up to about 70% of the GLG. These two factors, GLGs width and translucent layer width, indicate that the sex and age of the animal influence the deposition of GLGs. Incremental layers are also present, averaging 12 per GLG, and seem similar to incremental layers described in other marine mammals. A plot of the relationship of percent growth of the last GLG to time of death suggests that the deposition of GLGs is relatively constant, at least during the first half of the year, and that North Atlantic Ocean *Tursiops* give birth in the fall as well as in the spring.

INTRODUCTION

Owen (1945) documented the existence of "concentric layers of dentine" in odontocete teeth and, similarly, the "appearance of opaque striae or conecntric layers" (pg. 512) in polished sections of pinniped teeth. Lankester (1867) also noted a layering pattern in the teeth of a beaked whale, *Ziphius sowerbiensis* (=*Mesoplodon bidens*), but did not suggest a reason or use for them. In 1950, Scheffer suggested using these layers as a method of age determination when he found layers in the dentine and cement of a fur seal, *Callorhinus ursinus*, canines that corresponded to the known age of seals branded as pups and recovered up to eight years later. Laws (1952, 1953) suggested using dentinal layers in odontocete teeth as a measure of age when he found layers in sperm whale teeth similar to those in pinniped teeth. In the following years, dentinal growth layers (growth layer groups) were discovered and used as age indicators in *Stenella coeruleoalba* (Nishiwaki and Yagi, 1953,

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1954), *Berardius bairdii* (Omura *et al.*, 1955), and sperm whales (Nishiwaki *et al*, 1958).

In 1959, Sergeant described growth layer groups (GLGs) in the teeth of four bottlenose dolphins, *Tursiops truncatus*, from Marine Studios in Florida. Two of the animals were born and died in captivity; two were wild caught and died in captivity. In each case the number of GLGs in the teeth corresponded with the known or estimated age of the animals based on their time spent in captivity. The oldest animal was estimated to be 15.5 years old and had 17 GLGs. He also examined a few teeth from the pilot whale, *Globicephala melaena*, and one each from the Atlantic whitesided dolphin, *Lagenorhynchus acutus*, and the common dolphin, *Delphinus delphis*. All of the species demonstrated layering in the dentine similar to that of *Tursiops*, which was identical to that described by Nishiwaki and Yagi (1953) for *S. coeruleoalba*.

Since Sergeant's paper, dentinal GLGs have been used extensively for age determination of odontocetes. Most of the work has centered on direct application of the technique, predominantly for species involved in fisheries, for example, sperm whale (Gambell and Grzeborzewska, 1967; Best, 1970; Gambell, 1977), delphinids (Sergeant, 1962, 1973; Kasuya, 1972, 1974, 1976; Sergeant *et al.*, 1973; Kasuya *et al.*, 1974; Perrin *et al.*, 1976, 1977; Ross, 1977), the white whale (Sergeant, 1962), Baird's beaked whale (Kasuya, 1977), harbor porpoise (Nielsen, 1973; Gaskin and Blair, 1977) and Dall's porpoise (Kasuya, 1978). However, some aspects of age determination and age related factors visible in the teeth of odontocetes have been examined but not satisfactorily explained in many species. Some of these include description and clear identification of GLGs, formation rate of the layers, identification of accessory layers and the more recently discovered incremental layers, and sexual dimorphism.

Since GLGs were first suggested as a means of aging odontocetes much controversy has prevailed over the meaning of these layers, but for the most part the assumption has been that GLGs are deposited annually. However, in a few species evidence based on relative age indices indicates that GLGs are not annual. In the white whale, *Delphinapterus leucas*, Sergeant (1962) and Brodie (1969) have shown circumstantially that more than one GLG is deposited at least during the early growth of the animal, although Khuzin (1961) felt that only one GLG is formed each year. In the narwhal, *Monodon monoceros*, a species related to the white whale, Hay (in press) also feels that more than one GLG is formed. Kleinenberg and Klevezal (1962) found twice as many dentinal GLGs as maxillary bone layers in the Black Sea dolphin, *Delphinus delphis*. In sperm whales, Ohsumi *et al.* (1963) determined that one GLG is formed each year based on 11 whales captured seven to 16 years after being tagged, a rate which Best (1970) confirmed when he followed the growth of the tooth from samples which extended over six months of the year. Berzin (1961, 1964) and Gambell and Grzegorzwerska (1967), however, concluded from samples taken throughout the year that two GLGs are deposited annually. Perrin *et al.* (1977) suggest three possible hypotheses of rate of deposition of GLGs in the spinner dolphin, *Stenella longirostris*, after examining 2500 specimens, none of which is that just one layer is set down per year. They accepted the hypothesis that 1.5 GLGs are deposited in the first year, while one GLG is deposited in all other years, rather than a rate of 1.5 GLGs per year or 1.5 GLGs per year until puberty and one GLG per year thereafter.

Direct evidence on the rate of dentinal growth has supported an annual formation rate. Sergeant's (1959) finding that the number of GLGs in *Tursiops* teeth correlated with the known age or minimum known age for each animal offered the first measure. Similarly, in 1973, Sergeant *et al.* reported that in three captive *Tursiops* from Marineland of Florida the number of GLGs was approximately equal to the known age of the animal. Klevezal and Kleinenberg (1967) examined captive and wild specimens from nine orders of mammals, including cetaceans, and concluded that GLGs are an annual event. Tetracycline injected specimens have also supported an annual deposition rate of GLGs (Best, 1970; Gurevich and Stewart, in press; Perrin and Myrick, in press).

Divergencies of opinion on the periodicity of GLG formation within a species probably can be attributed to the presence of accessory layers, thin layers similar to the hypomineralized layer of the GLG. Klevezal and Kleinenberg (1967) proposed that all odontocetes form one GLG per year but in some species accessory layers are especially distinct, being almost indistinguishable from an annular layer, so that it appears as though two GLGs are deposited annually in some species. More commonly, however, accessory layers are not quite so distinct and may influence the count of GLGs to the extent that repeated counts on the same tooth by one person or several people tend to give different results (Kasuya *et al.*, 1974; Perrin, 1975; Kasuya, 1977; Kimura, in press; Perrin and Myrick, in press). The clear distinction between an accessory layer and a GLG boundary layer is still not defined in some species, for example, the killer whale (*Orcinus orca*), nor is the cause of accessory layers known.

Before the discovery of "annual" growth layers in dentine, research in dental histology had shown the existence of smaller incremental growth patterns. In 1934, Schour and Smith showed that in the continuously growing rat incisor 16 micrometers of dentine are laid down in 24 hours. In 1935, Schour and Steadman described an incremental stratification, also in the rat incisors, which appear as a succession of dark (well calcified) and light (less calcified) layers, the width of each pair totaling 16 μm. Further examination (Schour and Hoffman, 1939) showed the same 16 μm incremental mineralization pattern in 17 other species including fish, a shark, crocodile, mastodon, and several species of mammals, including man. These lines were later interpreted as incremental lines of von Ebner. Yilmaz, Newman and Poole (1977) described daily von Ebner lines in pig dentine where each band consisted of parallel light and dark portions in thin sections of teeth. These were probably equivalent to 5 μm daily von Ebner lines described by Krauss and Jordan (1965) and Newman and Poole (1974). Von Ebner lines have recently been described from the teeth of *Stenella*, where about 365 lines were counted within GLGs (Myrick, in press). Although their deposition rate has not been measured directly, the frequency of these von Ebner lines in the dolphin teeth suggests that they represent a daily growth pattern.

In 1970, Scheffer found still a different frequency incremental layer in thin sections of a *Dugong* tusk. He described a pattern consisting of "coarse layers" and "fine layers". Kasuya and Nishiwaki (1978) found the same pattern in captive dugongs where they defined coarse layers as annual growth layers or GLGs. There were 10 to 15 fine layers within each coarse layer, so the fine layers were suspected to be deposited on a monthly or lunar cycle. Kasuya (1977) found the "long cycles" (GLGs) in Baird's beaked whale teeth to contain many (11.0 to 13.4) "short cycles". Myrick (in press) noted incremental layers of the same periodicity in thin sections from teeth of several species of dolphins. In addition, scanning electron micrographs of a small sample of etched *Tursiops* teeth also showed subannual incremental layers, larger than von Ebner lines, which probably represent the same growth pattern seen by Myrick and Kasuya, appearing as a finer mineralization pattern within the mineralization pattern of the GLG (Hohn, in press). Although the deposition rate of these incremental layers has not been measured directly, this circumstantial evidence suggests a "monthly" growth or mineralization cycle which may be useful for more precise age determination, possibly back-dating the month of birth in young animals, or a better understanding of the mineralization patterns in dentine.

Sexual dimorphisms have also been described in the teeth of some odontocetes. The identification of sexual dimorphism in the growth of teeth has two advantages. First, skeletal material currently in museum collections that is missing data on sex may be more useful for systematic or other research if the sex can be determined from the material on hand, such as the teeth. Secondly, differences in the growth of teeth or other mineralized tissue probably reflects differences in habits or intrinsic factors between the sexes. These differences may help explain the cause of formation of growth layers.

