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Toothed Whales and
Sirenians

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Cover photographs:

'Scrimshaw' – the art of carving on a whale tooth; and an etched sperm whale toothing showing growth layer groups.

Photographs by courtesy of the Institute of Oceanographic Sciences, UK.

Growth of Odontocetes and Sirenians: Problems in Age Determination

PROCEEDINGS OF THE INTERNATIONAL CONFERENCE ON DETERMINING AGE OF ODONTOCETE CETACEANS [AND SIRENIANS]

La Jolla, California
September 5–19, 1978

Edited by
W. F. Perrin and A. C. Myrick, Jr
National Oceanic and Atmospheric Administration, National Marine Fisheries Service,
Southwest Fisheries Center, La Jolla, California 92038

Introduction

Age determination is a tool central to the development of estimates of life history parameters needed for assessment and management of cetacean stocks. While many published studies have been based on age estimated from growth layers in hard tissues, mainly teeth, there nonetheless exists considerable controversy about the significance of the growth layers, especially in tropical forms. There has been no assurance that different investigators are looking at the same structure when they talk about 'one growth layer'. There has been no accepted theory of the etiology, chemistry, or mechanism of growth layer deposition in odontocetes. Considering this situation, the comparative population dynamics of small cetaceans rests on an insecure foundation. The purpose of this conference was to bring together cetologists currently working on the life histories of odontocetes and workers in other, ancillary fields who could be expected to contribute to a potentially integrated approach to the problem.

The conference had two parts: an open symposium convened September 5–7, 1978, at the Scripps Institution of Oceanography, La Jolla, and a 9-day laboratory workshop held immediately following the symposium at the Southwest Fisheries Center. Participation in the workshop was by invitation.

The original intent was to limit the coverage of the conference to odontocete cetaceans, but during last-minute preparations for the conference it became clear that techniques currently being developed for age determination of sirenians are of potential value for studies of odontocetes. For this reason, two contributions on sirenians are included in this volume; one of them, by H.D. Marsh was presented in the symposium. Also, teeth of the dugong, *Dugong dugon,* were examined during the workshop.

PARTICIPANTS[1]

Lucie Arvy
Laboratoire d'Histoenzymologie
Faculté de Médicine
45 Rue des Saints-Pères
Paris VI, France

Jeffrey L. Bada
Scripps Institution of Oceanography
University of California at San Diego
La Jolla, California 92093, USA

Alan N. Baker
National Museum of New Zealand
Private Bag, Wellington
New Zealand

Alan Boyde
Department of Anatomy & Embryology
University College London
Gower Street
London WC1E 6BT, England, UK

Stephen Brown
Scripps Institution of Oceanography
University of California at San Diego
La Jolla, California 92093, USA

Ivar Christensen
Institute of Marine Research
P.O. Box 1870
5011 Bergen-Nordenes
Norway

Robert Clarke
People's Trust for Endangered Species
Unidad de Ciencias Marinas
Universidad Autonoma de Baja California
Apartado Postal 453
Ensenada, B.C.N., Mexico

L. H. Cornell
Sea World Inc.
South Shores Road
San Diego, California 92109, USA

Daryl P. Domning
Department of Anatomy
College of Medicine
Howard University
520 'W' St., NW
Washington, DC 20059, USA

Floyd E. Durham
University of Southern California and
Los Angeles County Museum
11932 Roseglen Street
El Monte, California 91732, USA

Charles W. Fowler
National Marine Mammal Laboratory
National Marine Fisheries Service
7600 Sand Point Way, NE
Building 32
Seattle, Washington 98115, USA

Ray Gambell
International Whaling Commission
The Red House, Station Road, Histon
Cambridge CB4 4NP, England, UK

William Gilmartin
Naval Ocean Systems Center
San Diego, California 92152, USA

Merrill E. Gosho
National Marine Mammal Laboratory
National Marine Fisheries Service
7600 Sand Point Way, NE
Building 32
Seattle, Washington 98115, USA

---

[1] Authored papers, participated in workshop, or acted as session chairmen.

Helen Grue-Nielsen
Institute of Comparative Anatomy
University of Copenhagen
Universitetsparken 15, DK-2100
Copenhagen, Denmark

Vladimir S. Gurevich
Hubbs/Sea World Research Institute
1700 South Shores Road
San Diego, California 92109, USA

James Harvey
Oregon State University
Marine Science Center
Newport, Oregon, USA

Keith Hay
Environment Canada
Fisheries & Marine, Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue,
Province of Quebec, H9X 3L6, Canada

Aleta A. Hohn
Department of Zoology
University of Maryland
College Park, Maryland 20742, USA

Clifford A. Hui
Naval Ocean Systems Center
San Diego, California 92052, USA

B. Jensen
Game Biology Station
Rønde, Denmark

Hiroshi Kajimura
National Marine Mammal Laboratory
National Marine Fisheries Service
7600 Sand Point Way, NE
Building 32
Seattle, Washington 98115, USA

Toshio Kasuya
Ocean Research Institute
University of Tokyo
1-15-1, Minamidai, Nakano-Ku
Tokyo 164, Japan

Bryan J. Kemper
Offshore Environment Division
Indian and Northern Affairs
2065 Woodcrest Road
Ottawa, Ontario K1A 0H4, Canada

Alan J. Kenyon
Sloan-Kettering Institute for Cancer Research
Donald S. Walker Laboratory
145 Boston Post Road
Rye, New York, 10580, USA

Makoto Kimura
National Marine Fisheries Service
Southwest Fisheries Center
8604 La Jolla Shores Drive
P.O. Box 271
La Jolla, California 92038, USA

V.L. Kirby
Department of Biology
San Diego State University
San Diego, California 92115, USA

Galina A. Klevezal'
Institute of Development Biology
Academy of Sciences of the USSR
26 Vavilov St.
117334 Moscow, USSR

V.M. Kozicki
Environment Canada
Fisheries and Marine
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue
Province of Quebec, H9X 3L6, Canada

Christina Lockyer
Sea Mammal Research Unit, NERC
Madingley Road, Cambridge CB3 0ET
England, UK

Helene D. Marsh
Department of Zoology
School of Biological Sciences
James Cook University of North Queensland
Townsville, Queensland 4811
Australia

Patricia M. Masters
Scripps Institution of Oceanography
University of California at San Diego
La Jolla, California 92093, USA

Bruce R. Mate
Oregon State University
Marine Science Center
Newport, Oregon, USA

James G. Mead
Division of Mammals
U.S. National Museum of Natural History
Smithsonian Institution
Washington, DC 20560, USA

Edward D. Mitchell
Environment Canada
Fisheries and Marine
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue
Province of Quebec, H9X 3L6, Canada

Nobuyuki Miyazaki
Department of Zoology
National Science Museum
Hyakunincho Shinjuku
Tokyo 160, Japan

Albert C. Myrick, Jr
National Marine Fisheries Service
Southwest Fisheries Center
8604 La Jolla Shores Drive
P.O. Box 271
La Jolla, California 92038, USA

Masaharu Nishiwaki
Department of Marine Sciences
University of the Ryukyus
Shuri Naha
Okinawa 903, Japan

Seiji Ohsumi
Far Seas Fisheries Research Laboratory
1000 Orido, Shimizu
Shizuoka-ken 424, Japan

Obla Paliza
Unidad de Ciencias Marinas
Universidad Autonoma de Baja California
Apartado Postal 453
Ensenada, B.C.N., Mexico

William F. Perrin
National Marine Fisheries Service
Southwest Fisheries Center
8604 La Jolla Shores Drive
P.O. Box 271
La Jolla, California 92038, USA

Kenneth V. Pierce
National Marine Mammal Laboratory
National Marine Fisheries Service
7600 Sand Point Way, NE
Building 32
Seattle, Washington 98115, USA

John H. Prime
Sea Mammal Research Unit, NERC
Madingley Road, Cambridge CB3 0ET
England, UK

George A. Sacher
Division of Biological and Medical Research
Argonne National Laboratory
Argonne, Illinois 60439, USA

Joan E. Sawyer
Zoological Society of San Diego
Balboa Park
San Diego, California 92103, USA

Victor B. Scheffer
14806 SE 54 Street
Bellevue, Washington 98006, USA

David E. Sergeant
Environment Canada
Fisheries and Marine
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue
Province of Quebec, H9X 3L6, Canada

Martin Sheldrick
Department of Zoology
British Museum (Natural History)
Cromwell Road
London SW7 5BD, England, UK

Brent S. Stewart
Hubbs/Sea World Research Institute
1700 South Shores Road
San Diego, California 92122, USA

Laurie J. Stuart
Moss Landing Marine Laboratory
P.O. Box 223
Moss Landing, California 95039, USA

Marshall R. Urist
UCLA Bone Research Laboratory
Rehabilitation Center
1000 Veteran Avenue
Los Angeles, California 90027, USA

James R. Zweifel
National Marine Fisheries Service
Southwest Fisheries Center
8604 La Jolla Shores Drive
P.O. Box 271
La Jolla, California 92038, USA

PROCEEDINGS OF THE SYMPOSIUM

Talks presented in the symposium and represented here by papers or abstracts are listed in the Table of Contents. Talks presented but (for various reasons) not included in this volume are listed below.

Arvy, L. – Biology of bone-osteoporosis in *Physeter macrocephalus* Linnaeus (Cetacea, Odontoceti, Physeteridae).

Durham F.E. – The aging of bowhead whales based on using bone layering ovarian scars, baleen, sutural fusion and body length.

Kasuya, T. – The life history of Dall's porpoise, with special reference to the stock off the Pacific coast of Japan. (Published in *Sci. Rep. Whales Res. Inst.*, 30: 1–64, pl. 1–5.)

Kemper, J.B. – Implication for management of narwhal: age determination techniques.

Mate, B.R. and Harvey, J. – Acid etching and acetate peels for microresolution of dental laminations.

Sawyer, J.E., Kirby, V.L. and Gilmartin, W.G. – Reproductive studies in the female Atlantic bottlenose dolphin.

Urist, M.R. – Biogenesis of bone.

ACKNOWLEDGEMENTS

This conference had its origin in the recommendations of a meeting of an FAO-ACMRR *ad hoc* advisory group on small cetaceans in 1974 in La Jolla. A plan and budget for the conference were drafted at a meeting of a research-planning subcommittee of the small cetaceans group (W.F. Perrin, R.L. Brownell, Jr, J.G. Mead and T. Kasuya) in Washington, DC in 1975. The conference began to assume reality in 1977 when the US Marine Mammal Commission proffered substantial funds for its support. Additional funding was soon forthcoming from the Southwest Fisheries Center of the National Marine Fisheries Service, the government of Australia (via the International Whaling Commission) and the People's Trust for Endangered Species.

The coordinating committee for the conference (A.C. Myrick, Jr, P.A. Sloan and M. Kimura) had a great deal of help from many people, including R.M. Allen, M. Di Bona, L.B. Dick, M.L. Dik, L.J. Hansen, L. Kadubec, K.S. Raymond, L.L. Vlymen, L.C. Prescott, J.A. Shoemaker and J. Webber. D.E. Sergeant, C.W. Fowler and M.R. Urist chaired sessions of the symposium. Others who helped make the conference possible were I. Barrett, J.F. Carr, R. Gambell, R.J. Hoffman, S.J. Holt, E.D. Mitchell, N. Mendes and J.R. Twiss, Jr. We thank these people and others we may have missed.

W.F. Perrin and A.C. Myrick, Jr.
La Jolla, California, 1980

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Report of the Workshop

A. INTRODUCTION

Techniques of age determination from examination of layers vary greatly from worker to worker. Some techniques have been developed for one particular species but may be useful for other species. The purpose of this workshop was to bring together experts in the life history of cetaceans so that could work side by side in the laboratory. The charges to the participants were:

1. Demonstrate techniques of specimen preparation.
2. Demonstrate methods of reading specimens (counting age-related layers or groups of layers).
3. Carry out and evaluate the results of cross-reading experiments, in which as many participants as practicable read each adequate series of prepared specimens.
4. Attempt to achieve some level of standardization of techniques, through review of one's own methods as well as review and evaluation of others' methods.
5. Review and assess new methods that may possibly be better than those currently in wide use.

The workshop was organized into two four-day cycles of three simultaneous taxon-oriented sessions each. The six sessions were on beaked whales, sperm whales, narwhals and white whales, large delphinids (mainly pilot whales and killer whales), dolphins, and porpoises. Each session had the use of laboratory space, microscopes, diamond-blade saws, glassware, reagents, stains, etc. Each four-day cycle was planned to consist of two days of intensive laboratory work within the three sessions, one day of demonstrations across the sessions, and one day of reports and discussions; but, in actuality, laboratory projects continued throughout the ten days of the workshop and, in some cases, beyond the end of the meeting. A brief meeting was held each morning to lay plans and assess progress.

Widely used methods of preparation of specimens for age determination available to the workshop for evaluation included:

1. Acid-etching of half-teeth to produce a relief of ridges and valleys corresponding to layered structure in the teeth.
2. Preparation of sawed and/or ground longitudinal thin sections of teeth or bone for examination under a conventional light microscope, without further preparation.
3. Preparation of decalcified and stained thin sections of teeth and bone for examination under a microscope.
4. Time-marking of teeth in live animals with tetracycline, so that growth during a known elapsed time can be identified in teeth collected subsequently.

The workshop also reviewed several relatively new and/or underutilized techniques:

1. Use of a polarized-light microscope for examination of thin sections (see Myrick, this volume).
2. Use of 'acetate peels' to replicate the etched surface of a tooth.
3. Use of microradiography to visually resolve layer structure in thin sections (see Hohn, this volume).
4. Use of the scanning electron microscope to examine surface features of etched or otherwise prepared specimens (see papers by Boyde and Hohn, this volume).
5. Use of the phenomenon of racemization of amino acids in inert protein to biochemically estimate absolute age (see paper by Bada, Brown and Masters, this volume).

Major responsibilities for sections of the report other than the session reports were as follows:

Introduction	W.F. Perrin
Terminology	C. Lockyer, A. Boyde and W.F. Perrin
Recommendations	C. Lockyer and B.S. Stewart
Catalog of techniques	H.D. Marsh
Literature cited	J.B. Kemper and W.F. Perrin
Glossary	A.C. Myrick, Jr

The session reports vary in organization and content, because of variation in the status of knowledge for the respective taxa. For some species, e.g. the sperm whale, there has been a relatively long period of use of age determination as a tool in the study of biology and in management, whereas for other species and whole groups, such as the beaked whales, knowledge of life history is rudimentary and age determination has received relatively little attention.

B. TERMINOLOGY

The participants in the workshop agreed that varying use of imprecisely defined terms has led to confusion among workers and in the literature as to exactly what features of hard tissues are being counted or calibrated. This varying usage and resulting confusion may be at the root of much of the seeming disagreement among workers about layer deposition rates among the odontocete species, e.g. the recurring 'one layer per year' vs. 'two layers per year' controversy of long standing (see Kimura, present volume). Prior to 1968, a controversy existed concerning deposition rate of layers in the teeth of the sperm whale, *Physeter macrocephalus*. In that year the International Whaling Commission (IWC) sponsored a special meeting of sperm whale experts from several nations, the goal being to resolve seeming differences in tooth-reading methods and to settle on a mutually-agreed and well defined terminology (IWC, 1969). The present group considered the sperm whale terminology emanating from that meeting, with an eye to adopting it for use in work on all odontocetes. The 1968 sperm whale terms and definitions follow:

> *Lamina:* a translucent or opaque zone in the dentine. The *translucent zone* appears clear or light in transmitted light, dark in reflected light, and forms the ridge in acid-etched teeth. The *opaque zone* appears dark in transmitted light, light in reflected light, and forms the groove in acid-etched teeth. A *growth layer* (layer) consists of two adjacent laminae, one translucent, the other opaque.

The group agreed that it became apparent during the laboratory sessions of the workshop that the sperm whale terminology is not adequate for general use in studies of odontocetes, for two reasons: (1) the definitions, while ostensibly precise, do not fully reflect actual accepted practices in reading teeth of sperm whales, and (2) the variety of dentinal structure among odontocete species does not permit uniform use of a single set of definitions.

The first problem, that of a discrepancy between tooth-reading practice and the definitions of the terms, has to do with designation of particular dentinal features as 'accessory layers', 'accessory laminae', or 'double laminae'. As pointed out by Gambell (1977), the usual practice is not to include 'irregularly appearing and fine translucent lines' in the count of growth layers. Furthermore, it developed during the laboratory session on the sperm whale (see below) that some features meeting the 1968 definition of 'growth layer' (consisting of a translucent zone and an opaque zone) were usually excluded from the count, not necessarily because of thickness or degree of definition but because of position relative to other, largely similar features. As Gambell (1977) pointed out, some teeth contain more 'double laminae' (two sets of zones counted as one growth layer) than others. The decision as to which features are included in the count as 'growth layers' is made on the basis of an inspection of the overall pattern of layer spacing in the tooth, rather than strictly on the basis of internal structure of the features. As Gambell (1977) phrased it, 'A certain subjective skill is then involved in the layer counts.' What is being sought, then, and used as a guide to counting is a more or less regular pattern of recurring groupings of 'laminae'. The group agreed that the 1968 sperm whale terminology does not adequately take into account this, as it were, spectral analysis of the assemblage of layers in the whole tooth.

The second problem, that of differences among species in the internal histology of countable features, must be addressed by use of generalized terms that are not histologically specific.

Considering the above-discussed factors, the group promulgates the following terminology.

Incremental growth layers are distinct layers parallel with the formative surface of a hard tissue (dentine, bone, cement and their subtypes) which contrast with adjacent layers. The contrasting nature may be:

(a) translucency and opacity of a section examined by transmitted light,
(b) dark and light (correspondingly less light-scattering and more light-scattering) layers seen on a surface with incident illumination (reflected light),
(c) high or low relief of the surface following the application of an etching technique, the surface being examined by incident light (microscopy) or scanning electron microscopy,
(d) more or less intense staining of a decalcified cut surface, where haematoxylin, a basic stain, is the most commonly used stain.

Incremental growth layers have also in the past been called 'laminae', 'bands', 'zones', and 'lines' (given enough magnification, a 'line' is resolvable into a layer).

Growth layer groups (GLGs) are groups of incremental growth layers which may be recognized by virtue of a cyclic repetition, generally at constant or regularly changing relative spacing in any of the above-discussed characters delineating the layers. Such a cyclic repetition of incremental growth layers must involve at least one change, i.e. between translucent and opaque, dark and light, ridge and groove, more stained and less stained, but may involve more than one change.

The term *growth layer group* thus is a generic term, to be defined in each instance of use. Use of the term in a particular study of a particular species should if possible include photographs or other illustrations of the structure and spacing of *growth layer groups* as defined in that study.

In species where growth has been studied by adequate means and using an adequate sample, it may be possible to define *growth layer groups* or series of groups which correspond to the amount of the hard tissue formed during one year.

The group agreed to adhere to the promulgated terminology in the body of the present report. Additional terms, of a more standard nature, are defined in the Glossary.

Usage of common and scientific names follows the list promulgated by the International Whaling Commission Subcommittee on Small Cetaceans and subsequently adopted by the Commission (IWC, 1977). A recent change in usage of scientific names was from *Physeter catodon* to *Physeter macrocephalus* for the sperm whale (IWC, 1979).

C. SESSION REPORTS

1. BEAKED WHALES

a. Participants

J.G. Mead *(Chairperson)*, L.J. Stuart *(Rapporteur)*,
A.A. Hohn, T. Kasuya, H.D. Marsh, E.D. Mitchell,
N. Miyazaki, A.C. Myrick, Jr and M.C. Sheldrick.

b. Introduction

The cetacean family Ziphiidae, the beaked whales, consists of a group of medium-sized whales about which very little is known. There are five genera, summarized as follows. The genus *Hyperoodon* contains two species, the northern bottlenose whale, *H. ampullatus,* restricted to the North Atlantic and fairly well known from studies based upon the Norwegian fishery, and the southern bottlenose whale, and *H. planifrons*, a virtually unknown species of the southern oceans. *Berardius* likewise consists of a pair of northern and southern species, of which the Baird's beaked whale, *B. bairdii,* of the North Pacific has been the object of a Japanese fishery and is fairly well known, while its very similar counterpart, Arnoux's beaked whale, *B. arnuxii,* is virtually unstudied. *Ziphius* is a monotypic genus containing only the cosmopolitan Cuvier's beaked whale, *Z. cavirostris,* for which there is a small body of knowledge based largely on stranded individuals. *Tasmacetus* likewise contains a single

NOTE. Dugong teeth were also examined in this session. Preparation techniques are described in Appendix 1 and structure of dugong teeth is discussed by Marsh (this volume), and Domning and Myrick discuss layering in periosteal bone in manatees (this volume).

species, Shepherd's beaked whale, *T. shepherdi*, of the southern oceans, of which about ten individuals are known from strandings alone. Lastly, the genus *Mesoplodon* contains about a dozen species, for which a very small amount of information has been derived, from stranded individuals.

Erupted teeth are reduced to a single pair in the mandible of all genera except *Berardius*, which has two pairs, and *Tasmacetus*, which has full mandibular and maxillary dentitions. Unerupted (vestigial) teeth are variably present in many species. Teeth are strongly sexually dimorphic in all species, being larger in males and generally not even erupting in females (except for *Berardius* and *Tasmacetus*). In males of most species the teeth are relatively much larger than in other odontocetes and are generally strongly laterally compressed.

c. Materials available and organization of the session

The best series of material available was that which Christensen brought from his (1973) studies of the Norwegian fishery for *Hyperoodon ampullatus*. This consisted of a series of 125 ground thin sections, with length, sex, and reproductive data available for all specimens. Accordingly, the session participants decided to concentrate initially on this species as a baseline for interpreting the lesser known beaked whales. Two series of slides were selected, an age-graded series of males and females and a random sample of both, for a total of 23 specimens. These slides were read by all members of the group, and it was readily apparent that the group agreed on what constitutes a growth layer group and that our reading techniques were compatible.

The next best series consisted of thirteen teeth of *Ziphius cavirostris* from stranded animals. Most of these were intact teeth, although Mitchell and Mead had brought a few slides, so preparation of acid-etched halves of teeth was begun, more or less concurrently with the reading of the *Hyperoodon* series. A set of acid-etched and pencil-rubbed sections and a set of ground thin sections were produced and ultimately read by the group. Some experimentation with stained decalcified sections was carried on by Marsh and Kasuya and continued by Mead after the session adjourned.

Kasuya produced a set of representative stained and ground sections of *Berardius bairdii* which, along with the data in his paper (Kasuya, 1977), formed the basis for discussion of this species.

There was an assortment of material available for other species, which was read and discussed on an opportunity basis. Preparation of a variety of materials for *Mesoplodon* species was pursued by Mead and Boyde on a continuing basis, including teeth from a specimen collected during the workshop. Some of this prepared material was examined by other members of the group, but without the same level of consistency as was applied to the *Hyperoodon* and *Ziphius* series.

d. Methods used in the workshop

Unstained ground sections

Growth layer groups were usually counted using a dissecting microscope with transmitted or obliquely reflected light. Cemental layer groups were also counted using a compound microscope, at a magnification of 60X–150X with transmitted light. In some cases it was found useful to have the condenser off-center to yield oblique illumination.

Stained, decalcified ground sections

The preparation method used was a modification of that used by Kasuya (see 2.c.i. in Catalog of Techniques below). Sections were prepared in this manner for teeth of *Berardius bairdii, Ziphius cavirostris*, and Gervais' beaked whale, *Mesoplodon europaeus*. Teeth were prepared dry after cleaning by boiling. Using a 15-cm diamond saw, each tooth was bisected longitudinally in the lingual-buccal plane and a 2-mm thick section taken from the cut surface. In the case of large teeth such as those from *B. bairdii* and an old male of *Z. cavirostris*, sections were cut in half for ease of further processing. One side of the section was then polished in water with whetstones (1200 and 4000 grit). The dried, polished surface was mounted on a 1-mm *Perspex* slide, using quick-setting cyano-acrylate monomer glue. The mounted section was then ground down, using the whetstones, to a final thickness of 40 μm to 70 μm (70 μm in the case of larger teeth).

Alternatively, the cut surface of the half or quarter tooth was polished and glued to the slide before the thin section was cut. In this case a ½-mm section was cut directly and ground down as above. In another approach, a tandem-bladed diamond saw (see Catalog of Techniques) was used to cut a lingual-buccal medial section ½ mm to 1 mm in thickness directly from the whole tooth and the section was mounted and ground as before. Sections thinner than ½ mm from large teeth tended to buckle. Decalcification and staining were as described below in 2.c.i. of Catalog of Techniques. Observations of the growth layer groups were made with a microscope using transmitted light at powers from 10X to 100X.

Etched bisected teeth

Teeth from *Berardius bairdii; Ziphius cavirostris;* Stejneger's beaked whale, *Mesoplodon stejnegeri*, and *M. europaeus* were treated in this manner. Each tooth was bisected longitudinally in the lingual-buccal plane, the cut surface polished as above, and etched, washed and pencil-rubbed as described in 2.b of Catalog of Techniques below.

e. Findings and recommendations

i. Northern bottlenose whale, *Hyperoodon ampullatus*

Teeth of this species are simple elongate cones, ranging from 30 mm to 50 mm in length. The teeth of males are slightly larger than those of females, and in this species, as in most ziphiids, tooth eruption occurs only in males. No etched or stained material was available.

The *enamel* is lacking or very thin and not seen in most sections. When present, the enamel is commonly partly covered by cement. The *prenatal dentine* is consistently of a more uniform texture and less opaque than postnatal dentine. As noted by Christensen (1973), the prenatal dentine generally has a light yellow or brown color, depending on the light source. The *neonatal line* is generally well defined, consisting of a thin translucent layer, variably followed by a thick opaque layer. It was most clearly seen near the apex of the tooth. A *dentinal growth layer group* generally consists of a wide layer of intermediate density, followed by a thin translucent layer, a thin opaque layer and another thin translucent layer. In most specimens, however, only one of the translucent layers is readily visible, so that the commonest sequence seen is a wide layer of intermediate density followed by a thin translucent layer and a thin opaque layer. Growth layer groups ranged in thickness from about 0.4 mm to 0.6 mm in early groups to about 0.3 mm in the last few groups deposited. Accessory layers are present in the dentine of this species and, according to Myrick, averaged about 13

Table 1

Counts by several observers of growth layer groups in the teeth of 23 specimens of *Hyperoodon ampullatus* and one specimen of *H. planifrons*[1]
Observers indicated by initials

Specimen number	Sex	Bodu Ln.(cm)	GLGs in dentine											GLGs in cement									
			NM	EDM	TK	MCS	JGM	ACM	AAH	IC*	LJS	Range	Ave.	NM	EDM	TK	MCS	JGM	ACM	AAH	LJS	Range	Ave.
H. ampullatus																							
L71/110	F	534	3	1.8	2	1.7	2.2	–	–	2	2	1.7–3	2.0	2	2	2	2	2	–	–	2	–	2.0
L71/7	F	605	4	2.9	2.5	2	2.7	4	3.7	3	–	2–4	3.1	4	3	3	2	2.8	4	3	–	2–4	3.1
L71/120	F	598	3	3.9	2.3	3	2.2	–	–	4	4	2–4	3.2	3	3	6	3	2.5	–	–	–	2.5–6	3.5
L71/8	F	650	4	3.5	2.8	4	4	5	4.8	4	–	2.8–5	4.0	4	3.5	3	3	3.5	5	4.8	–	3–5	3.8
L71/123	F	680	7	8	7.9	9	(6)	–	–	7	–	7–9	7.5	7	5+	9	9	(6)	–	–	8	6–9	7.8
L71/9	F	740	10	8	9	8	8.5	13+	10+	9	–	8–13+	8.8	9	7	9	–	(9)	8	9	–	7–9	8.5
L71/16	F	735	9	11.5	12	13	9.5	–	–	12	11	9–13	11.1	8	9	13	7	5+	–	–	7	7–13	8.8–
L71/1	F	760	13	13	12.5	13	12.6	13	13.5	14	–	12.5–14	13.1	12	–	–	–	4+	5	–	–	5–12	8.5–
L71/19	F	717	15	16	16	15	14.8	–	–	15	16	14.8–16	15.4	11+	7	–	7	6+	–	–	9	7–11+	7.7+
L71/118	M	564	3	2+	–	–	1.3	–	–	2	3	1.3–3	2.3	2	2	–	–	1.5	–	–	3	1.5–3	0.9
L71/113	M	573	3	1.5	3.2	–	1.9	–	–	2	2	1.5–3.2	2.2	–	1	6	–	–	–	–	2	1–6	3.0
L71/3	M	685	6	5.5	5.2	5	5.2	4	6	6	–	4–6	5.4	6	6	5	5	5.5	3	3	–	3–6	4.8
L71/13	M	820	8	8	7.8	–	7.6	–	–	8	8	7.6–8	7.8	7	7	8	7	6.5	–	–	8	6.5–8	7.3
L71/121	M	766	10	10	–	–	9.9	–	–	10	10	9.9–10	10.0	9	9	–	–	–	–	–	12	9–12	10.0
L71/10	M	800	12	12	12	12	12	12	12.5	12	–	12–12.5	12.1	12	6+	–	–	10	11	12	–	10–12	11.3–
L71/4	M	810	12	12	12	12	11.5	12	12	12	–	11.5–12	11.9	12	7+	11	–	11.5	12	12	–	11–12	11.7–
L71/11	M	842	13	13	13.5	12	12.9	–	12.9	12	–	12–13.5	12.8	12	10+	13	–	12.5	–	12	–	12–13	12.4–
L71/5	M	775	13+	13	13	10	13	10	11.9	15	–	10–15	12.4	12+	–	–	8	9	6	8+	–	6–12+	7.7+
L71/21	M	810	17	16	16	–	15.8	–	–	16	17	15.8–17	16.2	15	11	15	10	(10)	–	–	14	10–15	12.5
L71/17	M	864	17	17	16	–	14.8	–	–	17	17	14.8–17	16.7	16	17+	14	–	(14)	–	–	20	14–20	16.0+
L71/6	M	822	19	–	19	18	18.9	17	19	19	–	17–19	18.6	19	–	18	14	(18)	23	13+	–	14–23	18.4
L71/128	M	812	18	17	18	–	16+	–	–	22	17	17–22	18.4–	16+	9+	15	–	14	–	–	12	12–16+	13.7+
L71/127	M	887	–	18	24	–	(19)	–	–	23	18+	18–24	21.0	15	15+	–	–	(16)	–	–	18	15–18	16.3
H. planifrons																							
BMNH·1949·7·15·1	F	–	20.5	–	19	10	(22)	–	–	–	–	10–22	17.9										

NOTES FOR TABLES 1 – 27. The following notation is used in counts of growth layer groups (examples in brackets).

Whole number (no decimal) = count, or range, to nearest whole Growth Layer Group [25 or 20-25].

One decimal place = to nearest 1/10 or, in some cases, to nearest 1/4 GLG [24.2].

Whole number and plus sign = partial count of GLGs (not partial GLG; not included in averages; except that when a value with a plus exists which is higher than the average, a plus sign is added to the average) [25+].

Whole number(s) in parentheses = estimate of number (or of range) of GLGs (in bad section or tooth of poor readability) (midpoint of range included in averages) [(25) or (20-25)].

Whole number, plus sign and number in parentheses = partial count of GLGs and estimate of remaining, non-countable GLGs [25+(5)].

Two whole numbers separated by dash = range within which true value thought to lie (midpoint included in averages).

Dash = no data [–].

*Initials with asterisk are of participants with prior experience in reading teeth of the species considered in the table.

per growth layer group, corresponding well to the hypothesis that at least some of the accessory layers represent increments of growth during lunar months. Irregular dentine is commonly formed in the base of the tooth, but groups can generally be read through it. The inclination of incremental growth layers from the long axis of the tooth increases with time, resulting in the later layers in very old teeth being roughly perpendicular to the long axis of the tooth. The pulp cavity becomes filled in old animals, but dentine deposition continues. A complete age record can apparently be obtained from the dentine of even very old animals, as no major discrepancies were found between dentinal and cemental counts. In general, the teeth were very easy to read, due to the regularity and definition of the growth layers. This is evident in the generally excellent correspondence of readings by different observers (Table 1). Quite commonly, the best definition was seen in middle to late layers, with problems due to lack of definition or marked accessory layers tending to occur in early layers and to compression of late layers in very old specimens.

The *cement* is thicker than that seen in most delphinids, but not as thick as in other ziphiids. The cement in older males is considerably thicker than that in females of comparable age. In females, in which the teeth do not erupt, cement occurs well up the crown of the tooth, overlapping the enamel. A series of regular growth layer groups can be seen in the cement, consisting of the same general sequence of opaque and translucent layers as seen in the dentine. Cemental growth layer groups are about 0.25 mm thick. They occur in an overlapping sequence, progressing towards the root of the tooth. This appears to be the general situation in ziphiid teeth, particularly in males, where the cement is thicker and this condition is more readily apparent. It may well be related to the progressive eruption of teeth that occurs in males. Thus, it is necessary to examine the entire length of the tooth in order to obtain a complete count. In general, the cement was more variable from specimen to specimen, and counts were not as consistent as those obtained from the dentine (Table 1).

Unstained thin sections are quite adequate for counting of growth layer groups in the dentine in this and the following species, and although material was not available, it is suspected that stained or etched preparations would work as well. Although groups were readable in the cement of some specimens, these would probably be better shown in stained material. Since dentine deposition is continuous, cement readings are not critical.

Although Christensen (1973) has done an excellent job of correlating available biological data to support the hypothesis that one growth layer group per year is laid down, it would still be desirable to obtain known-age or time-marked individuals to confirm this. It would also be very useful in future studies if more young individuals were available, so that an attempt might be made to identify year classes by analysis of length frequency distribution. Some of these data might be obtainable from an analysis of stranded specimens.

ii. Southern bottlenose whale, *Hyperoodon planifrons*

A single tooth of this species was available for examination (Table 1). This tooth was larger than those of *H. ampullatus* (73 mm) and was from an old female. A ground thin section was the only preparation examined.

The *enamel* was apparently lacking in this specimen. The *prenatal dentine* is not qualitatively different from the postnatal dentine. Both tissues exhibit a finely globular texture, different than in *H. ampullatus,* and difficult to assess on the basis of a single specimen.

The *neonatal line* is an ill-defined translucent layer in this specimen under conventional light microscopy. The *postnatal dentine* is similar to that in some specimens of *H. ampullatus.* The sequence of elements in a *growth layer group* consists of a wide layer of intermediate density, followed by a narrow opaque layer and a narrow translucent layer. Growth layer groups are about 0.6 mm thick in the first few groups, decreasing to about 0.5 mm at the pulp cavity. This particular section is relatively thick, hence the opaque layer is more readily discerned and was used as the marker layer for counting. *Accessory layers* are present and, according to Myrick, average about 13 per growth layer group. Irregular dentine is a problem in the basal half of the tooth, where layers can be counted only near the cement-dentine junction. The pulp cavity is open in this specimen, and, as in *H. ampullatus,* probably does not close. As in females of *H. ampullatus, cement* occurs well up the crown of the tooth. Layering in the cement overlaps towards the root, as in *H. ampullatus,* and is best defined at a point along the middle of the tooth, probably below the neonatal root level. A few early growth layer groups were probably not seen owing to a lack of resolution, but the count of 26 cement layer groups is probably more reliable than the dentinal layer group count of about 20 groups. The sequence of elements of layering in the cement is the same as that seen in the dentine, i.e. a wide layer of intermediate density followed by a narrow opaque layer and narrow translucent layers. Growth layer groups are about 0.2 mm thick. As the groups become thinner in later years, the structure degrades into subequal pairs of opaque and translucent layers. The opaque layer is consistently easier to follow as a marker.

Material of this species is very difficult to obtain, and, while extrapolation of hypotheses based on *H. ampullatus* is probably valid, it would be very useful to have additional information from *H. planifrons.* Robert Clarke has considerable unpublished data on the single specimen examined (see Fraser, 1964).

iii. Cuvier's beaked whale, *Ziphius cavirostris*

Teeth of young individuals of this species consist of an elongate cone (much more elongate than in *Hyperoodon*) slightly tapered at the base. This general shape persists in older females, with the cement being relatively thin. In older males, however, the cement is extremely thick, with the tooth assuming a pyriform shape. Eruption is progressive in males, with extensive cement deposition accompanied by extreme tooth wear, which removes most of the dentinal portion of the tooth in old individuals. The teeth do not erupt in females.

Teeth were available from a limited series of museum specimens of stranded animals. All were relatively old animals, and the following conclusions are somewhat restricted by a lack of immature specimens, particularly of males. Both ground thin sections and etched preparations were available for the teeth of five females. Decalcified and stained sections were also available for a few of these same teeth. Preparations of seven male teeth were limited to acid-etched teeth and a single ground thin section.

The *enamel* is totally lacking due to wear in males and is present as a very thin layer partly overlaid by cement in females. In some specimens the *prenatal dentine* is more opaque than the postnatal dentine, while in others it is of a similar density. It is of a more uniform texture than the postnatal dentine, with no apparent accessory layers. The

Table 2

Counts by several observers of growth layer groups in the teeth of 17 specimens of *Ziphius cavirostris*

Specimen number	Sex	Body Ln.(cm)	Prep. method	GLGs in dentine										GLGs in cement									
				LJS	TK	NM	HM	AAH	EDM	JGM	MCS	Range	Ave.	LJS	TK	NM	HM	AAH	EDM	JGM	MCS	Range	Ave.
USNM20971a	F	–	Untreated thin sections	18	29	19	24	(21)	(33)	23+	13	18–33	22.4+	(17)	20	18	17	22	13+	13	(14)	13–22	17.3
USNM20971b				–	30	20	24	22	(31)	–	18	18–30	24.2	–	–	17	17	18	11+	–	(10)	10–18	15.5
USNM20993a	M	–		–	24	16+	–	12±1	(13)	12+	11	11–24	15.0	13	12	16	–	20±1	19+	17±1	10	10–20	14.7+
USNM20993b				–	31	16+	–	(13)	(19)	–	11	11–31	18.5	–	14	16	–	19	20+	–	12	12–20+	15.3+
USNM504347a	(F)	767		25	26	23	23	22	24	(22)	22	22–26	23.4	–	21	18	–	19	–	(20)	(10)	10–21	17.6
USNM504347b				–	26	20+	–	–	–	–	14	14–26	20.0	–	17	16+	–	–	–	–	–	–	–
JGM205	F	(600)		16	29	25	14	21	(15)	13+	15	14–29	19.3	12	25	16	–	12+	8+	12	5+	12–25	16.3-
BMNH1963·11·8·1	–	–		14	14	13	–	15.2	19	14	14	13–39	14.7	13	14	13	–	14	16±1	15	10	10–16	15.8
RNP327	M	–		–	18	13	–	12±3	(17)	13+	14	12–18	14.8	13	13	14	–	9+	15±1	14	12	12–15	13.5-
USNM312888	M	540	Etched half-teeth	19+	26	22+	13+	14	20+	19+	18+	14–22+	20.0+	–	21	–	–	–	17+	–	(7)	–	14.0+
USNM504294	M	579		16	16	17	16	14	17+	17	18	14–18	16.3+	–	–	–	–	13	9+	8	–	8–13	10.5
USNM504347	(F)	767		24	21	25	23	22	25	24	23	21–25	23.4	–	22	–	–	19	15+	20	(13)	13–22	18.5
BMNHSW1932·8	M	817		–	33	14+	7	–	10+	–	–	7–33	20.0	30	26	34	31	37	33	39	32	26–39	32.8
BMNHSW1933·4	M	798		–	29	29	16+	(20)	23+	15	19	15–29	22.4+	15	14	–	17	22	18	16	18	14–22	17.1
M446	(M)	–		–	–	17+	12	–	10+	–	(7)	7–17+	9.5+	62	63	59	63	63	62	63	61	59–63	62.0
RNP3	M	–		–	16+	24	15	16	17+	–	–	15–24	18.3	34	27	39	40	31	36	37	36	27–40	35.0
UZMON1	–	–		–	11+	16+	–	–	–	–	–	–	–	34±2	44+	37	39	28	35±2	42	40	28–44	36.4+
USNM20971	F	–	Decalcified and stained thin sections	–	–	–	–	–	7+	–	–	–	–	–	15	–	–	–	11	9	–	9–15	11.7
USNM20993	M	–		–	17+	–	–	–	11+	–	–	–	–	–	17+	–	–	–	28	14±2	–	14–28	21.0
USNM504294	M	579		–	14+	–	–	–	15	–	–	–	–	–	18	–	–	–	14	13	–	13–18	15.0
USNM504347	(F)	767		–	23	–	–	–	13	14+	–	13–23	18.0	–	21	–	–	–	20	15	–	15–21	18.7
JCM205	F	(600)		–	–	–	–	–	–	–	–	–	–	–	15	–	–	–	12	11	–	11–15	12.7
LACM52438	(F)	–		–	–	–	–	–	7+	–	–	–	–	–	14	–	–	–	13	(14)	–	13–14	13.7
BMNHSW1932·8	M	817		–	20+	–	–	–	20+	–	–	–	20.0+	–	33+	–	–	–	28	39	–	28–39	33.5
RNP3	M	–		–	–	–	–	–	16+	–	–	–	–	–	28	–	–	–	36	34	–	28–36	32.7
UZMON1	–	–		–	13+	–	–	–	15+	–	–	13+–15+	–	–	36	–	–	–	33	33	–	33–36	34.0

neonatal line is present as a thin translucent layer, variably followed by a thin opaque layer. In one specimen it consists of a thin opaque layer bounded on either side by a thin translucent layer. The neonatal line is consistently easy to identify but is commonly worn away in old males. A *growth layer group* in the postnatal dentine consists of a thick layer of intermediate density followed by a variable combination of adjacent thin opaque and translucent layers. Pairs of opaque and translucent layers are common, and there is some indication of a triplet consisting of a thin translucent layer bounded on either side by thin opaque layers. Thickness of growth layer groups ranges from about 0.6 mm to 0.3 mm. *Accessory layers* are very common, particularly in the first two growth layer groups in females and in the later groups in males. It appears that in females the first two growth layer groups are relatively thick, while those from the third layer on are less than half as thick as the first two. From that point on, there is a regular progressive decrease in thickness of the growth layer groups. Towards the base of the tooth, the groups become more irregular and difficult to count. The pulp cavity becomes reduced to a narrow channel filled with irregular dentine in old males, in which cement deposition is extreme. Counts of dentine groups were consistently easier when started from the middle groups, rather than from the oldest, as the cycles of deposition are somewhat easier to define in the mid-regions of the tooth. As is evident from Table 2, dentinal growth layer groups were not always well defined and easy to count. Acid-etched preparations were consistently easier to count than were thin sections (untreated, or decalcified and stained).

As noted above, *cement* deposition differs greatly between the sexes. In females, cement is deposited as thin continuous layers over most of the tooth. Growth layer groups consist of alternating opaque and translucent layers. In several teeth, the first two groups were about twice as thick as subsequent groups, corresponding to the situation seen in the dentine. The layering in the cement in females could be read in all preparations but was best in stained sections and acid-etched preparations, where it gave more consistent counts than did the dentine. In males, the early growth layer groups in the cement are thin and difficult to discern. They become progressively thicker up to about the twentieth group, where they are about 1.5 mm thick, then gradually diminish in thickness. *Accessory layers* are a consistent problem in discerning growth layer groups. The cemental groups in males are well defined in etched preparations. Relatively thick (200–300 μm) decalcified and stained sections also give good results for males. As noted for the sperm whale (session report below) there are areas in the cement of some male *Ziphius* teeth where prominent marker layers occur every five or six growth layer groups. These are of unknown significance.

It appears that etched preparations are the best technique for visualizing growth layer groups. Marsh, for *Dugong dugon,* has had some success utilizing thick sections (about 0.5 mm) which were decalcified and stained on the surface of one side, and it is possible this may work well for male *Ziphius* teeth.

As material of this species is virtually limited to stranded specimens, it is unlikely that any known-age or time-marked material will be available in the foreseeable future. It is therefore most important that full utilization be made of young specimens in an attempt to ascertain periodicity of growth layer groups, both by length-frequency definition of age classes and by examining the nature of the last formed dentinal layer. Due to problems of sample size, it is expected that the latter will be the more productive approach. At present there are no data relating to periodicity of growth layer groups in this species.

iv. Baird's beaked whale, *Berardius bairdii*

In this species there is a pair of laterally compressed teeth at the anterior end of each mandibular ramus, for a total of four teeth. The anterior tooth is the larger of the two, and sexual dimorphism is slight. Kasuya (1977) provided an extensive discussion of age determination in this species.

Decalcified and stained sections from five specimens, ground sections from two of these plus one additional specimen, and two etched preparations were examined.

The *enamel* is thick and frequently covered with cement in posterior unerupted teeth and worn but generally present in erupted anterior teeth.

The *prenatal dentine* is generally of a more uniform texture than the postnatal dentine but, as shown in Kasuya's photos, sometimes shows accessory layers. Kasuya (1977) found a mode of 14 accessory layers in the prenatal dentine. The prenatal dentine is generally more opaque in ground sections and stains darker than the postnatal dentine. The *neonatal line* is a generally well-defined, thin, translucent non-staining layer, variably followed by a thin opaque layer. *Growth layer groups* in the postnatal dentine consist of a thick layer of intermediate density, bounded by a thin translucent layer, variably accompanied by a pair of adjacent thin opaque layers. Numerous accessory layers are present in the postnatal dentine. The teeth are very unusual in that the pulp cavity is very large and is rapidly occluded by an irregular mass of vaso-orthodentine, associated with numerous secondary centers of dentine deposition (pulp stones). Deposition of orthodentine ceases in the denticular portion of the tooth after accretion of a maximum of about six dentinal growth layer groups. There commonly is a residual pulp cavity following closure of the root of the tooth, but this cavity becomes gradually filled with irregular dentine. Thus, dentine is of marginal use for age determination in all but young animals. Dentinal growth layer groups are not well defined and are more or less equally readable in specimens prepared by all three preparation techniques (Table 3).

Deposition of *cement* occurs over much of the tooth surface and is thickest in the basal half of the tooth. Growth layer groups consist of a pair of moderately defined, subequal opaque and translucent layers, in which can be seen numerous fine accessory layers. Kasuya (1977) found a mode of 12 to 14 of these layers per growth layer group. Younger groups are thicker (about 0.5 mm) than older groups but easier to count. In general, cement groups can be read in ground sections, but are better seen in stained sections and still better seen in acid-etched preparations. The cemental growth layer groups are much easier to count than the dentinal groups.

Although Kasuya (1977) presented evidence for one growth layer group per year in this species, based on the nature of the last-formed group and the number of short cycle elements (presumed lunar month layers or layers reflecting an endogenous physiological cycle of about one month) in it, this sample was small and limited to only part of the year. It is recommended that larger samples be studied, with particular emphasis on identification of age class by analysis of length frequency distributions of young animals, nature of the last-formed layer in animals taken in summer months (if possible), and correlation of growth layer group counts with ovarian data. As this species is the subject of a

Table 3

Counts by several observers of growth layer groups in the teeth of six specimens of *Berardius bairdii*, five specimens of *Mesoplodon stejnegeri* and five of *M. europaeus*

Specimen number	Sex	Body Ln.(cm)	Neon.ln. visible?	Dentine still growing?	GLGs in dentine									GLGs in cement								
					NM	LJS	TK[1]	MCS	JGM	AAH	EDM	Range	Ave.	NM	LJS	TK[1]	MCS	JGM	AAH	EDM	Range	Ave.
Berardius bairdii																						
					Untreated thin sections																	
55	F	640	–	–	2	–	2	2	2	1.5	1	1–2	1.8	2	2	2	2	2	1.5	(1)	1–2	1.8
53	M	780	–	–	5	4	3	4	1.5	1.5	1	1–5	2.9	5	4	3	2	(1)	–	(1)	1–5	2.7
49	M	1070	–	–	–	–	5	–	–	–	–	–	–	20	23	25	–	–	–	–	20–23	22.7
60	F	1100	–	–	–	–	–	<1	–	–	–	–	–	33	35	39	–	–	–	–	33–39	35.7
57	M	1010	–	–	–	–	6	–	–	–	–	–	–	–	32+	31+	25+	–	–	–	–	–
41[2]	M	1090	–	–	4	–	–	–	(2)	(1.5)	19+	1.5–19+	2.5	19	–	40+	–	(17)	(30)	(16)	16–40+	20.5+
					Etched half-tooth																	
57	M	1010	–	–	–	–	–	–	5+	5+	11+	–	–	–	–	(28)	–	23±2	29	26	23–29	26.5
					Decalcified and stained thin sections																	
55	F	640	–	–	–	–	2	–	1	0.5	1	0.5–2	1.1	–	–	–	–	1	–	(1)	–	1.0
53	M	780	–	–	–	–	3	–	(1)	5.5	1	1–5.5	2.6	–	–	–	–	1.5	–	(1)	1–1.5	1.3
49	M	1070	–	–	–	–	4	–	–	4.5	(5)	4–5	4.5	–	–	–	–	–	17	11	11–17	14.0
60	F	1100	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	31	(35)	18	18–35	28.0
57	M	1010	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	32±2	–	10+	–	–
Mesoplodon stejnegeri																						
					Untreated thin sections																	
USNM504329a	M	448	Yes	Yes	3	2	–	–	1.8	1.5	1.7	1.5–3	2.0	2	2	–	–	–	1.5	(4)	1.5–4	2.4
USNM504329b					3	2	–	–	–	–	2.0	2–3	2.3	3	2	–	–	–	–	4	2–4	3.0
USNM504330a	F	488	Yes	Yes	3	2.8	–	–	2.6	2.8	2.7	2.6–3	2.8	3	3.1	–	–	2.8	3	10+	2.8–10+	3.0+
USNM504330b					3	2.8	–	–	–	2.8	2.5	2.5–3	2.8	3	3.1	–	–	–	2.5	(3)	2.5–3.1	2.9
USNM504331	–	459	Yes	Yes	3	2	–	–	1.6	1.9	1.9	1.6–3	2.1	3	2.5	–	–	1.6	1.5	5	1.6–5	2.7
					Etched half-teeth																	
USNM252497	M	–	Yes	No	–	–	–	–	4.5	–	4	4–4.5	4.3	–	–	–	–	10.5	–	30+	10.5–30+	–
USNM143132	M	–	Yes	No	–	–	–	–	–	–	4+	–	–	–	–	–	–	18±1	–	10+	–	–
Mesoplodon europaeus																						
					Untreated thin sections																	
UNSM504349	F	420	Yes	No	–	–	–	–	–	7	(8)	7–8	7.5	–	–	–	–	–	–	4+	–	–
USNM504473a	(F)	452	Yes	No	13	10	–	–	–	8±4	9+	8–13	10.3–	12	–	–	–	–	26±4	31+	12–31+	19.0+
USNM504473b					13	12	–	–	–	–	13+	12–13+	12.5+	–	–	–	–	–	–	48+	–	–
USNM504473c					–	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
USNM504610	F	458	Yes	No	11	9	–	–	–	9±2	9	9–11	9.5	9+	–	–	–	(26)	4+	23+	–	–
					Decalcified and stained thin sections																	
USNM504473	–	–	–	–	–	–	–	–	(23)	–	–	–	–	–	29	–	–	(25)	(28)	(24)	24–29	26.5
USNM504610	–	–	–	–	9	–	–	–	(19)	–	–	9–19	14.0	–	(8)	–	–	(34)	(46)	(20)	8–46	27.0

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[1] Readings of *Berardius* teeth made in 1977.

[2] The results for this specimen probably reflect a mix-up in labeling.

fishery, it may also be possible to obtain time-marked animals.

v. Other beaked whales, *Mesoplodon* spp.

Teeth of whales of this genus are extremely variable in shape from species to species and extremely sexually dimorphic, even more so than in *Ziphius*. The simplest and smallest teeth are found in True's beaked whale, *M. mirus*, where they are flattened cones 3 cm or 4 cm long. *M. europaeus;* Sowerby's beaked whale, *M. bidens; M. stejnegeri;* and the strap-toothed whale, *M. layardi*, form a series in order of increasing length and lateral compression of the teeth. Boyde (1968) described the histology of the tooth of *M. layardi*.

The largest tooth sectioned in the workshop was from a male *M. stejnegeri* and had a greatest dorsoventral height of 15 cm, an anteroposterior length of 7.5 cm and a lateral thickness of 1.5 cm. Ground thin sections were available for teeth of four specimens of *M. stejnegeri*, three of *M. europaeus*, one of *M. mirus*, and seven of *M. bidens*. Decalcified and stained sections were available for two specimens of *M. europaeus*, and etched preparations were available for five of *M. stejnegeri* and three of *M. europaeus*. A single fresh tooth of *Mesoplodon* spp. was collected during the workshop. Boyde and Mead produced a variety of preparations from this specimen.

The *enamel* is thin but commonly present even in erupted teeth of old males. The *prenatal dentine* is generally more uniform in texture than the postnatal dentine and normally of the same density, although the prenatal dentine was decidedly more opaque in one specimen of *M. europaeus*. The *neonatal line* is generally a thin translucent layer of variable definition that is readily identifiable. In some specimens of *M. stejnegeri* it is followed by an adjacent thin opaque layer.

Growth layer groups are extremely variable in definition (Table 3) and structure. In the postnatal dentine they were ill-defined in the *M. europaeus* and *M. mirus* specimens at hand. In *M. stejnegeri*, the growth layer groups are composed of a thick layer of intermediate density bounded by a variable thin opaque or translucent layer. In *M. bidens*, the group consists of a thick layer of intermediate density followed by a single or two closely-spaced thin translucent layers. In some instances, where the double translucent layers are relatively far apart, it is difficult to recognize that they constitute a pair and not separate cycles. In all species examined, the pulp cavity becomes relatively occluded at an early age, resulting in the subsequent deposition of irregular dentine or vasodentine in which no layers can be discerned. This age varied from a single discernible growth layer group in a male *M. bidens* to about six in a female *M. europaeus*. In general, occlusion seems to occur sooner in males. As the tooth elongates by cement deposition in males, deposition of vasodentine continues in a somewhat modified and elongate pulp cavity, in which no layers are discernible. Dentine is unsatisfactory for age determination in all but very young individuals.

Deposition of *cement* in *Mesoplodon* teeth differs greatly between the sexes and appears to be entirely responsible for the great difference in size and shape of male and female teeth. As in *Hyperoodon*, cement deposition occurs in a series of overlapping growth layer groups which are much thicker near the base of the tooth. In females, the cement groups remain relatively thin, while in males they became increasingly thick in basal portions of the tooth, where much of its bulk is composed of vasocementum. In both sexes, the group consists of a thick intermediate layer bounded by a thin, variably defined translucent layer. In females, the cemental layer groups are very difficult to count in ground sections or etched preparations, and are moderately well defined in decalcified and stained sections. In males, particularly older individuals, initial growth layer groups are best visualized in ground sections, while later groups are best seen in etched preparations. In general, both are necessary to obtain a complete count, although more sophisticated etching procedures coupled with surface coating may reveal all groups.

Counts of cemental growth layer groups are necessary for older animals and are generally better than dentinal counts, even in young specimens. For females, stained sections gave the best counts, while etched preparations seem more promising for males.

As material for these species is generally obtainable only from stranded animals, samples are small, and it is difficult to assemble much data for any one species. It is recommended that a more consistent effort be made to obtain growth layer group counts and other biological information (such as reproductive data) from the few specimens which do become available. In the past these have largely been treated as curiosities, and very little of this valuable information has been preserved.

f. Summary

It was generally concluded that teeth of all the species examined could be read for growth layer groups, though it was abundantly clear that some were much more difficult than others. For all species except *Hyperoodon ampullatus* and *Berardius bairdii*, there is an extreme lack of biological data with which to correlate the results of counts of growth layer groups.

Based upon the limited results, it does appear that some generalizations can be made. Growth layer groups in both the dentine and cement generally consist of a thick layer of intermediate optical opacity, followed by a variable combination of thin opaque and translucent layers. Apparently as a result of extreme sexual dimorphism (except in *Hyperoodon* and *Berardius*) overlapping cemental layering is laid down and forms the only complete growth record. In general, etched preparations give the most satisfactory results for these species. Based upon extensive experience by dental histologists, it is apparent that much better preparations can be made from teeth which are handled and preserved wet than can be produced from the traditional boiled and dried material upon which most workers have relied in the past.

2. SPERM WHALE, *PHYSETER MACROCEPHALUS*

a. Participants

S. Ohsumi *(Chairperson)*, C. Lockyer *(Rapporteur)*
A. Boyde, R. Clarke, R. Gambell, M.E. Gosho,
M. Kimura, G.A. Klevezal', M. Nishiwaki, O. Paliza,
W.F. Perrin and J. Prime.

b. Introduction

Gambell (1977) summarized and reviewed work on age determination of the commercially important sperm whale, *Physeter macrocephalus*.

c. Materials available

Material provided to the workshop by members included 300 maxillary teeth from the southern hemisphere; two sets

Table 4

Counts by several observers of dentinal growth layer groups in etched teeth, and in a photograph of a thin section of a tooth, of sperm whales, *Physeter macrocephalus*, from the southeast Pacific

M = mature, I = immature, P = pubertal or pregnant, L = lactating, O = ovulating and R = resting, with number of ovarian corpora following maturity symbol

				GLGs in dentine												
Whale No.	Sex	Body Ln.(m)	Maturity	GAK*	OP*	RG*	MN*	JP	CL*	RC	MK	MEG*	SO*	WFP	Range	Ave.
PA18	M	13.2	M	14	16	–	16	–	–	–	–	18	17	–	14–18	16.2
PA25	M	12.6	M	20–22	(22)	25	21	27	–	–	–	24	24	23	20–27	23.4
PA91	M	15.6	M	37	(42)	–	–	43	45	41	–	38	43	–	37–45	41.3
PA374	M	8.8	I	–	–	–	–	–	–	–	–	6–9	5	–	5–9	6.3
PA424	M	11.2	P	11	12	12	14	13	–	–	10	–	14	–	10–14	12.3
PA440	M	16.6	M	–	(25)	–	–	41	(34)	27	–	55	–	–	27–55	36.4
PA602	M	10.3	P	11	13	13	12	12–13	12	–	11–12	11	11	10	10–13	11.7
PA615	M	12.5	–	–	22	22	29	25	–	–	–	18	–	–	18–29	23.2
PA628	M	12.5	P	16	17	18	20	21	17	–	–	–	–	18	16–20	18.1
PA956	M	16.1	M	19	24	25	25	23	24–25	–	–	26	23	24	19–26	23.7
Pi9	M	14.0	–	17	21	21	23	19–20	(20)	21	21	20	16	20	17–23	20.0
Pi259	M	13.2	–	–	21	25	(24)	–	25	27	–	–	26	21	21–27	24.1
Pi370	M	13.4	–	19	21	20	23	–	22	21	20	21	19	16	19–23	20.2
Pi399	M	13.4	–	18	19	22	20	22–23	–	18	–	21	18	18	18–23	19.6
T20	M	11.6	M	12	15	15	14	17	14	–	–	14	13	15	12–17	14.3
T25	M	9.7	P	11	13	13	15	11–12	13	12	11	11	11	–	11–13	12.2
T30	M	9.6	P	–	12	11	9	9	10	12	–	9	9	–	9–12	10.1
T36	M	12.2	M	–	–	20	20	18–19	20	–	–	17	–	–	17–20	19.1
PA168	F	9.7	P8	18	22	23	25	25	24	–	–	25	27	–	18–27	23.6
PA170	F	9.1	L4	13	16	16	15	–	15	16	13	–	16	–	13–16	15.0
PA462	F	9.8	L5	–	–	17	20	<18	17	–	–	17	17	16	16–20	17.3
PA510	F	10.3	P6	–	16	17	–	16	15	–	–	17	16	–	15–17	16.2
PA770	F	9.8	P7	19–20	–	25	21	20	20	–	–	23	–	–	19–25	21.1
Pi18	F	9.1	O3	9	–	12	11	11	10	–	–	12	12	–	9–12	11.0
Pi62	F	9.9	P7	19	–	23	(24)	22–23	23	–	–	20	20	–	19–23	21.6
Pi95	F	9.0	P3	11	–	–	13	12	–	11	–	11	12	–	11–13	11.7
Pi233	F	8.9	R1	(8–9)	–	9	10	9	–	9	–	–	9	–	8–10	9.1
Pi241	F	10.0	L/P8	15	17	17	–	15	17	–	–	17	–	–	15–17	16.2
I126	F	9.9	R3	–	49	–	51	45–46	45	–	–	43	–	–	43–51	46.7
I136	F	9.0	L?	13	13	14	–	14	14	12	–	15	–	–	12–15	13.6
I159	F	9.5	P3	13	16	–	–	(17)	18	16	–	14	–	–	16–18	15.7
I208	F	10.3	L9	–	(36)	–	54	38–39	38	–	–	36	–	–	36–54	40.5
T1	F	9.6	L4	–	21	–	23	24	23	25	–	–	21	–	21–25	22.8
T2	F	9.9	L5	13	18	16	–	16–18	16	16	–	14	14	–	13–18	15.5
T15	F	10.0	P7	17	22	23	19	19–20	–	20	17	20	17	–	17–23	19.4
T18	F	9.1	L3	12	17	17	18	16	–	–	–	13	11	–	11–18	14.9
T27	F	10.4	L6	–	28	20	22	24	20	–	–	18	–	–	18–28	22.0
Reader variability:																
Sample size				25	29	28	29	31	27	16	7	31	26	10		
Mean difference from average score				−2.12	−0.03	+0.80	+1.62	+0.71	+0.08	−0.17	−1.04	−0.26	−0.47	−1.20		
Standard deviation				1.16	2.97	1.25	2.88	1.54	1.43	2.76	1.29	2.62	1.73	1.52		
Counts from thin-section photograph				20.5	21	–	20.5	20	20	21	21	20	20.5	–	20–21	20.5

of mandibular and maxillary teeth from the north Pacific, all uncut; mandibular teeth from approximately 25 males and 25 females from the southeast Pacific which had not been boiled and which were already cut and acid-etched (Clarke *et al*, 1968); 24 mandibular teeth from Australian waters, of which 17 were already cut. There were three mounted tooth sections for microscopic examination. A photograph was provided for one of these sections. From the north Atlantic, three teeth were available from a 14.5 m male, two uncut and one cut and etched. Data concerning sex, length, date of capture and position of tooth in jaw series were available for these materials, together with some additional biological data. Because only teeth were available for examination, the group did not consider other hard tissues, e.g. bone, eye lens, although these have been used in the past.

d. Organization of the session

The work of the group was guided by the charges to the participants (see Introduction), and these were used as an agenda. Techniques of specimen preparation were compared and evaluated. The various techniques are detailed in Section E. Catalog of Techniques. Methods of reading were demonstrated and evaluated, cross-reading experiments were carried out, and an attempt was made to standardize scoring techniques.

e. Review of preparation techniques

The members communicated their usual procedures for collecting and preparing teeth. Nishiwaki and Lockyer collect the first mandibular tooth in accordance with the recommendations of the IWC Age Determination Meeting, Oslo, 1968 (IWC, 1969). Nishiwaki, in addition, takes routine collections of maxillary teeth from the mid/posterior jaw, selecting the straightest unerupted one. Klevezal' reports that Soviet scientists collect the first mandibular tooth. Clarke and his associates in 1958–62 collected the tenth mandibular tooth or the nearest undamaged tooth from all whales; they selected the tenth tooth because the first few teeth in the row are much smaller than the rest.

The group concluded that the first mandibular tooth is preferable for age determination, as it tends to be relatively unworn. However, in whales which have obviously worn mandibular teeth, it is preferable also to collect a straight, unerupted maxillary tooth.

In connection with the discussion of past treatment of sperm whale teeth, Boyde commented that the boiling of teeth denatures and extracts (gelatinises) collagen from the dentine and cement, and it is known that this process can damage the cemental layers and the pulp edge. Cleaning in an enzymatic preparation (e.g. of trypsin) gives better results.

The group decided that acid etching, according to the method described in Section E. Catalog of Techniques (10% formic acid for 30 hours at room temperature), is considerably better than other methods of preparation for age determination. For standardization of methods, it is preferable that all workers use one method for age determination. It is also better in routine age determination to examine the actual tooth rather than photographs or acetate peels.

For demonstrating techniques the group selected the first mandibular tooth and fourth and tenth maxillary teeth from one whale and the first mandibular tooth and ninth maxillary tooth from another. The demonstrations included cutting and etching and were followed by cross-reading experiments. In addition, first mandibular teeth from whales taken in Australian waters were selected for etching from seven specimens of known sex and length, and four other specimens from stranded whales.

All teeth cut during the workshop were mounted using adhesive dental wax on blocks, as the special vices (see Catalog of Techniques) usually used were not available.

f. Cross-reading experiments

Cut and etched teeth supplied from the southeast Pacific were read and scored by all members of the group (Table 4). This sample consisted of 37 teeth of both sexes.

The ranges of scores among the observers were generally small. The greatest ranges were observed for teeth having high scores, specifically Nos. PA 440 with a range of 27 to 55 and Pi 208 with a range of 36 to 54. Otherwise there was good agreement between the 11 different scorers.

Also shown in Table 4 are the mean differences from the average scores for individual scorers, together with the standard deviation of their scores. It is seen that, relative to the average scores, some scorers tend to overestimate and others to underestimate. Of 11 scorers, seven scored < ± 1.0 difference from the average count, and of the remaining scorers the greatest differences were +1.62 and –2.12. This result suggests that there is good agreement in the method of counting dentinal growth layer groups.

Also shown in Table 4 is the result of examining a thin mandibular tooth section. This was read after discussion on standardization of counting growth layer groups. Agreement among scorers was excellent.

Of the teeth scored in Table 4, 14 teeth which showed most variation in results were selected for re-examination by seven observers. The results are shown in Table 5. The original and revised average scores are given for each tooth, and the ranges and averages for the original and revised counts. The ranges for the revised counts were smaller in 12 cases, larger in one case, and the same in one case, i.e. the precision of the counts increased. Increased precision is also reflected in the absolute value of observer's mean-difference-from-average score; this measure went down for five observers

Table 5

Revised counts for selected teeth from Table 4

Whale No.	Revised counts of GLGs							Range		Average	
	GAK*	OP*	MN*	JP	CL*	RC	SO*	Revised	Original	Revised	Original
PA25	20	22	–	23	–	–	24.5	20–23	20–27	22.4	23.5
PA91	40–42	45	–	41	45	40	42.5	40–45	37–45	42.4	42.5
PA440	–	25	–	34	35	–	–	25–35	25–41	31.3	33.3
PA615	–	21	23	22	–	–	–	21–23	22–29	22.0	25.3
PA956	21	25	24.5	24	25	–	22.5	21–25	19–25	23.7	23.1
PA168	20	23	24	25	23	–	18+	20–25	18–27	23.0	22.8
PA770	18	–	22	21	21	–	–	18–22	19–25	20.5	20.1
Pi62	19	–	22.5	23	22	–	23.5	19–23.5	19–24	22.0	21.7
I159	11	15	–	17	13	12	–	11–17	13–18	13.6	16.0
I208	–	38	44	42	38	–	–	38–44	36–54	40.5	41.6
T2	12	17	–	16	16	13	12.5	12–17	13–18	14.4	15.7
T15	18	18	22	19	–	17	16	14–22	16–22	17.7	18.8
T18	13	17	15.5	14	–	–	14.5	13–17	11–18	14.8	14.8
T27	–	22	25	22	21	–	–	21–25	20–28	22.5	23.0
Reader variability								*Average absolute mean difference from average score*			
Sample size	10	12	9	14	10	4	7				
Mean difference from average score											
Revised	–2.10	–0.03	+1.53	+1.01	+0.51	–1.53	–0.20		Revised	0.99	
Original	–2.86	–0.92	+2.82	+1.26	+0.37	+0.68	–1.16		Original	1.44	

Table 6

Comparison of counts of growth layer groups in maxillary and mandibular teeth from the same sperm whale

Whale No.	Sex	Body ln.(m)	Tooth position	GLGs in dentine								
				MN*	JP	CL*	RC	MK	MEG*	SO*	Range	Ave.
T19	M	14.4	MD1	27	23	28	20	–	36	–	20–36	26.8
			MX9	32	29	28	34	29	33	–	29–34	30.8
13N/348	M	11.1	MD1	24.3	28	29	32	28	31	25	24.3–31	29.9
			MX4	24	31	24	28	21	30	26	21–31	26.3
			MX10	37	48	36	32	–	40	27	27–48	38.0

and up for two and decreased in the average from 1.44 to 0.99. The averages went down in nine cases, up in four, and were the same in one; the revised counts were generally lower than the original counts. This result indicates that initial counts of growth layer groups in difficult-to-read sperm whale teeth may be too high. It also appears that some few teeth, such as PA 440 in Table 5, are exceptionally difficult to interpret and may not be suitable for accurate aging.

The results of readings of the mandibular and maxillary teeth from individual whales are shown in Table 6. There is considerable variation in counts by different readers. It is clear that counts for teeth from a single whale vary not only between different readers but also between different teeth by one reader. These results are not consistent with the previous findings of Nishiwaki *et al* (1958), who found that the mandibular and maxillary dentinal layer counts were similar in individual whales. However, the sample sizes considered here in Table 6 are very small, and it was noted that in the maxillary teeth the pulp cavity was in the process of closing. The group recommends that further comparisons between different teeth from complete sets be made. At present, it would appear that choice of tooth could bias age readings.

The most significant effect of different scorers counting consistently higher or lower than each other is on the preparation of age-structured population models, where certain age-parameter values could be affected.

g. Standardization of scoring techniques

It was decided that the *neonatal line* should in all cases be determined (if possible) after cutting the tooth but before etching. Its position should be noted by measuring the distance from the tip of the tooth (crown). As the neonatal line by definition occurs at birth, when age is zero, this layer is not counted in age determination. In appearance, it is usually a diffuse ridge in the etched tooth and may be darker in color in the unetched tooth. However, it is frequently absent in mandibular teeth of older animals, where the crown is worn. The position of the neonatal line is demonstrated in Figure 1.

Two forms of dentinal *growth layers* were recognized: (a) translucent in transmitted light and forming the ridge in etched teeth; and (b) opaque in transmitted light and forming the groove in etched teeth. The simplest *growth layer group* consists of a pair of adjacent layers, one translucent and one opaque.

The position of the first translucent layer (or ridge, in etched teeth) after the neonatal line should be investigated. The spacing of these layers relative to subsequent spacing may give a clue as to the timing of birth, as the first groove and ridge in etched teeth may represent only part of a full growth layer group in terms of time.

The method of counting growth layer groups is demonstrated in Figure 1. Because of incomplete growth layer group formation on the pulp cavity edge, knowledge of the time of year of birth and time of year of capture are required in order to assess whether or not the whale's age is directly represented by the total number of ridges in etched teeth.

The presence of closely spaced double ridges in etched teeth was discussed. These are identified as a main layer and accessory layer and scored as one growth layer group only if the spacing between these two is immediately adjacent to and considerably less than the spacing between other adjacent

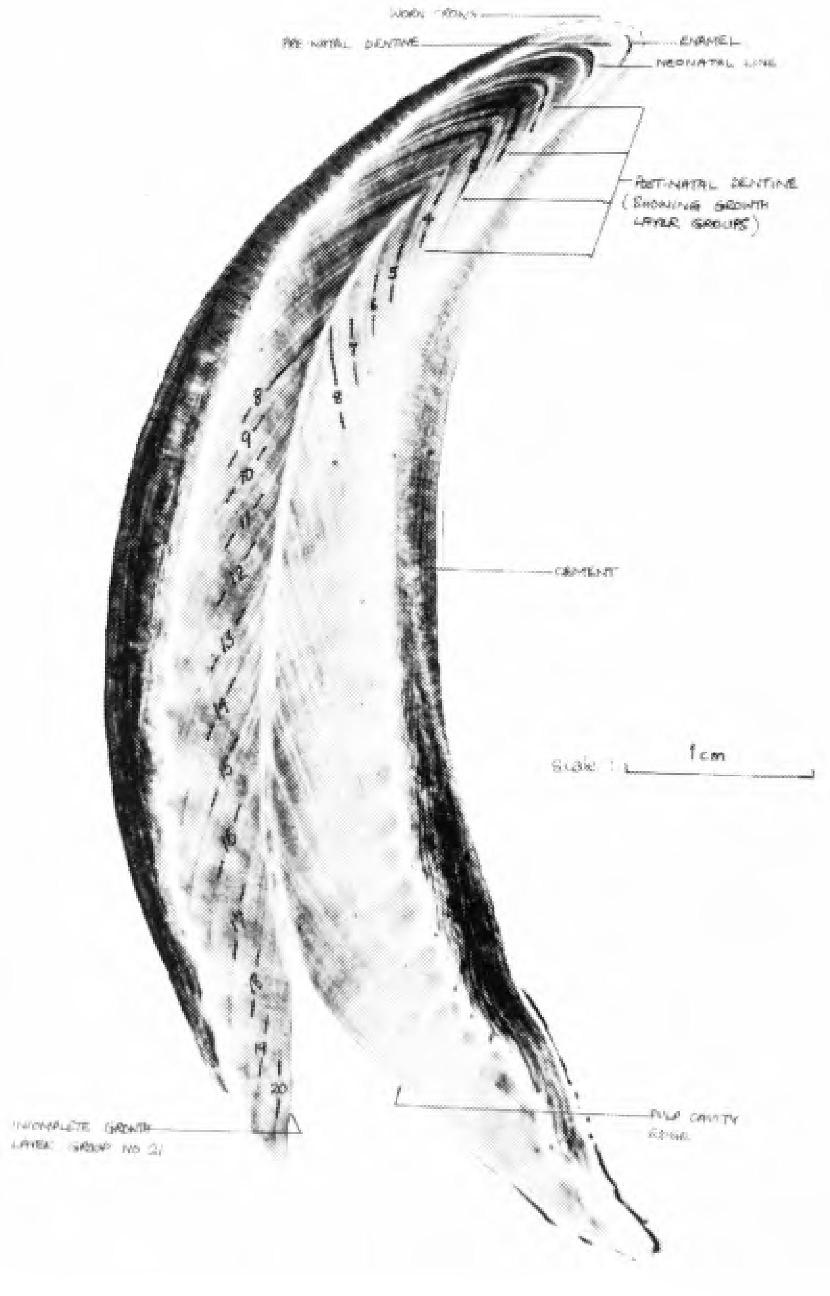

Fig. 1. Photograph of thin section of mandibular tooth of a sperm whale illustrating dentinal growth layer groups (counts at foot of Table 4).

groups. Periodically, more prominent layers are observed in teeth of females, perhaps every two to eight growth layer groups, but these have not been found to show any correlation with gestation or lactation (Klevezal′ and Tormosov, 1971).

Cemental growth layer groups appear to form in a similar pattern to the dentinal groups. However, they are mostly useful when examined in very thin (20 to 50 μm) sections which have been decalcified and stained with haematoxylin. Like the dentinal groups, the cemental groups form over the entire length of the tooth below the crown, and therefore, the cementum must be examined over the length of the tooth. Because of the size of sperm whale teeth, this procedure is probably not suitable or convenient. However, the cemental growth layer groups should be examined in teeth in which the pulp cavity is closed.

h. Rate of growth layer formation

At the IWC Age Determination Meeting, Oslo, 1968 (IWC, 1969), it was assumed from the evidence of histological studies, and especially tag recapture studies, that most probably one growth layer group forms annually. Also, Best (1970) and Gambell (1977) published further evidence to suggest that one growth layer group forms annually. Gambell studied the layer formation on the edge of the pulp cavity over a ten-month period in one area off Australia. Lockyer (Abstract in this volume) presented a review on the current status of the rate of growth layer formation and concluded that one growth layer forms annually. There are still some problems in the knowledge of the rate of growth layer formation, especially in accessory layer formation, but there is now substantial evidence to suggest that one growth layer group forms annually.

There are problems in the study of the exact timing of the layer formation, in that the boiling of teeth denatures the collagen and so affects the pulp cavity edge. As all teeth in any sample are similarly treated, they can be used to obtain a relative formation rate. However, the group would recommend that unboiled teeth (such as those collected by Clarke and his associates from the southeast Pacific) would be suitable for timing of growth layer group formation. Clarke proposed to follow up this suggestion, especially inasmuch as his sample covers a twelve-month period for both sexes. Lockyer also intended to continue such studies using teeth collected from Australia, but these teeth have been boiled.

i. Recommendations

In addition to the more general recommendations listed elsewhere in this report, the group makes the following specific recommendations for sperm whales:

i. Calves should be marked. This will yield valuable long-term results from the number of dentinal layers accumulated. The short 'Discovery' type .410 mark (Clarke, 1971) should be used.

ii. The use of radio-active tracer techniques (see General Recommendations) is especially recommended for examination of teeth from sperm whales taken in the north Pacific.

iii. Complete series of mandibular and maxillary teeth from individual whales of various ages should be compared to check the uniformity of growth layer group formation. Differences in growth layer group formation between sexes in different areas should be investigated, with special attention to more prominent growth layer groups and irregularities in female teeth.

3. NARWHAL, *MONODON MONOCEROS*, AND WHITE WHALE, *DELPHINAPTERUS LEUCAS*

a. Participants

I. Christensen *(Chairperson)*, V.M. Kozicki *(Rapporteur)*
H.N. Grue, D.E. Sergeant, K. Hay, V.S. Gurevich,
A.J. Kenyon, A. Boyde and J.B. Kemper.

b. Introduction

The dentition and structure of the dentinal and cemental growth layer groups of the white whale have been described by Sergeant (1973).

Although six pairs of maxillary dental papillae and two pairs of mandibular dental papillae are present in embryos of the narwhal, only the anterior two pairs of maxillary papillae persist and develop. Of these, the posterior pair of teeth are very small and nonfunctional in postnatal animals, while the anterior pair produces the 'tusks'. The left anterior tooth of males erupts as a long, tapering, sinistrally-spiralled tusk, while the right anterior tooth, up to 29 cm long, remains embedded in the maxilla. Both anterior teeth (up to 23 cm long) of female narwhals usually remain embedded in the skull, although the left tooth infrequently erupts as an elongated tusk. Individuals of both sexes may occasionally have two erupted tusks, and a few males are tuskless.

The unerputed teeth of old male and female narwhals, positioned horizontally in the maxillae, are long and tapering, and terminate posteriorly in a 'knot' or 'butt' consisting of dentine and cementum at the root. This tooth lengthens very rapidly during early postnatal life due to dentinal deposition but, as its axial growth rate decreases, the 'knot' appears and enlarges, due to both dentine deposition and formation of enveloping cementum. Around the age of sexual maturity, cementum covers the entire tooth, including its proximal or root end, and the tooth is then said to be occluded (i.e. no further growth).

On the other hand, the erupted tusk appears to grow and elongate continuously throughout life by deposition of dentine and cementum. Therefore, erupted tusks have greater potential value for determining age than the unerupted tusks. Emphasis in this workshop was placed on these organs, although the periosteal bone of the mandible is laminated and may be useful for determining age.

c. Materials available

For the narwhal, teeth were available from 45 specimens from a wild, harvested narwhal population which summers in the Lancaster Sound area of North Baffin Island. All specimens were derived from the native hunt, which occurs from mid-June to late-September. Unerupted tusks from males and females, mandibular sections and field data were available from a wide range of size and age classes. In addition, more complete materials were available from two males and two females collected from this area in August 1978. These included two large erupted tusks, an abnormal tusk in the lower mandible, and bullae and periotics from one male and two females, as well as the unerupted tusks and jaw sections.

For the white whale, unstained thin ground sections and longitudinally bisected teeth were available from seven specimens. In addition, several intact teeth were available.

d. Methods used in the workshop

i. Narwhal

A variety of potential age determination techniques was tested on the various tissues.

Unerupted tusks were bisected longitudinally on a band saw or circular saw; the cut surface was then polished with No. 600 waterproof sandpaper. Several of these tusks were embedded in *Bioplastic* prior to cutting. The half-teeth were exposed to 10% formic acid for 40 hours, washed in running water for two hours, dried and then read with a binocular microscope or the unaided eye. The specimens prepared in this manner included an ontogenetic series. The results were not entirely satisfactory. The acid etching highlights the ridges present in the dentine but fails to satisfactorily differentiate between major growth layer group boundaries and the numerous accessory layers which are present in the shaft (rapidly growing portion) of the tooth. Accessory layers are less common in the posterior, or butt, of the tooth, hence etching works well in this area. In some cases, etching tended to obscure the neonatal line.

