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A Report on Patterns of Deposition of Dentine and Cement in Teeth of Pilot Whales, genus *Globicephala*

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ABSTRACT

The present study reports on two aspects of pilot whale tooth structure: (1) deposition rate of dentinal and cemental laminae, and (2) patterns and possible significance of mineralisation anomalies. (1) Teeth from seven known-history captive northeast Pacific short-finned pilot whales, mostly having received tetracycline treatments, were examined. Numbers of cemental and dentinal growth layer groups (GLGs) were observed to be equivalent in teeth of age <15 yrs. Deposition rate was one GLG per yr, and confirmed the suitability of tooth GLGs for age determination in this species. (2) Mineralisation anomalies, their incidence and biological significance, were investigated in the teeth of the known-age short-finned pilot whales and in the teeth of northeast Atlantic long-finned pilot whales collected from Icelandic strandings in 1982 and 1986, and from a Faroese fishery-caught school in 1986. The following anomalous characters were recorded in the teeth: (a) *pulp stones*: discrete nodules containing concentric rings in the dentine; (b) *marker lines*, both in dentine and often cementum: discrete laminae which are regular yet noticeably different in appearance from the usual in morphology and affinity for stain; (c) *mineralisation interference*: irregularities in the lamina formation engendered by differential inhibition/activation of odontoblasts at the mineralisation front, causing realignment of the dentinal tubules and disrupting regular lamination patterns without preventing continuous formation; (d) *dentinal resorption*: actual erosion and frequent repair of existing regular laminated dentinal tissue; (e) *cemental disturbance*: any anomalous appearance of the usual regular laminated cemental tissue. Occurrence of these anomalies was scored by sex, current age, reproductive maturity, age at which the anomaly appeared, and in which calendar year. Comparisons of duplicate teeth from the same individual indicated that mineralisation anomalies must be of systemic origin, because type, pattern and age of occurrence of anomalies were similar. Results showed that appearance of pulp stones in the dentine tend to be associated with age at which puberty usually occurs. Marker lines increased with age after one year; there was also significant association with certain calendar years in Icelandic samples. Mineralisation interference increased with age reaching 60–100% incidence in animals >14 yrs in all samples. The incidence appeared related to age rather than reproductive maturity. Cemental disturbance was low in Icelandic whales, but reached *ca* 50% incidence in Faroese whales, in which it was both age- and maturity-related. Dentinal resorption was not evident in any immature animals. It reached a peak in old males, yet did not appear to greatly affect females until age >25 yrs. Dentinal resorption was common in the Faroese sample, and occurred in almost 100% of the adult males. In Icelandic animals it was <10% in specimens up to age 25 yrs. In very old animals (30+ yrs) incidence reached 20% to 40%. Observations from the known-history captives indicated that likely stressors, directly or indirectly responsible for certain mineralisation anomalies, may include sexual maturation, pregnancy and/or parturition, periods of starvation, as well as changes in health and life style.

KEYWORDS: PILOT WHALES; AGEING; MORPHOLOGY/ANATOMY; REPRODUCTION

INTRODUCTION

There have been several previous studies investigating the use of teeth for determination of age in short-finned pilot whales, *Globicephala macrorhynchus* (Kasuya and Matsui, determination for odontocetes is given in IWC (1980). The laminations in the dentine and/or cementum have generally been defined in terms of differing (high or low) mineralisation density and/or quality (Boyde, 1980); contiguous pairs comprising a growth layer group (GLG), as defined in IWC (1980). This is, of course, a simplification of the real situation where accessory laminae of varying mineralisation density and thickness are frequently observed. Myrick (1984) has addressed many of these issues. The main problem is the interpretation of laminae in relation to real time, i.e. what constitutes a daily, lunar and annual growth layer. The true incremental rate of growth of laminae has been investigated and established directly for some marine mammal species, but not pilot whales, by studying teeth from animals of known age or history (Hohn *et al.*, 1989), and the use of tetracycline to 'time-mark' hard tissues, i.e. dentine and cementum in teeth and bone (Yagi *et al.*, 1963; Best, 1976; Domning and Myrick, 1980; Gurevich *et al.*, 1980; Myrick *et al.*, 1984; 1988; Myrick and Cornell, 1990). Klevezal' (1980) concluded that in odontocetes, growth layers in both dentine and cementum are the result of seasonal growth rhythms, and that the special pattern of an annual GLG is determined by 'the form of the intraseasonal growth rhythms of an individual'. This had already been established for terrestrial mammals (Grue and Jensen, 1979). This theme of 'individuality' has been explored further by Akin (1988) for spinner dolphins (*Stenella longirostris*), where GLG patterns and general tooth morphology were found to be correlated with stock and geographical location. Klevezal' and Tormosov (1971) had previously used characteristics of the dentinal layers to distinguish between groups of sperm whales (*Physeter macrocephalus*).

However, an approach which assumes a constant regular growth pattern is over-simplified. Detailed examination of teeth of many mammals reveal that events may occur which appear to create systemically rather than locally caused disturbances in the normal regular deposition rate of laminae in the teeth (Myrick, 1988). In cetaceans such disturbances, which may be termed *mineralisation anomalies*, range from 'foreign' inclusions in the teeth, such as bone – true osteodentine (Best, 1966), to tooth-originating materials such as 'pulp stones', frequently labeled as 'osteodentine' (Boschma, 1938; Nishiwaki *et al.*, 1958). Further complexities include distinct laminae, 'deeply dark-stained layers (DSL)' defined by virtue of their unusual appearance and affinity for stain (Klevezal' and Myrick, 1984; Myrick, 1991); and various degrees of interference in mineralisation as well as actual resorption of dentinal and cemental tissue (Boschma, 1950; Myrick, 1988). Akin (1988) tried to use many of these characteristics to discriminate stocks but with equivocal results.

The purpose of this study has been twofold. Firstly, the aim has been to determine the true rate of incremental deposition of dentinal and cemental laminae in the teeth of pilot whales, in order to interpret GLGs correctly for age. Secondly, the aim has been to progress beyond this stage, and compare teeth extracted from (a) the same individual to investigate conformity of GLG patterns, and (b) different individuals derived from various sources and localities to study easily identifiable mineralisation anomalies which might have biological significance.

MATERIAL

One tooth was available for each of seven northeast Pacific short-finned pilot whales captured in California waters, six of which had received tetracycline medication during periods of their captive lives. Teeth were collected from these animals at death. Each animal had been maintained in captivity in Sea World establishments, up to the time of

In addition, teeth were available for each of 235 northeast Atlantic long-finned pilot whales, and duplicate teeth were examined for ten of these. This sample comprised 91 whales captured on 11 September 1986 in the Faroese drive fishery at Sandur on the island of Sandoy (*c.* 61°30′N, 0°11′E), 36 whales stranded on 21 August 1982 at Rif in Iceland (*c.* 64°56′N, 23°50′W) and 108 whales stranded on 26 October 1986 at Thorlakshofn in Iceland (*c.* 63°51′N, 21°22′W).

METHODS

All teeth were supplied cleaned, either dry (Faroes) or in alcohol (Sea World captives and Iceland). To my knowledge, none of the teeth had been boiled or treated in any harsh way. Once received, Sea World teeth excepted, they were transferred to distilled water and then to 10% neutral formalin solution for at least 24 hours for fixing, before then being transferred again to distilled water.

Histological preparation

The teeth were initially dried and mounted with **Lakeside* thermoplastic cement (no. 70C) on wood blocks designed to fit in the chuck of an **Isomet* low-speed rotary diamond saw machine. The Sea World teeth were oriented in such a way that the cut was made slightly off-centre, through the crown, pulp cavity and root of the tooth, so that the final cut was almost exactly central. The tooth was then realigned relative to the cutting blade, using a micrometer travelling screw gauge, so that the next parallel cut through the tooth would result in a section about 100μm thick. This section was then allowed to dry and subsequently mounted on a slide using a clear permanent mounting medium, **Protex*.