Van Utrecht (1969) found a sexual dimorphism in the extent that the neonatal line overlaps the cementum in teeth of the harbor porpoise, *Phocoena phocoena*, and a small sample of the white-beaked dolphin, *Lagenorhynchus albirostris*. Although Nielsen (1972) confirmed this finding in the harbor porpoise, it has not been described in other odontocetes. Another sexual dimorphism in the teeth of *Phocoena* is found in the ratio of the width of the opaque layer to the width of the translucent layer (Gaskin and Blair, 1977). In males, this ratio remains constant while in females it decreases steadily with age. Gaskin and Blair (1977) also found differences in the amount of dentine deposited annually, which they attributed to size differences of the sexes at any given age. Similar or additional sexual dimorphisms in the teeth have not been described in other odontocetes.

The purpose of this study is to describe dentinal GLGs and accessory layers, including their appearance and position, in western North Atlantic Ocean bottlenose dolphins; to examine age-related changes in GLG deposition and any sexual dimorphisms in dentinal characteristics; and to construct a growth curve for coastal North Atlantic Ocean bottlenose dolphins.

MATERIALS AND METHODS

The Sample

Teeth were collected by the Smithsonian Institution's Marine Mammal Salvage Program (MMSP) from *Tursiops* stranded along the Atlantic coast of the United States between South Carolina and New Jersey. 118 of these animals have complete data, including sex and total length. Two specimens were missing at least one piece of data and were included for GLG counts but not in other analyses.

Preparation of Sections

At least one tooth was taken from each specimen. A medial longitudinal cut was made on each of the teeth on a Buehler Isomet 11–1180 low speed saw using a rotating diamond blade with a saw speed of 5 to 6 on a relative scale of 1 to 10 (up to 300 rpm), following the method of Hohn (in press). One of the half-sections from each tooth was prepared for scanning electron microscopy. These were soaked in 5% formic acid for 3 hours, rinsed in water for at least one-half hour to remove the acid, cleaned in an ultrasonic cleaner with acetone for 15 to 60 seconds to remove extraneous surface particles, dipped in 70% ethanol to dehydrate and air dried. These etched sections were examined by scanning electron microscopy (SEM) (Hohn, in press). During an SEM session, a standard set of micrographs was taken which included serial micrographs of the entire tooth at low magnification (15 to 20×), requiring up to five micrographs, and higher magnifications (greater than 25×) for difficult to count, narrow GLGs, GLGs bordering the pulp cavity, and possible incremental layers. Specimens were prepared for SEM not more than two or three days before each session to help reduce their absorbing moisture.

For each tooth, a 150 μm thin section was also prepared. In most cases, the cut surface of the remaining half section was glued to a microscope slide and all but 150 μm cut off leaving the thin section adhering to the slide. The thin sections were removed by dissolving the adhesive in acetone, then mounted for examination on a clean slide.

Data Collection and Analysis

GLG counts were made for all of the specimens from the SEM micrographs and from the thin sections using a dissecting microscope with transmitted light (at 25×). The number of GLGs was plotted against total length for each animal. The total length of animals in each of the first 3 age classes (GLGs) was compared for males and females to examine differences in growth rates. The sample sizes were too small for statistical analyses for animals with more than four GLGs.

Measurements for GLG and translucent layer widths were taken for 19 females and 19 males from the thin sections using a dissecting microscope (at 50×) equipped with an ocular micrometer and a polarizing filter. Measurements of the first few GLGs were made near the base of the neonatal line on the concave side of the tooth. In sections in which it was difficult to measure at this point because of unclear boundaries, the GLG width was determined closer to the crown or tip of the tooth. The width of the first GLG is approximately the same from the base of the neonatal line to below the crown so a slight change in position did not affect the measurements. Later layers were measured where they were most clearly defined but in the upper half of the length of the GLG before it becomes compressed in the root, and below the apical end where the layers are especially wide. Sections that did not allow determination of boundaries of adjacent GLGs were not included.

Preliminary examination suggested the growth of the tooth by width of GLGs to be different in males and females. To test this, the width of each GLG was determined for each sex and compared using a Mann-Whitney Rank Sum test. In addition, the ratio of width of the translucent layer to entire GLG was plotted against GLG number, again considering males and females separately.

The optical density and extent of growth of the last GLG were determined and plotted against time of death for 16 males and 12 females whose teeth had fewer than four GLGs. Older animals were excluded because the GLGs become too narrow, and sometimes the boundaries too irregular, to get accurate measurements of a partial layer.

Incremental layers and accessory layers were treated separately. The number of incremental layers per GLG was determined where all of the increments of a GLG could be distinguished. The widths of individual increments were measured by position within different GLGs, i. e., on a ridge or in a groove for GLG 1, 2, or 3, or beyond GLG 3, using the SEM micrographs. The distribution and appearance of irregular accessory layers was noted. For accessory layers that appeared more regularly, their position within the GLG was measured.

Five variables were tested specifically for possible sexual dimorphism related to the neonatal line. First, the extent of the neonatal line beyond the bottom of the enamel was measured in thin sections on the concave and convex side of 42 teeth from mixed males and females. This is similar to the study by van Utrecht (1969). In addition, the width of the prenatal zone at the base of the enamel was determined, also from thin sections on both sides of the tooth. Finally, the width of the translucent neonatal line was compared in females and males. Means for each measurement were compared for males and females using a Student's t-test.

RESULTS

Age and Growth

A scatterplot of 64 females, 54 males and two specimens of unknown sex shows the general growth curve for *Tursiops* from the western North Atlantic Ocean (Fig. 1). For each of the first few age classes, including neonates, and for a cumulative average for animals with more than four GLGs, the mean total length is not significantly different for males and females using a Mann-Whitney test (Table 1). The minimum and maximum total lengths within each age class indicates the large overlap in total length of individuals in different age classes. The decrease in growth,

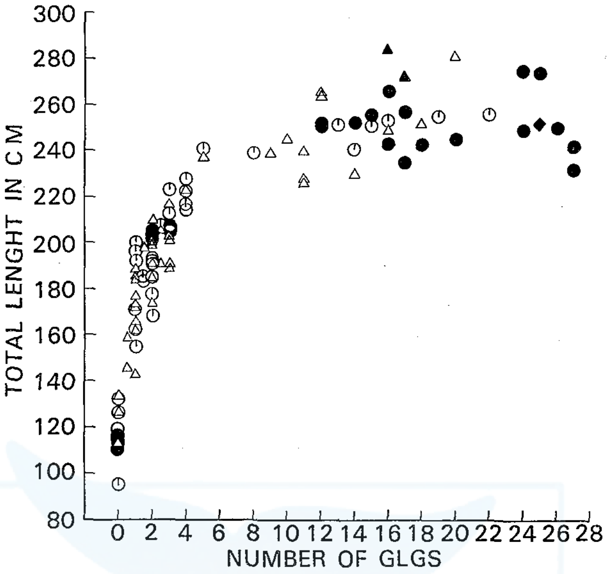

Fig. 1. Scatterplot of the number of growth layer groups (GLGs) to the nearest half-GLG and total length for 120 western North Atlantic Ocean *Tursiops*. Circles are females, triangles are males, diamonds are animals whose sex is unknown. Solid symbols represent a closed pulp cavity.

TABLE 1. SOME DESCRIPTIVE STATISTICS FOR TOTAL LENGTHS OF *TURSIOPS* BY NUMBER OF GLGS AND SEX

No. of GLGs	Sex	N	Total Length (cm) Mean	Min.	Max.
0	F	9	116	95	132
(Neonates)	M	9	118	110	134
0.5	F	0			
	M	2	153	146	159
1	F	6	180	155	200
	M	9	172	143	189
1.5	F	2	184	183	185
	M	1	198		
2	F	11	183	168	205
	M	8	196	174	210
2.5	F	2	207	206	207
	M	1	191		
3	F	6	210	205	223
	M	8	203	189	217
4	F	4	220	214	227
	M	2	220	216	223
More than 4 GLGs	F	24	249	232	275
	M	14	250	226	284
	F & M	38	250	226	284

large ranges in total length, and small sample size for animals with more than four GLGs allows for the determination of average "adult" total length of approximately 250 cm.

Description of GLGs

For the most part, GLGs in thin sections of teeth of these *Tursiops* appear as described in other odontocetes, particularly delphinids and *Tursiops* (Sergeant, 1959), as alternating translucent and opaque layers (see Perrin and Myrick, in press). The prenatal zone is a relatively uniform area easily distinguished by its lack of substructure, e. g., incremental and accessory layers, seen in post-natal dentine.