Longitudinal and transverse thin (40 μm) sections of the tip of an unerupted tusk were made and hand-ground for examination of cementum under a compound microscope.

Decalcified thin sections (40 μm) of the tip and posterior (root) end of unerupted tusks were obtained and stained with Mayer's Haemalum and mounted for examination of the growth layer structure of the cementum.

The two *erupted tusks* were sawn longitudinally on a diamond saw with a movable carriage. Only a narrow wedge was cut from each tusk, in order to preserve the shape and overall appearance of these specimens for subsequent display. About ten hours were required to cut each tusk. The wedge-shaped longitudinal sections of the erupted tusks were sanded with No. 400 waterproof sandpaper and subjected to acid etching. A solution of 30% phosphoric acid was applied to one cut surface of one tusk for one hour. Shorter exposures were found to be inadequate. In addition, a 10% solution of HCl was used on a second specimen. Both solutions were applied using a sponge; the surface was kept wet by constant application of the acid. After this procedure, the surface was washed with water for two minutes and then was air-dried for eight hours. The etched surface was rubbed with a wide carpenter's pencil, of medium hardness, which revealed the growth layer groups and accessory bands. Phosphoric acid proved to be better than hydrochloric acid in differentially defining growth layer groups and accessory lines.

Eight small sections of various growth layer groups were removed from one tusk for racemization analysis (see Bada *et al*, this volume). These samples spanned the entire range of growth layer groups present in the tusk from the 1978 (most recent) group to the oldest dentine near the tip of the tusk. A sample of the tip of the right (unerupted) tooth was subject to racemization analysis to correct for low or irregular temperature in the erupted tusk. Additional samples were taken from the surface of the pulp cavity of the erupted tusk for SEM examination, the results to be reported elsewhere.

Tympanic bullae and *periotic* specimens were embedded in *Bioplastic* and thin-sectioned (200 μm) using a double-bladed diamond saw. Sections in various planes were made.

Mandibular sections were taken between the mental foramina, embedded in *Bioplastic* and thin-sectioned (about 100 μm) using a *Gillings-Hamco* thin-sectioning machine. The sections were decalcified, stained with haematoxylin (see Catalog of Techniques for details) mounted in a glycerine-alcohol solution, and examined by means of a compound microscope or a binocular microscope. One mandibular section from one of the large tusked males was made available for later analysis in a scanning electron microscope.

ii. White whale

The unstained, thin longitudinal sections were examined with dissecting microscopes (10X and 20X) and a compound microscope with a viewing screen (30X). The intact teeth were longitudinally bisected. The polished surface of the bisected tooth was found to be quite difficult to read, so the half-teeth were etched in 10% formic acid. However, etching did not improve readability.

e. Description of the tissues

i. Narwhal

The *growth layer groups* are variable in internal structure. The relatively translucent prenatal dentine is followed by a thin translucent neonatal line and then somewhat more opaque postnatal dentine. Typically, early growth layer groups consist of a thick, uniform layer of intermediate opacity, then a thin translucent, a thin opaque, and a thin translucent layer. The thin opaque layers are white in reflected light, while the thin translucent layers are dark in reflected light. Later-formed growth layer groups in the knot (root) consist of a thick, relatively dense layer and a thin, less dense (translucent) layer. The thin layers correspond to the ridges of acid-etched teeth.

In the erupted tusks, the acid etching and pencil rubbing visualized the growth layer groups and many accessory layers within the groups. Both tusks had been depositing dentine in fairly regular layers about 4 mm thick. Upon superficial examination six to nine accessory layers can be seen within a growth layer group.

Cementum surrounds the entire unerupted tusk from root to tip (except when the pulp cavity is open), but it is extremely thin. The proximal cement at the root is contorted and marbled in appearance and does not present a layered appearance in either reflected or transmitted light. Boyde notes that *enamel* is not present at the tip of the tooth.

ii. White whale

Sergeant (1973) presented photographs of longitudinally bisected teeth of an ontogenetic series of white whales. The teeth are robust and reach a maximum length of about 8 cm in large males. The enamel is very thin and easily worn away in early postnatal life. The prenatal dentine is uniform and moderately opaque in thin (100 μm) sections. The neonatal line is thin and translucent.

Growth layer groups in the postnatal dentine generally consist of a thick, translucent layer, itself divided by thin opaque layers and a thick opaque layer. The dentinal growth layer groups are 0.2 to 0.4 mm thick and are relatively uniform in thickness throughout life.

The *cementum* is thick and has structure similar to that of the dentine. The cemental growth layer groups are 0.12 to 0.24 mm thick.

There is a correspondence in the number of cemental and dentinal growth layer groups, except that there may be one or two more dentinal growth layer groups. Either structure, as well as a series of wave-like projections between them, may be used for age determination. The pulp cavity remains open throughout life, and up to 40 or 50 growth layer groups may be present. The main problem in age determination is the severe wear of the crown of the tooth in older animals, affecting both dentine and cementum. Sexual dimorphism exists in the teeth: females have thinner cementum than males, and later dentinal growth layer groups may also be very thin in females, making their ages more difficult to determine.

f. Cross-reading experiments

i. Narwhal

Five observers read the acid-etched unerupted tusks which represented an ontogenetic series for both males and females (Table 7). Some of the readers tended to count accessory layers, while others did not. The counts tended to increase with body length, confirming the supposition that the growth layer groups represent increasing age to at least the point of occlusion of the tooth. In the six teeth examined from postnatal females, the range of the values tended to be small, about one-third of the mean, and a good correlation between the length of the animal and the mean growth layer group count was observed. The six male teeth presented to the readers posed more problems than did the female teeth. Counts of growth layer groups in males tended to be lower than female counts at small body lengths; i.e. males may have higher growth rates.

Eight observers read a series of nine teeth from seven different adult or near-adult animals (Table 8). Some of the teeth were etched; the unetched half of the same tooth was available for comparison and was used by some, but not all, observers. For the few female animals available, both right and left teeth were used. The means of the readings for the two tusks in each case were close to each other, although the range of estimates among readers was large. Accessory layers were a major problem, particularly in the second and third growth layer groups.

ii. White whale

The group concentrated on cross-reading of the thin longitudinal sections only. The number of dentinal and cemental growth layer groups was counted independently by each participant; these counts are listed in Table 9. At the time of reading, no data on body length or sex were available. The readers were in reasonably good agreement; however,

Table 7

Counts by several observers of growth layer groups in an ontogenetic series of etched half tusks (unerupted) from thirteen narwhals, *Monodon monoceros*

Specimen No.	Sex	Body ln.(cm)	Sexual maturity	Neonatal line visible?	Dentine still growing?	GLGs in dentine KH*	CL	VMK	JBK	IC	Range	Ave.
MM28	F	178	Calf	Yes	Yes	0	–	0	0	0	–	0
MM37	F	230	Immature	No	Yes	3	4	4	2.5	3.5	2.5–4	3.4
MM–P10–75	F	284	Immature	Yes	Yes	6	4	(5)	4.5	3.5	3.5–6	4.6[1]
MM43	F	312	Immature	Yes	Yes	9	8	8	7	10	7–10	8.4
MM–P7–75	F	326	Immature	Yes	Yes	11+	12	12	9.5	9.5	9.5–12	10.8
MM62	F	388	Mature	Yes	No	10	15	14	10	13	10–15	12.4
MM59[2]	F	265	Immature	Yes	Yes	6	–	6	1.5	–	1.5–6	4.5
MM–P19–75	M	262	Immature	Yes	Yes	2	1	1	1.0	1.5	1–2	1.3
MM–P23–75	M	287	Immature	Yes	Yes	7	6–7	8	6	6.5	6–8	6.7
MM–5–74	M	324	Immature	Yes	Yes	12	–	12	9	–	9–12	11.0
MM–P15–76	M	381	Mature	Yes	Yes	11	12–13	12	10	11	10–13	11.3
MM–A2–76	M	373	Mature	Yes	No	15	19	16	13	14	13–19	15.4
MM67	M	442	Mature	Yes	?	13	12	12	10	12.5	10–13	11.9

[1] Tooth not bissected evenly.
[2] Not etched.

Table 8

Counts by several observers of growth layer groups in etched half-tusks (unerupted) from nine adult or near-adult narwhals

Specimen No.		Sex	Body ln.(cm)	Dentine still growing?	GLGs in dentine VMK	DES*	IC	KAH*	HNG	VSG	JBK	AB	Range	Ave.
1423	l.	F	–	–	12	9	10	11	9	9	10	8–9	8–12	9.8
	r.			No	12	9	–	10	9	9	8	11	8–12	9.7
1424	l.	F	–	Yes	13	–	14	9	7	–	7	8	7–14	9.7
	r.			–	12	8	–	9	7	7	7	9	7–12	8.4
MM–5–74		M	324	Yes	14	12	13	12	13	11	10.5	10.5	10.5–14	12.0
MM–P21–76		M	329	Yes	12	10	12	9–10	(7)	10–11	–	–	7–12	10.2
1444	r.[1]	M	(436)	–	18	13–15	17	13	12	17	12	12–13	12–18	14.4
MM–P30–76		M	451	No	16	(15)	19	16–18	–	(17)	–	–	15–18	16.8
1443	r.[1]	M	452	Yes	10	10	9	11	9	11	10	9	9–11	9.9

Table 9

Counts by several observers of growth layer groups in thin-sections of teeth of seven white whales, *Delphinapterus leucas*

	GLGs in dentine									
Specimen No.	IC	DES	VMK	KAH*	HNG	JBK	VSG	TK	Range	Ave.
DES62–50	12	12	12	13	11+	11+	12+	11	11–13	12.0
DES63–60	20	30	31	22	25	26	28	–	20–31	26.0
DES62–90	5	7	8	7	6–7+	6+	7+	5	5–8	6.4
EDM1417	6	6	6	7	5.5	6.5	6	6	5.5–7	6.1
EDM1418	12	10	–	10	(14–17)	9	13.5	9	9–17	11.3
EDM1419	29	22	26	24	24	24	22	–	22–29	24.4
EDM1420	10	9	9	7	8	7	7.5	7	7–10	8.1

they felt that the greatest uncertainty was with the first few growth layer groups, which are relatively thick and often have prominent accessory layers.

g. Discussion and recommendations

i. Narwhal

The use of the teeth of narwhals to assess age by counting growth layer groups is based upon the assumptions that growth layer groups and accessory layers can be distinguished, and that the deposition of dentinal growth layer groups continues until closure of the tooth by cementum cuts off the supply of nutrients to the pulp cavity, thereby halting dentinal layering; or, alternatively, cessation of dentine growth allows cementum to grow over the last-formed dentine and seal off the pulp cavity. These assumptions are discussed below.

(1) The poor resolution of growth layer group boundaries in the early-formed tissues contributed to the variability of the length/age data, along with, of course, individual variation in growth rate. Clearly, some observers counted layers that were judged by others to be accessory layers. Acid etching tended to obscure the neonatal line. More experimentation with different acids and techniques is required.

(2) Hay (this volume) has suggested that the embedded tooth continues to deposit dentine until occlusion occurs by enveloping cementum. He stated that occlusion should occur at about the attainment of sexual maturity in males and females and that this age differs between the sexes. Not enough female specimens were examined during the workshop to demonstrate this finding adequately. The onset of sexual maturity in males occurs over a narrow range of body lengths but over a rather wide range of growth layer group counts (Hay, this volume). The extent of closure of the pulp cavity was found to be very difficult to ascertain, and it was not possible to determine at what point GLG readings become unreliable. The problem of ascertaining closure was discussed at some length following the reading of the acid-etched teeth. Since etching is strongest at the edges of the tooth (because the acid attacks these areas from two sides), a translucent layer develops at the periphery of the tooth. At the posterior edge of the tooth, this layer may be mistaken for cementum. Etched half-teeth should always be compared with the unetched half of the tooth when assessing occlusion.

Because of these technical problems, the members of the group agreed that the following work, in addition to the work outlined in the General Recommendations section, is needed to develop a reliable technique of age determination:

(1) Field collectors of unerupted tusks should assess the degree of closure by examining the root end of these teeth and noting the presence or absence of *in situ* pulp.

(2) SEM and light microscopy studies of the tip and posterior (root) end of apparently occluded tusks should be conducted to determine whether or not growth layer groups continue to develop in the cementum at these locations and to ascertain the microstructural nature of dentinal occlusion.

(3) In the absence of a reliable method of age determination using tusks, other hard tissues, including the mandible and auditory bullae, should be investigated for their potential value in age determination.

(4) Haematoxylin (or other) staining of decalcified histologically prepared thin sections of the mandible and dentinal root of the unerupted tooth should be investigated as a means to enhance the definition of growth layer groups in these tissues.

(5) Additional tests on acid etching of cut and polished, longitudinally-bisected, unerupted teeth should be conducted to distinguish between growth layer groups and accessory layers.

ii. White whale

In addition to the general recommendations concerning time-marking of wild and captive animals and use of various biochemical age determination techniques, the group recommends the following:

(1) An extensive ontogenetic series of teeth should be examined to define the growth curve with greater precision than has been done.

(2) All of the teeth from several various-sized animals of both sexes should be examined to assess the validity of determining age from a single tooth.

(3) 150 to 200 μm longitudinal, unstained thin sections mounted on glass slides are recommended for dentinal growth layer group counts (for routine age determination).

(4) The structure of the cementum should be examined in stained thin sections of teeth.

4. LARGE DELPHINIDS

a. Participants

J.G. Mead *(Chairperson)*, K. Hay *(Rapporteur)*,
S. Ohsumi, M. Nishiwaki and D.E. Sergeant.

b. Introduction

Delphinid whales include species of the Delphinidae ranging in maximum body length from approximately 2.5 m (the melon-headed whale, *Peponocephala electra*, and the pygmy killer whale, *Feresa attenuata*) to 7 m to 9 m (the killer whale, *Orcinus orca*). Much less is known of their biology than that of the dolphins, because of their larger size, fewer numbers, more secretive, pelagic mode and the accompanying greater difficulty in capturing and maintaining them in captivity. Relatively few are taken in commercial fisheries, although some have been the subject of local fisheries.

Generally, delphinid whales possess fewer teeth than do the dolphins. However, except for their larger size, the teeth of large delphinids are similar to those of the dolphins in most respects. They are simple, slightly-recurved conical teeth consisting of complexly layered dentine covered coronally by a mantle of enamel and surrounded basally by thin, poorly layered cementum. Their greater size permits superficial examinations of the dentinal layers in etched half-sections or ground thin-sections with the aid of low-power magnifiers. With the prominent exception of the long-finned pilot whale (Sergeant, 1962), large delphinids have not been studied intensively. This is due largely to the dearth of available materials representing most other species.

In addition to the problems common to all delphinids, such as pulp-cavity closure, complexity of dentinal tissue and thinness of cemental layers, determination of age using layering in the teeth of large delphinids involves several special problems. First, the teeth are relatively large, therefore preparation is time consuming, and the materials do not lend themselves easily to techniques developed for smaller delphinids. Second, the sample sizes are usually small, and ontogenetic series are difficult to construct for most species. Third, the dentinal tissue in several species, e.g. *Orcinus orca*, is extremely dense and difficult to examine in thin sections. Fourth, with one exception *(Globicephala melaena)* GLGs have not been defined for large delphinids.

c. Materials available

Teeth of the long-finned pilot whale, *Globicephala melaena;* the short-finned pilot whale, *Globicephala macrorhynchus;* and the killer whale, *Orcinus orca*, were available for study. These included eight specimens (seven with biological data) of *G. melaena* from Newfoundland, ten specimens of *G. macrorhynchus* from Japan, 52 specimens of *G. macrorhynchus* collected from stranded animals on the southeast coast of the United States (all with sex and length), and two teeth from a specimen of *G. macrorhyncus* which died in captivity. *Orcinus orca* material included teeth from a 5.53 m female taken in Norway, an animal stranded at Tierra del Fuego, and several other specimens obtained from various museums. In addition, a tooth from 'Old Tom', a killer whale which frequented Eden, Australia, was available. Teeth were available from one false killer whale, *Pseudorca crassidens* and one Risso's dolphin, *Grampus griseus.*

d. Methods used in the workshop

Thin ground sections of the teeth of eight specimens of *G. melaena* from Newfoundland were examined. In addition, a polished thin section of a tooth of one specimen was decalcified and stained (see 2.d. in Catalog of Techniques). A half-tooth of this specimen was also prepared.

Thin ground sections (40 to 70 μm) of teeth of *G. macrorhynchus*, from Japan, had been decalcified and stained with haematoxylin. Thin ground longitudinal and transverse sections of the teeth of *G. macrorhynchus* from the United States were also prepared.

All killer whale teeth were bisected, and one-half of each tooth was treated with 10% formic acid for 15 hours, except one specimen that was treated for only eight hours. A thin ground section of this tooth was decalcified in 10% formic acid for three hours and stained with haematoxylin. A thin ground unstained section of this tooth was also prepared. One killer whale tooth was bisected, one half acid-etched and a thin ground section produced from the other half. Stained sections were found potentially useful for counts of cemental layers in both species. A decalcified stained thin section of the cementum of one specimen was also prepared.

e. Tooth structure and growth

i. Long-finned pilot whale, *Globicephala melaena*

The *prenatal dentine* is relatively translucent, the *neonatal line* is a thin translucent layer, and the *postnatal dentine* consists of a regular series of thick opaque and thin translucent layers. The *growth layer groups* are not difficult to count, and *accessory layers* are usually readily discernible. The *cementum* is very thin. It is thickest at the root; the cemental growth layer groups are thus thin and compressed and difficult to count in unstained thin ground sections.

Initial longitudinal growth of the teeth of *G. melaena* is extremely rapid (for the first few growth layer groups); it ceases early (after two or three layer groups), and further layer groups are deposited more or less parallel to the long axis of the tooth. However, the pulp cavity closes early in life, after about ten to twelve GLGs have been deposited. Thus, the dentine is of limited value in determining maximum age of most specimens. Cemental layers can be seen on thin ground sections using the compound microscope, but they are not particularly clear or countable.

Sergeant (1962) carried out considerable work on age determination in this species. All of the teeth erupt at the same time, and all teeth contain about the same number of growth layer groups. Sergeant *(loc. cit.)* used ground transverse sections about 25 μm thick. The workshop observations of longitudinal thin sections of pilot whale teeth confirmed his observations. Since axial growth of the tooth is complete very early in life, thin transverse sections taken from the mid-point of the tooth were found to be most suitable for counting GLGs.

The following description (for comparative purposes) of growth layer structure in *G. melaena* is paraphrased from Sergeant (1962): the prenatal dentine is thin, uniform, and appears translucent. The neonatal line is translucent and thin. The first postnatal growth layer consists of a thick opaque layer followed by a thin translucent layer. This first growth layer group is extremely thick compared to subsequent groups and may contain a thin, weak or ill-defined translucent layer (within the thick opaque layer zone). Sergeant (1962) suggested that this growth layer group represents the first two years of life, when the whale is suckling and receiving constant nutrition; the presence of a weak translucent layer within the first postnatal opaque layer would thus represent the termination of the first year of life. The second and subsequent postnatal growth layer groups have the same structure, except that the accessory translucent layer is absent. The earliest-formed layer groups consist of thick opaque and thin translucent layers, but these groups are similar in thickness in the most recent layer groups. Occasionally, the translucent layers are bounded by thin highly translucent layers, giving a double-layer effect.

Sergeant (1962) also found that dentine deposition may cease due to closure at the base of the root, even when a definite pulp cavity is still present. The maximum number of dentine growth layer groups laid down in his sample was ten to fifteen, with a mean of twelve, similar to the results using the longitudinal sections (Table 10).

Irregular dentine with ill-defined, unreadable growth layer groups is laid down near the pulp cavity in the teeth of old animals. This dentine is clear and devoid of dentinal tubules. It occurs infrequently, and this phenomenon was not seen in the samples of *G. melaena* teeth examined in the workshop.

Sergeant's (1962) observations on cemental layering in *G. melaena* have been confirmed by the workshop investigators. The growth layer groups are extremely thin and compressed. Sergeant used 15 µm-thick transverse sections and studied them under a compound microscope. As in the dentine, cemental growth layer groups consist of alternating opaque and translucent layers. The cementocytes also present a laminated appearance which is not necessarily coincident with the growth layer groups. The numbers of dentinal and cemental growth layer groups are equal in the teeth of young whales (prior to closure of pulp cavity). A maximum of 50 cemental growth layer groups was counted by Sergeant in the teeth of old pilot whales. Sergeant's (1962) illustrations of thin ground sections of cementum at high magnification show that layering is ill-defined, but groups could be read in about 50% of the teeth; the accuracy of repeated counts was 10% to 20% (Sergeant, 1962).

The etched tooth of *G. melaena* contained six (obs. KAH) or twelve (obs. JGM) dentinal growth layer groups. A decalcified, stained thin section of this specimen did not provide satisfactory results.

Problems in counting growth layer groups in the *G. melaena* specimens included excessive thinness of sections, frequent double layering, and apparent accessory layers in the early layer groups. Thin, undecalcified longitudinal or transverse unstained sections generally seem to be most suitable for this species. Decalcification and staining of thin sections may be useful for defining and counting cemental layers and should be investigated.

ii. Short-finned pilot whale, *Globicephala macrorhynchus*
Decalcified, haematoxylin-stained thin longitudinal and transverse (about 30) sections of the teeth of *G. macrorhynchus* showed that the *prenatal dentine* was stained, the thin *neonatal line* was clear and unstained, and that the postnatal dentine consisted of alternating thick stained and thin unstained layers. *Dentinal growth layer groups* are more numerous than in *G. melaena*, being deposited for most of the life of the animal. Closure of the pulp cavity is thus much delayed. The growth layer groups are clearest and easiest to read in the apical region of the tooth, since apposition of dentine is greater here than in the root. As in *G. melaena*, longitudinal growth occurs only for two or three layer groups; thereafter, layer groups are deposited as a series of

Table 10

Counts by several observers of dentinal and cemental growth layer groups in teeth of twelve short-finned pilot whales, *Globicephala melaena*

Specimen No.	Sex	Body ln.(cm)	Neonatal line visible?	Dentine still growing?	GLGs in dentine								GLGs in cement						
					KAH	SO	MN	DES*	JGM*	EDM	Range	Ave.	KAH	SO	MN	JGM*	EDM	Range	Ave.
19	F	315	–	Yes	6	4	6+	4	5.5	7	4–7	5.3	–	–	–	6	4+	–	–
22	F	216	–	Yes	0	0	0	1	0.2	1.1	0–1.1	0.4	–	–	–	–	0	–	–
IX	M	574	No	No	12	17+	18+	(12+)	(13)	17	12–18+	14.0	(20)	–	–	(26)	13+	13–26	–
X	F	460	–	No	8	19+	16+	12+	(9)	9	8–19+	8.7	(12)	–	5	(16)	7+	5–16	11.0
XI	F	404	Yes	No	9	16+	16	8+	8	11	8–16+	11.0	(10)	18	–	(10)	9+	10–18	12.7
XII	–	–	Yes	No	(10)	9+	20+	(17)	7	13	7–20+	11.8	–	18	–	–	3+	–	–
XVI	M	282	–	Yes	1.5	3	2	2	1.5	2.7	1.5–3	2.1	–	–	–	2	1	1–2	1.5
Display 61	–	–	–	No	–	7	12	–	–	11	7–12	10.0	–	–	14	–	7+	–	–
UZMCN2	–	–	–	No	–	11	11	–	–	12	11–12	11.3	–	–	13	–	11+	–	–

nested cones parallel to the long axis. Cross sections can thus be used in routine age determination.

Although dentine is apparently deposited until old age in *G. macrorhynchus*, the innermost dentine (after growth layer group ten or so) consists of somewhat irregular, very thin and wave-like growth layer groups (secondary dentine with enclosed cementocytes in at least some specimens). These layer groups, especially those just adjacent to the pulp cavity, may be quite obscure and thus difficult to count in old whales. Sergeant (1959) has also noted this phenomenon in *G. macrorhynchus.*

The *cementum* of the tooth of *G. macrorhynchus* shows a pattern of layering similar to that in the dentine. However, the cementum is very thin, and the growth layer groups are thin, very compressed, and difficult to resolve using a binocular dissecting microscope. Resolution is somewhat better using a compound microscope. In older animals, the cementum is considerably thicker at the root; growth layer groups here are thicker and readily counted. In one old animal, cementum lined the entire pulp cavity subadjacent to the dentine. This cementum contains many layers and probably prevents further dentine deposition. An initially thick translucent layer in the cementum complicates interpretation of the first cemental growth layer group.

In general, transverse thin sections of the teeth of *G. macrorhynchus* proved easier to prepare than longitudinal thin sections, and counts of growth layer groups are more reliable. However, the first layer group did not always appear in a transverse section, because the sections were made well below the neonatal line. The first few layer groups and most recent layer groups of old animals also show some irregularity and present counting difficulties.

iii. Killer whale, *Orcinus orca*

The pattern of tooth growth in *Orcinus orca* is similar to that of *Globicephala;* initial axial growth is rapid and layers are deposited as a series of elongated nested cones. However, there is a slight tendency towards flattening and compression of the most recent *growth layer groups* in the occluded teeth of old whales, unlike the pattern in *Globicephala.* In some specimens it was obvious that this shift in pattern of deposition had taken place even though a small pulp cavity was still present. It is therefore likely that deposition of later dentinal layers may be independent of the existence of a pulp cavity and that presence of a pulp cavity may not necessarily indicate that dentine deposition is occurring.

The *cementum* of the teeth of killer whales is extremely thin, but growth layer groups were observed in the decalcified, stained sections. These were quite obscure, however, and difficult to count. With better preparation techniques, cemental layer counts may be possible.

The results from acid-etching of teeth of *Orcinus orca* were generally very poor. Accessory layers are numerous and frequently obscure any annual pattern of growth layers. A few teeth were readable but most were not. Tooth wear and deposition of irregular osteodentine are often seen in old teeth and are obstacles to age determination. A tooth from the killer whale 'Old Tom' from Eden, Australia (see Mitchell and Baker, this volume), was also examined by acid etching. The tooth was occluded and osteodentine was present in the root. Counts ranged from 13 to 18 dentinal growth layers; the cementum was impossible to read.

Thin ground and thin, decalcified and haematoxylin-stained sections were prepared from two killer whale teeth. Results for one were poor; dentinal growth layers were barely discernible and a proper count of the growth layers was not possible. About 19 layers were present in the cementum. The number of dentinal layers counted was 19 to 25, for the thin stained and unstained sections, respectively. The other tooth contained 15 to 29 growth layers when acid-etched; a stained thin section contained about 20 to 29 cemental layers. These layers, however, were not clear, and it would probably be better to prepare decalcified, thin sections (15 to 20 μm) in order to enhance these layers.

iv. False killer whale, *Pseudorca crassidens*

Very little information is available for this species, although large samples are occasionally available from mass strandings. *Enamel* in *Pseudorca crassidens* is relatively thick in the single specimen at hand. This is probably characteristic of the species. *Prenatal dentine* was more translucent than the postnatal dentine and was separated from it by a thin, opaque, *neonatal line.* The pattern of postnatal dentine deposition is similar to that described for *Globicephala.* A *growth layer group* consists of a thick layer of intermediate optical density, followed by a thin opaque layer and a variable narrow translucent layer. Numerous *accessory layers* were present and many lead to confusion for some specimens. The growth layer groups diminished in size slightly with age, with the pulp cavity filling up as in *Orcinus* and the last layer groups approaching a horizontal orientation (perpendicular to the long axis of the tooth). The pulp cavity was apparently nearly closed in this specimen, and although the section was slightly off center, no irregular dentine was seen.

Cemental growth layer groups overlapped towards the root of the tooth, as seen in ziphiids. It is therefore necessary to have the full length of the root for examination; cross sections would be unsatisfactory. The cemental layering in the single specimen examined was very irregular in places and did not permit an accurate count. Readability of these layer groups might be improved in stained preparations.

v. Risso's dolphin, *Grampus griseus*

In *Grampus griseus* the *enamel* was also relatively thick in the single specimen of this species which we examined. The *prenatal dentine* was more uniform in texture and more opaque than the *postnatal dentine,* and was set off from the latter by a well-defined translucent *neonatal line. Growth layer groups* in the postnatal dentine consisted of a thick layer of intermediate optical opacity followed by a thin translucent layer. Numerous *accessory layers* were present. Dentinal deposition appears to be essentially similar to that seen in *Globicephala,* although the available *Grampus* section was slightly off center, and it was difficult to assess the nature of the last dentinal layer groups. *Cement* deposition appeared to be similar to that seen in *Globicephala,* although growth layer groups were not readable in the section at hand. It is expected that those preparation techniques suitable for *Globicephala* would work as well on *Grampus.*

f. Cross-reading experiments

Several observers (up to seven) read series of teeth for *G. melaena, G. macrorhynchus* and *O. orca* (Tables 10, 11 and 12, respectively). The results reflect the above-discussed great difficulty in defining and counting GLGs (dentinal and cemental) in teeth of these large delphinids caused by the presence of numerous and variable accessory layers that often obscure the pattern of layering. Agreement among observers was extremely poor; the ranges of counts were

Table 11

Counts by several observers of growth layer groups in dentine teeth of ten long-finned pilot whales, *Globicephala macrorhynchus*, from Japan, and seventeen from the southeast coast of the US

Two teeth were read for some specimens

Speci-men No.	Sex	Body ln.(cm)	Neonatal line visible?	Dentine still growing?	GLGs in dentine									GLGs in cement							
					KAH	SO	MN	DES*	JGM*	TK	EDM	Range	Ave.	KAH	SO	MN	JGM*	TK	EDM	Range	Ave.
Japan																					
2	M	467	Yes	No	16+	28+	18	–	20+	24	20+	18–28+	21.0	–	20	14	(22)	39	9+	14–39	23.8
4	M	359	–	Yes	10	11	11	–	9.2	10.5	10.3	9.2–11	10.3	8	9	–	(9)	10	7+	8–10	9.0
7	M	354	–	Yes	10	9	10	–	10	9	9	9–10	9.5	8	9	–	–	10	4+	8–10	9.0
9	F	369	Yes	No	–	29+	19	–	19+	29	22+	19–29+	24.0	–	22	22	(20)	41	25+	20–41	26.3
13	M	448	Yes	No	15	30	16	–	26	24	22+	15–30	22.5	–	26	16	19+	32	19+	16–32	24.7
14	M	454	Yes	Yes	27	29	22	–	22	23	20+	22–29	24.6	–	18	12	18+	26	19	12–26	18.8
21	F	374	–	Yes	25	27	27	–	24	20	22+	20–27	24.6	–	13	–	10+	24	11+	13–24	18.5
25	F	334	–	Yes	21	28	22	–	18	20	19±2	18–28	21.8	–	–	–	11+	30	13+	–	–
26	M	235	–	Yes	1.5	2	2	–	1.5	1.5	2	1.5–2	1.8	1.5	1	–	1.5	2	1+	1–2	1.5
27	M	258	–	Yes	3.5	3	3	–	3.2	3.5	4	3–4	3.4	–	3	–	(3)	3	3	–	3.0
*Southeast coast of US***																					
196	F	386	–	No	–/27	14+/25+	9/–	–	11+/27+	–	–	9–14+/–	–	–	–	26/–	5+/(14)	–	–	–	–
197	F	325	–	Yes	4/–	7/–	5/–	4/–	7.5/–	–	–	4–7.5/–	5.5/–	–	–	–	3.2/–	–	–	–	–
198	F	336	–	Yes	3/7	7/7	10/–	5/(4)	8.2/8.7	–	–	3–10/4–8.7	6.6/6.7	–	–	–	–/3.2	–	–	–	–
199	F	343	–	Yes	17/19	14/13	25/–	15/12	14.7/16.6	–	–	14–25/13–19	17.1/15.2	–	–	–	–/(9)	–	–	–	–
200	M	535	–	Yes	12/9	13+/8+	18/–	9+/9	(17)/15	–	–	12–17/9–18	15.7/11.0	–	–	–	–/(12)	–	–	–	–
201	F	392	–	Yes	22/–	21/18+	22/–	9+/9	(13)/(14)	–	–	13–22/9–18+	19.5/11.5	–	–	–	–/(14)	–	–	–	–
202	F	351	–	No	11/–	18+/27+	20/–	–	13+/(25)	–	–	11–20/–	11.0/–	–	–	–	(23)/–	–	–	–	–
203	F	378	–	Yes	–	29+/31+	49/–	–	(36)/(39)	–	–	36–49/–	42.5/–	–	–	–	(16)/(25)	–	–	–	–
204	F	384	–	No	–	29/25+	40/–	–	(36)/(30)	–	–	29–36/–	35.0/–	–	–	–	–/13±1	–	–	–	–
205	F	305	–	Yes	–	8/8	10/–	–	9.5/9.5	–	–	8–10/8–9.5	9.2/8.8	–	–	–	–/4	–	–	–	–
206	M	263	–	Yes	–	1/1	4/–	–	(3)/(2)	–	–	1–4/1–2	2.7/1.5	–	–	–	–/2	–	–	–	–
207	M	488	–	Yes	–	6+/14	18/–	–	8+/(20)	–	–	–/14–20	–/17.0	–	–	–	–/14	–	–	–	–
208	M	481	–	Yes	–	12+/14	21/–	–	(13)/12	–	–	13–21/12–24	17.0/13.0	–	–	–	–	–	–	–	–
209	M	411	–	Yes	–	6/–	12/–	–	10/–	–	–	6–12/–	9.3/–	–	–	8/–	–	–	–	–	–
210	F	385	–	Yes	–	7/6	11/–	–	9/(11)	–	–	7–11/6–11	9.0/8.5	–	–	13/–	–	–	–	–	–
211	M	371	–	Yes	–	8+/11+	10/–	–	–/16	–	–	–		–	–	8/–	–	–	–	–	–
212	F	359	–	Yes	–	11/6	12/–	–	10/9	–	–	10–12/6–9	11.3/7.5	–	–	–	–	–	–	–	–

**GLGs in Dentine/Cement

Table 12

Counts by several observers of growth layer groups in dentine of teeth of 23 killer whales, *Orcinus orca*

Specimen No./ tooth position	Neonatal line visible?	Dentine still growing?	Prep. method	GLGs in dentine								
				KAH	SO	MN	JGM	TK	EDM	VMK	Range	Ave.
'24 years'	Yes	No	Stained	15+	(24)	26	20+	28	23+	18+	24–28	26.0
D118/LL5	No	Yes	Etched	19+	(28)	38+	(38)	26	40	28+	26–40	33.0
D118/RL10	Yes	(Yes)	Etched	14+	30+	43+	20+	27	20	29	20–43+	25.3
D119/RL7	(Yes)	(No)	Etched	25+	(39)	–	(24)	29	42	–	24–42	33.5
I.C.	Yes	Yes	Etched	(15)	32	54	(16)	29	30	15	15–54	27.3
LACM 781	Yes	Yes	Etched	2	2.2	8	2	2	2.5	2	2–8	3.0
MNHNH2430	(Yes)	Yes	Etched	20+	29+	–	(26)	22	14+	19	19–29+	22.3
RNP31	Yes	Yes	Etched	15	33	25+	(15)	24	25+	13	13–33	20.0
RNP31	Yes	Yes	Stained	–	28	–	–	23	19+	–	23–28	25.5
UZM1	Yes	(Yes)	Etched	15+	22	29	(20)	21	23+	15	15–29	21.4
UZM1	Yes	(Yes)	Stained	–	24	–	–	10+	8+	–	–	–
UZM3	No	No	Etched	11+	16+	10+	10+	11	8	9+	8–16+	9.5
UZM9	Yes	(Yes)	Etched	17+	16+	–	–	19	11+	–	–	–
UZM9	Yes	(Yes)	Thin-sect.	10+	11+	15+	12+	18	11+	13	13–18	15.5
UZM11	Yes	(Yes)	Etched	0.5	0.3	3.5	2	2	2	2	0.3–3.5	1.8
UZM12	Yes	Yes	Etched	7	29	36+	(13)	16	20+	12	7–36+	15.4
UZM13	Yes	Yes	Etched	3	2.6	11	3	3	2.2	3	3–11	4.0
UZM24	Yes	Yes	Etched	34	36	51	40±3	37	39+	32	32–51	38.3
UZM38	Yes	No	Etched	(10)	18+	35	9+	18	10+	9	9–35	18.0
UZM5a	Yes	Yes	Etched	4	22	28	(10)	9	21+	12	4–28	14.2
UZM5b	Yes	Yes	Etched	4	23	28+	(12)	8	(30)	14	4–30	15.2
UZM5c	Yes	Yes	Etched	3.5	29	34	(11)	11	25	13	3.5–34	18.1
Old Tom	Yes	(Yes)	Etched	15+	24+	(35)	(18)	33	14+	17	17–35	25.8

greater than for any other odontocete group studied during the workshop. The disagreement was especially great for the etched teeth of *O. orca*, for which estimates varied by a factor of four or more for several specimens. For the pilot whales, the counts from the decalcified and stained specimens from Japan (Table 11) were slightly less variable than those from untreated sections, but still not satisfactory. These results demonstrate clearly that present preparation techniques are inadequate and that age estimates based on them are unreliable.

g. Recommendations

i. Preparation techniques for the pilot whales need to be improved. Untreated sections should not be used for age determination. Decalcification and staining of thin sections should be further researched and improved, for both dentine and cementum. Cemental preparations are necessary in age determination, since pulp-cavity closure occurs early in life (at about 12 growth layer groups) in *G. melaena* and later in *G. macrorhynchus.*

ii. More work on age determination of *Orcinus orca* must be carried out. The initial studies carried out in the workshop suggest that dentine cannot be used in most teeth and that preparation of decalcified, thin stained sections of cementum is a more promising approach.

iii. Initial age studies of a wild population of *Orcinus orca* may provide a useful starting point in elucidating age determination in this species. Emphasis must be placed on practical aspects such as preparation techniques, interpretation, and reading.

5. DOLPHINS

a. Participants

A.C. Myrick, Jr and G.A. Klevezal' *(Co-chairpersons),*
J. Prime *(Rapporteur),* A. Boyde, R. Clarke,
V.S. Gurevich, A.A. Hohn, T. Kasuya, M. Kimura,
V.M. Kozicki, C. Lockyer, H.D. Marsh, E.D. Mitchell,
N. Miyazaki, W.F. Perrin, B.S. Stewart and D.E. Sergeant.

b. Introduction

Dolphins possess simple, slightly-recurved, cone-shaped teeth consisting internally of layered dentine, covered coronally by a thin mantle of prismatic enamel and laterally by layered cementum. Age estimates are best obtained by preparing a mid-longitudinal thin section of the tooth and counting repeating or semi-repeating 'growth layer groups' within the layered tissue with the aid of a microscope.

There are a number of special problems associated with the use of dental layering to determine age of dolphins. First, in many species the teeth are small, and dentinal deposition may occlude the pulp cavity and cease before the animal dies. Thus counts of dentinal growth layer groups in teeth of older adults may obtain only minimum age estimates. Second, although cemental deposition is thought to continue throughout ontogeny, the cementum may be unlayered,

NOTE: Two sessions were originally convened, one on temperate dolphins, under Klevezal', and a second on tropical dolphins, under Myrick. When it became apparent during the sessions that this was an artificial dichotomy having little or nothing to do with patterns of layer deposition, the sessions were merged.

poorly-layered, or too thin to 'read'. This makes difficult efforts to correlate growth layer groups in dentine with those in cementum. Third, dentinal growth layer groups are complex units consisting of so-called 'accessory layers' of variable intensity. This has led to considerable confusion over what criteria should be used in defining growth layer groups to be counted. Fourth, a number of diverse tooth-preparation and layer-counting techniques are in use. These emphasize different criteria, producing different interpretations of age. Little progress has been made toward the inter-calibration of these methods, or in determining which may be most reliable. Finally, as is true for odontocetes generally, although age estimates frequently have been couched in terms of years, accumulation rates of dental layers are not known. Growth layer groups have not been calibrated to absolute time.

The exercises carried out in the session on dolphins addressed various aspects of the above problems.

c. Materials available

i. Unstained thin (100 to 300 μm) longitudinal tooth sections mounted in *Permount* were available for ontogenetic series of spotted dolphins, *Stenella attenuata,* and eastern and whitebelly spinner dolphins, *S. longirostris* subspp. from the eastern tropical Pacific. Unstained thin sections were also available for eleven bottlenose dolphins, *Tursiops truncatus,* nine of which were of known age, known minimum age or had been marked *in vivo* with tetracycline; for four common dolphins, *Delphinus delphis,* similarly treated and for a series of *Tursiops* teeth which had been microradiographed (see paper by Hohn, this volume).

ii. Decalcified and stained thin sections were available for 20 spotted dolphins, *S. attenuata,* from Japan, 20 striped dolphins, *S. coeruleoalba,* from Japan, 10 rough-toothed dolphins, *Steno bredanensis,* from Japan and 8 common dolphins, *Delphinus delphis*, from the Black Sea.

iii. Whole teeth were available for several additional species of small delphinids, in the synoptic osteological collection of the Southwest Fisheries Center.

d. Description of structures

The purpose of this exercise was to identify and describe histological features of the tooth relevant to counting of growth layer groups, using the age series of unstained thin sections of teeth of *S. attenuata* and *S. longirostris* and the

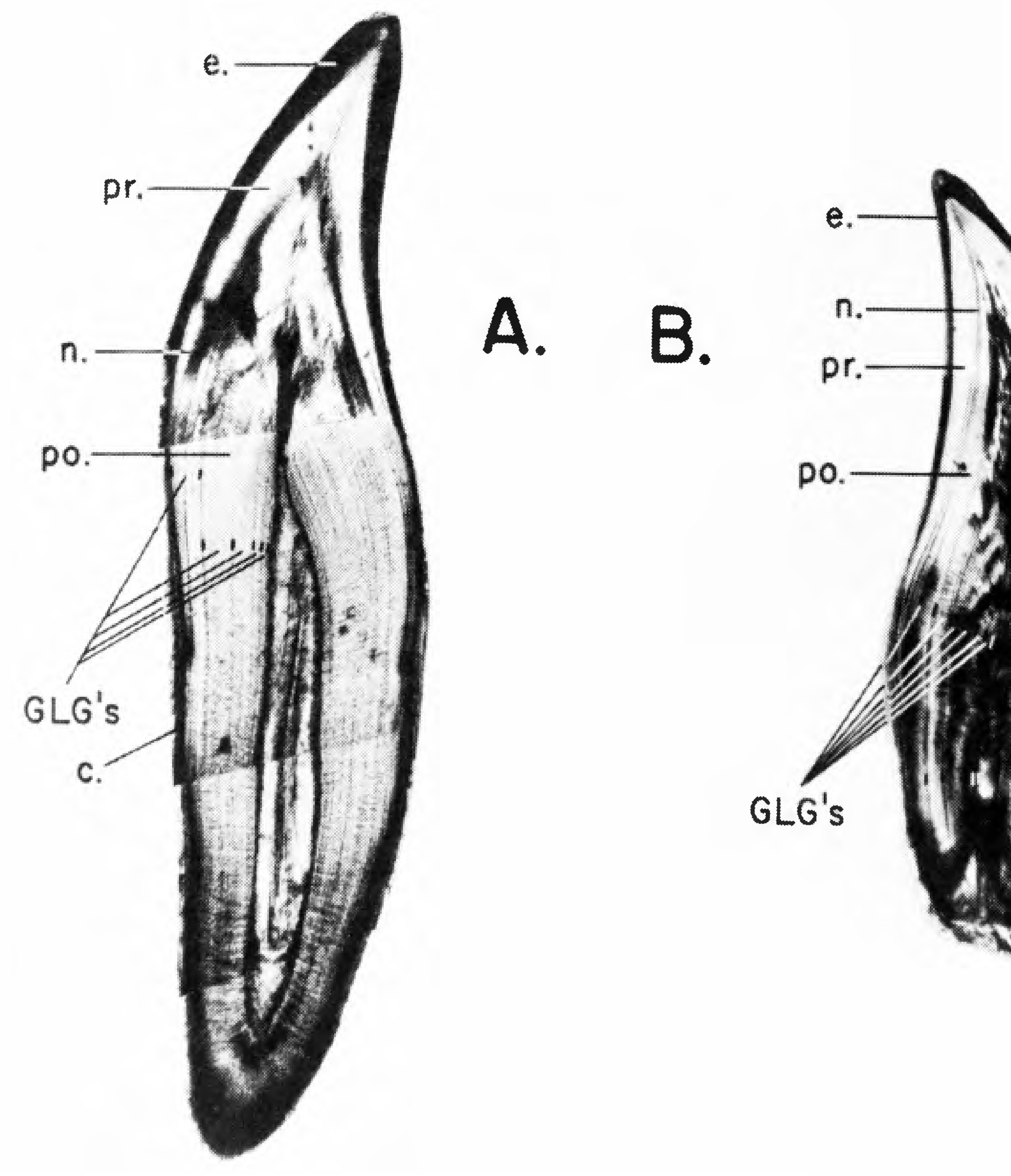

Fig. 2. Photographs of 200–μm-thick sections of undecalcified teeth of *Stenella* spp. (x57). Abbreviations: c. cementum, e. enamel, n. neonatal line, po. postnatal dentine, pr. prenatal dentine.
A. *Stenella attenuata* (SWFC #DBZ096). Tooth contains 5 GLGs.
B. *Stenells longirostris* (SWFC #RCD156). Tooth contains 7 GLGs.

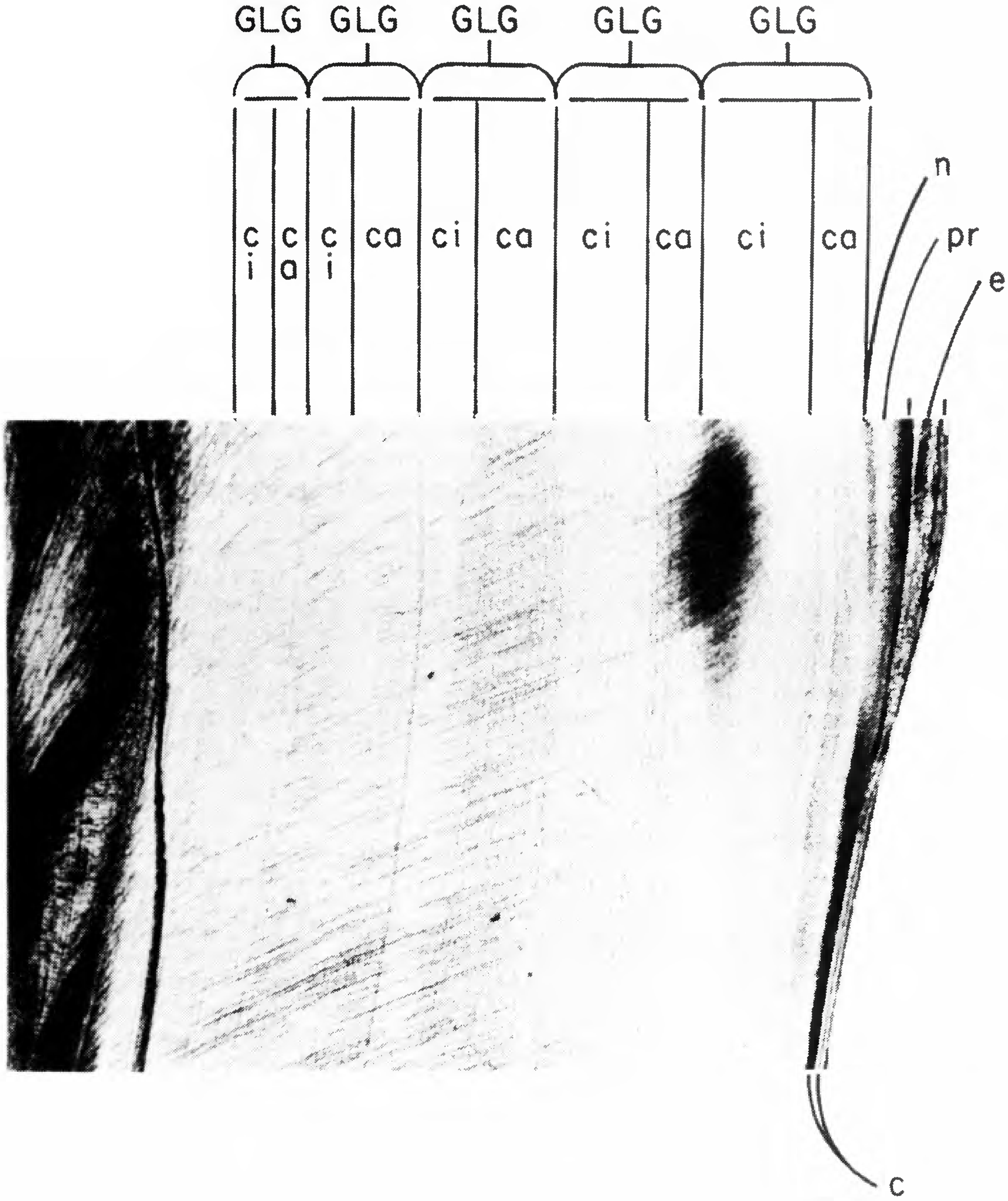

Fig. 3. Photograph of a 200-μm-thick, undecalcified section of a partial tooth (SWFC #0028) illustrating the typical structure of growth layer groups in the dentine of *Tursiops truncatus* as described in the text (x57). Abbreviations: e. enamel, c. cementum, GLG. growth layer groups 1–5 (ca. components containing alternating opaque and translucent layers, ci. component of intermediate to slightly opaque optical density with fine substructure), n. neonatal line, pr. prenatal dentine, po. postnatal dentine.

series of decalcified and stained sections of *S. attenuata, S. coeruleoalba, D. delphis* and *Steno bredanensis* and the autoradiographed specimens and other unstained preparations of *Tursiops* teeth. For the most part, low-power dissecting microscopes or compound microscopes at 30X to 50X were used to carry out the investigations. A compound microscope with a closed-circuit television camera and one with polarizing and phase contrast capabilities were also available.

In *Stenella* spp., the *enamel* in unstained sections tends to be more yellow than the dentine when viewed with transmitted light under a dissecting microscope. The *prenatal dentine* that is deposited initially tends to be more transparent and amorphous than that deposited later. Immediately external to the neonatal line, the prenatal dentine is less transparent and shows fine layering. The *neonatal line* is marked by a thin, internal, brightly translucent layer followed by a thin opaque layer. The *postnatal dentine* is finely layered. Under a compound microscope at low magnification (30X to 50X), each *growth layer group* (Figure 2) consists of a cycle of layers, as follows: (1) thick translucent layer; (2) bright thin translucent layer; (3) thick translucent layer which tends to become darker towards the internal edge. Because of the dark edge of the last type of layer, each growth layer group appears to be bounded by thin opaque layers. The typical pattern of the growth layer group is most evident in the first three or four groups, after which the pattern becomes more difficult to discern. In the more recently deposited growth layer groups in older animals, the third type of layer described above is compressed, and the growth layer groups appear as translucent layers containing strong accessory layers bounded by dark edges.

In the decalcified and stained preparations of *Stenella* spp., *Delphinus* and *Steno*, most of the *enamel* had been lost. Where present, it can be seen as an unstained tissue.

Prenatal dentine typically appears as a moderately or lightly stained tissue with little inner structure. The *neonatal line* is marked by a very lightly stained or unstained layer. The *postnatal dentine* consists of alternating structures stained with varying intensity. The boundary layer(s) of each *growth layer group* is interpreted as the region which stains with greatest intensity. The change in spacing of these lines with age is similar to, but not always identical to, that observed in the unstained preparations.

Examination of unstained thin sections of teeth of *Tursiops truncatus* showed that a typical growth layer group consists of a thick layer of intermediate to slightly opaque optical density with fine substructure, followed by a slightly thinner layer containing an alternating sequence of two to five opaque and translucent layers (Figure 3). In general, the former component of the growth layer group tends to be more translucent in older growth layer groups and more opaque in younger groups. Each in the series of these growth layer groups appears as being bordered by thin opaque margins. More recently deposited growth layer groups in adults tend to have the thick opaque component reduced in thickness and the alternating-layered component proportionately thicker.

Comparison of the microradiographs of thin sections of *Tursiops* teeth with the sections as seen with light microscopy, showed that the neonatal line changes from translucent to opaque under radiophotography, whereas the optical density of the layers of the first growth layer groups are reversed (opaque to translucent and *vice versa*). This is the reverse of the situation described in *Phocoena* by Grue (see Porpoises section below). It is concluded that there may be differences between species regarding the transparency and degree of mineralization of layers.

Comparison of the microradiographs of thin sections of *Tursiops* teeth to the original ground thin sections using light microscopy showed the neonatal line to be translucent in ground thin sections and dark (radiolucent) in the microradiographs. The optical density of layers of subsequent growth layer groups is correlated with the mineral density, i.e. opaque layers are radiopaque and translucent layers are radiolucent, but under light microscopy the radiopaque layers appear light and the radiolucent layers appear dark. This is the reverse of the situation described in *Phocoena* by Grue (Nielsen, 1972); there may be differences between species regarding the optical density and degree of mineralization of layers.

Although counting growth layer groups was secondary to the objective of observing structural development and defining patterns of growth layer groups, the number of growth layer groups counted shows a good correlation in the larger series of unstained sections with body length in the smaller size groups, i.e. up to about 140 cm to 150 cm (Figures 4 and 5). In this range, there is a gradual increase in the mean number of growth layer groups counted, with

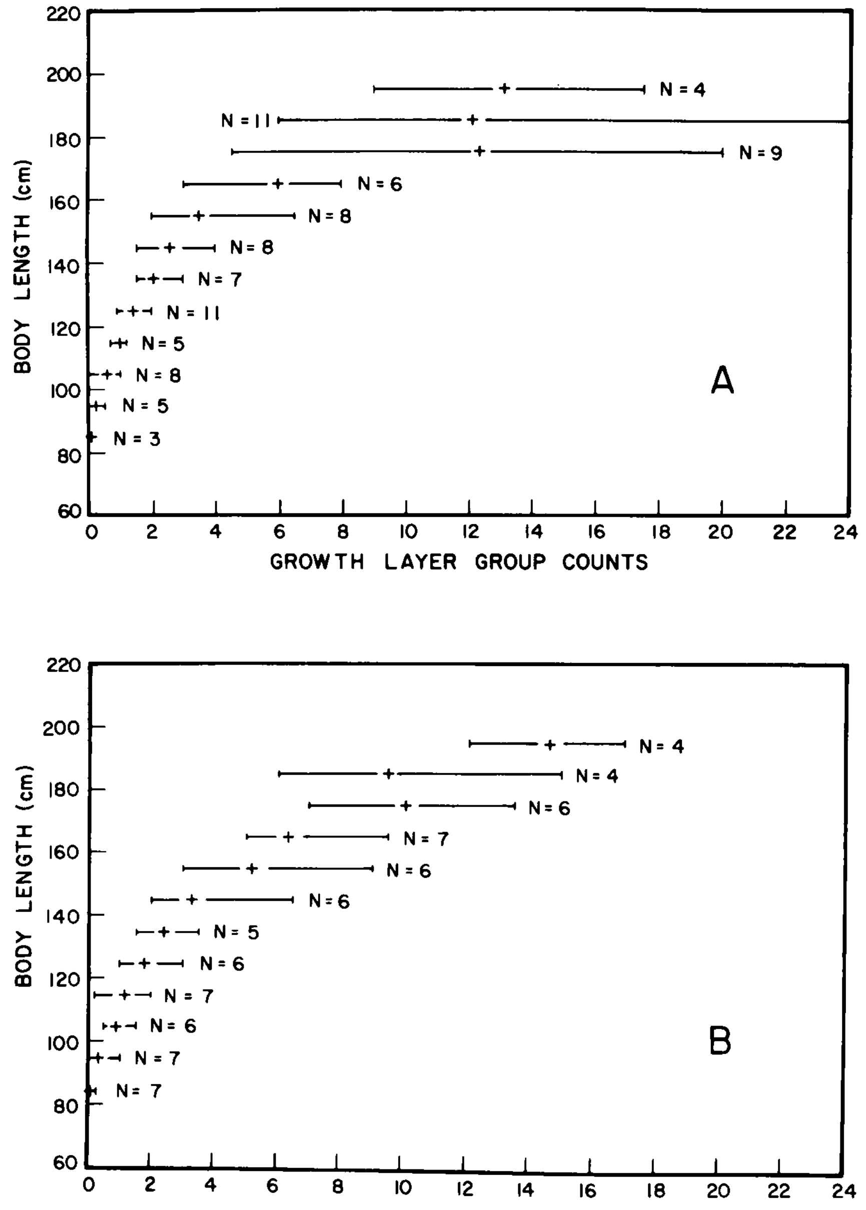

Fig. 4. Variation in counts among several observers of dentinal GLGs in teeth plotted against body lengths of 13 males (A) and 13 females (B) of *Stenalla attenuata* from the eastern tropical Pacific. Bar indicates range of GLG counts. Cross indicates range mean. N = number of observers.

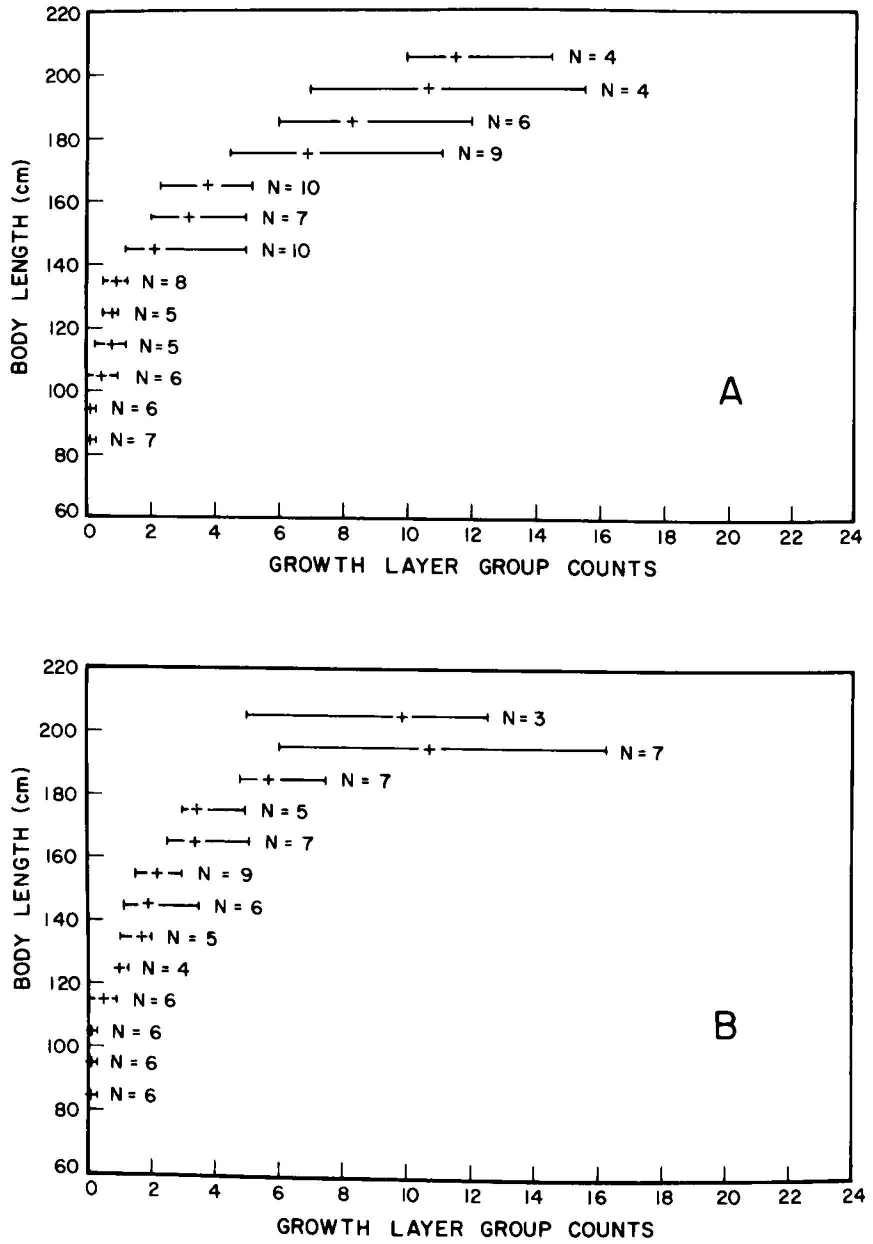

Fig. 5. Variation in counts among several observers of dentinal GLGs in teeth plotted against body lengths of 12 males (A) and 12 females (B) of *Stenella longirostris* from the eastern tropical Pacific. Bar indicates range of GLG counts. Cross indicates range mean. N = number of observers.

Table 13

Counts by several observers of growth layer groups in decalcified and stained sections of teeth of eight common dolphins, *Delphinus delphis*, from the Black Sea

Specimen No.	Sex	GLGs in dentine							
		GAK*	DES	VMK	CL	VSG*	WFP*	Range	Ave.
1 X 12	F	15	15	16	16–17	15+	16	15–17	15.7
1 X 9	M	17	16	18	19	17	–	16–19	17.4
1 X 16	F	13	14	13	12	15	16	12–16	13.8
VII 9	F	15	16	15	16	16	16	15–16	15.7
VII 5	F	18	19	18	18	17	18	17–19	18.0
VII 3	F	12	12	13	13	13	14	12–14	12.8
VII 6	F	16	17	17	18	17	15	15–18	16.7
VII 4	F	6	6	6	6	6	6	–	6.0

a relatively small range of values. Although the mean number of growth layer groups counted increases with size in larger, presumably older animals, there is a far greater range of values for each size stratum. This increased variability is probably largely due to individual variation in adult size, but it may also be partially due to the facts that identification of the later-formed growth layer groups in larger dolphins is much more difficult than the earlier layers and that accessory layers can be mistaken for growth layer groups.

e. Cross-reading experiments

i. Common dolphin, *Delphinus delphis* (decalcified and stained sections)
There was excellent agreement among the participants in counting growth layer groups (Table 13). Disagreement lessened after discussion and re-examination, from a maximum of ± 2 groups to ± 0.5 groups. The cementum was too thin to allow resolution of growth layer groups.

ii. Spotted dolphin, *Stenella attenuata;* striped dolphin, *S. coeruleoalba;* and rough-toothed dolphin, *Steno bredanensis* (decalcified and stained sections)
The results for these three species are presented in Tables 14 to 16 (pp. 26-8) Although in many cases the range of counts for the dentine is wide, the majority of counts for each specimen are within two growth layer groups of the mean, with only one or two counts significantly increasing the range. In comparison with the far greater range of counts found in the unstained ontogenetic series, these results are extremely encouraging. It seems that decalcified and haematoxylin-stained sections (see Catalog of Techniques, below) will significantly assist the counting of growth layer groups by both experienced and inexperienced observers.

Fewer members of the group counted the cemental growth layer groups in the stained sections. Generally, the results indicate that cemental counts are less reliable than dentinal counts. Although the range of cemental counts was small in a few specimens, in the majority of cases it was far greater than dentinal counts for the same specimen. The group concluded that although cemental counts may sometimes be used to confirm dentinal growth layer group counts, the dentine appears to be the more reliable. Improvement of preparation and intercalibration between dentinal and cemental growth layer groups may eventually resolve the problem.

iii. *Tursiops truncatus* (six unstained thin sections and one etched half-tooth)
Agreement among the readers was very good (Table 17, p. 29), with correspondence to ± 2 growth layer groups. A half-tooth of *Tursiops truncatus* was etched with 5% formic acid for two hours and the readings compared with those of undecalcified thin sections (Table 17). The counts were most consistent among observers for the etched half-tooth.

f. Rate of deposition of growth layer groups

The purpose of these exercises was to examine the relationships between counts of growth layer groups and true age in years.

i. Known-age and known minimum-age specimens
Thin ground sections of teeth from nine specimens of *Tursiops truncatus* (Table 18, p. 29) were examined under dissecting microscopes with transmitted and/or reflected lights. Counts of growth layer groups (Table 19, p. 30) for young animals (less than four growth layer groups) were in close agreement with ages in years. The counts for older animals varied widely among the observers and largely disagreed with age in years. The ranges of counts exhibited a general increase with increase in known age. For the oldest known-age animal (21 years), however, two observers obtained counts of 20 and 21 growth layer groups.

In one tooth of 'Moe B', an animal known to have been 3.3 years old at death, numerous accessory layers were visible within the growth layer groups. Members of the group agreed on a count of 12 such layers within the second growth layer group.

Correspondence between counts of growth layer groups and age in years in the youngest and oldest specimens of *T. truncatus* supports the thesis of deposition of one growth layer group per year in this species. The accessory layers within growth layer groups may represent lunar growth increments (Myrick, this volume). The group felt that correspondence between count of layer groups and age in years would probably be closer in the mid-age range animals in decalcified and stained sections, because such layer groups are clearer and subject to less individual variation in interpretation.

ii. Specimens marked with tetracycline
Sections of teeth from four tetracycline-injected specimens of *Delphinus delphis* and three of *Tursiops truncatus* (Table 20, p. 31) were available for examination with ultraviolet light

[to p. 29]

Table 14

Counts by several observers of growth layer groups in dentine and cement of decalcified and stained sections of teeth of twenty spotted dolphins, *Stenella attenuata*, from Japan

Specimen No.	Sex	Body ln.(cm)	Neonatal line visible?	Dentine still growing?	GLGs in dentine										GLGs in cement					
					TK*	MK*	HDM	NM*	ACM*	WFP*	JP	BSS	Range	Ave.	TK*	NM*	ACM*	WFP*	Range	Ave.
1	M	148	Yes	Yes	1.5	2	–	0.5	4	2	2.5	2.2	0.5–4	2.0	2	1	3	3	1–3	2.3
7	M	168	Yes	Yes	4.5	3	2.3	2.3	5	(3)	4	4.2	2.3–5	3.5	5	3	3	2	2–5	3.3
34	M	176	Yes	Yes	11	4	–	7.5	10	7.5	8.3	7.5	4–11	8.0	11	7	10±1	7	7–11	8.8
35	M	186	Yes	Yes	12	5.5	–	11.5	12	11	10	11.7	5.5–12	10.5	12	9	7	(9)	7–12	9.3
30	M	195	Yes	Yes	13	6	–	11.5	10	8	10	9.2	6–13	9.7	13	11	9±2	12	9–13	11.3
31	M	200	Yes	Yes	15	6	–	12.5	13	8	14.5	12.1	6–15	11.6	18	14	13	13	13–18	14.5
29	M	205	Yes	Yes	11	7	–	9.5	11	8	9.5	9.2	7–11	9.3	19	12	11±3	17	11–19	14.8
17	M	214	Yes	?	15	7	–	15.5	15	10+	11+	11	7–15	12.7	21	15	15±2	15	15–21	16.5
25	M	203	Yes	?	15	7	–	14.5	20	9.5	13	14.3	7–20	13.3	30	23	20±4	22	20–30	23.8
38	M	204	Yes	?	–	–	–	–	–	–	–	–	–	–	16	15	7	(12)	7–16	12.5
24	F	139	Yes	Yes	1.5	2	–	1.8	2	0.5	1.5	2.1	0.5–2.1	1.6	2	1	1	1	1–2	1.3
19	F	157	Yes	Yes	1.5	3	1.8	1.8	2	1.8	1.8	2.1	1.5–3	2.0	2	1	1	1.5	1–2	1.4
14	F	163	Yes	Yes	4.5	4	–	3.5	4	3.5	3	4.3	3–4.5	3.8	5	3	3	3	3–5	3.5
5	F	175	Yes	Yes	8	4.5	–	7.5	12	6	7	10.1	4.5–12	7.9	8	7	9	6	6–9	7.5
40	F	183	Yes	Yes	11	5	–	10.5	9.5	7	10.5	9.1	5–11	8.9	11	9	8	7	7–11	8.8
8	F	198	Yes	Yes	10	6.5	–	10.5	10	10	9	8.5	6.5–10.5	9.2	13	12	9	10	9–13	11.0
2	F	189	Yes	Yes	13	6	–	12.5	12	13	11.5	11.1	6–13	11.3	13	12	12	13	12–13	12.5
9	F	195	Yes	Yes	12	6	–	12.5	13	13	12	12.9	6–13	11.6	20	13	12	13	12–20	14.5
36	F	198	Yes	Yes	19	6	15.0	18.5	19	16	18	16.2	6–19	16.0	32	19	18	16	16–32	21.3
41	F	203	Yes	Yes	14	7	–	11.5	13	12	10.5	13.5	7–14	11.6	18	15	–	(10)	10–18	14.3

Table 15

Counts by several observers of growth layer groups in dentine and cement of decalcified and stained sections of teeth of twenty striped dolphins, *Stenella coeruleoalba*, from Japan

Specimen No.	Sex	Body ln.(cm)	Neonatal line visible?	Dentine still growing?	GLGs in dentine											GLGs in cement					
					VSG*	TK*	MK*	HM	NM*	ACM*	WFP*	JP	BSS*	Range	Ave.	TK*	NM*	ACM*	WFP*	Range	Ave.
309	M	158	Yes	Yes	–	1.5	2	0.5	1.5	1.5	1.5	1	3.1	0.5–3.1	1.6	1	1	1	(1)	–	1.0
308	M	164	Yes	Yes	3.5	1.5	2	–	2.0	2	2	2.5	1.5	1.5–3.5	2.1	1	1	–	1	–	1.0
328	M	205	Yes	Yes	–	7	7	–	6.5	–	7	6.5	6.8	6.5–7	6.8	7	6	–	3	3–7	5.3
331	M	207	Yes	Yes	–	5	5	–	4.5	7	4.5	4.5	3.5	3.5–7	4.9	5	5	6	(3)	3–6	4.8
337	M	214	Yes	Yes	–	9	9	–	8.5	9	9	8	9.4	8–9.4	8.8	9	9	7	–	7–9	8.3
329	M	221	Yes	Yes	–	9	9	–	8.5	8.5	9	7.5	8.1	7.5–9	8.5	9	8	8	9	8–9	8.5
312	M	227	Yes	Yes	–	15	10	–	15.5	13	13	15	15.3	10–15.3	13.8	15	15	13	17	13–17	15.0
353	M	231	Yes	?	10.0	23	10+	–	15.3	23	24	13	11.5	10–24	17.1	43	24	23	24	23–43	28.5
313	M	238	Yes	?	–	20	11+	–	16.5	17	18	15	12.1	12.1–20	16.4	24	16	17	18	16–24	18.8
390	M	240	Yes	Yes	–	20	12+	–	18.5	17	18	17	–	17–20	18.1	22	20	14	19	14–22	18.8
399	F	140	Yes	Yes	–	0.5	1	–	1.0	1.5	0.6	1	1+	0.5–1.5	0.9	1	1	0	1.5	0–1.5	1.0
325	F	167	Yes	Yes	2.5	1.5	2	0.5	2.0	1.5	2.0	1	3+	0.5–3+	1.6	2	2	0	1.5	0–2	1.1
360	F	172	Yes	Yes	3.5	1.5	2	2.0	2.0	2	1.5	2	2+	1.5–3.5	2.1	2	2	2	3	2–3	2.3
383	F	188	Yes	Yes	–	5	4	–	8.5	9	10	8	9+	4–10	7.4	5	8	(5)	(5)	5–8	5.8
362	F	199	Yes	Yes	–	6	6	–	4.0	7	6.2	4.5	5+	4.5–7	5.6	6	4	6	(4)	4–6	5.0
335	F	217	Yes	Yes	–	11	9	–	8.5	14	9	7.5	8	7.5–14	9.6	11	0	0	0	0–11	9.5
391	F	224	Yes	Yes	–	18	11	–	10.5	16	13	11	11+	10.5–18	13.3	34	17	16	14	14–34	20.3
326	F	222	Yes	Yes	–	14	11	–	9.5	12	10	9.5	8+	9.5–14	11.0	14	8	7	8+	7–14	9.7
323	F	226	Yes	Yes	–	18	11	–	13.5	18	(11)	(11)	9+	11–18	13.8	19	14	12	12	12–19	14.3
348	F	229	Yes	Yes	–	–	14	–	15.5	25	14	16	12+	14–25	16.9	43	28	25	29	25–43	31.3

Table 16

Counts by several observers of growth layer groups in the dentine and cement of decalcified and stained sections of teeth of ten rough-toothed dolphins, *Steno bredanensis*, from Japan

Speci-men No.	Sex.	Body ln.(cm)	Neonatal line visible?	Dentine still growing?	GLGs in dentine												GLGs in cement					
					VSG	AAH	TK	MK	NM*	ACM	MN	WFP	JP	BSS	Range	Ave.	TK	NM*	ACM	WFP	Range	Ave.
3	F	240	Yes	Yes	25	24	24	24	25	23	25	24	22	24	22–25	24.0	20	18	–	(7)	7–20	15.0
4	–	–	Yes	?	15	13	18	14	18	16	19	12+	13	19	13–19	16.1	25	25	30	(27)	25–30	26.8
25	M	225	?	?	24	21	25	18	24	20±3	23+	15+	19	25	18–25	22.0	33	32	24±3	23	23–33	28.0
A4	M	230	Yes	Yes	20	17	18	23	19	19	19	17.5	17.5	17.5	17–23	18.8	19	22	18±2	17	17–22	19.0
A8	M	230	Yes	Yes	13	13.5	14	17	17	21	17	16	14	17	13–21	16.0	15	19	17±2	19	15–19	17.5
A10	F	225	Yes	Yes	11.5	14.5	14	12	15	15	14	15	12.5	13	11.5–15	13.7	–	17	10	14	10–17	13.7
A11	M	222	Yes	Yes	16.5	16	17	15	18	19	18	19	16	17	15–19	17.2	18	22	–	14+	18–22	20.0
A17	M	240	Yes	Yes	10	12.5	14	11	16	15	26+	12+	14+	16	10–26+	12.1	12	15	10	8	8–15	11.3
A19	M	228	Yes	Yes	16.5	19	18	–	19	17	20	17	16.5	(14)	14–20	17.4	17	20	17	(13)	13–20	14.0
A20	F	240	Yes	Yes	35	35	30	24	35	26±3	31	26	32	20.1	20.1–35	29.4	29	33	29	24	24–33	28.8

Table 17

Counts by several observers of dentinal growth layer groups in unstained thin sections of teeth of four bottlenose dolphins, *Tursiops truncatus*

Specimen No.	Sex	Body ln.(cm)	GAK*	CL	VMK	DES*	DES¹	Range	Ave.
211	F	165	1.5	1.5	2.5	1.5	1.25	1.5–2.5	1.8
181	F	202	3+	5	5	5	5	–	5.0
RF	F	220	13–14	11	14	11	11	11–14	12.1
245	M	230	18	18	18	19	19	18–19	18.7

[1] Previously published readings; not included in ranges and averages.

under a compound microscope. The specimens of *Delphinus delphis* were marked with tetracycline by intramuscular (IM) injection as part of an experiment (reported by Gurevich *et al*, this volume) to calibrate dentinal and periosteal layering with time. These authors concluded that the dentinal tissues of some of these animals were not marked because dentinal deposition had ceased (due to their advanced ages). The teeth of Nos. 502, 505 and 506 (Sea World serial numbers) showed well-defined fluorescent marks in the layered dentine. In Nos. 502 and 506 the marks are in the third-youngest translucent layer, separated from the pulp cavity by two opaque layers. In No. 505 the mark is in the third-youngest opaque layer. The two younger opaque layers are separated by three translucent layers. For No. 502, the time represented between treatment date and date of death is ten and two-thirds months. For No. 505, the time represented between treatment date and tooth-extraction date is one year and three months. For No. 506, the time represented between treatment date and date of death is slightly less than one year. The data for all three specimens suggest the possibility that in *Delphinus delphis* dentinal opaque layers accumulate at a rate of slightly more than two per annum. In light of the results obtained from Klevezal''s staining technique (see Catalog of Techniques), by which each growth layer group produces two stainable layers in *Delphinus delphis*, it may be concluded tentatively that the two opaque layers internal to the tetracycline mark reflect an accumulation rate of approximately one growth layer group per annum. This would also be in keeping with the results above suggesting a one-growth-layer-group rate for *Tursiops*.

For two of the time-marked specimens of *Tursiops*, dates of treatments were not known, but the configuration of the multiple fluorescent marks is interesting in that they may be followed down to the base of the root and seen as unbroken lines that enter and delineate layers in the cementum. This indicates that the same pulse that influences layering in the dentine also operates in the cementum and that tetracycline injection may be useful in calibrating the cemental layers as well as the dentinal layers (Myrick, this volume).

g. Summary and recommendations

i. Based upon the results of the exercises discussed above, it is apparent that the dentinal patterns of dolphins are basically similar to each other – varying from one species to another only in detail. The *prenatal dentine* may be characterized as a slightly stainable, poorly layered tissue demarcated internally by a *neonatal line* consisting of a thin translucent layer, immediately followed by a thin opaque layer. The *growth layer groups* within the postnatal dentine have two major components: (1) a thick layer of intermediate optical density that is generally more translucent toward the external margin and more opaque toward the internal margin and which has lightly-layered internal structure, and (2) a thinner, more translucent layer that ends in a thin opaque sublayer. In older individuals the more recent growth layer groups become more compressed in the first component, and the second appears proportionately more prominent. Details of the pattern vary with species. However, it would appear that much of the variation and disagreement in the literature concerning deposition rates and the nature of growth layers arises not only from limitations of particular techniques of preparation and examination of specimens but

Table 18

Known-age and minimum known-age specimens (thin ground longitudinal sections of teeth) of *Tursiops truncatus* available for study at the workshop

Specimen name or no.	Sex	Date of capture	Date of birth	Date of death	Age
'Rollie'	M	–	6 Mar. 55	1 Sep. 55	6 months
'Maggie'	F	–	23 Feb. 53	12 Jul. 55	2 years 4.5 months
'Mona'	F	27 Apr. 46	–	28 Aug. 55	⩾ 9 years
'Pinger'	M	–	Nov. 70	Feb. 74	3 years 3 months
AB 718	–	1 Dec. 67	–	31 Dec. 77	⩾ 10 years
AB 722	–	27 Dec. 67	–	25 Aug. 77	⩾ 10 years
'Splash I'	M	Apr. 52	–	9 Feb. 59	⩾ 7 years
'Moe-B'	M	–	14 Apr. 75	4 Jul. 78	3 years 3 months
'Spray'	F	–	26 Feb. 47	21 Mar. 68	≅ 21 years

Table 19

Counts by several observers of growth layer groups in the dentine of nine known-age or known minimum-age specimens of *Tursiops truncatus*

Specimen name or no.	GLGs in dentine															
	BSS*	MK	VSG*	AAH*	RC	TK	JP	NM	ACM	GAK*	CL	VMK	DES*	Range	Ave.	Known age
'Rollie'	0.5	0+	–	0.5	–	0.5	0+	0.5	0.5	–	–	–	–	–	0.5	6 months
'Maggie'	2.3	3	3.6	2.3	–	3	2.5	2.1	2.5	2.5	2.5	2.5	2.5	2.1–3.6	2.6	2 years 4.5 months
'Mona'	9.9	18	12.3	14	–	12	15.5	15.9	–	–	–	–	–	9.9–18	13.9	15 years
'Pinger'	3.3	3	3.3	3.3	3	4	3	2.5	3.0	–	–	–	–	3–4	3.2	3 years 3 months
AB718	8	10+	–	8	–	–	11	–	–	–	–	–	–	8–10+	9.0	⩾ 10 years
AB722	10	9+	–	12	–	–	15	–	–	–	–	–	–	10–15	12.3	⩾ 10 years
'Splash I'	8.3	4	6.5	9	–	7	8.5	8.0	8.0	8	9	10	10	4–10	8.0	⩾ 7 years
'Moe-B'	3.1	3.5	3.0	3.3	3.3	3	3.2	2.4	3.3	–	–	–	–	2.4–3.5	3.1	3 years 3 months
'Spray'	–	–	–	–	–	–	–	–	–	20	–	–	21	20–21	20.5	≅ 21 years

also from variable interpretation of what constitutes a growth layer group, the entity being counted. It is therefore very important that the structure of what is being counted be histologically defined in detail for the species, or even population, under consideration in a particular study. The description should be based on an ontogenetic series.

ii. The evidence for *Tursiops* strongly indicates that one growth layer group is deposited annually. The data for *Delphinus* suggest a similar deposition rate, although the structure of the growth layer group is more complex than in *Tursiops.* Adequate calibration of the rate of deposition of growth layer groups remains to be done for the other dolphins. The group strongly endorses the relevant recommendations promulgated for odontocetes in general (see General Recommendations), in particular those having to do with known-age specimens and *in vivo* time-marking (Myrick, this volume).

iii. It is suggested that multiple tetracycline markings be made at every opportunity in concert with periodic tooth extractions for live captive animals. Special emphasis in such experiments should be placed on young animals. Samples of teeth from all animals held in captivity should be collected, sectioned, and examined under ultraviolet light for fluorescent marks and the information correlated with veterinary data. When feasible and where the probability of recovery is considered likely, programs of time-marking of wild animals should be carried out (Myrick, *loc. cit.*).

iv. It was the opinion of the group that decalcification and staining gives the best results for small delphinid teeth. Etching of a longitudinally sectioned tooth is a good method for the larger dolphins with larger teeth such as *Tursiops*, and thin undecalcified sections are useful (but rated third in preference) if the longitudinal section is accurately cut or ground to include the largest possible longitudinal section of the pulp cavity. Because of the extreme difficulty of distinguishing strong accessory layers from the opaque boundary layers of the growth layer groups, the examination of undecalcified and unstained thin sections is not recommended unless high-quality compound microscopes, preferably with polarizing light capabilities, are available (Myrick, *loc. cit.*). Such material is suitable for dentinal counts only; cemental growth layer groups should be counted in very carefully prepared decalcified and stained specimens.