All other teeth were aligned on the chuck and cut in a similar plane, but about 0.5–1.00mm off-centre. The resulting larger pieces from these and the Sea World teeth, i.e., the portion containing or nearest the central zone, were then freed from the cement and decalcified as follows. Teeth were sorted according to appearance, into approximate age categories of neonate, young, young adult, old and very old, mainly to select for tooth size and volume. The teeth were then placed in perforated plastic histological baskets with labels, and decalcified in **RDO*, a commercially prepared mixture of acids, for 4–32 hrs, depending on the tooth volume and in accordance with manufacturer's recommendations. Decalcified teeth were quite flexible and rubbery in texture, and were rinsed in running water for several hours, whence they were re–immersed in distilled water.

The teeth were sectioned on a CO_2-freezing stage of a sledge microtome at 30–35μm thickness. Sections selected as most central and complete were then stained in histological baskets in Haematoxylin for approximately 2hrs. The sections were then 'blued' in weak ammonia solution, rinsed in distilled water, and dehydrated in 70% alcohol before mounting on 5% gelatin-coated slides. The sections were then dried on a slide warmer and finally permanently mounted using *Protex*. All *Protex*-mounted sections required several days on a slide warmer to completely harden.

Examination

The sections were all examined using a **Zeiss* microscope at x15-x500. The lowest magnification was adequate for dentinal GLG examination, but x125-x500 was essential for cemental examination. Decalcified and stained sections were examined in plain transmitted light. Tetracycline-treated sections were examined using transmitted plain, polarised and reflected ultra violet (UV) light.

Table 1

Data for Sea World captive short-finned pilot whales. Note that all dates are given as month/day/year.

s: oral tetra- ne medication dosage in g	Date of death	Length at death in cm	Weight at death in kg	Maturity at death and/or history since capture	History of health since capture	Time in captivity in yrs.	Estimated age at death in yrs.
le no. 7401, mature pregnant female caught on 10.23.74, 411.5cm at capture, weight 562.7kg on 01.07.76							
.74; 10g BID .74; 11.5g .74; 14.5g .81; 25g BID	10.05.81	421	788.6	Aborted foetus on 11.06.74; 160cm calf born on 05.25.81, but died few days later Progesterone study indicated 3-6 oestrous cycles; ovaries senescent!	Body weight fluct-uations in last few months of life; 863.6kg on 09.30.81, then stopped eating; cause of death - chronic kidney disease	6.95	Minimum 14; could be quite old i.e. 30+ if reproductive history correct
le no. 7501, immature male caught on 11.14.75, 348.1cm at capture							
eatments	04.10.76	379	375	Testes of small size and immature	Generally good; cause of death bronchopneumonia	0.42	Approx. 4-5, from body size
le no. 7603, immature male caught on 12.20.76, 322cm on 01.20.77, weight 404.5kg on 01.20.77							
.78; 29.5g	05.20.78	328	522.7	Testes small and immature	Good feeding except anorexia Feb. 1978; weight fluctuating from 536-582kg between 02.20.78 and 04.21.78; loss of weight up to death; kidney infection in Feb. and May 1978; cause of death uremia, septicaemia	1.42	Approx. 3-4 from body size
e no. 7802-H, probably immature female caught on 11.20.78, 335cm at capture							
80-02.08.80; D 81-04.28.81; D 83 continuing) 83-04.04.83	10.13.83	396	643	One large corpus luteum on left ovary; no pregnancies	Variable feeding habits; stopped eating 09.15.83; rapid weight loss from 718kg on 09.16.83; previous good health; cause of death kidney infection and septicaemia	4.875	Approx. 8-10; probably first ovulation
e no. 8001, immature male caught on 01.08.80, 274cm at capture							
80-05.12.80; ID/intra- larly	05.15.80	283	229	No information	No health crises until one week prior to death; weight loss; cause of death pneumonia, kidney and liver disease	0.35	Approx. 2-3 from size and year of birth=1978
no. 8003, female caught on 12.16.80, 340.5cm at capture, weight 477kg							
80; 10g SID 80; 5g BID	07.05.83	374	682	One large corpus luteum on left ovary; large follicle; no foetus	Weight loss to 432kg by 04.16.81; health good there-after; cause of death lung disease	2.56	Approx. 7-9 from size; probably first ovulation
no. 8227, immature male caught on 12.10.82, 316cm at capture							
82; 8g	12.19.82	316	370.5	Immature	Not feeding prior to death; cause of death due to complications of natural parasite overload	0.03	Approx. 3 from size

SID = single dose daily; BID = two doses daily.

All known-history teeth were photographed and identifiable GLGs and other structures (fluorescent tetracycline bands, accessory laminae, etc.) were measured in terms of thickness and distance from the neonatal line (NL) and pulp cavity edge, using an eye-piece micrometer, calibrated with a 1mm micrometer slide at each magnification. The position of such features was verified by photographic measurement. For all teeth examined, the total numbers of dentinal and cemental GLGs were recorded, as well as the position and nature of any anomalies in the teeth. The site of measurement was at 90° to the axis of the GLGs on the shoulder of one 'limb' of the tooth, level with, or just below the apex of the pulp cavity (Myrick *et al.*, 1988).

All Icelandic and Faroese teeth were examined without reference to biological data. Total dentinal and cemental GLGs, and the anomalies as specified below were recorded. Many of the teeth were also photographed. The captivity information for the Sea World specimens was required to establish a calibrated model of pilot whale tooth layering. This information was kept to a minimum, i.e. whether or not the animal had been given tetracycline and the length of time in captivity.

A summary of tooth preparation techniques for both UV and plain light examination is provided in Appendix 1.

Criteria used for classifying anomalies

(1) Pulp stones
These are discrete nodules containing concentric rings in the dentine (Fig. 1a).

(2) Marker lines
These are discrete laminae found in dentine and often cementum which are regular yet noticeably different in appearance from the normal boundary layers in morphology and affinity for stain (Fig. 1b). They are equivalent to the DSLs and maturational layers described by Klevezal' and Myrick (1984).