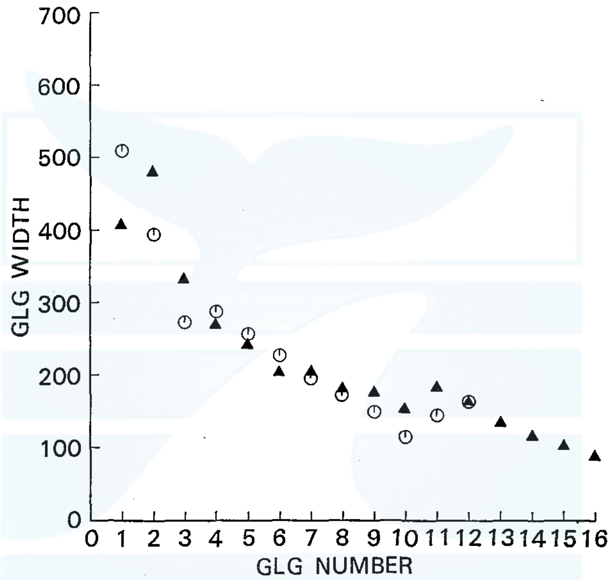

Fig. 2. Average width of GLGs for females (circles) and males (triangles), in micrometers. Measurements based on thin sections of teeth from *Tursiops*.

The neonatal line is a narrow translucent layer usually followed by a narrow, intense opaque layer. The first GLG begins after the neonatal line with a wide, variably opaque layer riddled with accessory layers, and ends with a narrow translucent layer often less apparent than some of the accessory layers. The second GLG is similar to the first in having the wide, variably opaque layer followed by a narrow, relatively, but indistinctly, translucent layer. The third and sometimes fourth GLGs have more distinct opaque and translucent layers and, although accessory layers can still be seen, they are not as overwhelming as in the first two GLGs. Subsequent GLGs have better demarcated opaque and translucent layers, less variability in mineral densities within a layer, and fewer accessory layers.

The same basic description is applicable to etched half-sections where opaque layers are equivalent to grooves and translucent layers are equivalent to ridges.

However, the numerous accessory layers in the first few GLGs are usually not as prominent in the etched sections. The exception is when the mineral density differences, normally accentuated by etching, are small across a GLG (usually in the first two GLGs), then incremental layers and accessory layers in the opaque zone of the GLG are almost indistinguishable from the translucent (boundary) layer of the GLG. This also occurs in thin sections. Furthermore, in etched sections, there are not clear boundaries between GLGs, i.e., there is not an abrupt end to the translucent layer or ridge. Rather, the topography of the surface might be likened to rolling hills with a rounded crest and slopes, and valleys inbetween. This does not allow for clear points or ends of layers from which to make measurements.

In general, the widths of GLGs decrease as the animals get older (Fig. 2). The first few GLGs are the largest and the last few are the smallest (Table 2). More specifically, in females, the first GLG is the widest averaging 509 μm, the

TABLE 2. SUMMARY OF DESCRIPTIVE STATISTICS FOR TOTAL GLG WIDTHS MEASURED FROM THIN SECTIONS OF TEETH FROM *TURSIOPS*. SIGNIFICANT DIFFERENCES DETERMINED USING A MANN-WHITNEY RANK SUM TEST. MEASUREMENTS IN MICROMETERS

GLG No.	Sex	N	Mean Width	Min.	Max.	Significant Differences
1	F	19	509	418	570	$p<.005$
	M	19	407	342	475	
2	F	12	393	285	475	$p<.05$
	M	11	480	380	608	
3	F	6	272	190	342	$p<.05$
	M	10	331	190	418	
4	F	6	285	228	380	
	M	7	269	209	342	
5	F	6	253	171	342	
	M	6	241	152	380	
6	F	6	225	152	285	
	M	6	203	152	266	
7	F	5	194	152	266	
	M	6	203	152	247	
8	F	5	171	95	247	
	M	6	181	133	228	
9	F	5	148	95	228	
	M	6	174	114	228	
10	F	4	114	76	190	
	M	5	152	114	209	
11	F	2	143	114	171	
	M	4	181	133	209	
12	F	2	162	133	190	
	M	4	162	152	190	
13	M	3	133	95	171	
14	M	2	114	95	133	
15	M	4	100	76	135	

TABLE 3. SUMMARY OF DESCRIPTIVE STATISTICS FOR TRANSLUCENT LAYER WIDTH DIVIDED BY GLG WIDTH (=PERCENT TRANSLUCENT LAYER) FOR EACH GLG MEASURED FROM THIN SECTIONS OF TEETH FROM *TURSIOPS.* SIGNIFICANT DIFFERENCES DETERMINED USING A MANN-WHITNEY RANK SUM TEST. RESULTS EXPRESSED AS PERCENTS

GLG No.	Sex	N	Mean Width-%	Min.	Max.	Significant Differences
1	F	19	9	1	17	
	M	19	10	4	14	
2	F	10	15	4	33	
	M	11	14	4	32	
3	F	6	39	18	67	$p<.10$
	M	9	23	5	50	
4	F	6	40	17	60	
	M	6	33	13	50	
5	F	6	41	22	56	
	M	6	41	10	75	
6	F	6	48	33	36	
	M	6	41	14	56	
7	F	5	48	38	58	
	M	6	45	36	50	
8	F	5	50	33	60	
	M	6	49	23	57	
9	F	5	57	40	71	
	M	6	48	33	67	
10	F	4	57	50	67	$p<.10$
	M	6	45	27	56	
11	F	2	73	67	78	$p<.05$
	M	4	44	27	57	
12	F	2	71	70	71	$p<.05$
	M	4	52	38	63	
13	M	3	51	50	52	
14	M	2	47	43	51	
15	M	4	50	50	51	

second is smaller at 393 μm, with subsequent GLGs decreasing in width. An exception is a slight increase in size at GLGs 11 and 12 which might be an artifact of small sample sizes (N=2). In males, the trend is similar except that the second GLG, which is 480 μm, is larger than the first, which is 407 μm. The third GLG is smaller than the first and subsequent GLGs follow the same pattern as in the teeth of females. Again, there is a slight increase in size at GLG 11. Measurements of the widths of GLGs 1, 2 and 3 are statistically significantly different between males and females (Table 2).

The width of the translucent layer follows the opposite pattern. GLGs set down in older animals have wider translucent layers or the width of the translucent layer to the entire GLG (percent translucent layer) increases (Table 3). In females there is a leveling off of percent translucent layer at GLGs 6 through 8, then an increase for subsequent GLGs. In males, the percent translucent layer increases initially, then remains fairly constant after GLG 4. This gives GLGs in the teeth of male *Tursiops* a more even appearance in size and spacing, which is especially apparent in SEM micrographs (Plate I, Fig. 1).

The translucent layer widths between females and males are statistically significantly different at GLGs 1, 3, 6, and 12. The percent translucent layer is significantly different between females and males at GLGs 3, 10, 11, and 12 (Table 3). There are no measurements for females beyond GLG 12.

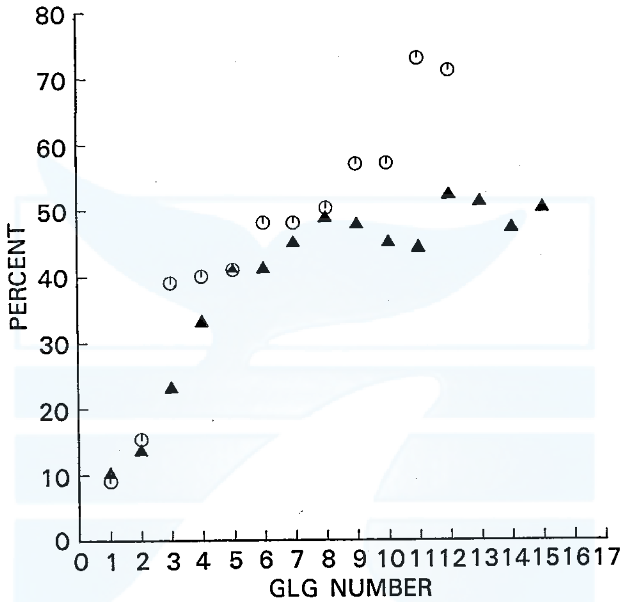

Fig. 3. Average percent translucent layer for each GLG. Circles are females, triangles are males.

Last GLG

A plot of percent growth of the last layer against time of death for 28 female and male *Tursiops* shows most of the points to set roughly on a line representing the expected growth of the current GLG if the deposition of dentine is constant and the new GLG begins in late January or early February (Fig. 4, Table 4). For the cluster of 14 points which occurs during February and March, with four additional points in May, the extent of growth of the GLGs increases proportionally with time along this line. Some of the points which do not fit this line fall on or close to another line representing the expected tooth growth of animals whose new GLG begins in September. However, there are only six specimens representing this group.