6. PORPOISES

a. Participants

H.N. Grue *(Chairperson)*, M. Sheldrick *(Rapporteur)*,
I. Christensen, T. Kasuya, J.B. Kemper and L.J. Stuart.

b. Introduction

The teeth in *Phocoena* spp. and *Phocoenoides dalli* are relatively small and unusually shaped for odontocete teeth. In *P. phocoena*, the teeth have a spade-shaped crown, and those at both ends of the tooth row are smaller and may not erupt. The teeth of *P. dalli* are yet smaller than those of *Phocoena* spp. and are rudimentary, their function apparently taken over by the gums (Miller, 1929). The internal structure of porpoise teeth is also unusual in appearance among cetacean teeth, probably because of their shape and

Table 20

Sample of teeth from tetracycline-injected dolphins available at the workshop

Specimen No.	Species	Date(s) treated	Treatment method	Date tooth extracted	Date of death	Time bracketed
502	*Delphinus delphis*	7 Feb. 77	Intramuscular	–	29 Dec. 77	11 months
504	*Delphinus delphis*	7 Feb. 77 & 28 Apr. 78	Intramuscular	28 Apr. 78	–	14 months
505	*Delphinus delphis*	7 Feb. 77 & 28 Apr. 78	Intramuscular	28 Apr. 78	–	14 months
506	*Delphinus delphis*	7 Feb. 77	Intramuscular	–	Jan. 78	11 months
722	*Tursiops truncatus*	–	Oral	–	–	?
718	*Tursiops truncatus*	–	Oral	–	–	?

small size, and for this and other reasons there has been some controversy about the interpretation of internal structure of the teeth of *P. phocoena* and especially about the nature of the growth curve for that species (see Fisher and Harrison, 1970 – proposed growth curve; Gaskin and Blair, 1977, Perrin *et al*, 1976–criticism of growth curve of Fisher and Harrison, 1970; Nielsen, 1972; Stuart and Morejohn, this volume). Three of the participants in the porpoise session of the workshop (Grue, Kasuya and Stuart) have worked or are working extensively with porpoise teeth, and the workshop provided a valuable opportunity for these workers to explore standardization of techniques of preparation and scoring of teeth and other hard tissues and to reach broader agreement on interpretation of results.

c. Materials available

Prepared and unprepared materials from four species of *Phocoena* and from the Dall's porpoise, *Phocoenoides dalli* were available to the group for study. The group decided to work with the two species for which adequate series of prepared tooth sections were available, the harbour porpoise, *Phocoena phocoena* and Dall's porpoise, *Phocoenoides dalli.*

i. Harbour porpoise, *Phocoena phocoena*

Teeth were available from 214 animals from three regions: the northeast Atlantic (Danish waters), the northwest Atlantic, and the northeast Pacific. The material consisted of prepared and unprepared specimens. Also available were one skull with mandibles, six additional mandibles, two bullae, three humeri, and three caudal vertebrae of animals from the northeast Pacific.

The teeth from the northeast Pacific (from 84 animals) had been half-ground according to the method of Stuart (see Catalog of Techniques). The material from the northwest Atlantic consisted of thin sections of undecalcified teeth from 44 animals. These teeth (from the US Museum of Natural History) had been longitudinally sectioned with an *Isomet* double-bladed diamond saw at a private laboratory in San Diego, California; thickness of the sections was judged to be 50 to 70 μm. The sections were mounted in *Permount.* The prepared material from the northeast Atlantic consisted of 30 stained sections of decalcified teeth prepared by Grue (see Nielsen, 1972 and Catalog of Techniques). Included in the material from Denmark were four animals of known minimum age. These animals were caught at an age of one year, judged from the date of capture and length of the animals. They were kept in captivity at the Marine Bio-Acoustical Investigations, Denmark for from 6 to 39 months. Of the animals, one was treated orally twice and one three times with oxytetracycline (DUMEX) for therapeutic reasons. A microradiograph of a transverse thin section of a tooth from one of these animals was also available for study.

ii. Dall's porpoise, *Phocoenoides dalli*

Decalcified and stained thin sections from 99 animals from the northwest Pacific were available. They were prepared by Kasuya (see Catalog of Techniques). Two unprepared teeth were also available.

iii. Other spp.

Very limited material was also available for the spectacled porpoise, *Phocoena dioptrica,* and the cochito, *P. sinus,* but was not utilized during the workshop. Thin sections of undecalcified teeth from 21 specimens of Burmeister's porpoise, *Phocoena spinipinnis,* from the southeast Pacific were available (from the US Museum of Natural History). Longitudinal sections had been prepared as for the *Phocoena phocoena* teeth from the northwest Atlantic. The preparations proved to be inferior and were not dealt with further.

d. Methods used in the workshop

All the prepared teeth had been sectioned longitudinally, such that the section passed through the pulp cavity. Preparation techniques were demonstrated to the group for unstained half-teeth by Stuart and for stained and decalcified teeth by Kasuya and Grue (for exact descriptions see Catalog of Techniques).

The half-teeth were examined under a dissecting microscope with reflected light. A film of water on the ground surface increased clarity of structures. Dentinal structures in the unstained thin sections and in the decalcified and stained sections were examined with a dissecting microscope using transmitted light. For examination of cementum in the latter, a compound microscope was used at 100X magnification.

Three teeth of the half ground teeth of *P. phocoena* from the northeast Pacific were etched using three different treatments: 10% formic acid for 2 hours, 10% formic acid for 1 hour and sodium hypochlorite for 20 minutes, and 0.5 EDTA (pH 7.4) for 80 minutes and sodium hypochlorite for 20 minutes. All three were then coated with gold palladium to enhance surface relief.

Several sections (100 to 150 μm) of the mandible from a northeast Pacific harbour porpoise were taken from just posterior to the symphysis. Sections of an auditory bulla from the same animal were also taken. The sections were cut using a double-bladed *Isomet* 11-1180 low speed diamond saw. One section of the mandible was further ground, decalcified in 10% formic acid and stained with Mayer's haemalum. Two sections, one from the mandible and one from the bulla, were decalcified in 5% nitric acid, sectioned on a freezing microtome and stained with Mayer's haemalum.

By means of grinding, longitudinal sections of the teeth of the two tetracycline-marked animals were prepared to a thickness of approximately 60 μm. The sections were viewed under a Zeiss photomicroscope with fluorescent vertical illuminator, using reflected light (Mercury burner HBO-100 w/2 with filter-reflector combination for violet).

e. Structure of the tooth

i. Harbour porpoise (Figures 6, 7, and 8)

The spade-shaped crown is covered by a thin layer of *enamel* usually of a yellow color. The root is covered by a thin layer of cementum and is usually straight in mandibular teeth but in maxillary teeth may curve in two planes (not a spiral).

The *prenatal dentine* usually appears globular and is moderately stained in stained sections. In half-tooth preparations it does not appear globular and is opaque (white under reflected light).

The *neonatal line* in stained sections consists of a thin unstained layer bounded by a very thin heavily stained layer next to the prenatal dentine. In the half-tooth preparations, the neonatal line is a well-defined translucent layer (dark under reflected light). The neonatal line became a wide groove in the formic-acid etched half-tooth and a narrow ridge in the EDTA-treated specimen.

A *growth layer group* in *postnatal dentine* in a stained preparation consists of a thick, moderately stained layer and a thin unstained layer. In the thick layer several fine sublayers of varying stainability may be seen. The thin unstained layer usually appears as a single unstained layer often divided from the thick, moderately-stained layer by a very thin heavily-stained layer, but it can also consist of two or more thin stained layers divided by very thin moderately-stained layers (Figures 6 and 7). Towards the crown, the growth layer groups may be thicker than in the root, and a wave-like appearance of the thin unstained layer is often seen in this expanded region. In the first growth layer group, the moderately-stained layer is usually thicker than in successive growth layer groups and often appears globular. With increasing numbers of growth layer groups, the difference in thickness between the two main layers in a growth layer group is diminished, and in old animals the unstained layer may be the thickest. After the first two postnatal growth layer groups, the relative stainability of the two main layers of the group may change, but a definite pattern exists by means of which observers usually are able to count the number of growth layer groups present. Growth layer groups are usually observed most clearly in the cervical part of the tooth, and counting is most easily done along a longitudinal axis through the center of the pulp cavity.

The postnatal dentine forming the root is laid down parallel to the long axis of the tooth. With increasing numbers of growth layer groups the size of the pulp cavity is reduced, but an open pulp cavity seems to be persistent even in old animals.

In ground half-teeth, the growth layer groups in the postnatal dentine consist of a thick opaque layer which may have some thin translucent layers within it, and a well-defined translucent layer which is much thinner than the earliest-formed opaque layers (Figure 8). The translucent layer may be double, with a thin opaque layer between the two translucent layers. The opaque layers tend to become thinner with increasing age of the animals, but the translucent layers do not change as much in thickness. This results in approximately equal thicknesses of the composite layers in the later-formed growth layer groups, as in stained sections. Growth layer groups are usually easier to read in the coronal part of the tooth, where they tend to spread out due to the bulbous nature of the crown. The fine accessory layers tend to show up in this portion also.

It is apparent that the same structural units are being counted as growth layer groups in the stained sections and in the unstained half-teeth.

The apparent dentinal structure differed among the three etched preparations. In the tooth etched in formic acid for two hours, three pairs of ridges and grooves appeared, whereas five growth layer groups had been counted in the half-ground tooth before etching. In the tooth etched in formic acid for one hour and treated with sodium hypochlorite, six major sets of ridges and grooves were visible, a good match with the 6.5 growth layer groups counted before etching. In the preparation treated with EDTA, the ridges and grooves were not sufficiently defined, probably due to insufficient exposure to EDTA.

The maximum number of dentinal growth layer groups in *Phocoena phocoena* seems to vary from region to region. In animals from the northeast Atlantic the maximum number observed was 8 (Nielsen, 1972), in animals from the northeast Pacific the maximum number was 10 (Stuart and Morejohn, this volume), whereas in animals from the northwest Atlantic 13 growth layer groups have been reported, with an average maximum suggested to be 8 to 10 (Gaskin and Blair, 1977) or 6 to 9 (Gaskin, 1977). Nielsen (1972) assumed that after development of 7 to 8 growth layer groups, further dentine was laid down as osteodentine in which no growth layer groups could be seen. Gaskin and Blair (1977) did not agree with this finding, as they were able to count up to 13 growth layer groups in northwest Atlantic specimens. Examination of additional material from the northeast Atlantic during the workshop confirms the development of osteodentine in which growth layer groups are not recognizable (Figure 6). This material shows, however, that the more recently deposited dentine is of the normal type in the distal part of the root (towards the crown), while in the apical part osteodentine is deposited. This indicates that the formation of readable growth layer groups exceeding the maximum number suggested by Nielsen (1972) should be possible in the distal part of the root, but it has not yet been observed in northeast Atlantic specimens.

A *cemental growth layer group* in a stained section consists of a rather thick layer of lightly-stained cementum followed by a very thin intensely-stained layer. The intensely-stained layer is most clearly observed near the crown. Within the thick lightly-stained layer, several very thin heavily-stained layers may occur, and it is often difficult to distinguish them from the thin intensely-stained layer which marks completion of the cycle, especially in young animals. By following the layers along the root, it is usually possible to assess the number present. As in dentine, the first growth layer group is usually thicker than successive layer groups. The thickness of a particular growth layer group in the cementum is much less constant than in the dentine and may vary substantially along the root. In old animals excessive cementum growth may form bulbs on the sides of the root proximally (Figure 7). Cemental growth layer groups are not visible in ground half-teeth.

ii. Dall's porpoise

The morphology of the teeth of this species has been recently figured and described by Kasuya (1978). In a decalcified and stained section, the *prenatal dentine* is seen as a stain-

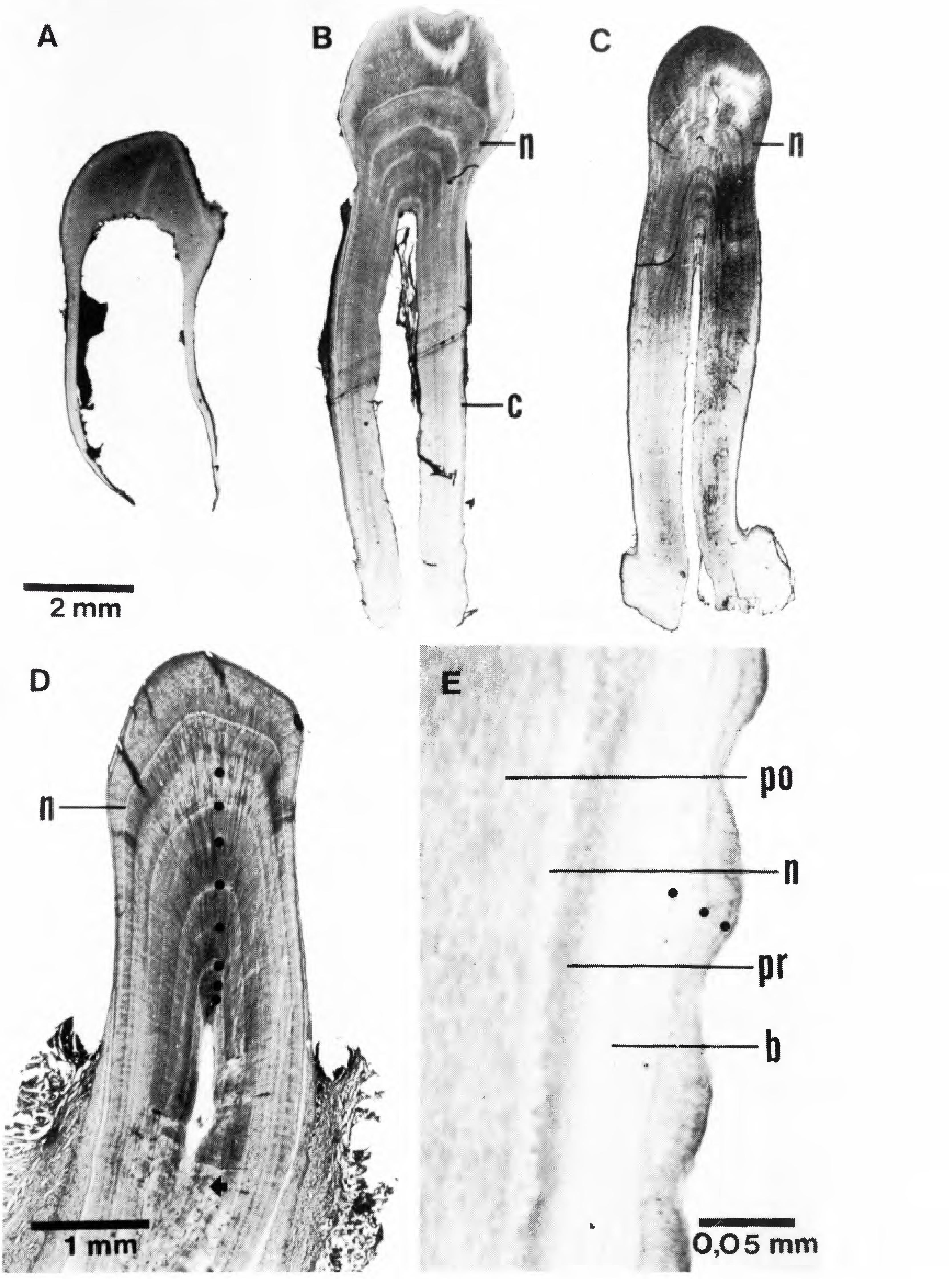

Fig. 6. 30-μm-thick longitudinal sections of decalcified and stained teeth (Mayer's haemalum) of *Phocoena phocoena* from the NW Atlantic. Abbreviations: b. dentino-cemental border, c. cementum, n. neonatal line, po. postanatal dentine, pr. prenatal dentine.

A. No. B 10. Male, newborn. Body length 75 cm, body weight 6.6 kg. Neonatal line not yet formed. No cementum.
B. No. DR 44. Female, body length 132 cm, body weight 30 kg, four complete growth layer groups in dentine; the fifth under development.
C. No. CN 153. Male, body weight 56.5 kg, eight growth layer groups in dentine. Excessive cementum growth on the tip of the tooth.
D. No. 071. Female, body weight 56 kg, eight growth layer groups in dentine. Osteodentine in the proximal part of the tooth (arrow).
E. B 5. Male, body length 140 cm, body weight 38 kg, three prominent layers in the cementum. Dentine.

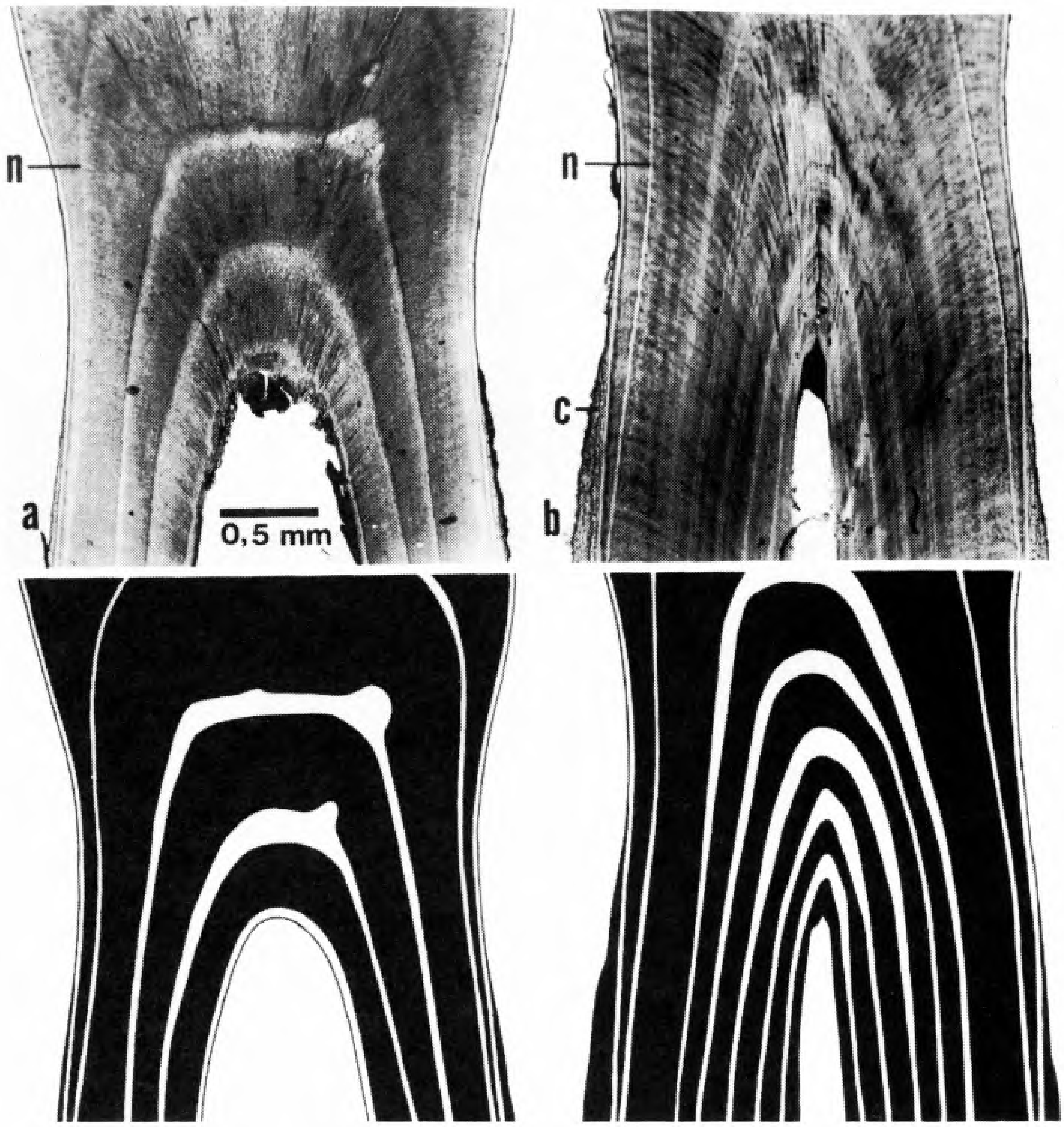

Fig. 7. 30-μm-thick longitudinal section of decalcified and stained teeth of *Phocoena phocoena* from the NW Atlantic with schematic interpretations of layer patterns. Abbreviations defined in Fig. 11.
A. No. A 19. Three growth layer groups.
B. No. CN 150. Male, body weight 50 kg, six growth layer groups in dentine.

able layer with about ten vague accessory layers in it. The *neonatal line* is visible as a thin unstainable layer bounded by narrow stainable layers. The first postnatal dentinal layer is stainable, but in successive layers the stainability generally decreases and irregular accessory layers become conspicuous. In this species, dentine deposition appears to cease early in life (Kasuya, 1978). The reading of dentinal growth layer groups would not seem to be useful for age determination in *P. dalli*. *Cemental growth layer groups* are usually observed most clearly at the level of the proximal end of the prenatal dentine as alternations of faint stainable and unstainable layers. Individual variation of cementum thickness is great, and thin accessory layers are often found in the thicker growth layer groups. Recognition of the accessory layers is often difficult, but by careful scanning along the cementum it may be possible to distinguish accessory layers from growth layer groups.

f. Layering in bone

Some but not all of the six observers could see layering in the thin sections of the mandible and bulla in both undecalcified and decalcified and stained tissue. Exact counting of layers was difficult, because the sections were too thick. Sufficiently thin sections (15 μm) of decalcified tissue could not be made due to limited capabilities of the freezing microtome (cryostat) available. Further investigation is necessary to assess if growth layer groups in the mandible and bulla are useful for age determination in *P. phocoena*. For mandibles, stained sections of decalcified tissue were better than undecalcified sections. For the bulla, no agreement on preferable technique was reached.

g. Cross-reading experiments

i. Harbour porpoise

A set of tooth sections from ten animals was selected from each of the three geographic regions (northwest Atlantic, northeast Pacific, and northeast Atlantic). Biological data were available for some of the northeast Atlantic animals during the session. The three sets of teeth were read by six individuals of varying experience in reading growth layers in teeth (one had no experience). The sets were read twice, before and after discussion of the general composition of a growth layer group. Results of readings are listed in Tables 21 and 22 (pp. 36-7).

The available material from the northwest Atlantic consisted of unstained thin sections of undecalcified teeth. The sections were not satisfactory from a technical point of view, because the pulp cavity was not properly exposed. The six observers agreed that correct counts could not be achieved on this material.

The material from the northeast Pacific (Table 21) consisted of ground half-teeth and the material from the northeast Atlantic (Table 22) of stained sections of decalcified teeth. Readings for both showed good agreement between observers, although some teeth were more difficult to read than others. Stuart reported having found better agreement of repeated readings of half-ground teeth with use of a dissecting microscope of higher optical quality than those provided at the workshop, in conjunction with optical-fiber lighting. No significant increase in range with increasing number of growth layer groups was observed. Discussion of reading technique did not seem to improve readings of dentinal growth layer groups.

The section for one animal in the sample from the northeast Atlantic was judged nonreadable by three observers. This particular animal was held in captivity for 39 months.

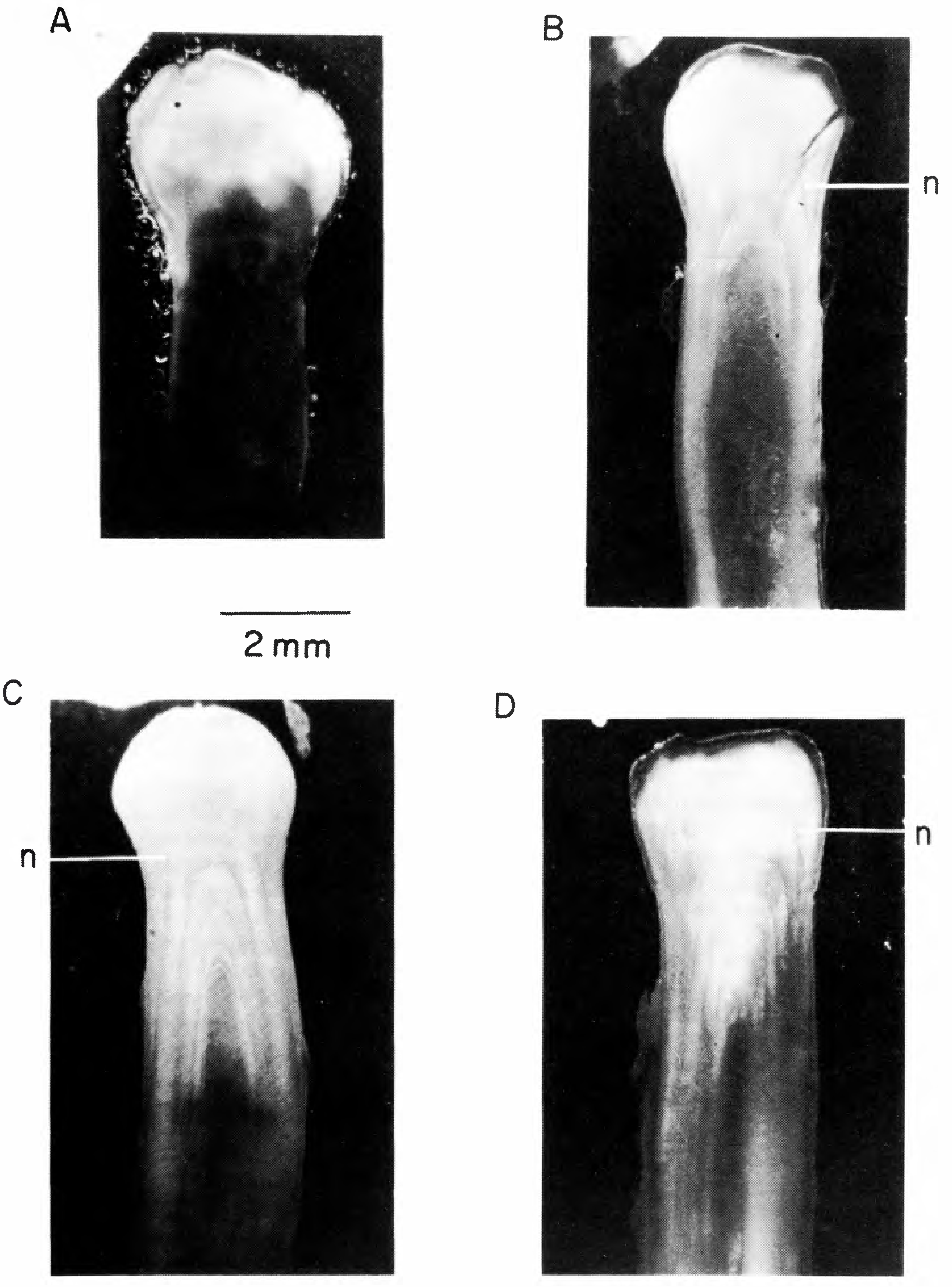

Fig. 8. Ground half-teeth of *Phocoena phocoena* from the NE Pacific. Abbreviation: n. neonatal line.
A. No. CAS 15949. Female, body length 99 cm. Neonatal line formed but difficult to see in this photograph. Part of opaque layer in first growth layer group has been formed.
B. No. C 35. Male, body length 136 cm, body weight 31.9 kg, two complete growth layer groups formed.
C. No. CAS 16603. Male, body length 146 cm, body weight 36.74 kg, four complete growth layer groups formed.
D. No. UCB 21509. Sex unknown, six complete growth layer groups formed 29.

Table 21

Counts by several observers of growth layer groups in the dentine of ten harbour porpoises, *Phocoena phocoena*, from the northeast Pacific

Counts in ground half-teeth before (a) and after (b) group discussion of counting procedures

Specimen No.	Sex	Body ln.(cm)	GLGs in dentine							
			HNG*	JBK	TK	MCS	LJS*	IC	Range	Ave.
CAS16603 a	M	146	4.5	3.5	4	4	5	4	3.5–5	4.2
b			4	3.5	3	6	3.5	4	3–6	4.0
CAS12769 a	F	163	6.5	6.5	5	4	6.5	4	4–6.5	5.4
b			6.5	6.0	5	8	5	6.5	5–8	6.2
SJSU1514 a	–	–	1.5	1.0	1.5	1.5	2	1.5	1–2	1.5
b			1.5	0.5	1	0.5	1.5	0.5	0.5–1.5	0.9
CAS13931 a	M	132	3.5	2	3	3	5	2.5	2–5	3.2
b			3.5	2.5	3	2.5	3	2.5	2.5–3.5	2.8
CAS13930 a	M	141	3	3	5	–	6	–	3–6	4.3
b			4	2+	4	3.5	4	(4)	3.5–4	3.9
CAS16572 a	F	137	2.5	2.5	3.5	3	3.5	3	2.5–3.5	3.0
b			2.5	2.5	3.5	3	3.5	3	2.5–3.5	3.0
CAS13923 a	F	78	0	0	0.5	–	0	0	0–0.5	0.1
b			0	0	0.5	0	0	0	0–0.5	0.1
CAS15671 a	F	–	0.5	0.5	1	1	1	0.5	0.5–1	0.8
b			0.5	0.5	1	1.5	0	0.5	0–1.5	0.7
CAS15987 a	F	158	6	3.5	6	–	6	–	3.5–6	5.4
b			6	4.5+	6	5	7	4.5	4.5–7	5.7
CAS15948 a	M	111	1	0.5	1	0.5	1	0	0–1	0.7
b			1	(0.5)	1	1	1	0.5	0.5–1	0.8

The first year in captivity, it suffered from various ailments including pneumonia, and it is suggested that the reason for lack of variation in dentinal structures during that period might be due to either pathological phenomena or disturbance of normal dentine deposition during a rather long treatment with tetracycline (given daily for two periods of ten consecutive days with an interval of 29 days).

In the material from the northeast Atlantic cemental layers were also counted (Table 22). The group found that it was difficult to assess the number of growth layer groups in the cementum, and the range of the readings indicates that in the present material (30 µm sections) precise counts were not possible. Cemental layering should be further investigated using thinner sections (12 to 15 µm).

ii. Dall's porpoise

Tooth sections from ten animals were selected and the cemental growth layer groups counted by six observers before and after discussion of general composition of a growth layer group. Biological data for these animals were not available during the workshop session. Results of the two readings are given in Table 23. Although discussion of reading techniques increased the accuracy of multiple observer readings, repeated counts made by the same person showed as much as a 20% change. The number of growth layer groups in cementum appears to be not sufficiently reliable for age determination in *P. dalli* at present.

h. Readability of specimens in relation to preparation technique

In order to compare readability of the growth layer groups in teeth prepared by the three different techniques (half-ground teeth, thin sections of undecalcified teeth, and stained sections of decalcified teeth) three teeth from a specimen of *P. phocoena* of known minimum age, 22 months, were prepared differently and read by six observers. Results of readings are listed in Table 25. The range of the counts were equal for the three teeth, and there was little difference between counts of each reader. The results of this test are of questionable value, however, because the readers knew that the teeth were from the same animal before reading.

i. Rate of deposition of growth layer groups

i. Known- and minimum-age specimens

Six observers counted dentinal growth layer groups in specimens of *Phocoena phocoena* from the northeast Atlantic considered to be of minimum known age (Tables 24 and 26). From the data of capture and length of the animals, all four were estimated to be born in the year previous to the year of capture. Based on the reading results, there is substantial evidence that deposition of one growth layer group per year occurs in the first two years of life. This is in accordance with the findings of Nielsen (1972) and Gaskin and Blair (1977). There were some disagreements among readers in determining when a full growth layer group is present,

Table 22

Counts by several observers of growth layer groups in the dentine and cement of ten specimens of *Phocoena phocoena* from the northeast Atlantic

Counts in sections of decalcified and stained teeth before (a) and after (b) group discussion of counting procedures

Specimen No.		Sex	Body ln.(cm)	GLGs in dentine								GLGs in cement						
				HNG*	JBK	TK	MCS	LJS*	IC	Range	Ave.	HNG*	JBK	TK	MCS	LJS	Range	Ave.
A18	a	F	–	0	0.5	1	0.5	0.5	0.5	0–1	0.5	0	–	0.5	0	0	0–0.5	0.1
	b			0.5	0.5	0.5	0.5	1.5	0.5	0.5–1.5	0.7	0	–	0	0	1	0–1	0.2
B7	a	F	127	1.5	1.5	1.5	1.5	2	1.5	1.5–2	1.6	2	2.5	2	2	2	2–2.5	2.1
	b			1.5	1.5	1.0	1.5	1.5	1.5	1.0–1.5	1.4	2	(3)	2	1.5	1	1–3	1.9
B1112F	a	M	137	1.5	1.5	2	1.5	2	2.5	1.5–2.5	1.8	2	1.5	2	2	3	1.5–3	2.1
	b			1.5	1.5	1.5	1.5	2	2	1.5–2	1.7	2	5	4	2	2	2–5	3.0
TIM	a	M	–	1.5	2	3	2.5	–	2	1.5–3	2.2	1	–	3	–	–	1–3	2.0
	b			1.5	2.0	2	2	2	2	1.5–2	1.9	1	2	4	2	1	1–4	2.0
A19	a	–	132	3.5	3	3.5	3.5	2	3	2–3.5	3.1	2	–	3	–	3	2–3	2.7
	b			3.5	3	3.5	3	3.5	3	3–3.5	3.2	2	3	5	3	3	2–5	3.2
Dr.44	a	F	152	4.5	4.5	5	4.5	5	4.5	4.5–5	4.7	4	4	5	3	5	3–5	4.2
	b			4.5	4.5	5	4.5	4.5	4.5	4.5–5	4.6	4	8	7	4	6	4–8	5.8
B6	a	F	138	4	2	4	4.5	–	4.5	2–4.5	3.8	4	3.5	4	–	5	3.5–5	4.1
	b			4	–	3.5	–	–	4.5	3.5–4.5	4.0	3	7	7	–	4	3–7	5.3
B3	a	M	161	3.5	3.5	6	4	3	4	3–6	4.0	3	3.5	6	3	3.5	3–6	3.8
	b			3.5	3.5	4	3.5	3.5	4	3.5–4	3.7	3	5	–	(2.5)	5	2.5–5	3.9
B2	a	F	–	5.5	5	8	5.5	4.5	4.5	4.5–8	5.5	5	–	9	5	5	5–9	6.0
	b			5.5	4	5	5	4.5	4.5	4–5.5	4.8	5	5	7	(4)	6	4–7	5.4
CW153	a	M	–	8.5	8	–	8	7	7.5	7–8.5	7.8	8	7	–	6	5	5–8	6.5
	b			8.5	5+	9	8	8.5	7	7–9	8.2	8	6	8	(7)	8	6–8	7.4

Table 23

Counts by several observers of growth layer groups in the cement of teeth of 10 Dall's porpoises, *Phocoenoides dalli*

Counts before (a) and after (b) group discussion of counting procedures

Specimen No.		Sex	Body ln.(cm)	GLGs in dentine							
				HNG	JBK	TK*	MCS	LJS	IC	Range	Ave.
131	a	M	166	6	0.5	6	3	5	5	0.5–6	4.3
	b			6	5	4	4	6	4	4–6	4.8
138	a	M	178	5	(0.5)	2	2.5	3	3	0.5–5	2.7
	b			5	3	5	1	6	4	1–6	4.0
133	a	F	184	1	–	3	0.5	5	–	0.5–5	2.4
	b			2	–	5	–	(3)	–	2–5	3.3
140	a	M	171	0	0	2	0	0	–	0–2	0.4
	b			2	–	1	0	0	–	0–2	0.8
151	a	F	178	(0)	–	7	0	5	5	0–7	3.4
	b			–	(2)	7	–	5	–	2–7	2.7
182	a	M	186	1	0.5	6	–	7	7	0.5–7	4.3
	b			4	6	4	–	6	4	4–6	4.8
183	a	F	162	1	–	3	2	5	7	1–7	3.6
	b			4	4+	4	4	4	0	0–4	3.2
72–284	a	F	110	0	–	0	–	0	–	–	0.0
	b			0	(1)	0	0	0	0	0–1	0.2
155	a	M	176	5	0.5	8	2	7	6	0.5–8	4.8
	b			6	5	8	5	8	6	5–8	6.3
163	a	M	188	1	–	3	0	–	–	0–3	1.3
	b			2	5	5	5	2	1	1–5	3.3

but this seemed to be due to slightly different reading techniques.

ii. Tetracycline-marked specimens

Teeth from two labelled individuals of *Phocoena phocoena* (Table 27) were ground to 60 μm (longitudinal sections). Under reflected light the three fluorescent lines previously seen clearly in a transverse section from the first specimen using transmitted light (Nielsen, 1972) were very faint. In the tooth from the second specimen, no fluorescence from tetracycline could be seen. Failure of the tetracycline marks to show clearly during the workshop probably was caused by lack of suitable filters.

NOTE: After the workshop, both sections were examined by Grue under transmitted ultraviolet light with filters BG 12 and 530. A marked difference in distinctness of the fluorescent lines was seen in the first specimen, being most clear under transmitted light. As noted above, GLGs were not clearly delineated in this tooth, neither in decalcified and stained sections nor in undecalcified thin sections, thus it is not possible to establish the exact relationship between GLGs and tetracycline marks in this specimen. The section from the second specimen under transmitted light showed one very clear fluorescent line corresponding to the first treatment, whereas the second treatment given three months before death showed faintly. The second optically translucent layer was under development when the animal died, and both marks are located in this layer, the first at the beginning of the layer and the second near the pulp cavity. The drug was administered 180 days and 70 days before the animal died, indicating that the translucent layer takes at least 110 days to form.

j. Recommendations

i. The best preparation technique and method of counting layers in hard tissues should be established for all species individually in this group.

ii. Further examination of bullae and mandibles by means of stained sections of decalcified tissue should be made for comparison of layering in these bones with dentinal and cemental layers. This is especially important for *Phocoenoides dalli.*

iii. The possibility that early cessation of dentine deposition occurs while cementum deposition continues throughout life, as suggested by Kasuya (1978) for *Phocoenoides dalli,* should be investigated for other species in the group. For this purpose vital staining would be of value.

iv. For vital staining of animals in which growth layer groups are studied in decalcified tissue, marking of the organic phase is required. The procion dyes seem to be suited for experiments in this field. (For references see Catalog of Techniques.) Marking of the organic phase would make it possible to intercalibrate layering in dentine, cementum, mandibles and bullae with the actual age of the animal.

v. For maximum readability of growth layer groups in thin sections of undecalcified teeth use of water or glycerine as a mounting medium is recommended. Canada balsam and *Permount* should be avoided, as they may reduce readability.

Table 24

Data for five minimum-age specimens of *Phocoena phocoena* available at the workshop

		At capture			At death			Tetra-cycline
Specimen No.	Sex	Body ln.(cm)	Weight (kg)	Est. age (mo.)	Body ln.(cm)	Weight (kg)	Est. age (mo.)	Marked ?
'Gulle' B6	F	114	23	11	152	57	51	Yes
'Tim'	M	–	–	12	–	–	25	No
'Snoopy'	M	111	26.0	12	127.5	33.5	23	Yes
B11	M	118	29.9	10	137	37	18	No
B7	F	115	36.6	10	127	33.6	18	No

vi. Neither Nielsen (1972) nor Gaskin and Blair (1977) found that cemental layering provided a predictable criterion for age determination in *Phocoena phocoena*, as no definite correlation between number of growth layer groups in dentine and cementum could be found. Further investigation of decalcified and stained sections of teeth no more than 12 to 15 μm thick is recommended, however, before a definite rejection of the use of cementum structures for estimating age in *P. phocoena* is made.

vii. The etching technique shows promise for age determination in this group. A broad range of treatments in varying combinations of acids and hypochlorite and in EDTA should be carried out experimentally.

D. GENERAL RECOMMENDATIONS

In addition to the specific recommendations included above in the reports of the sessions on particular taxa and the detailed technical recommendations below in the Catalog of Techniques, the following general recommendations were agreed upon by the group.

1. Ideally, at the start of any large-scale investigation, a reference collection of teeth from an ontogenetic series of near-term fetuses, neonates, immature and mature animals, preferably of both sexes, should be available.
2. Examinations of materials from specimens of known age and known minimum age are an important approach to calibration of hard-tissue layering with time. Such materials and associated data as are available should be collected and sequestered by a few specific repositories where they may be examined upon request by investigators. Two such repositories suggested by the group are the Sea Mammal Research Unit, Cambridge, and the Southwest Fisheries Center, La Jolla, California.
3. For known-age and known minimum-age specimens, there should be cross-checking of growth layer group counts between as many experts in age determination as possible

Table 25

Counts by several observers of growth layer groups in teeth of a specimen of *Phocoena phocoena* ('Snoopy') prepared with three different techniques

Estimated age is 22 months (Table 24)

		GLGs in dentine							
Tissue	Preparation method	IC	MCS	JBK	LJS*	HNG*	TK	Range	Ave.
Dentine	Decalcified and stained section	2	2	1.5–2.5	2	1.5	2	1.5–2.5	1.9
	Half-tooth	1.5	2	1.5	2	1.5	2.3	1.5–2.3	1.8
	Untreated section	1.5	2	–	2	1.5	2.5	1.5–2.5	1.9
Cement	Decalcified and stained section	1	1	1	2	1	2	1–2	1.3
	Half-tooth	1	2	2	1	2	1	1–2	1.5
	Untreated section	2	3	3	3	3	2	2–3	2.7

Table 26

Counts by several observers of growth layer groups in teeth of five minimum-age specimens of *Phocoena phocoena* in Table 24

	GLGs in dentine							
Specimen name or no.	IC	TK	JBK	MCS	LJS*	HNG*	Range	Ave.
'Gulle'	4.5	3.5	–	–	–	4	3.5–4	4.0
'Tim'	2	2	2	2	2	1.5	1.5–2	1.9
'Snoopy'	2	2	1.5–2.5	2	2	1.5	1.5–2.5	1.9
B11	2	1.5	1.5	1.5	2	1.5	1.5–2	1.7
B7	1.5	1.0	1.5	1.5	1.5	1.5	1.0–1.5	1.4

Table 27

Data for two specimens of *Phocoena phocoena* marked with oxytetracycline at the Marine Bio-Acoustic Investigations, Strib, Denmark

					Tetracycline treatment			
Specimen name	Date	Est. age (mo.)	Body ln.(cm)	Weight (kg)	No.	Dose (mg/kg)	Length (days)	
'Gulle' B6	7 May 66	11	114	23	–	–	–	(captured)
	19 Jul. 66	13	–	–	1	~90	10	
	8 Sep. 66	17	121	31	–	–	–	
	Oct. 66	18	–	–	2	~65	10	
	17 Nov. 66	19	126	44	–	–	–	
	22 Jun. 67	27	144	45	–	–	–	
	27 Jul. 67	28	–	–	3	~80	15	
	5 Aug. 69	51	152	57	–	–	–	(died)
'Snoopy'	5 Jun. 71	12	111	26.0	–	–	–	(captured)
	6 Jul. 71	12	–	31.8	–	–	–	
	12 Aug. 71	13	119	29.4	–	–	–	
	21 Oct. 71	16	120	37.0	–	–	–	
	2 Nov. 71	16	–	–	1	20.3	–	
	24 Feb. 72	20	–	–	2	20.3	–	
	26 Apr. 72	22	128	40.0	–	–	–	
	28 Apr. 72	22	–	39.0	–	–	–	
	29 Apr. 72	22	–	37.0	–	–	–	
	1 May 72	22	–	35.8	–	–	–	
	5 May 72	22	127.5	33.5	–	–	–	(died)

and using as many preparation techniques as are appropriate.

4. Young wild animals should be captured, measured, tagged and released, to provide long-term information on growth rates. If possible, a tooth should be collected when the animal is tagged and measured.

5. Examinations of tetracycline-engendered marks in layered hard tissues rank among the most useful methods in evaluating layer accumulation rates. Biochemical time-marking coupled with periodic harvesting of teeth may yield the critical data necessary for calibration of dentinal layer groups and for cross-calibration of dentinal, cemental and periosteal layering. Efforts should be made to utilize captive animals in tetracycline-marking and tooth-harvesting experiments. This method is particularly suitable for captive animals but could also be applied to wild tagged animals. Further, veterinary records of scientific and commercial aquaria should be researched to identify tetracycline-marked animals, dosages, treatment dates and dates of death. If such marked material is located, negotiations with its custodian should be initiated in order to obtain samples and data for the scientific community. Oceanaria should be encouraged to keep detailed clinical records on each animal injected with antibiotics and other materials capable of being used in marking hard tissues. Any odontocete kept in captivity for a period of more than one year, or receiving any injection of antibiotic or other marker, should have teeth extracted and retained for scientific study upon death in captivity.

6. There is need for further histological and physiological studies of types not usually carried out by investigators of life history, including:
    a. Detailed ultra-microscopic examination of the structure of dentine, with special reference to changes caused by acid etching and other treatments.
    b. Investigation of the histochemical origins of irregularities in growth layer groups and their possible correlation with biological cycles and events in the life of the animal.
    c. Investigation of histological correlates in layered structures of teeth and bone.

7. The study of the racemization rate of aspartic acid in teeth and eyes of cetaceans (Bada *et al*, this volume) has promise as a useful method to develop age-determining techniques. Efforts should be made to get known age specimens into the hands of investigators exploring this technique.

8. As the report of the IWC Age Determination Meeting, Oslo, 1968 (IWC, 1969) suggested, workers in age determination of sea mammals should investigate the use of radio-active tracer techniques, based upon the incorporation of fission, fallout radio-nuclides, such as strontium 90. These may give a dateline in teeth of suitable age with dentine layers formed in the 1950s.

E. CATALOG OF TECHNIQUES

1. SELECTION, COLLECTION, STORAGE AND PRELIMINARY EXAMINATION OF TEETH

a. Selection

Cetaceans are largely homodont, so some of the factors causing problems in selection of teeth in other mammals (Morris, 1972) do not apply for most species. However, in general, teeth should be selected which are as little worn (the presence of some enamel or fetal dentine is desirable) and as straight as possible. Teeth selected for examination of cemental layering can be worn at the tip. In dolphins and porpoises, the best teeth (largest and intact) are usually those at the center of the lower tooth row. In sperm whales the first mandibular tooth is usually selected when the tooth crown is not worn. However, in whales which have obviously worn mandibular teeth, it is preferable to collect a straight erupted tooth or unerupted maxillary tooth as well. In the narwhal, the commercial value of the erupted tusk usually necessitates the collection of unerupted tusks. When beginning work with a species that has not been well studied previously, samples of teeth from throughout the jaws should be surveyed for several individuals of differing age and sex before teeth in a particular position are settled on for the overall study.

b. Collection

Prolonged boiling of teeth should be avoided if possible, as this denatures protein and may damage the dentine and cementum. If necessary, large teeth can be cleaned with proteolytic enzymes (Luther, 1949) or an ultrasonic cleaner (Spence and Tonkinson, 1969). Mead and Boyde report that a tooth of *Mesoplodon* sp. cleaned with trypsin was much easier to process and read than other *Mesoplodon* teeth which had been boiled.

c. Storage

Labelled teeth should be stored in 70% ethanol. Teeth should never be stored dry, because they crack, nor in solutions containing glycerine, which is messy and difficult to remove. Prolonged storage in unbuffered formalin is not recommended. It decalcifies the teeth and may inhibit staining.

d. Preliminary examination

Teeth should be examined externally and measured before they are cut. Signs of wear, the state of the pulp cavity, the state of the surface and external growth layers (if any) should be noted. It may also be useful to photograph teeth at this stage as a permanent record.

2. PREPARATION AND EXAMINATION OF TEETH FOR AGE DETERMINATION

Although growth layers may be visible on the external surface of some teeth, it is generally necessary to use either longitudinally bisected half-teeth, or longitudinal thin sections for age determination.

a. Equipment for cutting and sectioning teeth

i. Hacksaw

Sperm whale teeth can be held in an ordinary vice and cut with a hacksaw, but this method yields a very rough surface and should not be used if a diamond saw or band saw is available.

ii. Diamond saws

A variety of diamond saws can be obtained from lapidary supply companies. The size of the saw to be used depends on the size of the tooth. The *Buehler Isomet* low-speed saw has been found extremely satisfactory for most small teeth. Blades are available from 3 in to 5 in (76 mm to 127 mm) in diameter. The 3-inch diameter blade is 0.006 in (0.153 mm) thick, and the 4-inch diameter blade is 0.012 in (0.305 mm) thick. The saws are fairly expensive, but a similar saw was made in the Geology Department, James Cook University, Queensland, Australia, for about US $50.00 in 1977. The *Buehler* blades are thin, yet rigid and are sintered. The diamonds are impregnated in a continuous rim running about ¼ in (0.64 cm) down the sides of the blade, so that the blade polishes as it cuts. Extra grinding or polishing may not be necessary. Tandem blades separated by shims of the desired section thickness can be used to cut a section directly, but this results in a section too thick for some microscopic studies.

Larger saws are needed for larger teeth. Lockyer cuts mandibular teeth from sperm whales longitudinally along the mid-line through the crown and apex of the pulp cavity, using a diamond saw with a 3-phase electrical motor. Very large and/or curved teeth are cut using a commercial-type band saw. The teeth are held in a specially designed vice which holds the tooth vertically in relation to the direction of rotation of the blade. Details of construction of the vice can be obtained from the Sea Mammal Research Unit, Cambridge, England.

In cutting sperm whale teeth, Nishiwaki first marks the central axis on the outside of the tooth for the cutting line.

Marsh embeds dugong teeth in the desired orientation in blocks of ice before cutting with a 10 in (25.4 cm) diameter diamond saw. The flat bottom of the block of ice can be braced against the movable carriage of the saw to maintain the preferred orientation.

The large erupted tusks of the narwhal are sawn longitudinally on a diamond saw with a movable carriage on which the tusks are mounted. A quarter-round wedge is cut from each tusk, to preserve the shape and overall appearance of the tusk for subsequent display. About ten hours are required to cut each tusk (Kemper and Mitchell, this volume).

iii. Band saws

A commercial band saw may be the most suitable for cutting large curved teeth.

iv. Freezing microtomes

Most workers seem to prefer freezing microtomes to cryostats for sectioning decalcified teeth or bone. Grue recommends a microtome manufactured by *Reichert-Jung*. It is usually possible to buy a carbon dioxide-freezing stage for attachment to a heavy duty sledge microtome, e.g. by *Leitz*. Wedge-shaped knives should be used for bone and tool-edged for teeth.

v. Special microtomes for cutting undecalcified teeth or bone

Saw microtomes, e.g. by *Leitz*, are available to cut sections from 50 μm to 100 μm thick or thicker from undecalcified teeth or bone. *PMW* manufactures a microtome which can be used to cut undecalcified sections 1 μm to 999 μm thick from specimens of maximum length 450 mm, maximum width 150 mm.

b. Preparation of half-teeth

Teeth which have been bisected longitudinally through the pulp cavity can be subjected to a variety of techniques in order to count growth layer groups, usually in the dentine. Half-teeth can be prepared by grinding or sawing. The cut surface is usually polished or etched before examination.

i. Polished preparations

Stuart uses the following method to prepare mandibular teeth from *Phocoena phocoena*:

Teeth are mounted on petrographic microscope slides (which are easier to handle for grinding than are biological slides, because they are shorter and wider) by heating *Lakeside 70* thermoplastic resin on the slides and placing the tooth on the resin before complete cooling and hardening occurs. The slide is held while the tooth is ground by hand. The tooth is ground to near its center with silicon carbide 600 grit powder with water. Aluminium oxide 302½ abrasive powder is then used to polish the tooth to the center of the pulp cavity, while checking frequently to make sure that the center is not missed. This process takes five to ten minutes. For older teeth in which the pulp cavity is very small, it takes longer to reach the center, because more checking with a binocular microscope is needed to insure that the pulp cavity is not lost. The polished surface is examined under a binocular microscope. A film of water on the surface of the half-ground tooth and/or oblique illumination increases visibility of growth layers. This method is suitable for reading dentinal layers, but it should not be used for counting cemental layers.

ii. Etched preparations

The method used to cut the tooth depends on the size and shape of the tooth (Section 2.a). It is generally necessary to polish the cut surface before etching. Wet and dry sandpaper (400 grade or finer) or a flat whetstone is recommended.

A variety of demineralization reagents can be used to etch the tooth, and experimentation with reagents and times is suggested for initial work with a particular species. Examples of successful techniques follow (user's names in parentheses).

Sperm whale (Lockyer). The sanded half-tooth is placed cut surface down in 10% formic acid for 30 hours at room temperature. The surface is kept clear of the base of the container by means of small pegs, and the acid is poured in to cover about 0.5 cm to 1.0 cm of the sides of the tooth. After pouring the acid, the container is agitated briefly to clear air bubbles.

Narwhal (Kemper). Thirty per cent phosphoric acid or a 10% solution of HCl is applied with a sponge to the cut surface for one hour at 20°C. Shorter durations are inadequate. The surface is kept wet by continuous application of the acid.

Dugong (Marsh). Half-teeth are immersed in 5% HCl in 70% ethanol for three hours at room temperature. Mitchell (1978) obtained good results by immersion in 5% formic acid for 1½ hours at room temperature.

Beaked whales (Marsh). The cut surface is etched for two hours in 10% formic acid at room temperature. Etching in 5% HCl for two hours has been found to be unsatisfactory.

Pilot whales (Mead). Two hours in 10% formic acid at room temperature etches the teeth satisfactorily. Boyde and Jones (1974) suggested that etching times can be reduced if the demineralization front is subsequently removed with 5% sodium hypochlorite. Preliminary experimentation with this technique done by the sperm whale group and the porpoise group indicated that the demineralization time is indeed reduced; more experimentation is needed before a schedule can be recommended. Boyde also suggests the use of 5% lactic acid and 0.5 M EDTA at pH 7.4 as etching reagents, preferably followed by treatment with sodium hypochlorite.

Harbour porpoise (Stuart). Treatment for 80 minutes in EDTA was tried during the workshop, but little effective etching was accomplished. Much longer treatment is recommended.

After etching, the tooth can be washed in running water to remove the etching reagent. Brain (1966, p. 143) detailed a procedure for testing for the removal of acid reagents from a specimen. The tooth must then be dried thoroughly, cut surface uppermost, at room temperature for several hours. The etching agent is volatile and evaporates. The layers in the tooth are then thrown into a relief of ridges and troughs.

Rubbing the surface with pencil or crayon perpendicular to the ridges is recommended for emphasizing the relief in large teeth, e.g. those of sperm whales and dugongs. Jeweler's rouge, because of its smaller grain size, may be preferable for smaller teeth (Pierce and Kajimura, this volume).

Metal-coating, e.g. with gold-palladium used in preparation of material for scanning electron microscopy, has been used with partially encouraging results on the teeth of *Phocoena phocoena* (Session 6 report above).

The type of microscope needed for counting the growth layer groups depends on the size of the tooth. Magnification may not be necessary; e.g. most sperm whale teeth can be read with the naked eye. A hand lens or a binocular microscope may be used. Obliquely reflected light is desirable, whatever the magnification.

iii. Use of acetate peels

A permanent record of the etched pattern of ridges and grooves may be made by lifting the graphite or rouge off the tooth with transparent adhesive tape. Alternatively, an 'acetate peel' can be made by applying acetone to the etched surface and allowing a thin acetate sheet (obtainable from suppliers to architectural draftsmen) to melt over the ridges and grooves of the etched surface. After the acetone has evaporated, the sheet bears a negative copy of the etched surface.

c. Preparation of undecalcified unstained sections

The method used varies with the size of tooth and the equipment available. Intending workers should consult the literature on preparing undecalcified bone sections, e.g. Sissons (1968) and Wallington (1972). Sections are generally 30 μm to 300 μm thick and should be longitudinal and through the centre of the pulp cavity.

i. Sections prepared by grinding

Kasuya prepares ground sections of small teeth directly using whetstones (1,200 grit for grinding, 4,000 grit for polishing) or waterproof sandpaper (400 grade) for grinding and fine carborundum powders on a glass plate or two pieces of plate glass roughened with abrasive (Sissons, 1968) for polishing. Special lapping machines have also been developed for tooth or bone sections, e.g. see Massey and Malcolm (1976). Section thickness can be checked using the focusing dial on a microscope, an engineer's micrometer or dial gauge or a paper thickness gauge.

ii. Sections prepared by sawing and grinding

Small teeth may be attached to a wooden block using dental impression compound (e.g. *Kerr's Greenstick,* available from all dental supply houses) or a contact adhesive. Special chucks have been developed for use with the *Buehler Isomet* saw. Although small teeth may be embedded in thermoplastic (such as *Lakeside 70*) or epoxy resin prior to cutting (Sissons, 1968; Hill, 1973), this can make orientation of the cut difficult.

Using a diamond saw (see 2.a. above), each tooth is bisected longitudinally and a section taken from the cut surface. Sections as thin as 30 μm to 50 μm can be prepared this way. In the case of large teeth, such as those from some beaked whales, each section can be further bisected longitudinally for ease of further processing. Section thickness recommended by Kasuya for observation of dentinal growth layer groups follows:

Globicephala macrorhynchus	40 µm to 80 µm
Tursiops truncatus	40 µm to 60 µm
Peponcephala electra	50 µm to 60 µm
Stenella attenuata	40 µm
Stenella coeruleoalba	40 µm
Pontoporia blainvillei	30 µm
Dugong dugon	60 µm to 90 µm

One side of the section is then polished in water, e.g. with whetstones (1,200 and 4,000 grit). The dried, polished surface is mounted on a 1 mm thick Perspex slide using quick-drying cyano-acrylate monomer glue. The mounted section is then ground down, using the whetstones, to the desired thickness. Alternatively, the cut surface of the half- or quarter-tooth can be polished and glued to the slide before the thin section is cut. Special chucks are available to hold a slide while a thin section is being cut, e.g. the *Buehler 11 to 1,118* vacuum chuck. In this case a ½ mm thick section can be cut directly and ground down as above. For larger teeth, Kasuya uses *Perspex* slides. With glass slides, when the tooth contracts as it dries or swells when polished in water, it parts from the slide or causes the section, or even the slide, to crack.

iii. Sections prepared by sawing only

If a tandem-bladed diamond saw (see Section 2.a. above) is available, a section of the desired thickness can be cut directly from the whole tooth. In the case of larger teeth sections, they should be cut ½ mm to 1 mm in thickness, as thinner sections tend to buckle. The section can then be ground to the desired thickness (Section 2.c.ii).

iv. Mounting of sections

Sections are usually mounted in a synthetic resin such as *Permount. DPX* is unsuitable except for thin sections. Mounting in glycerine (temporary mount) or other media of low refractive index increases contrast between the layers. Kasuya advises that the adverse effect on readability by balsam or *Permount* is very great for dolphin teeth and strongly recommends use of glycerine or water. For a full discussion of the properties of the various mounting media see Lillie and Fulmer (1976).

v. Examination of sections

Examination with a quality compound microscope is essential for some specimens and for cemental layers. The use of polarized and/or phase contrast facilities and the petrographic microscope can be extremely useful (Myrick, this volume). A variety of microscopes should be tried when beginning a study of untreated sections.

d. Preparation of stained sections of decalcified teeth

Stained sections are almost essential for counting cementum layers, except perhaps in male specimens of *Ziphius* and *Berardius* (Session 1 above). For some species, e.g. small delphinids, they may also be the preferred method of counting dentinal layers.

As the stain colors the ground substance of the tooth, it must be applied to decalcified sections. One of the haematoxylins is usually used without a counterstain, but other reagents which stain protein or acid mucosubstances may be worth investigation (Thomas, 1977).

There are basically two approaches: the section can be prepared first and then superficially decalcified, or the whole tooth can be decalcified and then sectioned. In both cases observations of the growth layers are made with a microscope using transmitted light at powers from 10X to 200X.

Techniques which have been used successfully by participants in the workshop are outlined below. The type of haematoxylin and mountant chosen reflect individual preferences and are not necessarily the only ones suitable. For a full discussion of haematoxylins see pp. 204–8 and of the various mounting media pp. 111–22 in Lillie and Fullmer (1976).

i. Preparation of the section and subsequent decalcification

Kasuya prepares the sections as for undecalcified unstained sections (Section 2.c.) to a final thickness of 10 µm to 15 µm *(Phocoenoides)*, 15 µm to 20 µm *(Stenella)*, 20 µm to 30 µm *(Pontoporia)*, about 30 µm *(Globicephala)* or 40 µm to 70 µm *(Berardius)*. In general, the larger the tooth the thicker the section. The sections are decalcified in 5% formic acid at room temperature (25°C). Time varies with the size of the tooth from several hours for *Phocoenoides* to 24 hours for *Globicephala* and *Berardius*. Kasuya reports that for the latter, good results are obtained by immersion in 10% formic acid for four hours at 20°C. The sections are then washed in running water for several hours or overnight.

The sections are stained in haematoxylin. Kasuya recommends Mayer's haematoxylin for 30 min to 60 min. They are then washed in running water for three to ten hours, dehydrated in a graded series of ethanols, cleared in xylene and mounted in a medium such as Canada balsam or *Permount* (but see 2.c.iv. above). Excess stain can also be removed quickly with acid alcohol (1% HCl in 70% ETOH). The haematoxylin can be blued quickly by immersion in tap water to which a few drops of ammonia have been added. Kasuya uses the disappearance of enamel as an indiciation of complete decalcification. This cannot be used for *Phocoenoides* teeth, in which the enamel does not dissolve in acid.

ii. Decalcification of the whole tooth before sectioning and staining

It is recommended that users of this technique refer to publications which discuss all aspects of the preparation of undecalcified sections. Brain (1966) and Wallington (1972) are highly recommended in this regard. The method is most suitable for small teeth.

The whole tooth is first placed in the decalcifying agent. For a discussion on the relative merits of decalcifying agents see Brain (1966), Lillie (1951), Clayden (1952) and Wallington (1972). If it is desired to retain the organic matrix of the enamel, Brain (1966) recommends decalcification in 4N sodium acetate hydrochloric acid buffer solution, pH 3.5. However, Boyde (unpublished) has found that the residual enamel after decalcification by this method is a poorly soluble calcium phosphate (probably $Ca(H_2PO_4)_2\ H_2O$) and not organic matrix. Klevezal' and Lockyer and Crawley (unpublished) and Grue all recommend 5% HNO_3. Grue recommends that 100 ml of 5% HNO_3 be used per gram of tooth and advises that constant agitation of the decalcifying fluid decreases the time required. The end point of decalcification should be determined by x-ray or chemical means (Brain, 1966, pp. 121–8; Wallington, 1972, pp. 14–5) in the first instance.

Suggested decalcification times using 5% HNO_3 for adult teeth are five hours *fide* Grue for *Phocoena* teeth (fluid agitated), 16 to 20 hours *fide* Klevezal' for *Delphinus delphis* (without agitation), 48 to 60 hours *fide* Lockyer and Crawley (unpublished) for *Lagenorhynchus* (unagitated). In general, times for teeth of adults are much longer than for those of juveniles.

The tooth is next washed in running water, for six to eight hours (Klevezal'), overnight (Grue), or 48 hours (Lockyer and Crawley, unpublished). Brain (1966, p. 143) details a procedure for testing for the removal of acid reagents from a specimen.

The tooth is now ready for sectioning. It can be stored in distilled water for up to 24 hours. Longer storage may decrease stainability. If longer storage cannot be avoided, a crystal of thymol should be added to prevent the growth of mould (Grue).

The decalcified tooth is sectioned with a freezing microtome (Section 2.a.). Sections may be mounted on the chuck with water or a special mounting medium such as *Tissue Teck OTC* medium. The tooth is sectioned longitudinally, and several sections from the centre of the tooth are retained and transferred to distilled water using a brush. Recommended section thickness is 12 μm to 15 μm for cement and no more than 30 μm for dentine.

The sections can then be mounted on slides before subsequent processing, or they can be processed and then mounted, using one of several alternative techniques:

> *Technique of Grue.* The sections are floated on a bath of 5% gelatin in distilled water at 40°C and transferred to an acid-cleaned slide with a brush. The section is pressed flat on a slide, using filter paper and then dried vertically in an oven at 40°C overnight. After cooling to room temperature, the sections are rehydrated in distilled water for 5 min, stained in Mayer's haematoxylin for 35 min to 45 min – depending on the age of the stain (heating stain to 30°C reduces time) – and blued in 1% $NaHCO_3$. After washing in running water for 30 min, the sections can be temporarily mounted in a medium such as *Aquamount*, or they can be dehydrated in graded ethanols, cleared in toluene and mounted in a synthetic resin such as *DPX*. The stained sections should be stored in the dark, although even this may not prevent fading of the stain after several years.
>
> *Technique of Lockyer and Crawley (unpubl.).* Sections of the tooth are adhered with distilled water to a slide coated with 5% w/V gelatin and set on a slide warmer for 48 hours. The slide is then dried for 30 min at 30°C. The sections are stained with newly-ripened Ehrlich's haematoxylin for 90 min, washed carefully with tap water, differentiated in acid alcohol, washed again in tap water, blued in ammonia vapour, and, finally, washed in distilled water. The slide is dried for 30 min, mounted in *Euparal* and placed on a slide warmer for 48 hours.
>
> *Technique of Klevezal' (mounting after processing).* The sections are stained with Ehrlich's haematoxylin (5 min to 30 min). The time of staining depends on the age of the haematoxylin. The older the haematoxylin, the more rapid is the staining and the better is the result. It is recommended to use haematoxylin three months old or older. The stained sections are rinsed with distilled water and washed in running water for 10 to 15 min. They are then passed through a series of glycerine solutions, first in 25% for 5 min, then in 50% for 5 min and finally in 75% for 5 min. All the sections are then examined by eye or under low magnification, and two to three of the best ones (in relation to stain quality and pulp cavity position) are selected for mounting. The selected sections are placed on a slide in pure glycerine and covered with a cover glass. This temporary preparation can be kept in good condition for one to two years.

iii. Staining partly-decalcified teeth

For large teeth, it may be useful to stain partly-decalcified thick sections. In this method, the contrast within each growth layer group caused by the degree of staining is enhanced by the relief produced by etching.

Sections approximately 500 μm thick are prepared as described in Section 2.c.ii., and etched, e.g. for one hour in 5% HCl. After thorough washing, the sections are stained with haematoxylin (e.g. Mayer's), differentiated if necessary in acid alcohol (1% HCl in 70% ethanol), blued in tap water to which a few drops of ammonia have been added, washed and dried. The sections are not covered with a coverslip and are examined with reflected light. Wetting the surface may assist reading.

This method is useful for determining the nature of the last growth layer group in large teeth, e.g. dugong teeth, which are routinely scored from etched half-teeth (Marsh, this volume). In this case, only the area near the pulp cavity need be sectioned.

e. Techniques previously found useful but not tested in this workshop

i. Microradiography

Low voltages are suitable for demonstrating growth layer groups when used in conjunction with fine-grain photographic emulsions. Brain (1966) gives details of how to modify a clinical x-ray unit for microradiography and of its mode of operation.

> Absorption [of x-rays] by the atoms of a particular element is proportional to its atomic number. The absorption by any substance will therefore depend on the types and relative proportions of the atoms present in its molecules (Brain, 1966).

Thus, different tissue components are distinguished by differential absorption where they differ in composition and density. Microradiography can be used to demonstrate the variations in mineral content between the various layers which comprise a growth layer group.

Care should however be taken in attempting to correlate changes in the degree of mineralization (radiopacity) with changes in structure (optical density) or in stainability. Grue doubts that it is advisable to generalize about this from species to species or even from specimen to specimen within some species. Yamamoto *et al* (1962) also found that the staining reaction of cementum with carbol-fuschin does not always correspond with microradiographic observations.

Another caution has to do with the 'absorption-edge problem' when dealing with low kV x-rays. For example, Boyde advises that calcium absorption falls tremendously below 4.037 kV – its critical absorption edge – and the 4.5 kV level used by Grue (Nielsen, 1972), unless very

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NOTE. When using HNO_3 it is important to avoid acid which has developed a distinct yellow color. This is due to the slow formation of nitrous acid, which increases the speed of decalcification and can cause problems with subsequent staining. The discoloration can be minimized by storing the acid in a brown bottle in the dark and completely prevented by adding 2 grams of urea to each liter of acid. The yellowing is only temporarily arrested, and as soon as the yellow begins to redevelop more urea should be added (Clayden, 1952).

carefully measured, is dangerously close to the absorption edge.

A polished thin section is examined in the microradiographic method. Hohn has found that 150 μm thick sections work well for *Tursiops truncatus.* 600 μm thick sections have been used for *Dugong.* For *Phocoena phocoena,* 50 μm thick sections are suitable. As debris from cutting will be exposed during microradiography and possibly block the structure of the section, it should be cleaned with an ultrasonic cleaner.

The unmounted section is placed on the x-ray plate (e.g. *Kodak* high resolution) which is in a vacuum, keeping the thin section in contact with the emulsion during exposure. Hohn uses a *Picker Mini-shot (Model I)* x-ray unit modified for low voltages and long exposures for 90 min at 11 kV and 2.5 mA for *T. truncatus* and for 3 hours at 13 kV and 1.5 mA for *Dugong.* The x-ray source to film distance is 20 cm. Nielsen (1972) used *Phillips MRC,* maximum intensity 12.5 Å, radiation for 90 min at 1 mA and 4.5 kv. The image is examined with a compound microscope and transmitted light.

ii. Scanning electron microscopy (SEM)

Hohn found that teeth from *T. truncatus* which have been bisected longitudinally must be cut in half to yield a sample of the proper size to fit on some SEM stages. The specimen is etched using one of the techniques outlined in Section 2.b.ii. above. It is then washed in distilled water or ETOH and cleaned in an ultrasonic cleaner.

The section is glued on a slide plate as specified in operational instructions for the SEM. Hohn recommends use of *Elmer's* glue and plating with gold-palladium. Low magnifications allow all the growth layer groups to appear on one exposure. Tilting the section to a 45° angle increases the contrast.

f. Cross calibration of techniques

When more than one of the techniques outlined above are used on teeth from animals of the same species, it is important to be conservative about equating the changes as a result of one technique (e.g. stainability) with those seen as a result of another (e.g. ridge and groove pattern, in etching). If it is desired to compare such changes, a masking technique should be used which allows one half of a tooth to be treated with one technique and the other half with the second technique. Boyde *et al* (1978) recommended use of colored cosmetic nail varnish (removable with acetone/amyl acetate) or a solution of polymethylacrylate (*Plexiglas/Perspex*/amylacetate) in chloroform (removable with chloroform) as masking agents.

i. Cross calibration of structures seen in etched and unetched half-teeth

For comparison of structures seen in etched and unetched half-teeth (technique used by Marsh for *Dugong*), the half-tooth is prepared and polished (Section 2.b.i.), and half the surface is coated with histological wax (e.g. *Paraplast*) by dipping in a beaker of wax. A straight edge can be obtained with masking tape. The tooth is then etched, washed and dried (Section 2.b.ii.) and the wax removed with a solvent such as xylene. The etched half of the tooth is then rubbed with pencil to highlight the ridges. The correspondence between the banding seen in reflected light and the ridge-groove pattern can then be observed.

ii. Cross calibration of layers observed in unstained undecalcified sections with those seen in stained decalcified sections

For comparison of growth layer groups observed in untreated thin sections with those seen in stained decalcified sections, a thin section is prepared as outlined in Section 2. Part of the section is masked with wax. Kasuya suggests wax of plant origin with a low melting point. Marsh found that dental wax is superior to paraffin. It is important that the junction of the masked and unmasked areas be a sharp straight line, which should preferably run at right angles to the growth layer groups rather than down the middle of the tooth. Marsh achieves a sharp straight edge by pouring the wax onto the tooth behind a razor blade held at an angle of about 30° to the tooth surface along the desired junction line.

The section is next decalcified in 10% formic acid for two to three hours (longer if necessary), washed well in running tap water and stained with haematoxylin as in Section 2.d.i. To remove the wax, the stained section is passed through the following processes:

(Absolute alcohol) → (Absolute alcohol) → (Xylene) → (Xylene)
→ Mounting in Canada balsam ↓
Mounting in glycerine ← (Absolute alcohol) ← (Absolute alcohol)

A permanent mountant such as *Permount* decreases the contrast of the undecalcified portion of the tooth, particularly in *Stenella* spp. Kasuya improves the contrast by mounting in glycerine or by mounting only the stained portion and leaving the undecalcified portion uncovered. The use of a compound microscope is recommended for examination of the section.

3. PREPARATION OF BONE FOR AGE DETERMINATION

Klevezal' does not recommend the aging of odontocetes using growth layers in bones as a routine method. It can however be used as a subsidiary method.

Processes of bone resorption and remodelling destroy the inner growth layers. Klevezal''s experience suggests that inexperienced readers, using untreated thin sections for bone layer reading, may get a false result because of the orientation of the primary osteonal systems, which can look like layers. It is especially important for workers using bone sections for aging purposes to be familiar with the development and structure of 'plexiform' bone (Enlow, 1963), since surface-parallel slabs could easily be mistaken for growth layers representing regular growth increments. The significance of these surface-parallel slabs for age determination is not known. Decalcified and stained sections are preferred.

The inner wall of the mandible has usually been examined (e.g. Klevezal' and Kleinenberg, 1967). Hay takes bone from between the mental foraminae in the narwhal. Klevezal' suggests that in large mammals such as odontocetes and mysticetes it may be more fruitful to investigate bones which grow with negative allometry with respect to the growth of the animal (Klevezal', 1972).

In specimen preparation 1 cm to 2 cm thick samples of bone are removed with a saw, decalcified, sectioned and stained with haematoxylin.

4. VERIFICATION OF THE TIME INTERVAL REPRESENTED BY THE GROWTH LAYER GROUP

a. Time-marking of teeth

In order to verify the number of growth layer groups laid down per unit of time, a variety of chemicals, such as tetracycline (Nielsen, 1972), alizarin red (Klevezal', this volume), lead acetate (Yagi *et al*, 1963) have been administered to mammals in order to mark the hard tissue laid down at the time of administration.

i. Use of tetracycline

Tetracyclines are the most versatile of all vital dye-markers of teeth and bone growth. They may be administered in every convenient way (intravenously, intramuscularly, intraperitoneally and orally) for a given animal. Even small doses, e.g. 1 mg/kg in the rat (Boyde, unpublished), are enough to mark the growing parts of teeth and bone, making them yellow in ultraviolet light. For a detailed description of use of tetracyclines as markers of growth in bones and teeth see Milch *et al* (1958), Harris *et al* (1962) and Hansson (1967).

The toxicity of tetracyclines is comparatively low and well estimated. After intraperitoneal introduction LD_{50} for mice is 330 mg/kg, for rats, 320 mg/kg. A dose of 20 mg/kg is considered completely harmless. For intramuscular administration, 10 to 20 mg/kg of body weight is recommended by Klevezal', 20 mg/kg by Grue. Klevezal' advises that tetracycline administered intramuscularly is absorbed or excreted within eight hours. At Sea World of San Diego, four common dolphins, *Delphinus delphis,* were treated with good results (Gurevich *et al,* this volume). The animals were given total dosages of 3 gms *Oxytetracycline* 100, via three intramuscular injections administered in one treatment session. Dosages varied from 39 mg/kg to 54 mg/kg. Each injection contained 1 gm of *Oxytetracycline* 100 (50 cc), to which was added approximately 2 cc of 2% lidocaine solution. Injections were administered in the dorsal musculature 5 cm to 10 cm anterior to the base of the dorsal fin, just off midline. To facilitate treatment, animals were removed from their holding tanks and restrained until injections were completed.

Tetracycline can also be given orally, e.g. in fish. Doses higher than those used in injection procedures are required, and there is need for experimentation. Klevezal' reports that orally administered tetracycline is likely to be absorbed over a longer period, e.g. 24 hours, than is injected tetracycline.

A tooth should be extracted immediately before tetracycline is administered, so that a comparison can be made between layering in marked and unmarked teeth to see if tetracycline affects the layering process. This can be done by using a dental tooth extractor on a restrained animal.

The teeth from injected animals should be stored in the dark, dry or in ethanol, *not in formalin,* which is acidic and may demineralize the teeth. Boyde has successfully kept tetracycline-labelled teeth for several years in glutaldehyde in cacodylate buffer, with yellowing of the material but no loss of tetracycline fluorescence. Boiling for five minutes does not affect subsequent fluorescence. As decalcification removes the tetracycline, undecalcified and unstained sections must be prepared as outlined in Section 2.c. The sections are examined in a compound microscope equipped with an ultraviolet illuminator system.

ii. Use of procion red

Procion dye *(Procion red – 88N, Imperial Chemical Industries Limited – (ICI))* is taken up by collagen and so is not lost in the procedures used in conventional histological decalcification techniques (Prescott *et al,* 1968; Golland and Grand, 1968). Procion dyes are not acutely toxic when given intravenously. When given intraperitoneally they produced peritonitis and ascites (Golland and Grand, 1968). The injectable solution is made up of 10% procion red in 0.85% NaCl and filtered through millipore filters with pore diameters of 0.8 μm to 0.22 μm. The solution should be prepared on the day of use, because the dye easily hydrolyzes. 150 mg/kg body weight of procion red has given good results in staining bone and dentine in rabbits and rats and dentine and cementum in cats (Grue). It is injected intravenously.

The dye can be seen in decalcified tissue, sectioned at 7 μm to 15 μm, in ordinary light but will also fluoresce. Use of an HBO ultraviolet lamp with an FITC 6 filter as the excitation filter and the combination 53 and 47 as the barrier filter is recommended.

iii. Use of alizarin

Alizarin red S has been injected intraperitoneally usually in multiple dosages (see Hoyte, 1960). Klevezal' advises that it sometimes causes ascites in rodents. It may be less harmful if injected subcutaneously (Lowe, 1971).

b. Seasonality of layer deposition

If teeth are available from a sizeable sample of animals which died in all or most of the months of the year, indirect evidence of the time interval for the deposition of each growth layer group can be obtained by examination of the nature of the layer being laid down at the time of death and plotting the frequency of layer deposition against month of death. In this case, it is essential that the teeth be read without knowledge of the date of death. This technique has been widely used, e.g. by Ohsumi *et al* (1963); Gambell and Grzegorzewska (1967); Gaskin and Blair (1977); Gambell (1977) and Marsh (this volume).

c. Aspartic acid racemization

Another potential way to determine absolute age is based on the amount of aspartic acid racemization that has occurred in the teeth or eyelens nucleus (Bada *et al,* this volume). For this technique, eye lenses should be collected within 30 hours of death and frozen. Imbedded teeth are best. Teeth should not be boiled.

This method depends for success on knowledge of:

(a) precise temperature of the teeth during life,
(b) date of death of the cetacean providing the tooth sample, and
(c) calibration of 'ratio age' with the true age for some known age animals.

5. APPROACHES TO COUNTING

There was discussion at the workshop about how counting of growth layer groups should be approached. At one extreme, teeth can be read 'blind', that is, with no reference to any of the biological information about the animal from which they came. At the other extreme, teeth can be read in rough age-ranked order, after the animals have been relatively aged with reference to all other information available. In choosing between these approaches, some factors should be taken into consideration, Differences in growth layer group counts between animals of the same sex and of similar body length, can be due to three causes:

(a) blunders – mislabelling of teeth, clerical errors, etc.,
(b) errors in counting, and
(c) biological variation – development variation, individual variation, geographical variation, successional variation.