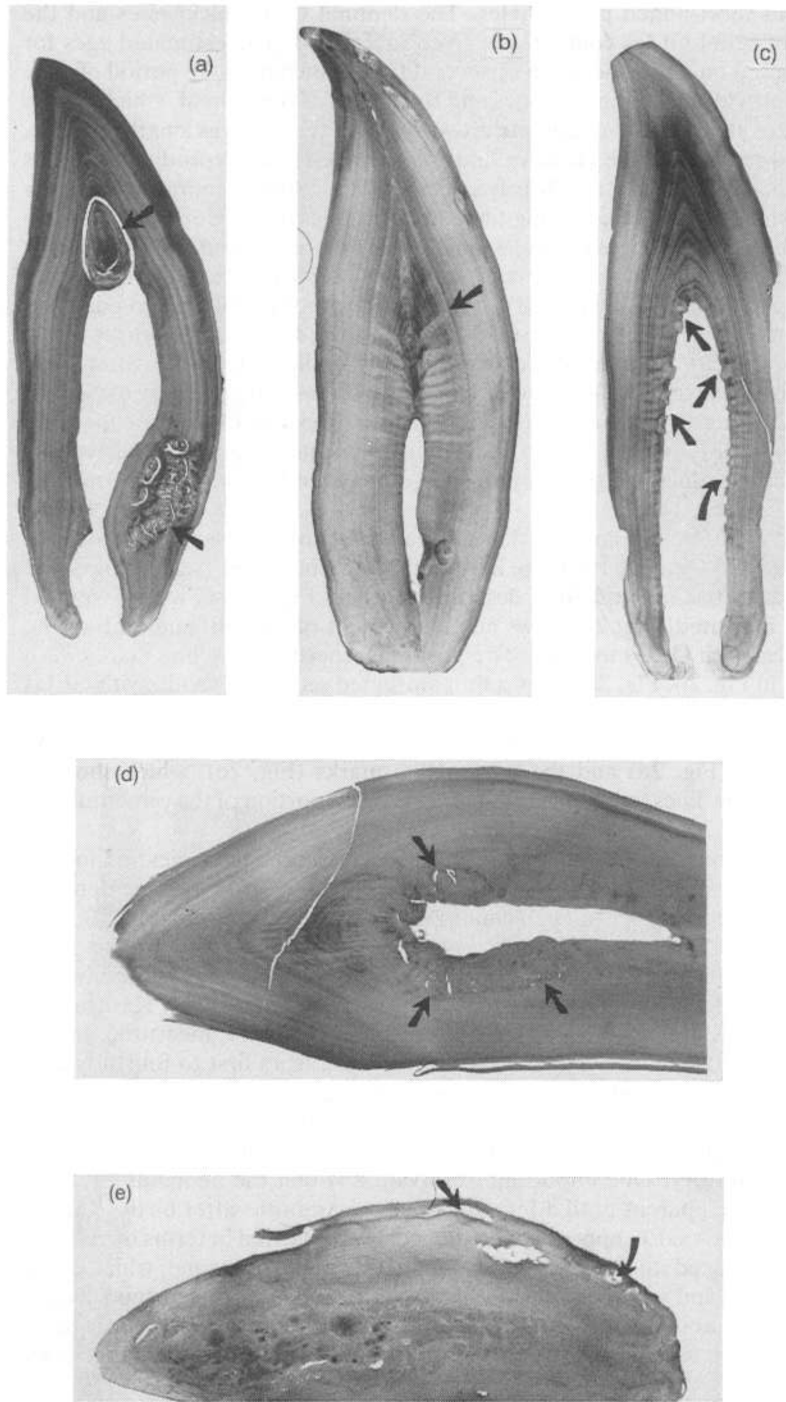
(3) Mineralisation interference
This refers to irregularities in the lamina formation emanating from differential inhibition and/or activation of odontoblasts at the mineralisation front (normally, the pulp cavity edge), causing realignment of the dentinal tubules and resulting in wavy lines, squirls and asymmetry (Fig. 1c), which disrupt usual patterns yet do not prevent continuous lamina formation (Myrick, 1988).

(4) Dentinal resorption
This refers to erosion and frequent repair of existing regular laminated dentinal tissue, resulting in an amorphous and/or globular appearance (Figs 1d and e), frequently with holes, cutting across and into regular tissue (Myrick, 1988).

(5) Cemental disturbance
This refers to any anomalous appearance of the usual regular laminated cemental tissue (Fig. 1e), including mineralisation interference and resorption (Myrick, 1988).

RESULTS AND DISCUSSION

Incremental studies

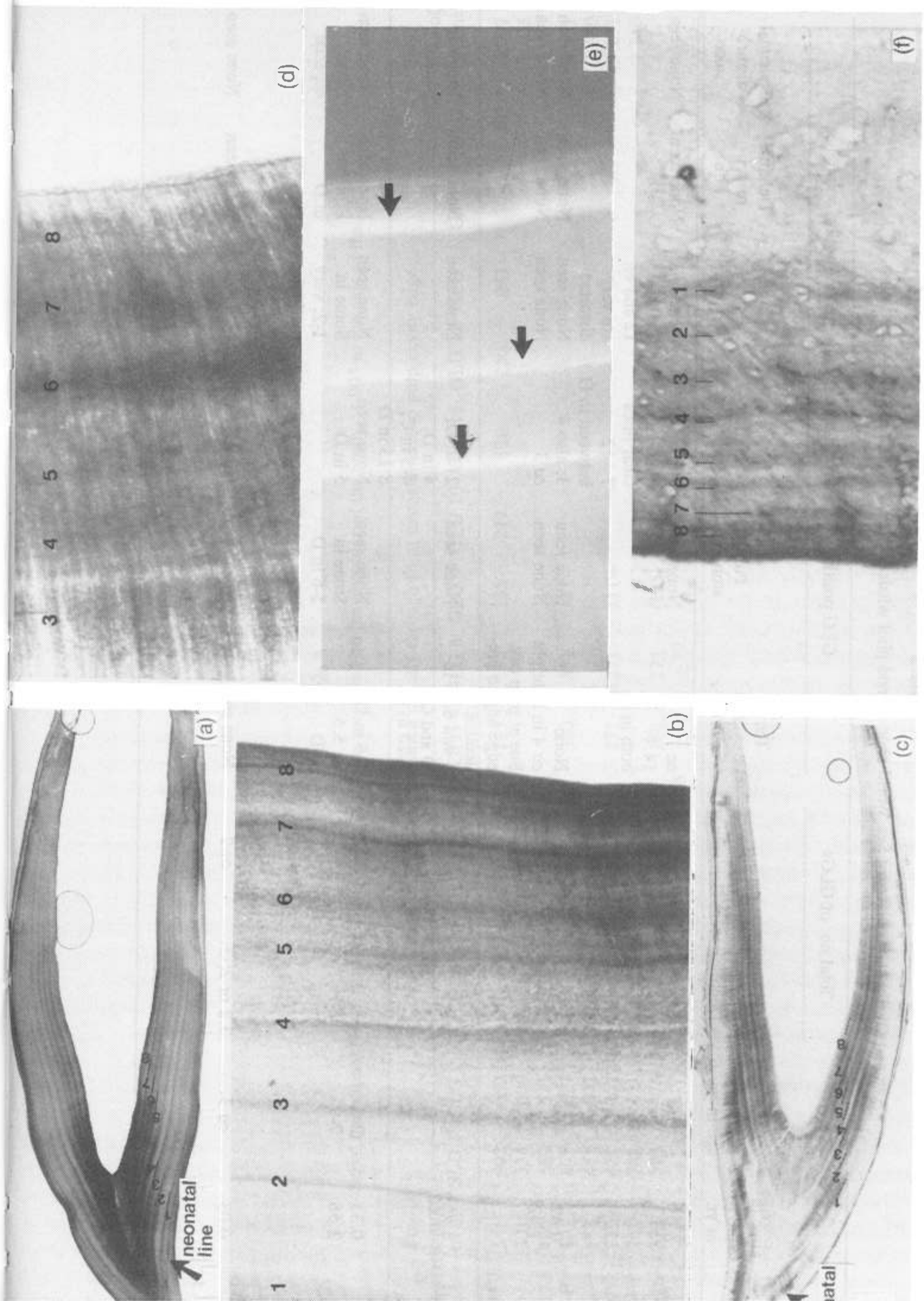


. Thin stained sections of decalcified pilot whale teeth, demonstrating various mineralisation anomalies.
p stones in dentine of Th-18, 369 cm female long-finned, from Iceland, age 9 yr;
rker line in dentine of Th-2, 419 cm mature female long-finned from Iceland, age 22 yr;
eralisation interference in dentine of 7401, 421cm mature female short-finned from California, age 14+ yr, note also the presence of several marker lines;
tinal resorption in dentine of S-2W, 575cm mature male long-finned from Faroes, age 30 yr;
nental disturbance in cementum of S-184, 462cm mature female long-finned from Faroes, age 24 yr; note also the presence of severe mineralisation
rference, dentinal resorption and pulp stones.