In most cases the last layer is opaque (Table 5). The translucent layer borders the pulp cavity in four of the specimens, and one of these is a translucent accessory layer and not a GLG boundary layer. Two of the GLG translucent layers

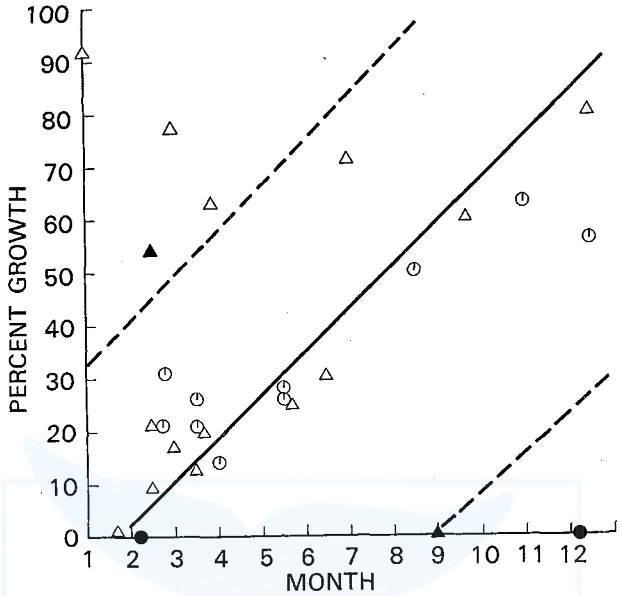

Fig. 4. Scatterplot of the percent growth of the last GLG in teeth of young *Tursiops* on approximate date of death. Percent growth calculated for each animal from actual width of the last layer divided by the average total width of the GLG. Solid line presents expected growth of the GLG throughout the year if the GLG begins in late Jan.-early Feb. and grows at a constant rate. Hatched line represents the same for GLGs which begin in September. Circles are females, triangles are males. Hollow symbols indicate an opaque last layer, solid symbols represent a translucent last layer.

occur in late January to early February and one occurs in September. The accessory layer that falls in mid-March is an animal whose dentinal growth pattern falls on the line for GLGs which begin in the fall.

Non-GLG Layered Structures

In addition to GLGs three layered structures can be differentiated in the teeth of *Tursiops*. Incremental layers are relatively evenly spaced layers which are found throughout the GLGs and are most easily seen in SEM micrographs of etched sections. Accessory layers are layers of opposite mineral density that would be expected within a GLG with an even mineral density. The third structure is similar to an accessory layer because it appears in a layer of opposite mineral density of the dentine adjacent to it.

Incremental layers could be counted and measured from some of the SEM micrographs (Plate I, Fig. 2). The average number of incremental layers per GLG is 11.8 for three GLGs from 39 specimens (Table 5). Of the three GLGs in which incremental layers could fairly consistently be counted, GLG 2 was the easiest for counting. Incremental layers are rarely visible in the compressed GLGs set down

TABLE 4. PERCENT GROWTH OF THE LAST FORMED GLG. GROWTH OF THE LAST GLG AND TIME OF YEAR OF DEATH FOR SOME *TURSIOPS* MALES AND FEMALES. PERCENT LAST LAYER CALCULATED FROM EXPECTED AVERAGE SIZE OF THE FULLY FORMED GLG. OPTICAL DENSITY OF THE DENTINE BORDERING THE PULP CAVITY IS BASED ON THIN SECTIONS USING TRANSMITTED LIGHT

Catalogue No.	No. of Complete GLGs	% Growth Last GLG	Approx. Date of Death	Optical Density
Females				
504565	1	31	late Feb	opaque
504590	1	26	mid-March	opaque
504403	1	26	15 May	opaque
CWP094	1	37	22 May	opaque
SEAN3331	1	50	mid-Aug	opaque
504539	1	56	late Dec	opaque
504550	2	0	early Feb	translucent
504592	2	14	30 March	opaque
504399	2	28	15 May	opaque
504583	2	21	early Mar	opaque
395179	2	63	4 Nov	opaque
504528	2	0	early Dec	translucent
504549	2	21	mid-Feb	opaque
Males				
504578	0	54	late Feb	translucent*
JGM401	0	80	14 Dec	opaque
504536	1	1	21 Jan	opaque
504553	1	9	mid-Feb	opaque
504567	1	21	late Feb	opaque
504218	1	17	1 March	opaque
395790	1	25	22 May	opaque
504535	1	92	early Jan	opaque
504561	2	77	early Mar	opaque
504591	2	25	early Mar	opaque
504290	2	35	mid-June	opaque
504122	2	60	21 Sept	opaque
504551	3	13	11 Feb	opaque
504765	3	13	14 Mar	opaque
CWP084	3	44	late Mar	opaque
504291	3	71	27 June	opaque
504313	3	0	3 Sept	translucent

* translucent accessory layer.

in the teeth of "old" animals, e.g., beyond GLG 10, in etched sections. Even in the first few GLGs incremental layers were not always clear enough to count. On the etched surface of the tooth, they were most clear on the "slopes" rather than on the top of a ridge, where one prominent very wide increment often turned out to be two, or in the grooves, where it is was "dark". The incremental layers have the same three-dimensional pattern as GLGs and are only visible when there is a contrast between the ridges and grooves of adjacent increments.

TABLE 5. NUMBER OF INCREMENTAL LAYERS PER GLG IN *TURSIOPS* DENTINE

Catalogue No.	GLG 1	GLG 2	GLG 3	>3 GLGs
291472	12–13	12	12	10–11
504122	9	13	—	—
291431	12	11	13	8–9
291403	—	12	10	11–12
291402	—	13	—	9
12277	—	11	—	—
291462	12	11	—	—
CWP088	—	—	—	11 11
191456	14	—	—	9
504534	14	12	12	—
504864	12–13	—	11–12	—
504726	—	11	10	—
291498	—	11	10	10–12
291466	—	—	11–12	—
395430	—	—	—	10
395179	12	—	—	—
504561	—	11	—	—
JGM399	11	—	—	—
291426	—	15	11	—
		Summary		
Means	12.2	11.8	11.3	10.8
Std. dev.	1.6	1.2	1.1	1.2

The widths of the incremental layers varied slightly (Table 6). Their mean width in GLG 1 was 26 μm and 23 μm, for ridges and grooves, respectively, 28 μm and 26 μm in GLG 2, 27 μm and 24 μm in GLG 3, and 23 μm and 20 μm for measurements made from GLGs later than three. It appears, in general, that increments in the grooves are smaller than those on the ridges in the same GLG and that the increments become smaller with successive GLGs.

Conversely, accessory layers tend to be variable in position and intensity, but are usually thin translucent layers or lines within the opaque layer of the GLG. In addition, in each of the first two GLGs there is an especially wide, prominent layer about halfway through the GLG (Plate II, Fig. 1). In the first GLG, this accessory layer occurs an average of 50% through the GLG in females and 64% of the width of the GLG in males. It occurs approximately in the middle of the second GLG in females and males. In some teeth these accessory layers are more conspicuous than the GLG boundary layer. This is particularly true since in the first two GLGs the translucent boundary layer is very narrow. In GLGs beyond number three, accessory layers become less frequent and the center, wide accessory layer is not as pronounced. Accessory layers are not found in the compressed GLGs set down in old animals.

A third non-GLG layered structure might also be called an accessory layer. These layers occur adjacent to any accessory or GLG boundary layer as very in-

TABLE 6. WIDTHS OF INCREMENTAL LAYERS IN *TURSIOPS* DENTINE MEASURED FROM SEM MICROGRAPHS OF ETCHED SECTIONS. MEASUREMENTS ARE SEPARATED BY THEIR LOCATION WITHIN A GLG AND BY GLG NUMBER. MEASUREMENTS IN MICROMETERS

Catal. No.	GLG 1		GLG 2		GLG 3		>3 GLGs	
	Ridge	Groove	Ridge	Groove	Ridge	Groove	Ridge	Groove
291472	38.8	35.4	34.6	28.9	25.4	26.6	20.3	—
504122	29.8	20.0	26.0	24.0	—	—	—	—
291431	34.5	25.0	—	31.8	—	36.3	23.9	—
291403	—	—	27.5	25.0	—	—	—	17.5
291402	—	—	25.8	20.0	—	—	25.0	22.8
12277	—	—	32.0	30.0	32.5	22.5	—	—
291462	29.3	32.5	31.5	—	—	—	—	—
CWP088	—	—	—	—	—	—	30.8	—
							16.8	—
291456	—	—	—	—	—	—	20.3	—
504534	22.1	18.9	19.8	—	—	17.1	—	—
504864	22.5	15.0	20.3	20.2	—	16.7	—	—
504726	—	—	—	35.7	28.6	—	16.3	—
291498	—	—	18.4	15.2	18.0	—	—	—
291475	—	—	31.0	22.8	—	—	—	—
291466	—	—	—	—	40.0	20.0	26.6	—
395179	14.3	11.9	—	—	15.1	—	—	—
504561	—	20.0	14.4	—	—	—	—	—
JGM399	—	28.6	47.7	28.6	—	—	—	—
504532	—	—	29.9	29.2	—	—	27.8	—
291426	31.3	—	—	29.8	—	28.0	22.5	—
395430	—	—	20.0	—	—	—	—	—
				Summary				
Means	26.2	23.4	27.6	26.2	26.6	23.9	23.0	20.2
Std. Dev.	7.5	8.4	8.7	5.7	9.2	7.0	4.7	—
Overall mean		26.1						
Std. Dev.		7.0						

tense narrow bands of the opposite mineral density as the structure they abutt. Most often, this layer is opaque and follows a translucent layer. It is very noticeable following the neonatal line, the wide central accessory layer, and some of the irregular accessory layers throughout the GLGs. When this structure occurs by thin translucent boundary layers, it is sometimes more prominent than the translucent layer so more easily used as the boundary layer (Plate II, Fig. 2).