Reading teeth with reference to biological information including relative age criteria can minimize factors (a) and (b) but it can also lead, through conscious or unconscious bias introduced by knowing how old the individual 'should be' based on length, sex and other information, to a serious underestimation of biological variation and possibly even a completely invalid 'growth curve'.

The following overall approach is recommended:

(1) Select the most appropriate tooth or bone, and develop a technique or techniques to demonstrate growth layer groups clearly.

(2) Describe the growth layer groups. If the sample permits, a subsample constituting an ontogenetic series consisting of large fetuses, neonates and immature individuals, preferably of both sexes, based on body length should be assembled. This series can then be studied to determine the nature and thickness of, e.g. the prenatal dentine and neonatal line, and the growth layer groups in dentine and/or cementum can be described.

(3) The entire sample of tooth or bone preparations should then be read in random order. It may be a good practice to label preparations with random numbers (each corresponding to a specimen number) while this is being done. Each preparation should be read independently several times by an observer, or several observers fully conversant with the results of (2), until adequate repeatability is achieved.

(4) If anomalies are found when the readings are checked against biological data, a careful check should be made for blunders, e.g. clerical errors or mislabelling. If these are not discovered, the preparation should be read again by an independent observer again without reference to the biological information, and if the anomaly persists, it should be accepted as probably reflecting biological variation.

6. STATISTICAL TREATMENT OF DATA

It was decided at the workshop that the sample sizes for the cross-reading experiments were generally too small to justify statistics beyond ranges and averages. However, the use of statistics on growth layer group counts is advisable if sample sizes are large enough, and the following discussion is included to encourage future workers to use statistics to make more objective assessments of (1) readability of individual teeth or bones, and (2) readability of the teeth or bones of a whole population.

a. Establishing readability of an individual specimen

Each specimen should be scored independently several times (at least six or seven times if statistics are to be used) and the mean standard deviation of the score calculated. The coefficient of variation (standard deviation divided by mean) is a dimensionless measure of the readability of the specimen. Specimens which have an unusually large coefficient of variation should be discarded as unreadable.

b. Measurement of the readability of the teeth of a population

It can also be useful to compare the readability of teeth of different populations or different species. It would be, for example, valuable to compare the readability of teeth from a tropical population with those from a temperate population.

The error mean square, obtained from a one-way analysis of variance over all the growth layer group scores for all the specimens in the sample, is a good estimate of the error variance of the scores. The coefficient of variation for the whole population is then estimated by dividing the square root of the error mean square by the mean of the scores. This gives a measure of the readability of the teeth or bones of the whole population.

7. UNEXPLORED TECHNIQUES

Several potential techniques were suggested at the workshop as possibly worthy of development.

(a) Development of an automated system of reading.

(b) Computer enhancement of photographs of growth layer groups to reduce confusing background noise.

(c) Statistical spectral analysis of digitized analogue representation of growth layers (height, spacing of etched surface, variation of optical density in transmitted light, etc.) to identify significantly cyclic features.

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G. GLOSSARY

Accessory layer (lamina, line, band, *incremental growth layer*). Any single layered component of a *growth layer group* which is discernible from adjacent layers in a hard tissue.

Cementum. A calcified tissue of mesodermal origin, often containing osteocytes (cementocytes) and forming layers over the basal portion (root), and, in some species, over part of the coronal portion of a tooth (see Figure 9). Serves as 'attachment bone' of the tooth.

Vasocementum. Cementum containing vascular channels (i.e. for blood vessels).

Crown. The coronal (or distal) part of a tooth that in life protrudes from the gingival surface and that is demarcated by an enamel covering in most species (see Figure 9).

Dentine. A cellular, calcified tissue of mesodermal origin that constitutes most of the tooth body (root and crown), except in ziphiids. It is covered on the crown by enamel and basally by cementum.

Irregular dentine. Dentine in which few or no tubules occur, typically confined to certain areas of the pulp cavity wall.

Orthodentine. Dentine containing tubules that radiate from a single pulp cavity. It is formed internal to the enamel by a process of matrix synthesis and mineralization induced by inward-migrating odontoblasts (see Figure 9).

Osteodentine. Dentine in which whole cells have been included, i.e. similar in structure to bone; usually occupying the pulp chamber.

Postnatal dentine. Orthodentine lying internal to the neonatal line; deposited after birth. Typically, postnatal dentine shows well-developed layering patterns.

Prenatal dentine. Poorly-layered orthodentine lying external to the neonatal line; deposited before birth (see Figure 9).

Vasodentine. Dentine containing vascular channels (i.e. for blood vessels).

Enamel. Acellular (prismatic) highly mineralized secretion of ectodermal origin forming an external covering for the dentine (see Figure 9). May be itself in part covered with cementum.

Eruption. Migration of a tooth to a position external to the gingival surface.

Growth layer group (GLG). A repeating or semi-repeating pattern of adjacent groups of incremental growth layers

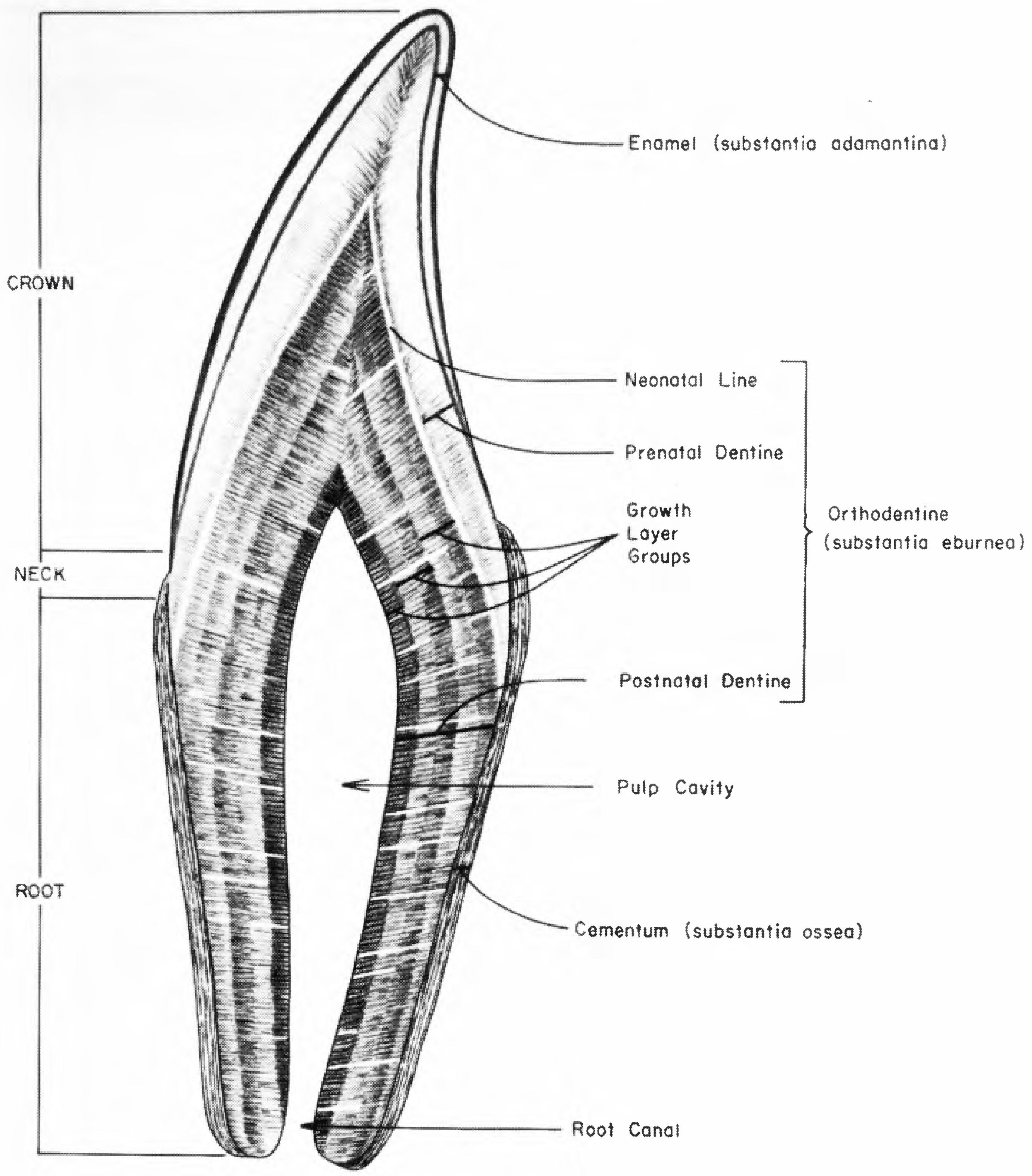

Fig. 9. Illustration of terms in the Glossary.

within the dentine, cementum, or bone which is defined as a countable unit. Such a unit must involve at least one change, i.e. from transparent to opaque, dark to light, ridge to groove, intensely stained to lightly stained, but may involve more than one change (see Figure 9).

Homodont. Referring to a series of teeth with the same morphology.

Incremental growth layers ('laminae' – 'bands' – 'zones' – 'lines' – 'accessory layers'). Discernible layers occurring parallel to the formative surface of a hard tissue (dentine, cementum, bone) which show any contrast with adjacent layers.

Isodont (haplodont). Having pointed, conical teeth.

Knot. The root or basal terminus of the tusk (i.e. of a narwhal).

Monophyodont. Possessing only one (permanent) set of teeth, i.e. without deciduous precursors.

Neck. The region of a tooth immediately below the enamel that separates the anatomical crown from the root (see Figure 9).

Neonatal line. A particularly well defined growth layer of the orthodentine that separates prenatal dentine from postnatal dentine (see Figure 9). It is believed to be the product of disturbances of the nutrition of the animal in the immediate post-partum period.

Periodontal membrane (ligament). Connective tissue containing collagenous fibers that pass from the cement of a tooth to the *lamina dura* of the tooth socket.

Pulp cavity. The central conical or cylindrical chamber of a tooth bounded by dentine which, in life, contains the pulp consisting of connective, sensory and nutritive tissues (see Figure 9).

Occlusion of pulp cavity. The condition in which the pulp cavity has become filled with dentine, and/or primary dentinal accumulation has ceased.

Pulp stones (pearls). Globular masses of secondary dentine that form in the region of the pulp cavity.

Root. That part of a tooth typically covered by cementum and contained within the tooth socket below the gingival surface (see Figure 9).

Vestigial tooth. An unerupted tooth that remains imperfectly developed and/or functionless.

A Review of Studies to 1970 of Growth Layers in the Teeth of Marine Mammals

Victor B. Scheffer
Marine Mammal Commission (Retired)
14806 S.E. 54th Street, Bellevue, Washington.
and
Albert C. Myrick, Jr
National Oceanic and Atmospheric Administration, National Marine Fisheries Service
Southwest Fisheries Center, La Jolla, California

ABSTRACT

This is a review of the discoveries of layers in teeth of marine mammals and subsequent researches that led to the widespread use of dental layers for age determination of marine mammals. Zoologists during the 19th century noted the existence of layers in marine mammal teeth, but it remained for Boschma, in 1938, to associate dental layers with age, and Chapskii, in 1941, to recognize the value of layers to wildlife research. Methods for aging and analyzing samples from marine mammal populations were developed in the early 1950s and have since been applied to most extant species of toothed marine mammals. A systematic account of the first 40 years of age determination research on 36 species of Cetacea, Pinnipedia, and Sirenia, is presented.

INTRODUCTION

> In the central blinds of bone, as they stand in their natural order, there are certain curious marks, curves, hollows, and ridges, whereby some whale-men calculate the creature's age, as the age of an oak by its circular rings. Though the certainty of this criterion is far from demonstrable, yet it has the savor of analogical probability.
> (Melville, 1851, p. 324)

Age composition is one of the life history parameters needed to assess the dynamics of mammalian populations. In the past, criteria such as body length, eye lens weight, degree of closure of cranial sutures, tooth wear, and the number of corpora albicantia have been used to estimate *physiological age* in many species. For marine mammals, growth layers deposited in the teeth are a more useful criterion because they indicate *chronological age.* Tooth-age determination has become standard procedure in stock assessment and management decisions for marine mammals. This paper is an account of the pioneering efforts and progress up to 1970 in estimating the age of marine mammals by counting dental layers.

HISTORICAL SUMMARY

Zoologists of the 19th century described or illustrated growth layers in teeth of marine mammals without seeming to understand their significance. Owen (1845) and Eschricht (1845, 1862) mentioned concentric layers in teeth of odontocetes. Witness also the sketches of a beaked whale tooth in Lankester's 1867 paper and the delicate lithographs of sperm whale and killer whale teeth in Van Beneden and Gervais' 1880 monograph (plates 20 and 49). The first person to correlate growth layers with age in years was Boschma, who in the late 1930s examined a longitudinal section of a sperm whale tooth.

Chapskii, in 1941, was among the first to foresee the value in wildlife research of counting layers. Through examination of cross sections of walrus premolars he estimated ages up to 27 years. The value of growth layers was recognized in 1948 by Scheffer (1950), who developed a technique for aging otariids. In the same year, Laws (1952), studying elephant seal teeth from South Georgia, developed a technique for aging phocids. In his 1952 paper, Laws stated that

> a superficial examination of material in museums indicates that the teeth of a number of other vertebrates, living and fossil, have internal rings or external ridges which, like those found in seals, may possibly be used for determining age (p. 974).

Nishiwaki and Yagi (1953) were first to use dental layers in determining age of cetaceans. After the early 1950s, research using this method of age determination increased rapidly, and by the late 1960s the method had been applied to most species of toothed sea mammals.

SYSTEMATIC ACCOUNT

(Terminology follows IWC recommendations)

Cetacea: Physeteridae

***Physeter macrocephalus* (sperm whale)**
Boschma (1938) made a thorough study of the teeth of the sperm whale (our Fig. 1). He examined polished, longitudinal sections of a tooth from the Antarctic and was struck with the regular distribution of osteodentinal 'pearls' parallel to, and sandwiched between, the layers of orthodentin (p. 243). He likened the series to the 'winter-rings in the scales of fishes' and surmised that the animal must have been at least seven years old.

Nishiwaki *et al* (1958) made the first intensive study of growth zones in sperm whale teeth. In 40 whales, they found 21 to 28 teeth in each lower quadrant. In 27 of the whales, there were no visible upper teeth; in five there were from two to thirteen visible upper teeth, and in eight the presence of upper teeth was uncertain or unrecorded. The authors studied teeth numbers seven to ten in the lower series, the largest. They took teeth from 37 males and 51 females. With saw, rasp, and grindstone they made longitudinal sections and counted 'laminations' in the dentine. Laminations in the cementum were often worn or obscure, and were not

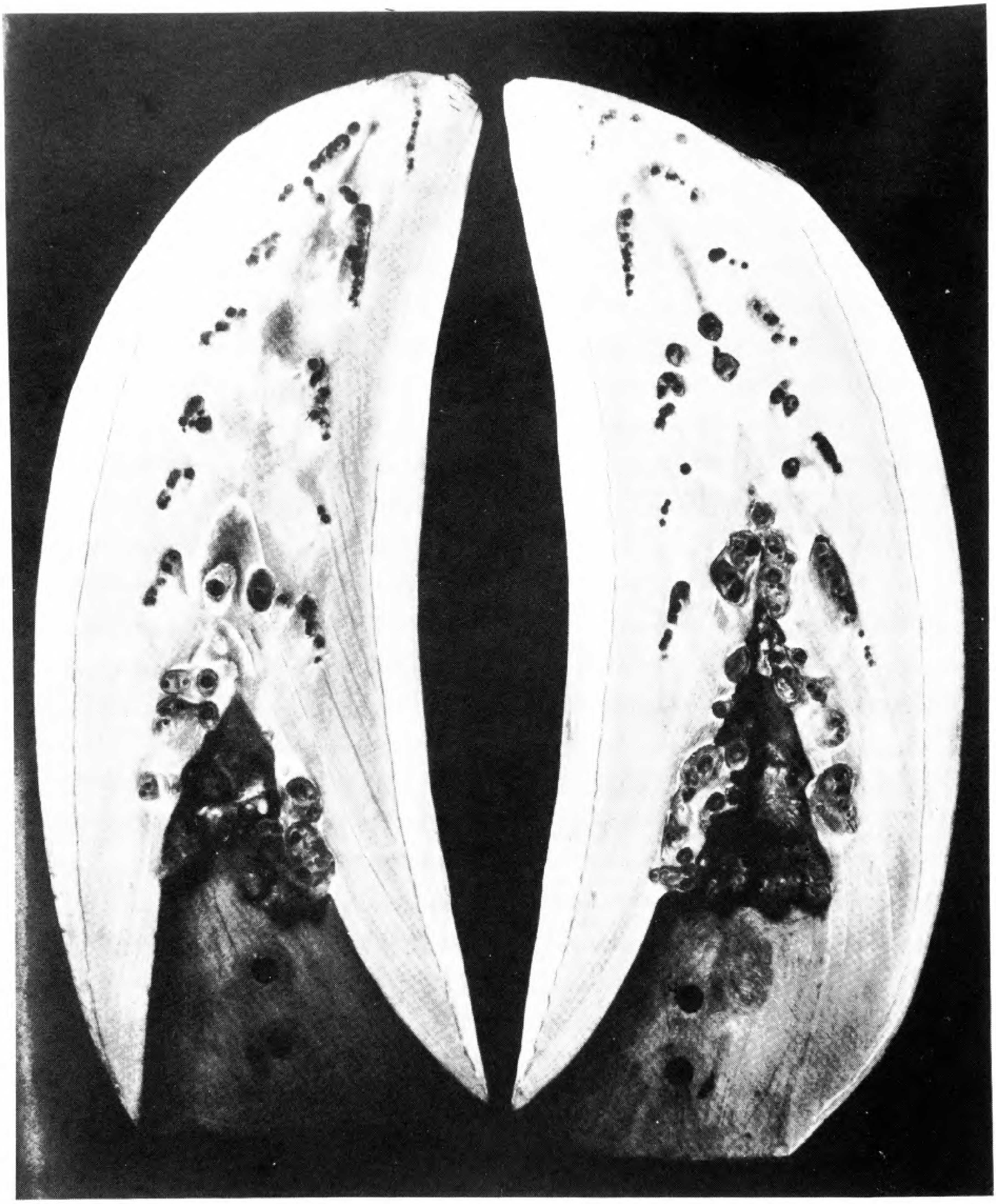

Fig. 1. "Longitudinal sections of a tooth of a sperm whale . . . showing successive masses of osteodentine" (Boschma, 1938, caption for pl. 13). Note the thick cementum, showing as a white border on the right and left sides of each section.

useful indicators of age. The authors concluded tentatively that two laminations are laid down in dentin each year. On this basis, males, with up to 48 laminations, should attain sexual maturity at age four or five years, with nine or ten laminations. Females, with up to 70 laminations, should attain sexual maturity at age four. A 'lamination' as used here is a seasonal zone, and two zones make an annual layer. Ohsumi *et al* (1963) were later to show that one layer, consisting of a dark and a light zone, is deposited annually.

In material from 24 sperm whales, Nishiwaki *et al* (1961) studied the relationship between the number of layers in mandibular bone and in teeth. Layering in bone does not present a permanent record.

As the bone tissue is resorbed from the mandibular

> canal, the laminations will disappear at different rates according to the positions on the mandible (p.502).

Layers are most persistent near the middle of the mandible, behind the symphysis. In females there is evidently a one-to-one relationship between the number of layers in bone and dentin until the period when about 13 layers have been formed. Thereafter the number in the bone remains constant at about 14, while that in the dentin continues to increase. Although the authors had jaws from only three adult males, with 14 or 15 bone layers, they stated that

> 18 laminations were observed in a mandible of an Antarctic male sperm whale, so it is probable that more laminations remain in the male than in the female (p. 503).

Berzin (1961a) reported that

> the age was determined from the teeth in 306 male and 61 female sperm whales. Assuming the annual deposition of two layers of dentin in the teeth (winter and summer), it can be calculated that the males reach sexual maturity at an age of four and a half to five years and a body length of 9.5 m to 10 m. The seventh and ninth pairs of teeth are the first to be cut, while the front and back are the last (at ten to eleven years). The pulp cavity of the first pair becomes filled in at the age of 30 to 35 years. The similarity in calcification of the two layers (winter and summer) indicates that the sperm whale does not have a prolonged winter fast . . . (p. 17279).

The oldest males and females were 28 to 32 years old, respectively.

Berzin (1961b) reported further details of his study of the 61 females. In sexually mature animals four to five years old or older he found one 'corpus luteum vestige' [corpus albicans] for each two layers of dentin. This relationship confirmed his opinion that two layers are deposited annually. In older females, however, the age estimated from tooth layers was greater than that estimated from corpora, from which he concluded that the cycle of ovulation is interrupted from time to time in older whales. The oldest specimen was 'barren', age 29 from the evidence of the ovaries and age 30-plus from the evidence of the teeth.

Omura *et al* (1962) described the skeleton of a male sperm whale 46 feet long. The animal was evidently not fully grown; the vertebral epiphyses were not completely ankylosed. The authors stated that 'the body length of male sperm whales at the attainment of physical maturity is thought to be between 52 and 53 feet' (p. 36). A longitudinal section of an upper tooth of this animal showed '32 laminae'.

Laws (1962, Fig. 8) reproduced a photograph of a thin longitudinal section of a lower tooth with '36 incremental layers' in dentin and cementum (representing about 18 years). The cementum layers show exceptionally well in this figure.

Ohsumi *et al* (1963) used maxillary teeth of the sperm whale in a study of growth layers. Most of the maxillary teeth, similar in the two sexes, remain hidden in the gum through life where they are protected from wear and damage. The authors saved one buried tooth from near the middle of an upper tooth row from each of 448 whales taken in the North Pacific in 1960–61. They also studied the teeth of eight whales which were marked in 1952–60 and recovered in 1962. In longitudinal sections the important layers were:

(1) the forming layer, next to the pulp, consisting of predentin and dentin, and

(2) the next-oldest layer, i.e. the one of the preceding season (our Fig. 2).

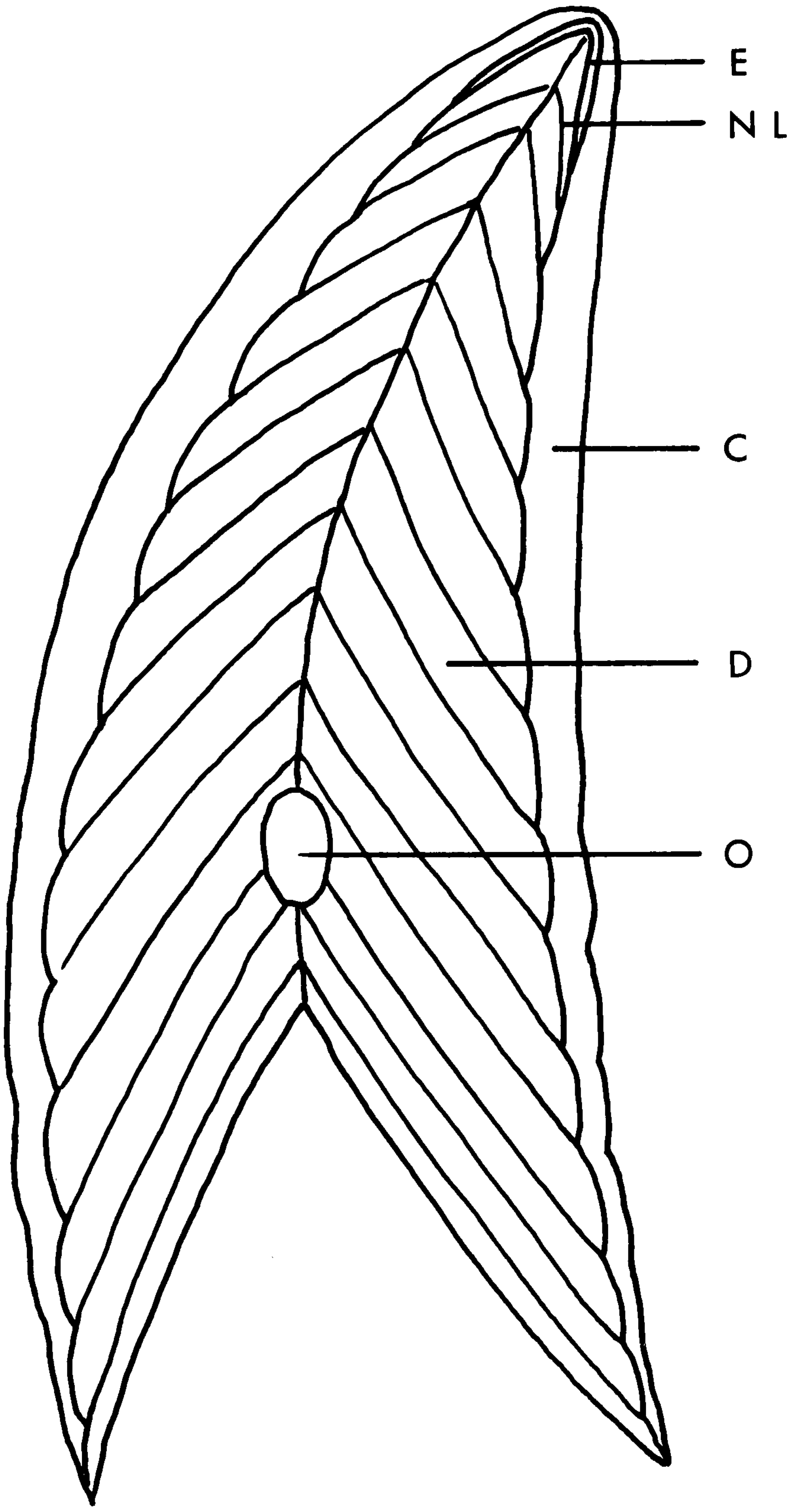

Fig. 2. Diagram of an unerupted maxillary tooth of an adult sperm whale; longitudinal section. E = enamel, representing a very small portion of the tooth, NL = neonatal line, C = cementum, D = dentine, O = osteodentin. (After Ohsumi *et al.*, 1963, Fig. 1).

A 'layer' was defined as the zone between narrow 'dark bands'.

The dark and light layers are seen by reflected light as translucent and opaque layers by transmitted light. Since the translucent layer is anisotropic in polarized light and is weakly stained by carbol-fuchsin, the authors concluded that it has a better developed crystalline structure than the thick opaque layer.

With increasing age, the thickness of the newest layer tends to decrease. Mean layer thickness in males is slightly greater than in females. In the authors' sample, the mean number of layers in males was 18.7 (5 to 35) and in females 14.4 (4 to 27).

By plotting the distance between the latest dark layer and the pulp against the date of capture, the authors deduced that the dark layer is laid down in midwinter, or January. In certain individuals, however, the layer may begin to form as early as mid-October and in others it may not be completed until April. 'The dark band [layer] . . . seems to be accumulated as the result of a good nutritious condition' (p.32).

Partly from these measurements of seasonal progression in thickness of dentin and partly from study of marked whales, the authors concluded that only one dark layer a year is deposited. A female about 37 feet long was marked at sea on 17 June 1953 and killed on 30 August 1961, when her length was verified as 35 feet. An upper tooth showed 14 layers and her ovaries two corpora albicantia. The evidence fits the assumption that she was nearly adult when marked (6 years, 6 layers) and fully adult when killed (14 years, 14 layers).

Gambell and Grzegorzewska (1967), using a sample of mandibular teeth from 659 sperm whales from South American and Antarctic waters, analyzed the rate of layer formation. They concluded that a layer containing a light and a dark band was laid down twice a year.

Cetacea: Monodontidae

Delphinapterus leucas **(white whale)**

Yablokov (1958, pp. 41–3) figured the teeth of the white whale but did not discuss growth layers.

Sergeant (1959) examined teeth of several hundred white whales taken in the northeastern Canadian Arctic. He made longitudinal thin sections and counted up to 50 layers in the dentin. He tentatively concluded that two complete layers (four zones) are deposited yearly.

The relationship between layers and age is still unclear (Sergeant, 1962a, p. 4).

> Soviet workers, on the basis of length-frequencies, believe beluga to become white in colour and the females to mature sexually as early as the third year, males in the fourth. The discovery of growth-layers in the teeth has begun to allow accurate age determination; but the rate of deposition of the layers, and hence the absolute age which they represent, has not yet been determined.

Cetacea: Ziphiidae

Berardius bairdii **(Baird's beaked whale)**

Omura *et al* (1955, p. 107) illustrated a longitudinal section of a tooth of a Baird's beaked whale and suggested the possibility of estimating age by growth layers (our Fig. 3).

The anterior teeth are peculiar in that the pulp cavity becomes filled with osteodentin and very little orthodentin.

> Outside the teeth a layer of cement is formed yearly, making a coating of cement from which, we think, it is possible to determine the age (p. 107).

There appear to be four layers of cementum on the tooth illustrated.

Yablokov (1958, Fig. 5) illustrated a longitudinal section of a *Berardius* tooth but did not show growth layers.

Mesoplodon bidens **(Sowerby's beaked whale)**

Lankester (1867, pls. 5 and 6) illustrated one of the teeth of *Ziphius sowerbiensis.* He showed six or seven layers of dentin and cementum, but did not comment on the possibility of their being annual layers.

Ziphius cavirostris **(Cuvier's beaked whale)**

Kenyon (1961) sectioned teeth of two *Ziphius cavirostris* – a 658 cm-long female having 24 to 28 growth layers and a 544 cm-long male having 13 growth layers. He thought that each layer represented a year's growth.

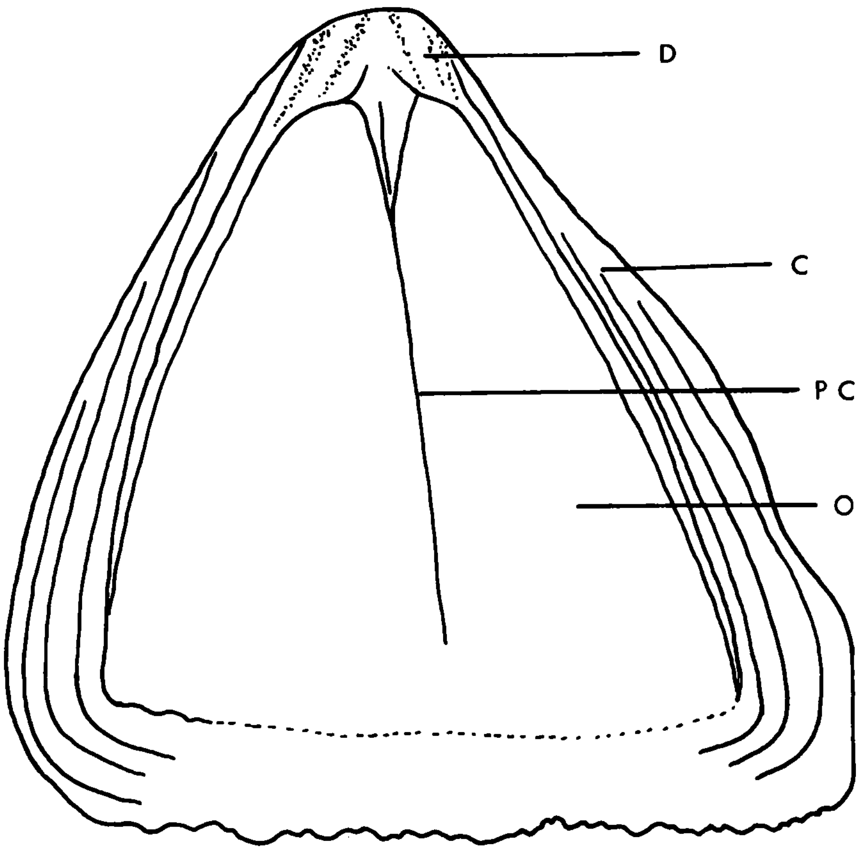

Fig. 3. Diagram of a tooth of a Baird's beaked whale about 4 years old; longitudinal section. D = dentin, C = cementum, PC = pulp canal, OD = "osteodentine". (After Omura *et al.*, 1955, Fig. 26).

Hyperoodon planifrons **(southern bottlenose whale)**

One of the earliest illustrations of layers in odontocete teeth was presented by Eschricht (1845). There is no indication that he was aware of the significance of the layers.

Cetacea: Delphinidae

Orcinus orca **(killer whale)**

Sergeant (1959) examined the teeth of a killer whale from eastern Canada. He stated briefly that the growth pattern was similar to that in *Globicephala* and *Tursiops.*

Globicephala spp. **(pilot whales)**

Sergeant (1959, 1962b) made a detailed study of teeth from at least 694 long-finned pilot whales *(G. melaena)* collected in 1951–59 from Newfoundland. Both dentin and cementum are layered.

> The tooth of a pilot whale is relatively short, and a single cross-section cut in the upper part of the root will pass through all growth layers and enter the pulp cavity. A longitudinal section shows the varying thickness of each growth layer from its apex in the crown to its termination in the root, and the extent of the pulp cavity. [Ten to fifteen layers are deposited before the cavity fills.] However, transverse and longitudinal sections were found to give similar readings of numbers of dentine layers, and the more easily cut transverse sections were employed for routine ageing (1962b, p. 7).

The sequence of zone formation in the dentin is given below. Translucent dentin is said to be poorly calcified. 'This has recently been confirmed by radiography of thin tooth sections' (1962b, p.13).

1st layer. A zone of prenatal dentin followed by a narrow, translucent zone, the neonatal line, which is formed at birth, about mid-August.

2nd layer. An opaque zone passing into a translucent zone, the two representing the nursing period of nearly two years.

3rd and subsequent layers. An opaque zone passing into a translucent zone. Sergeant tentatively assumed that the opaque zone is laid down in August-January and the translucent zone in February-July. The translucent zone coincides with the six-month mating period which peaks in April-May. The largest whales had from 30 to 50 layers.

Although Sergeant had no known-age pilot whales to substantiate his conclusion, he assumed that one layer represents one year. On this basis, the mean age at sexual maturity of females is six years, or the same as the known age at maturity in captive *Tursiops* (interesting, in view of the great difference in size of the two animals; but, see Sacher, this volume).

He also examined the teeth of two short-finned pilot whales, *G. macrorhynchus*, from Florida. The teeth were

> considerably larger than those of *G. melaena* of the same body size. In section they show much thicker dentine, with early deposition of the secondary dentine. . . Growth layers appear to be more frequent than in *G. melaena* of the same length. . . The wide difference in tooth structure between these closely related species is noteworthy (1959, p.285).

***Lagenorhynchus acutus* (Atlantic white-sided dolphin)**
Sergeant (1959) examined the teeth of one white-sided dolphin from the eastern coast of Canada. He found the growth pattern similar to that in *Globicephala* and *Tursiops.*

***Delphinus delphis* (common dolphin)**
Sergeant (1959) examined the teeth of one common dolphin from eastern Canada and found the layered pattern similar to that in *Globicephala* and *Tursiops.*

Kleinenberg and Klevezal' (1962) estimated age in Black Sea common dolphins by comparing the number of dentinal layers in the teeth with the number of bone layers in the lower jaw and the number of corpora albicantia. They examined 33 dolphins taken in July and September 1961. They decalcified entire(?) teeth and cut longitudinal sections. Evidently one layer of bone is laid down each year and 'the number of layers in the dentine of the tooth exactly doubles the number of layers in the lower jaw plus or minus one' (translation, p. 6). Two light and two dark layers are laid down each year in the dentin. The oldest dolphin in the collection was an 11-year male with 11 layers in the jaw bone and 22 zones in the teeth. The authors concluded that most females attain sexual maturity at age two.

***Tursiops truncatus* (bottlenose dolphin)**
Sergeant (1959) was fortunate in obtaining teeth of two bottlenose dolphins of known age, born in the aquarium at Marine Studios, Florida. One was six months old and the other 28½ months. He also obtained the teeth of two old males and one old female, both of unknown age, though held for known periods in the aquarium.

According to Sergeant *(op. cit.)* the growth of the root by increments of cone-shaped dentin resembles that of *Stenella.* The neonatal line, laid down shortly after birth, is clear and unstainable. Following the neonatal line are alternating zones of wide, dense, heavily staining dentin and narrow, clear, poorly staining dentin, repeated at regular and gradually decreasing intervals until the pulp cavity is reached. Two zones, or one layer, are deposited annually.

The daily rate of deposition of dentin as seen in a mid-longitudinal section was estimated to be:

in a known-age male, age 6 months	2.0 microns
in a known-age female, 28½ months	
during the first year only	1.8 microns
during the second year only	1.7 microns
in a male with 8 layers	1.0 microns
in a male with 10 layers	0.8 microns
in a female with 17 ± layers	0.2 microns

The rate of tissue deposition in the female seems to be considerably slower than in the male.

***Stenella coeruleoalba* (striped dolphin)**
Nishiwaki and Yagi (1953) were pioneers in the search for age indices in the teeth of cetaceans. They sought first to learn the rate of deposition of dentin in the teeth of the striped dolphin, a species commonly taken in Japanese waters. In 1951 they injected lead acetate into four captives and released them in Mito Aquarium. The dolphins would not feed; the first one to die survived only five days and the last one fourteen days. From each dolphin they removed the 20th tooth from the posterior end of the jaw in each quadrant (our Fig. 4). They sectioned the teeth transversely

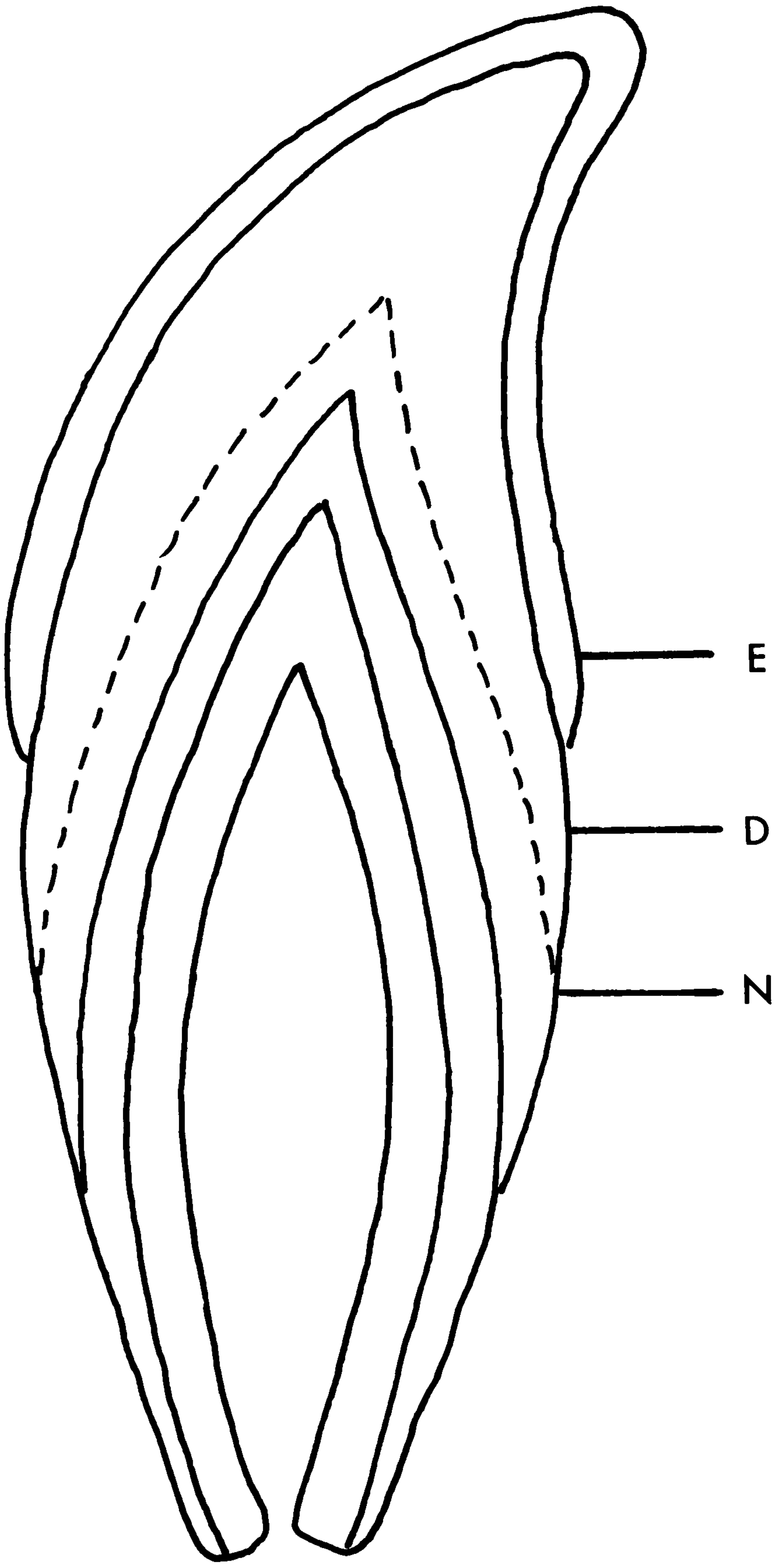

Fig. 4. Diagram of a tooth of a striped dolphin (*Stenella coeruleoalba*) 3 years old; longitudinal section. E = enamel, D = dentin, N = neonatal line (After Nishiwaki and Yagi, 1953, Fig. 3).

or longitudinally. A distinct lead line was visible in the teeth of two animals that lived seven and fourteen days.

Later, the authors examined teeth from 35 males, 55 females, and 16 fetuses collected in 1951. They concluded that the typical zones in a longitudinal section of the root of a large tooth are, in order of deposition: homogeneous fetal dentin; a neonatal line, poorly calcified and unstainable with hematoxylin; and 'striped' dentin with two to eight stripes, immediately external to the pulp cavity.

On the basis of their 1951 sample, plus 98 sets of teeth collected in December 1951 and in 1952, the authors explored further the relationship between dentinal layers and age (Nishiwaki and Yagi, 1954). The number of layers showed a close relationship to body length. The logarithm of the number of dentinal layers showed a positive linear relationship to body length; the shortest individuals had one layer and the longest individuals had eighteen layers. Well-stained zones are deposited in, or shortly before, December and again in May-June. 'Thus it seems by these facts that the deep stainable layer might be made twice a year, but we cannot say at present anything with certainty' (p. 403).

Phocoena phocoena (harbour porpoise)

Sergeant (1959) examined the teeth of two harbour porpoises from eastern Canada. The growth pattern resembles that in *Globicephala* and *Tursiops.*

> In addition . . . the spade-shaped crown, typical of the group [subfamily Phocoeninae], is composed of thick, prenatal dentine and of enamel (p.285).

Pinnipedia: Otariidae

Allodesmus kelloggi (an extinct sea lion)

Mitchell (1966, p. 21) counted 'more than thirteen pairs of light and dark zones' in sectioned teeth of adult male sea lions from the Temblor Formation, late middle Miocene, He saw ridges on the surface of one postcanine.

Eumetopias jubatus (Steller sea lion)

Fiscus (1961) examined the right upper canines of 59 Steller sea lions taken in Alaska in 1958 and found up to 19 growth layers in males and 22 in females. He prepared longitudinal sections by the method of Wilke, Niggol and Fiscus (1958, MS).

Thorsteinson and Lensink (1962) studied another collection taken in Alaska in 1959 from 'harem bulls' only. The teeth were examined by Lensink and Fiscus; 'disagreements . . . occurred in 46% of the sample, but most involved differences of one year and were readily reconciled' (p. 356). The population had a mean age of 10.8 years (6 to > 17).

Spalding (1964) counted layers in the cementum in thin longitudinal sections of upper canines of 138 female sea lions. The first dark cemental zone is deposited in August and September in an animal about 15 months old. It is followed by a light zone, then a dark zone in late summer or fall of the second and succeeding years. He had only one known-age specimen, 16 months old.

Zalophus californianus (California sea lion)

Scheffer (1950, p. 2) saw 'dubious' external growth ridges on the teeth of the California sea lion. Orr *et al* (1970) counted from one to fifteen annual zones on longitudinal sections of upper canines of 35 male sea lions from the Gulf of California.

Otaria flavescens (southern sea lion)

Laws (1962) described growth layers in teeth of the southern sea lion on the basis of several canines collected in the Falkland Islands in late summer. He used longitudinal thin sections, some decalcified and stained. In his Figure 5, the zone pattern looks like a cross section of an old oak stump with numerous fine, regular layers. He suggested the possibility of lunar periodicity in feeding, with two dark and two light zones deposited each month. He measured the thickness of individual deposits and sketched a theoretical time schedule. The peak of deposition was in July (mid-winter) and the depression was in September-October, the breeding season.

Callorhinus ursinus (northern fur seal)

In 1948, Scheffer (1950) examined teeth of known-age, tagged northern fur seals and found external ridges encircling the root, each ridge representing the accelerated growth of one winter-spring season. In 1950, biologists of the Pribilof seal industry standardized a technique for counting external ridges on the right upper canine tooth. They estimated age to ten years and pooled older ages as 'ten-plus'. The age composition of a fur seal population was first published by Wilke (1952, Table 2), for 451 seals taken in waters off Japan.

Wilke *et al* (1958, unpubl.) developed a better technique. They sliced each tooth longitudinally with a bandsaw, ground and polished the flat face, and counted zones in reflected light.

Kubota *et al* (1961, 1963) described development of growth zones on the upper canines (our Figs 5 and 6), as follows:

(1) At birth (e.g. in July) the root may not be visible beyond the enamel. The prenatal dentin, all or most of it within the crown, is light colored, the first postnatal dentin is darker; the light and dark zones meet at the 'neonatal line' (which is often not visible).

(2) (In summer and fall the root grows slowly.) 'Interglobular spaces' appear in the peripheral dentin, i.e. the region away from the pulp.

(3) (The growth rate accelerates in spring.) About the end of April in the life of the pelagic yearling a narrow 'transparent zone' begins to form in the dentin. It terminates on the surface of the root, at the crest of the ridge which marks the end of the seal's first year (in June or July).

Scheffer measured the apical growth on 25 known-age 3-year-old females taken at sea between 23 January and 3 October. He concluded that the ridge does not start to round off, and the summer-fall valley does not start to form, until June. The first indication of a thin shelf representing the beginning of the next ridge is formed in August.

Yagi *et al* (1963) administered lead acetate and tetracycline to a 4-year female fur seal in an aquarium. The seal lived 162 days after the first injection. Both drugs left traces in the dentin which were demonstrable by histochemical methods.

Scheffer and Kraus (1964) studied the morphology and development of seal teeth. Among their conclusions are the following:

(1) Dentin gradually fills the root canal until, at ages varying from 3 months (for small incisors) to more than 20 years (for the large upper canines of the male), the root is completely closed.

Fig. 5. Upper canine teeth of two female northern fur seals: (left) age 11, taken in mid-August; (right) age 12, taken in mid-July; longitudinal thin sections by reflected light. Numbers indicate the (translucent) dentin deposited in spring. (From Kubota *et al.*, 1963, pl. 1.)

(2) Growth layers in the upper canine of a seal held in captivity from age 3 months to 58 months were abnormal, though could be grouped into five annual series.

(3) 'Ridges on the surface of the growing root, finer than [yearly] annuli and spaced between them, are thought to originate in individual periods of intensive feeding' (p. 309).

Scheffer and Peterson (1967) continued the study of fine ridges. During the 16-week suckling period, about ten or eleven zones are laid down in the dentin of the pup, each representing one period of suckling followed by a fast. The zones are permanent.

Arctocephalus australis (South American fur seal)

Mansfield (1958a, p. 39) saw concentric rings in cross sections and surface ridges on canine teeth of ten South American fur seals.

> In the older specimens in which the pulp cavity was almost occluded, a . . . pattern of alternate light and dark rings of dentine was found. As in the Weddell seal a pair of these rings was assumed to represent one year of the life cycle, and the specimens were aged on this basis, the age . . . was found to agree within a year with the age determined by the number of external ridges on the teeth. These ridges are well marked, and the teeth resemble those of the Alaskan fur seal for which the method has been proven valid.

The source of the ten specimens was not given, though Mansfield wrote (in letter of 17 February 1967) that 'they most probably came from J.E. Hamilton's Falkland Islands fur seal collection in the British Museum.'

Arctocephalus pusillus (Cape fur seal)

Rand (1956, p. 12) found that in teeth of the Cape fur seal 'the numerous annuli showed no discontinuous pattern that

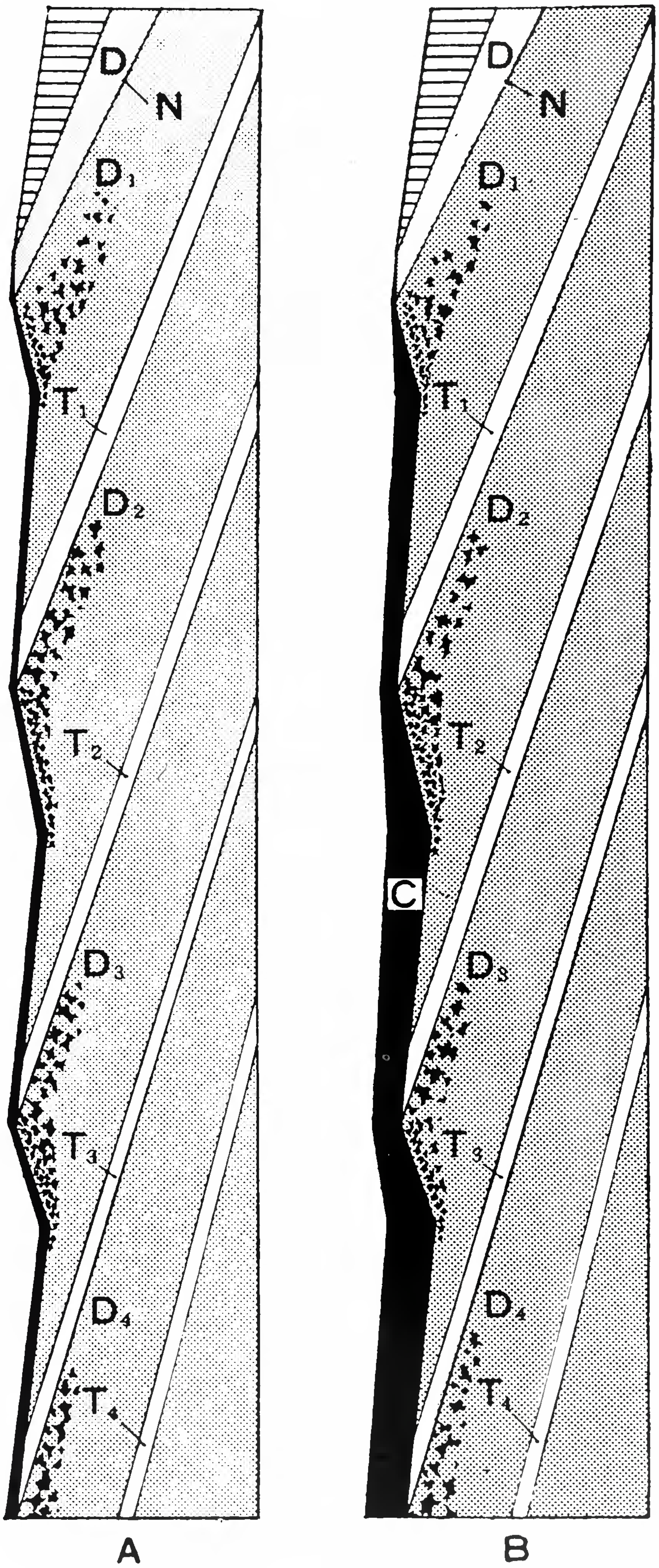

Fig. 6. Diagram of upper canine teeth of northern fur seals, (A) "young" and (B) "old"; longitudinal thin sections by transmitted light. C = cementum, D = prenatal dentin, N = neonatal line, D 1 to 4 = opaque dentin deposited in 1st to 4th years; T 1 to 4 = "transparent" [translucent] dentin deposited in 1st to 4th years. (From Kubota *et al.*, 1961, pl. 7.)

could be correlated with definite annual or other recognizable time units.'

Pinnipedia: Odobenidae

Odobenus rosmarus (walrus)
It has long been known that ridges surround the root of the tusk in some old walruses, particularly old males (our Fig. 7). The ridges are partly obscured by cementum but are revealed when the cementum is chipped away or when the tusk is sectioned lengthwise. Cemental layers are also deposited on the premolars.

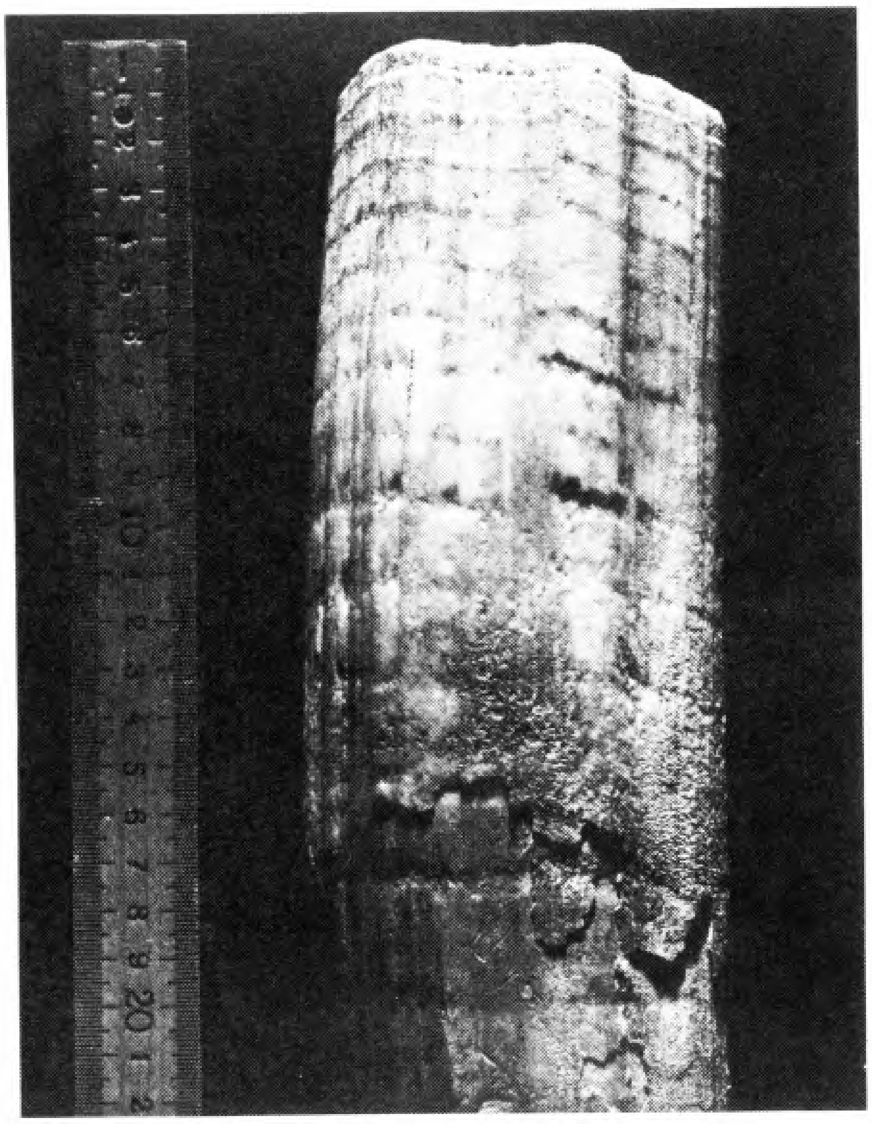

Fig. 7. Tusk of an old male Pacific walrus showing annual ridges. Date of death unknown, a subfossil from beach sands of St. Paul Island, Alaska. A very large tusk, total length along convex surface 917 mm (36.1 inches). (VBS photo 2509A.)

The pioneer work of Chapskii (1941) on walrus teeth has been mentioned. Mohr (1943, p. 259) stated that

> longitudinal and transverse sections through the teeth of walruses of known age reveal that the cemental hyperplasia takes place in the form of yearly rings, similar to the otoliths and bones of fish.

In 1952, Brooks and Fay independently undertook studies of the Pacific walrus *(O.r. divergens)* (Brooks, 1954, unpubl.; Fay, 1952, unpubl.). They focused on interpretation of the cemental layers of the premolars. Brooks concluded that one dark ring or none is deposited in the first year and two dark rings (summer and winter) in each year of life after the first. He correlated estimated age with number of cementum rings on the premolars and with tusk length. In his Figure 9, for example, he indicated that an 8-year-male with tusks one to fifteen inches long has fifteen rings.

Fay (1956, unpubl.) described criteria for each year class from newborn to age four, using length of tusk, wear of enamel on the tusk, degree of staining of the teeth, cranial and body size, observation of the adult female (presumptive mother) with the young animal, and reproductive history of the mother as revealed by autopsy. Available to him were teeth from seven Pacific walruses and nine Atlantic walruses of known age, raised in zoos. He counted the cemental layers on sections of the second upper premolar or the first lower premolar. In the Pacific walrus, two or three zones of cementum are deposited the first year, decreasing to one layer per year at time of sexual maturity. In the Atlantic walrus *(O.r. rosmarus)*, one or two layers are deposited before sexual maturity and one thereafter. In very old Pacific walruses, as many as four initial layers of cementum have disappeared through wear.

Longitudinal sections of the tusk of the Pacific walrus show up to 30 marginal ridges and indicate an age of 30-plus years. In specimens collected in May, the ridge is at the apex of the root. In specimens taken in the ensuing twelve months the zone between the ridge and the apex of the root widens progressively at a rate such that by the next May the ridge is again at the apex. Thus, the ridge is clearly an annual mark. Although the ridge count may be indicative of minimum age in years, the cemental layer count in the premolar roots is more consistent.

In the spring and summer of 1960, Samuel J. Harbo began a study of walrus in the Bering Strait and vicinity (Harbo, 1961). He collected the teeth, mainly lower canines, of 886 walruses. He standardized a method of sectioning the teeth longitudinally and of counting zones in cementum. He used the formula: two zones per year through the fifth year and one zone per year in the sixth and subsequent years; plus a factor varying from one to three years to correct for loss through attrition. He concluded that the oldest males and females were 28 and 26 years old, respectively, but according to Burns (1966, p. 5)

> . . . closure of the root apex most commonly occurs between the fifth and sixth years of life . . . it is probably not possible to determine the age of very old individuals.

Mansfield (1958b) studied 176 walruses from the northwestern Atlantic. He was able to classify calves, yearlings, and 2-year-olds, but not older animals, by body and tusk measurements. With regard to cemental layers on the premolars of the male, he stated:

> It is assumed that after the second year, one well defined layer of cementum is laid down annually, and this continues throughout the life of the animal (p.39).

He was confident that he could age males, but not all females, from counts of cementum layers on the premolars.

Pinnipedia: Phocidae

***Phoca vitulina* (harbour seal)**
Mansfield and Fisher (1960) examined an upper canine tooth of an old male harbour seal which had been held in captivity from its first summer. In thin cross sections they counted 18 to 20 cemental layers. They learned later that the seal was 19½ years old.

> There were no clearly marked dentinal layers . . . but examination of other canines from both male and female harbour seals has shown that, where dentinal layers are present, they agree in number with those in the cementum (p. 93).

Each cemental layer contains a wide opaque zone and a narrow translucent zone. The narrow zone is

> laid down in the spring and early summer and may represent a period of minimal feeding during the breeding period and the moult (p. 93).

In 1969, Michael Bigg completed a study of harbour seals from British Columbia. He wrote (1969, p. 8) that translucent layers are laid down in the cementum between late autumn and late spring, and opaque layers between late spring and late autumn.

Phoca hispida **(ringed seal)**
McLaren (1958a) made cross sections of teeth from more than 750 ringed seals and estimated age from counts of dentin layers. The oldest seal, a male, had more than 43 layers. He validated his method by counting bands on the claws. Sections of the teeth show the following sequence:

> *Mid-July to mid-March of the following year* – translucent dentin is deposited.
>
> *Mid-March to mid-July* – opaque dentin is deposited. It becomes increasingly reflective and vacuolar in June and early July when the seals are fasting, basking on the ice, and molting.

Tikhomirov and Klevezal′ (1964, Fig. 1) published a photograph of a cross section of a ringed seal tooth, as did Klevezal′ (1964, Fig. 1).

Phoca groenlandica **(harp seal)**
Fisher (1952) was first to estimate ages of harp seals by growth layers in the teeth. The oldest in a sample of 375 seals was about 28 years old. Fisher and Sergeant (1954, p.1) stated that

> because the reading of annual rings is not always exact, the totals for the ages of nine years and up are smoothed [on a graph] by fives.

And Sergeant and Fisher (1960, p. 36) noted that

> the relative strength of a year class can be detected [through tooth reading] up to five or six years; after this age, errors in age determination . . . obscure the real fluctuations in year-class strength.

According to Fisher (1954) the zones of dentin are laid down as follows. The *translucent* zone is laid down in November-March during the period of active feeding on the southbound migration and during the breeding season. The *opaque* zone starts to form during the molt (April-May) and continues to form during the northward migration and summer wandering in the Arctic (June-September).

Rasmussen (1957) published a photograph of a thin cross section of a 7-year-old harp seal tooth. He described the formation of 'white' zones laid down in feeding periods, mainly during summer, and 'dark hyaline' zones laid down in fasting periods such as molting and breeding periods in early spring.

> As the animal becomes older, the pulp cavity becomes filled with dentine, and in old animals the cavity is completely closed. This sets a limit on how far we can trace the age of an animal. With reasonable accuracy, we can read ages of 15 to 17 years, but we have specimens showing clear readings up to 27 years (p.52).

Phoca fasciata **(ribbon seal)**
Tikhomirov and Klevezal′ (1964, Figs. 2 and 5) illustrated cross sections of ribbon seal teeth. They counted cemental layers but concluded (p. 19) that age determination from claws is easier and more practical, because these do not wear appreciably with age.

Klevezal′ (1964) was interested in determining the age of sexual maturity in the ribbon seal. From counts of dentinal layers in cross sections of canines she showed that maturity in both sexes is reached by the end of the third or fourth year.

Erignathus barbatus **(bearded seal)**
Allen (1880, pp. 654–655) described the dentition of the bearded seal as

> teeth small, molars spaced, slightly implanted, early becoming defective by attrition; partly deciduous in old age, . . . Quite early in life the teeth become much worn, and in old age the crowns of the three middle molars become often wholly worn away, leaving only the fangs [roots], and even these sometimes in part disappear.

McLaren (1958b) concluded that he could not determine age from the teeth but that growth increments in claws were useful as age indicators.

Tikhomirov and Klevezal′ (1964, p. 19) concluded that bone strata in the lower jaw are the best indication of age.

Cystophora cristata **(hooded seal)**
Rasmussen (1957) made the first systematic collection of hooded seal teeth in Denmark Strait in 1954. (Sealing crews had saved the lower jaws.) A male pup in the natal 'blueback' pelage was tagged in 1951 and recaptured at age five as a 'bladdernose' in 1956. Presumably its teeth were used to calibrate the age-reading method. In a sample of at least 25 males and 22 females, the oldest male was 25 years old and the oldest female 24 years.

In his report on a sample of some 6,000 hooded seals, Rasmussen (1960) provided the following description of the canines:

> In the tooth's dentine we find alternating dark and light bands. As the seal gets older, layer after layer of white dentine is laid down round the pulp canal divided by dark layers. The pulp canal is eventually filled with dentine and in very old animals the central part will be almost completely closed. In hoods [sic] a thick layer of cement is also laid down annually on the tooth's outer surface. This layer may become very thick, especially in older animals. In this outer layer we can find closely spaced rings which in numbers agree with those we find inside the tooth itself. By counting both the inner and outer rings one can get a good check on the accuracy of age determination, especially in older animals (1960, p. 14, in translation).

A study by Øritsland of the breeding of the female hooded seal was based principally on examination of ovaries from 371 animals, all aged by dentin layers in the canine teeth (Øritsland, 1964, p. 14). The seals were collected in waters of Jan Mayen and Denmark Strait. Thirteen (or 3.5%) were described as age '21+'.

'Hansi', an adult male hooded seal, died in Tiergarten Bremerhaven in 1954 after 14 months in captivity (Mohr, 1966). Mohr photographed a thin cross section of one of Hansi's lower canines and estimated his age at 14 to 15 years. The photograph shows a very wide series of cemental layers (our Fig. 8).

Halichoerus grypus **(gray seal)**
Hewer (1960, 1964) examined teeth, mostly lower canines, of 295 gray seals. He had known-age specimens from classes 0 to 1 year, 6 years, 12 years, and 27 years. Since the pulp canal closes in the fifth or sixth year, he counted both dentinal and cemental layers up to about the fifth year, then cemental layers only (our Fig. 9).

Deposition of cementum in the gray seal is complex. Cementum first appears on the root of the lower canine

> about three to four months after birth, somewhat unevenly in two bands on the root. Afterwards, these bands coalesce (*op. cit.*, 1960, p. 960).

Fig. 8. Lower canine of a hooded seal, male 14–15 years old; thin cross section. DC = junction of dentin with the very thick cementum. (After Mohr, 1966, Fig. 2.)

For estimation purposes, October is assumed to be the mean month of birth; thus the first cementum appears in January or February.

The next layer of cementum is accumulated in September when the seal completes its first year of growth. All annual layers can be identified by the more translucent appearance toward the outer (alveolar) edge. In the second year, cementum spreads rapidly over the apex of the root, stopping all further growth of the dentin toward the apex, but deposition of dentin continues as a series of decreasingly smaller cones for another four or five years.

Monachus schauinslandi (Hawaiian monk seal)

Kenyon and Fiscus (1963) examined the right upper canines of two adult Hawaiian monk seals, a male estimated at 20 years old and a female at 11. There were no surface ridges; the pulp canals were closed; on longitudinal sections the dentin showed [annual] layers and layered cementum. The cementum on the male tooth was up to 7 mm thick and on the female tooth up to 3 mm. The layers of cementum did not decrease in thickness with advancing age.

The monk seal does not migrate.

> It may be concluded that . . . fasting, probably during molt, causes visible annular [i.e. annual] interruptions in the deposition of cementum and dentin which may be used as an indication of age . . . The pulp canal of the canines appeared to have filled completely at four or five years as indicated by visible layers in the dentin (p. 281).

Mirounga leonina (southern elephant seal)

Laws proposed a new method of age determination for

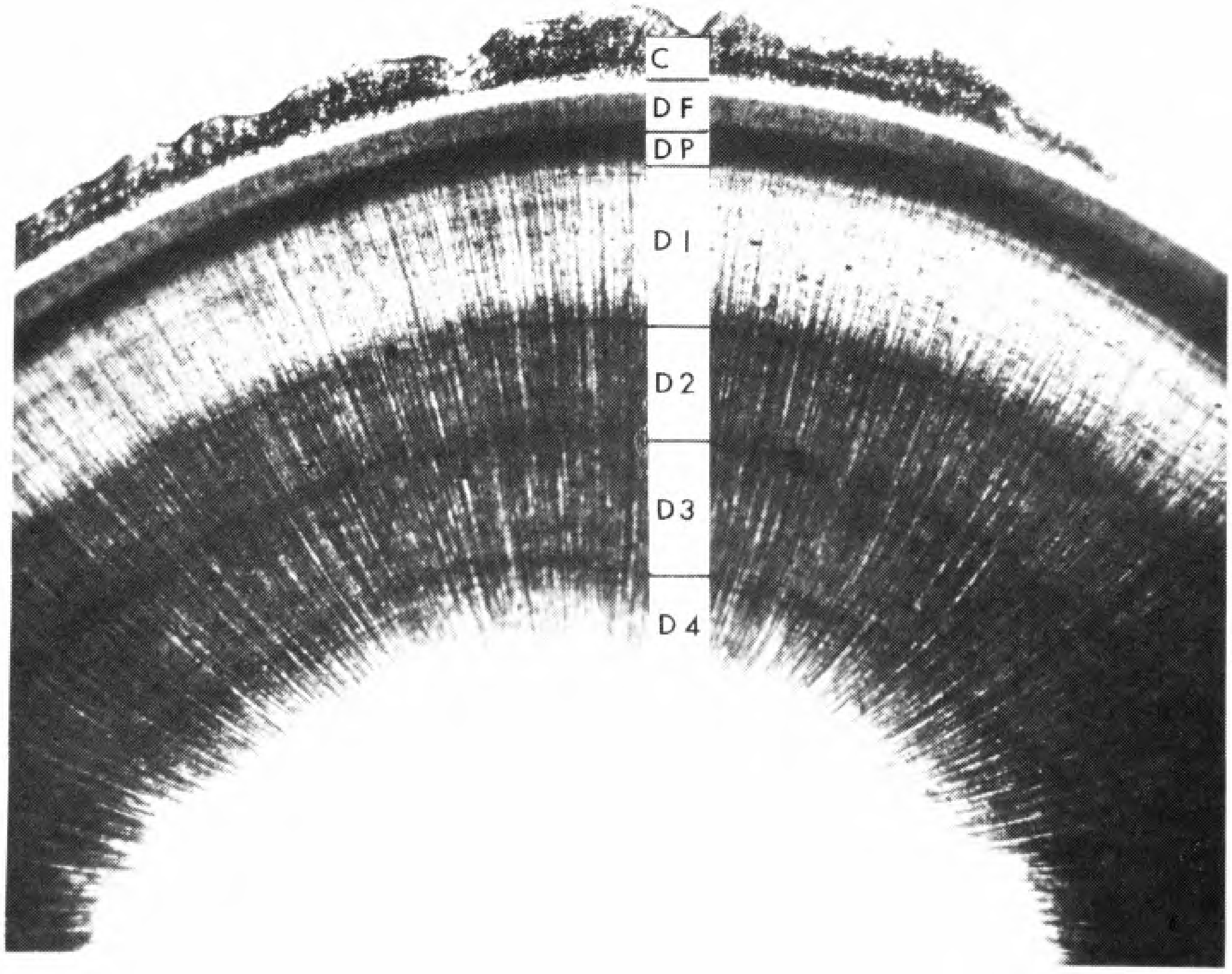

Fig. 9. Lower canine tooth of a gray seal, age 3 years, 8 months; thin cross section by transmitted light. C = cementum (separated from dentin by a clear artifact), DF = fetal dentin, DP = pup dentin, D 1 to 4 = dentin of 1st to 4th years. (After Hewer, 1964, pl. 1, Fig. 3.)

mammals largely from studies of elephant seals at the South Orkneys (1952, 1953a). He continued to study elephant seal teeth, with a special interest in microstructure and its metabolic implications (1953b, 1959, 1960, 1962).

> The canine teeth . . . grow continuously throughout life; the pulp cavity remains open and attrition is negligible (1953a, p. 3).

At the level of the alveolar aperture all annual layers are exposed.

The permanent canines erupt at an average age of eight days (lower) and ten days (upper). Inside the fetal dentin, a sharp neonatal line serves as a starting mark for counting annual layers. It is followed by a variable series of zones laid down through adolescence to sexual maturity. Maturity may occur at the end of the third year in males and the second year in females. In the fourth and later years, four zones a year are laid down rather consistently in both sexes. These include two zones of translucent dentin ('columnar') alternating with two zones of opaque ('marbled').

Although Laws did not have known-age seals older than pups, he proved indirectly that the four-zone group must represent one year's increment (1953a, p. 6). The first recaptures of known-age elephant seals, 18 branded individuals, were reported by Carrick and Ingham (1960, p. 329).

Laws (1953a, p.4; 1960, p.10) suggested the following development of hard and soft layers of dentin:

(1) a hard zone is deposited in the spring breeding period (mid-September to mid-November);
(2) a soft zone is deposited in the early summer feeding period (November and December);
(3) a hard zone is deposited in the late summer and autumn molting period (January to early May); and
(4) a soft zone is deposited in the winter feeding period (mid-May to mid-September).

Carrick and Ingham (1962) gave a detailed description, with 69 photographs and 2 diagrams, of canine tooth structure in the elephant seal. They used known-age animals, 20 males and 17 females branded as pups on Macquarie Island in 1951–59 and killed in 1956, 1957, 1959, and 1960. The oldest was 8 years, 3 months old. All four canines were saved and were treated by various methods designed to reveal dentinal layers to best advantage without the labor of cutting thin sections (our Fig. 10).

The findings of Carrick and Ingham differed from those of Laws for certain reasons:

(1) at South Georgia, where Laws worked, the male and female seals mature sexually at two and four years, respectively (Laws, 1960, p. 472; at Macquarie they mature at three to five and six years, respectively.
(2) Carrick and Ingham had the advantage of known-age specimens, many of them representing the younger ages. Furthermore, some of the marked animals had been seen and identified in the wild before death, and had been reported as 'ashore in May', 'pre-molt in February', or otherwise.
(3) Carrick and Ingham sectioned the teeth at the top of the pulp cavity where the zones are widest, and they stained the sections; both procedures contributed to a clearer picture of the zones. Carrick and Ingham concluded that 'the pattern of calcification is less simple than Laws suggests' (1962, p.111).

Lobodon carcinophagus (crabeater seal)

Laws, (1953a, Fig. 4) illustrated a cross section of a lower canine of a male crabeater seal estimated to be six years old. Later, he estimated the ages of 76 crabeaters, some found dead after an epizootic. The oldest were 19 years old (Laws and Taylor, 1957). Laws studied the same sample again (1958) in greater detail. The sequence of zone formation is approximately as follows:

(1) Thick fetal dentin laid down in the first six weeks of pregnancy.
(2) A sharp neonatal ring, about September.
(3) A (presumed) weaning ring five or six weeks later.
(4) A series of fine, irregular, post-weaning layers becoming vacuolated toward the end of the first year, in September or October (spring). The vacuolated zone is white or gray by reflected light and is the most useful zone for indicating age, for it appears each year in spring.

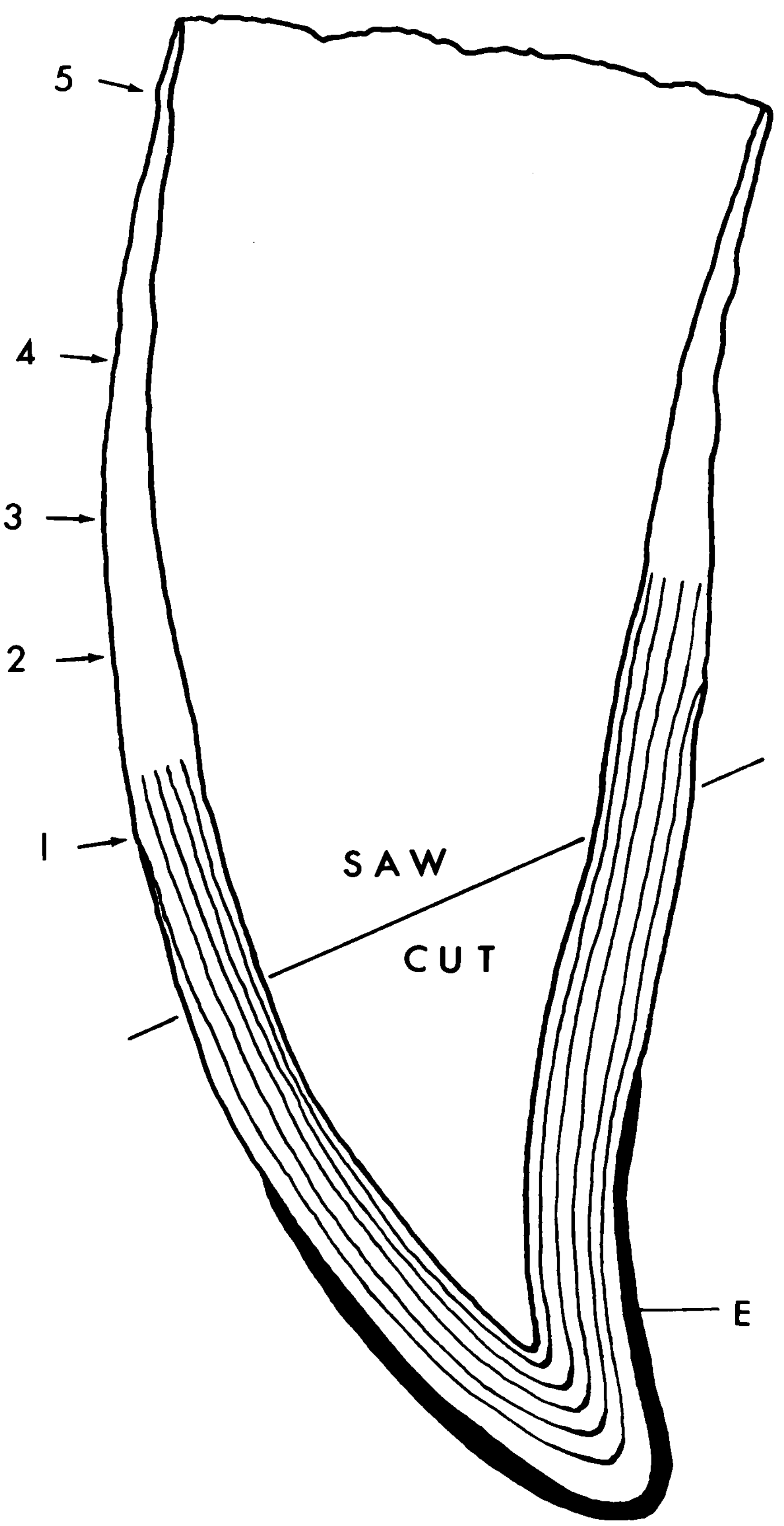

Fig. 10. Lower canine of an elephant seal showing where a typical saw cut is made at the level of the gum to obtain the crown and a portion of the root containing all annual layers. Longitudinal section, diagrammatic; 67-month ("6-year") male. E = enamel; arrows show approximate position of apex at birthdays 1 to 5. (After Carrick and Ingham, 1962, pl. 4.)

***Ommatophoca rossii* (Ross seal)**

Laws (1953a, p. 9) saw concentric rings on cross sections of a Ross seal tooth.

***Hydrurga leptonyx* (leopard seal)**

Laws (1952) saw concentric rings on cross sections of leopard seal teeth and subsequently (1953a, Fig. 4b) illustrated a section from a canine of a 6-year-old female.

Paulian (1955) counted 'stries d'accroissement' on teeth of six leopard seals from Kerguelen Islands. He felt certain of the ages of three males, one, two, and three years old, but less certain of the ages of three other seals about five to eight years old.

Laws (1957) estimated the ages of 39 leopard seals; the oldest was a male of 16-plus years.

***Leptonychotes weddelli* (Weddell seal)**

Laws (1952, 1953a) saw concentric rings on cross sections of teeth of the Weddell seal.

Mansfield (1958a) examined the teeth of six male and twelve female Weddell seals from the South Orkneys (our Fig. 11). He estimated the age of the oldest male at 16 years; of the oldest female at 14 years. From outside in, by reflected light, the sections show:

(1) wide, dark zone (translucent) dentin deposited in fetal and suckling life;
(2) a wide, light zone of opaque dentin assumed to represent the winter period of feeding at sea;
(3) another translucent zone, now called 'columnar' dentin, assumed to represent the summer period of breeding and molting, with irregular feeding;
(4) another opaque zone, and so on.

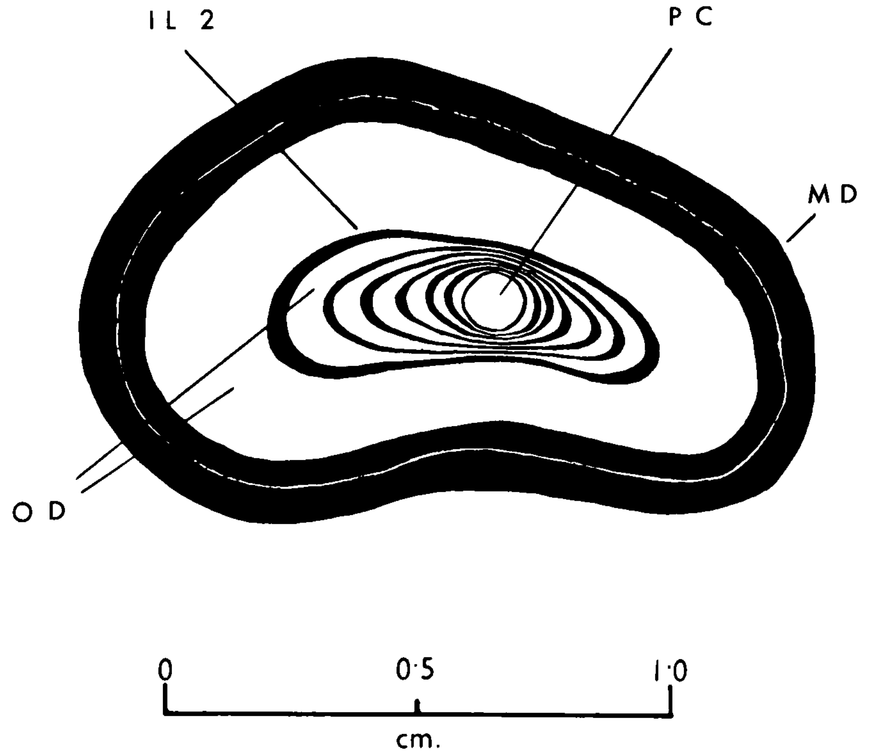

Fig. 11. Diagrammatic cross section of a canine tooth of a female Weddell seal about 8 years old, by reflected light. PC = pulp cavity, MD = "mantle" or translucent dentin presumably laid down up to the end of lactation, OD = opaque dentin, IL 2 = inner limit of 2nd year's growth. (After Mansfield, 1958a, Fig. 17.)