2 for the Sea World short-finned pilot whales. The dentinal GLG thicknesses and the average thickness of serial GLGs counted are given in Table 3. The estimated ages for these animals are based on a consideration of several factors including the period of time in captivity, which provides a 'minimum' age; and the length of the animal, which can be related to known sizes at age and approximate growth parameters, such as lengths at birth, sexual maturity and maximum size (Kasuya and Matsui, 1984) and reproductive cycles (Kasuya and Marsh, 1984; Marsh and Kasuya, 1984; 1986). Some reported values for short-finned pilot whales off Japan and long-finned pilot whales in the North Atlantic are given in Table 4. The estimated ages in Tables 1 and 2 are thus approximate and probably under– rather than over-estimated, because the 'ages' reported for the free-living populations are largely based on unverified GLGs for interpretation of age. In addition, the length of the female reproductive cycle (3–5 yrs, long-finned; 6–7 yrs, short-finned) is irrelevant for captive–held females where pregnancy is unlikely to occur after each ovulation. Interestingly, specimen 7401 had a hybrid calf (cross with *Tursiops truncatus*) on 25 May 1981, indicating an unusual reproductive history. Presence of corpora lutea and albicantia in the ovaries is only a certain indication of ovulation and not necessarily pregnancy unless actually documented; all pregnancies may not have reached term (e.g. see specimen 7401 in Table 1).

The decalcified stained section and the thin untreated section of specimen 7802-H (a 396cm female at death, 13 October 1983, age 8.5yr) are shown in Fig. 2. The photographic sequences demonstrate the methodology described earlier. Fig. 2a shows the stained section with GLGs indicated. Fig. 2b shows an enlargement of the lefthand limb of the section (Fig. 2a), also with GLGs marked. The positions where tetracycline marks were noted are indicated in Fig. 2b. Fig. 2c shows a thin untreated section of tooth, with GLGs indicated. Figs 2d and 2e show an enlarged (to same scale) portion of the lefthand limb of Fig. 2c; in plain light (Fig. 2d), and the same section in reflected UV light (Fig. 2e). The positions of the GLGs (Fig. 2d) and the tetracycline marks (Fig. 2e), which show as brightly fluorescing yellow lines, are indicated. Fig. 2f shows a portion of the cementum on the lefthand limb of Fig. 2c with the cemental GLGs indicated.

The growth in tooth structure, GLGs and the periods of tetracycline medication for the Sea World short-finned captives are shown in Fig. 3. The data indicate average dentinal growth for each GLG, decreasing from (specimens nos 7401, 7603, 7802H and 8003; Fig. 3; 593–520µm in yrs 1–4, to 368–267µm in yrs 5–8. The annual growth rate from tetracycline history is *ca* 360µm in ages 3–4 yrs, and *ca* 343µm from age 5–9 yrs; from yrs 7–14, the average rate is 238–247µm, and *ca* 333µm in the age range 6–9 yrs. Kasuya and Matsui (1984) reported a progressive decrease in thickness of GLGs, measured on the tooth shoulder as follows: 950µm, 400µm, 330µm and 300µm from first to fourth 'year', until only about 100µm by age 30 GLGs. The most rapid growth rate appeared to be between 0.25 and 0.5 GLG when the teeth erupt and the calf commences taking solid food. These data are compatible with the observed data in Table 3, and the known growth rate from tetracycline history. One important observation is that the neonatal line (16–64µm) does not become apparent until a few weeks or even months after birth (Kasuya and Matsui, 1984). The first GLG appears to be the most complicated in terms of growth, frequently with a pronounced stainable accessory lamination at the mid-zone, which could be misinterpreted as the end of the first GLG. This phenomenon has been reported by Hohn (1980) and Kasuya and Matsui (1984). These characteristics may reflect the rapidly changing growth rates and feeding criteria of the calf, as well as behavioural changes and stresses.



Tooth sections from 7802-H, captive 396cm mature female short-finned pilot whale from California. a. Decalcified stained section of whole tooth with ...inal GLGs identified; b. Enlargement of the GLGs in the lefthand limb of Fig. 2a, with GLGs identified; c. Thin untreated section of whole tooth with ...inal GLGs identified; d. Enlargement of the GLGs in the lefthand limb of Fig. 2c, with GLGs identified; c. The same section as in Fig. 2d, but viewed ...reflected UV light, and showing the fluorescent tetracycline marks which are indicated; f. Enlargement of the cemental GLGs in the cementum of Fig. ...th GLGs identified.

Table 2

Age and GLG interpretation of Sea World short-finned pilot whale teeth; D = dentine, C = cementum.

le .	Time in captivity in yr.	Estimated age in yr.	Total no. of GLGs: Dentine	Total no. of GLGs: Cementum	GLG position of tetracycline marks and mineralisation anomalies: Tetra-cycline marks	Pulp stone(s)	Marker line(s)	Mineral-isation inter-ference	Dentinal resorp-tion	Cemental disturb-ance
	6.95	>14	14.25-14.5	14	*ca* 7.5; 14.1? both in D	None seen	4-5; 7; 12; many clear lines 7 - 12 between in D	4 and on; 7 and on; 12 and on are very distorted	None seen	None seen
	0.42	4-5	4.5	4+	None	None seen	3; also 4	None seen	None seen	None seen
	1.42	3-4	4	3-4	*ca* 4 in D bright line at pulp cav. edge adj. to pre-dentine	None seen	*ca* 3	None seen	None seen	None seen
-H	4.875	8-10	8.5	8 or 7?	5 and 6.25 in D and C; 8.25 in D	None seen	2? in D; 4 in D; 4-5 in C; 8.15 in D	None seen	None seen	Irregular pattern
	0.35	2-3	3	2 or 3	2.8 in D	None seen	None seen	None seen	None seen	None seen
	2.56	7-9	8+	8	*ca* 5.5 in D	Stone in 5-6 in D	5 in D	Some in 1-2; very markedly distorted after 5	Possible in D	None especial
	0.03	*ca* 3	3.2 or 2; 3 is very pronounced - is artifact?	*ca* 2	None seen	None seen	None seen	None seen	None seen	None seen

Table 3

Serial measurements of dentinal GLG thickness in μm, measured on the shoulder of the tooth, at 90° to the axis of the GLGs.

le .	Pre-dentine to nl	Thickness of GLG in μm, measured serially through from neonatal line to pulp cavity edge: GLG 1	GLG 2	GLG 3	GLG 4	GLG 5	GLG 6	GLG 7	GLG 8	GLG 9	GLG 10	GLG 11	GLG 12	GLG 13	GLG 14	GLG 15	Total span of dentine
	541	902	632	256	271	316	150	195	195	135	211	120	150	241	301	391+	5,007
	301	534	609	662	541	361+											3,008
	406	587	571	722	481												2,767
-H	301	475	627	542	523	428	380	380	352	143+							4,151
	481	451	872	782													2,586
	211	421	301	571	782	361	271	271	271	211+							3,671
	361	782	421	421	181+												2,166
age	372	593	576	565	520	368	267	282	273	(135)	(211)	(120)	(150)	(241)	(301)		
	±114	±182	±180	±182	±182	±56	±115	±93	±79								

Table 4

Reported age and growth parameters for short-finned pilot whales off Japan and long-finned pilot whales in the North Atlantic (Sergeant, 1962; Martin *et al.*, 1987; Martin and Rothery, 1993; Kasuya *et al.*, 1988; Desportes *et al.*, 1993 and Bloch *et al.*, 1993).

	N. Pacific *G. Macrorhynchus*	N. Atlantic *G. melas*
Length at birth	140cm	178cm
Length at sexual maturity, males	394-525cm (x=422)	430-490cm
females	316cm	366-378cm
Age at sexual maturity, males	16yrs	12-14.3yrs
females	7.5-11.5 (x=9)yrs	6-8.7yrs
Maximum length, males	525-580cm	630cm
females	405cm	550cm
Maximum age, males	45yrs	46yrs
females	62yrs	59yrs

Life-history events with age and tooth structure for the short-finned captives are indicated in Fig. 3. In all whales maintained in captivity for about one year or longer, it is clear that some type of anomaly formed in the tooth within *ca* 9 months after initial capture. In four of five whales (7501, 7603, 7401 and 7802H), a marker line is present. In the remaining whale (8003), a pulp stone appeared within a few months of capture, and mineralisation interference, already present, continued. The pulp stone formation coincided with a period of weight loss. All animals experience a period of nutritional stress shortly after captivity, both due to poor feeding and adaptation to a change in diet. Specimen 7401 aborted a foetus within a few months of captivity, the timing coinciding with the formation of a marker line. Specimen 7802H formed a marker line about the time of probable maturation and first ovulation, marked by a large corpus luteum on the ovary (Table 1). Although marker lines appear both before and after capture, their appearance does not seem to be random. They frequently mark the start of a period, often prolonged, of mineralisation interference or more severe disturbance in GLG formation, e.g. specimens 7401 and 8003. The period of severe mineralisation interference in specimen 7401 commenced just prior to and continued throughout her pregnancy that resulted in the live birth of a hybrid calf. The mother died shortly afterwards from illness.