Sexual Dimorphism

Measurements of four of the five variables specifically tested for possible sexual dimorphism similar to that found by van Utrecht (1969) proved not to be significantly different (Table 7). The difference in the fifth variable, neonatal line width, was highly significant (p<.005) using a t-test with males having a wider neonatal line than females.

TABLE 7. SUMMARY OF MEASUREMENTS FOR POSSIBLE SEXUAL DIMORPHISMS IN THE TEETH OF *TURSIOPS*. MEASUREMENTS IN MICROMETERS

Variable	Sex	N	Mean	S. E.
Extent neonatal line extends beyond enamel, convex side*	F	13	418.0	64.2
	M	18	471.8	52.5
Extent neonatal line extends beyond enamel, concave side*	F	17	429.2	54.4
	M	18	405.3	53.3
Width of prenatal zone, convex side*	F	13	58.5	4.4
	M	18	54.4	4.3
Width of prenatal zone, concave side*	F	16	54.6	5.2
	M	18	48.1	4.6
Neonatal line width convex side**	F	21	27.0	0.8
	M	22	37.3	1.3

* Not significantly different using Student's t-test.

** Significant at $p < .005$.

DISCUSSION

Age and Growth

The growth rate of *Tursiops* is similar to that of many other odontocetes. During its first year, an average western North Atlantic *Tursiops* increases its length by 53%, approximately the same increase found for the same age animals of *Stenella attenuata* (Perrin *et al.*, 1976), *S. coeruleoalba* (Kasuya, 1972, 1976), *Globicephala melaena* (Sergeant, 1962), *Tursiops* from Florida (Sergeant *et al.*, 1973), and *Tursiops* from South Africa (Ross, 1977). The rate of growth decreased during the second year of life, averaging only a 10% increase in total length in these *Tursiops* and an average of 10 to 15% in the other species mentioned above. After three GLGs the growth rate slows considerably.

Cetaceans have been described as having high rates of growth while they are young (Bryden, 1972). However, there is a great deal of variability in total lengths of individuals in any age class. In *S. longirostris*, animals with one GLG range from about 100 cm to 135 cm in total length, those with two GLGs range from 110 to 145 cm, with equivalently large variation in older animals (Perrin *et al.*, 1977). In pilot whales, *G. melaena*, neonates range from 165 to 195 cm in total length (Sergeant, 1962). A sample of harbor porpoises from the Bay of Fundy ranged from 75 to 105 cm at birth, 105 to 130 cm at one year, 118 to 135 cm at two years, 125 to 145 cm at three years, again continuing this variation in length within each age category for older animals (Gaskin and Blair, 1977). The large variability and overlap of different aged animals illustrates that, although it is useful to construct growth curves and use mean total lengths for examining growth rates, it is not reliable to use total length to determine the age of an individual.

In the western North Atlantic *Tursiops* an especially large range in total length exists in each age class. This may be due to strandings of individuals from two different population of *Tursiops*, coastal and offshore, where the offshore animals seem to be larger than those found along the coast (J. G. Mead, unpub. data). At this time, however, it is not possible to differentiate to which population a young stranded animal belonged, so all specimens less than 280 cm have been included.

Differences in adult total length or rate of growth between males and females have been found in some cetaceans. When it occurs, generally males are larger than females as adults in odontocetes (e.g. pilot whales and sperm whales), while in mysticetes males are smaller than females as adults (e.g. humpbacks and fin whales) (Bryden, 1972). But delphinids generally have not been found to be dimorphic in adult total length (True, 1889; Sergeant *et al.*, 1973). The results from this study seem to support this.

Two studies have been published on the growth rate of wild *Tursiops*. Sergeant *et al.* (1973) looked at *Tursiops* from Florida waters. Although they reported birth to occur at 100 cm, they present no data for neonates. The growth curve, based on 22 males and 24 females, is similar to that of western North Atlantic *Tursiops* but with younger animals (less than five GLGs) apparently smaller in Florida. The apparent difference between the two groups may be due to differences in our definition of GLGs. The asymtotic length of males from Florida was found to be 270 cm and that of females to be 250 cm. The coastal *Tursiops* in this study appear to have the same asymptotic length for males and females at 250 to 260 cm. Ross (1977) gives some biology of *T. aduncas* from South African waters. The age of birth ranged from about 84 cm to 112 cm. Females reached sexual maturity at a smaller length than males. The largest female was 252 cm and the largest male 254 cm which makes them smaller than the western North Atlantic *Tursiops*. Although the *Tursiops* from Florida waters might be the same type as those from further north along the coast, those from Africa are probably not.

GLG Widths

As a dolphin tooth grows and deposits more dentine, the pulp cavity becomes increasingly smaller until it finally occludes and no more dentine deposition can occur. Correspondingly, as the pulp cavity becomes smaller, the GLGs become narrower. This decrease in GLG width could be solely the result of the new forming GLG being compressed into a smaller pulp area. If this is the case then each GLG would be a little smaller than its preceding GLG and a plot of the relationship between GLG width and number would be approximately a straight line with a negative slope. If this relationship follows a different pattern then it would suggest that there are other factors influencing the deposition or mineralization of dentine.

There are some general patterns that can be found in a plot of average GLG width against GLG number that suggest that physiological factors affected by the age or sex of the animals are important in GLG growth. These patterns can be visualized in Fig. 2. First, there are significant differences between males and females in the widths of the first two or three GLGs. It is particularly interesting that the second GLG is, on the average, wider than the first GLG in teeth from males, and that the third GLG decreases so that it is narrower than the first. Secondly, the changes in width of the first three GLGs is large, but subsequent GLGs show a marked decrease in this rate, i.e., GLGs following number three are not much narrower than their adjacent preceding GLG. This appears to be an affect of age of the animals since it occurs in females and males. Again it is interesting that this abrupt decrease in GLG deposition rate occurs at the same time that the growth curve of *Tursiops* (Fig. 1) shows a noticeable decrease in rate of growth. Thirdly, the average width of each GLG is larger in females than in males from GLGs four to six and smaller in GLGs which follow. The effect is an almost constant decrease in width of GLGs four through ten in females. In males, the rate of change in width of GLGs appears smaller than in females for GLGs six through ten. In an etched section of tooth, these GLGs appear evenly spaced and sized across the surface and almost can be considered diagnostic to determine teeth from males. Similarly, the characteristic constant decrease in size of GLGs in teeth from females is apparent in etched sections. Perrin *et al.* (1977) plotted the percent decrease in width from one GLG to its adjacent GLG in the spinner dolphin beginning at GLG 3 and continuing to GLG 16. They found that each GLG average more than 95% of the width of the preceding GLG. They did not consider females and males separately, which in *Tursiops* teeth gives different results, as discussed above. A progressive decrease in width of GLGs has also been described from the harbor porpoise (Nielsen, 1972; Gaskin and Blair, 1977), striped dolphin (Kasuya, 1972), and the pilot whale (Sergeant, 1962). Christensen (1973) found an initial decrease in bottlenose whales followed by constant thickness after the fourth GLG. Except for Gaskin and Blair (1977), measurements of widths were not given, nor were specific differences by sex or age noted. The mean widths of GLGs given by Gaskin and Blair (1977) show that GLGs are wider in teeth from female harbor porpoises than those from males.

The translucent layer can also be used as an indicator of physiological changes affecting GLGs. The absolute value of the width of the translucent layer is probably not an important parameter by itself, except to help determine the time span over which it is deposited, because it is dependent on the GLG number and width. The percent translucent layer is a more useful quantity. As with GLG width, the trend in percent translucent layer is different in females and males although significant differences occur only in GLGs 3 and 10 through 12. In females, the percent translucent layer increases almost continuously with age. Beyond GLG 10 more than half of the GLG consists of the translucent layer, reaching about 70%. This trend appears as an initial rapid increase in percent translucent layer which levels almost as a plateau (between GLGs 3 through 8) then increases rapidly again after GLG 8. This is noticeably different than males where an initial rapid increase in percent translucent layer leads to a nearly constant level, with some fluctuation. The translucent layer comprises only about 50% of the GLG after GLG 8. The change in percent translucent layer around GLG 8 through 10 in females and males may be related to physiological changes associated with the onset of sexual maturity, since this seems to be approximately the age at which the animals become sexually mature (Harrison, 1969; Ridgway, 1972).