Sirenia: Dugongidae

***Dugong dugon* (dugong)**

Pocock (1940) used body length and cranial sutures as criteria of physiological age in dugongs. Scheffer (1970) studied dentinal layering in the tusk, using etched, half-tooth and longitudinal thin sections. Dentin seems to be deposited with annual, monthly, and perhaps even daily periodicity.

CONCLUSION

Research interest in the layering of marine mammal teeth began to quicken about thirty years ago. Now the tooth layers of thousands of individuals are routinely read each year as a procedure in life-history analysis. Despite their wide use as age indicators, tooth layers remain poorly understood with respect to their etiology, biochemistry, and physiology. The problems of preparing teeth for examination and examining and interpreting teeth layers will be dealt with in the papers which follow.

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Histological Studies of Dental Tissues of Odontocetes

Alan Boyde
University College London, Gower Street, London WC1E 6BT, England

ABSTRACT

This article discusses the nature of incremental lines in enamel, dentine and cement with special reference to the odontocetes. Some peculiarities of the dental histology of this group are also described.

Incremental lines, which are most probably due to a circadian rhythm of ameloblast secretory activity, are found in both the deeper, prismatic and superficial prism-free layers of enamel, which is formed mainly during the foetal period.

Incremental lines in dentine may show variations in optical translucency, (radio-) density, the proportions of the matrix which are mineralized or not mineralized (interglobular dentine), the size distribution of the large mineral particle clusters or calcospherites, the degree of fusion of neighbouring calcospherites, the degree of mineralization within the calcospherites, the amount of peritubular dentine, the proportion of ground substance (proteoglycans stainable with haematoxylin) to collagen, the proportion of carbonate to phosphate in the apatitic mineral component, the proportion of space occupied by the dentinal tubules and their side branches and the orientation of the dentine tubules. All of these factors may correlate with variations in the rate of formation of new matrix, coupled with factors controlling the mineralization process. Evidence is presented which suggests that both matrix formation and mineralization may both come to a complete stop at certain stages during both dentine and cement formation in some odontocetes.

Apart from the marked growth layers, odontocete oddities include the high proportion of prism-free enamel, the depth of dentine occupied by von Korff fibres, the presence of collagen parallel with the tubule axis in two walls of the tubules in some species, and the high proportion of lateral and anastomotic branches of dentine tubules. The beaked whales may possess both vasodentine and vasocementum in addition to the more common dental tissues.

INTRODUCTION

'What is at the origin of the systemic physiological disturbance giving rise to the lines?' If that could be answered, the correlation between season and growth layers could be made. For certain terrestrial mammals and for pinnipeds, gross seasonal changes in feeding habits correlate well with gross fine structural changes in the dental tissues, but perusal of this volume will indicate how limited is the equivalent data for cetaceans.

'What are the cellular mechanisms by which the putative systemic physiological modulations are transduced into structural change in the hard tissue matrix and mineral components?' We are far from answering this question for odontocetes. However, we are also far from being able to describe the interplay of (1) body-level changes in factors such as general nutrition and blood levels of calcium, magnesium. phosphate, active vitamin D metabolites ($1.25(OH)_2D3$), parathyroid hormone, calcitonin, prostaglandins, growth hormone, sex hormones and other steroid hormones, (2) the consequent modulations of cell behaviour, and (3) the consequent changes in hard tissue structure and/or chemistry for man or laboratory mammals.

However far from the goal of understanding 'physiological mechanisms and correlates of layer deposition in (hard) tissues of mammals', the acquisition of some understanding of the normal range of appearance seen in the formative areas of the hard tissues and of the changes in structure which correspond to the gross growth layers seems to be entirely pertinent to the aims of this workshop and symposium, and, in this spirit, I offer this brief descriptive review of some features of odontocete dental histology. The figures appear on pp. 70-87.

DESCRIPTION

Enamel

Enamel in odontocetes has a simple structure. The parallel prisms run straight, they do not decussate (Figs. 1 and 2). They are bounded by incomplete circular sheaths and stacked in hexagonal arrays (Figs. 4, 5 and 6). Separate interprismatic and prismatic areas occur, partly separated by sheaths, though there is little difference between these areas (Fig. 4) except in their sites of origin (Boyde, 1976). In many odontocete species there is a thick layer of prism-free enamel (Fig. 2). In some species, this type of enamel occupies the whole thickness of the tissue.

A recent hypothesis suggests that variations in the rate of enamel secretory activity in the ameloblasts over a 24-hour period would induce variations in the width of the prisms at the expense of the intervening interprismatic phase, and changes in the carbonate to phosphate ratio in the apatite-like mineral phase (Boyde, 1979) (Fig. 3). Both effects would help to explain the visibility of the 'cross striations' by optical microscopy and by back-scattered electron imaging in the SEM. Nevertheless, whether or not the prismatic markings of the enamel are easily seen, the daily incremental growth layers, that is, the cross striations, perpendicular to the prism direction and nearly parallel to the tooth surface, are most often quite clearly marked (Figs 1 and 2).

The cross striations of the enamel prisms – and the equivalent, presumably daily, layer lines found even in the non-prismatic enamel – may be used to determine the interval between the onset of enamel formation in a tooth and the time of death that occurs whilst enamel is still forming (Boyde, 1964). Unfortunately, most cetaceans have thin enamel and form their teeth in such a way that most, if not all, of the enamel is laid down before birth.

From the practical viewpoint, therefore, we may neglect the usefulness, except in studies to verify foetal age-growth classes, of the internal structure of enamel in odontocete age determination.

Dentine

(1) *Collagen.* Orthodentine in odontocetes has been studied far more by ageing experts than by developmental histologists (Christensen, 1973; Gambell, 1977; Grue-Nielsen, 1973;

Kimura, MS; Klevezal' and Kleinenberg, 1969; Klevezal', 1973; Nishiwaki and Yagi, 1973; Ohsumi *et al,* 1973; Sergeant, 1959). Many interpretations of the adult odontocete dentine structure are therefore indirectly based on developmental studies made in other mammals (Laws, 1952; Lowe, 1967; Morris, 1972; Scheffer, 1950; Yilmaz *et al,* 1977). I believe that considerable efforts should be devoted towards understanding the normal features of the development of this tissue in each and every species if modulations of its structure are to be used in the determination of age (Craighead *et al,* 1970; Jensen and Brunberg-Nielsen, 1968; Kimura, MS: Klevezal', 1973; Mitchell, 1963; Morris, 1972; Yilmaz *et al,* 1977).

Odontocete dentine differs qualitatively from that of other mammals in the proportion of collagen which is present as large (more than one micron) diameter bundles of parallel fibrils (Figs. 7, 8 and 12). Typically, large diameter bundles called 'von Korff fibers' are restricted to the most peripheral dentine layer called 'mantel dentine' in other mammals (Lester and Boyde, 1968). They may extend through the thickness of the dentine in the sperm whale (Figs. 20, 28 and 29) and narwhal, where they are located in the walls of some of the dentine tubules. In other words, the dentine tubule wall may have collagen parallel with the tubule axis, a circumstance which is exceedingly rare in other mammals. It has yet to be described how this arises and how the presence of this unusual phase affects the process of mineralization of the dentine. Such radial fibers may function to strengthen the tissues by tying together the feltwork of fine fibrils with an orientation parallel to the formative surface of the pre-dentine (Figs. 8, 9 and 16). The latter phase forms all of the 'circumpulpal' dentine in other mammals and is still the predominant phase in odontocetes. The role of the radial, tubule-wall collagen may be of greater importance in the apparently poorly mineralized dentine of the sperm whale and narwhal, since a lesser degree of mineralization would permit greater flexibility and ease of rupture in the calcified connective tissues.

(2) ***Mineralization.*** Mineralization of odontocete dentine occurs by formation of clusters of calcium phosphate (Hydroxyapatite) crystals close to and in the plane of the mineralizing front (Figs. 10, 11, 17, 24, 28, 33 and 35). These clusters may originate as membrane-bound extrusions – 'matrix vesicles' (Anderson, 1976; 1978) – liberated by the odontoblasts. They grow by 'infecting' concentric tissue layers with the mineral deposition process, thereby often achieving a roughly spherical form which matches their name – calcospherites (Figs. 10 and 11).

Gross variations in the degree of mineralization of the dentine, correlating to some extent with the light-optically visible growth layers, can be explained in some cases by the failure of all the calcospherites to fuse. Thus classical interglobular areas (IGD) of unmineralized dentine may be present (Figs. 19, 26 and 27). It is, however, unclear that variations in the proportion of IGD provide explanations for all aspects of the growth layering seen in odontocete dentines, and it will be necessary to investigate whether there are not significant variations in the degree of mineralization within them. It is generally assumed that poorly mineralized dentine as a whole consists of patches of well mineralized dentine mixed with patches which have not mineralized (IGD).

(3) ***Mineral density.*** Electron microscopical evidence so far suggests that IGD contains no crystals, whereas mineralized areas are so dense that the individual crystals can be resolved only with difficulty (Fig. 27). However, there may be important differences in the density of mineralization within areas which were taken to be uniform in the past. More careful electron microscopy may contribute to this point. The continuity of crystals at the macroperiod boundaries within the collagen (Fig. 22)—whether they are waisted at these points or not—the diameters of the crystals in collagen, and the packing density of crystals in the ground substance between the collagen, need to be examined minutely.

X-ray microscopy of plane-parallel ground sections shows that the incremental lines in both cementum and dentine correspond to variations in the density of the tissue, most probably due to variations in the degree of mineralization (Fig. 30). However, the results do not contribute to the question of the qualitative versus the quantitative aspects of the mineral distribution.

(4) ***Light-optical characteristics.*** Incremental lines in dentine are most commonly studied by light-optical methods, when the relative opacity and translucency of the tissue is scrutinized. Translucency is an expression of structural homogeneity. Both homogeneously non-mineralized (or experimentally demineralized) tissue and well-mineralized tissue are translucent.

Local refractive index changes due to inhomogeneities like the calcospherites in surrounding non-mineralized dentine matrix, lead to light scattering. The light-scattering properties of the tissue will depend on the size of, and the distance between, the scattering particles, as well as on the refractive indices of the two components, that is, mineralized dentine and surrounding non-mineralized dentine matrix. (The refractive index difference will change when the tissue is dried, or the water is substituted with another solvent such as ethanol, xylene, or canada balsam.) Two layers of dentine would show different light scattering properties yet have the same degree of mineralization if layer A had fewer, larger calcospherites and layer B had more, smaller ones. Furthermore, two layers may have similar light-scattering properties and different degrees of mineralization—in the extreme, the difference may be between 0% and 100% mineralization. An examination of optical characteristics of native dentine may, therefore, not provide the necessary means of distinguishing between different types of layer, and this is probably the reason behind the present widespread use of 'etching' (i.e. partial demineralization) and histological staining procedures in the study of growth layers.

(5) ***Demineralization ('etching').*** 'Etching' with, for example, formic acid, dissolves the calcium phosphate below the cut and polished sectioned surface of the tooth. The depth to which demineralization progresses will depend upon diffusion-transport properties of the local area in the tissue, and may thus be influenced by the number, size and orientation of spaces such as cell process spaces and non-mineralized spaces between mineralized areas within the tissue. However, it is probable that the main factor influencing the progress of demineralization is the degree of mineralization of the tissue; more heavily mineralized patches will be less porous, have more mineral to be transported out of them, and give rise to local high calcium and phosphate ion products which can lead to local remineralization. All these factors lead to a slower progress of demineralization. The production of the surface relief which perhaps partly justifies the use of the term 'etching' depends upon air drying and the associated shrinkage. The superficial layer of demineralized dentine

shrinks as it dries down on to the 'demineralized front' within the tissue, and because this front is irregular, so is the surface of the dried demineralized tissue. 'Etching' can be simply and effectively improved by dissolving away the superficial demineralized layer with, for example, a strong NaOCl solution. The demineralized layer actually serves to obscure the sharper detail present at the demineralization front.

(6) *Cellular space.* The size and packing density of calcospherites in dentine are clearly not the only factors affecting the degree of mineralization and the light-scattering properties of this tissue. The proportion of cell-process-occupied space is a major factor affecting the net degree of mineralization in all calcified connective tissues, and the orientation and division of the cell space component affect the optical properties. The sub-cemental granular layer of Tomes in the peripheral dentine of human tooth roots is an example of a hypomineralized (non-incremental) layer of complex origin. There is a poor degree of mineralization of the matrix, and there is an increased cell-volume-space component to reduce the proportion of the tissue which could be occupied by mineral. Cetacean dentines frequently show examples of incremental layer lines which have an increased cell-space fraction, either due to simple dilatation of the dentine tubules or an increase in the number or the dimensions of their branches.

(7) *Mineralization and the peritubular dentine.* It is generally accepted that intertubular dentine reaches its level of mineralization at the mineralizing front and that there is no maturation phase analogous to that which can be demonstrated in lamellar bone and dental enamel in this tissue. Probably, the levels of mineralization do not change subsequent to the passage of the mineralization front in this tissue. However, the level of mineralization within dentine can be altered by the deposition of peritubular dentine. Peritubular dentine (PTD) is a highly mineralized phase which, in mammals other than cetaceans, generally does not contain collagen. Its formation increases the net mineral content, and by reducing the cell-space fraction, increases the optical translucency. In man, PTD does not form locally within regions of interglobular dentine. If this is also true in cetaceans, the formation of PTD would be expected to exaggerate, rather than diminish, the prominence of layering due to differences in the degree of mineralization (amount of IGD).

(8) *Tubular orientation.* Some layer lines in dentine are associated with a change in orientation of all the dentine tubules (Fig. 18). Such changes might reflect a variation in the rate of matrix synthetic activity on the part of the odontoblasts. Some layering phenomena detected by oblique incident illumination are evidently not incremental in origin, but reflect the varying behavior of large groups of odontoblasts within a vast sheet of these cells. The checker-board appearance seen in transverse sections of elephant tusks and, occasionally, in sperm whale dentine has this origin. Alternate longitudinal columns of odontoblasts have an alternate sense of a component of movement, translocating across the surface which they are forming. Some groups of cells are moving up the tooth, and others down, so that the net statistical movement is zero. Layering due to cell movement patterns is often not parallel with the incremental growth planes and could, conceivably, interfere with the ordinary business of counting incremental growth layers.

(9) *Differential rate of formation.* Changes in the rate of formation of dentine may be associated with changes in the chemistry of both its organic and the inorganic components as well as the directional properties of the major cell process spaces. Practically important in this regard is the ratio of the basopholic glycosaminoglycan (proteoglycan, sulfated acid mucopolysaccharide) ground substance component of the matrix to the acidophilic collagen. Several growth-layer counting problems have only been practically tackled through the use of haematoxylin staining, of either 10 to 20 μm-thick decalcified sections or the superficially decalcified surfaces of thick sections or half-teeth. Comparative studies of mineralized connective tissues suggest that more basophilic layers containing more ground substance in proportion are formed both when the rate of formation of the tissue stops and when new formation commences after a quiescent period. Circumstantial evidence strongly supports this interpretation of the haematoxylin staining lines in mammalian cement and dentine (Craighead *et al*, 1970; Grue-Nielsen, 1972; Jensen and Brunberg-Nielsen, 1968).

(10) *Mineral composition.* It is not known whether the dentine mineral component also varies chemically in growth layers of different density and optical character, but, by analogy in enamel, at least the daily growth layers might vary in their carbonate content. The simplistic viewpoint is that carbonate is a large component of the hydroxypatite-like mineral in bone, cement and dentine. CO_2 is an end product of metabolism, and local pCO_2 would be higher in more metabolically active tissues. Thus dentine formed during more rapid growth periods might contain more carbonate and this might have direct effects on the refractive index of the tissue, or an indirect effect due to some modulation of the process of nucleation and growth of calcospherites.

(11) *Physiological correlates of dentinal layering.* There is a paucity of data concerning the physiological correlates of layer deposition in cetacean dentines. If certain structural or chemical findings in the dentine of a whale match those in the dentine or bone of a laboratory (Yilmaz *et al*, 1977) or well studied terrestrial mammal, where the physiological correlates, such as changes in vitamin D, fasting versus feeding (Laws, 1952; Scheffer, 1950), parathyroid hormone and sex hormone status can be well characterized, then we may attempt to make inferences by analogy. Although this approach will not get us very far, let us examine the conclusion to which it can lead for odontocete dentine.

Systemic origin of layering

Any method of low-resolution microscopical examination will show a similar pattern of incremental layers in the dentine of teeth which were forming at roughly the same rate and exactly the same time in one individual. Because no two growth layer groups are identical, subtle dissimilarities can be used to cross-match the growth layers between the teeth of one individual (Boyde, 1964). The observations can only be interpreted by assuming that the dentine growth layers are related to changes in factors not related to individual teeth or related groups of teeth.

Mineralization under systemic control

SEM examination of mineralizing-front preparations shows that the greater part of the entire surface within one tooth or groups of teeth forming at the same time in an individual shows similar appearances regarding the size, range and morphology of the calcospherites at the mineralizing front

(Figs. 11, 17, 26 and 34). This suggests, theoretically, that one of the factors under systemic control is the pattern of mineralization of the dentine, and practically, that for simple conical teeth, one area of the forming front of the dentine may be sampled and the results used to characterize all the forming areas in that tooth and similar regions of teeth in similar stages of development.

Matrix and mineral deposition sometimes stop

SEM examination of mineralizing-front preparations occasionally shows that the collagenous component of the dentine is more or less fully mineralized (Fig. 25). Calcospherites are not seen, because mineralization has progressed to a limit or stop, and that arrest can only be due to the fact that there is no more matrix to be mineralized. The observation establishes that dentine matrix production may come to a complete halt and then start again. This is often tacitly assumed to be the case by wildlife biologists, but is the reverse of the assumption most generally used by dental histologists.

Rate of formation varies

Interpretation of SEM appearances of dentine mineralizing fronts suggests similarities of the dentine matrix with rapidly formed foetal-type bone during rate-reduced phases and with adult lamellar bone during active growth phases. Thus it is characteristic of foetal bone-mineralizing fronts that one cannot recognize the collagen pattern in the distribution of the mineral–and this is the most common circumstance in odontocete dentine formation–whereas in adult bone one can. 'Collagen fibrils' can be recognized in stop-phase preparations (Fig. 25).

Mineral deposition sometimes stops while matrix formation continues

Anorganic preparations of dentine sometimes almost fall apart, and are often easily cleaved, along incremental planes where mineralization is defective. SEM studies of the faces of such cleavages show calcospherites on both sides, indicating that arrest of mineralization also occurs independently of arrest of matrix formation.

Collagen-to-ground-substance ratio under systemic control

SEM examination of cleaned predentinal surfaces in forming odontocete teeth show the collagen phase detail obscured by the overlying and embedding ground substance to varying degrees. Together with the light microscopic findings of varied basophilic staining lines (Grue-Nielsen, 1972), this suggests that variations in the ratio of collagen to ground substance in dentine are also under systemic control.

Secondary dentine, vasodentine

Much of the dentine occupying the pulp chamber is composed of orthodentine pulp stones encapsulated in a mass of orthodentine (sometimes osteodentine or vasodentine). In the ziphiids, vaso-orthodentine commonly succeeds the orthodentine of the denticle which forms the original center of the tooth, and this may be followed by vasodentine before the greater part of the tooth comes to be formed of cementum (Boyde, 1968).

Pit-like and slit-like mineralization deficiencies in primary dentine

In narwhal tusks (Figs. 32 and 36) and sperm whale teeth, local defects occur in the dentine opposite pits or grooves in the predentinal surface, i.e. in relation to localized areas where the rate of matrix formation by the odontoblast has at some time been less than average. Calcospherites fail to form in these areas, and they are bounded in the completed tissue by calcospherites (Fig. 36). They are, in effect, very large interglobular dentinal areas with a radial distribution of IGD, in contrast to the circumferential distribution of IGD in incremental lines. In narwhal material, some of the depressions in the formative surface opposite which these radial non-mineralized rows form are longitudinal helical grooves (Fig. 33) so that longitudinal radial sectors of badly mineralized dentine may form (Figs. 32 to 36). Others, as in the sperm whale, are pits and give rise to cylindrical regions of defect, running parallel with the dentine tubule axis.

Cement

The bone-like nature of cement makes it easier for the hard-tissue histologist to accept that some of the surface parallel markings (e.g. more or less radiolucent, more or less optically translucent, more or less basophilic (Grue-Nielsen, 1972; Jensen and Brunberg-Nielsen, 1968; Klevezal' and Kleinenberg, 1969), represent phases of arrest of growth. By analogy with the analysis of bone growth, the most commonly used material would be haematoxylin (± eosin) stained, decalcified, microtomed sections. Basophilic lines are called *resting* or *cementing* lines and represent periods of arrest of growth, correlating in SEM studies with regions where the intrinsic matrix collagen fibers, parallel with the surface of the cementum, are mineralized to their limits, or mineralization may have progressed slightly beyond into a superficial seam of predominantly ground substance matrix, the latter being the basophilic material.

The extrinsic, penetrating Sharpey fibers (Boyde and Jones, 1968) may mineralize to a level above that of the surrounding intrinsic fibers (Fig. 15). Conversely, active growth phase mineral fronts are characterized by the Sharpey fiber insertions being represented as pits (Fig. 41), since the mineralization proceeds more actively in the newly formed intrinsic fibers, the latter showing as incomplete entities due to the active progress of mineralization within them.

Decalcified, stained sections (or stained surfaces of superficially demineralized polished ground sections) are particularly suited to studies of cementum because of the slower rate of deposition of this tissue and the consequent closer spacing of the growth lines. Lower power microscopy is imperative in their study, but several-fold magnification is further necessary because, like bone, not all of a cementum surface may be forming at once, and it is necessary to gain a general picture of the patch-like nature of the distribution of new cemental layers (Craighead *et al*, 1970; Jensen and Brunberg-Nielsen, 1969; Mitchell, 1963).

The patchiness of cement (and bone) formation varies strongly from species to species. Counting cement layers may have the advantage over counting dentinal layers in that cemental formation evidently continues in some species for considerable periods after dentine formation has stopped. It must, however, be realized that the most important evidence for the continued formation of cementum is the finding of larger numbers of cement layers in larger teeth from (generally) larger or, on the basis of other circumstantial evidence, older animals. Evidence for the presence of active cemental formation should be carefully assessed for each species before it is taken into account. One simple observation that indicates that cementum formation is in progress in a single, isolated specimen is the SEM finding of a mineralizing front with Sharpey fiber pits (Fig. 41). On the other hand, finding a resting, mineralized front with protruberant Sharpey fibers (Fig. 15) does not show that formation has stopped forever (Boyde and Jones, 1968).

In cetaceans with larger amounts of cementum and higher cemental growth rates, the lines may be so widely placed as to permit their study in polished, sectioned surfaces or thick ground sections with the naked eye or a weak magnifying glass. In ziphiid whales, where the cementum may make up the bulk of the tooth, the cement lines may be easily distinguished with the naked eye, particularly in the grossly expanded, possibly vascular cementum (Boyde, 1968) (Fig. 38) and the highly cellular cemental regions (Figs. 37 and 40).

As a general observation, we can note that the lower-density layers in the cementum of large odontocete teeth commonly have a high cell-space volume fraction, i.e. at times which are probably periods of rapid intrinsic matrix formative activity, cementoblasts more commonly become included into the tissue and so differentiate into cementocytes.

SUMMARY

This article has considered some of the many kinds of structural variation within the successive layers of tissue which give rise to the growth or incremental layering which is such a prominent feature of odontocete dental tissues. Future work should be aimed at determining the degree of cross-correlation between several parameters, including the chemistry of the organic matrix and the mineral components, the various reasons for differences in the density of distribution of the mineral component (such as differences in the density, size and number and degree of fusion of calcospherites in dentine), and variations in the proportion and arrangement of the spaces occupied by cells and their processes in the cellular calcified tissues. These factors should in turn be correlated with the methods used by the field biologist, including relative optical translucency, haematoxylin-staining intensity and degree of air-drying shrinkage following superficial demineralization (e.g. formic acid 'etching'). Finally, one would hope for correlations between all these structural parameters and the biological factors which lay at their origin. By analogy with other mammalian species which may be more conveniently studied, we know that possible seasonal variations in many dietary and hormonal factors need to be investigated before a satisfactory explanation for growth layering can be put forward.

Odontocete dental tissues also provide strong contrasts to those of other mammalian orders, which challenge the calcified tissue biologist for explanations. How far these structural peculiarities match the peculiar environment and physiology of the cetacean also remains to be investigated.

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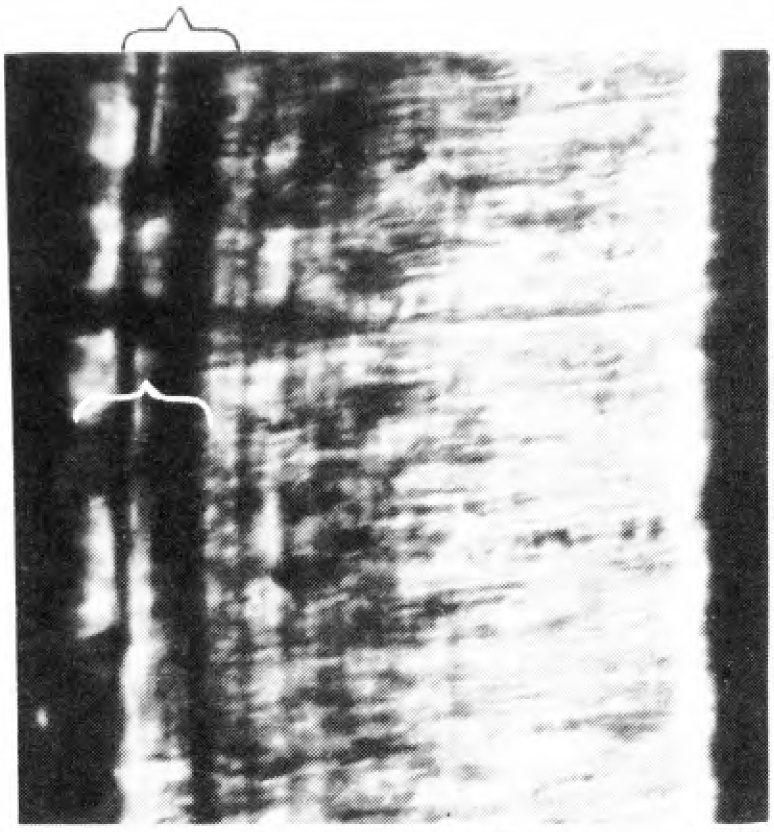

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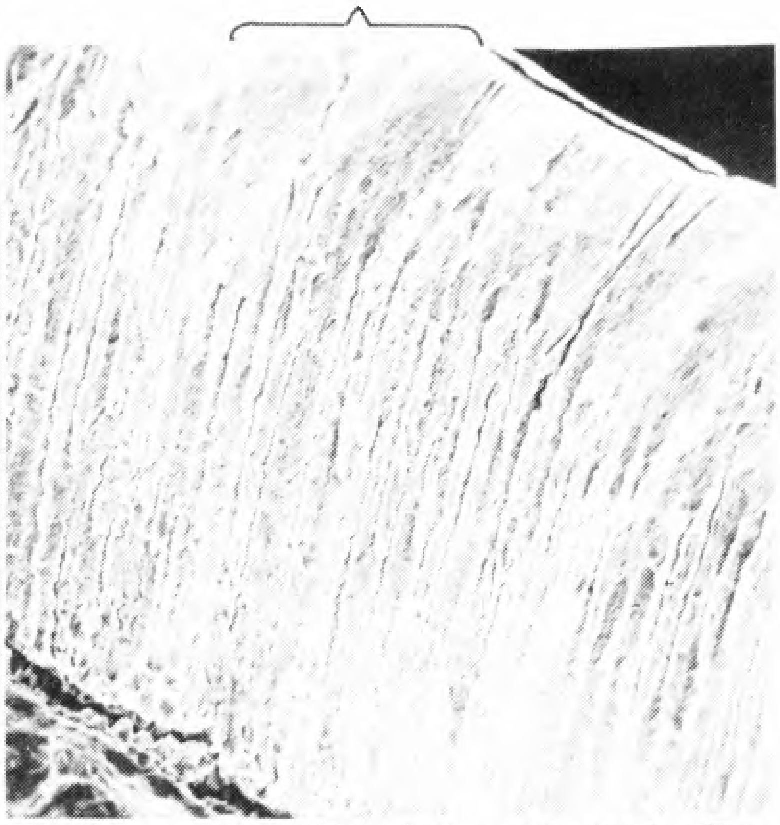

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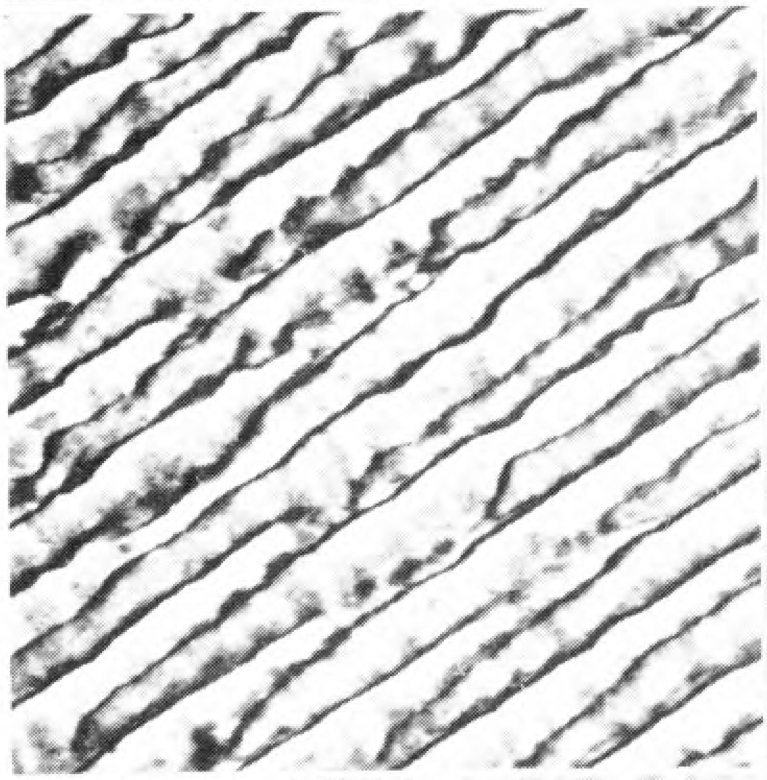

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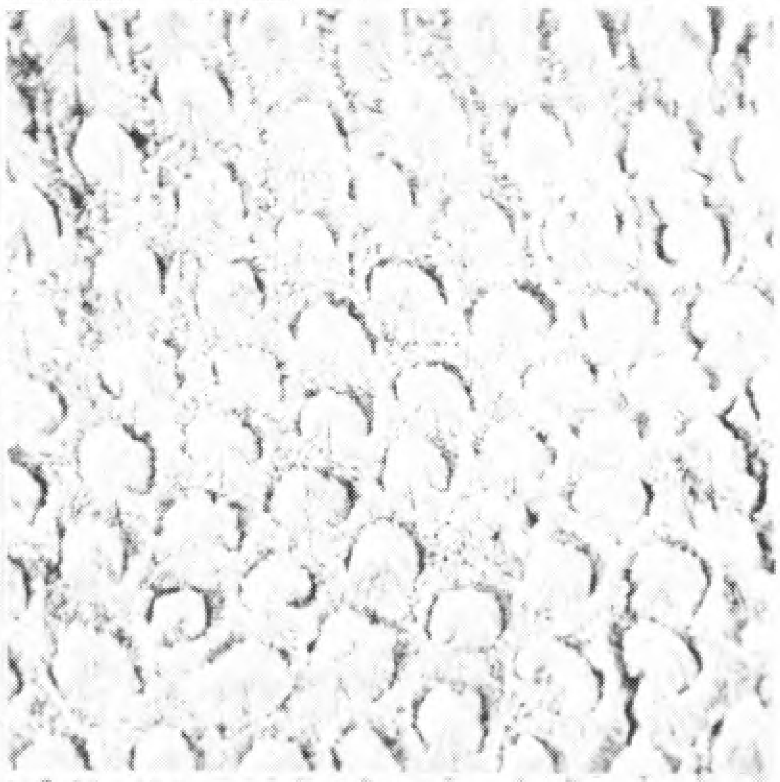

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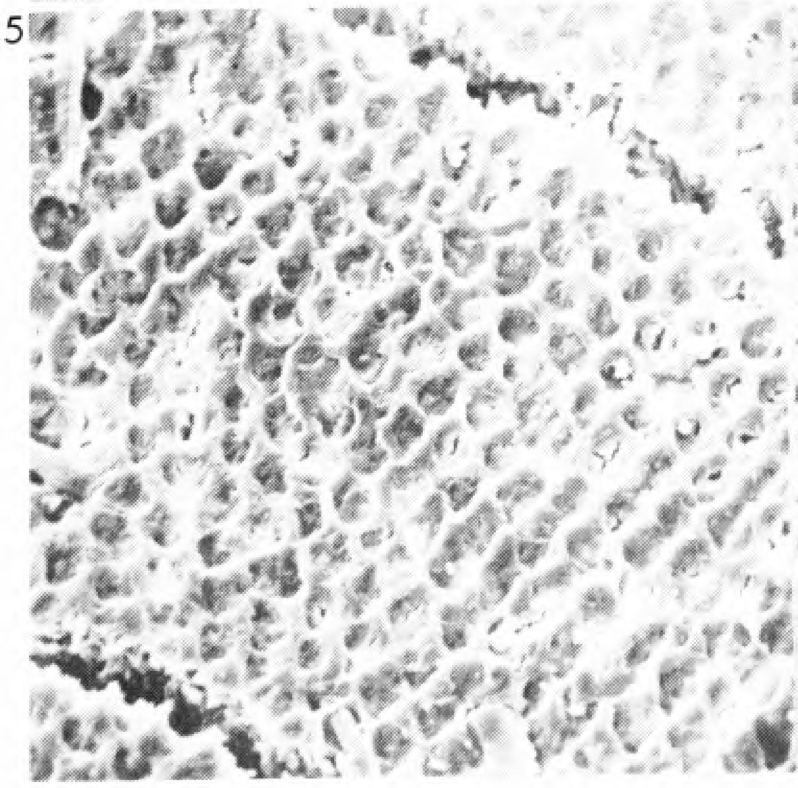

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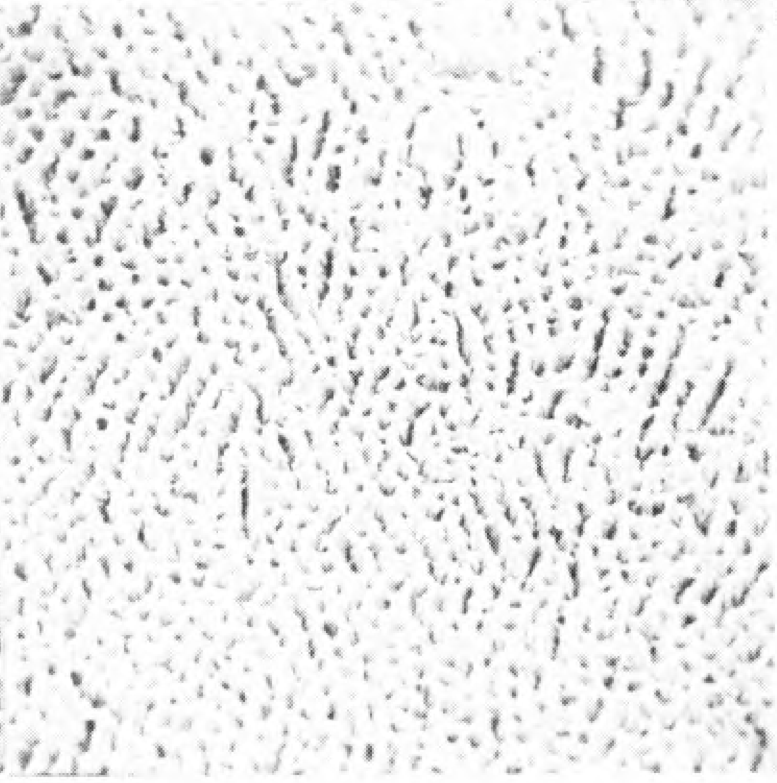

Fig. 1. TS crown of *Globicephala melaena* tooth, between crossed polarizers. Note relative thickness of prismatic and non-prismatic (bracketed) surface layer of enamel. Fine incremental lines probably reflecting circadian rhythm of enamel formation are present throughout the thickness of the enamel.

Fig. 2. LS of the same tooth (*Globicephala*) etched 1% H_3PO_4 for 20 secs., Au coated, SEM 10 kV, showing relative depths of prismatic and non-prismatic (bracketed) layers. Fieldwidth 106 μm.

Fig. 3. LS of human tooth, surface cut with Reichert-Jung rotary diamond micromiller and carbon coated. SEM 25 kV backscattered electron image, showing cross-striations of the enamel prisms (which are circadian increments of enamel) as variations in density. Fieldwidth 90 μm.

Fig. 4. *Globicephala*, polished surface TS prisms etched 90 secs. 2% H_3PO_4. SEM 10 kV. Fieldwidth 38 μm.

Fig. 5. Foetal tooth from *Stenella longirostris*, SEM 10 kV. Showing developing enamel surface. Fieldwidth 74 μm.

Fig. 6. Anorganic developing enamel surface in foetal tooth, *Stenella longirostris*. Fieldwidth 160 μm.

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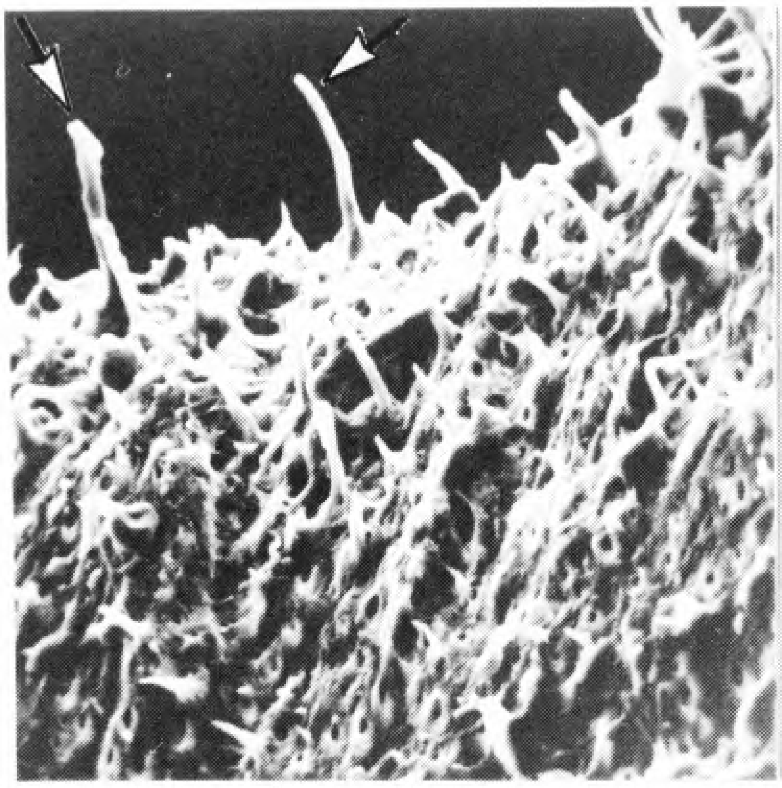

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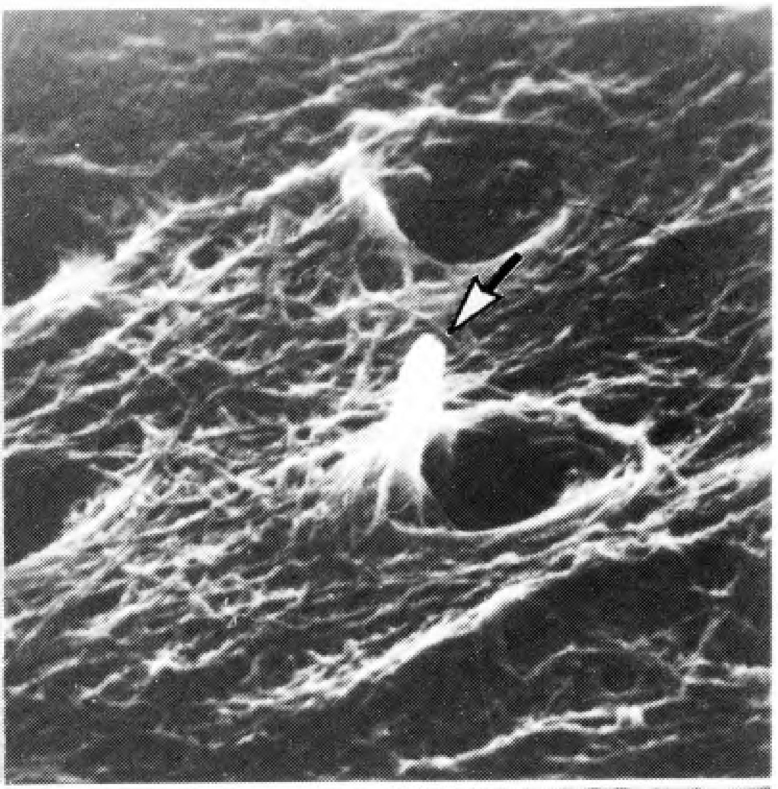

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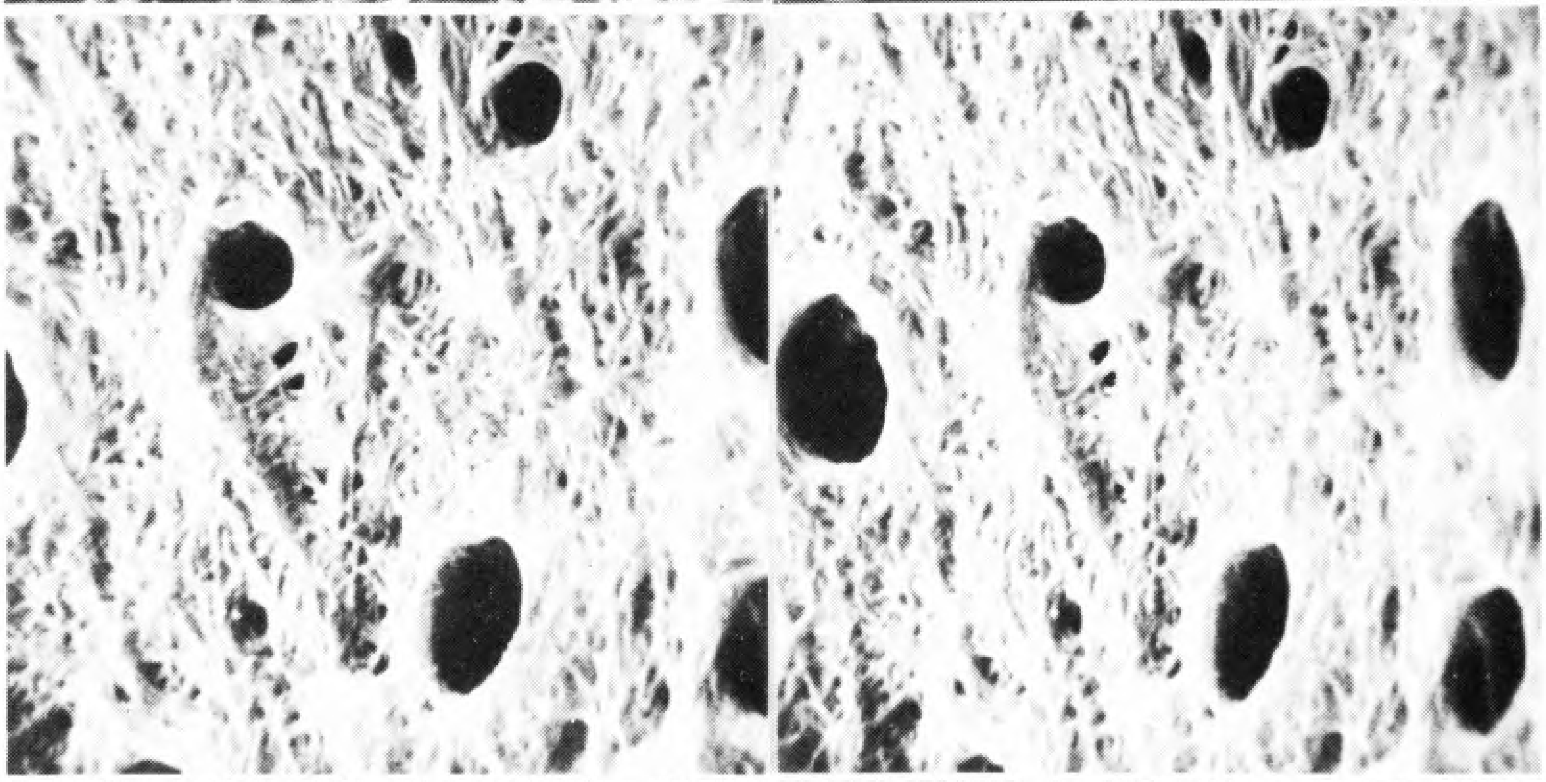

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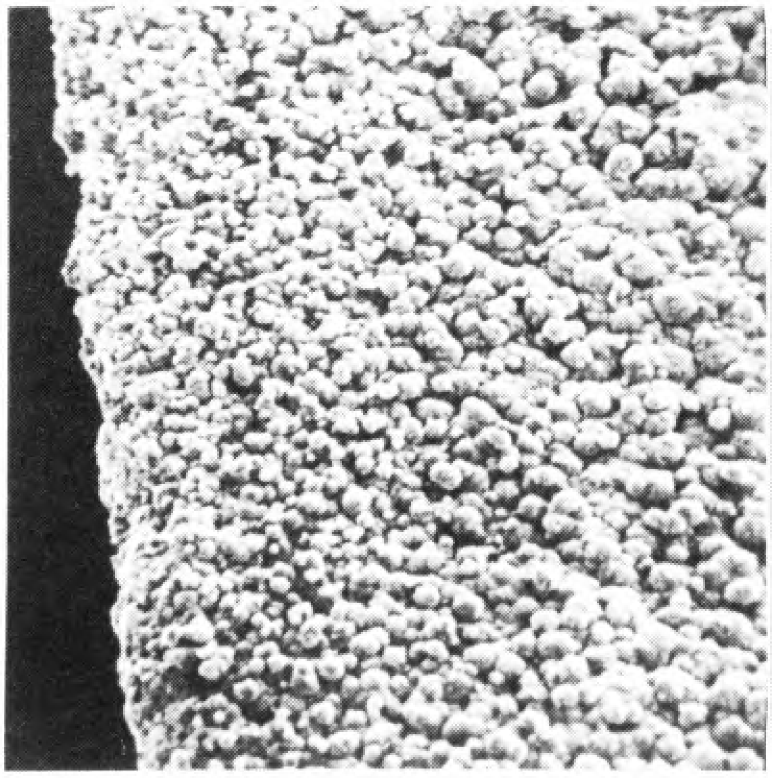

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Fig. 7. Newborn *Tursiops truncatus*. Tooth stripped of soft tissues, showing von Korff fibres (arrowed) projecting from the predentine surface close to the growing end of the tooth. Fieldwidth 82 μm.

Fig. 8. Similar preparation (*Tursiops*) away from growing end showing single collagen fibre bundle (arrowed) in dentine tubule opening, perpendicular to forming dentine surface (a von Korff fibre). Fieldwidth 11 μm.

Fig. 9. Similar preparation (*Tursiops*) showing typical features of circumpulpal dentine formation in later stages. Note fine feltwork of collagen fibrils. Stereo-pair, tilt angle 10°. Fieldheight 11 μm.

Fig. 10 and 11. *Tursiops* developing tooth treated with hot 1,2 ethane diamine to expose the mineralizing front of the dentine. Fig. 10: close to the growing edge of the tooth numerous small calcospherites are found. Fig. 11: At 2 mm and all points further from the growing edge, the distribution of sizes of calcospherites is much as shown here. Note the increase in size of these features, many of which span several dentine tubule openings. Fieldwidths both 245 μm.

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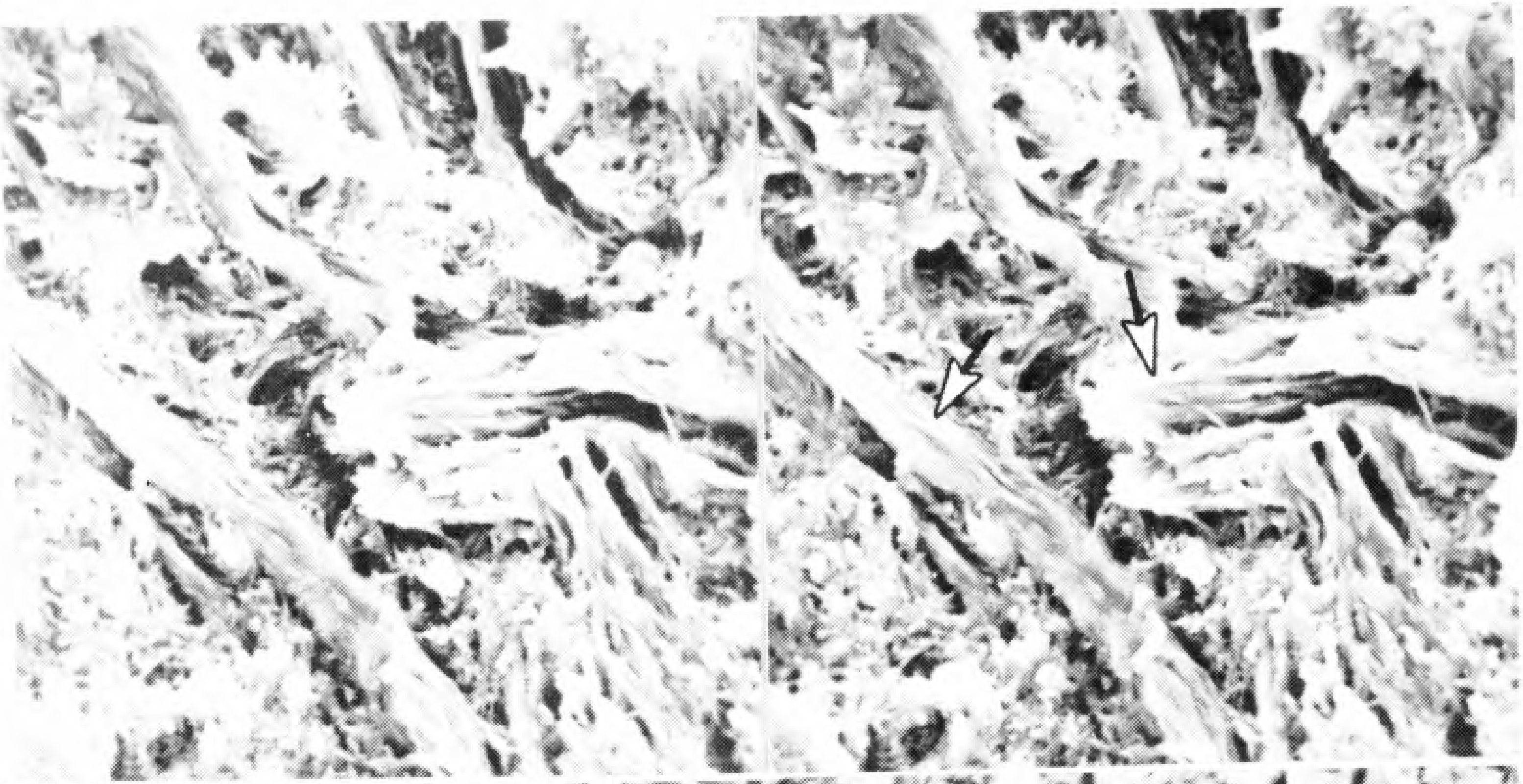

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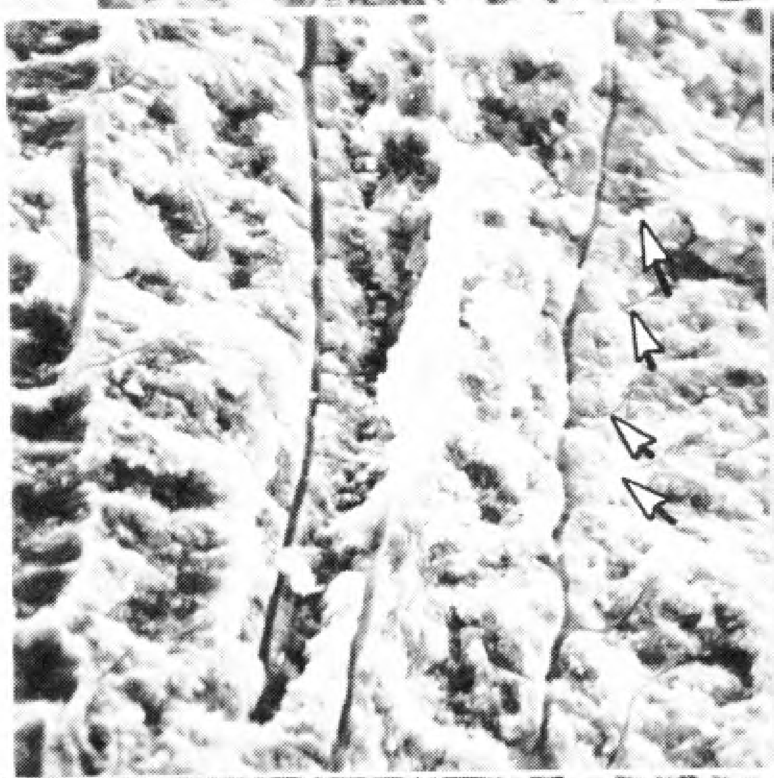

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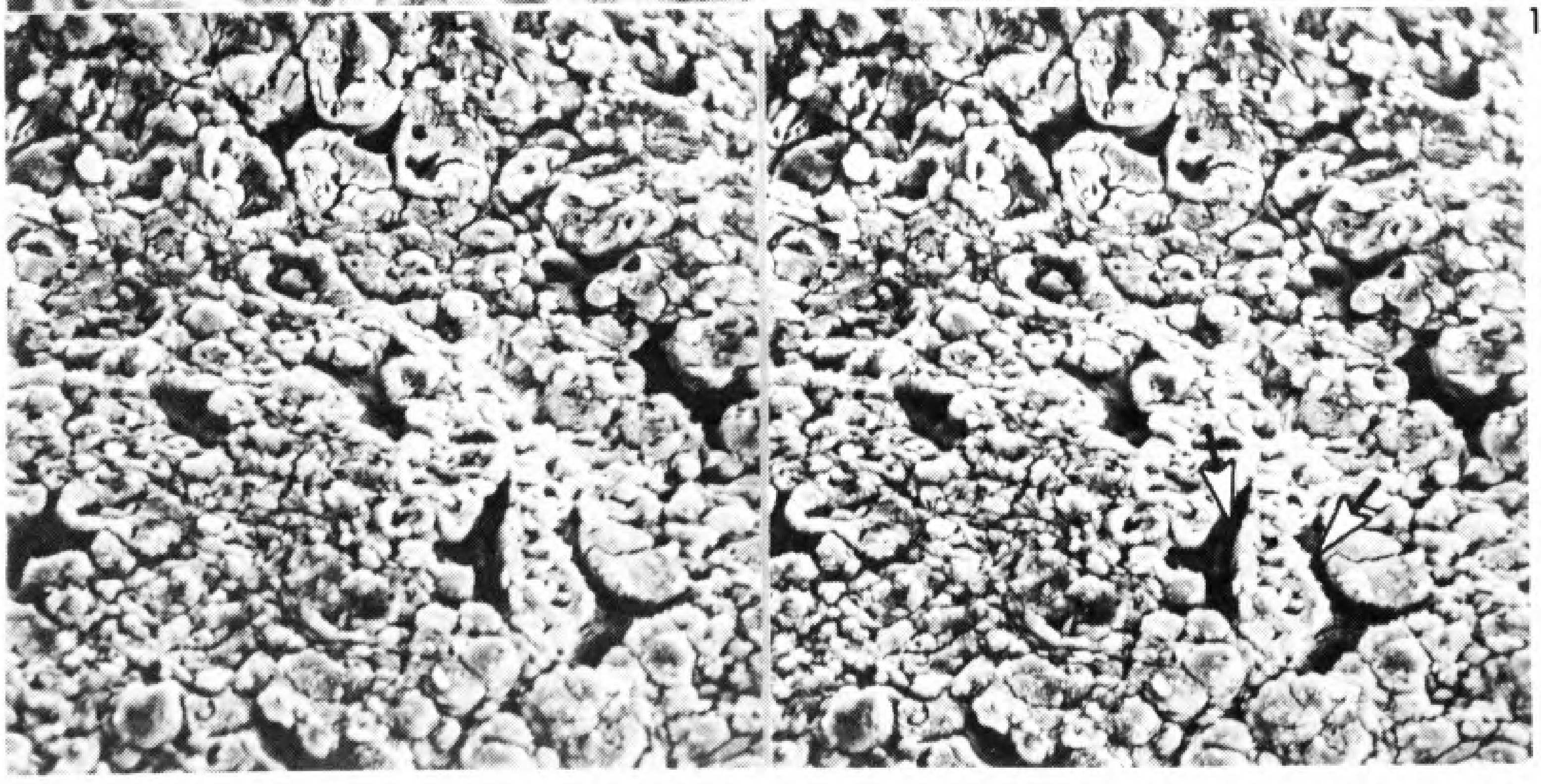

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Fig. 12. Fractured *Tursiops* tooth near enamel dentine junction showing large diameter collagen fibre bundles (von Korff fibres – arrowed). Stereo-pair tilt angle 10°. Fieldwidth 27 μm.

Fig. 13. Fractured *Tursiops* dentine, showing numerous side branches (arrowed) of major dentine tubules. Fieldwidth 27 μm.

Fig. 14. Fractured *Tursiops* dentine, showing presence of afibrillar peritubular dentine zones. Fieldwidth 27 μm.

Fig. 15. Anorganic surface of cement in *Tursiops* tooth, showing Sharpey fibres as projections, and half-formed cementocyte lacunae (arrowed), nestling between the Sharpey fibres. This is a *resting* (i.e. *not* actively forming) surface. Stereo-pair, tilt angle 10°. Fieldwidth 74 μm.

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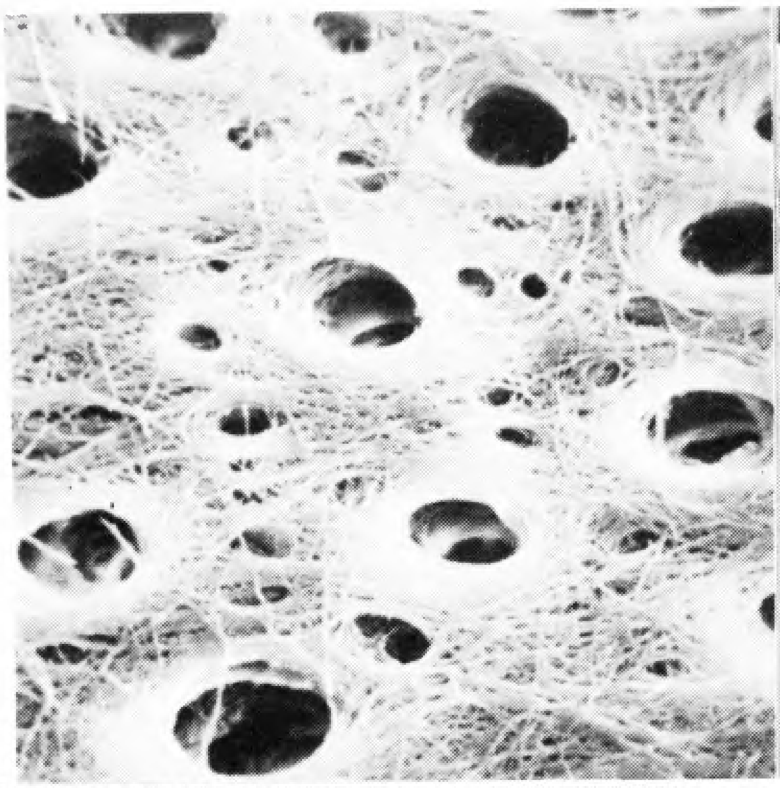

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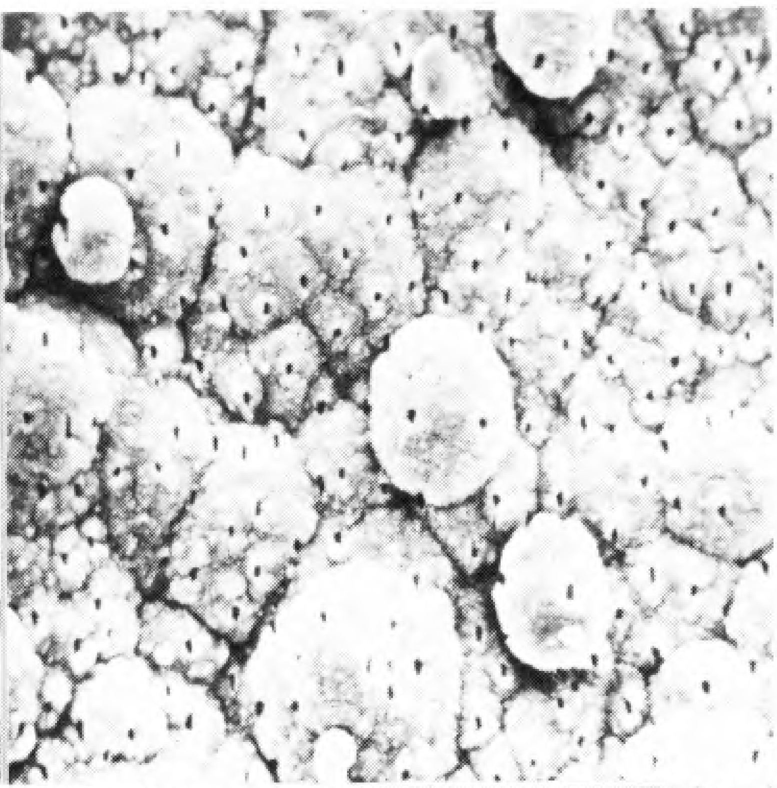

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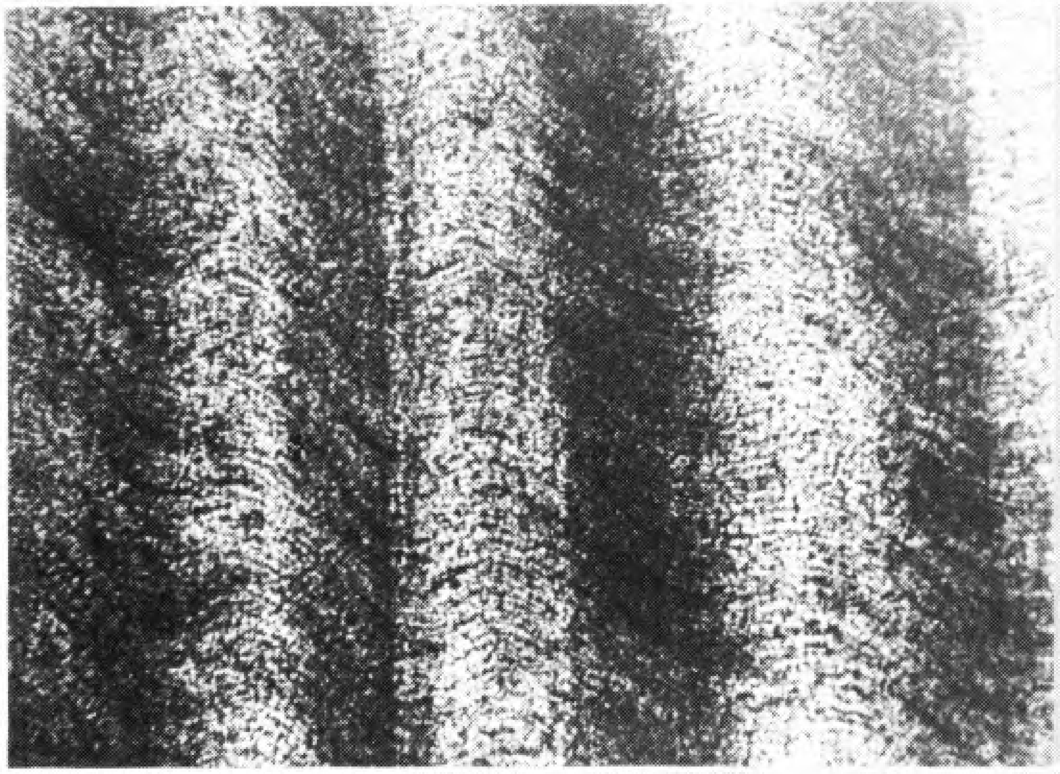

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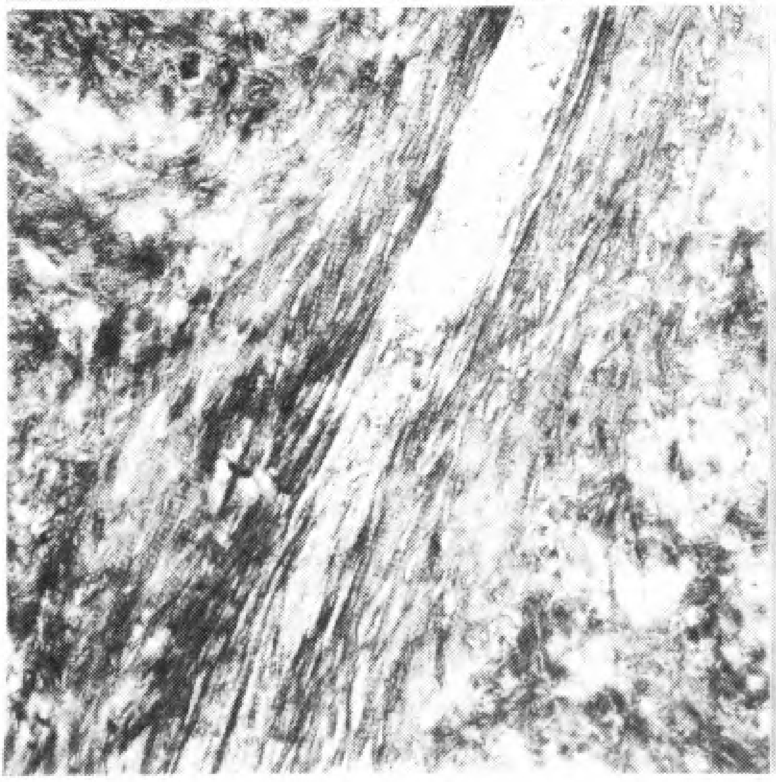

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Fig. 16. Surface of predentine in *Globicephala* tooth, showing fine mesh of collagen fibrils. Fieldwidth 16 μm.

Fig. 17. *Globicephala* tooth treated 30 mins with 5% NaOCl to expose the mineralizing front. Fieldwidth 80 μm.

Figs 18 and 19. LS *Physeter* tooth viewed between cross polars showing curvatures of the dentine tubules almost parallel to the incremental line direction. The small size of the calcospherites can be seen in Fig. 19. Fig. 18, x100, Fig. 19, x250.

Figs 20 and 21. TEM micrographs of ultra-thin sections of OsO_4 fixed demineralized *Physeter* dentine. Note the large amount of collagen parallel with the tubule wall in the tubule cut longitudinally in Fig. 20, and that this LS collagen component is only seen on two sides of the near transversely cut tubule in Fig. 21. Fieldwidths: Fig. 20, 5.5 μm. Fig. 21, 2.5 μm.

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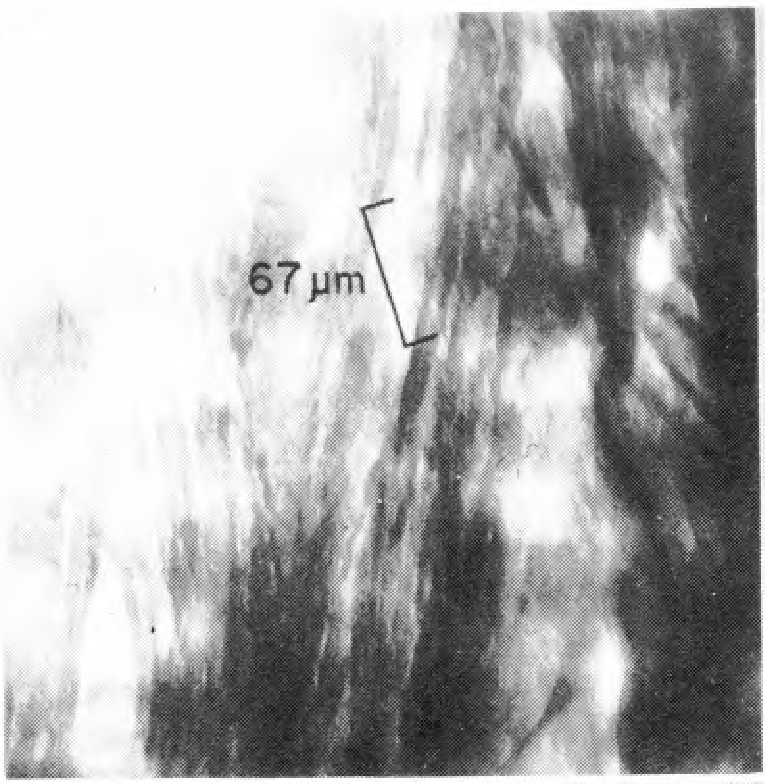

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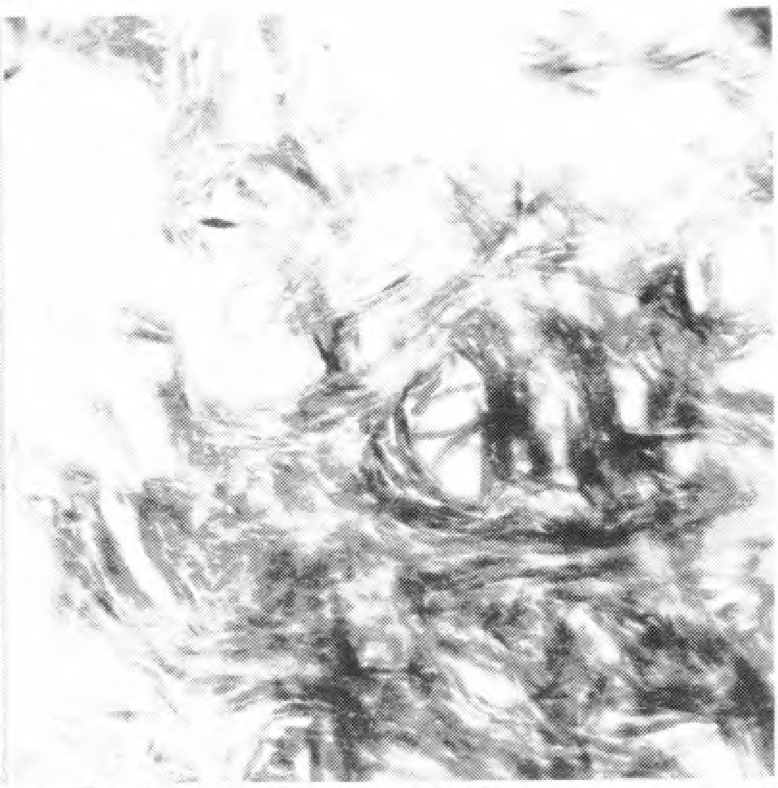

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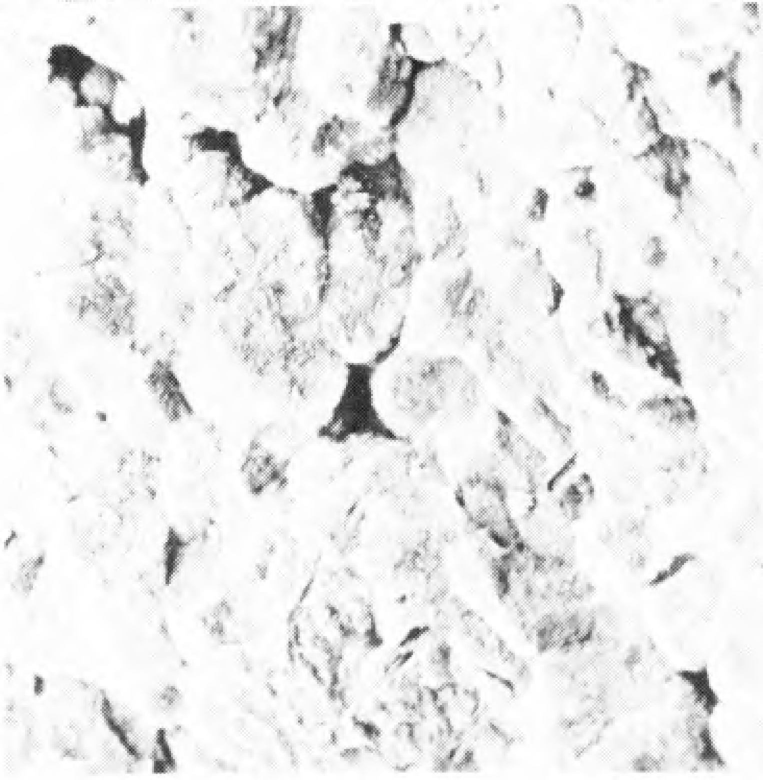

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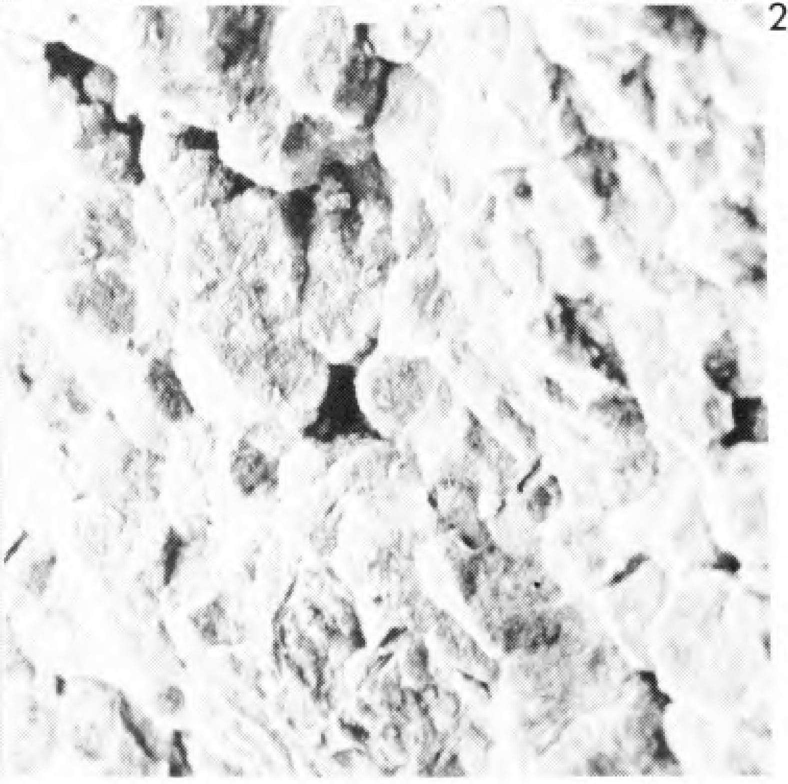

Figs 22 and 23. Ion beam thinned sections of intact *Physeter* dentine, unfixed, unstained. It is possible to recognize the 67 μm collagen macroperiod in Fig. 22. The mineral component is visualized as pairs of dark lines, which the author interprets as showing mineral enshrouding the collagen microfibrils and which are continuous for lengths much greater than the macroperiod. Fieldwidths: Fig. 22,405 μm. Fig. 23,726 μm.

Fig. 24. Anorganic mineralizing front preparation of *Physeter* dentine, typical of appearances found in animals captured at various times throughout the year; (this one, a 12 m male, was taken off Durban in March 1967). Note calcospherites and opening of dentine tubules. SEM 10 kV. Fieldwidth 74 μm.

Fig. 25. Similar preparation (*Physeter* tooth treated for 30 mins. with 5% $NaOCl$ showing completely mineralized collagen fibrils (which run mainly in longitudinal axis of tooth) indicating an arrest in the process of dentine matrix formation and mineralization. Fieldwidth 42 μm.

Fig. 26. (Stereopair) Fractured anorganic *Physeter* dentine, showing the interfaces between calcospherites in the dentine, and an interglobular zone where the calcospherites had failed to fuse. Fieldwidth 60 μm.