Thus, it appears that these mineralisation anomalies are not random but are often linked with life-history events. Observations from these known-history captives indicate that likely stressors (directly or indirectly) giving rise to mineralisation anomalies include sexual maturation, pregnancy and/or parturition, and periods of starvation or nutritional stress, as well as changes in health and life style (change from free-living to captive).

Mineralisation anomalies

(1) Comparison of teeth from single individuals

Table 5 details GLG counts and anomalies for 10 individuals for which at least two teeth were examined. These results show not only that different teeth from the same individual have similar GLG counts, but more significantly, that they usually show similar growth anomalies and patterns. This is similar to findings reported by Myrick (1988) for several dolphin species. The most variable character in terms of position and age of occurrence is the pulpstone. However, this may depend on the section of the tooth as their discrete

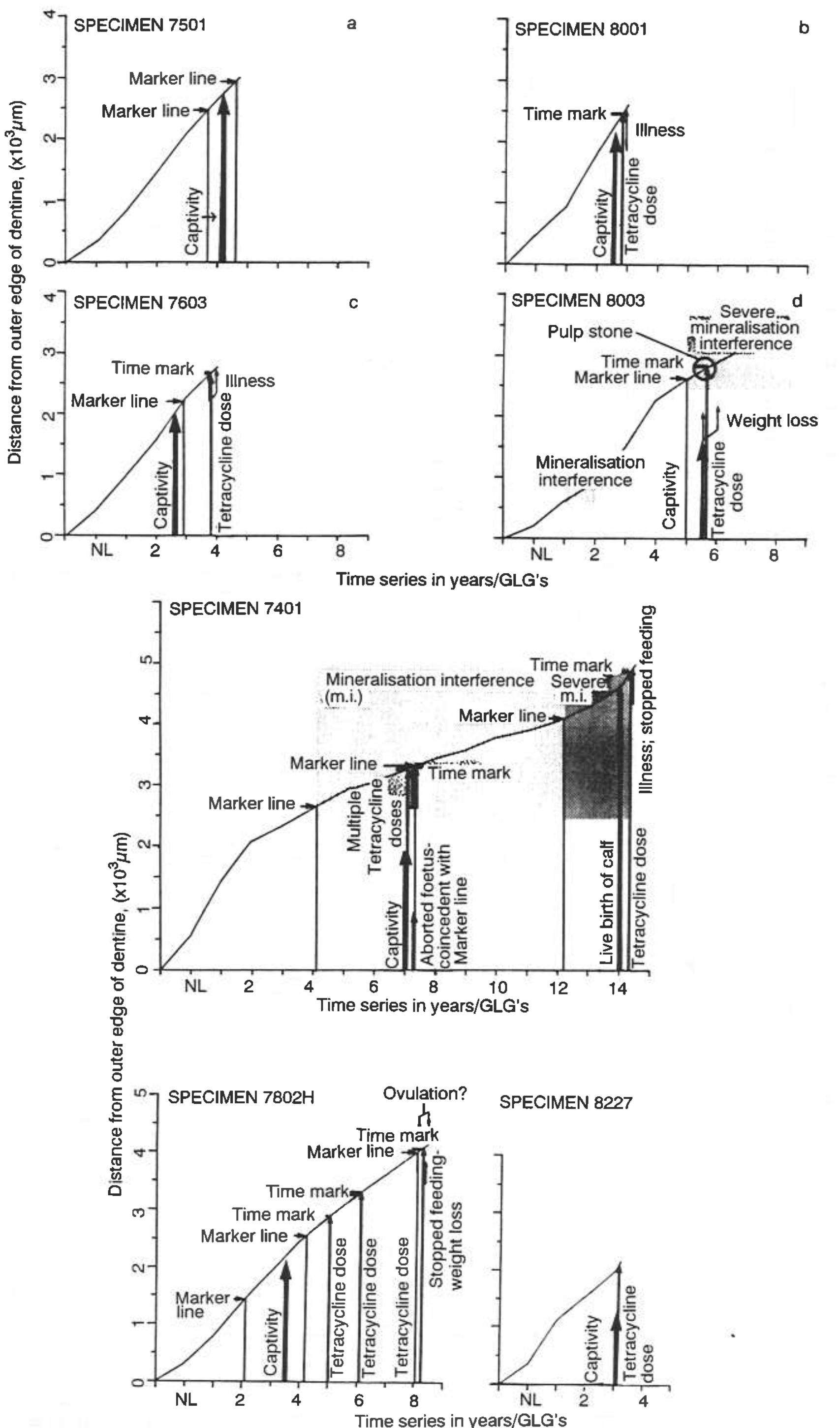


Table 5

Details of age and anomalies observed in duplicate teeth from long-finned pilot whale individuals. R = Rif, Th = Thorlakshofn.

Whale no.	Sex	Length in cm.	Maturity	Age in GLGs/years: Dentine	Age in GLGs/years: Cementum	Presence and position (GLG) of mineralisation anomalies: Pulp stones	Marker lines	Mineral-isation inter-ference	Dentinal resorp-tion	Cemental disturb-ance
-3	F	412	+ pregt	17+2	19	-	+ 9,11,12	+	-	-
-3				19	19	+ 7	+ 8/9,12	+	-	-
-14	M	516	+ ?	19-23	23-25	-	+ 3,6	-	-	-
-14				23	24-25	-	+ 3,6	-	-	-
-16	F	405-407	+ pregt	24	23	+ ?	+ 2,11,14,?16,17,19	+	-	-
-16				24	22+	+ 11	+ 2,11,14,16,17,19	+	-	-
-21	M	501-505	+ ?	11	10/11	+ 8	+ 4,8	+	-	-
-21				11	11	+ 8	+ 4,8	+	-	-
-25	F	432-434	+ lactg	16-19	19	+ 7/6	+ 5,8,16?	-	-	-
-25				18	19-23	-	+ 5,8,16/15	-	-	-
-25				17+	19	+ 10	+ 5,8,??10,16/15	-	-	-
-26	F	410-411	+ lactg	11	11-13	+ 7	+ 1,2	+	-	-
-26				11	11-13	+ 9	+ 1,2	+	-	-
h-2	F	419	+ ?	22	18+	+ 7,17	+ 5,7	+	+ ?	-
h-2				22	20	+ 12	-	+	+ ?	-
h-3	F	400	+ ?	12	12	+ 6,7	+ 5,6,10,11	+?	-	-
h-3				12	12	+ 9	+ 1,4/5	+	-	-
h-3*				11+?	10+	?	+ 1,5	+	-	-

Very poor preparation of tooth.

anticipated, e.g. nos Th-2 and Th-3. Again, this may be a real phenomenon or perhaps it is more likely to be artifactual. It is clear that the importance of obtaining good central sections well prepared histologically, so that there is no distortion of laminations, cannot be stressed enough (see also Hohn *et al.*, 1989). From the overall similarity between duplicate teeth, we may conclude that, in principle, any tooth taken from an individual pilot whale can be used for age determination.