The changes at GLGs 3 to 4 may be influenced by the decreasing growth rate at that age. The large percent translucent layer in females beyond age ten can be speculated about with even less certainty. The most obvious explanation since there are such large differences between males and females would be that the wide translucent layer, which is hypomineralized (Hohn, in press), is the result of a calcium drain caused by reproduction. At this time, there is no direct evidence for this. Gaskin and Blair (1977) noticed a progressive increase in proportional thickness of the translucent layer with age in harbor porpoises but only in females, so they attributed it to calcium mobilization. They constructed a regression line through a scatterplot of the proportion of opaque layer to translucent layer but did not mention specific differences at any age. However, their results, along with mine, indicate that percent translucent layer is worth examining in other species for possible dimorphism and explanation for the formation of the layers.

Last GLG

Most of the strandings of *Tursiops* occurs during February and March. Since calving also occurs during this season, it should be about this time when the neonatal line appears in the teeth. If the deposition of dentine is fairly constant, animals born in the late winter to early spring would have completed each GLG during that season in successive years and then started another GLG. Each month more of the new GLG would have accumulated following a hypothetical line representing constant growth by percent GLG formed throughout the year, one twelfth or 8% monthly, as in Fig. 4. Most of the 28 animals examined for percent growth of the last GLG follow this hypothetical line, at least during the spring of the year when most of the animals strand. However, this line begins in January or early February, earlier than when neonates are generally found. By late February or March, 10 to 20% of the new GLG has already formed, the amount that would be expected at a constant deposition rate of dentine with the new GLG beginning in January. This would suggest that the end of the previous GLG, which is the translucent layer, occurs in December or January. Scatter around this line is probably due to individual variation in time of birth and actual widths of GLGs, and possibly to abnormal physiological states of the animals before they strand.

Tursiops may have two seasons of parity: one in the spring and another in the fall (Townsend, 1914; Harrison, 1969; Ridgway, 1972; Ross, 1977). In particular, Townsend (1914) believed that North Atlantic Ocean *Tursiops* give birth during both seasons. Ross (1977) has found stranded neonates in the fall and spring which suggests more than one or an extended breeding season. But neonates have not been found through the MMSP during the fall, even though many are picked up during the spring. However, if the growth of the tooth can be used as an indicator of season of birth, i.e., animals born in the spring have formed one GLG the following spring as previously discussed, then *Tursiops* which have formed 50% of a GLG in the spring must have started that GLG during the fall. Similarly, an animal whose last GLG ends in the fall, probably also began deposition of GLGs during the fall. A hypothetical line drawn to represent the expected growth of teeth which begin GLGs in the fall should parallel the equivalent line for animals whose GLGs begin in the spring, but off-set by approximately six months. This hypothetical line, represented in Fig. 2, has several points which fall on or close to it. This would suggest that these animals were also born in the fall rather than in the spring as we would have anticipated based on stranding records. This may provide evidence to support Townsend's (1914) idea of two seasons of calving for *Tursiops* in the North Atlantic Ocean.

Although growth layers in teeth and bone have been used for about 30 years for age determination, there have been only speculative suggestions as to the cause of the rhythmic deposition of the layers. The environmental or endocrinological mechanisms influencing or regulating the mineralization pattern in hard tissue is unknown. In several species of odontocetes, the optical or mineral density of the GLG layer bordering the pulp cavity when an animal dies has been used as an indicator of the physiological or nutritional state of the animal. Sergeant (1959) found the "clear zone" (translucent layer) to begin in February and occur through April in captive *Tursiops* from Florida. Sergeant *et al.* (1973) said they could not determine the density of the last layer in mostly wild *Tursiops* from Florida based on their sample size (N=62). Sergeant (1962) found the opaque zone to occur adjacent to the pulp cavity in pilot whales, *Globicephala melaena*, from Canadian waters caught in the summer. In a mass stranding of pilot whales, *G. macrorhynchus*, in South Carolina during mid-October, the opaque layer bordered the pulp cavity (unpub. data). Nishiwaki and Yagi (1954) found that the well stained (=opaque) layers occur in December and May through June in *S. coeruleoalba*. Similarly, the opaque layer borders the pulp cavity in June through February in harbor porpoises from the Bay of Fundy (Gaskin and Blair, 1977), in the summer in Baird's beaked whales from the North Pacific (Kasuya, 1977), in May and June in bottlenose whales from the North Atlantic (Christensen, 1973), and in June through November in dugongs from the equatorial and North Pacific (Kasuya and Nishiwaki, 1978). In summary, the opaque layer appears to border the pulp cavity at least during the summer months, and usually longer, in most species. Conversely, the translucent layer is more often at that point during the winter or early spring. In *Tursiops*, the translucent layers, as accessory layers or GLG boundary layers, are deposited in the fall (September, N=1), and in late January, early February, or March (N=3). However, all the animals examined were young which is when the translucent layer is very narrow. As males and females age, a higher proportion of each layer becomes translucent, so the time of deposition of each layer of a GLG would depend on the age of the animal, at least in *Tursiops*. This, again, assumes that the dentine continues to be deposited at a constant rate and only the mineralization pattern changes. The GLG number at which previous researchers determined the optical or mineral density of the layer bordering the pulp cavity, or changes in the relative amount of translucent dentine, has not been mentioned. The collection of enough data of this kind may help us to understand the cause of layering.

Non-GLG Layered Structures

Incremental layers. It is difficult to count incremental layers from SEM micrographs because the small mineral changes throughout an increment and between increments does not always produce enough contrast in etched sections to make individual increments clear (Plate III). In addition, increments are difficult to see on the "back side" of a ridge or in a groove where it is "dark" because fewer electrons reach the collecting plate. The effect is probably that counts are slight underestimates of actual numbers of incremental layers.

Partially for the same reasons, it may not be accurate to measure widths of incremental layers from etched sections. The etching process produces the ridge and groove pattern in GLGs and in incremental layers, so there is no longer a flat surface. This is compounded by the tilting of the sections in the SEM chamber. Although the quantitative effect of these factors is unknown, the average number of increments per GLG (12) multiplied by the average width of the increments (26 μm) is not equivalent to the average width of the first three GLGs measured from thin sections (approximately 420 μm). Measurements are probably an underestimate of actual sizes of increments. SEM of etched sections, however, seems to provide the best way to visualize incremental layers.

The appearance of incremental layers in *Tursiops* dentine supports the idea of Schour and others that dentine grows or is mineralized in spurts or increments. The frequency of approximately 12 increments in one annular layer or GLG suggests that this sub-annual growth is similar to that described in dugongs (Kasuya and Nishiwaki, 1978), Baird's beaked whale (Kasuya, 1977), and dolphins of the genus *Stenella* (Myrick, in press). However, the width of the increments has not been determined for those species. The average size (26 μm) of incremental layers in *Tursiops* dentine seems much too large to represent daily von Ebner's lines of 5 μm width described by Krauss and Jordan (1965) and Newman and Poole (1974) in human teeth, or von Ebner's lines in pig dentine (Yilmaz, Newman, and Poole, 1977). If incremental lines in *Tursiops* are approximately monthly, daily increments would have to average 0.87 μm, measured from SEM micrographs. Myrick (in press) has found daily increments in dolphin dentine, but has not determined the width of either these von Ebner's lines or the "monthly" growth increments. Schour and Hoffman (1939) measured the "calcification" rhythm in 17 species of vertebrates and found it to recur at intervals of 16 μm (no time interval was mentioned). They were counting accentuations (light or more calcified areas) which alternated with dark areas. It is possible that "monthly" increments in *Tursiops*, which are the result of mineral differences, are similar to the pattern found in other vertebrates by Schour and Hoffman (1939).

One of two basic assumptions seems to be made when considering the deposition or mineralization of dentine. The first presumes the increments are time-regulated by some extrinsic or intrinsic factor so that the common denominator is the number of increments set down during a given period of time. The second assumption is that these processes continue at a constant rate regardless of the age or the species of the animals. The latter would seems to be supported by Schour and Hoffman's (1939) observations of the 16 μm calcification rhythm and researchers looking at constant sizes of von Ebner's lines (Krauss and Jordon, 1965; Newman and Poole, 1974). However, the authors do not specify the age of the dentine where the measurements were made, e.g., immediately subjacent to the enamel or several years growth away from the enamel. Nor do they mention making a series of measurements throughout each tooth, except over short periods of time such as days or weeks. Although my measurements of incremental layers are subject to error, as discussed previously, the trends suggest that the increments decrease in size in GLGs set down later in an animal's life. Increments in GLGs beyond number three are smaller than those in the first three GLGs. This may be an artifact of etched sections. However, in order for the number of increments to remain the same in all of the GLGs, the increments must become narrower as the GLGs become narrower; again with the assumption that the number of incremental layers is relatively constant, which seems to be more supported by the data from *Tursiops* teeth.