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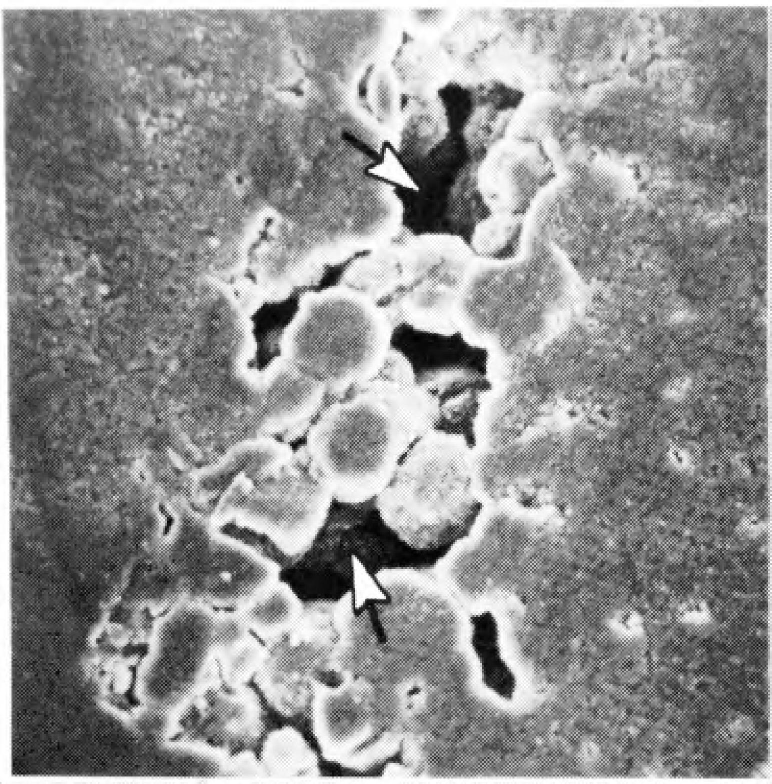

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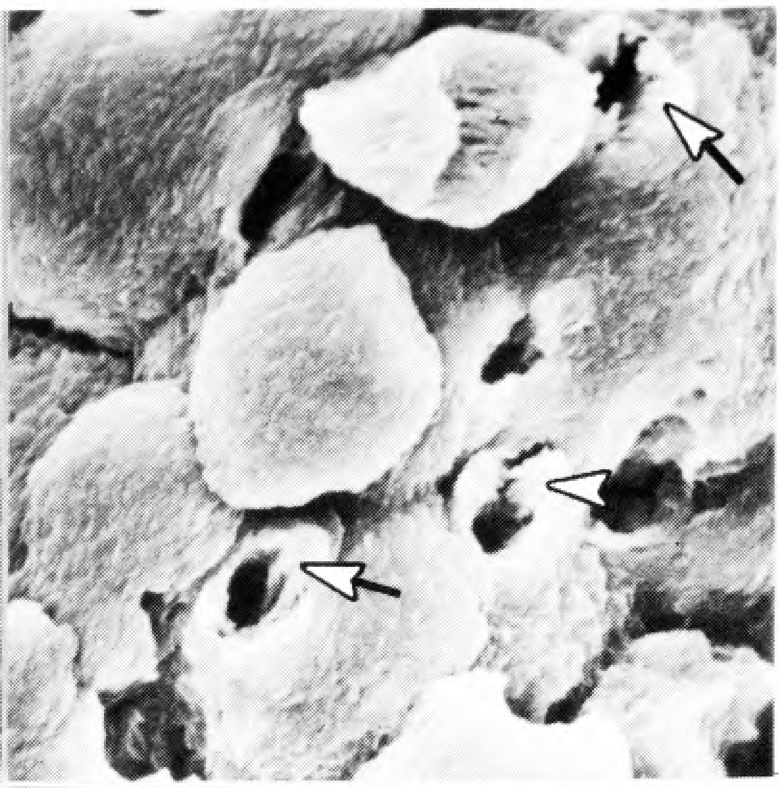

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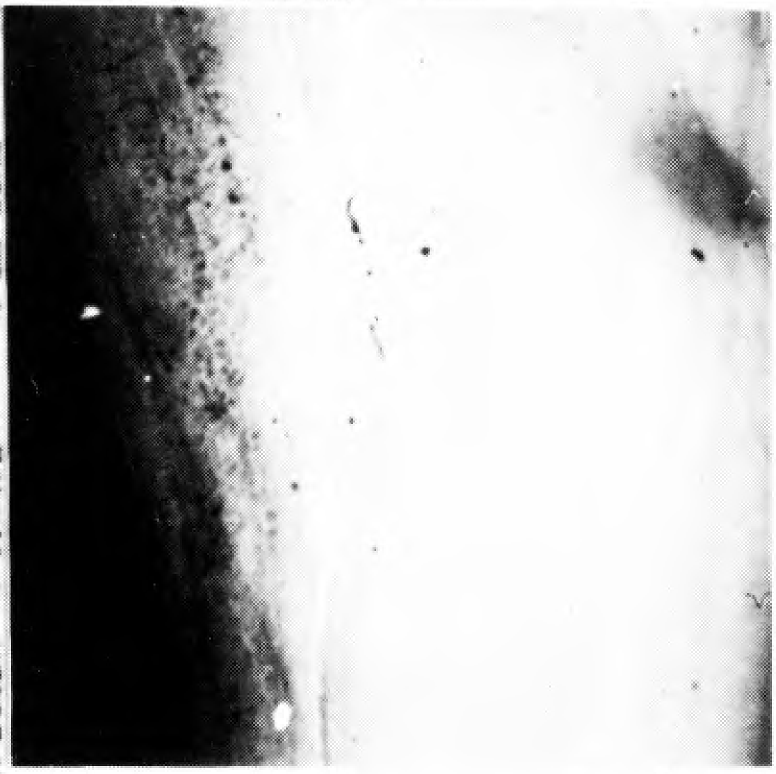

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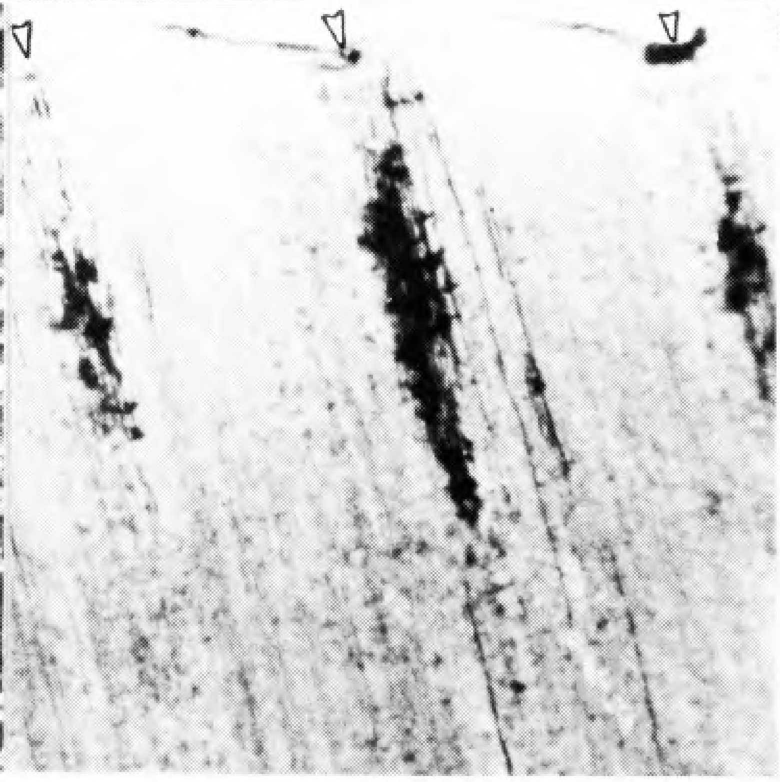

Fig. 27. Radially cut surface of *Physeter* dentine treated with NaOCl to remove unmineralized collagen, showing interglobular dentine spaces (arrows) where calcospherites (separate mineralizing centres) had failed to fuse. Fieldwidth 77 μm. X 1250.

Fig. 28. Anorganic forming surface of *Physeter* dentine showing mineralization within longitudinal collagen fibre component (arrowed) at the level of the openings of the dentine tubules in the mineralizing front. Fieldwidth 16 μm. ×6000.

Fig. 29. Fractured *Physter* dentine, parallel with long axis of tubules, showing longitudinal fibre component in oral and aboral sides (arrowed) and transverse fibrils in lateral walls of tubules. Fieldwidth 17 μm.

Fig. 30. Microradiograph of LS of *Physeter* tooth, showing varying radiodensity of the incremental layers.

Fig. 31. Fractured *Mesoplodon layardi* dentine, showing peritubular zones. SEM 10 kV. Fieldwidth 71 μm.

Fig. 32. Narwhal tusk dentine cut TS, ordinary light micrograph showing relationship of radial mineralization deficiencies to depressions (arrowed) in the formative surface of the tissue (top). Fieldwidth 500 μm, approximately.

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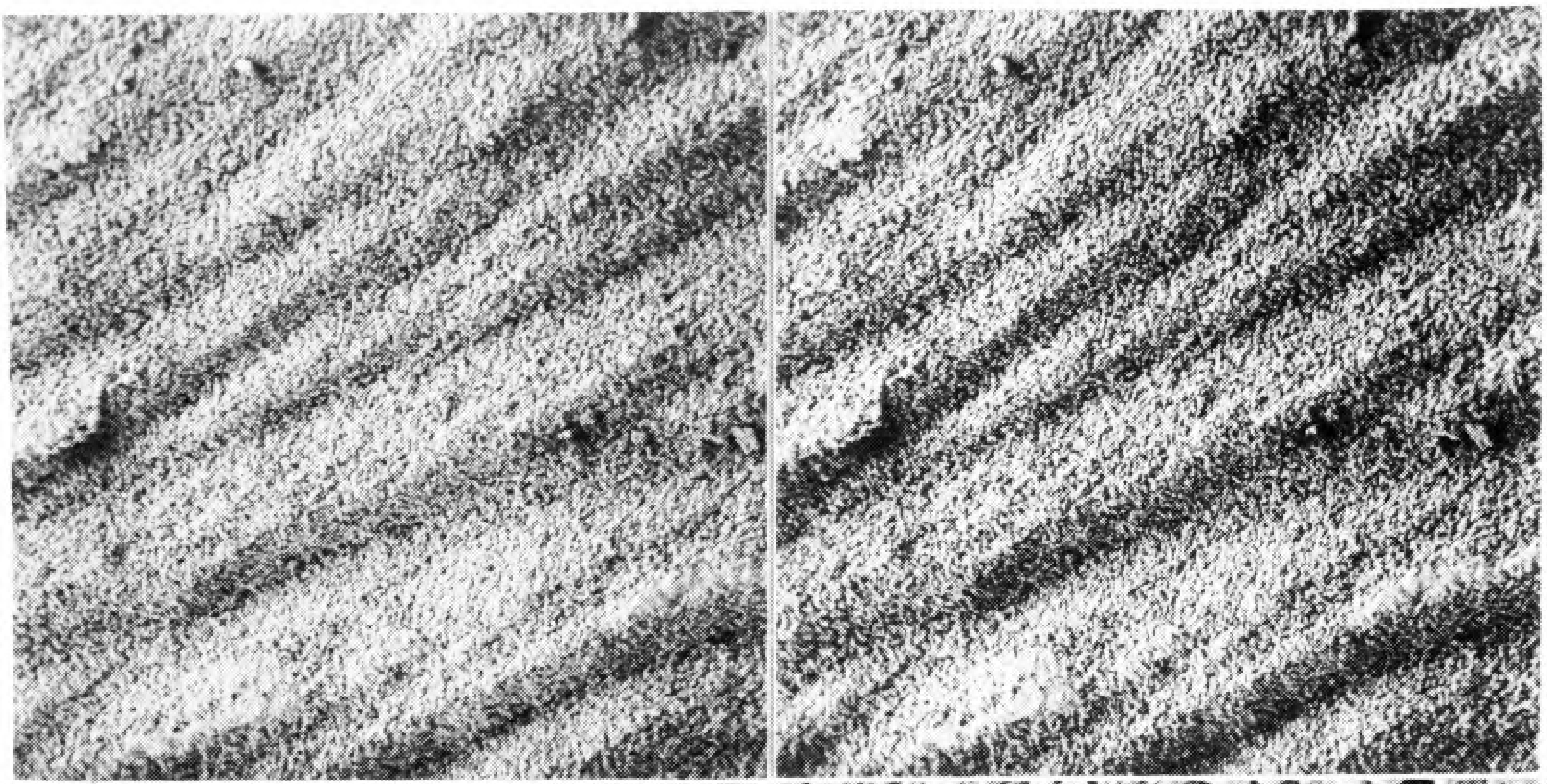

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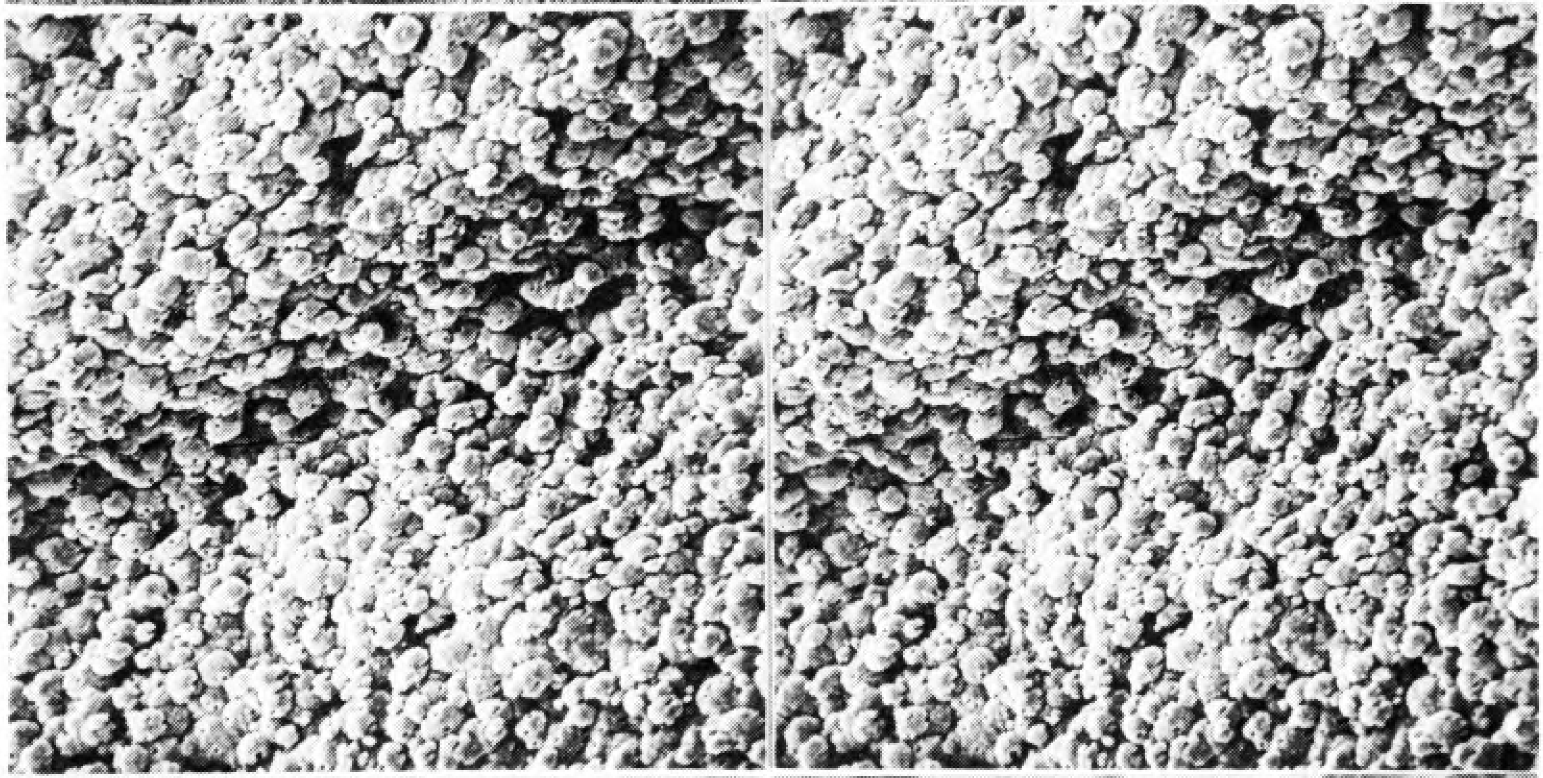

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Fig. 33. 1,2 ethane diamine extracted narwhal tusk dentine, showing longitudinal grooves in the (formative surface) mineralizing front, SEM 10 kV. Stereo-pair. Fieldwidth 1350 μm.

Fig. 34 and 35. Successive higher magnifications of same, showing bottom of groove where calcospherites fail to fuse properly, leaving longitudinal radial sheets of interglobular dentine beneath this surface. Fieldwidths: Fig. 34, 225 μm; Fig. 35, 22 μm.

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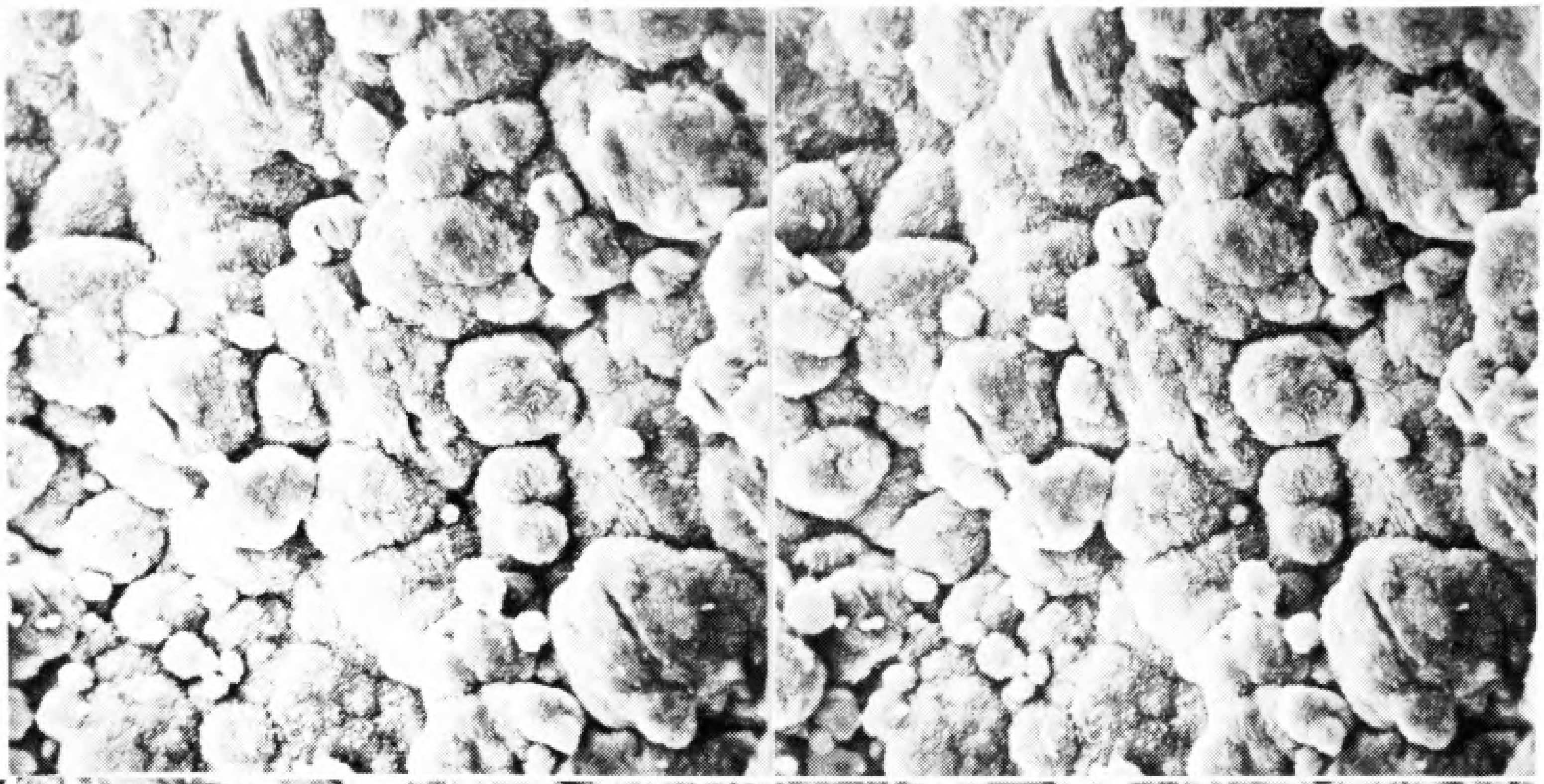

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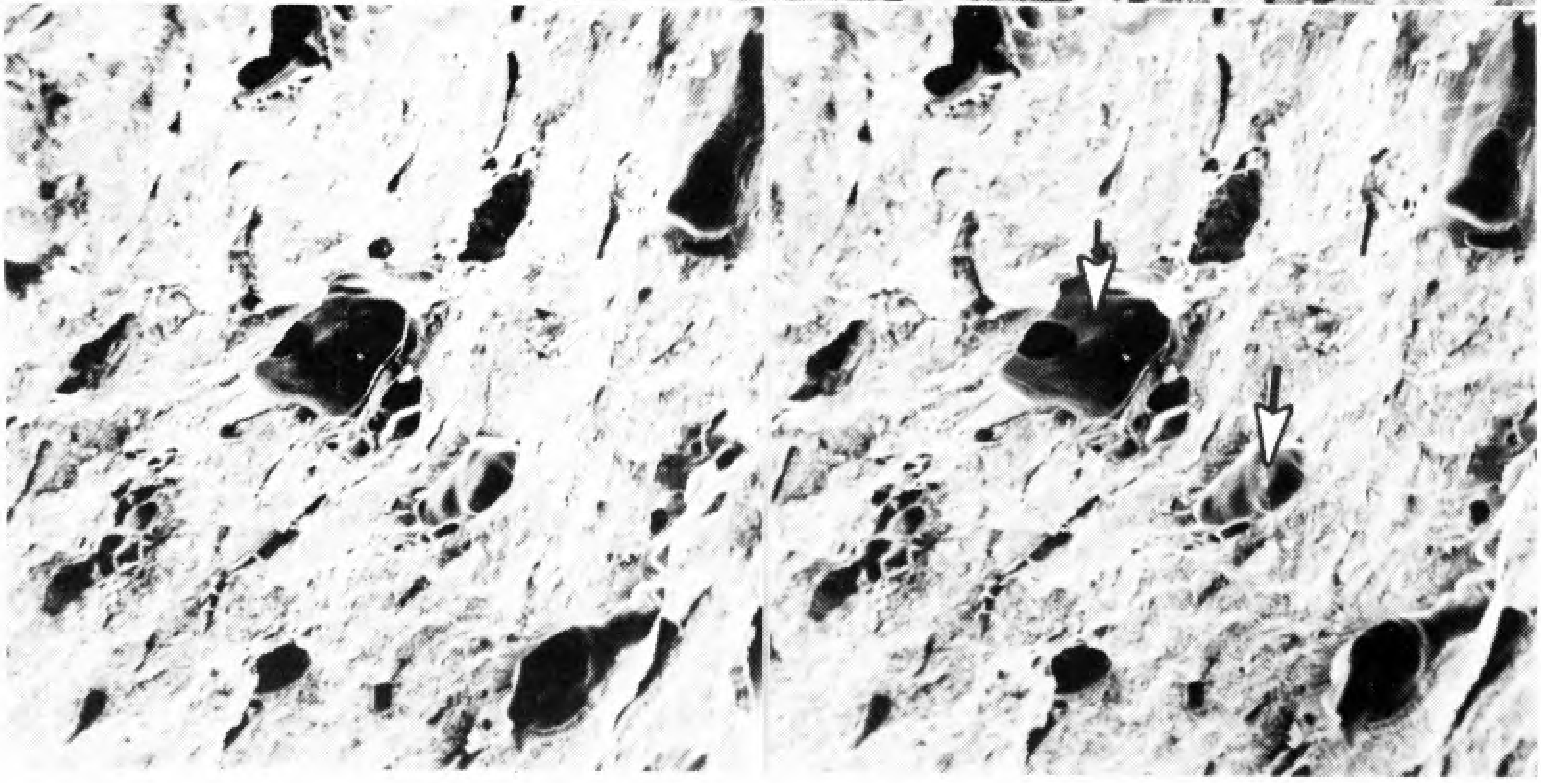

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Fig. 36. Anorganic narwhal tusk dentine, separated along one such longitudinal mineralization deficiency, showing calcospherites bordering the non-mineralized area. Fieldwidth 68 μm.

Fig. 37. Fractured narwhal tusk cement. Stereo-pair SEM showing cementocyte lacuna. Fieldwidth 25 μm. X3900.

Fig. 38. Fractured *Mesoplodon bidens* vascocement showing smooth dense walls (arrowed) of presumed vascular channels in this voluminous tissue. Fieldwidth 665 μm. x430.

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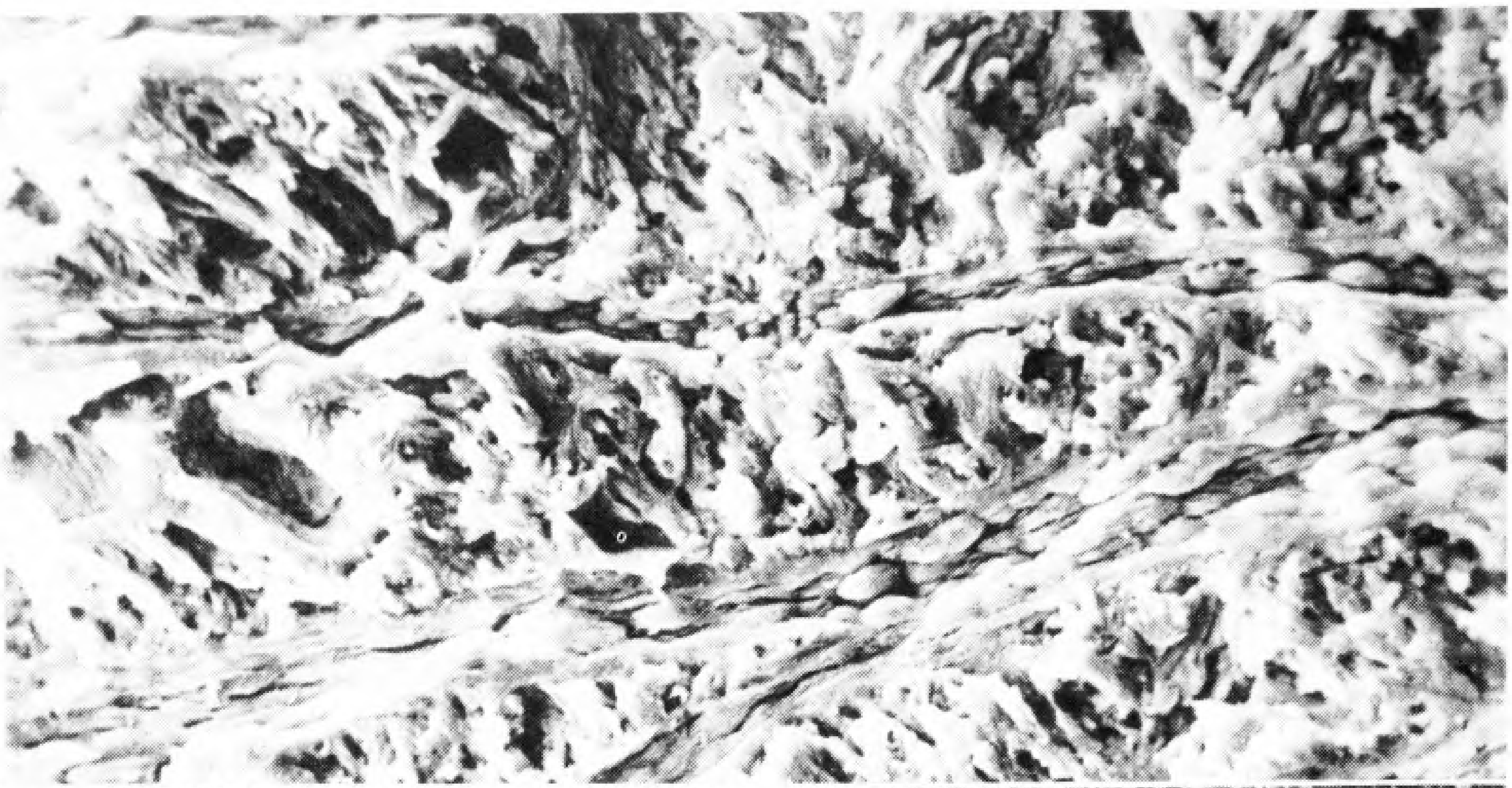

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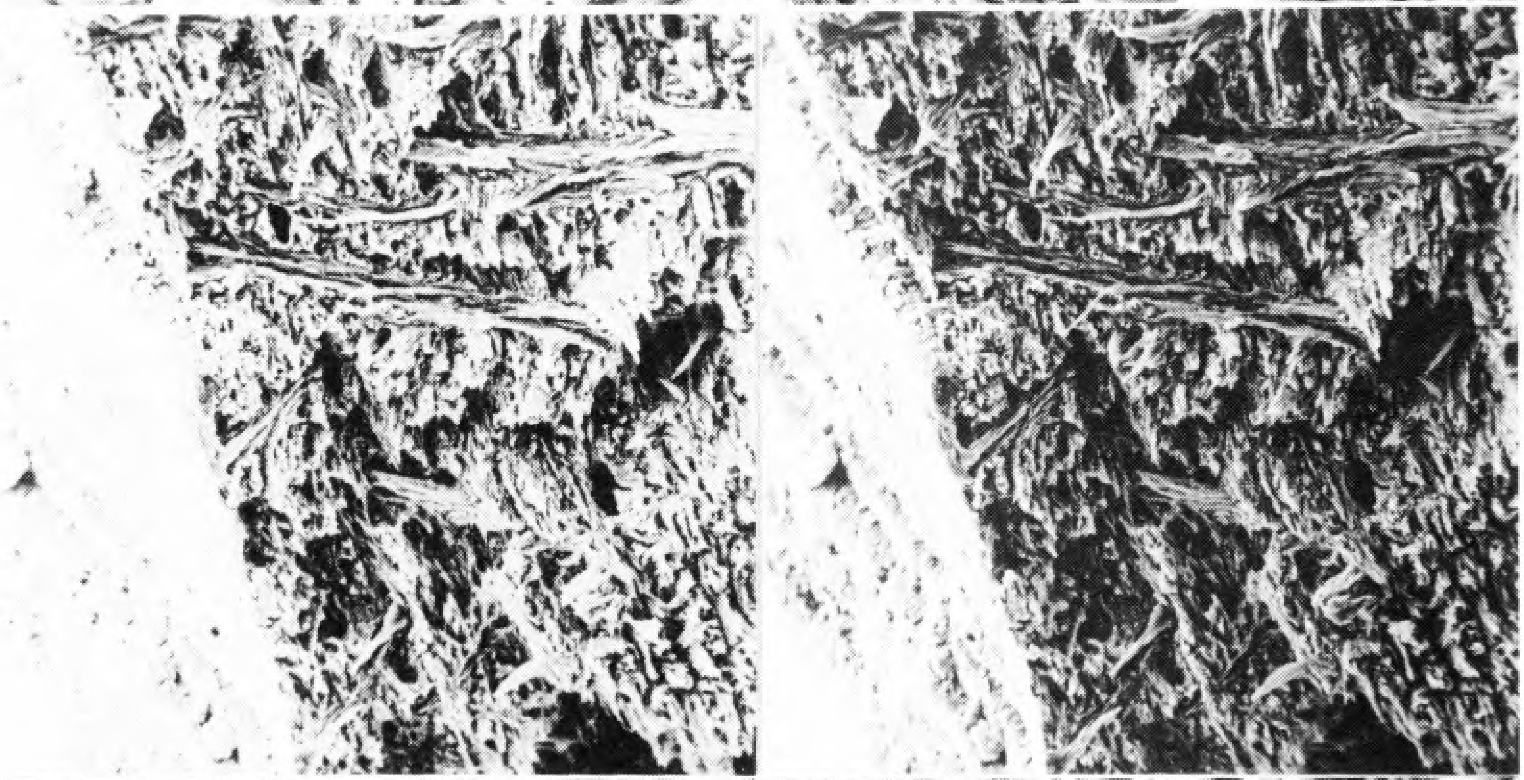

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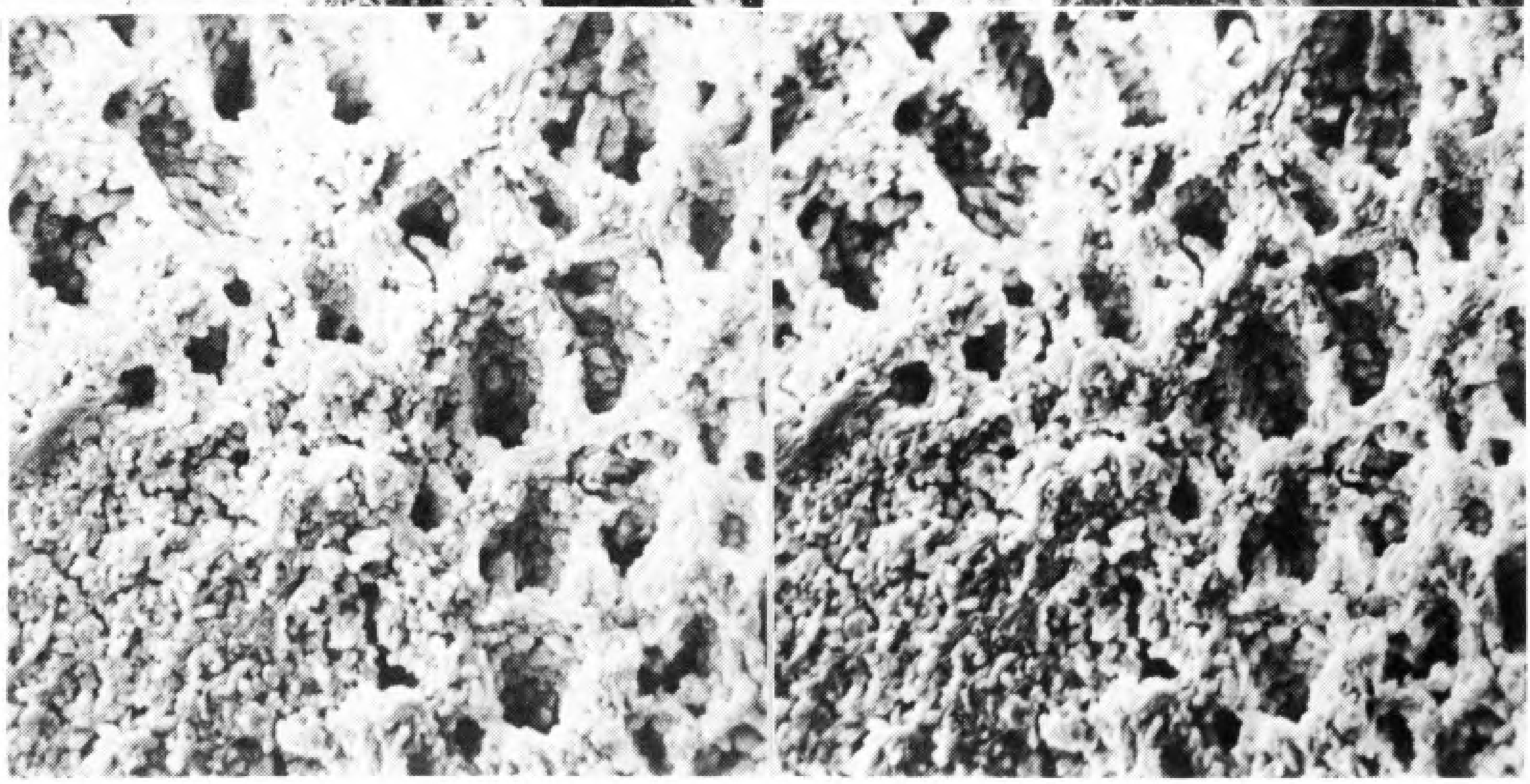

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Fig. 39. Anorganic *Physeter* cement fractured along the long axes of the Sharpey fibres, showing the extent of mineralization within the Sharpey fibres the lozenge-shaped particles are mineralized zones (calcospherites of a sort!). Central portions of large Sharpey fibres frequently remain unmineralized. Fieldwidth 110 μm.

Fig. 40. Fracture perpendicular to worn coronal cementum surface of *Physeter* tooth. Note how incremental layers wear to leave stepped surface (left); and the decussation of the Sharpey fibres within the tissue. Fieldwidth 197 μm.

Fig. 41. Anorganic forming cementum surface in sperm whale tooth showing pits which are depressions in the mineralization front located in the Sharpey fibres. The mineral particle clusters typify actively progressing mineralization in bone-like tissues. (Compare these formative surface appearances with a resting surface – *Tursiops*, Fig. 15.) Fieldwidth 80μm.

Layers in the Hard Tissues of Mammals as a Record of Growth Rhythms of Individuals

G.A. Klevezal'
N. K. Koltzov Institute of Development Biology, USSR Academy of Science
Vavilov Street 26, Moscow, USSR

INTRODUCTION

As far as age determination is concerned there is hardly a group of mammals which has gained more attention than marine mammals. Age determination of mammals using annual growth layers in dentine, cementum and periosteal bone tissue began to be applied on a large scale when annual growth ridges were described around the roots of the teeth in the fur seal *Callorhinus ursinus* (Scheffer, 1950), and annual growth layers in the dentine of the elephant seal *Mirounga leonina* (Laws, 1952). Soon after that, annual growth layers were found in dentine and cementum of many other pinnipeds and in cetaceans and used for age determination (Laws, 1953, 1957, 1958; Rasmussen, 1957; McLaren, 1958; Nishiwaki and Yagi, 1953; Nishiwaki *et al*, 1958; Sergeant, 1959). Annual growth layers in the hard tissues of terrestrial mammals were described several years later (see reviews by Sergeant (1967) and Klevezal' and Kleinenberg (1967)) and they began to be used on a large scale for age determination only five to ten years ago.

Being engaged in the investigation of both marine and terrestrial mammals we can see that many difficulties which we meet when aging whales we meet also when aging animals of other species such as ungulates, rodents and carnivores. But of course in working with animals such as rodents there are many opportunities to study the process and the mechanisms of annual growth-layer formation which we do not have when working with whales. We can easily experiment, i.e. we can mark and kill as many animals as necessary. As a result, we may clarify, at least to some extent, the causes of typical difficulties and we suppose that our conclusions may be extrapolated to whales as soon as it is shown that the basic mechanisms of growth-layer formation are the same for all mammals, both marine and terrestrial.

Below we consider those patterns of annual layer formation in hard tissues which seem to be the same for mammals of different systematic and ecological groups.

METHODS

During annual layer investigations we usually use decalcified and haemotoxyline-stained sections. As a rule in such sections an annual layer consists of a wide poorly stained zone and a narrow deeply stained one (or deeply stained line in an annual bone layer). Inside the wide zone there may be regularly or irregularly appearing narrow zones (lines) stained as deeply as the primary one, or more lightly stained accessory laminae. But sometimes, e.g. in the case of sperm whale teeth, we investigated thin ground sections in transmitted light, where annual layers consist of alternation of thin translucent and wide opaque zones with more opaque and translucent accessory laminae within the latter.

GROWTH OF AN ANIMAL AND ANNUAL LAYER FORMATION

Annual layers are not formed and preserved in all the teeth or in all the bones of mammals, but where we find them we can see certain parallels in their formation in different tissues. Some authors believe that annual layers are formed as a result of seasonal changes of the growth rate of a given structure – tooth or bone (Scheffer, 1950; Mansfield, 1958; Klevezal', 1963 etc). We have suggested that the annual growth layers in the mammalian hard tissues are correlated with seasonal changes in the growth rate of an individual as a whole (Klevezal' and Kleinenberg, 1967). In this feature they are similar to shells of molluscs and to scales, bones, and to otoliths of fishes, and we can categorize all of them under the name 'recording structures' (Mina and Klevezal', 1970), enlarging the field for possible extrapolations.

Comparing the patterns of annual layers in the tissues of different bones and teeth of an individual, I have noted that (i) the wider the annual layer, the more accessory laminae there are; (ii) the number and the location of the accessory laminae are similar in the annual layers formed in different structures of an animal if these layers are of similar width (Klevezal', 1970). All the recording structures of an individual register the changes in the individual growth rate, but some of them register only comparatively large changes and some, both large changes and comparatively small ones. In the first case, recording structures are less sensitive. This suggests that sensitivity of a recording structure depends mainly on its own growth rate (Mina and Klevezal', 1970). An annual layer itself we consider as a record of a year growth cycle of an animal: its narrow zone being a record of an autumn-winter growth retardation; its wide zone, a record of spring-summer rapid growth; and accessory laminae, records of intraseasonal growth changes.

The experimental data show us that changes in an animal's growth rate, even when artificially induced, are adequately registered in the growth of the teeth. It was shown, for instance (Johannessen, 1964), that in rats whose growth was inhibited or stimulated by hormones, the changes of the dentine increments coincided with the changes of an animal's growth rate (Fig. 1).

We experimented with mammals that usually hibernate (hamsters, *Mesocricetus brandti,* and dormice, *Dyromys nitedula*), preventing them from hibernating in winter. The presence or absence of growth was ascertained both by the changes of body weight and by the inclusion of tetracycline in the growing bone. The dormice stopped growing in the beginning of the winter and began to grow again in the spring. A normal narrow zone was formed in the periosteal bone tissue.

The hamsters grew rapidly all winter; the body weight increased during the autumn-winter period. Tetracycline

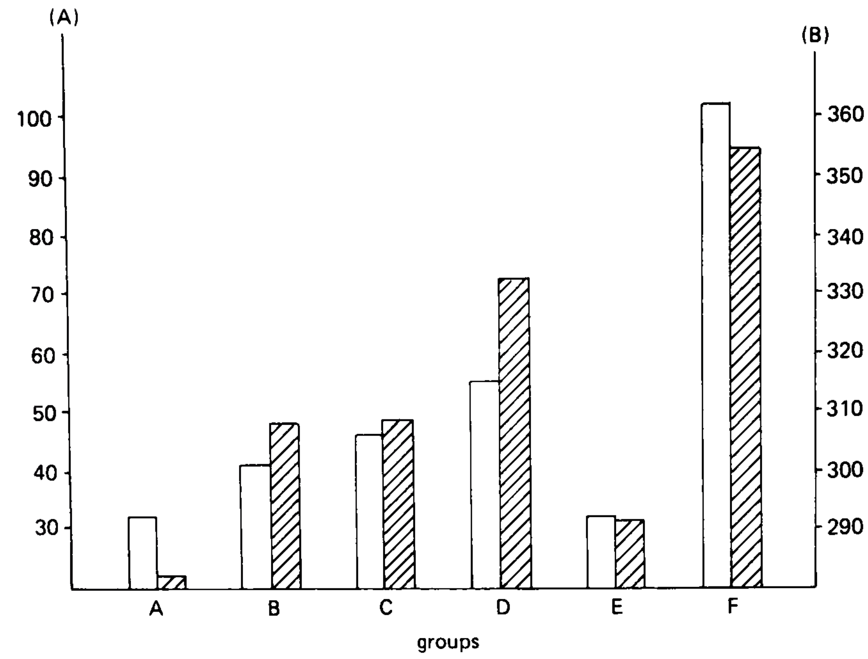

Fig. 1. Relationship between body weight increments from 21st to 41st days of life (A) and thickness of molar dentine on 41st day of life (B) in rats of different experimental groups (using the data of Johannessen (1964)).

injections in October, January, and March identified the growth at the time of the injections, and the wide increment formed during the winter could be seen in the ground sections of teeth and bones (Fig. 2). No narrow zone delimiting an annual layer was formed, either in the cementum or in the bone tissue. Its absence confirms that winter growth retardation, usually recorded, did not take place in our experiment. In this experiment, the animals of only one of the two species responded to the alteration of environmental conditions. Nevertheless, we can see that even when individual growth rhythms were changed artificially they were adequately registered by the recording structures.

There is no reason to believe that the mechanisms of layer formation in marine mammals are substantially different from those in terrestrial mammals. Consequently, it is possible to suggest that the pattern in marine mammals is also primarily the record of growth peculiarities of an animal and through them one may interpret other physiological processes. Evidently Scheffer (1975) was right in assuming that, in the fur seals, annual periods of moulting, fasting of adults during breeding periods, etc. were reflected in the dentinal layer pattern. It is quite plausible that in fur seal sucklings the small dentinal layers are formed in connection with the rhythms of feeding (Scheffer and Peterson, 1967), and that there are records of diurnal and lunar periods of growth in the growth layers found in dugong teeth (Scheffer, 1970). I suggest that in many other cases the layered patterns of recording structures are closely connected with growth rhythms of individuals, e.g. it may be so in the case described by Kasuya (1977) who wrote that in *Berardius bairdii* 'short cycle layering is probably caused by a genetic physiological cycle of about 29.4 days on the average.'

SIGNIFICANCE OF THE GROWTH RATE OF A RECORDING STRUCTURE

The statement that the growth rate of a recording structure determines its sensitivity is quite applicable to the dentine and cementum of mammalian teeth. It follows that the wider the annual increment, the more accessory laminae are formed in the layer. These accessory laminae make age determination difficult, but they may be used to study intraseasonal growth-rate changes. Working on the dentine or cementum, we can choose the tooth and its part which has the growth rate most convenient for our particular aims.

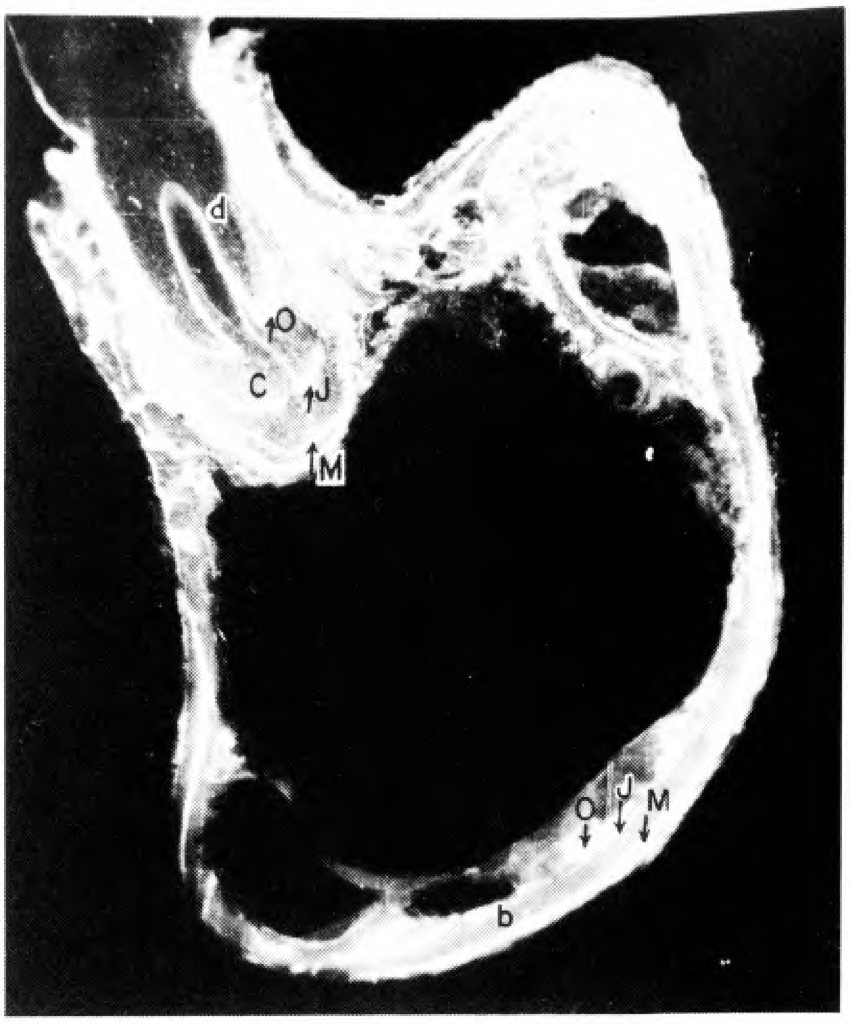

Fig. 2. Ground section of mandible of nonhibernated specimen of *Mesocricetus brandti* injected by tetracycline in October, January and March. Ultraviolet light. O, J, M – tetracycline marks in molar cementum (c) and bone (b). d – dentine.

The situation is a little different in investigations of bone. Here the layers are formed in the periosteal zone as the bone grows in width. In the process of remodeling of the bony tissue, periosteal bone is replaced by osteons and previously formed layers disappear. In many species of mammals long bones and mandibles retain periosteal bone with annual layers long enough to be used for age determination. In other species however, porous or reticular tissue, i.e. without layers, is formed. We can see such a picture in big ungulates, some carnivores and baleen whales. In baleen whales annual layers were not found in mandibles (Klevezal' and Kleinenberg, 1967), limb bones, vertebrae, ribs or rostral bones (Klevezal' and Mitchell, 1971).

I tried to examine the causes of this phenomenon. Having compared the growth rates of marine and terrestrial mammals, some with and some without annual layers in their bony tissue, I came to the conclusion that Amprino (1947) was right in his assumption as to the correlation between growth rate of the bone and its structural characteristics. Porous or reticular bony tissue without annual layers is formed in large, fast-growing animals, and compact periosteal bone, in small animals and in large, but slowly-growing animals (Klevezal', 1972). The indices that I used to characterize animal size and growth rate gave significantly smaller values for rodents and small carnivores (with bony layers) than for large carnivores and ungulates (without bony layers). Because of the way in which the indices were calculated, it was not possible to compare terrestrial mammals with cetaceans, but the 'intercetacean' comparison gave the same results: the indices for baleen whales were much greater than those for the sperm whale and other odontocetes, all of which have bony layers (Klevezal', 1972).

If the structure of a newly forming bony tissue is really determined by the growth rate of a given bone, then in large, fast-growing animals the layered periosteal tissue most probably is formed in the bones growing with negative allometry, i.e. more slowly than an organism as a whole. We have found annual layers in *Bulla tympani* of baleen whales (Klevezal' and Mitchell, 1971), it being a bone fitting well the description given above.

Thus, in some cases, the absolute growth rate of a structure determines its morphological characteristics and as a consequence, the possibility of its use as a recording structure. If we want to determine the age of large, fast-growing mammals by layers in the bony tissue it is of little use to look for annual layers in big, isometrically-growing bones of their skeleton.

SEASONAL GROWTH RHYTHM AND ANNUAL LAYER CHARACTERISTICS

The seasonal growth cycle in mammals usually includes a phase of relatively rapid growth (in the spring-summer period) and a phase of retardation or complete cessation of growth (in the autumn-winter period); in recording structures a wide and a narrow zone is formed, respectively. I have studied seasonal rhythms of growth in the suslik, *Citellus undulatus*, using alizarin to reveal the growth of the skeleton (Klevezal', 1978). The suslik is a hibernator; in the region where I worked; it is active from April to September. In the young of the year the bones grew during the whole period of active life and only in late August, before the beginning of hibernation, the growth stopped. In the next year of life the growth began in the end of May and stopped in early July, 1.5 months earlier than in the young of the year, long before the hibernation (Fig. 3). It is important to note that after the second year of life, growth did not cease; growth continued in older animals also.

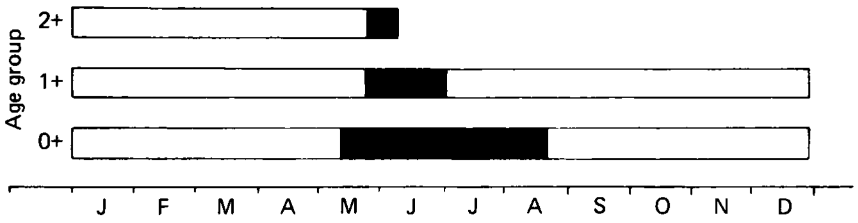

Fig. 3. Duration of growth phase (black) in annual growth cycle of *Citellus undulatus* of different age groups.

These data confirm our assumption that in mammals, as in other animals (e.g. fishes), the length of the season of growth (and thus the period of formation of a wide zone in an annual layer) decreases as an individual becomes older (Mina and Klevezal', 1976). The young grow and the wide zones in the annual layers are formed during the whole period when external factors permit. In older individuals the period of growth is shorter and wide zone formation is narrower than would be permitted by external factors. This conclusion seems to be applicable to mammals of different species. It is possible that accessory laminae, which are usually more numerous and more frequent in annual layers during the early years of life of individuals, are formed not only because the growth rate of recording structures was higher, but also because the period of growth was longer.

These data show us also that the growth of an animal in a given year may stop not because some environmental influences make growth impossible, but because of some innate regulation. Changes in the innate regulatory mechanisms may result in the appearance of the additional period of intensive growth in a year. This could produce 'double layers' in the bone or tooth. 'Double layers' may form in the hard tissues of mammals, both marine and terrestrial. In such cases there are two narrow zones instead of one in an annual layer.

I have found 'double layers' in the osseous tissue of the majority of field mice, *Apodemus agrarius*, that we studied from the Moscow district, in the osseous and cemental tissue in 50% of the marmots, *Marmota baibacina*, from Tian-Shan, in osseous tissue in 60% of minks, *Mustela vision*, from a breeding farm, in the cementum in 52% of moose, *Alces alces*, from the European part of the USSR, in the cementum in 32% of adult wolves, *Canis lupus*, and in 13% of martens, *Martes martes*, from different parts of the USSR. According to Lockard (1972) 'double layers' were observed in the cementum in 39% of female deer and in 28% of male deer, *Odocoileus virginianus*, from different states of the USA. Hall-Martin (1976) found them in the cementum in 30% of *Giraffa camelopardalis* from South Africa. The possibility of the 'double layer' formation was proved beyond doubt by studies on mammals which are known not to live more than 1.5 years (Klevezal' and Mina, 1973) and on longer living mammals of known age (Lockard, 1972; Hall-Martin, 1976).

'Double layers' are found in both males and in females which had been pregnant and which had been 'resting' during the year of layer formation (Klevezal' and Mina, 1973). Lockard (1972) found them in a castrated male deer so their formation is not determined by sex hormones. We have shown that in moose and wolves the formation of 'double layers' cannot be connected with the particular conditions of the year of their formation (Klevezal' and Mina, 1973).

Usually in long-lived animals, the first annual layer includes one narrow zone which often is not distinct (Fig. 4), but two narrow zones may appear in the next annual layers. Such a pattern was observed by Lockard (1972) in *Odocoileus virginianus*. According to our data among 54 wolves, from different parts of the USSR, which had two or more annual layers in the cementum of the canines, only one had two narrow zones in the first indistinct annual layer, ten wolves had all 'double layers' (except for the first one) and others had no 'double layers' (eight specimens) or very few of them. In the last case several first layers were not 'double' but the next ones were. Among ten moose from the middle-European part of the USSR, which had 4 to 16 annual layers in the incisor dentine, only two individuals had 'double layers' and in these all the layers were 'double' except the first two.

It seems probable that in long-lived mammals the pattern of seasonal growth rhythm changes with age in the following way. In the first year of life an individual grows as long as is permitted by external factors. In very big and very long-lived animals, such as cetaceans, the picture may not change for several years up to the period of sexual maturation when, as a rule, the growth rate of an individual decreases. Then the length of the phase of growth in a seasonal cycle decreases every year and individuals may have not one but two checks of growth in a year (e.g. in winter and in late summer) and correspondingly two periods of growth (in spring-summer and in autumn periods). Often such a form of growth rhythm is not inherent in all members of a population and seems to be determined by some genetic factor. The visible manifestation of such a rhythm is the formation of 'double layers'.

The appearance of 'double layers' varies in accordance with the duration of the second growth period. If the second period of growth is short, the annual layer has two closely-spaced narrow zones (Fig. 4) and this 'double layer' will be always counted as one annual layer. If the second period of growth following a short cessation of growth is relatively long, the pattern may be interpreted easily as two annual layers (Fig. 4).

The formation of 'double layers' in the dentine was described in several species of whales. Working with the Black Sea common dolphins, *Delphinus delphis,* we found that two layers in a year were formed in all the animals under study (Kleinenberg and Klevezal', 1962). In white whales, *Delphinapterus leucas,* many individuals were found to have 'double layers' (Sergeant, 1973). In some cases different interpretations of the pattern of an annual layer in whales' dentine seem to cause differences in estimates of the accumulation rate of the dentinal layers (Klevezal' and Kleinenberg, 1967; Kasuya, 1972).

If the 'double layers' are not always identified as such, if they are not formed in all the individuals of a population or not in every layer, then we may get an average number of layers per year between one and two. When 'double layers' are formed only in some of the members of a year class of a given population it may be useful to try a method for their identification used for a similar purpose in ichthyology (Mina, 1973). The method includes construction of a curve plotting along the x-axis the distance from some fixed point of the tooth section to narrow zones, and along the y-axis, the frequency of zones at a given distance. Since narrow zones delimiting 'real' annual layers are present on all the sections but 'secondary' zones occur on some of them, the entire curve must be polymodal, the highest of the peaks marking the position of the 'real' annual-layer borders.

INTERPOPULATIONAL DIFFERENCES OF THE ANNUAL LAYER PATTERNS

In many cases it is difficult to determine the age of some marine and terrestrial mammals because of variability and the absence of distinct layers in recording structures of animals in some populations. Sometimes the layers on the ground sections cannot be seen, but can be made visible using some other method. However, more often the layers remain indistinct despite the method used. Our experience in aging terrestrial mammals shows, in a very general way, that the indistinct and variable annual layers are usually formed in regions of non-contrasting climate where there are no pronounced differences between warm and cold or between dry and humid seasons (Klevezal' and Mina, 1973). In aging terrestrial mammals in such cases it is most helpful to change the recording structure used for age determination. For example, where annual layers are indistinct in the dentine, we often may find a better record in the cementum or in the periosteal bone. In some of our previous papers we tried to show that each recording structure is characterized by specific properties and there are cases when one of them is more useful than others (Mina and Klevezal', 1970). If the layers are equally poorly visible in all the recording structures there is nothing to be recommended at the present time. In such cases it is more honest to denote the age less precisely, i.e. not as 5, 10, or 15 years, but as the most probable age interval (e.g. 3 to 6, 7 to 12, or 10 to 20 years).

Variation of the annual-layer patterns, although a nuisance in age determination, may be useful when we try to decide if different populations mix with one another. We used the characteristics of the annual layers to estimate the degree of isolation of sperm whale groups in the southern hemisphere (Klevezal' and Tormosov, 1971). A group of sperm whales (males only) from each region was characterized with the percent of individuals having the distinct annual layers in the mandibular tooth dentine, and the percent of those which had dentine with indistinct pattern of layers (Fig. 5). Should there be free exchange of individuals between groups, the percent of individuals with a given type of layers should be the same in different groups. We have found that this was not the case. A significant difference was found between sperm whales from the southeastern Indian Ocean, where the individuals with distinct layers were scarce, and from the south Atlantic where such individuals were abundant. There was no difference between groups from the southeastern and southwestern parts of the Indian Ocean. We deduced from these data that the sperm whales from the south Atlantic did not mix with the sperm whales from the Indian Ocean whereas the groups of sperm whales in the Indian Ocean possibly do exchange their members. Clarke and Paliza (1972), having analyzed morphometrical data, come to the similar conclusion that there is genetic exchange between groups of sperm whales in the Pacific and Indian Oceans, but that the Atlantic sperm whales are isolated from them.

Studying interpopulational differences in sperm whales' annual layer characteristics, we assumed that the differences were determined by different environmental conditions in the regions where the sperm whales lived. Later, we conducted some experiments to clarify the influence of climate on the characteristics of the annual layer in field mice, *Apodemus agrarius.* Mice from the Moscow district having distinct annual layers were placed under conditions where seasonal contrasts were poorly pronounced. They continued forming distinct annual layers in the bone tissue for two generations and only then appeared some deviations in several individuals (Klevezal' and Mina, 1973). In another experiment the field mice, from a region with non-contrasting climate, having indistinct annual layers, were placed under conditions of contrasting climate. After only one year in the new conditions these mice had distinct layers in the bone tissue. So we have had to conclude that distinct rhythms of growth and the distinct annual layers may be observed in two quite different situations. In some cases they are determined endogenously and in some cases they are induced exogenously.

Assuming the principles of determination of annual layer patterns to be common for all mammals, we can make some statements concerning our sperm whale data. The formation of indistinct layers is determined endogenously (genetically). Some tooth sections contained indistinct and distinct layers separated one from another by irregular intervals. Such cases were more often observed in the Indian Ocean sperm whales than in those from the Atlantic (Klevezal' and Tormosov, 1971). I interpret this pattern as a record of *irregular* external influences (e.g. changes of temperature during migrations) on individuals which are not genetically predisposed to distinct seasonal rhythms of growth causing distinct layer formation. A section of a tooth with equally distinct layers leads to the consideration of two possible explanations. It may be a manifestation of a genetical predisposition to distinct seasonal growth rhythms or it may be a pattern marking individuals which are not genetically predisposed to distinct growth rhythms, but are regularly influenced by rhythmic external factors. Our data do not permit us to choose one of these explanations.

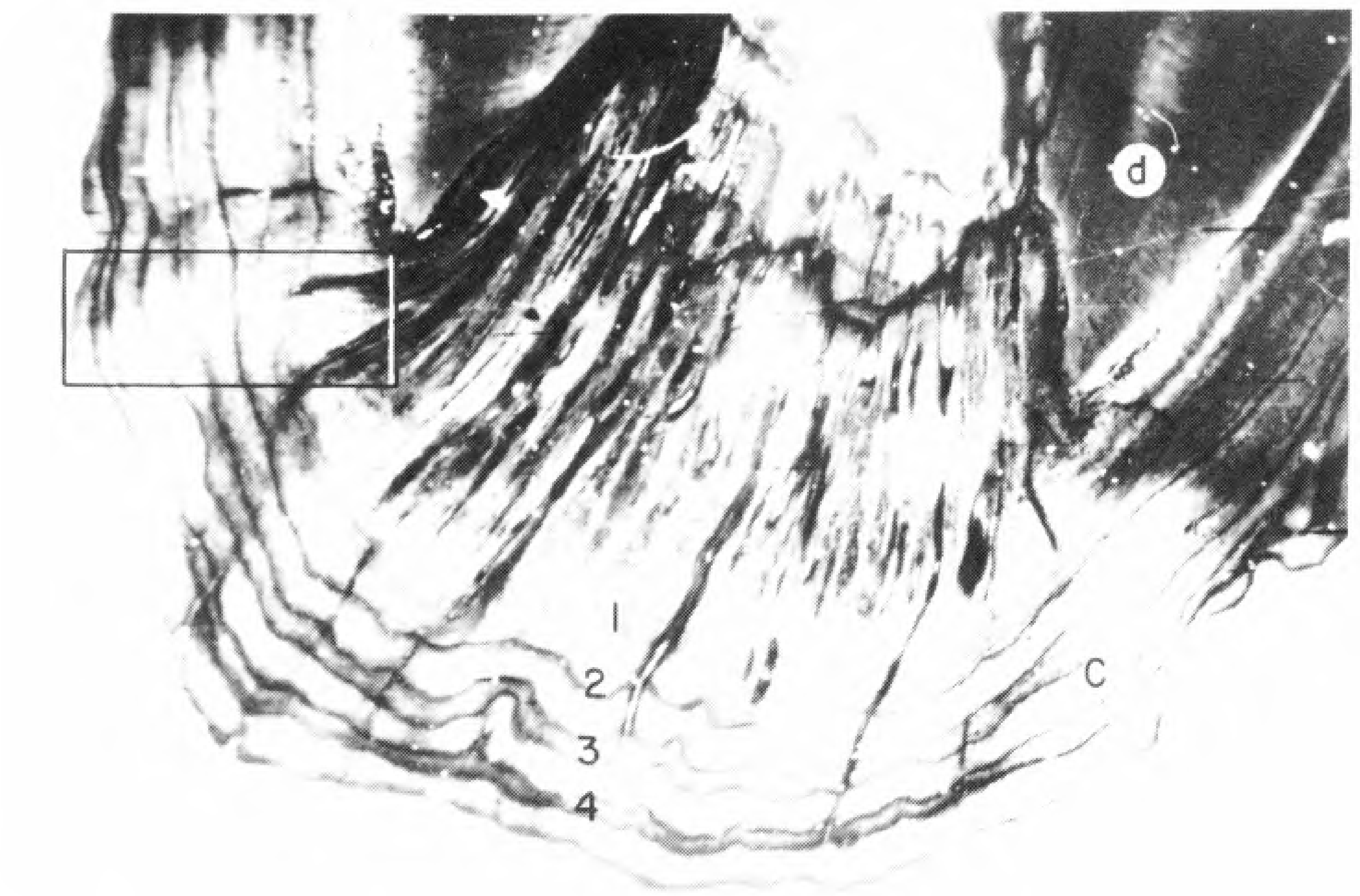

Fig. 4. Stained section of a canine root of *Canis lupus*. Four annual layers are present in the cementum (1–4). Narrow zone of the first layer is indistinct. Third and fourth annual layers have two narrow zones ("double layers"). In some areas (e.g. in square) third annual layer may be wrongly counted as two. c – cementum, d – dentine.

CONCLUSION

I believe that some general statements are possible about causes and mechanisms of annual layer formation in recording structures of mammals. These statements have to be taken into account by those who use annual layers in investigations on mammals whatever the systematic or ecological group with which they work. Some of the statements are formulated below.

(1) Annual layers in dentine, cementum and periosteal bone tissue are a record of seasonal growth rhythms or an individual, the special pattern of an annual layer being determined by the form of the intraseasonal growth rhythms of an individual.

(2) The sensitivity of a recording structure such as dentine, cementum or periosteal bone tissue is determined by its own growth rate.

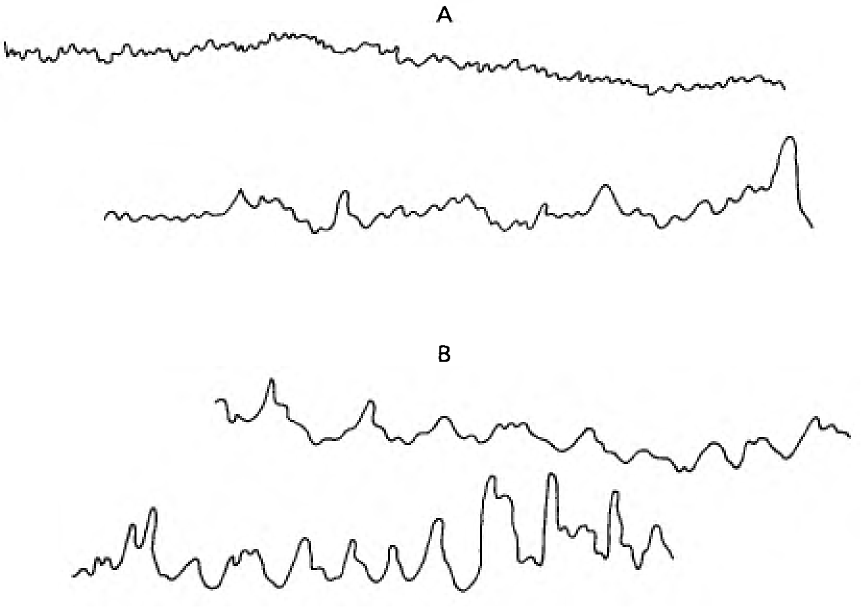

Fig. 5. Photodensigrams of dentine layers of sperm whale teeth. A – indistinct layers (Indian Ocean), B – distinct layers (Atlantic Ocean).

(3) The decrease in width of annual layers usually observed during the life of an animal is connected both with the decrease of the rate of growth during the growth period and with the decrease of the length of this period from year to year.

(4) Two narrow zones in an annual layer arise when there are two periods of growth retardation in a year. As a rule, such a form of a seasonal growth rhythm is found in some members of a population only, and is independent from peculiarities of the sexual cycle being determined by genetic factors.

(5) The distinctness of the annual layers and the degree to which their distinctness is correlated with the distinctness of the seasonal growth rhythm may be determined by both endogenous and exogenous factors.

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Some Approaches to Calibration of Age in Odontocetes Using Layered Hard Tissues

Albert C. Myrick, Jr
National Oceanic and Atmospheric Administration, National Marine Fisheries Service
Southwest Fisheries Center, La Jolla, California

ABSTRACT

Current methods in determining age of odontocetes rely upon untested assumptions. The relationship between layering and absolute age requires calibration. To achieve this goal, five approaches are suggested:

(1) the establishment of a reference collection for known-age, minimum known-age, and time-marked samples;
(2) the construction of developmental series of layered hard tissues representing individuals from various taxonomic and geographical stocks;
(3) the tagging and recovery of wild animals that have been time-marked;
(4) an extended program of multiple time-marking and extraction of teeth of live-captive animals; and
(5) the study of layered hard tissues in conjunction with clinical records of specimens treated with fluorescent antibiotics.

Pilot programs using these approaches are in various stages of implementation at the Southwest Fisheries Center, La Jolla.

INTRODUCTION

Recent studies of odontocete life histories have used counts of layers in teeth and in bones to estimate individual ages preparatory to constructing age-composition and growth models of dolphin populations. The models rely on the sweeping assumption that layers are deposited in hard tissues of odontocetes yearly in a manner analogous to the process of tree-ring formation.

Specifically, the working assumptions seem to be, first, that layering occurs at approximately constant rates; second, that yearly layers may be defined by convenient breaks that occur between dark and light zones in the tissue; and third, that the dark-light components of putative annual layers reflect physiological responses to seasonal change or to some other regular fluctuation in the external environment. Despite the 'soft' data used in interpreting layers in terms of age, it is also assumed that the results are sufficiently accurate for constructing meaningful life history models for various species of odontocetes.

Our present knowledge of the factors contributing to hard-tissue layering in odontocetes is insufficient to justify the use of the above assumptions as though they are proven hypotheses. The general physiology of cetaceans is poorly understood, and it is not known what causes layering in the hard parts of dolphins. Despite years of study, there exists little direct evidence that layering occurs at a constant or predictable rate, although it is true that a progressive increase in layers stands as some measure of increasing age. The few published studies that have used small samples of known-age teeth (Hui, 1978; Sergeant, Caldwell and Caldwell, 1973) or of single treatments to time-mark specimens (Best, 1976; Gurevich, Stewart and Cornell, this volume), have demonstrated only that the known elapsed time may be divided into a pattern of layers (i.e. that layers may be interpreted in such a manner as to obtain the age that is already known for the specimen). Results of a recent exercise reported by Kimura (this volume), involving experienced readers who counted dentinal layers of known-age specimens, underscore the problem that when the age is unknown, counts of number of layers are subjective and variable.

Although experience in defining growth layer groups (GLGs, terminology of workshop report, this volume) in teeth of specimens of known age is an important first step in the right direction toward calibration, such exercises are by no means a panacea for the problem of obtaining accurate age estimates. Nor should they seriously be regarded as more than a poor alternative to direct monitoring of layering rates in living animals. The exercises do not provide adequate support for inferring either that accumulation rates are constant, or, that 'annual' layers composed of dark and light subunits represent yearly records. Pulp-cavity occlusion, tooth wear, and poorly defined layering in dentinal, cemental, and periosteal tissues, as well as subjective interpretations of results using non-standardized techniques, may impair accurate reconstructions of life histories of even the most well known species.

This paper suggests various approaches that may be used to test the assumptions discussed above.

APPROACHES

Calibration of layered structure with absolute age (if indeed such is feasible) must be regarded as a goal of first priority and critical to accurate models of odontocete population dynamics. The appropriate tools are available for assessing the feasibility of real-time calibration, and opportunities now exist for applying these tools. The following approaches are suggested for assessment.

(1) The use of small numbers of specimens for which the layering period is known prevents confident conclusions about layering rates, but retrospective calibrations of large samples of such specimens could add measurably to this block of circumstantial evidence. A considerable amount of material exists from time-marked, e.g. tetracycline-injected, known-age (captive-born), and known minimum-age (wild-captured and held in captivity) animals representing a number of odontocete species, but at present it is held by a large number of individuals and institutions, some of whom do not fully realise its potential value in age/layer calibrations.

An international center (or centers) should be established as a permanent repository for all available time-marked, known-age, and known minimum-age specimens. The designated center would be charged with the responsibility of collecting and maintaining such specimens as a systematic reference library for use by all specialists engaged in age determination of odontocetes.

(2) A primary problem in calibration is the difficulty in defining countable units in layered tissue. Because hard-tissue layering patterns are complex and variable, it is important to understand to what extent differences in age, sex, and geographical stock influence such variability.

As available, teeth and samples of bone should be collected and prepared in thin section to construct ontogenetic series demonstrating developmental sequences of layer accumulation in individuals taken from discrete geographical and taxonomic stocks. The ontogenetic series would be ordered according to body length and sexual maturity and separated by sex. They should be curated centrally and be made available for study upon request.

(3) The possibility exists that layering rates and patterns among wild animals are different from those among animals maintained in captivity.

As economically and technologically feasible, experiments should be carried out to tag wild odontocetes *en masse* and time-mark their hard tissues by tetracycline injection. Eventual recovery of some animals through stranding salvage, incidental kill associated with commercial fisheries, or through other means would provide information on layering rates and patterns.

(4) Captive animals exist in numbers sufficient for comparative studies of layering rates and of factors that may influence layering in odontocetes.

Long-term studies should be carried out to time-mark and to periodically extract teeth of odontocetes maintained in captivity in order to monitor layering. Ideally, the experiments should be performed on reasonably large samples of dolphins representing both sexes and various age groups and should include controlled feeding exercises that would permit investigators to examine the possible effects changes in diet or in feeding frequency may have on layering. Experiments might be designed to alter water temperature, water salinity, or day length. Such experiments should require continuous observational records of any apparent changes in behavior, physiology, or proximate environment that may later be correlated with layering events. Eventually, results of experiments conducted near equatorial zones might be compared with results of experiments carried out on the same species in more temperate areas to examine the possibility that seasonality may alter layering patterns or rates.

(5) Many animals maintained in research and commercial oceanaria are treated routinely with medicines containing antibiotics, such as tetracycline, which combine with the hard tissues. When thin sections of layered tissue from these animals are examined microscopically under ultraviolet light, the markers fluoresce. By identifying the dates of the treatments, especially if the treatments were multiple, it is possible to calibrate the layered tissue with these fluorescent time envelopes, and thus, to calculate layering rates.

Veterinary records of marine mammals from commercial and scientific aquaria should be researched to identify preserved specimens that may have been incidentally time-marked, during medical treatment, with fluorescent or otherwise detectable drugs. If incidental marks are found to be multiple and incorporated into dentinal, cemental and periosteal layering systems, it may be possible to intercalibrate layers of all three systems with real time, allowing any system to be used alternatively. This would be valuable in cases of older specimens where dentinal deposition has ceased because of pulp cavity occlusion or where early periosteal bony layers have been resorbed.

PILOT PROGRAMS

Pilot programs with the above goals in mind are in various stages of implementation at the Southwest Fisheries Center in La Jolla.

(1) A literature search is underway to collect references to experiments or studies of known-age and time-marked specimens, or of captive animals for which time in captivity has been documented. Samples or specimen loans are being requested from individuals and institutions who are in charge of such specimens in order that we may prepare, examine and photograph them. Specimens that have been sequestered through donation will be organized into a permanent systematic collection. Photos and pertinent data for specimens in the collection and on loan to us will be maintained in a data file and eventually will be computerized. Requests for specimen donations or loans will be made also to acquaria and other institutions likely to have known-age or time-marked specimens in their charge.

(2) Sections of teeth and bone selected from early fetal through adult animals are being processed to construct comprehensive developmental series of males and females for *Tursiops truncatus, Delphinus delphis, Stenella attenuata* and *Stenella longirostris.* As adequate samples are accumulated, documented and catalogued, they will become available for study upon request.

(3) As part of school composition and cohesion experiments carried out on wild dolphins in the eastern tropical Pacific by NMFS scientists aboard the M/V *Queen Mary* chartered for dolphin/tuna research by the U.S. Tuna Foundation in September-October 1978, 331 offshore spotted dolphins, *S. attenuata,* were captured, measured, sexed, tagged, tetracycline-marked, and returned to the wild. Because this species is associated with the yellowfin tuna fishery, operations of which are being monitored by NMFS scientific technicians aboard many purse seiners, there is a good chance that at least some tagged specimens will be returned. Returns should enable us to compare hard tissue accumulation rates in wild dolphins with those in captive dolphins.

(4) A three-year project is being implemented, in cooperation with Hubbs/Sea World Research Institute, to monitor layering rates using tetracycline injections and tooth extraction. The study is using a large sample of healthy captive delphinids. In addition to developing data on layering rates, the study will attempt to identify such factors as age, sex, diet, handling and treatment, seasonal fluctuations in water temperature, and intergeneric vari-

ability, that may influence or alter layering patterns. The project will use a minimum of 12 specimens of *Tursiops truncatus*, representing approximately equal numbers of males and females and including animals of various relative ages. Animals of other species will also be used to develop data on delphinids in general.

Teeth will be prepared and examined under ultraviolet light to locate fluorescent marks, which will be used to calibrate growth layer groups in stained and unstained specimens.

(5) When microscopically examined under reflected ultraviolet light, thin-sectioned teeth from several captive specimens have revealed multiple fluorescent marks within their dentinal layers. Efforts are being made to obtain veterinary data for these specimens in an attempt to identify treatment dates, dosages, and the kinds of antibiotics used to treat these animals. In the absence of these data, I have identified growth layer groups to estimate the real time represented as bracketed by the fluorescent markers. This was done as a test of the assumption that growth layers are accumulated at a constant rate. Veterinary data, when obtained, will be used to test the estimates of time intervals represented between the markers.

CONCLUSION

The approaches and pilot programs outlined above offer means by which the long standing assumptions in age determination from layered tissues may be tested extensively. The progress made along these lines will depend upon the cooperation of members of the scientific community and of other persons in charge of animals, preserved skeletal materials, and medical records from commercial aquaria. Only through such cooperative efforts may we expect to address the many other difficult questions of dolphin life histories with some assurance.

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Acid Etching and Highlighting for Defining Growth Layers in Cetacean Teeth

Kenneth V. Pierce
and
Hiroshi Kajimura

National Oceanic and Atmospheric Administration, National Marine Fisheries Service
Northwest and Alaska Fisheries Center, National Marine Mammal Laboratory, Seattle, Washington 98115

ABSTRACT

A prerequisite for using the growth layers in dentine or cementum of marine mammal teeth as a method of age determination is the ability to accurately 'read' the growth layers.

The method used to prepare pinniped teeth for age determination was adapted for use on cetacean teeth. The teeth were sectioned along the longitudinal axis using a band saw and/or lapidary equipment and acid-etched in a 5% formic acid bath. After thoroughly rinsing and air-drying the tooth section, the etched surface was highlighted by applying a light coating of jeweller's rouge. The well-defined growth layers were then easily 'read'.

INTRODUCTION

Differential mineralization of mammalian dental tissues over a period of time produces a series of discernible growth layers. The use of growth layers as indicators of age has been accepted widely by researchers for a number of years (Klevezal' and Kleinenberg, 1967). However, in the past, it has been difficult to interpret these growth layers with a degree of accuracy acceptable to all researchers (Klevezal' and Kleinenberg, 1967; Anas, 1970; Best, 1970). Problems encountered in interpretation include: (1) lack of knowledge of the biological and environmental factors controlling deposition of dental tissue; (2) inability to routinely distinguish between annual (one year) growth layers and the so-called 'accessory bands' believed to represent differential growth rates over a period other than one year; (3) lack of animals of known age for comparative growth layer analyses; and (4) lack of consensus on tooth preparation methodologies and interpretation, in standardized terms, of observed results.

Marine mammals, especially tropical and migratory species, pose special problems because of the expense and difficulty in collecting biological and environmental data on them in the field and in maintaining them in captivity. A method of tooth preparation that facilitates accurate growth layer counts would be a valuable tool for aging studies pertaining to marine mammal populations. Personnel of the National Marine Mammal Laboratory, Seattle, Washington, using modifications of several existing techniques, recently developed an accurate method for defining growth layers in the teeth of pinnipeds (Pierce and Kajimura, unpublished manuscript). This paper describes the application of the method to teeth of odontocete cetaceans.

METHODS AND MATERIALS

This study was undertaken to determine the feasibility of using the pinniped tooth preparation method on cetacean teeth. When selecting teeth for processing, the largest teeth available from each specimen were selected. No attempt was made to determine which teeth in an individual specimen would produce the best results.

Teeth from the following cetacean species were processed:

Family Delphinidae	
Tursiops truncatus (Montagu, 1821)	Bottlenose dolphin
Delphinus delphis (Linnaeus, 1758)	Common dolphin
Lagenorhynchus obliquidens (Gill, 1865)	Pacific white-sided dolphin
Lissodelphis borealis (Peale, 1848)	Northern right whale dolphin
Grampus griseus (G. Cuvier, 1812)	Risso's dolphin
Pseudorca crassidens (Owen, 1846)	False killer whale
Globicephala macrorhynchus (Gray, 1846)	Short-finned pilot whale
Orcinus orca (Linnaeus, 1758)	Killer whale
Phocoenoides dalli (True, 1885)	Dall's porpoise
Family Monodontidae	
Delphinapterus leucas (Pallas, 1776)	White whale
Family Physeteridae	
Physeter macrocephalus (Linnaeus, 1778)	Sperm whale
Family Ziphiidae	
Ziphius cavirostris (G. Cuvier, 1823)	Cuvier's beaked whale

Prior to processing, all of the teeth had been boiled in a trisodium phosphate solution, approximately ¼ cup trisodium phosphate/1 gallon water (≅ 15 gm/l), air dried, and stored in boxes and plastic bags from three years to more than ten years.

Grinding

A *Craftsman*[1] lapidary grinder equipped with a 240 grit wheel, a 400 grit wheel, and a felt buffing wheel to which a tin oxide polishing compound was applied, was used to produce a longitudinal half-section of each tooth.[2] A half-section was produced by grinding along the mid-longitudinal axis of the tooth until the pulp cavity was completely exposed. In most cases, the initial grinding was accomplished with the 240 grit wheel. Subsequently, the ground surface

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[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

[2] This grinder has been subsequently replaced with a [1] variable speed grinder/polisher which is safer to use and allows more precision in grinding/polishing.

was polished for one to two minutes with the felt buffing wheel using tin oxide compound.

Very large teeth, such as *Orcinus* and *Physeter* teeth, were rough-sectioned with a band saw prior to grinding to reduce grinding time. As a safety precaution, before sawing, the teeth were positioned vertically in plastic laboratory weighboats – available from most scientific supply houses – partially filled with fibreglass. The fibreglass provided the teeth with 'safety handles' which could be easily removed after sawing was completed by lightly tapping them with a hammer. The smaller odontocete teeth were initially ground with the 400 grit wheel to save time. For easy handling, these teeth were attached to glass slides using *Histoclad*[1] histological mounting media prior to grinding. It was necessary to allow the mounting media to cure for several days before grinding to insure a secure bond between the tooth and the glass slide. Teeth were left attached to the glass slides throughout the process described below or removed by placing them in xylene or tolulene overnight.

As the teeth of some specimens were curved in more than one plane, it was important to follow the curvature of each tooth as closely as possible when grinding. This was done by applying differential pressures with the finger tips while grinding. In cases of extreme curvature, the edge of the grinding wheel was used. The finished half-section surface represented the central mid-longitudinal axis of the entire tooth, including any curvature of the original tooth.