(2) Age-related characteristics

The sub-sample sizes from Rif, once sorted by age, were so irregular and small, that after a preliminary comparison of analyses with results for Thorlakshofn, and given the geographic proximity, samples from both locations were combined. Fig. 4 shows the comparison by age group of the five different anomaly characteristics defined earlier for the Icelandic and Faroese whales; both sexes are included. The levels of incidence of pulp

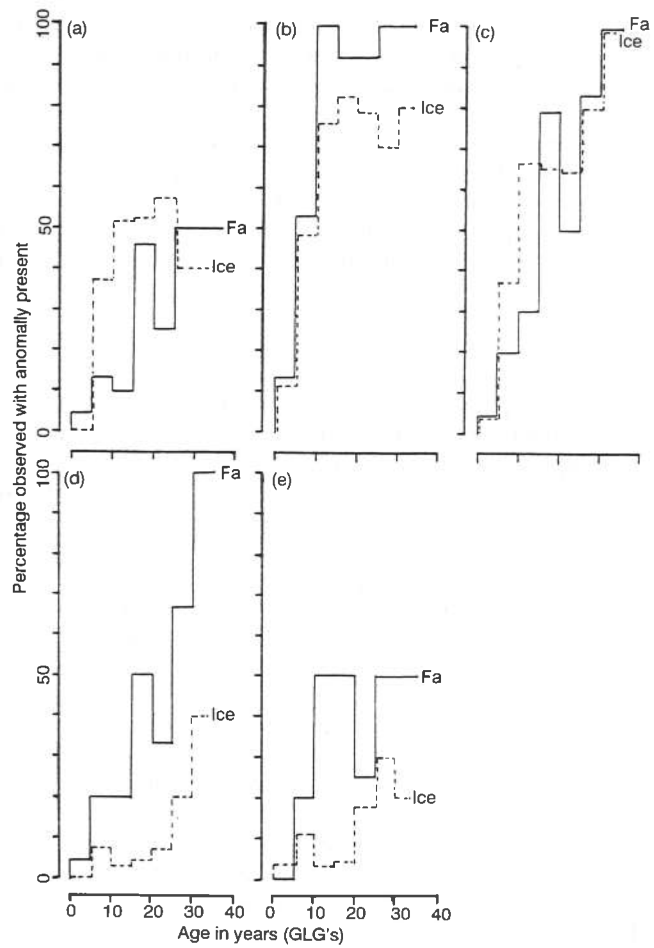


Fig. 4. Tooth mineralisation anomalies for sexes combined by age and location: Ice=Iceland; Far=Faroes.

stones, marker lines and mineralisation interference are similar in both locations, with an increase with age. The increase in pulp stones and mineralisation interference starts later in the Faroese whales, but the pattern is similar. However, the levels and patterns of incidence of dentinal resorption and cemental disturbance clearly differ between the two locations, with low overall levels for Iceland, but with dentinal resorption increasing steadily for the Faroese whales until there is 100% incidence in old animals. As might be expected, the incidence of all characteristics appear to be age-related, but each type has a different level of incidence: the pulp stones and cemental disturbance only reach *ca* 50% maximum in the oldest animals, whereas the other anomalies show extreme variations from 40–100% depending on locality.

The types of anomaly may be inter-related, and affected to varying degrees by the same internal and/or external factors. Thus each may represent a measure of the severity of response to such factors. I believe that the available evidence (e.g. see Myrick, 1988), particularly that detailing causes of changes in tooth structure (Johannessen, 1964; Nikiforuk and Fraser, 1979; Jensen *et al.*, 1981) suggests that the resorption and transformation of existing dentinal tissues is a response elicited by a severe long-term or continuing change in physiological state, probably with hormone imbalance, which either exacerbates any pre-existing hereditary-based tendencies or initiates a chronic hypocalcaemic condition. Other anomalies such as pulp stones and marker lines may be clear-cut responses to discrete short-term problems, even recurrent ones if recovery is prompt, in growth and nutritional status. Situations of intermediate severity may result in varying irregularities such as mineralisation interference. All these conditions assume a systemic cause rather than a purely local one specific to the tooth such as gum infection or parasites. Myrick (1988) has proposed theoretical models which show the effects of supra-threshold and chronic cyclic sub-threshold stressors on calcium ion balance and the mode of calcium ion recruitment into the blood. A recent study by Bengtson (1988) on fur seals (*Arctocephalus gazella*), has shown the occurrence of periodic starving/suckling laminae in mothers and their pups, which are associated with the nursing pattern. This would appear to be directly related to the periodic calcium and nutrient transfer.

Boschma (1950) investigated the problem of resorption along the lines of Colyer (1936) who believed that resorption was caused by physical external pressure, such as that from adjacent teeth. Such physical pressure can actually create resorption (Boyde, 1984), and the rate of erosion depends on the orientation and density of the mineralised tissue. Boschma stated that resorption could not be caused by pressures from adjacent teeth in sperm whales, but that pressures from opposing teeth in the upper jaw might lead to resportion. However, for one of the teeth he examined, he stated

'the abnormality of this tooth is of a similar kind as that previously described by other authors; the cause of the process is unknown. There is at least not any indication of a contact of this tooth with an antagonist in the upper jaw'.

Boyde *et al.* (1984) demonstrated that resorption resulting in typical 'Howship's lacunae', can be experimentally created *in vitro* using sperm whale dentine and osteoclasts from rabbit foetuses. Resorption *in vivo* by this mechanism is clearly a physiological process; perhaps systemic as well as local.

The evidence for a systemic cause of anomalies in pilot whales is strengthened by the appearance of the same anomalies in different teeth from the same animal (Table 5). This is discussed further under 'real time-related characteristics'.

Table 6 shows the mean age ±SE of occurrence of the first, second and subsequent pulp stones and marker lines for each of the three locations for long-finned pilot whales. The

Table 6

Mean age ± SE (GLGs/years) of occurrence of pulp stones and marker lines in long-fin pilot whale teeth.

	Mean position of recurrence in terms of age ± SE (GLGs/years) of mineralisation anomaly in the dentine.							
	Pulp stones		Marker lines					
ographic cation	1st	2nd	1st	2nd	3rd	4th	5th	6th
f, land	9.29±1.27	11.0	5.37±0.76	7.71±0.59	11.50±1.00	14.33±1.40	17.17±0.70	19.80±0.58
orlakshofn, land	6.54±0.53	5.57±1.41	4.91±0.45	8.66±0.67	11.68±0.71	12.25±1.48	18.50±1.94	31.00
ndur, roe Islands	10.04±1.32	9.71±2.39	5.87±0.46	10.68±0.65	15.20±1.09	20.25±2.14	20.00±4.00	17.00

for pulp stones and up to six times for marker lines. Klevezal' and Myrick (1984) noted up to five such marker lines in *Stenella*. The mean age for both first and second pulp stone occurrence is similar regardless of location, although the actual age is lowest for Thorlakshofn. The actual age range of *ca* 5.5–11 yrs suggests a possible link with onset of puberty/sexual maturity. However, it must be emphasised that pulp stones initially form within the pulp where they may remain free for a period of time before becoming incorporated into the dentine itself. Thus it is difficult to determine the precise age when the pulp stone was produced. Certainly the age may be earlier than that reported when first identified in the dentine. Many pulp stones contain what appear to be concentric GLGs. However, the deposition rate, if applicable, is unknown.

Results for the marker lines (Table 6), however, indicate a general recurrence at average intervals of usually around 3–5yrs (range 1–12 yrs), commencing at an earlier age (4–6 yrs) than the pulp stones, and continuing up to age >30 yrs. The irregularity of the mean ages which sometimes appear to decrease with time rather than increase, is caused by progressively decreasing sample sizes. The formation of marker lines may be influenced by a repetitive combination of internally generated 'crises' and/or external environmental ones. Alternatively, marker lines could be created as the result of relatively small changes in concert with normal biological rhythms coinciding chronologically.