The assumption of constant size of increments also seems to be disputed by the differences in width of incremental layers on the ridges and grooves in etched sections. Increments in the grooves measure, on average, smaller than increments on the ridges and slopes of the GLGs. This size difference may be a result of the collapse of the tissue when the mineral is removed or it may be a real difference in widths of increments in the hypomineralized and hypermineralized parts of the GLG. This should be checked in untreated dentine, possibly in thin sections using a petrographic microscope (Myrick, in press).

It is tenable that the mineralization or deposition of dentine could be regulated by monthly or lunar factors, that this produces the relatively constant number of increments per GLG in *Tursiops* teeth and possibly all species, that the size of the increments changes progressively with the age of the animal and possibly between the hypo- and hypermineralized layers of the GLGs, and that the incremental layers can be used to gain more information about the biology of species and the growth of mineralized tissue. However, this must be measured by direct marking of individuals, e.g., tetracycline, or by the examination of many more species before any conclusions are drawn.

Accessory layers. There appears to be two kinds of accessory layers as they have been described formally or referred to in the literature. The first are the narrow accessory layers which appear irregularly in GLGs. These are approximately the same size as incremental layers and can be distinguished from incremental layers only because their mineral density, seen as optical density, differs appreciably from that of the GLG layer in which they occur. The second kind are the very prominent, wider than incremental layer accessory layers which occur regularly, approximately in the center of the GLG. It is undoubtedly these prominent accessory layers which are responsible for the debate about number of GLGs set down annually in odontocete teeth. An unexperienced person would almost assuredly count these regular accessory layers as GLGs. For example, Leatherwood *et al.* (1978) counted five GLGs in thin sections of teeth from a young *Tursiops* (USNM 504122). In the same animal I counted a little more than two GLGs (Plate 5). The SEM micrograph shows only two GLGs (although the first prominent ridge is an accessory layer - the first translucent layer is hardly visible). Similarly all of their counts of GLGs from seven *Tursiops* used in both studies are in disagreement with mine, with their counts from the teeth of young animals doubling mine. Kleinenberg and Klevezal (from Klevezal and Kleinenberg, 1967) described an annual deposition of two GLGs in the teeth of the Black Sea dolphin, *Delphinus delphis*, which they later (1967) attributed to the presence of this accessory layer. In general, they agreed that GLGs are annual but this prominent center accessory layer often gives the impression that two GLGs are deposited.

Myrick (in press) refers to incremental layers in *Stenella* teeth as accessory layers. It seems that incremental layers are visible due to their mineralization cycle within GLGs. If this cycle is subject to extrinsic or intrinsic influences then incremental layers will be more obvious within a GLG when the expected mineral density is disrupted. This disruption would make the incremental layer appear as one of the narrow, irregular accessory layers. In the first two GLGs where the translucent layer is very narrow, these accessory layers would and do make it difficult to determine where the GLG ends. However, incremental layers by themselves do not usually interfere in the process of counting GLGs in *Tursiops* teeth, especially using SEM.

Regardless of the kind of accessory layer, accessory layers make counting GLGs very confusing in the first few GLGs. A person experienced in "reading" odontocete teeth can probably estimate very close to the actual number of GLGs in a tooth section, but identifying the exact end of a GLG becomes very difficult in some teeth. There is also a great deal of variability between animals. Accurate age determination of some species of odontocetes, e.g. *Tursiops*, depends on experience, from looking at many teeth from all ages of the animals to accurately identify accessory layers before trying to count GLGs.

Small scale mineral changes. The narrow opaque accessory-type layers which follow translucent layers appear to be the result of abrupt small-scale changes in the dentine. Irving and Weinmann (1948) described a similar occurrence in rat incisors. They injected rats with strontium to observe the effects on calcification in the teeth and found that the strontium caused a hypocalcified layer (16 μm wide) to occur. Immediately following this layer, a very evident narrow "calciotraumatic" line was found which sharply demarcated the hypocalcified dentine from the normal, calcified dentine. They attributed this line to be caused by a shock to calcium metabolism in the rat by the strontium. The same effect was found in rats after administration of calciferol or parathyroid hormone (Schour and Ham, 1934; Schour *et al.*, 1934), or immediately following parathyroidectomy (Schour *et al.*, 1937), where the parathyroid helps regulate mineralization processes. In addition, Kronfeld and Schour (1939) found the calciotraumatic line to occur following the neonatal line. It may be this same calciotraumatic line that is present in thin sections of *Tursiops* teeth following the neonatal line and other translucent zones.

There has been some question about the mineral density of the narrow layers in odontocete teeth, for example, the neonatal line. The consensus is that they are translucent and hypomineralized. But Nielsen (1972), Klevezal and Kleinenberg (1967), and Kasuya (1976) have suggested that the narrow layers are translucent but hypermineralized for some species. The wide layers are then relatively hypomineralized, so the relationship between mineral and optical densities is different from that of other odontocetes. Because of this, Nielsen (1972) has suggested that optical density cannot be used as an indicator of mineral density. However, it may be that where the translucent layer is very narrow, the opaque "calciotraumatic" line is more apparent and is considered the layer. In some tooth sections of *Tursiops* it is easier to use this opaque line than the preceding translucent layer to follow a GLG down the extent of the tooth. However, the neonatal is still considered to be translucent and hypomineralized and subsequent boundary layers are translucent and hypomineralized. The calciotraumatic line needs more examination for its occurrence and effect in odontocete teeth.

Sexual Dimorphisms

The sexually dimorphic trait found by van Utrecht (1969), i.e. the greater extent of the neonatal line in males, in teeth from 20 harbor porpoises, *Phocoena phocoena*, is not significantly different in the teeth of male and female *Tursiops*. Van Utrecht also mentioned that the same trait was applicable to *Lagenorhynchus albirostris*, a delphinid, but this was based on only three females and three males. Teeth of species from the family Phocoenidae are considerably different from those of the family Delphinidae. Instead of the nested "cone" appearance of GLGs in *Tursiops* teeth, the GLGs in *Phocoena* are bulbous in the crown or spatulate end, then continue in the narrower root parallel to the long axis, as in the handle of a spatula. The difference in growth between phocoenid teeth and delphinid teeth presumably allows for any dimorphic growth of the neonatal line relative to the top of the cement in *Phocoena* but not in *Tursiops*.

The second trait examined for possible differences between sexes of *Tursiops* seems related to the first. If the neonatal line was to extend further down the root of the tooth in male *Tursiops* as it does in male *Phocoena*, it might be because there was a greater amount of prenatal dentine in males. Having more prenatal dentine could have suggested that the neonatal line was deposited at a later time in males or that the tooth began development earlier. But neither trait was significant so should not be used to identify an animal of unknown sex in *Tursiops*.

The greater extent of the neonatal line could also be related to the width of the neonatal line. If the measurements were made from the outer edge of the neonatal line, a wider neonatal line would extend its length if the deposition of the neonatal line was initiated at the same time in males and females. In the case of *Tursiops*, the width of the neonatal line is significantly different in males and females. However, the greater neonatal line width in males is coupled with insignificant differences in either the extent of the neonatal line or width of the prenatal zone. This is probably because the neonatal line is very narrow, especially relative to its length, and the 10 μm mean difference in this width between males and females does not significantly alter the overall measurement of the extent of the neonatal line. In addition, it is very difficult to accurately measure the neonatal line width because it is so small and, although not difficult to identify, its boundaries are not always clear. Even though the width of the neonatal line proved to be a significantly different sexually dimorphic trait, I would not recommend that it be used as the sole identifier of the sex of an unknown specimen of *Tursiops*.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. SEM micrograph of etched longitudinal section of tooth from an old male *Tursiops* showing relative evenness in GLG width, translucent layer width, and spacing of GLGs. Magnification: ×17.

Fig. 2. Etched longitudinal section of tooth from a 2.5 year old male *Tursiops*. The small layers running parallel to the GLGs are incremental layers. Although more than two GLGs are present, the first is deceptively masked so that the actual boundary layer, marked by the arrow, is not the first prominent ridge. The beginning of the second prominent ridge serves as the boundary for GLG 2. nl—neonatal line, al—accessory layer, PC—pulp cavity
Original magnification: ×18.