Acid etching

Polished half-sections were placed in a 5% formic acid bath (Bow and Purday, 1966), the volume of acid being at least 20X volume of teeth. Progress in etching was monitored by removing the teeth from the acid bath every two to three hours, briefly rinsing in running water, placing them in acetone for several minutes (not exceeding three minutes) and then allowing the teeth to air dry for at least ten minutes. This procedure permitted accurate determination of the degree of etching achieved and prevented over-etching. The teeth of most adult cetaceans required eight to ten hours for proper etching. In a few cases, prolonged soaking in 20% formic acid (Bow and Purday, 1966) for periods up to one hour improved the etching achieved in the 5% formic acid. However, the teeth of young animals (less than four growth layers) and very small teeth were adequately etched in only two to six hours. Decalcification of *Phocoenoides* teeth occasionally occurred within five hours in 5% formic acid.

Formic acid was found to be the best decalcifier. Nitric acid and hydrochloric acid (Brain, 1966), produced less distinct layering and often caused the dental tissues to crack or separate. A commercial decalcifying solution was also tried but the etching time was longer, with no improvement in quality.

When a satisfactory degree of etching was achieved, the teeth were removed from the acid bath, rinsed overnight in running water and allowed to air dry for one day or more. The teeth must be rinsed thoroughly before final drying. If the teeth were not rinsed sufficiently, the residual acid caused gradual deterioration of the etched surfaces after it was dried.

Determination of etching endpoint

Ability to determine the acid etching endpoint is easily acquired with experience. When properly etched, the growth layers occur as distinct groupings of 'valleys' and 'ridges' on the sectioned surface of the tooth. Usually, a valley separates two prominent ridges and contains a series of less prominent ridges (ridges of lesser height). When surfaces are underetched, the distinct valley/ridge groupings do not develop. Over-etching causes the flattening out of the ridges, obscuring the valley/ridge groupings. The sectioned surface of a decalcified section is almost flat. Etching time depends upon the amount of mineralized tissue present in the tooth; therefore, etching time is critical (± one hour) only in small teeth and the teeth of young animals, where the amount of mineralized tissue is small. Insufficiently etched teeth can be returned to the acid baths at any time; but once they are over-etched, attempts to resurface the tooth section usually result in the destruction of the half-section.

Highlighting and examination

Prior to examining the etched surface, the growth layers were highlighted with jeweller's rouge. This was accomplished by rubbing jeweller's rouge over the etched surface with a dry finger and gently blowing off the excess. If both finger and tooth surfaces are dry the jeweller's rouge adheres only to the ridges. Jeweller's rouge was selected as the highlighting agent because it is inexpensive, easily obtainable, and produces more contrast between valleys and ridges, especially for photographic purposes, and causes less mess than graphite (Bow and Purday, 1966). Other fine-grained, coloured compounds such as cupric oxide have been found to provide good highlighting properties for etched growth layers (Bruce Mate, University of Oregon, Newport, OR, pers. comm.) but they were not used in this study.

A simple hand-held reading-glass magnifier provided sufficient magnification for accurate examination of the growth layers on most etched sections. The smaller dolphin/porpoise teeth were examined with the aid of a dissecting microscope capable of 30X magnification. On occasion, the dissecting microscope was also used to examine closely spaced growth layers in older animals.

RESULTS AND DISCUSSION

In most cases, cetacean teeth treated in the above manner evidenced distinct growth layers in both dentinal and/or cemental tissues (Figs 1–4). Exceptions were young animals and *Phocoenoides* teeth. The valley/ridge groupings were present but not well defined in the teeth of young animals. However, in older animals, the early growth layers were as well defined as later growth layers. Valleys and ridges were also discernible in the cementum of the *Phocoenoides* teeth, but, due to the small size of the teeth, the cementum tended to decalcify rather than etch. Decalcification of cementum tended to occur in other small dolphin teeth also, but to a lesser degree. The teeth from a young specimen of *G. griseus* failed to show growth layers. Whether the lack of discernible growth layers was a result of age or a species characteristic was not determined. Teeth of older specimens were not available for comparison.

Acid etching of cetacean teeth produced results similar to those for pinniped teeth. In both cases, growth layers were discernible as valley/ridge groupings; the valley separating two prominent ridges, with one to several less prominent (lower) ridges present within each valley remained fairly constant. In pinnipeds (primarily *Callorhinus*) we found the number of prominent ridges to correspond with known ages in years.

Ridges and valleys represent the degrees of mineralization of the dental tissues, the ridges being the least mineralized regions (Scott and Symons, 1961). In pinnipeds, poor

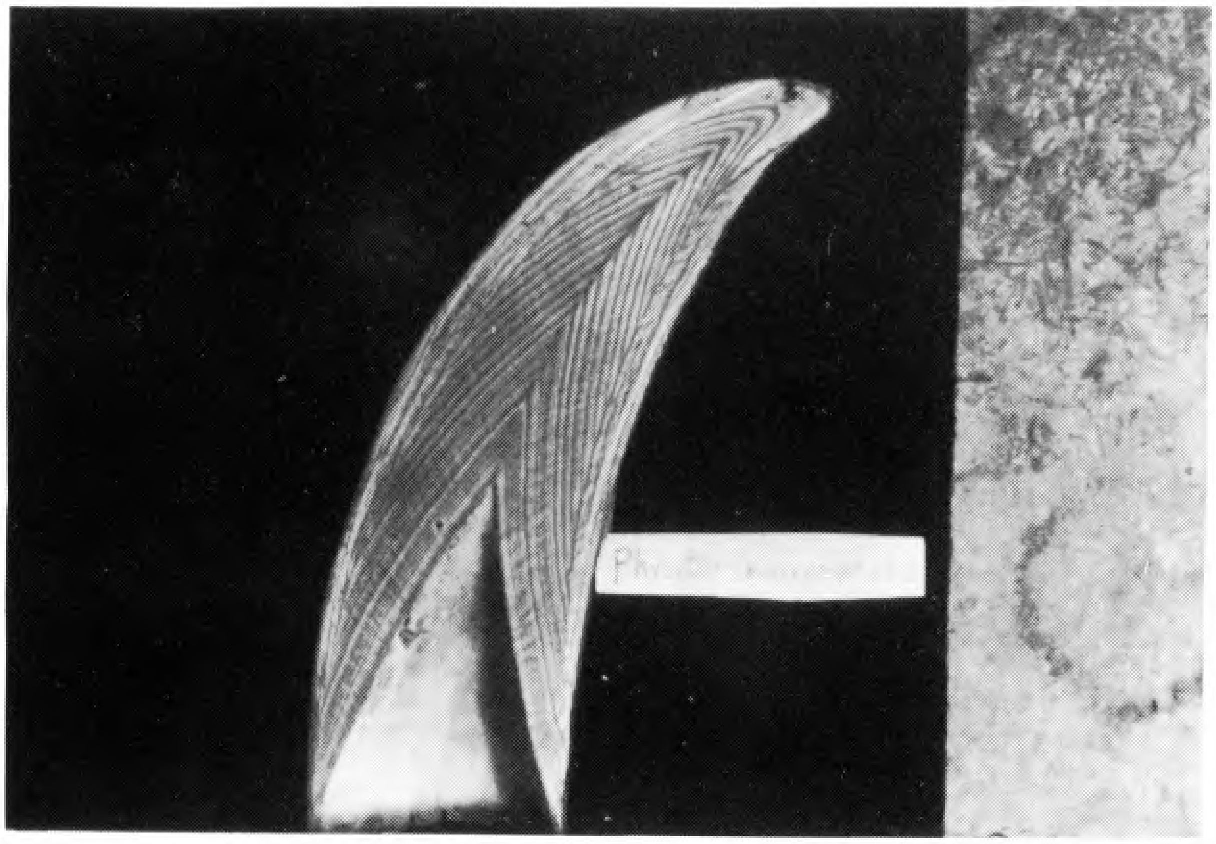

Fig. 1. Acid-etched highlighted tooth of 22/23 year-old sperm whale (*P. macrocephalus*).

mineralization of dental tissues is believed to represent periods of poor feeding (McLaren, 1958; Fisher, 1954; Laws, 1953; Kenyon and Fiscus, 1963). In *Callorhinus,* the prominent ridges are probably representative of the time spent on the breeding grounds, as this is a period of prolonged fasting (Scheffer, 1950). The prominent ridges and associated valley are considered to be annuli (representative of one year of growth). The less prominent ridges, because of their presence within the valley and their cyclic repetition, are considered accessory bands, for which the causal mechanisms are not fully understood. A similar interpretation has been applied to other pinniped species.

The valley/ridge grouping similarities between pinnipeds and cetaceans indicate mineralization patterns in cetaceans similar to pinnipeds, in that mineralization of dental tissue is cyclic. The prominent ridges probably correspond to annuli in pinnipeds, while the less prominent ridges correspond to the accessory bands. However, lack of biological and environmental data which affect dental tissue mineralization in cetaceans prevents the determination of the intervals of time associated with major growth layers, thus preventing actual age determination.

Our method, in itself, is not the final solution to aging cetaceans. Biological and environmental data presently available are not sufficient for correlating growth layers to age for most cetacean species (Kasuya, 1972; Sergeant, 1973a; Kasuya, 1976; Gambell, 1977), *T. truncatus* being an exception (Sergeant, 1973b; Hui, 1978). However, we believe our method does offer several advantages in accurately interpreting the growth layers, at least with larger teeth. Preparation of stained thin sections from decalcified teeth as reviewed by Klevezal' and Kleinenberg (1967) is widely used for

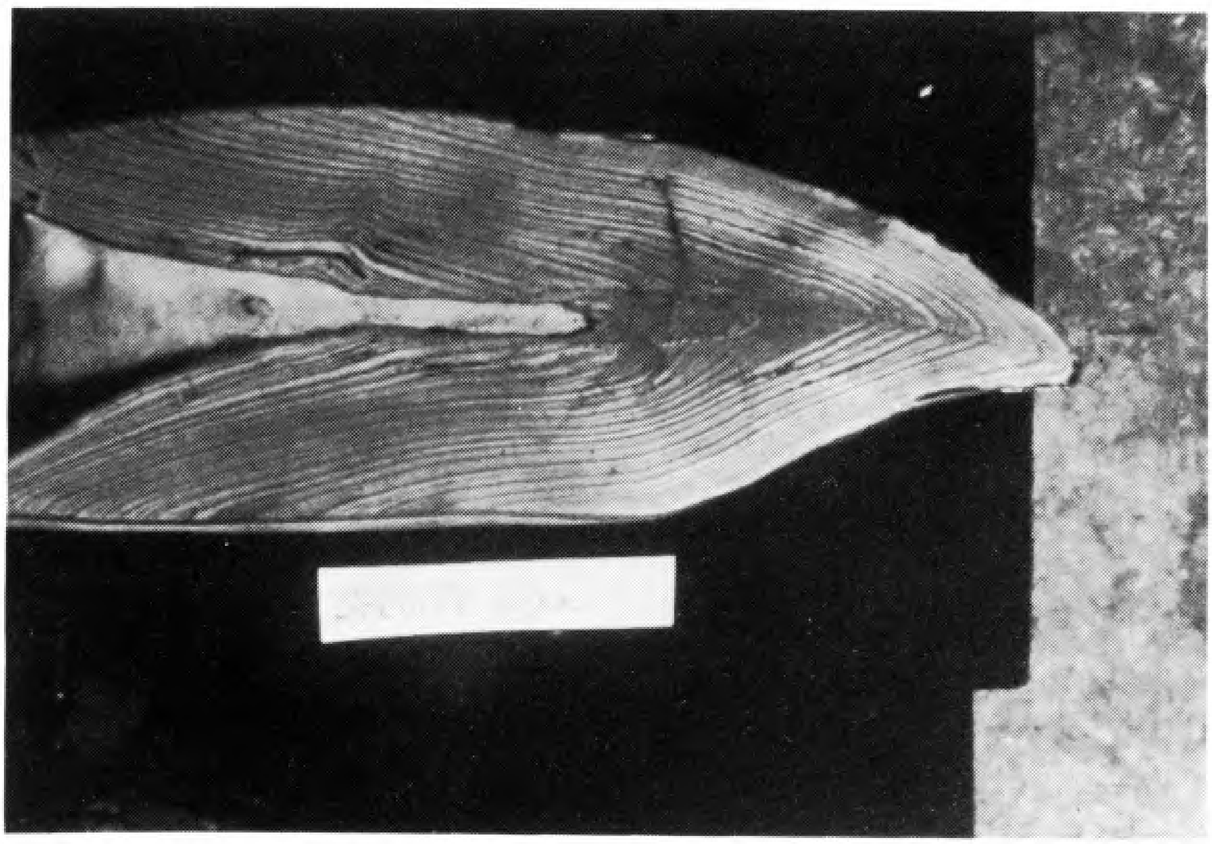

Fig. 2. Acid-etched highlighted tooth of 23 year-old killer whale (*O. orca*).

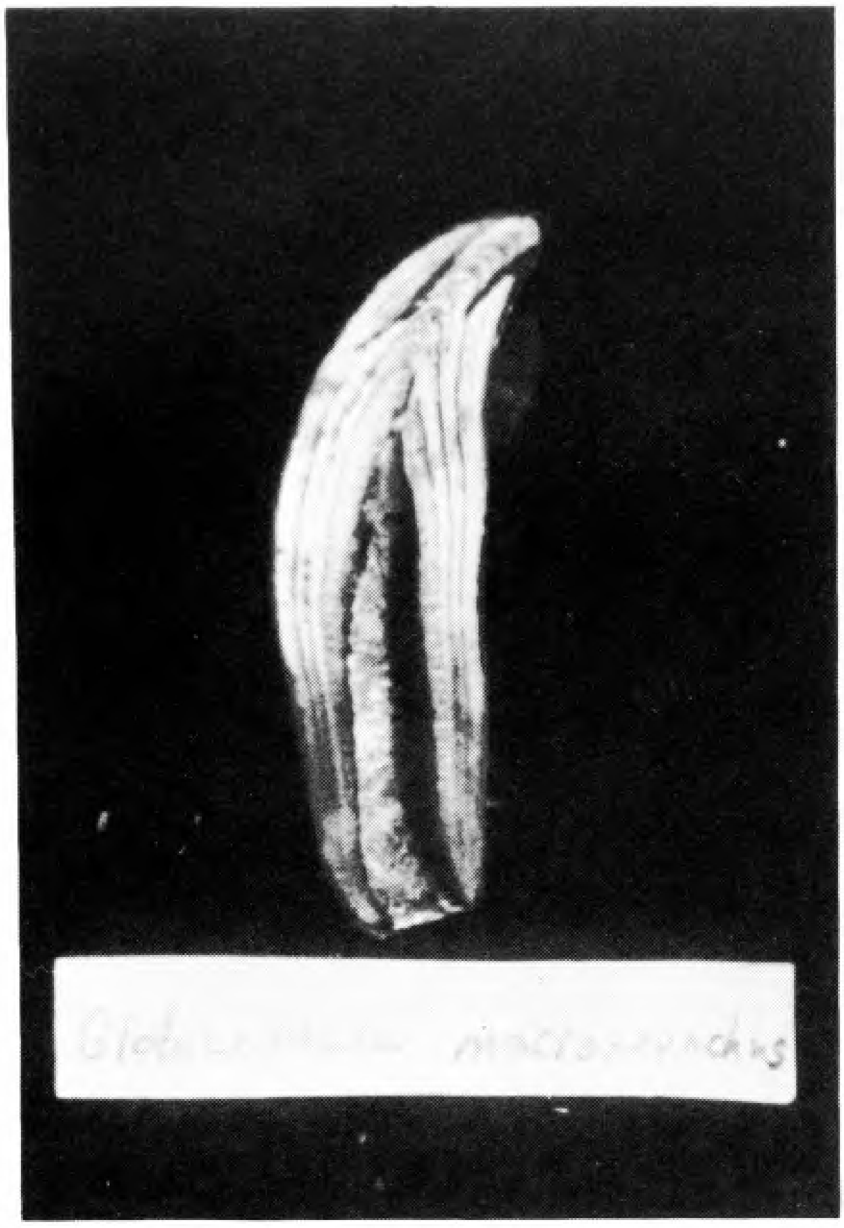

Fig. 3. Acid-etched highlighted tooth of 6 year-old shortfin pilot whale (*G. macrorhynchus*).

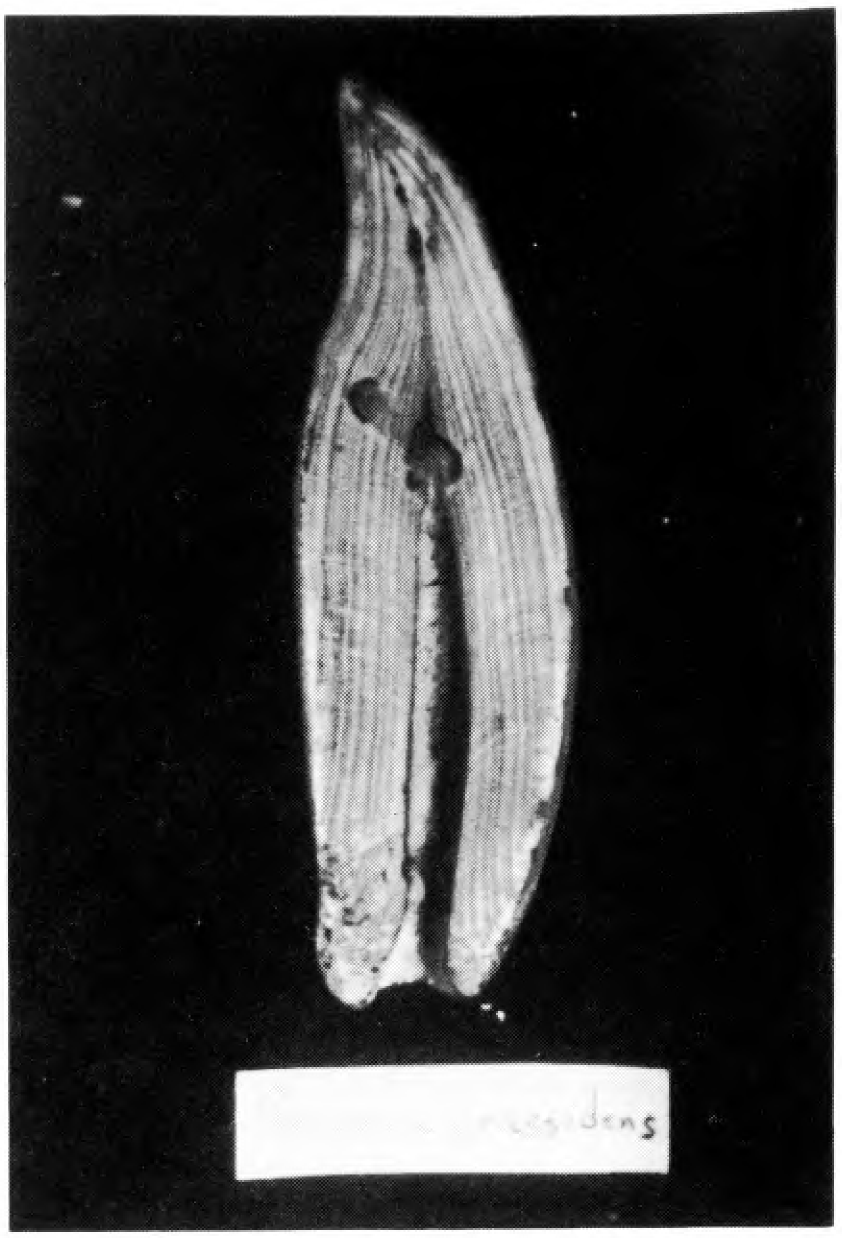

Fig. 4. Acid-etched highlighted tooth of 13 year-old false killer whale (*P. crassidans*).

aging many mammalian species. While stained thin sections produce good results with small teeth, the method is of little value in the preparation of large teeth, because the equipment used in sectioning is designed for handling small pieces of tissue (Eaton, 1977). The equipment is also expensive, and operators require experience in histological techniques. Other sophisticated techniques such as electronmicroscopy and X-ray photography, while producing excellent resolution of fine details, require even more costly equipment and highly skilled operators, making them of little value for routine laboratory use. Acid etching and highlighting require only some type of grinding device and a minimal amount of experience. Also, the correct choice of grinding equipment could make this method easily adaptable to work in the field or in the lab. Another advantage to acid etching is the ability to distinguish between major growth cycles (annular equivalent) and intermediate cycles (accessory bands) by the differential heights of the ridges. In our experience, the reduction in etching time from 30 hours used by Bow and Purday (1966) to eight to ten hours for cetaceans and 12 to 16 hours for pinnipeds enhanced the development of related groupings of ridges.

The acid etching method described above is applicable to a large number of marine mammals, with the exception of very small toothed cetaceans and very young animals. In these cases, other methods, such as staining of decalcified thin sections and length/weight or anatomical ratios data, when available, may provide equal or superior results.

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Examination of Layered Tissues of Odontocetes for Age Determination Using Polarized Light Microscopy

Albert C. Myrick, Jr.

National Oceanic and Atmosphere Administration, National Marine Fisheries Service,
Southwest Fisheries Center, La Jolla, California 92038

ABSTRACT

The polarized light microscope with a 1/4 λ plate greatly improves resolution of the microstructure in layered hard tissues of dolphins by revealing changes in crystallographic alignment not visible using more conventional instruments. In adult specimens of *Stenella attenuata* the prenatal enamel contains up to 240 striae that are thought to represent daily records of prenatal growth. Prenatal dentine contains approximately 60 incremental layers, the significance of which is unknown at present. In *Lagenorhynchus, Delphinus, Tursiops* and *Stenella* spp. the neonatal 'line' consists of several incremental layers, suggesting that post-parturitional trauma may be a prolonged event. In these species the postnatal dentine contains 13 regularly-spaced accessory layers in the first five or six growth layer groups (GLGs). These accessory layers are thought to reflect lunar cycles, and each contains an average of 28 microlayers (lines of von Ebner) that may represent daily growth increments. Cemental GLGs show fewer accessory layers than those of dentine. GLGs in periosteal bone consist of accessory layers that may reflect lunar cyclicity. It is suspected that layering in dentinal, cemental, and periosteal tissues is in response to the same cyclic influences.

INTRODUCTION

Investigators of dolphin life histories routinely use compound or dissecting microscopes to identify and count growth layer groups (GLGs) in thin sections of teeth and bones to determine relative ages. These instruments operate with plain light that is either transmitted through a section or reflected from the surface of a section to detect differential optical densities or topographic relief delineating GLG boundaries thought to represent uniform segments of time.

A prime cause of disagreement among investigators in counting layers has been the difficulty in deciding where to separate the layers. Also, there may be difficulty in distinguishing any regular layering at all in sections that have not undergone special preparation. The polarizing microscope assists in overcoming some of these problems by expressing structural and histological differences as highly contrasted patterns of light (i.e. as different colors or shades). It accentuates structure, not visible using more conventional instruments, that is of potential importance in studies of dental development, and it provides further bases for understanding and interpreting layering patterns for age determination.

This paper is an account of preliminary research conducted using the polarizing microscope in examination of the layered structure of hard tissues of dolphins as an aid to understanding more fully the macrostructures useful in estimating ages.

BACKGROUND

The petrographic polarizing microscope was developed initially to determine the mineral composition of rocks by examination of their crystallography. It is a compound microscope with light-polarizing and phase-contrast capabilities that can be used to define differential optical and crystallographic features of translucent objects prepared in thin section (Moorehouse, 1959). The technique has been used widely in comparative dental histological studies (e.g. von Keil and von Nolting, 1968; Peyer, 1968).

Dental and periosteal tissues of dolphins and other vertebrates contain the mineral hydroxyapatite. Because growth is inconstant, layers of various optical densities are formed, and crystal lattices of hydroxyapatite are deposited within the layers in orientations that tend to differ from increment to increment but which have a common alignment within a given growth increment. When layered tissue is examined with polarized light, each crystallographic alignment, and variations in the composition of the mineral phase and organic matrix are made visible.

MATERIALS AND METHODS

1. Ontogenetic series

Teeth and samples of periosteal bone, from the 'pan' region of the mandible and from the premaxillae at the base of the rostrum, were collected from approximately 50 female specimens of *Stenella attenuata* from the eastern tropical Pacific–the animals ranged from early fetal to old adult. Materials were prepared in undecalcified thin section ($\cong 250\ \mu m$) and arranged in order of body length to form developmental series. Each series was examined under polarized light in an effort to characterize developmental stages in the layering patterns and to inter-calibrate dentinal, cemental, and periosteal growth-layer-group (GLG) patterns.

2. Known-age specimens

Thin sections ($\cong 250\ \mu m$) of undecalcified teeth from captive-born (known-age) individuals of *Tursiops truncatus* were examined by polarized light microscopy, without my advance knowledge of their ages, in an effort to identify reliable structures that may be useful in determining absolute ages.

3. Tetracycline-marked specimens

Several teeth from specimens of *Tursiops truncatus, Lagenorhynchus obliquidens,* and *Delphinus delphis* containing tetracycline-marked tissues were examined by polarized light and under ultraviolet reflected light to intercalibrate postnatal dentinal and cemental layers. One of the known-age specimens was incidentally marked with tetracycline while *in utero.* The marked tissue was examined to identify the structural time of birth.

4. Preparation of samples

All teeth were sectioned mid-longitudinally using a *Buehler Isomet* saw equipped with two diamond-embedded blades separated by a 200-μm shim to produce a wafer 200 μm to 300 μm thick. Bone samples were sectioned transversely using the same equipment. Thin sections were mounted on glass slides with *Permount* and covered with glass coverslips.

5. Method of examination

All sections were examined at between 57 x and 1200 x using a *Zeiss* photomicroscope No. 472190 equipped with a rotatable polarizer and analyzer, a rotary stage, petrographic objectives, and a 1/4 λ (first order red) quartz plate. A fluorescent vertical illuminator with a filter-reflector No. 44-77-05 combination was used for examination of the tetracycline-marked specimens in ultraviolet light.

RESULTS AND DISCUSSION

1. Enamel

A. ***Ontogenetic development of layers.*** Enamel is deposited prenatally. Polarized light microscopy of this tissue in *Stenella attenuata* reveals up to 240 growth lamellae lying parallel to each other, arranged in an offlapping pattern downward from near the apex and oriented subnormal to the prismatic structure (Fig. 1). Analyses of the ontogenetic series indicate that these layers represent growth increments that first appear in teeth of fetuses at body lengths of between 25 cm and 28 cm. There is an increase in the number of layers with increasing body length. At 50 cm the enamel contains approximately 40 layers, at 70 cm there are 150 layers, and in teeth of full-term fetuses (approximately 82.5 cm) and postnatal animals including old adults (up to 220 cm) up to 240 enamel layers have been counted. This suggests that incremental growth in enamel probably does not continue after parturition.

B. ***Significance and possible applications of enamel layering.*** Perrin, Coe and Zweifel (1976) concluded that the average gestation period for *S. attenuata* is approximately 11.5 months (or 345 days). If the layers in the enamel represent daily growth, as much as 240 days (or 8 months) of fetal development could be accounted for. The unrecorded 3.5 months would represent the period from conception to the stage at which the tooth germ is developed immediately prior to layer formation.

If examination of larger samples substantiate these tentative findings, enamel layers may be used in conjunction with labelling of fetal hard-tissues in captive, pregnant females and with embryological data from terrestrial mammals to estimate more precisely the fetal growth stages and gestation periods of delphinid species.

2. Prenatal dentine

A. ***Ontogenetic development of layers.*** Prenatal dentine has been relatively ignored except as a 'landmark' tissue internal to which GLG counts are begun. It has been described as unlayered or granular in appearance, but polarized-light

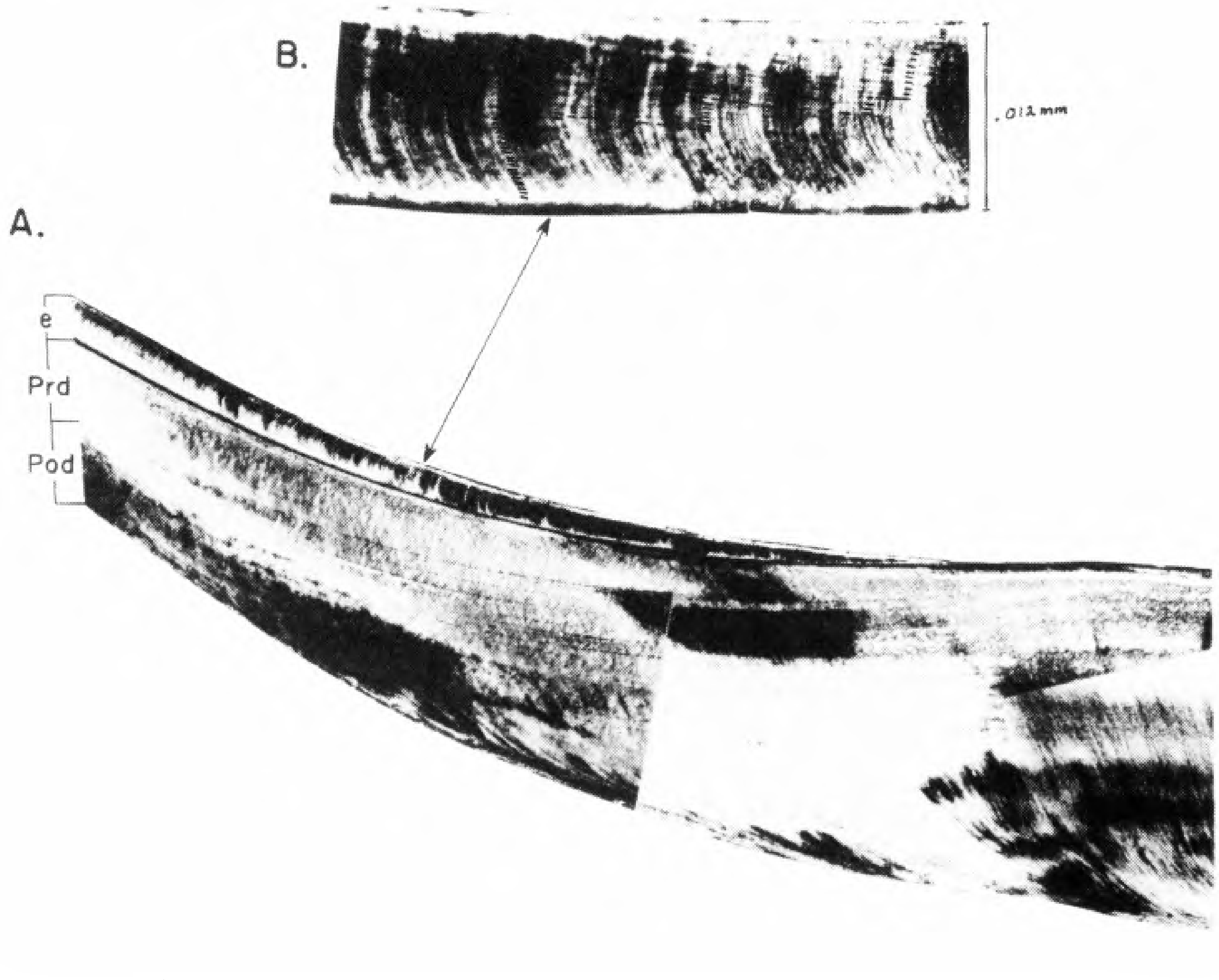

Fig. 1. Daily (?) layering in enamel in a tooth of a female of *Stenella attenuata* (TCF 060).
A. Half of a tooth section viewed with polarized light and showing layering in the enamel (arrow) (57x).
B. Highly magnified section of enamel as shown in A showing layers as indicated by marks. Because of the oblique (offlapping) arrangements of layers many transects at various intervals from apex to neck may be necessary to obtain full counts. A total of 223 enamel layers was seen in the tooth.
Abbreviations: e. enamel, n. neonatal line, prd. prenatal dentine, pod. postnatal dentine.

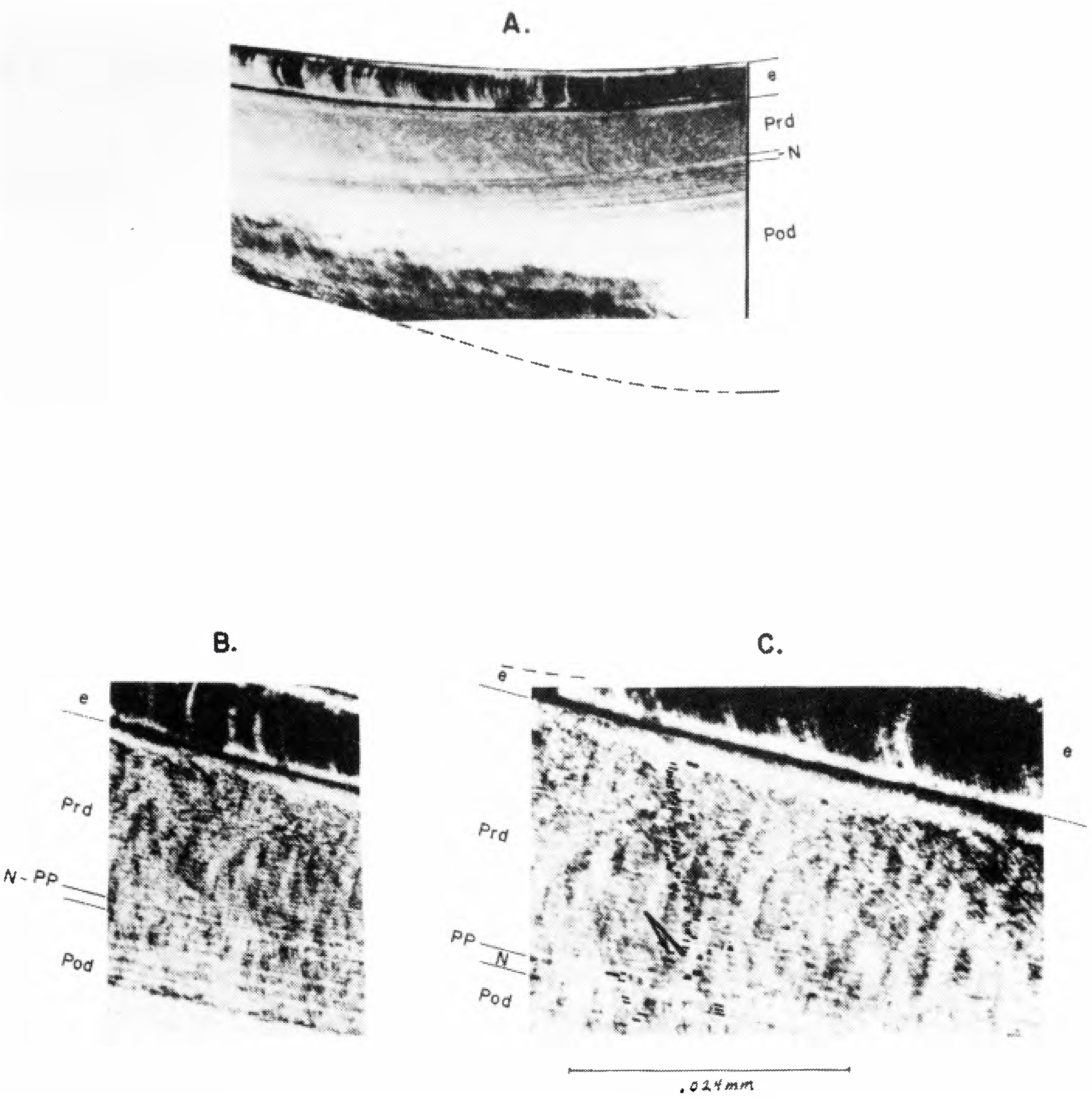

Fig. 2. Layering in prenatal dentine and neonatal line in *Stenella attenuata* (TCF 060).
A. Thin section of half of tooth as viewed with crossed polarizers and phase contrasted light showing prenatal dentine and neonatal line with layers not readily apparent (57x).
B. Magnified view (200x) of prenatal dentine as in A showing layering in prenatal dentine and neonatal line.
C. Highly magnified view (250x) of same section as in B with 60 counted layers in the prenatal dentine and 5 layers visible in the neonatal layer. Major layers often bifurcate in prenatal dentine (arrows). Note that layering is difficult to resolve in the earliest deposited tissue near the enamel. Abbreviations: PP. structural point of parturition, N neonatal line.

microscopy reveals that layers are fairly well developed in prenatal dentine (Fig. 2).

Layers first appear at the same stage of fetal development as do layers in enamel, i.e. at 25 cm to 28 cm. Unlike enamel layering however, prenatal layers seem to accumulate at a considerably lower rate. At 50-cm body lengths the teeth show only 25 prenatal dentinal layers, at 70 cm approximately 45 layers are present, and in the teeth of full-term fetuses and postnatal animals only 55 to 60 layers have been counted.

B. ***Significance of prenatal dentinal layering.*** The significance of layering in this tissue is not readily apparent. If prenatal dentinal growth occurs concomitantly with that of enamel (i.e. for a presumed period of approximately 240 days), then prenatal dentinal layers would be deposited at an average rate of one every four days. Such a rate would not seem to be correlated to any known exogenous cycle. On the other hand, distinguishing layers in this tissue is difficult, especially in the region of earliest deposition (Figs. 2B–C). It is possible that either some of the 'growth pulses' governing layering in enamel are not of sufficient intensity to produce visible layers in the prenatal dentine, or that another technique is needed to detect existing, but indistinct layers.

3. Neonatal line

The neonatal 'line' has been thought to demarcate the point of parturition–recording post parturitional trauma of the neonate as it is confronted with the external environment and a new mode of feeding. Polarized-light microscopy at 200 X shows that this 'line' is composed of an alternating series of three or more pairs of opaque and translucent layers bounded on either side by a rather bright translucent layer (Figs. 2B–C).

4. Postnatal dentine

A. ***Composition of GLGs.*** Microscopic examination (at 57 X) of postnatal dentine using plain transmitted or reflected light reveals repeating or semi-repeating patterns

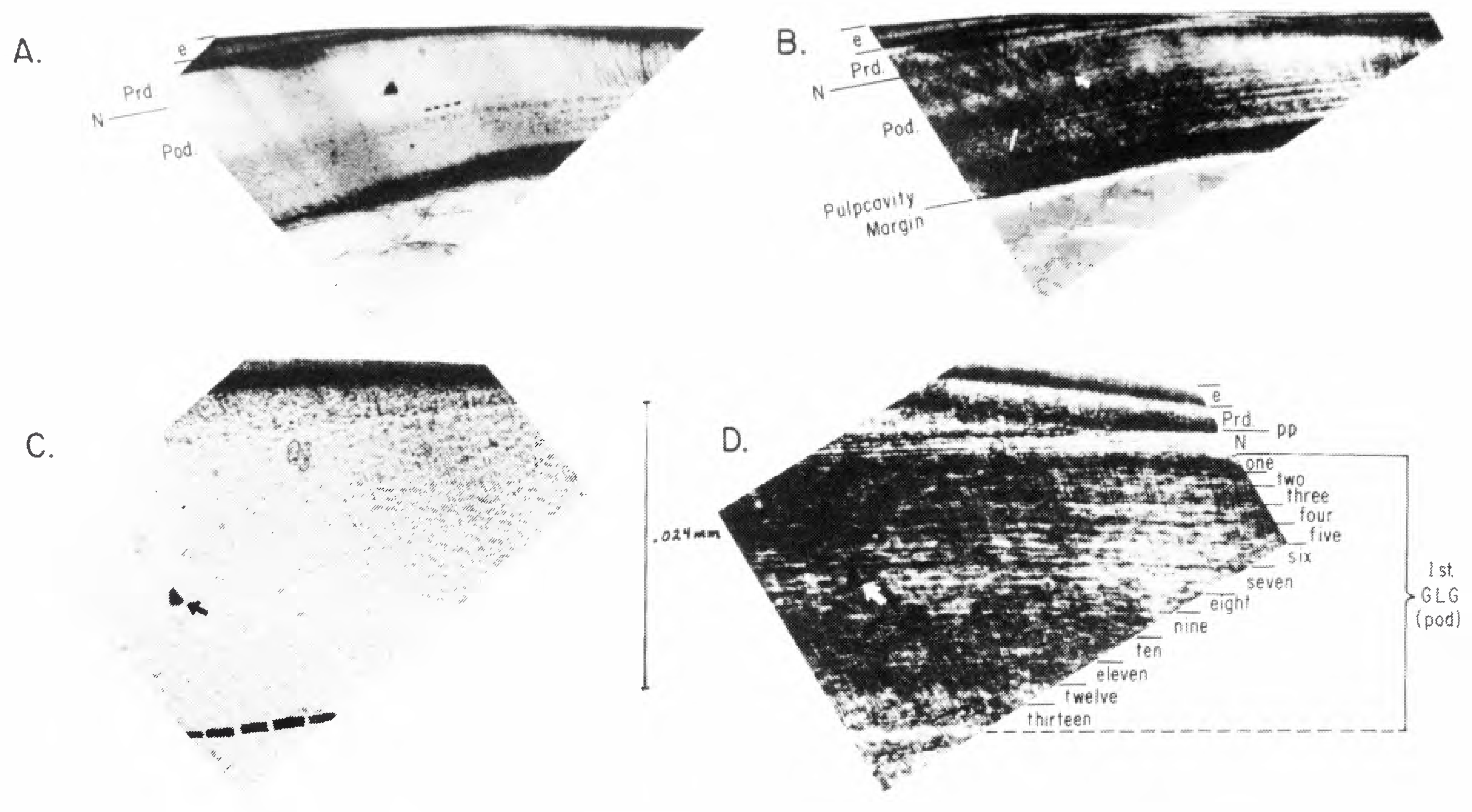

Fig. 3. Lunar layering in postnatal GLGs of a tooth of a specimen of *Stenella attenuata.*

A. Plain-light view of one-half of tooth section showing postnatal dentine. Dashed line indicates boundary between first and second growth layer groups. Triangle (an artifact) used as point of reference (57x).

B. Section as in A, viewed with crossed polarizers and phase-contrasted light. Note presence of multiple layers brought out by this technique.

C. Plain-light view of section as in Fig. A (250x). Arrow indicates triangle reference point.

D. Section as in C viewed with crossed polarizers and phase-contrasted light. Note layered components of neonatal line and 13 counted accessory layers within first GLG (bracketed). At this power the more intense aggregations of microlayers (lines of von Ebner) become apparent. Abbreviation: GLG = growth layer groups.

of accessory layers that may be used to define GLGs. Each accessory layer consists of a pair of components—a translucent layer and an opaque layer, which, in turn, are composed of fine dark and light incremental layers. With ordinary light microscopy, accessory layers are seen to occur in varying intensities, and commonly the accessory layers that mark the boundaries of GLGs are no more distinct than some of the more strongly developed accessory layers within the GLGs. Hence problems often arise in defining GLGs that introduce considerable subjective error into age estimates.

Polarized light microscopy of the tissue enhances resolution of accessory layers already optically apparent using more conventional microscopes and makes visible other accessory layers that cannot otherwise be detected easily. At 200 X the first (i.e. externalmost) GLG is shown to consist of 13 accessory layers excluding the one represented by the neonatal layer (Figs. 3D and 4). Although younger GLGs are deposited as increasingly thinner, more compressed layering sequences, the first three or four GLGs usually show 13 accessory layers also (Figs. 3 and 4). Younger GLGs usually are too thin to permit delineation of all incremental components, but in large, well layered teeth 13 accessory layers can be detected in the fifth and (rarely) sixth GLGs.

B. ***Possible significance of accessory layers.*** There are 13 lunar months (synodic cycles) in a year. It is possible that, as in many other marine organisms, the physiologies of odontocetes are or were influenced either directly or indirectly by lunar periodicity and that this periodicity is reflected in the layering patterns of hard tissues. If accessory layers correspond with lunar cyclicity, they could be used as countable units to determine age. To test this, accessory layers in the teeth of two known-age specimens of *Tursiops truncatus* ('Pinger' and 'Moe B') were counted without advance knowledge of their true ages. Teeth of both specimens contained between 42 and 45 accessory layers. 'Pinger' (SWFC 0007) was born in captivity at the Naval Ocean Systems Center, San Diego in November 1970 and died in February 1974 (Hui, 1978). 'Moe B' (SWFC 0008) was born at Sea World in San Diego on 14 April 1975 and died on 4 July 1978. Both animals were 3 years, 3 months old (or 43 lunar months old). The close agreement between the number of accessory layers counted and the age of the specimens in lunar months strengthens the hypothesis that accessory layers, when optimally viewed, may be used to estimate ages for young specimens.

C. ***Lines of von Ebner: daily(?) records.*** At high magnifications (800 to 1200 X), polarized-light microscopy of postnatal dentine reveals a system of minute incremental growth layers (so-called 'lines of von Ebner') within the accessory layers (Figs. 3D and 5). Often these structures are difficult to see, but in accessory layers where they are most distinctive, I have counted an average of 28 such microlayers. Because they are contained within accessory layers that I interpret to represent 28-day lunar cycles, I suggest that the lines of von Ebner in dolphin teeth may reflect daily growth.

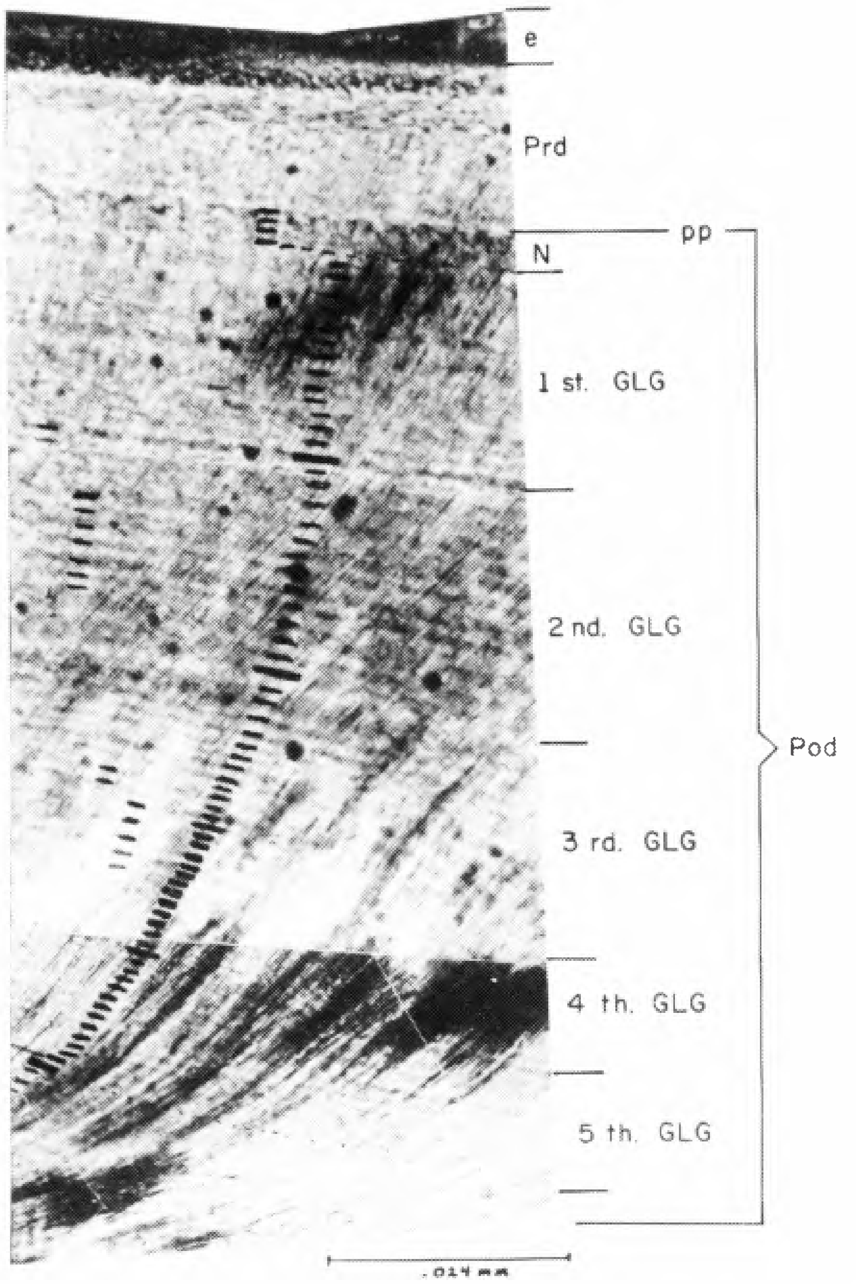

Fig. 4. Partial tooth section of a female specimen of *Stenella attenuata* (TCF 060) viewed with cross polarizers and phase-contrasted light. Section shows 13 regularly spaced accessory layers in each of the first five GLGs (each GLG defined by translucent boundary layers) (200x). Regular spacing and number of accessory layers indicated lunar cyclicity (see text).

5. Cementum

A. ***Resolution of GLGs.*** In the teeth of most species of small delphinids, cemental GLGs are difficult to define without special preparations because of the extreme thinness and poor layering of this tissue. Typically, cementocytes are stratified, but their parallel alignments do not correspond necessarily to cemental layering where it is distinguishable.

Compared to ordinary light, polarized-light microscopic examination provides somewhat better resolution of cemental layers because of the color contrast produced. Nevertheless, only decalcification and staining procedures (Kasuya, 1977) seem to enhance cemental layering adequately.

B. ***Accessory layers.*** When visible under polarized light, GLGs in cement exhibit fewer accessory layers than the 13 found in early dentinal GLGs. Tooth sections of captive specimens of *T. truncatus* clinically treated with tetracycline show fluorescent markers in the dental tissue when examined microscopically with reflected ultraviolet light (Figs. 6A, B, C and D). These markers extend down the dentinal layers, wrap around the bases of the teeth, and are continuous with markers within the cemental tissue (Figs. 6C and D). This indicates that the same mechanism which governs dentinal layering also influences cemental layering, but it is not known why fewer accessory layers are found in the cemental GLGs. The problem may be one of preparation and examination techniques (see Kasuya, 1977).

6. Periosteal bone

Polarized-light microscopy helps in delineating GLGs in transverse sections of periosteal bone from the 'pan' region of the mandible (Fig. 7) and from the premaxilla near the base of the rostrum (Fig. 8). A small ontogenetic series of bone samples compared with teeth from the same individuals indicates that periosteal GLGs seem to accumulate at the same rate as dentinal GLGs. In old specimens, some resorption of the early periosteal GLGs takes place, but relicts of these resorbed layers remain discernible with the polarizing microscope (Fig. 8).

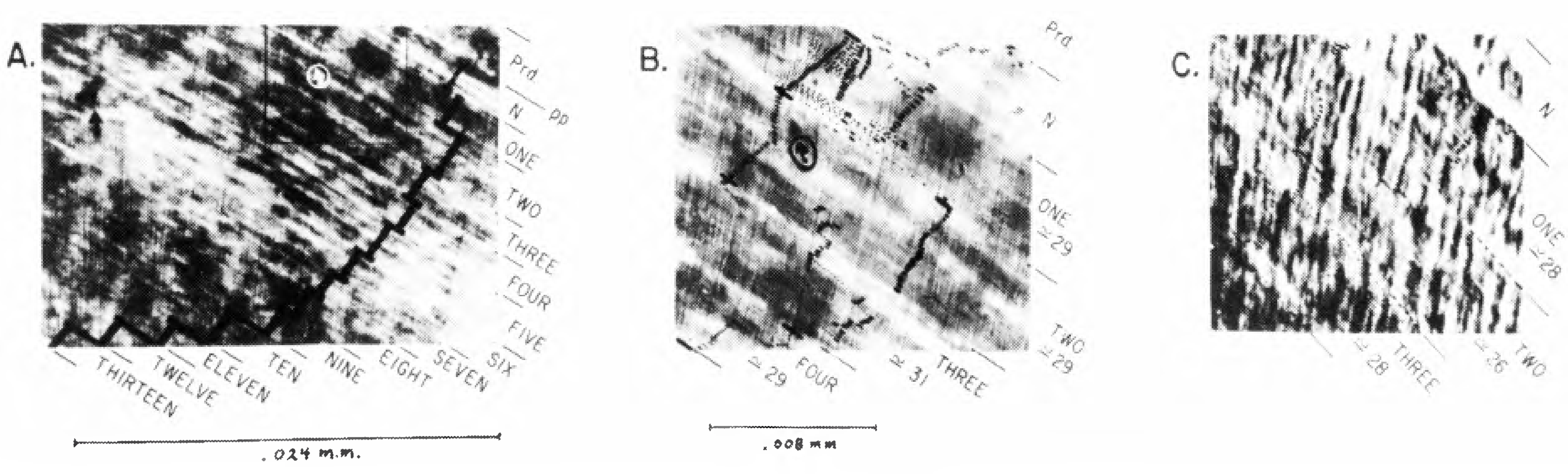

Fig. 5. Daily layering in postnatal GLGs in *Stenella attenuata*.
A. Magnified view (250x) of layers as shown in Fig. 3D showing 13 (bracketed) lunar layers within the first growth layer group.
B. Magnified view (800x) of inset in 5A, showing system of lines of von Ebner in first four accessory layers. Counts average 29 lines per accessory layer. This suggests that daily growth is represented. Circled arrow points to small triangle in second accessory layer used as point of reference.
C. Lines of von Ebner in another region of same tooth showing greater resolution produced by changing angle of section relative to polarizers. An average of 27 or 28 lines may be seen in first three accessory layers (800x).

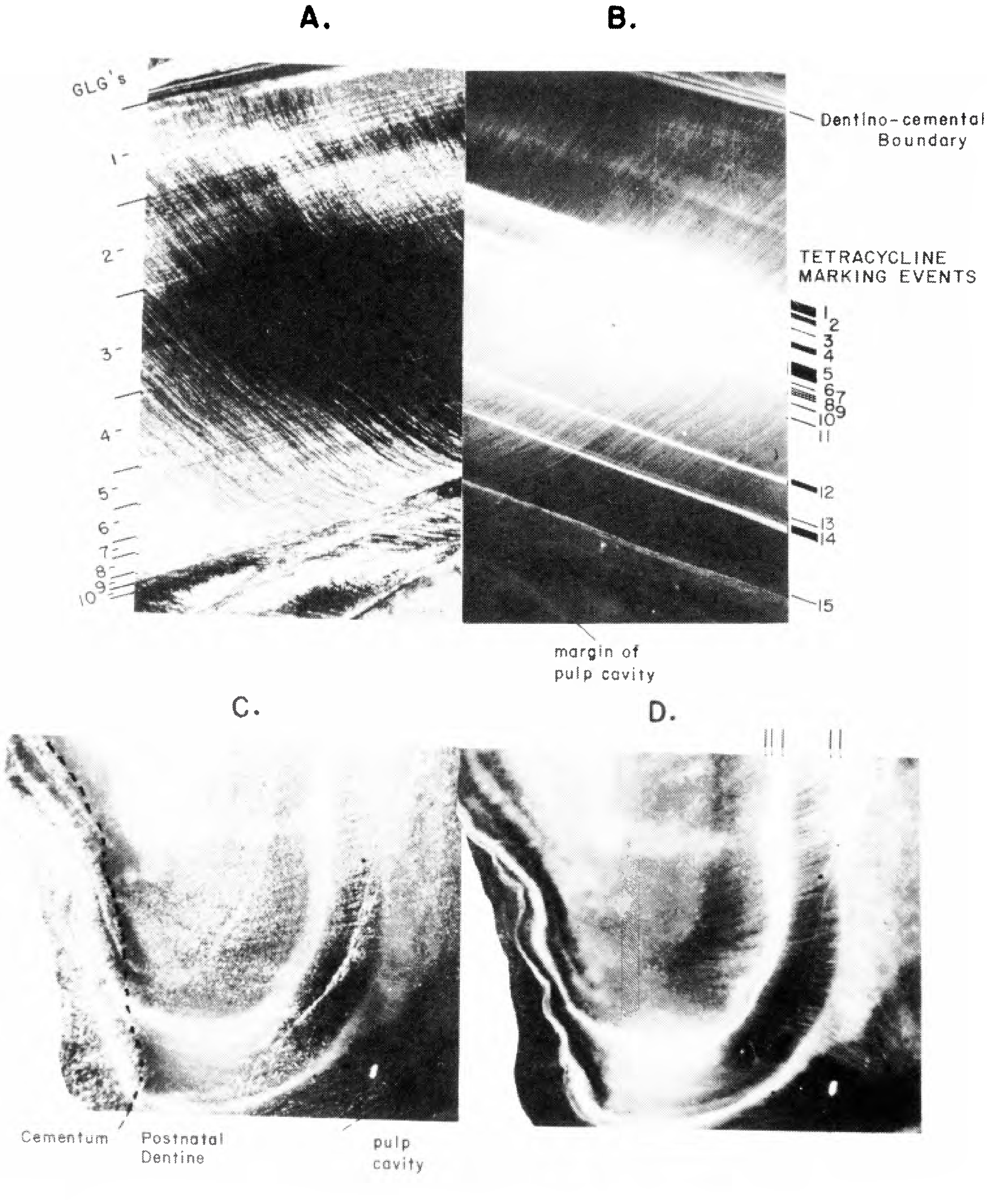

Fig. 6. Tetracycline-marked partial tooth sections of *Tursiops truncatus* (#AB718) showing fluorescent bands in relation to layers in postnatal dentine and cementum.

A. Dentinal tissue viewed using polarized light, showing GLGs.

B. Mirrored view of dentinal tissue as shown in A viewed under ultraviolet light showing flourescent bands (150x).

C. Base of tooth viewed using combination of reflected ultraviolet light and transmitted plain light showing relationship of fluorescent markers to layers in cementum and dentine (150x).

D. Base of tooth viewed under ultraviolet light showing continuity of flourescent markers in dentine and cementum (150x).

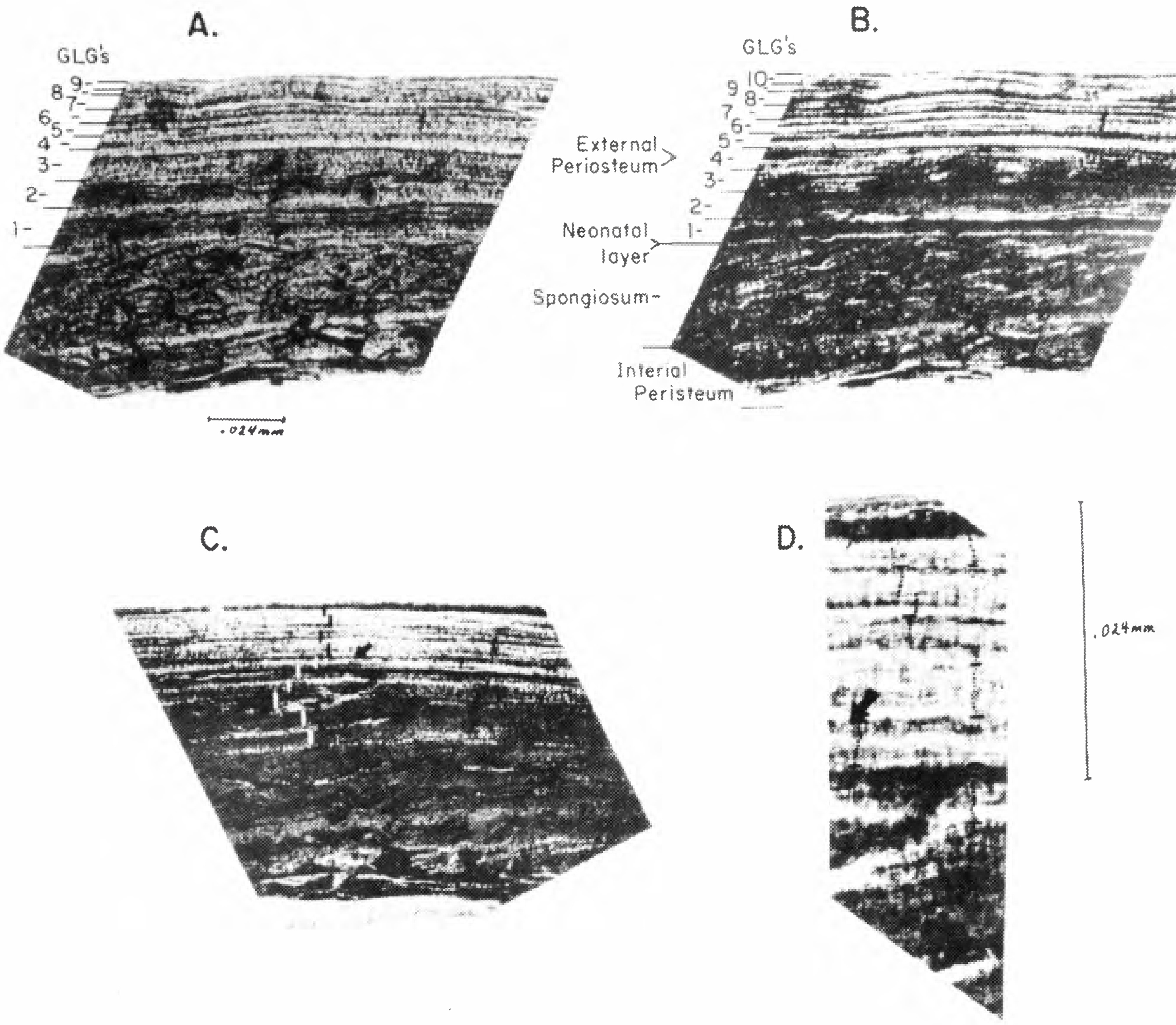

Fig. 7. Periosteal layering in mandible of *Stenella attenuata* female (TCF 060).
A. Transverse section of mandible from the "pan" region viewed in plain light (57x).
B. Section as in A showing strong contrast between GLGs produced by polarized and phase-contrasted light.
C. Another section of same bone viewed with polarized light. GLGs are marked. Arrow indicates point of reference between fifth and sixth GLG (57x).
D. Magnified (150x) area of section as in C showing 13 accessory layers per GLG.

The technique shows also that each periosteal GLG is composed of 13 accessory layers–most apparent in early layers (Fig. 7D). As in the dentinal GLGs the accessory layers within later GLGs in the bone become compressed. The presence of lunar-cycle layers in bone suggests that dentinal, cemental, and periosteal layering is influenced by a common mechanism. If this proves to be true, eventually all three layering systems may be used interchangeably to obtain maximum age estimates of very old individuals in which dentinal layering has stopped because of pulp-cavity occlusion.

SUMMARY

Polarized-light microscopy gives high resolution and color contrast to the ultrastructure in layered hard tissues of delphinids. Visual access to the ultrastructure is useful in gaining a more complete understanding of the factors influencing layering and layering rates. With the use of this method to examine ontogenetic series (in *S. attenuata*), and known-age and marked tissues of other delphinids – the following tentative conclusions were reached:

(1) Enamel layers occur that may represent daily growth records. Because enamel is developed prenatally, the layers may be used in concert with other reproductive and embryological data to estimate gestation periods and fetal age-growth stages in dolphins.

(2) The neonatal line is a complex layer, the initial component of which may mark the point of parturition. The presence of its other components may indicate that post-parturitional trauma is a prolonged event.

(3) The prenatal dentine has a layered pattern that commences consonantally with enamel layering, but its layering rate may be governed by different physiological factors.

(4) Each of the first five or six postnatal dentinal and periosteal GLGs are composed of 13 accessory layers that seem to correspond to lunar months. If this is true, the age of young animals may be estimated more accurately than in the past.

(5) Lines of von Ebner in postnatal dentine of delphinids may represent daily growth.

(6) Cemental, dentinal, and periosteal layering probably is influenced by the same physiological mechanism. This may eventually permit use of all three systems interchangeably in estimating ages of old animals.

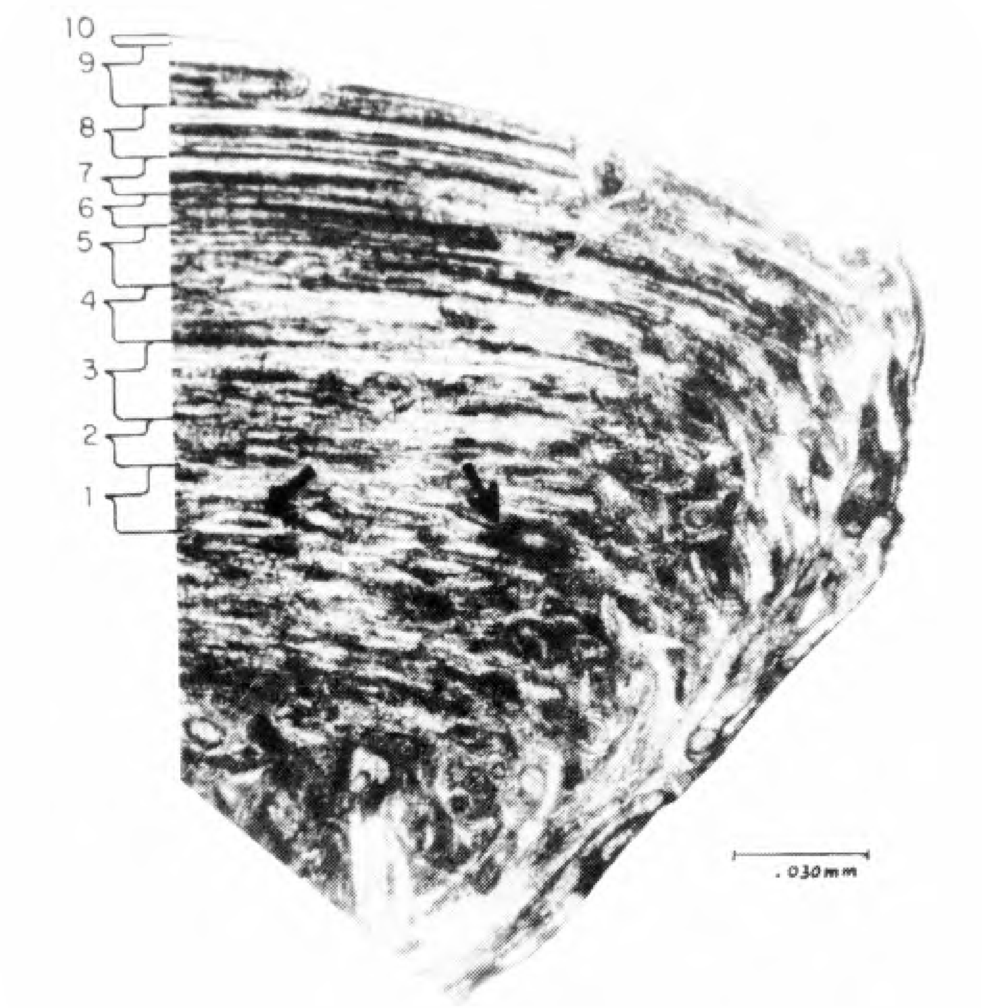

Fig. 8. Periosteal layering in premaxilla of *Stenella attenuata* female (TCF 060).

A. Transverse section of premaxillary bone taken near base of rostrum and viewed with polarized light, showing GLGs. The first GLG shows incipient resorption as indicated by presence of secondary Haversian systems (arrows) (80x).

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Age Determination of Marine Mammals Based on Aspartic Acid Racemization in the Teeth and Lens Nucleus

Jeffrey L. Bada*, S. Brown and P. M. Masters

Scripps Institution of Oceanography and Institute of Marine Resources
University of California, San Diego, La Jolla, California

ABSTRACT

Amino acid racemization dating was developed as a method for determining the ages of fossil materials. However, aspartic acid racemization has recently been found to take place with age in long-lived mammals in metabolically stable proteins, such as tooth enamel and dentine, and those present in the ocular lens nucleus. We have suggested that this racemization reaction could be used as a 'biochronological' tool for estimating ages in natural living mammal populations. This new aging method might be particularly useful for assessing the ages of marine mammals, which are difficult (or impossible) to age by other methods. We present here some preliminary results on the use of aspartic acid racemization aging technique which provides a potentially important mechanism for determining the age distributions in natural populations of these endangered animals.

INTRODUCTION

We would like to describe a new method for assessing the ages of living mammals that we have been working on in the Scripps Institution of Oceanography Amino Acid Dating Laboratory. However, before we begin the discussion of this method – amino acid racemization dating – it would be useful to give a brief historical account of the development of this technique because it is a nice illustration of interdisciplinary research, spanning the fields of organic chemistry, geochemistry, archaeology, biochemistry, and mammalian biology.

Amino acid racemization dating was originally developed as a method for assessing the ages of fossils, such as bones, shells and wood (for reviews, see Bada and Schroeder, 1975; Masters and Bada, 1978; Schroeder and Bada, 1976; Williams and Smith, 1977). This method is based on the fact that all of the amino acids (with the exception of glycine) commonly found in proteins can exist in two different isomeric forms called the D and L enantiomers. The chemical and physical properties of these enantiomers are identical, with the exception that they rotate plane polarized light in equal but opposite directions.

Around 1850, Pasteur first demonstrated that the amino acids which are present in the protein of living organisms consist of only the L-enantiomers; this is especially true in higher organisms. Since under equilibrium conditions both enantiomers would be present in equal abundances, a living organism maintains a disequilibrium state with the use of enzymes which stereoselectively utilize only the L-enantiomer. Thus, after an organism dies, or after a metabolically stable protein has been synthesized, the biochemical reactions which maintain the disequilibrium state cease, and a process called 'racemization' begins. In the racemization reaction, the L-amino acids are reversibly converted into the corresponding D-amino acids, and this reaction continues until there are equal amounts of both enantiomers present (i.e. D/L ratio = 1.0). Racemization was first found to take place in strongly acidic and basic solutions nearly 100 years ago, but it was only a few years ago that it was shown that this reaction also takes place at neutral pH at rates comparable with those in acid and base (Bada and Schroeder, 1975). In the last ten years, the possible use of the amino acid racemization reaction as a dating tool has been investigated.

The rate at which the racemization process takes place depends on the amino acid and, since racemization is a chemical reaction, the rate also depends on temperature; the higher the temperature, the faster the racemization rate. The half-lives racemization (i.e. the time required to attain a D/L ratio of 0.33) for free amino acids ranges from a few thousand years for aspartic acid at 25°C, to many millions of years for isoleucine at 0°C.

DATING FOSSIL BONES BY AMINO ACID RACEMIZATION

The racemization process has been found to take place in a variety of fossils over geological time, and this reaction can be used (with certain limitations) to estimate the age of fossil materials. The discussion here will deal primarily with bones, since the investigation of racemization in living mammals was a direct outgrowth of this work.

In order to understand the principles used in the racemization dating of fossil bones, it is necessary to discuss briefly the kinetics of the racemization reaction. (This discussion of kinetics is actually applicable to the dating of any fossil material.) The kinetic equation for the racemization reaction can be written as

$$\mathrm{Ln}\left(\frac{1+D/L}{1-D/L}\right) - \mathrm{Ln}\left(\frac{1+D/L}{1-D/L}\right)_{t=0} = 2\,k{\cdot}t \qquad (1)$$

where k is the interconversion rate of the L- and D-enantiomers, t the age of the sample, and D/L the amino acid enantiomeric ratio in the sample. When the extent of racemization is small (i.e. D/L enantiomeric ratio $\sim< 0.15$), this equation can be simplified to

$$\mathrm{Ln}\,(1+D/L) - \mathrm{Ln}\,(1+D/L)_{t=0} = k{\cdot}t \qquad (2)$$

The t=0 terms in equations (1) and (2) account for the fact that some slight racemization occurs during the processing procedure.

The above equations illustrate that there are two variables that determine the extent of racemization in a fossil: time (t) and k, which in turn is a function primarily of temperature. In order to determine one of the variables, the other must be known. For example, in order to date a bone, a value for k at the site must be available. The value of k at a particular locality is determined by a 'calibration' procedure

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*Alfred P. Sloan Fellow, 1975–1979

wherein a sample of known age from the area of interest is analyzed for its D/L enantiomeric ratio. The age and D/L ratio of the 'calibration' sample is substituted into equation (1) or (2), depending on the measured D/L ratio, and an *in situ* k value is thus determined. This k represents an average, integrated value over the age of the 'calibration' sample. Thus, any variations in the temperature or other environmental parameters of a locality are evaluated when the 'calibration' constant k is determined. Once k has been determined at a particular site, then this value can be used to date (with certain limitations) other samples from the general area.

The aspartic acid racemization reaction has been the most widely used in fossil-bone dating because it has the fastest racemization rate of the common stable amino acids. Thus, easily detectable aspartic acid racemization occurs during the time period datable by radiocarbon (i.e. the last $\sim$40,000 years). Some of the results obtained by using aspartic acid racemization and the 'calibration' procedure are given in Table 1. Using the 'calibration procedure', the racemization dating technique has been applied at over 35 sites throughout the world (see Masters and Bada, 1978, for recent summary).

Some of the more interesting samples we have analyzed are some paleoindian skeletons from sites near Scripps. The racemization ages of some of the skeletons range between 40,000 to 50,000 years, which contradicts the widely accepted theory that human beings first migrated into the New World only about 20,000 years ago or later. The racemization ages suggest that migrations into the New World occurred much earlier than originally believed. Because of these older dates for North American Indian skeletons, the racemization technique has received a barrage of criticism. However, we find it hard to accept that the North American ages are incorrect, because at every other site in the world where the racemization method has been applied, the ages have been found to be acceptable.

THE DISCOVERY OF RACEMIZATION IN LIVING HUMAN BEINGS

It was our contention that the major environmental factor that affects the racemization (i.e. k) in a fossil bone is the temperature to which it has been exposed, and that this temperature history could be adequately evaluated by using the 'calibration' procedure. However, other investigators have argued that variations in some environmental parameters, such as pH, ground water 'leaching', and the like, might affect the racemization rate in a manner that perhaps is not accounted for in the 'calibration' procedure (Hare, 1974; Von Endt, Hare, Ortner and Stix, 1975).

To demonstrate that temperature was indeed the major variable affecting racemization, we proposed that the k values determined by using well-dated Holocene (the present-day interglacial geological epoch) bones should correlate fairly well with the present-day air temperatures at the sites from which various samples were obtained (Bada and Helfman, 1975). We tested this by using several Holocene samples from sites with widely differing environmental characteristics in various areas throughout the world and found a good correlation between the k values and the site temperatures. At this point we also realized that if the k values at the various temperatures were indeed correct, then the racemization rate at 37°C (mammalian body temperature) should be sufficiently rapid that detectable racemization might also take place in living mammalian proteins which were metabolically stable (i.e. not regenerated throughout their lifetime). In order to test this, we required a protein that was laid down early in the developmental stage of a mammal's existence and remained essentially unaltered throughout the animal's life. Such a protein would thus be incubated at 37°C for many years; under these conditions, detectable racemization should take place if the rate constants we had determined at lower geological temperatures were accurate.

The most suitable proteins appeared to be those present

Table 1

Ages derived from aspartic acid racemization of various fossil bone samples

(Taken from Bada and Helfman, 1975 and Masters and Bada, 1978)

Site and location	Age (yrs)*	D/L aspartic acid	Aspartic acid age (yrs)
Muleta Cave, Mallorca, Spain	16,850 ± 200 (UCLA-1704D)	0.273†	$k_{asp} = 1.25 \times 10^{-5}\ yr^{-1}$
	18,980 ± 200 (UCLA-1704E)	0.293	18,600
	28,600 ± 600 (UCLA-1704A)	0.455	33,700
Murray Springs, Arizona	5,640 ± 160 (A-905A, B)	0.33†	$k_{asp} = 2.34 \times 10^{-5}\ yr^{-1}$
	11,230 ± 340 (A-805)	0.52	10,500
Tarkhan, Egypt			
Sample F1516	3200–2700 B.C.	0.450†	$k_{asp} = 9.31 \times 10^{-5}\ yr^{-1}$
Sample F1691	3200–2700 B.C.	0.470	3200 B.C.
Sample F1556	3200–2700 B.C.	0.453	2900 B.C.

* Notation in parentheses is radiocarbon laboratory identification number.

† Sample used to calculate k_{asp} 'calibration' constant.

in teeth, i.e. enamel and dentine, since they satisfied the criteria of being metabolically stable. Moreover, appropriate known-age teeth could be obtained from dentists. We thus contacted some local dentists, who began collecting teeth. These teeth were analyzed, and indeed we observed increasing racemization of aspartic acid in both enamel and dentine (Helfman and Bada, 1975, 1976), with increasing age of the tooth, exactly as we had expected from the geological and archaeological samples at lower temperatures. The results of the dentine analyses are shown in Figure 1.

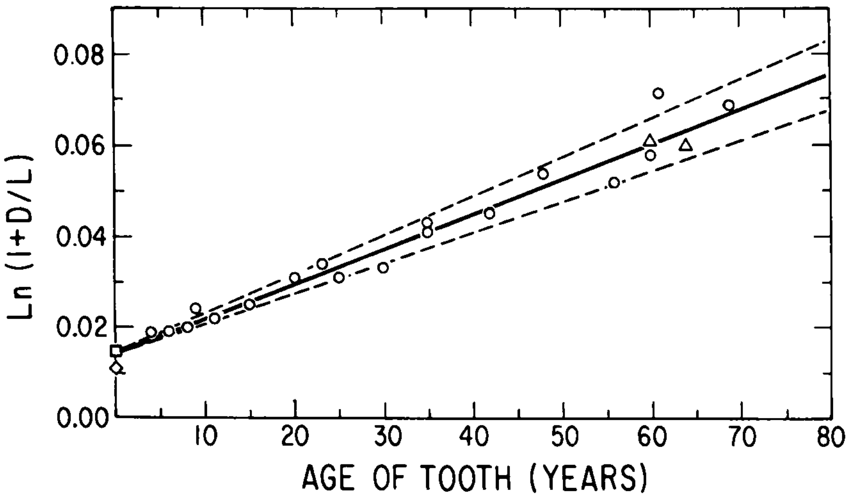

Fig. 1. A plot, in the form of equation (2), of aspartic acid racemization in human teeth of various ages. The ages of the teeth were corrected, depending on the type of tooth, from actual donor ages by the values listed in ref. 8. The slope (7.87×10^{-4} yr^{-1}) is equal to the k_{asp} value in human tooth dentine. The broken lines represent the k_{asp} values calculated for a ± 0.7°C difference in body temperature. ○ individuals of known age; △, exact ages of individuals is unknown but was estimated by dentists; ◇, albumin; □, bovine tendon collagen. (Reprinted from *Nature*.)

Besides simply confirming our prediction, the results on racemization in human teeth also launched a totally new area of research. First of all, we realized that racemization in mammalian teeth provided the basis of a 'biochronological' tool which could be used to assess the ages of living human beings as well as natural populations of living mammals (Helfman and Bada, 1975, 1976). One potentially fascinating application of this method concerned certain human populations in the world, especially in the Georgian Province of Russia; in Hunza, Pakistan; and in Vilcabamba, Ecuador, where individuals are claimed to have extraordinary longevities, in excess of 100 years. There is a major controversy as to whether these people are really as old as they claim, or whether they are misrepresenting their ages for the prestige of being the oldest living humans on earth. We proposed that racemization analyses could determine whether these people are really as old as they claim, provided we could obtain a suitable tooth from each individual. Unfortunately, this has not been easy to do and we still are attempting to obtain tooth samples.

Another interesting application of dating by this method would be the use of the extent of racemization in enamel and dentine to determine the age at death of human remains from ancient burials. The only requirement in this application is that the burial be fairly recent or that it be maintained at lower temperatures so that the extent of post-mortem racemization has been insignificant compared to that produced during the life of the individual. The racemization technique has recently been used to determine that an Eskimo woman, killed in a landslide about 1,600 years ago on St Lawrence Island, Alaska was ∿53 years old when she died; this age was in close agreement with that derived from other independent evidence (Masters and Zimmerman, 1978).

In addition to the 'biochronological' applications, the racemization in human teeth studies also has important implications concerning the aging process in mammals. If racemization occurs in all metabolically stable proteins, and if this racemization induced changes in the functionality of these proteins, then racemization might contribute to the complex aging mechanism in mammals (Helfman, Bada and Shou, 1977). However, we first had to demonstrate that racemization occurred in metabolically stable soft-tissue proteins. Recently we have shown that racemization also takes place with increasing age in the proteins present in the eye lens nucleus (Masters, Bada and Zigler, 1977, 1978). Several age-related changes take place in the proteins present in the lens nucleus, and it appears that some of these changes could be explained by racemization (Masters *et al*, 1978). Thus, in the lens nucleus, there does indeed seem to be some correlation between racemization and aging. Further research in this area should provide additional important information about what part in the aging process in mammals might be attributed to racemization.