Kasuya and Matsui (1984) show photographs of sectioned short-finned teeth illustrating what they define as 'secondary dentine'. This resembles mineralisation interference (Myrick, 1988) and usually began between ages 7–16 yrs for both sexes, coinciding with the usual timing of sexual maturation. Unlike the samples in this study, however, Kasuya and Matsui (1984) found differences in the timing of this character among adjacent teeth from the same individual. This suggests that correlation with maturation may only be general, and may in fact be more closely associated with the hormone changes during puberty which are often prolonged in males. Pulp stones and possible resorption can be seen in the 'secondary dentine'. Kasuya and Matsui (1984) also describe 'cellular dentine', layered tissue accumulating at the root and pulp cavity edge in teeth of older animals (>10 yrs). This appeared to be most extensive in old males. This has not been found in the long-finned pilot whales thus far examined.

(3) Sex-related characteristics

The incidence of the five characteristics by sex, all locations combined, is shown in Fig. 5. The levels and patterns of incidence in the two sexes are similar for pulp stones, mineralisation interference and marker lines, with the incidence generally increasing most in the years up to 10–14 yrs. Dentinal resorption and cemental disturbance are not really apparent until older ages, and then the incidence appears to be greater in males. This suggests that in general, susceptibility to the causative factors of these characters is not sex-specific. By contrast Myrick (1988) found that resorption was highest in female *Stenella longirostris*.

(4) Sexual maturity-related characteristics

Fig. 6 shows the incidence of the various characters by sexual maturity status for all locations combined. Dentinal resorption does not occur in the teeth of any immature animals, regardless of age. The reason for this clear demarcation may be connected with the hormonal changes that take place at the transition to adulthood. It is plausible that such hormonal upheaval and the physiological stresses which may accompany it and continue long afterwards, are capable of bringing about hypocalcaemia under certain

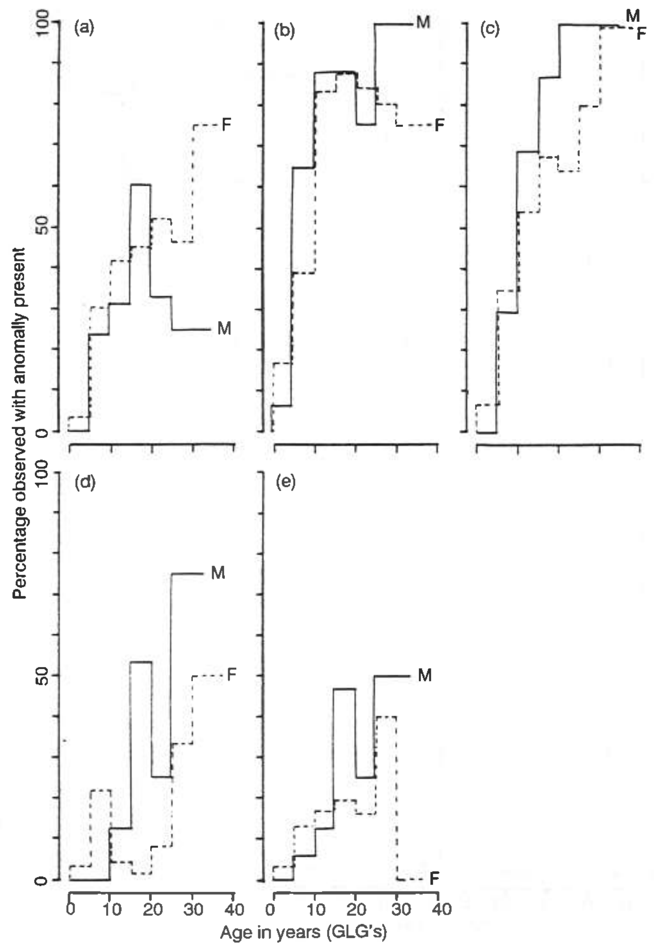


Fig. 5. Tooth mineralisation anomalies for Iceland and Faroes locations combined by age and sex: M=male; F=female. (a) Pulp stones; (b) Marker lines; (c) Mineralisation interference; (d) Dentinal resorption; (e) Cemental disturbance.

level of incidence of all characters is lower for immature (i.e. younger) animals. However, the incidence of marker lines with age is almost identical for both mature and immature animals and appears to increase steadily with age, until nearly all specimens are affected. This suggests that external (environmental) factors may be of relevance and a preliminary examination of this is given below.

(5) Real time-related characteristics

Two characters were selected for this analysis: pulp stones and marker lines. The information on year of stranding, age at death and sequential ages at which pulp stones and marker lines occurred, permitted a calculation of the calendar year in which the character appeared in the tooth. Initially, each anomaly and location was treated

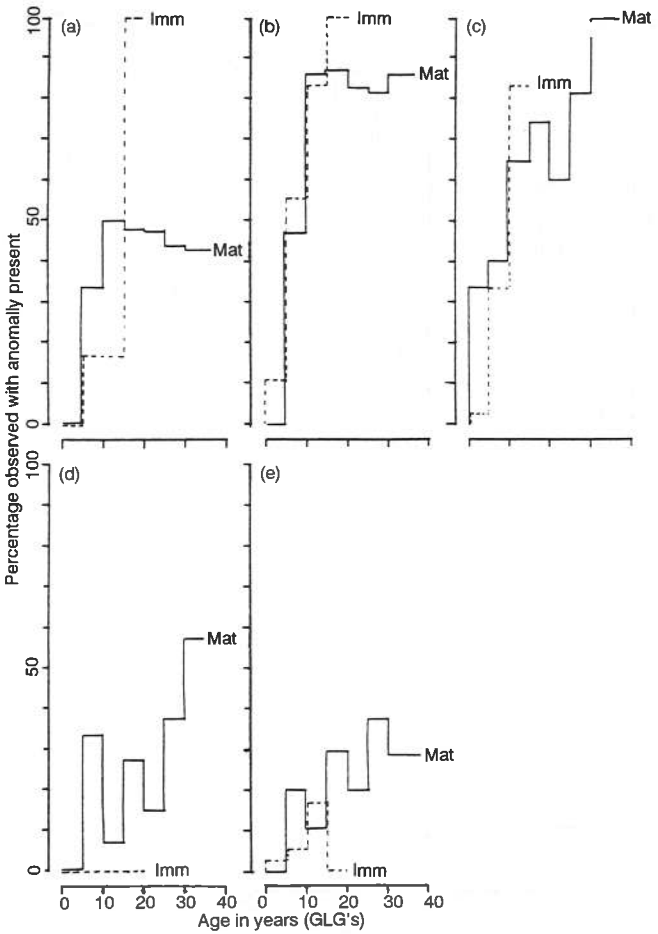


Fig. 6. Tooth mineralisation anomalies for Iceland and Faroes locations combined by age and sexual maturity status: Imm=immature; Mat=mature. (a) Pulp stones; (b) Marker lines; (c) Mineralisation interference; (d) Dentinal resorption; (e) Cemental disturbance.

year indicated that the Icelandic samples were similar and could thus be combined, whereas Sandur was more often different. Secondly, even when anomalies appeared in a particular year for several whales, the incidence was rarely high, indicating that in general only a minority were responding to whatever the causative factor might be. In addition an anomaly of some kind occurred almost every year. This might be explained partly by a lack of precision in age determination (e.g. ±1 or 2 GLGs). Chi-square tests on differences between proportions, showed no significant association between year and occurrence of pulp stones for either Iceland or the Faroes. However a significant association ($P<0.0001$, Chi-square=73.729, df=32) was found for marker lines for Iceland, although not for the Faroes. In particular, the year 1960 was prominent for Iceland when >40% teeth from whales in the sample (which would have been alive at that time) exhibited a marker line. A similar peak occurred in 1953 (Fig. 7).