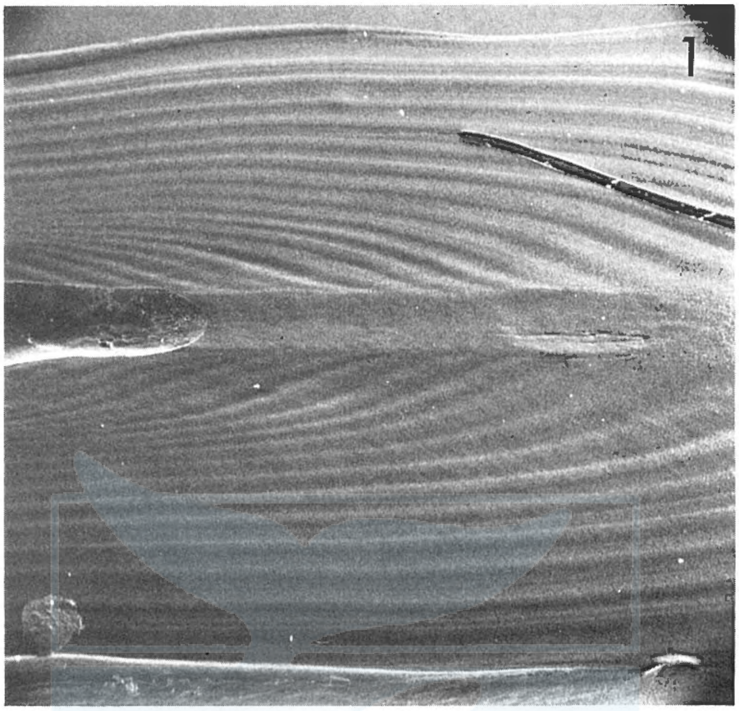
PLATE II

Fig. 1. Longitudinal thin section (transmitted light) of a young *Tursiops* tooth. The dark evident line following the neonatal line (nl) may be a " calciotraumatic line ". One GLG is complete, the second is beginning. The center accessory layer (al) is very evident and could easily be mistaken for an additional GLG.
Magnification: ×18.

Fig. 2. Longitudinal thin section of a tooth from a male which could have from 1.5 to 6 GLGs. This animal is probably just over 3 three years old. The GLGs are marked by arrows. Notice the accessory layers which occur centrally in the first two GLGs.
nl—neonatal line
Magnification: ×12.

PLATE III

Fig. 1. Etched section of same 2.5 year old male in Plate II, Fig. 2 at a higher magnification. Notice especially that it is difficult to separate individual incremental layers (il) because two adjacent layers with small mineral density differences appear as one.
nl—neonatal line, al—accessory layer
Magnification: ×50.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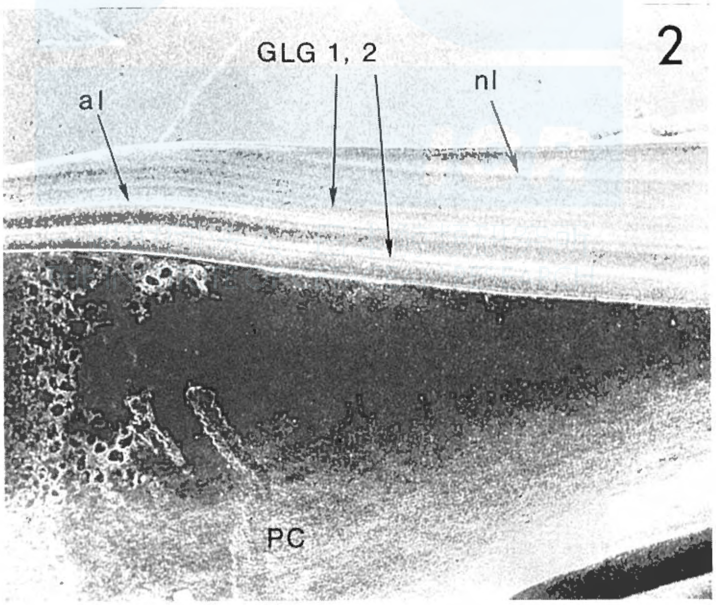
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al
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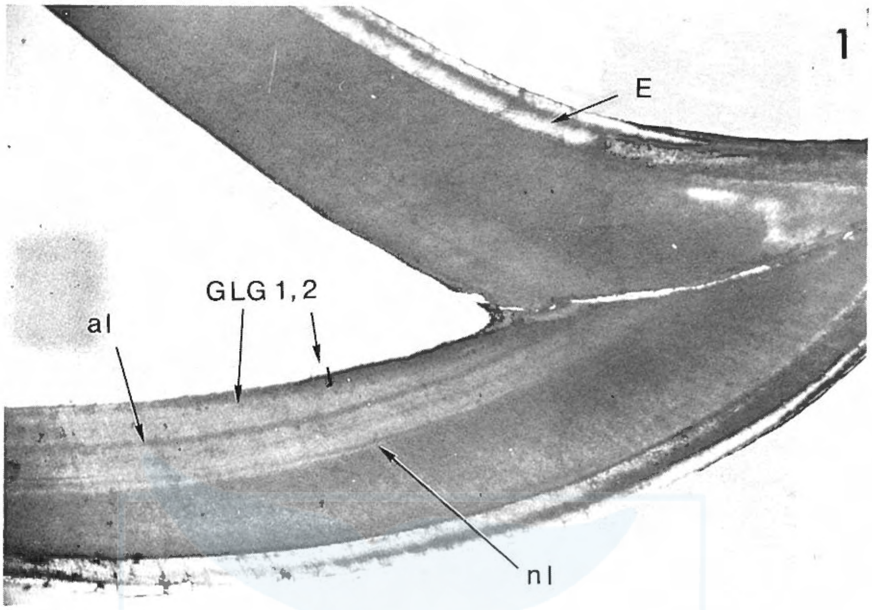
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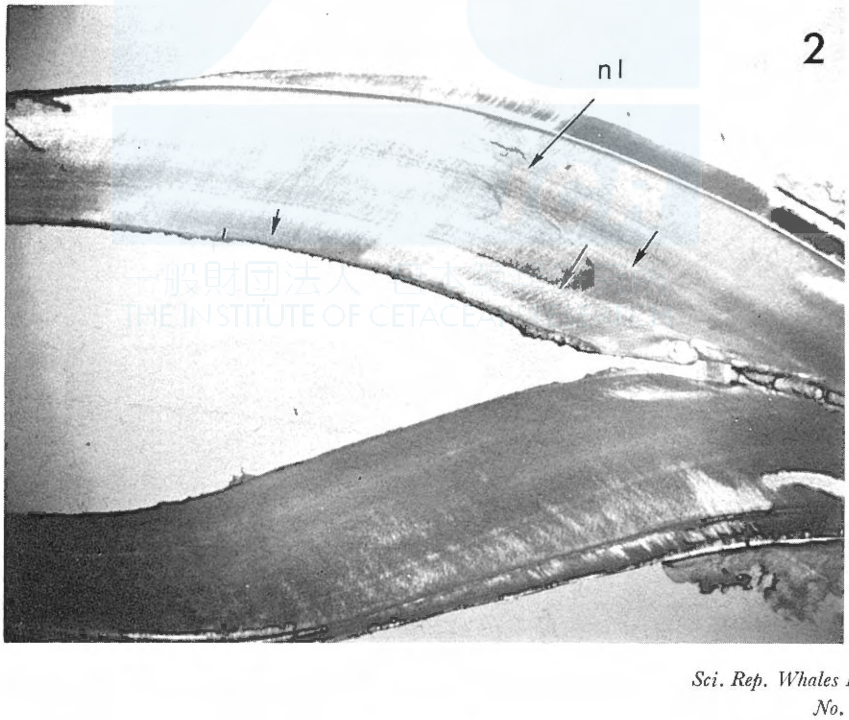
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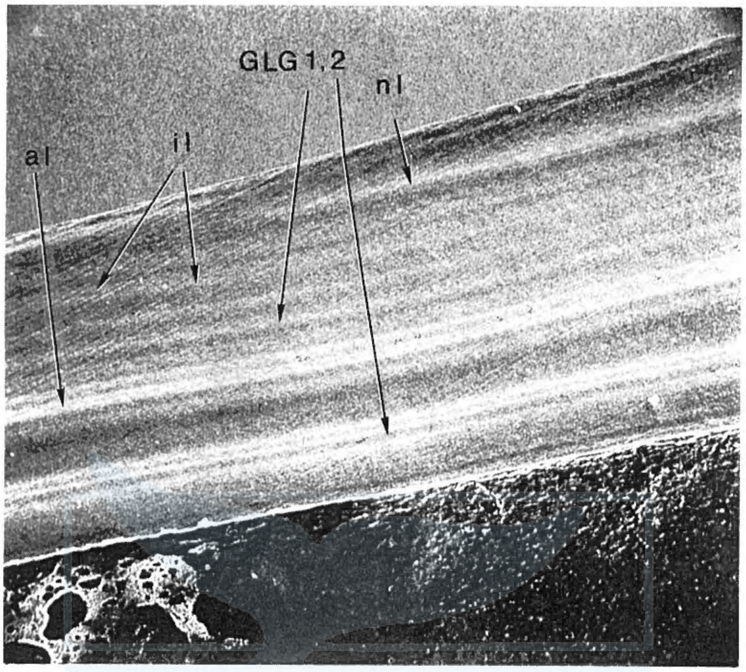
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