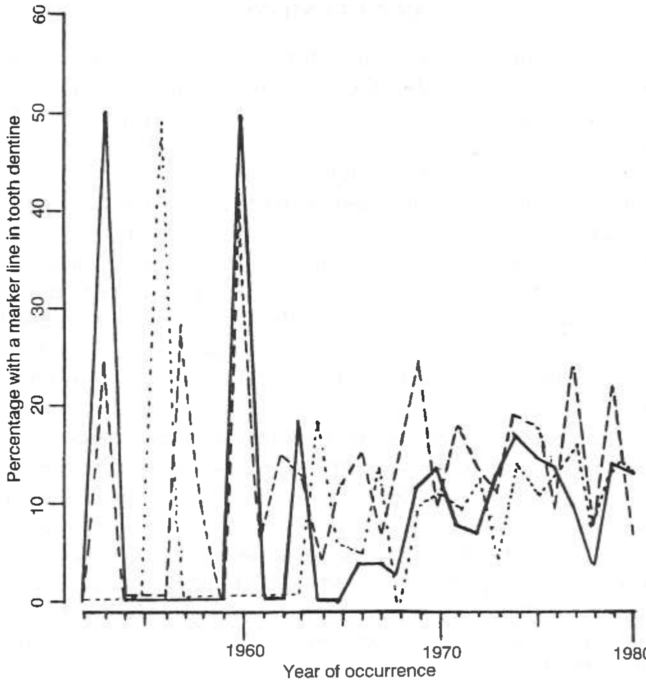


Fig. 7. Percentage frequency of tooth specimens with marker lines for both sexes, all ages and all maturity statuses combined, by year for each location: Thorlakshofn (—), Rif (- - - -), Sandur (.....).

the 1982–83 El Niño event. The anomalous layer, described as the 'El Niño mark' (ENM) comprised a pair of hypocalcified incremental layers about 75μm wide within the 1983 GLG. The feature appeared predominantly, but not exclusively, in adult females. Because the primary prey of the species was anchoveta (*Engraulis ringens*), the stocks of which collapsed during the El Niño period, Manzanilla ascribed dietary deficiencies as the cause of the tooth anomalies. Teeth of other species did not display ENMs, but were feeding on a different prey source. This is the first convincing evidence of a connection between tooth anomalies and environmental factors.

In the case of the Icelandic pilot whales, the 1953 and 1960 peaks (Fig. 7) appear to have no obvious environmental correlate. The diet of pilot whales is primarily squid, although other prey may be taken in times of need (Desportes and Mouritsen, 1993). A major problem is that the locality of the whales during those years may have been anywhere in the North Atlantic. A major climatic reversal in the northeast Atlantic commenced around 1960 (Dickson *et al.*, 1975), but the effects of this were experienced over a protracted period of years (Malmberg, 1985) and not as a sharp change as in the El Niño period.

Further investigation of environmental changes at those times may be worthwhile. It is interesting that none of the Faroese teeth had a marker line in those years suggesting that the Icelandic whales were affected by factor(s) that did not influence the Faroese group.

CONCLUSIONS

Examination of teeth from seven known-history captive northeast Pacific short-finned pilot whales, mostly having received tetracycline treatments, determined that in teeth of age <15 yrs, numbers of cemental and dentinal growth layer groups (GLGs) were equal. The incremental rate of deposition is one GLG per yr. Both dentinal and cemental GLGs may therefore be used directly for age determination in years.

Five types of mineralisation anomaly can be recognised in pilot whale teeth, and the incidence of these can vary with age, geographical origin, sex and maturity. Observations from the known-history captives indicate that likely stressors (directly or indirectly) include sexual maturation, pregnancy and/or parturition, and periods of starvation, as well as changes in health and life style, such as the transition from free-living to captive. *Pulp stones*, when present, tend to be associated with age at which puberty usually occurs, and the incidence does not greatly increase thereafter. *Marker lines* occur throughout life at all ages, after age one year, regardless of state of maturity, and therefore, as anticipated, incidence increases with age; there is also significant association with calendar year e.g. 1960 stands out in Icelandic samples, but not in the Faroese. *Mineralisation interference* increases with age reaching 60–100% incidence in animals over 14 yrs in both Icelandic and Faroese animals. The incidence is related to age rather than maturity. *Cemental disturbance* has a low incidence in Icelandic whales, and reaches *ca* 50% incidence in Faroese whales. This is also age– and maturity-related. *Dentinal resorption* has not been recorded in immature animals of either sex. Incidence reaches a peak in old males, yet does not appear to greatly affect females until age >25 yrs. Dentinal resorption occurs frequently in the Faroese animals, reaching almost 100% incidence in males. The incidence in Icelandic animals is <10% until age 25+ yrs when incidence rises from 20% to 40% in very old animals (30+ yrs).

The difference in these levels of incidence by location and sex indicates varying degrees of susceptibility to the causative factors. This may be genetically controlled, or simply a measure of local environmental or behavioural stress factors. These factors revealed in the teeth may explain differential mortality and sex ratios, but this requires further examination. Potential hypotheses include the possibility that the higher levels of resorption in teeth of adult males may be connected with the stresses of inter-male competition in reproduction. Reproductive stresses will, of course, differ between the sexes. The male is destined to compete regularly, probably annually, for procreative status in schools which are female dominated. The female however, may only reproduce every 3–5 yrs. If Klevezal' and Myrick (1984) are correct in their interpretation of 'parturition' laminae (DSLs), reproductive history may, in theory, be deduced from the teeth. Periods of nutritional and environmental hardship may be translated into unusual marks in the dentine, where growth ceases temporarily and mineralisation problems arise. Another possibility is that other stress factors, perhaps manmade, such as fishery conflicts, may lead to identifiable anomalies in teeth. Consideration of such hypotheses help to formulate questions that can be addressed experimentally.

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Appendix 1
HISTOLOGICAL PREPARATION OF TEETH

STEP 1 – Preparation for all methods.
1. Extraction of tooth from the mandible after rotting of the jaw.
2. Cleaning of the tooth by physical abrasion or enzyme digestion – definitely no boiling.
3. Mounting of dry tooth on wooden blocks using thermoplastic cement, in a plane to permit cutting through the crown to root axis.

STEP 2 – For examination of tetracycline presence with reflected UV light.
4. Cutting of section through centre line (crown and pulp cavity apices) at approximately 100µm, using an *Isomet* low-speed rotary diamond saw with micrometer travelling screw gauge.
5. Mounting of thin section on acid-cleaned glass slide with clear resin permanent mounting medium, *Protex* or *DPX*, under a glass coverslip and drying on a slide warmer for a few days.

STEP 3 – For examination of 'growth layer groups' (GLGs) in dentine and cement using transmitted plain light.
6. Decalcification of remaining larger portion of the tooth (near half) after detachment from the cement and wood block, in *RDO*, a commercial acid product for pathological use, for 4–32hr depending on tooth volume (5% nitric acid may also be used, but decalcification may take several days or weeks).
7. Complete rinsing of decalcified tooth portion in water for 24hr, followed by temporary storage in distilled water.
8. Fixation of decalcified material in 10% neutral buffered formalin.
9. Section cutting at a thickness of 30–35 micron, from the decalified tooth portion using a freezing stage on a sledge microtome, using *Tissue-Tek* as a mounting medium.
10. Transfer of selected sections to histological baskets and immersion in water using a paint brush, followed by staining in Haematoxylin stain for 2hr, subsequent water rinsing and 'blueing' in ammonia solution for a few minutes, then water-rinsing and partial dehydration in 50% and 70% alcohol.
11. Flotation onto and arrangement of stained sections on a 5% gelatin smear-coated glass slide under 70% alcohol and subsequent drying of slide and sections on a slide warmer.
12. Mounting of dried stained sections with clear resin permanent mounting medium *Protex* or *DPX* under a glass coverslip, and drying on a slide warmer for a few days.