THE USE OF ASPARTIC ACID RACEMIZATION IN ASSESSING THE AGES OF MARINE MAMMALS

As we have suggested elsewhere, the extent of aspartic acid racemization in enamel, dentine and the lens nucleus might be used to determine the ages of living mammals (Helfman and Bada, 1975, 1976; Masters *et al*, 1977). This new aging method would be particularly useful for marine mammals since the ages of these animals, especially cetaceans, are difficult or impossible to determine. During the last two years, we have carried out preliminary investigations of the extent of racemization in the teeth and lens nucleus of various marine mammals; we report here some of our findings.

Teeth investigations

One important aspect of using aspartic acid racemization in teeth to determine the ages of various mammals is to demonstrate that the racemization rate is the same in the teeth of all species. Unfortunately this is difficult to test, since it is rare to find animals with known ages of twenty to thirty years, the time required to observe detectable racemization in dentine (see Fig. 1). However, we were successful in obtaining teeth from a 27-year-old zebra from the Tulsa Zoological Park. The dentine from the tooth was isolated and analyzed as described elsewhere (Helfman and Bada, 1976) and was found to have a D/L aspartic acid ratio of 0.040. The aspartic acid racemization age equation derived from the human dentine studies is

$$t(yrs) = \frac{Ln(1+D/L) - 0.014}{7.87 \times 10^{-4}\ yr^{-1}} \tag{3}$$

and, substituting the Tulsa zebra D/L aspartic acid ratio into this equation, yields an age of ∿ 30 years. This is in reasonable agreement with the actual age of the animal. We have also analyzed teeth from other known-age mammals (a camel and a fur seal) and the racemization ages of these animals were also in fairly good agreement with each animal's actual age. Unfortunately, these other racemization analyses were of poor quality and we therefore consider them to have questionable validity. However, these results, along with the zebra results, suggest that the rate of racemization is approximately the same in the teeth of all mammals, although we must emphasize that because of the lack of teeth from a large number of known-age mammals, the conclusion is tentative.

Dolphins

We have carried out racemization analyses on a number of dolphin teeth for which dentinal growth layer counts have also been obtained. It was thought that perhaps racemization results might be useful in determining the rate of deposition of dentinal growth layers in dolphin teeth. Because of the complex growth pattern of dolphin teeth, special procedures were required in order to attempt to isolate the oldest dentinal sample. For *Tursiops truncatus,* one tooth from each animal was cut mid-sagittally with a small, high-speed hand drill. The whitish inner core, which was believed to be secondary dentine, was ground out with the drill. The crown of each tooth was removed by cutting at the point where the enamel layer terminated. The white enamel veneer was ground off, leaving only an amber dentine sample believed to be primary or neonatal in origin. The sample was then processed by using the procedures described elsewhere (Helfman and Bada, 1976).

For *Stenella attenuata,* the crown of a tooth from each animal was removed by cross-sectional cutting at the enamel line. These crown samples were thought to be comprised of ∿ 95% neonatal dentine (W.F. Perrin, pers. comm.), so no further processing was required; the crowns were processed as usual (*op. cit.*).

The results of the dolphin teeth racemization analyses are shown in Table 2. No racemization was detected in most of the teeth, which is actually the expected result, because the teeth were all from quite young animals. The only detectable racemization above the time zero (t=0) value of ∿ 0.014 was sample RSG-89. Substituting the measured D/L aspartic acid ratio for this sample into equation (3) yields an average of ∿ 17 years, which in turn implies a dentinal growth rate of approximately one layer/year. Although this growth layer rate is consistent with that obtained from tetracycline marking (Best, 1976), the racemization-derived rate must be viewed as very preliminary because it is based essentially on only one analysis. Further investigations of the extent of aspartic acid racemization in dolphin teeth might help substantiate this result.

Table 2

Extent of racemization of aspartic acid in dolphin teeth

Species	Sample No.	Dentinal growth layers†	D/L aspartic acid
S. attenuata	Newborn	0	0.014
S. attenuata	PQ-02	2.5	0.013
S. attenuata	CV-318	3	0.015
S. attenuata	CWO-63	6	0.015
S. attenuata	GMT-73	9.5	0.018
S. attenuata	RSG-89	16	0.028*
Tursiops	WFP-522	5	0.017

* Corrected because sample was hydrolyzed for seven hours, rather than six hours.

† Provided by W. F. Perrin with the exception of WFP-522 which was done by C. Hui.

Sperm whales, *Physeter macrocephalus*

We have also analyzed several sperm whale teeth in which dentinal growth layers had been counted. Again, special precautions were taken to isolate the oldest dentine sample. The overlying cementum layer was ground off with a hand drill. A section of dentine ∿ 1 cm long was then cut from as near the tip of the crown as possible. These dentine samples were processed, using standard procedures. Figure 2 shows the D/L aspartic acid ratios plotted in the form of equation (1), where the number of growth layers is used instead of time (t). There appears to be an excellent correlation between the extent of aspartic acid racemization in the sperm whale teeth and the number of dentinal growth layers, with the exception of the results from the maxillary teeth. The reason for the anomalous results for maxillary teeth is unknown, although it could be due to the fact that these teeth apparently have a more complex developmental pattern than the mandibular teeth. A least squares fit of the results shown in Fig. 2 yields a value of ∿ 1×10^{-2}/ growth layer for the slope of the line. The slope, in turn, is equal to

$$\text{Slope} = \frac{2 \cdot k_{asp}\ (yr^{-1})\ \text{in sperm whale teeth}}{\text{dentinal growth layer rate (layers/year)}}$$

where k_{asp} is the rate of conversion of L-aspartic acid into D-aspartic acid. Using the k_{asp} value in human dentine (i.e. $7.87 \times 10^{-4} yr^{-1}$) yields a dentinal growth-layer rate in sperm whale teeth of ∿ 0.2 layers/year. This growth-layer rate is considerably slower than the more generally accepted rate of ∿ 1 layer/year (Gambell, 1972). The reason for the extraordinarily slow rate derived from aspartic acid racemization measurements is unclear at the present time. One possibility is that the rate of racemization in sperm whale teeth is actually considerably faster than in human teeth. However, the results from the dolphin teeth analyses suggest that the racemization rate in cetacean teeth is similar to that in humans. If the k_{asp} value in sperm whale teeth is similar to that in dolphins and humans, then the high D/L aspartic acid ratios indicate that sperm whales could be considerably older than heretofore estimated. This conclusion, however, is not consistent with a large amount of other age information obtained by cetacean biologists.

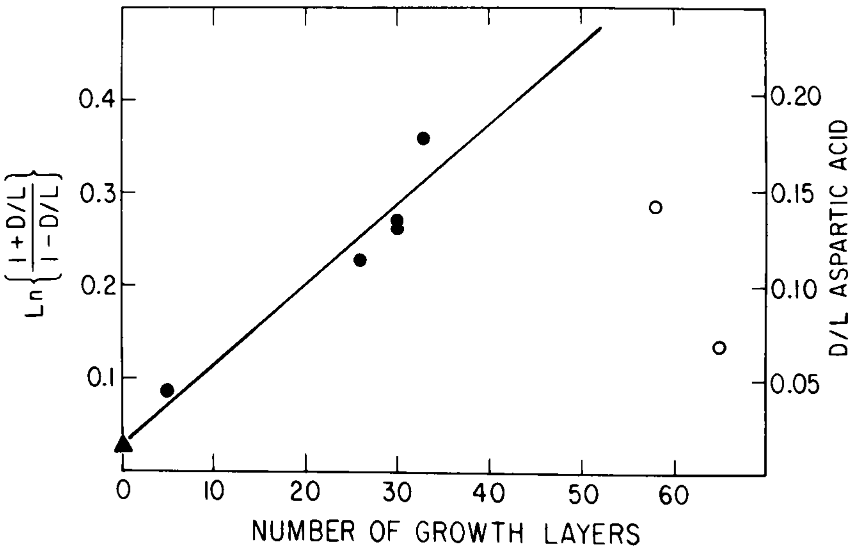

Fig. 2. A plot, in the form of equation (1), of aspartic acid racemization in dentine from sperm whale teeth for which dentinal growth layer counts had also been determined. The two samples with 30 growth layers are for two different teeth from the same animal and are thus indicative of the reproducibility of the D/L measurement. ●, racemization in mandibular teeth; ○, racemization in maxillary teeth; and ▲, racemization in the tooth of a newborn dolphin (see Table III).

At first it was thought that the high D/L aspartic acid ratios in the sperm whale teeth were due to the boiling of the teeth in various solutions after they were collected. However, the correlation shown in Fig. 2 apparently discounts this possibility, since it seems likely that the boiling would randomly induce racemization in the teeth and thus there should be no correlation between the extent of racemization and the number of growth layers. Determining the reason for the high extent of racemization in some sperm whale teeth will be an important area for further research.

Narwhals, *Monodon monoceros*

Recently we carried out an interesting series of analyses of both the erupted and unerupted tusks from a male narwhal. Approximately 40 growth layers were counted (by B. Kemper) in the exposed tusk. Assuming a growth-layer rate of ∿ 1 year, this would indicate that the animal was ∿ 40 years old. Several samples from individual growth layers in the exposed tusk were analyzed and were found to exhibit no detectable racemization with increasing numbers of growth layers; the average D/L aspartic acid ratio was ∿ 0.014, which is identical to the t = 0 term for human dentine. This is, in fact, the expected result, since the erupted tusk was exposed at ∿ 0°C or so (i.e. arctic sea water temperatures), and at these low temperatures no racemization would take place over a 40-year time span. However, in the unerupted tusk, which has been maintained at about 37°C, we felt there should be detectable racemization. The tip of the unerupted tusk was analyzed and found to have a D/L aspartic acid ratio of 0.044. Substituting this ratio into equation (3) yields an estimated age for the tip of the unerupted tusk of ∿40 years, which is in agreement with that deduced from the number of growth layers in the erupted tusk.

It is obvious from these preliminary results that racemization in the teeth of marine mammals may be a useful indicator of the animal's age. This technique is more useful for some species than for others. For example, dolphin teeth are very small, and it is difficult to isolate a suitable dentine sample for analysis, while sperm whale teeth and narwhal tusks, on the other hand, can be easily sampled.

Lens nucleus studies

The extent of aspartic acid racemization in the nucleus of the ocular lens provides another potential indicator of a mammal's age (Masters *et al,* 1977). However, as was the case in the teeth investigations, we first had to ascertain whether the rate of racemization in the lens nucleus was the same among various mammals. To test this, we required lenses from known-age mammals, which were not easily obtainable. However, we managed to collect some lenses from known-age animals (zebras, seals, and a gorilla) from zoos and marine aquaria, and the extent of racemization in the lens nucleus was investigated, using the procedures described elsewhere (*loc. cit.*). The results are shown in Fig. 3. A straight line with a slope (i.e. k_{asp} value) equal to 1.25×10^{-3} yr^{-1}, the k_{asp} value in the human lens nucleus, was drawn through the results for each species. As can be seen, this slope seems to fit the data reasonably well.

These results suggest that the k_{asp} values in the lens nucleus are similar in all mammals. Again we emphasize that this conclusion is tentative because it is based on only a limited number of known-age specimens. One interesting result that is apparent in Fig. 3 is that the t=0 term (i.e. the D/L aspartic acid ratio at time = 0) appears to be different for various species. For example, in the seal and zebra lens nucleus, the t=0 ratios are ∿0.03 and ∿0.02, respectively, while in the human lens nucleus the value is ∿0.055. The reason for this difference may be due to slight differences in the protein composition of the lens nucleus in the various mammals (Zigler and Sidbury, 1976). It is well known that during the acid hydrolysis step necessary to break down the proteins into their constituent amino acids, different proteins show varying degrees of acid catalyzed racemization (Manning, 1970). The different t=0 terms for racemization in the lens nucleus could be simply an artifact of the processing procedure. However, this means that in order to use the extent of racemization in the lens nucleus as an indicator of age, a reliable t=0 term must be available. This is easily obtained by analyzing a lens from a very young animal.

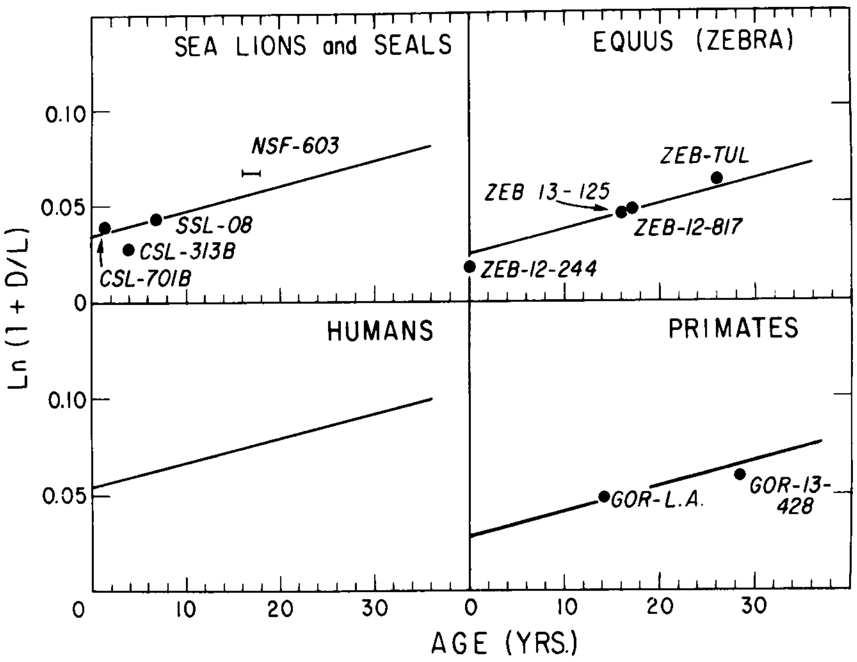

Fig. 3. A plot, in the form of equation (2), of aspartic acid racemization in the lens nucleus of several mammal species of known age. The slopes of the lines are all equal to the k_{asp} value (i.e., 1.25×10^{-3} yr^{-1}) determined in the human lens nucleus. The abbreviations used are as follows: NSF, northern fur seal; SSL, Steller sea lion; CSL, California sea lion; ZEB, zebra; and GOR, gorilla. The numbers are the Amino Acid Dating Laboratory identification numbers.

Stenella attenuata

To determine whether the extent of racemization in the lens nucleus might be used to assess the ages of marine mammals, we carried out some analyses on *S. attenuata*. The lenses from the dolphins were collected immediately after the death of the animals during tuna purse-seining operations and stored frozen until the time of analysis. The lens nucleus was isolated, processed, and analyzed, using the procedures described elsewhere. In Fig. 4, the extent of racemization in the lens nucleus of these animals is plotted in the form of equation (2) where the number of dentinal growth layers counted in the teeth of the subject animals are used instead of time. If we exclude sample JMC-636 (since there is some question about the validity of the number of growth layers which were counted), there is a good correlation between the extent of aspartic acid racemization in the lens nucleus and the number of growth layers. A least squares fit of data yields slope ≅ 2.5 (±1) x 10^{-3} growth layer. Since

$$\text{Slope} = \frac{k_{asp}\ (yr^{-1})\ \text{in } \textit{S. attenuata} \text{ lens nucleus}}{\text{dentinal growth layer rate (layers/year)}}$$

and if we use the k_{asp} value in the human lens nucleus, we obtain a dentinal growth layer rate for *S. attenuata* of ∿0.5 (± ∿ 0.3) layers/year. Considering the quality of the data, this rate is in reasonable agreement with a rate of ∿ 1 layer/year, deduced from other evidence (W. F. Perrin, pers. comm.). This result suggests that the extent of racemization in the lens nucleus in *S. attenuata*, and in other dolphin species as well, provides a rough cross-check on the estimated dentinal growth layer rate. Moreover, it provides a completely independent mechanism for assessing the age of these animals, especially those which are too old to be estimated from the number of dentinal growth layers (i.e. animals in which the substantial tooth wear has made a growth layer count impossible). For example, the extent of aspartic acid racemization in the lens nucleus of JMC-636 suggests that this animal is on the order of 20+ years old; an accurate age estimate from growth layer count was not possible for this animal because of the badly worn teeth.

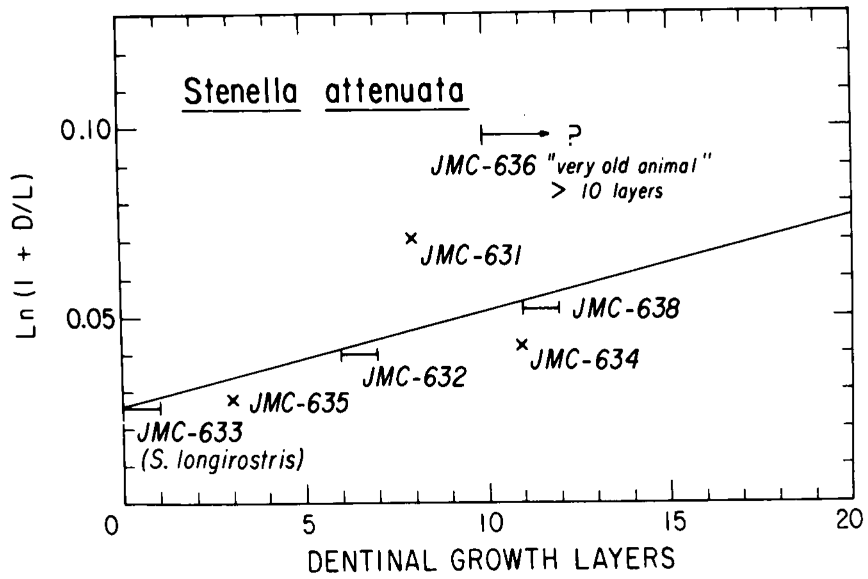

Fig. 4. A plot, in the form of equation (2), of aspartic acid racemization in the lens nucleus of *S. attenuata* where the number of dentinal growth layers (counted by W. F. Perrin) in a tooth from the subject animal is used instead of time. The identification numbers are those of W. F. Perrin.

Tursiops truncatus

We have also carried out aspartic acid racemization analyses of *Tursiops* lens nuclei but unfortunately these results were not as good as those obtained for *S. attenuata.* Two lens nuclei from animals that have been in captivity for more than ten years showed much less racemization than would be expected. The reason for the anomalously low racemization is unclear. Perhaps these lens samples were contaminated in some manner; or perhaps it might be due to some effect related to their captivity, such as their being given large doses of antibiotics.

These results present some preliminary evidence which suggests that the extent of racemization in the lens nucleus provides another potential method for assessing the ages of marine mammals. We hope that further investigations in this area will provide an indication of the reliability and limitations of this new aging method.

SUMMARY AND CONCLUSIONS

Although the amino acid racemization method was developed originally to determine the ages of fossil materials, it is now obvious that this method also has tremendous potential applicability in 'biochronology'. The extent of racemization in dentine and in the ocular lens nucleus can be used, with certain limitations, to assess the age distribution of natural living mammalian populations. This aging method should provide an excellent new tool for wildlife biologists who have been hampered in the past by their inability to determine accurately the ages of wild animals. In some cases it appears that the extent of racemization in the teeth would be a suitable indicator of age, while in other instances only the lens nucleus might be useful. This of course provides some definite limitations on the applicability of the technique, because although teeth could be extracted from anesthetized animals, the eye lens could only be obtained from dead animals. Moreover, our findings at this point have all been carried out on eyes that have been frozen immediately upon the death of the animal. The effects that might arise from collecting an eye lens several hours or days after the animal's death are uncertain at the present time, as are the effects of preserving the eye lens in a solution such as Formalin.

One interesting potential application of the racemization method is that all of the various procedures used in the aspartic acid racemization measurements could be easily modified so that they could be carried out under field conditions. Thus, another advantage of the racemization aging technique is that age estimates could be obtained in the field.

ACKNOWLEDGEMENTS

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Age Determination of the Narwhal, *Monodon monoceros* L.

Keith A. Hay*

Marine Sciences Centre, McGill University, Montreal, Province of Quebec

ABSTRACT

The growth layers which are visible on the polished cut surface of the longitudinally-bisected embedded tooth and in thin transverse sections of the periosteal zone of the anterolabial portion of the mandible of the narwhal are described and found to provide a valid index of age for this species. However, the embedded tooth occludes due to closure of the root by enveloping cementum after the deposition of 10 to 20 dentinal growth layers; mandibular periosteal growth layers must be used to estimate the ages of narwhals with occluded teeth. Up to 50 mandibular periosteal layers are deposited in male narwhals, which have a positive net accumulation of these layers throughout life, in spite of the resorption of several first-formed layers. Female narwhals deposit a maximum of 30 mandibular periosteal layers, suggesting that in older females approaching physical maturity there is no net accumulation of these layers. Dentinal and mandibular layers are equal in number (at least in males) until the time of occlusion of the embedded tooth. The time basis of the layers is not known with certainty. However, on the basis of a statistical analysis of the body length-frequency distribution, it is estimated that about three layers are deposited annually during the first two or three years of life. Comparison of the annual ovulation rate with the accumulation rate of corpora albicantia suggests that one growth layer is deposited annually in mature females.

INTRODUCTION

Development of a reliable age determination technique is essential to the formulation of a rational management strategy for any exploited population. The narwhal, *Monodon monoceros* Linnaeus 1758, is heavily hunted by the Inuit of northern Baffin Island in the Northwest Territories of Canada. As part of a study of the biology and life history of this species since 1963, I have investigated various methods of age determination of the narwhal, using the layered structure of both teeth and periosteal bone.

The use of growth layers in the teeth of pinnipeds for age determination was discovered by Laws (1952) and Scheffer (1950), and has been extended to odontocetes (e.g. Sergeant, 1959). Since that time much additional work on age determination has been accomplished for many odontocete species. Jonsgård (1969) has published an extensive review of age determination methods for marine mammals, and Klevezal′ and Kleinenberg (1967) reviewed age determination methods for all orders of mammals.

MATERIALS AND METHODS

The embedded (unerupted) tooth and distal portion of the mandible were removed from 62 narwhals netted during the summers of 1963–65 in Koluktoo Bay, near the head of Milne Inlet, Baffin Island and from 131 narwhals killed by the Inuit from 1974 to 1976 near the communities of Arctic Bay and Pond Inlet (Fig. 1). The netting technique and other background data on the earlier studies are given by Mansfield, Smith and Beck (1975). Further details of the hunting techniques and catch composition are presented by Hay (1979).

Typically, male narwhals have an elongated, sinistrally-spiralled tusk which emerges from the left maxilla and a smaller (up to about 29 cm in length) elongated tooth which is embedded in the right maxilla, while females have two of these embedded teeth, one in each maxilla, which attain a maximum length of about 23 cm. The embedded tooth is oriented horizontally within the maxilla, and, in adult whales, its anterior tip is located about 15 cm posterior to the anterior tip of the rostrum. The right embedded teeth of both male and female narwhals were used in this study.

The embedded teeth were bisected longitudinally using a jeweller's saw of carbon steel. The tooth was oriented so as to ensure a longitudinal, mid-sagittal cut. The cut surfaces were sanded with medium and 120 grade sandpaper to remove the saw marks; they were then polished with 220 and 320 grade waterproof sandpaper and placed in a mixture of equal parts of water, absolute alcohol, and glycerin for preservation. Examination of teeth and counts of growth layers were carried out using a magnifying lamp or binocular microscope. The nature of the most recent dentinal zone in the teeth of young animals (adjacent to the pulp cavity) was recorded.

The possibility of using periosteal layering in the mandible for age determination was investigated. Periosteal bone is thickest and the layers are therefore widest in the anterior part of the mandible, between the mental foramina. Transverse sections about 1 cm thick were taken from this region of the mandible, and were embedded in acrylic resin *(Ward's bioplastic;* Fig. 5a). Thin sections of about 100 μ were cut from these mandibular sections using a *Gillings-Hamco* thin-sectioning machine. These sections were stored in 70% ethyl alcohol and were examined using a *Leitz Wetzlar* trichinoscope to study and count the periosteal growth layers. A few thin sections of teeth were obtained and examined in the same way.

In addition, a few thin sections of an embedded tooth and some mandibles and the cut surface of a bisected embedded tooth were stained with silver nitrate by the modified von Kossa method (Drury and Wallington, 1967) to demonstrate the distribution of calcium salts and to investigate the physiological basis of hard tissue layering (i.e. differential mineralization). This method stains calcified tissues black.

RESULTS

The embedded tooth

Description of growth and growth layers – The embedded (unerupted) tooth of the newborn narwhal is about 9–10 cm in length and consists of an elongated cone of dentine, 2–3 mm thick at the apex in median longitudinal section. In this tooth (Fig. 3a) a small amount of postnatal dentine may be seen beyond the thin, dark, often double, neonatal line, which is easily distinguishable in all older teeth as well

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**Present address:* Northwest Atlantic Fisheries Center, Fisheries and Oceans Canada, St. John's, Newfoundland.

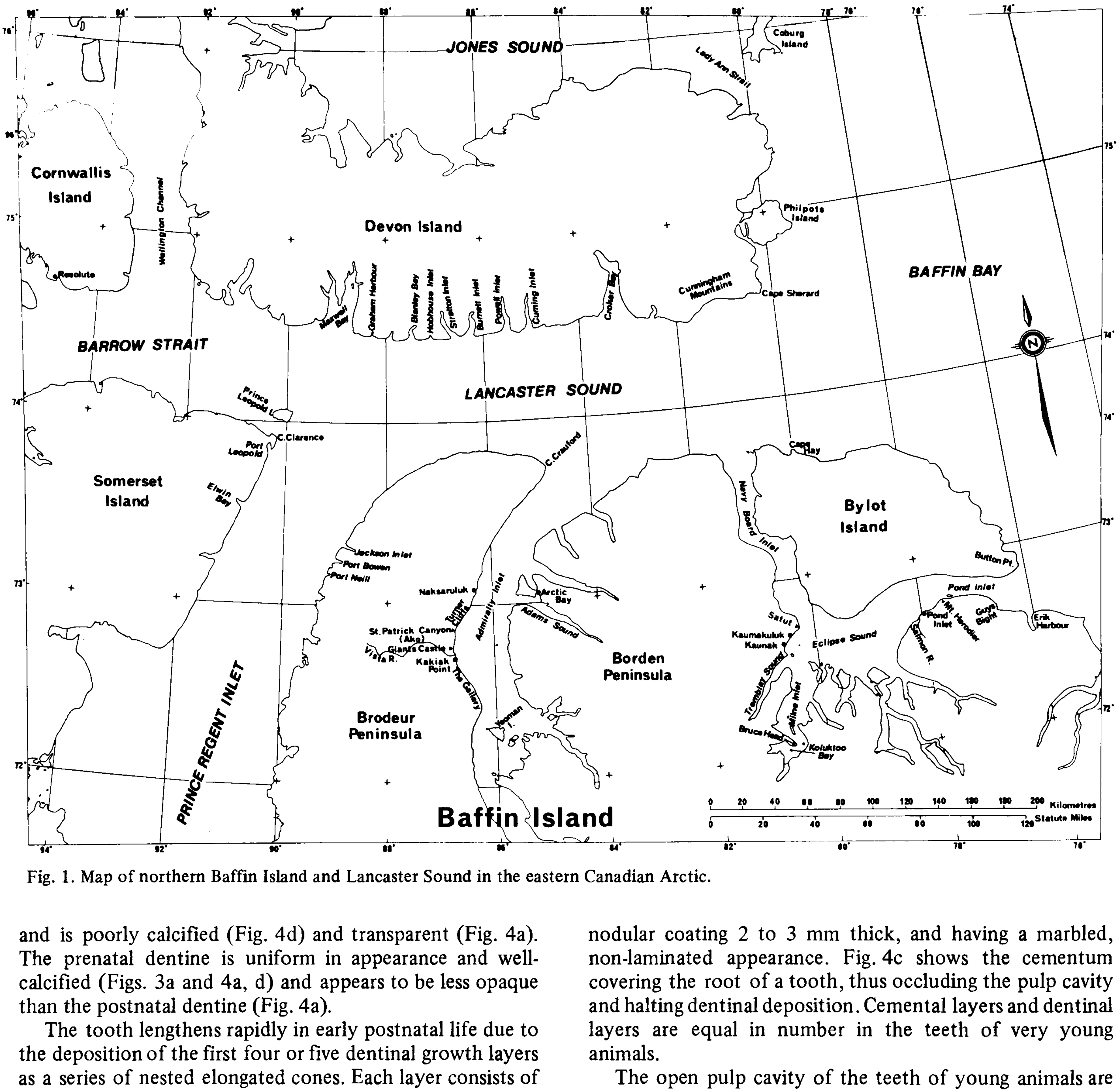

Fig. 1. Map of northern Baffin Island and Lancaster Sound in the eastern Canadian Arctic.

and is poorly calcified (Fig. 4d) and transparent (Fig. 4a). The prenatal dentine is uniform in appearance and well-calcified (Figs. 3a and 4a, d) and appears to be less opaque than the postnatal dentine (Fig. 4a).

The tooth lengthens rapidly in early postnatal life due to the deposition of the first four or five dentinal growth layers as a series of nested elongated cones. Each layer consists of a broad light band and a narrow dark band (Figs 3b–h). On closer inspection, each narrow dark band is composed of a wide bright or foamy white band bounded by a pair of narrow dark lines, as viewed in reflected light; however, examination of thin sections reveals that the foamy white band is extremely opaque optically and that the bounding narrow dark lines are translucent (Fig. 4b). Silver-nitrate staining of bisected teeth (Figs 4d–f) reveals that the narrow dark bands are poorly calcified compared to the wide bands. In young animals sampled during summer, a narrow dark band occurs at the edge of the pulp cavity (Fig. 3b).

Cementum also accumulates around the developing dentine of the embedded tooth, but it is not useful for age determination because of its extreme thinness (0.5–1.0 mm) and also because cemental layers are not uniformly represented along the length of the tooth, due to rapid elongation of the tooth during the first few years of life. In addition, cemental layering is difficult to resolve on the median longitudinal sections of embedded teeth. Moreover, the cementum in the root portion of the tooth is irregular, forming a nodular coating 2 to 3 mm thick, and having a marbled, non-laminated appearance. Fig. 4c shows the cementum covering the root of a tooth, thus occluding the pulp cavity and halting dentinal deposition. Cemental layers and dentinal layers are equal in number in the teeth of very young animals.

The open pulp cavity of the teeth of young animals are filled *in vivo* with pulp tissue. In older teeth, if deposition of dentine is still occurring, a small cone of pulp tissue remains at the proximal end of the tooth, while cementum slowly encroaches upon the dentine at the root.

Some dentinal layers contain faint zones which are considered to be accessory and are thus ignored in counts of dentinal layers. These accessory bands are variable between animals and layers, with regard to their presence and definition. They usually present no problems in counts of dentinal layers. However, the group which examined material from the narwhal at the Workshop on Age Determination of Odontocetes considered this question in detail (see Report of Session 3 of the Workshop).

The earliest dentinal layers are deposited at an acute angle to the axis of the rapidly elongating tooth; later layers are parallel and more compact. These later-formed layers form a knot of dentine at the root of the tooth; the proximal end of this knot usually curves medial from the long axis of the tooth, occasionally up to an angle of 90 degrees (Fig. 3). Small nodules of osteodentine occasionally form within the

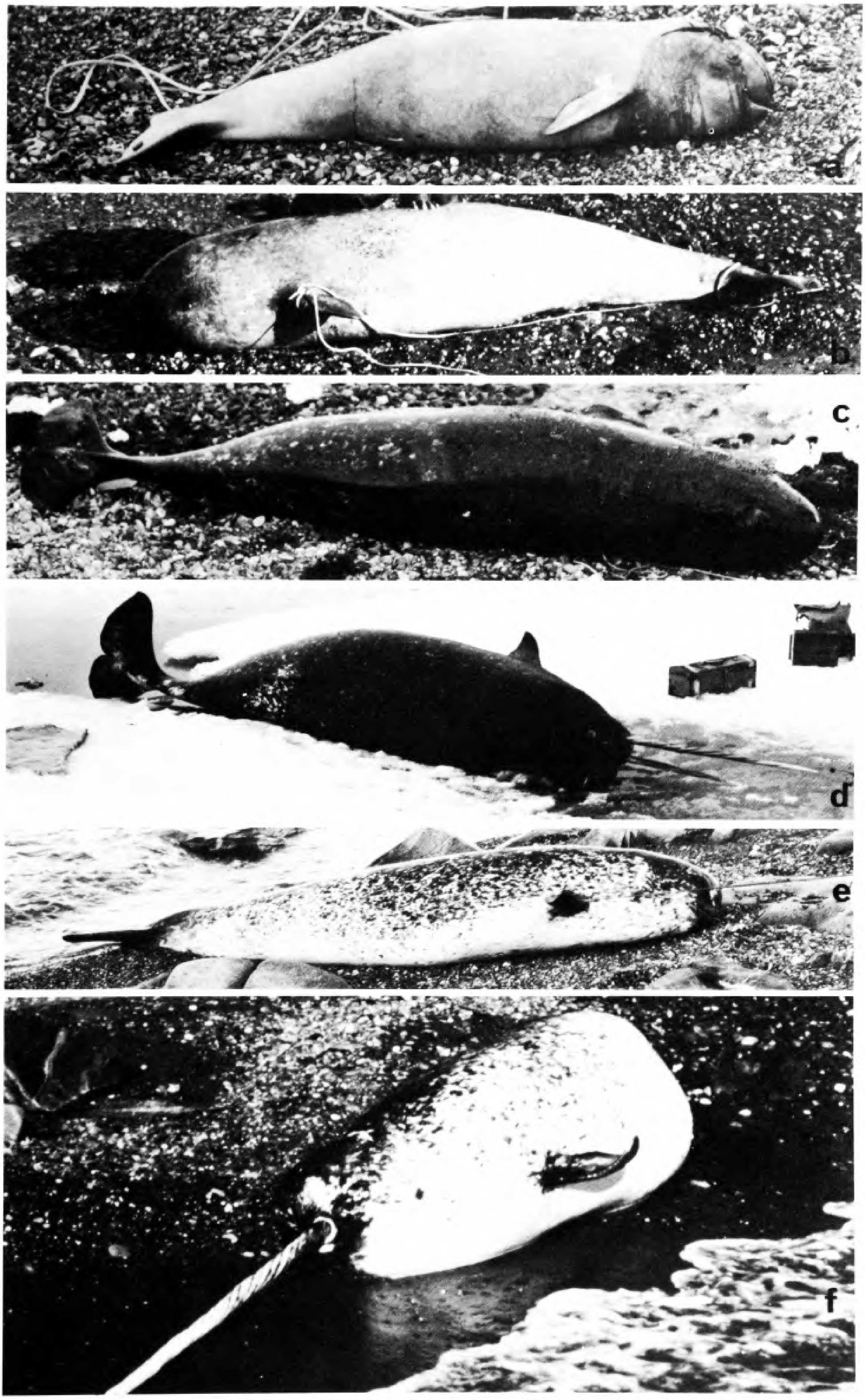

Fig. 2. External appearance of the narwhal.

(a) **neonatal male, 160** cm, **captured** at Kaunak on 29 **August,** 1975.

(b) yearling female, 241 cm, two dentinal layers, captured at Kaunak on 4 August, 1975.

(c) immature male, 336 cm, 12 dentinal layers, tusk not erupted, captured at Kaunak on 4 August, 1975.

(d) maturing male, 372 cm, 13 mandibular periosteal layers, two tusks erupted, captured at Pond Inlet floe edge on 19 July, 1976 (photo by H. Silverman).

(e) adult female, 376 cm, 14 dentinal layers, captured at Kaunak on 20 August, 1975.

(f) adult male, 452 cm, 20 dentinal layers (occluded), captured at Kaunak on 28 August, 1974.

Fig. 3. Polished surface of longitudinally-bisected embedded teeth of the narwhal.
(a) tooth of neonatal male, 160 cm, no postnatal dentinal layers.
(b) left tooth of female calf, 230 cm, four growth layers. A narrow dark band can be seen adjacent to the pulp cavity.
(c) proximal portion of right tooth of a young male, 324 cm, 12 growth layers.
(d) root of right tooth of a maturing male, 393 cm, with 17 growth layers.
(e) distal portion of right tooth of a maturing male, 443 cm, with 16 growth layers. Dentinal deposition had ceased, and 30 growth layers were present in the mandible.
(f) root portion of right tooth of same animal as in (e) above.
(g) root of right tooth of a mature female, 385 cm, 14 growth layers (occluded). Nineteen mandibular periosteal layers.
(h) distal portions of the teeth of an immature male, 262 cm, two growth layers.

orthodentine of the proximal portion of the knot, but do not interfere with counts of growth layers.

Occlusion and knot development -- In the teeth of older animals, after the pulp cavity has disappeared, dentinal layers continue to be deposited, but they comprise an enlarging knot at the root. Deposition continues until the encroaching cementum covers the entire root including its proximal end. In teeth with completely occluded pulp cavities, dentinal layer counts provide a minimum estimate of age. The mechanics of dentinal deposition in the knot are unknown, and the reasons for the persistence of pulp tissue at the proximal end of the tooth, long after the pulp cavity has disappeared, are also unknown.

Table 1 summarizes the changes in dentinal occlusion, knot development and closure of the pulp cavity with increasing body length and age. The onset of the formation of a knot (dentine and irregular nodular cementum at the

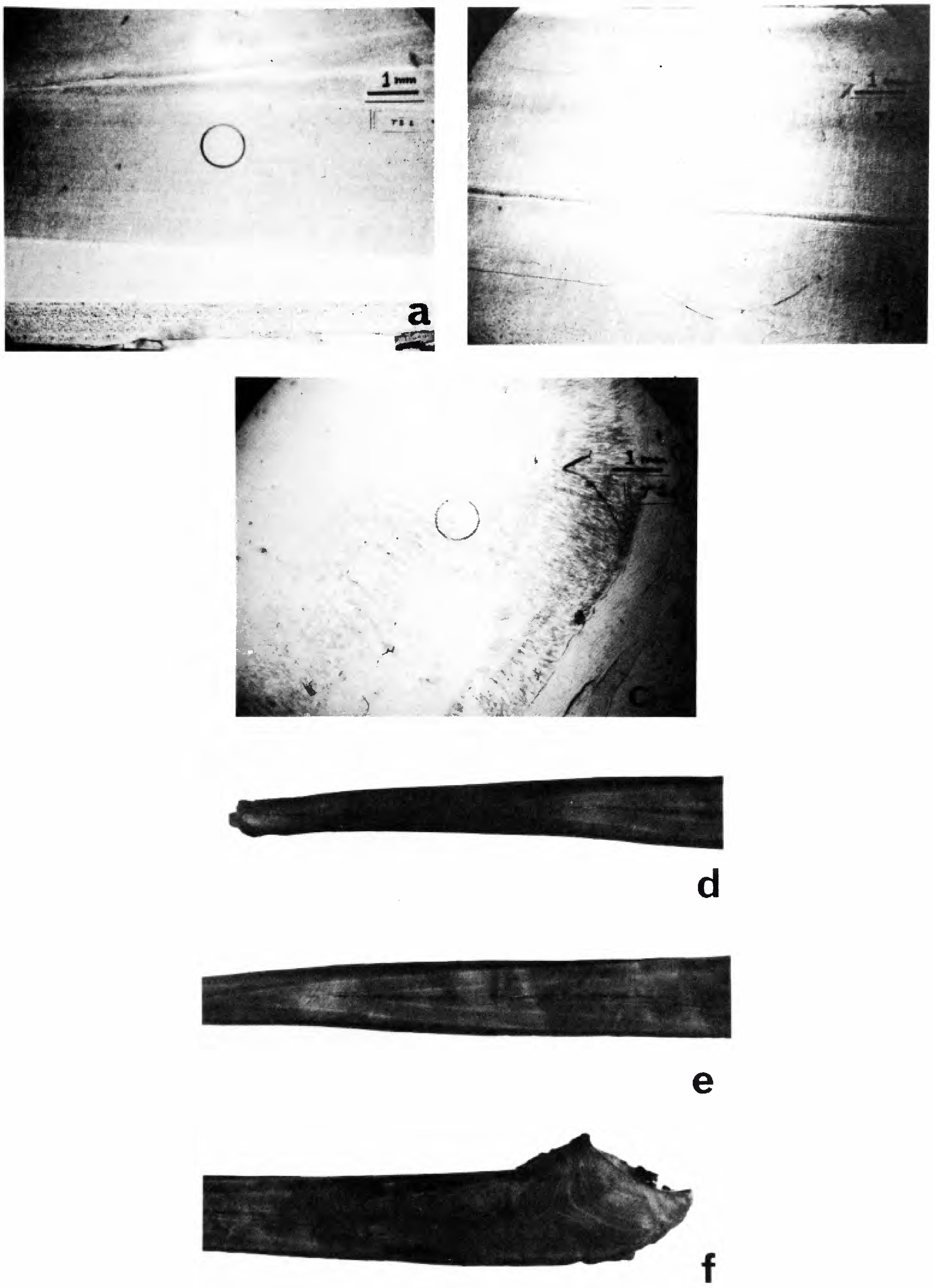

Fig. 4. Thin longitudinal, mid-sagittal sections of embedded teeth and silver nitrate-stained bisected embedded teeth.

(a) thin section of anterior portion of tooth. Note translucent neonatal line and optical difference between pre- and postnatal dentine.

(b) thin section of middle portion of tooth. Note optically dense narrow dark band bounded by thin translucent zones.

(c) thin section of root. Note cementum occluding root at the right.

(d) distal portion of silver nitrate stained tooth. The neonatal line is unstained. (MM-5-74, male, 324 cm, 12 dentinal layers.)

(e) middle portion of silver nitrate stained tooth. The narrow dark bands are weakly stained. (MM-5-74, male, 324 cm, 12 dentinal layers.)

(f) proximal portion of silver nitrate stained tooth. (MM-5-74, male, 324 cm, 12 dentinal layers).

Table 1. Dentinal development in the embedded tooth of the narwhal

	Males		Females	
	Body length (cm)	No. dentinal layers	Body length (cm)	No. dentinal layers
Sample size	75	77	94	97
Knot development				
Mean	290	7	300	9
Range	290–290	7–7	290–310	7–11
Pulp cavity closure[a]				
Mean	330	9	320	9
Range	310–390	7–15	310–330	9–9
Dentinal occlusion[b]				
Mean	400	16.0	370	13.8
Range	390–430	13–19	350–410	11–24
Age at sexual maturity[c]				
Mean	395	17	340	11.8

[a] No visibly open pulp cavity, but deposition occurring.

[b] Cessation of dentinal dêposition (cementum covers root).

[c] Number of composite growth layers from Hay (1979).

root) occurs at about six to seven layers in males and eight layers in females (Table 1). Maximum knot development in male teeth occurs at body lengths greater than 400 cm (17 to 18 layers); however, the teeth of a few large old males have poorly developed knots. In males, the pulp cavity closes at a body length of 320 cm (eight to nine layers), but a few teeth of males with up to 15 dentinal layers still have open pump cavities.

The dentinal knots of females develop in a similar way to those of males, but in general they are smaller. Pulp cavity closure occurs after nine layers at a body length of 320 cm. Table 1 demonstrates that the embedded teeth of females occlude at a younger age than those of males. However, a few larger females (380–400 cm) were still depositing dentine with 15 to 24 dentinal layers present in the embedded teeth. The rate of dentinal occlusion increases greatly after the attainment of sexual maturity in both sexes.

The average number of dentinal layers in the occluded teeth of 29 males is 16, corresponding closely to the average age at the attainment of sexual maturity by males (17 composite growth layers), while the occluded teeth of 37 females had an average of 13.8 dentinal growth layers, which is 2.0 layers greater than the average age of sexual maturity in females (Table 1).

Thus, the embedded teeth are potentially useful for age determination to a minimum age of 10 to 12 layers, although most teeth are useful until sexual maturity in both sexes. Several teeth may be useful beyond this age (up to 18 layers in males and 24 layers in females), due to variability in the age at which occlusion occurs (Table 1).

The most recent dentinal zone and season – It was possible to determine the nature of the most recent dentinal zone (narrow or wide) in the teeth of 47 young whales (24 males and 23 females). Overall, a narrow dark band was being deposited in the teeth of 81% of these whales (92% of males; 70% of females). The teeth of four males during July were depositing a narrow band 0.5–1.5 mm wide, while the teeth of four females during July were depositing a wide band of 0.05–2.00 mm thickness. In August and September, the teeth of 15 to 18 (83%) females and 18 of 20 (90%) males were depositing a narrow band, of thickness 0.3–1.5 mm. There was no detectable correlation between the thickness of the most recent dentinal zone and date of capture. Thus, the evidence from dentinal structure suggests that a narrow dark band is deposited in August and September, at which time these whales are feeding lightly (Hay, 1979) and depositing poorly mineralized but optically opaque dentine in their teeth (Fig. 3b). Unfortunately, this analysis provides no clue to the frequency of dentinal layering because of the short sampling season and high incidence of dentinal occlusion.

The mandible

Description of growth and growth layers – The layered structure of bone has received far less attention in age determination than have the dental structures. Laws (1960) pointed out the growth layers in the ventro-lateral area of the mandible of the sperm whale ***Physeter macrocephalus***, while Nishiwaki, Ohsumi and Kasuya (1961) and Brodie (1969) examined mandibular growth layers in the sperm whale and the white whale ***Delphinapterus leucas***, respectively. Kleinenberg and Klevezal' (1962) investigated mandibular periosteal layering in ***Delphinus delphis*** as a means of determining age in this species.

Layering was examined in thin sections taken from the mandible between the mental foramina, where the periosteal bone is thickest (Fig. 5a). The periosteal growth layers are most pronounced on the ventro-labial and mid-labial aspect of the mandible and are virtually absent in the dorsal and lingual regions. In all regions of the cross section, Haversian canals surrounded by concentric systems of osteocyte lacunae and resorption cavities are abundant (Fig. 5). In old animals, this secondary bone formation causes some discontinuity and irregularity of the periosteal layers, thus interfering with interpretation and counting.

The periosteum deposits intramembranous bone on the surface of the mandible, a discontinuous process as revealed by the laminated appearance of the bone (Fig. 5). The intramembranous trabeculae enlarge rapidly by further bone deposition, so that periosteal bone soon becomes compact.

The innermost, first-formed postnatal periosteal bone consists of alternating wide opaque and narrow translucent zones; these initial layers have a corrugated or uneven appearance (Fig. 5). Later zones are more compact and flattened as the appositional growth rate decreases. The narrow translucent zones are called adhesion lines by Klevezal' and Kleinenberg (1967). The corrugated appearance of the growth zones, particularly the first-formed, seems to be associated with the development of parallel arrays of the Haversian systems. The adhesion lines are relatively wide in the later-formed, more compact layers.

In many mandibular sections a presumed neonatal line is evident near the mandibular canal (Fig. 5c). This clear, narrow line separates the prenatal and postnatal bone which are optically translucent and opaque, respectively. However, this line disappears as periosteal bone formation progresses, due to resorption. In addition, there is substantial resorption of tissue within the periosteal bone. The presence of only a few growth layers and the thinness of bone on the lingual and dorsal aspects of the anterior mandible seem to be indicative of either negligible appositional growth and internal resorption or else a balance between relatively rapid rates of apposition and resorption. The retention of a few layers similar to the early labial layers in the lingual-dorsal region of the mandible favours the first alternative. Appositional growth exceeds resorption on the labial surface of the lower jaw, where old animals may lack the wide, irregular layers which are deposited in early postnatal life, and the layers adjacent to the mandibular canal are therefore compact and flattened. Further evidence for internal resorption in old animals is indicated by the fact that the layers adjacent to the enlarging mandibular canal

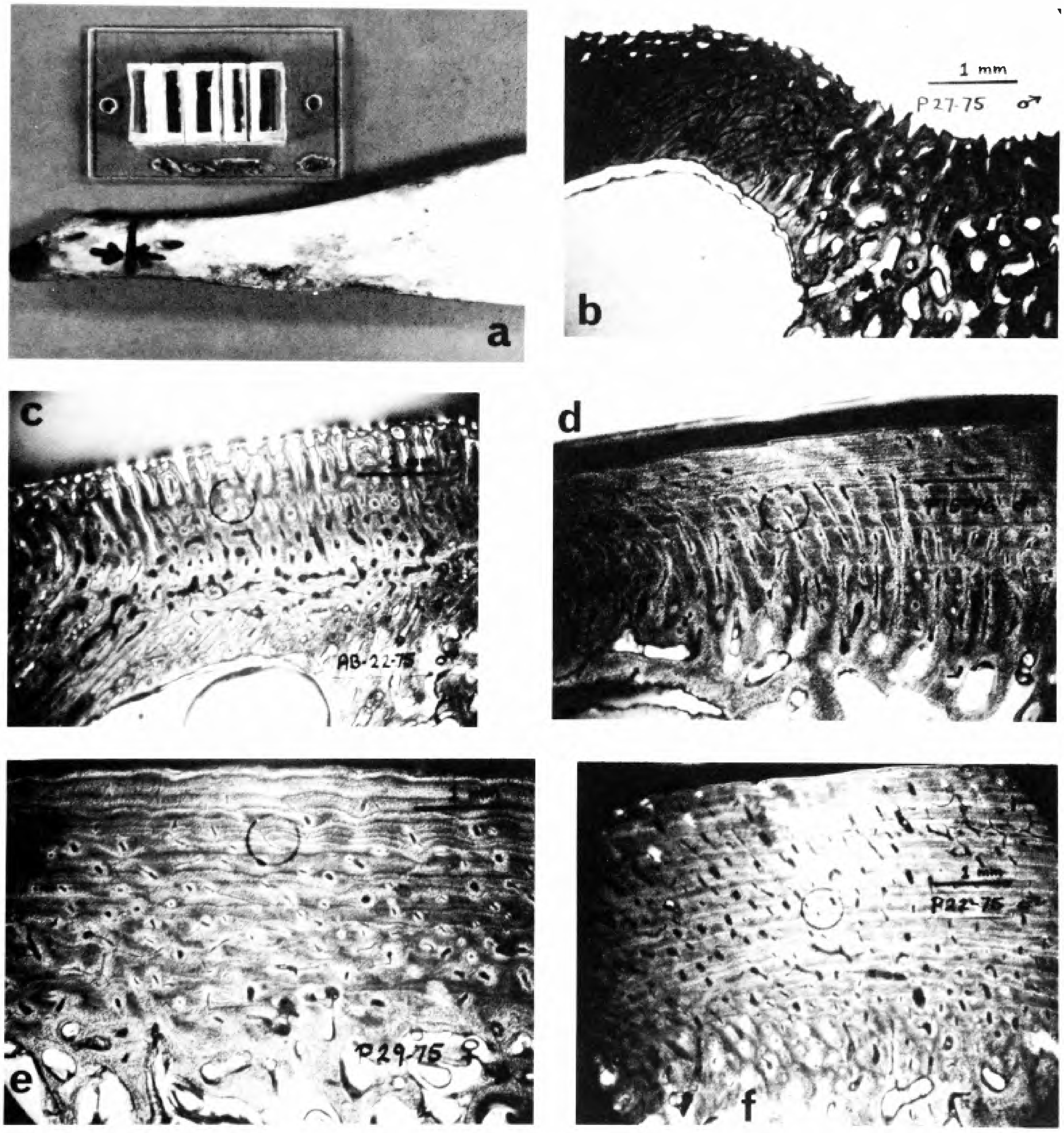

Fig. 5. Thin transverse sections of the ventro-labial portion of the anterior part of the mandible.
(a) location of section cut from the mandible (at arrows) and block of bioplastic containing four mandibular sections.
(b) neonatal male, 160 cm, no postnatal mandibular growth layers.
(c) suckling male, 230 cm, two mandibular layers. Note prominent neonatal line.
(d) maturing male, 381 cm, 14 mandibular layers.
(e) adult female, 382 cm, 12–13 mandibular layers.
(f) adult male, 440 cm, 35 mandibular layers. Note internal resorption.

are discontinuous or interrupted.

The rates of periosteal deposition and internal resorption by the marrow cavity are greater in the mid-labial area than in the ventro-labial area. In consequence, mid-labial growth layers are quite wide, but the neonatal line and several of the earlier layers may be removed by osteoclast activity. On the other hand, ventro-labial growth layers are more narrow, but the neonatal line is more often preserved in this area. However, the slow appositional growth rate results in extreme compression of the late-formed layers, which are thus difficult or impossible to count. In practice, counts of growth layers begin at the first adhesion line in the ventro-labial area and eventually shift to the mid-labial area when the zones of the former become too compressed. Unfortunately, for old narwhals in which the mandibular neonatal line cannot be detected, the counts of growth layers provide minimum estimates of age.

The most recent periosteal zone at the mandibular surface is usually an adhesion line. However, in a few young animals caught in spring, the most recent zone is opaque.

Treatment of thin sections of the mandible with silver nitrate reveals that the narrow adhesion lines are poorly calcified; they are probably formed during the mid-summer period of reduced feeding, at the same time as the narrow dark band in the embedded teeth.

Relation between dentinal and mandibular layers

Dentinal and mandibular periosteal layers are approximately equal in number until the accumulation of about 14 layers in males and 12 layers in females (Fig. 6); beyond these points, the number of mandibular layers exceeds that of dentinal layers due to dentinal occlusion. In females, the number of dentinal layers increases with the number of periosteal layers until the accumulation of 12 layers, when the incidence of dentinal occlusion increases, resulting in a much larger number of periosteal than dentinal layers in old

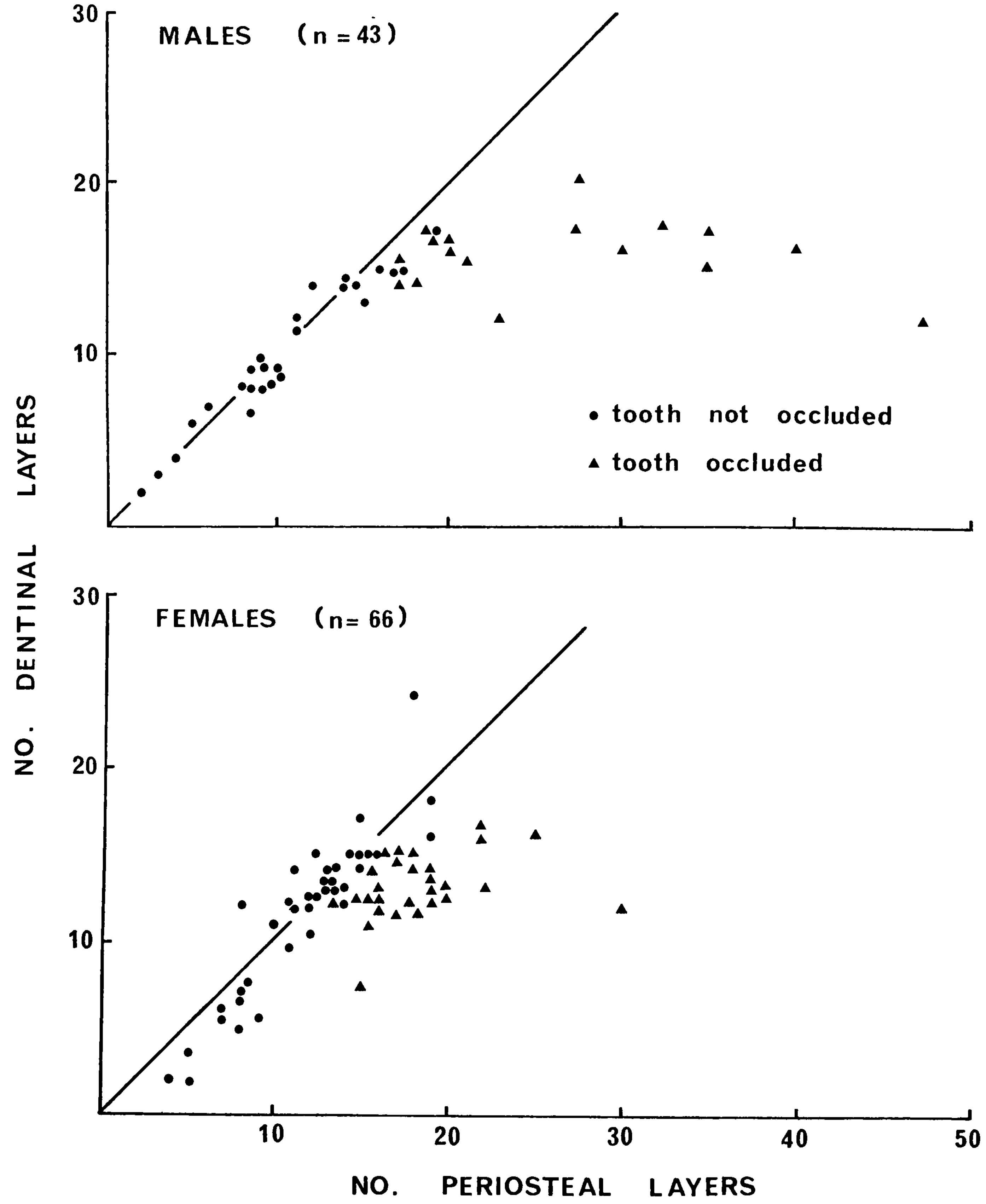

Fig. 6. Relation between number of dentinal layers and mandibular periosteal layers in the narwhal. The straight line represents a theoretical 1:1 ratio.

females. However, in young females with less than 10 layers, the number of periosteal layers is consistently greater than the number of dentinal layers (Fig. 6). The initial equality of dentinal and mandibular layers, with the exception of young females, permits the conclusion that the frequency of layering is the same for both tissues.

Frequency of dentinal and periosteal layering

Length-frequency distributions – The frequency distributions of body length of postnatal narwhals captured from 1963 to 1976 are presented in Fig. 7. Analysis of the length composition of the catch of each year showed that season (ice hunt vs open water hunt) and method of capture (netting vs hunting) had little influence on the catch composition. Netting seemed to catch relatively more calves, especially neonates, while the hunt at the floe edge did not produce any male calves; however, only 12 males were sampled at the floe edge in 1976. Thus, Fig. 7 is believed to represent the average narwhal catch composition. Females comprise 54.7% of the total catch, excluding the animals captured in 1976 at the floe edge where females were selectively sampled.

The dissection of the length-frequency distribution (Fig. 7) into the first four age-classes using the method of Cassie (1954) is presented in Table 2. Cassie's (1954) statistical technique involves plotting the cumulative frequency and body length on probability paper, and graphically extracting the mean body length and standard deviation of each year-class. It is useful only for the first few year-classes which grow rapidly and show large differences in body length. However, individual variation in growth rate and date of birth relative to sampling date causes a certain degree of overlap between the younger year-classes, except for the newborn class.

This analysis suggests that male and female growth rates are equal up to the fourth age-class (3-year-old animals), when males average 338 cm in body length and females, 332 cm. Table 2 shows that the annual increment in growth layers, derived from the length-age relations presented below, varies from 3.2 to 3.8 in the male and 2.9 to 3.8 in the female. It is suggested that three layers on average are deposited annually during the first few years of life. The higher increment of 3.7 to 3.8 layers in the third year of life suggests that 4-year-old or older narwhals are strongly overlapping with the 3-year-old class. The mean length of females of the second year-class is greater than that of males and corresponds to a greater number of growth layers; this probably results from the small sample sizes used in the analysis.

Although these results are statistically significant, their validity is suspect owing to the very small sample sizes (Fig. 7) and the fact that the values are extracted visually from graphical plots; hence, a certain, though small, amount of subjectivity is involved.

Ovulation rate – The annual ovulation rate is estimated to be about 0.7, and the accumulation rate of corpora albicantia is calculated as 0.76 per growth layer (Hay, 1979). Assuming that the accumulation rate of corpora albicantia may be a slight overestimate due to underestimation of the ages of some very old females (see below), it appears that one dentinal layer and one mandibular periosteal layer are deposited annually in mature females.

Postnatal growth

The growth curves of postnatal narwhals are presented in Figs. 8 and 9. Growth rates of both sexes are approximately equal until the accumulation of ten growth layers, after which the growth rate of females declines relative to males. There are a large number of females between 370 cm and 390 cm in body length (Fig. 9); these are probably females that are approaching physical maturity and which comprise the pronounced mode in the length-frequency histogram (Fig. 7). The number of composite growth layers refers to the number of mandibular periosteal layers if the tooth is judged to be occluded; otherwise, it is the number of dentinal growth layers.

DISCUSSION

The growth layer structure of the embedded tooth of the narwhal agrees closely with descriptions of these layers in other odontocetes and in pinnipeds. The narrow dark band in narwhal teeth, which is bright white in reflected light but optically opaque in transmitted light, fit the description of 'marbled' or 'interglobular' dentine which is poorly calcified but optically dense (Laws, 1962; Fisher, 1954). There seems to be a positive correlation between the translucency of dentine and its content of mineral salts (Bloom and Fawcett, 1975; Sognnaes, 1960; Klevezal' and Kleinenberg, 1967; Laws, 1962; Kubota, Nagasaki, Matsumoto and Tsuboi, 1961; Ohsumi, Kasuya and Nishiwaki, 1963). Grue-

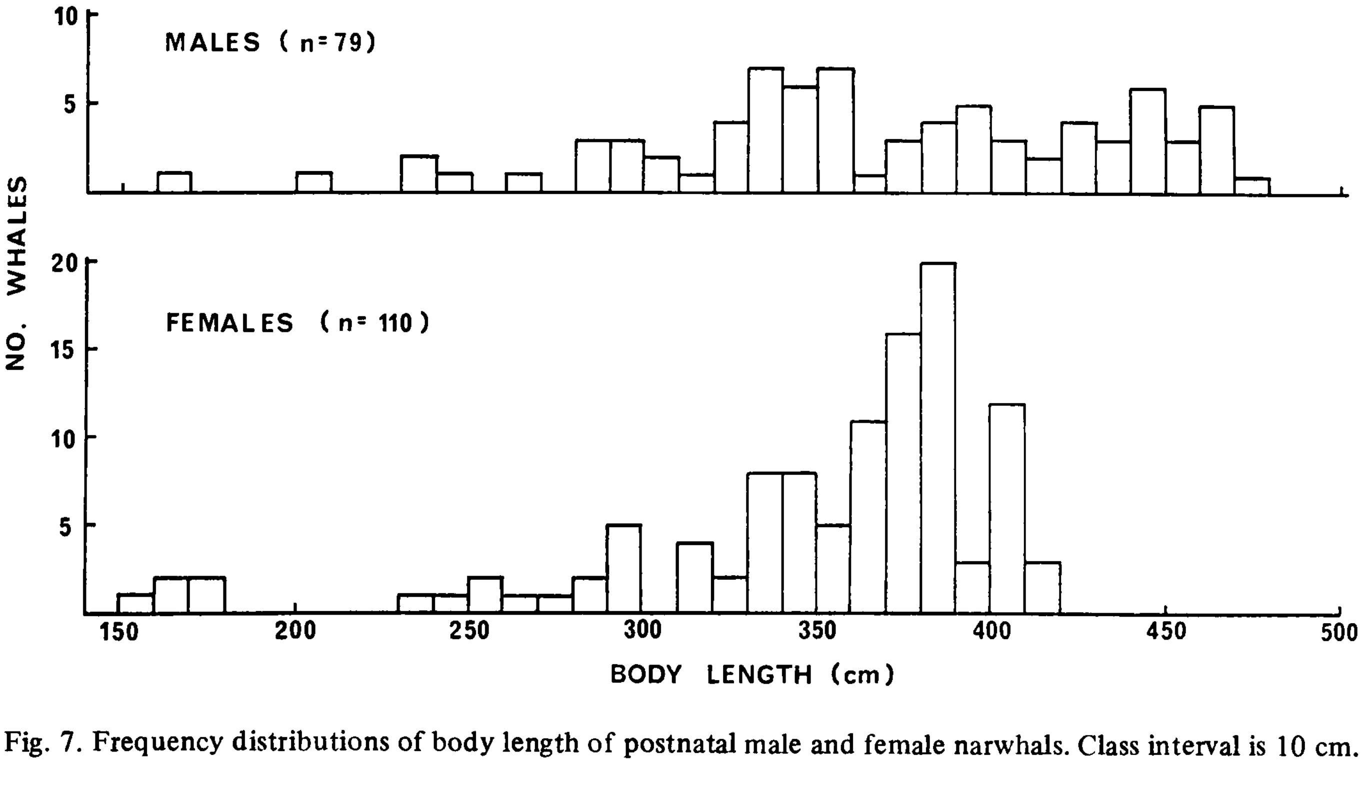

Fig. 7. Frequency distributions of body length of postnatal male and female narwhals. Class interval is 10 cm.

Table 2
Length-frequency analysis of the catch of narwhals, 1963–76, by the method of Cassie (1954), using 10-cm length class interval

	Year classes							
	Males				Females			
	0	1	2	3	0	1	2	3
Mean body length (cm)	160	235	290	338	163	247	290	332
Standard deviation (cm)		9.14	10.48	11.96	9.41	11.83	14.25	12.90
Length increment (%)		47.0	23.4	16.6		51.5	17.4	14.5
No. dentinal layers[a]	0	3.2	6.4	10.2	0	3.9	6.8	10.3
No. periosteal layers[a]	0	3.2	6.5	10.3	0	3.8	7.0	11.1
No. layers composite[b]	0	3.2	6.5	10.3	0	3.8	6.7	10.4
No. layers increment		3.2	3.3	3.8		3.8	2.9	3.7

[a] From length-layer curves.

[b] From length-layer curve which is a composite of periosteal and dentinal length-layer curves (Figs 8 and 9).

Nielsen (1972) has confirmed by micro-radiographic study of the teeth of *Phocoena phocoena* that the narrow, optically-translucent zones are radio-opaque to X-rays and therefore have a higher content of calcium salts than the more radio-translucent, optically-opaque zones. On the other hand, a few studies indicate that optical opacity and mineral content of the dentine are positively correlated (Sergeant, 1959; Gambell and Grzegorzewska, 1967; Schour and Hoffman, 1939). The relationships between optical opacity, mineral content, and other physico-chemical properties of the dentine are still unresolved problems in studies of odontocete age determination.

The use of dentinal growth layers to estimate the age of odontocetes is often limited by tooth occlusion which occurs after 10 to 20 (or more) growth layers in the embedded tooth of the narwhal. Dentinal occlusion has been noted in the sperm whale *Physeter macrocephalus* (Nishiwaki, Hibiya and Ohsumi, 1958; Best, 1970), the Atlantic pilot whale *Globicephala melaena* (Sergeant, 1962), *Berardius bairdii* (Kasuya, 1977), *Stenella attenuata* (Perrin, Coe and Zweifel, 1976; Kasuya, 1976) and *S. coeruleoalba* (Kasuya, 1976; Miyazaki, 1977). It is often difficult to assess the occurrence of dentinal occlusion (cessation of dentine deposition), a situation which decreases the reliability of estimates of life history parameters based on dentinal growth layer counts.

Various hypotheses have been advanced to explain the heterogeneous, layered appearance of the teeth of marine mammals. These include seasonal variations in hormone balance (Carrick and Ingham, 1962), endogenous annual rhythms (Gaskin and Blair, 1977), and specific genetic growth cycles with periodic '. . . enhancement of some of the short cycle layers. . .' by environmental change or seasonality (Kasuya, 1977). The most common explanation for the layered appearance of dentine and cementum relates to seasonal changes in nutritional state or feeding intensity (Laws, 1953; McLaren, 1958; Fisher, 1954; Mansfield, 1958; Scheffer, 1950) but little direct evidence is available. However, Scheffer and Peterson (1967) found a direct correlation between nutrition and postnatal dentinal layering in suckling pups of the Alaskan fur seal, *Callorhinus ursinus*. Between birth and weaning of the pup, each of an average of eleven nursing cycles (each cycle consisting of one nursing period and a period when the female goes to sea to feed) was recorded as a narrow layer within the postnatal dentine of the pup's canine tooth. Kubota *et al* (1961) believed that differential calcification of the dentine in the canine tooth of the fur seal reflects alternating periods of normal and disturbed calcium metabolism, which in turn reflects the nutritional state of the animal. For example, a deficiency of vitamin C leads to a reduction in the dentinal deposition rate and subsequent pathological hypercalcification (Shaw, 1955).

The deposition of well-calcified dentine in the teeth of odontocetes is generally attributed to good feeding conditions or increased feeding activity, while poorly mineralized dentine is deposited when feeding conditions are suboptimal or when feeding activity is reduced (Brodie, 1970; Sergeant, 1959, 1962, 1973; Ohsumi *et al*, 1963). The obscurity of the first several layers in the teeth of suckling animals is considered to be evidence for the importance of optimal nutrition in building well-calcified teeth (Brodie, 1970; Sergeant, 1962, 1973). On the other hand, Klevezal' and Kleinenberg (1967) and Kubota *et al* (1961) attributed the deposition of well-calcified dentine to seasonal reductions in body growth rate (and by inference, feeding rate) and deposition rate of dentinal organic matrix, assuming that the rate of mineralization is constant.

One can only speculate on the proximate factors which are responsible for differential calcification in the dentine of the narwhal's tooth. The available evidence suggests that low feeding intensity in mid-summer is correlated with the deposition of a narrow layer of poorly-mineralized dentine; this is corroborated by the observation that the narwhals are becoming thinner at this season (Hay, 1979). Reduced food intake in summer should result in a deficiency of vitamins and minerals needed for the elaboration of well-calcified dentine. These variations in mineral content are then expressed as differences in optical opacity of the dentine.

Mandibular periosteal layering in the narwhal is identical to that described for the white whale by Brodie (1969) and similar to that described for the sperm whale by Nishiwaki *et al* (1961). Peabody (1961) attributed the deposition of the narrow translucent laminae of the periosteal bone of poikilothermic vertebrates to reduced growth rate during winter or breeding cycles. More attention should be given to periosteal growth layers in the bones of odontocetes, especially those in which the teeth occlude early in life.

Until the time of dentinal occlusion, in the male narwhal the number of dentinal layers agrees closely with the number of periosteal layers (Fig. 6). This is in marked contrast to the conclusion that two dentinal layers and one mandibular periosteal layer are deposited annually in the white whale *Delphinapterus leucas* (Brodie, 1969) and in *Delphinus delphis* (Kleinenberg and Klevezal', 1962). On the other hand, Nishiwaki *et al* (1961) found that, in the sperm whale, dentinal and mandibular periosteal growth layers are equal in number until layer 14, after which the number of mandibular layers is constant because of a balance between apposition and resorption rates, while dentinal layers continue to accumulate.

The rate of dentinal layering in the teeth of odontocetes is still unknown, mainly because the relationship between the variously-defined growth layers and time (age) has not been examined directly. A rare exception is the work of Best (1976), who established that one dentinal growth layer is deposited annually in the teeth of captive *Lagenorhynchus obscurus,* using the technique of time-marking the teeth with tetracycline.

Based largely on indirect evidence, dentinal growth layers are believed to accumulate at a rate of one per annum in *Globicephala melaena* (Sergeant, 1962), *Tursiops truncatus* (Sergeant, 1959), *Stenella attenuata* (Kasuya, Miyazaki and Dawbin, 1974; Perrin *et al*, 1976), *S. coeruleoalba* (Kasuya, 1972), *S. longirostris* (Perrin, Holts and Miller, 1977), *Phocoena phocoena* (Gaskin and Blair, 1977; Grue Nielson, 1972), *Hyperoodon ampullatus* (Christensen, 1973), and *Berardius bairdii* (Kasuya, 1977). However, two dentinal growth layers are thought to be deposited annually in the teeth of *Delphinapterus leucas* (Brodie, 1971; Sergeant, 1973) and *Delphinus delphis* (Kleinenberg and Klevezal', 1962).

The rate of dentinal layering in the sperm whale *(Physeter macrocephalus)* is a matter of considerable controversy. Ohsumi *et al* (1963), considering the recruitment rate of the stock and Best (1970, 1974), considering the ovulation rate, concluded that one dentinal layer is deposited annually in this species. On the other hand, Nishiwaki *et al* (1958), considering young whales near weaning, and Gambell and Grzegorzewska (1967), analyzing the seasonal deposition of the most recent translucent zone, concluded that two dentinal layers are deposited annually. More recently, Gambell (1977) has concluded that southern hemisphere sperm whales deposit one dentinal layer per year, since all samples showed only one annual peak of formation of each type of lamina (translucent and opaque).

The conclusion that three dentinal and periosteal layers are deposited annually in the young narwhal may seem surprising. Brodie (1969) referred to secondary laminae in the early postnatal growth of the mandibular periosteal bone of the white whale *Delphinapterus leucas,* so that such a possibility exists for this species as well. Laws (1952, 1953) found that the frequency of dentinal layering was higher and more irregular in immature than in adult elephant seals *(Mirounga leonina)*, correlated with the more regular pattern of seasonal haul-out of the adults. Best (1970, 1974) considered that two dentinal layers are deposited during the first year of life in sperm whales, although subsequent layers are annual. On the basis of theoretical or indirect evidence, Perrin *et al* (1976) and Perrin *et al* (1977) have suggested that, in *Stenella,* the frequency of dentinal layer deposition may be higher during the first year of postnatal life than in subsequent years. These results do not necessarily confirm the above-stated conclusion, but they do indicate the possibility of a higher or more irregular frequency of deposition of dentinal layers in early postnatal life.

The growth curves for the narwhal (Figs 8 and 9) suggest that the number of composite growth layers is a valid index of age. However, the large number of females between 370 and 390 cm body length, with 14 to 20 composite growth layers, seems to indicate either the possibility of a cessation of periosteal activity or the attainment of a balance between the deposition and resorption rates of periosteal growth layers in old females approaching physical maturity. Thus, the ages of many older females may be underestimated and the true growth curve would be further to the right than that indicated in Fig. 9, especially after about 370 cm body length. On the other hand, there is no accumulation in old males over a narrow range of ages, indicating that periosteal layers probably continue to accumulate throughout the life of male narwhals.This is corroborated by the attainment of as many as 45 to 50 periosteal layers by males and only 30 by females (Figs 8 and 9). Kasuya *et al* (1974) also underestimated the ages of females of *S. attenuata* approaching or at physical maturity (12.5 years), at which time poorly calcified secondary dentine is deposited and age determination becomes difficult.

Sexual dimorphism in body size and growth rate is evident in the narwhal and many other odontocetes, males usually attaining a larger size than females (Christensen, 1973; Sergeant, 1962; Nishiwaki *et al* 1958; Best, 1970; Brodie, 1971; Sergeant, 1973). However, females of *Berardius bairdii* (Kasuya, 1977) and *Phocoena phocoena* (Gaskin and Blair, 1977) attain greater sizes than the males. Male narwhals do not demonstrate the accelerated growth at puberty found to be characteristic of the males of many other odontocetes (Fig. 8).

SUMMARY AND CONCLUSIONS

(1) The postnatal dentine of the embedded tooth of the narwhal consists of a succession of growth layers, each of which comprises one wide light zone and one narrow dark zone. These layers have a repeatable and consistent structure and are apparently related to age. However, dentinal deposition ceases due to complete coverage of the root by cementum at mean ages of 15 to 16 layers in males and 13 to 14 layers in females. The incidence of dentinal occlusion increases markedly after the mean age of sexual maturity in both sexes.

(2) A narrow dark band occurs adjacent to the open pulp cavity of the embedded teeth of most whales. This zone is poorly-mineralized and is deposited during August and September when narwhals are feeding lightly or not at all.

(3) The periosteal bone of the anterior portion of the mandible is also laminated. Mandibular periosteal layers are deposited throughout most of the animal's life and may be used to assess the ages of animals whose embedded teeth are occluded. However, the reliability of counts of periosteal growth layers is reduced, especially in old males, by internal resorption and removal of an unknown number of early layers by osteoclast activity.

(4) Prior to dentinal occlusion, dentinal and mandibular periosteal layers are equal in number, indicating that both provide a valid index of age, while in young females there are slightly more periosteal than dentinal layers.

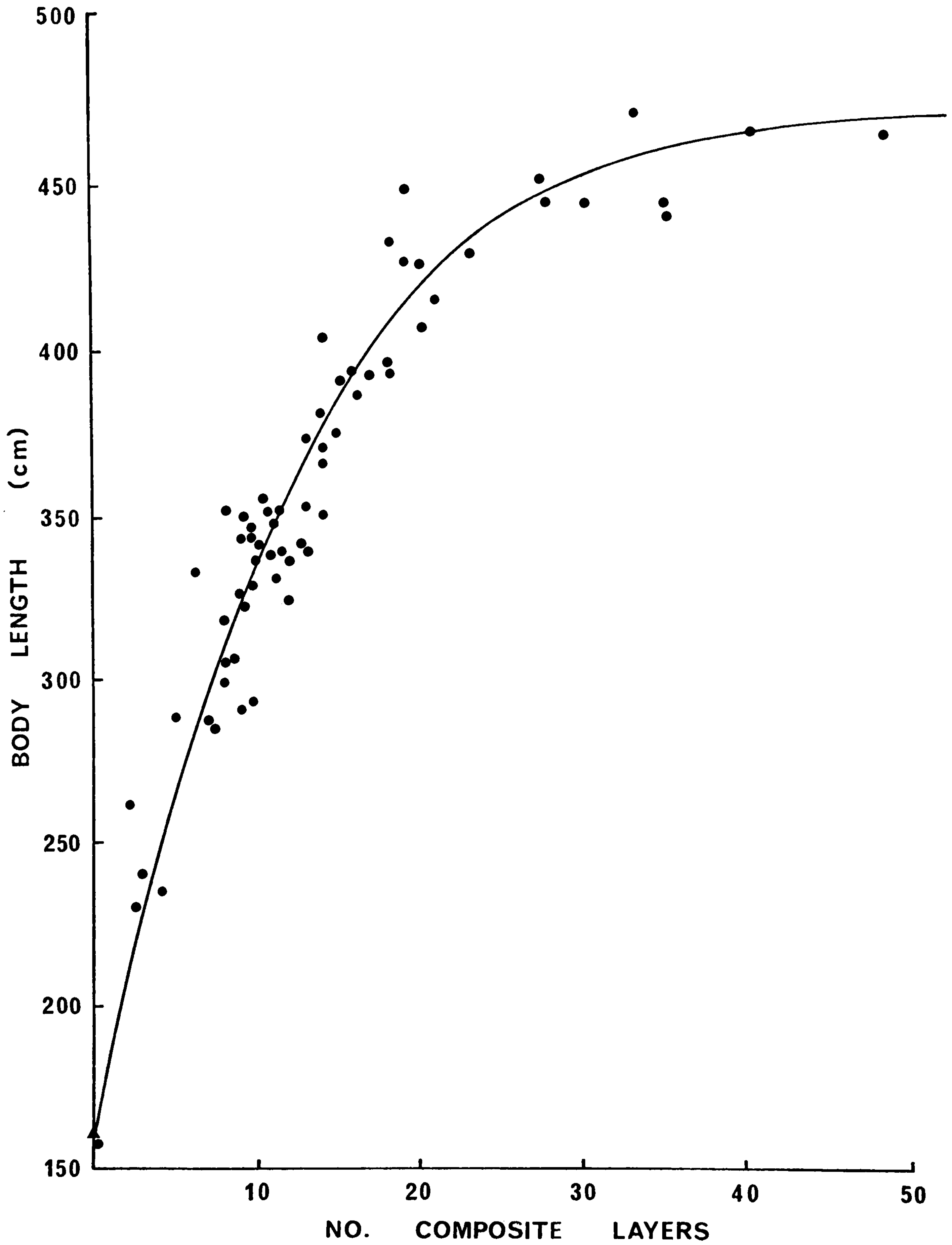

Fig. 8. Body length and age of 65 postnatal male narwhals. The triangle indicates the mean body length at birth. The curve is fitted by eye.

(5) The time basis of the growth layers is not known with certainty. Analysis of the length composition of the catch indicates that about three growth layers are deposited annually in young animals, and consideration of female reproductive data suggests that one growth layer is deposited annually in the tooth and mandible of adult females.

(6) Counts of mandibular periosteal growth layers are more useful for the estimation of the ages of male than for female narwhals. This is due to the accumulation of periosteal layers throughout the life of the male, whereas a balance between deposition and resorption of growth layers is achieved in the mandibles of old females approaching physical maturity. The maximum number of mandibular periosteal growth layers is about 50 in males and 30 in females.

(7) In view of the difficulties involved with the age determination techniques employed in this study (dentinal occlusion, bone resorption), other techniques (including studies of periosteal layering in bones other than the mandible) should be explored. Intensive tagging of calves and other young narwhals, in conjunction with the fishery prosecuted by the Inuit, is probably the most promising approach to elucidation of age determination of the narwhal.

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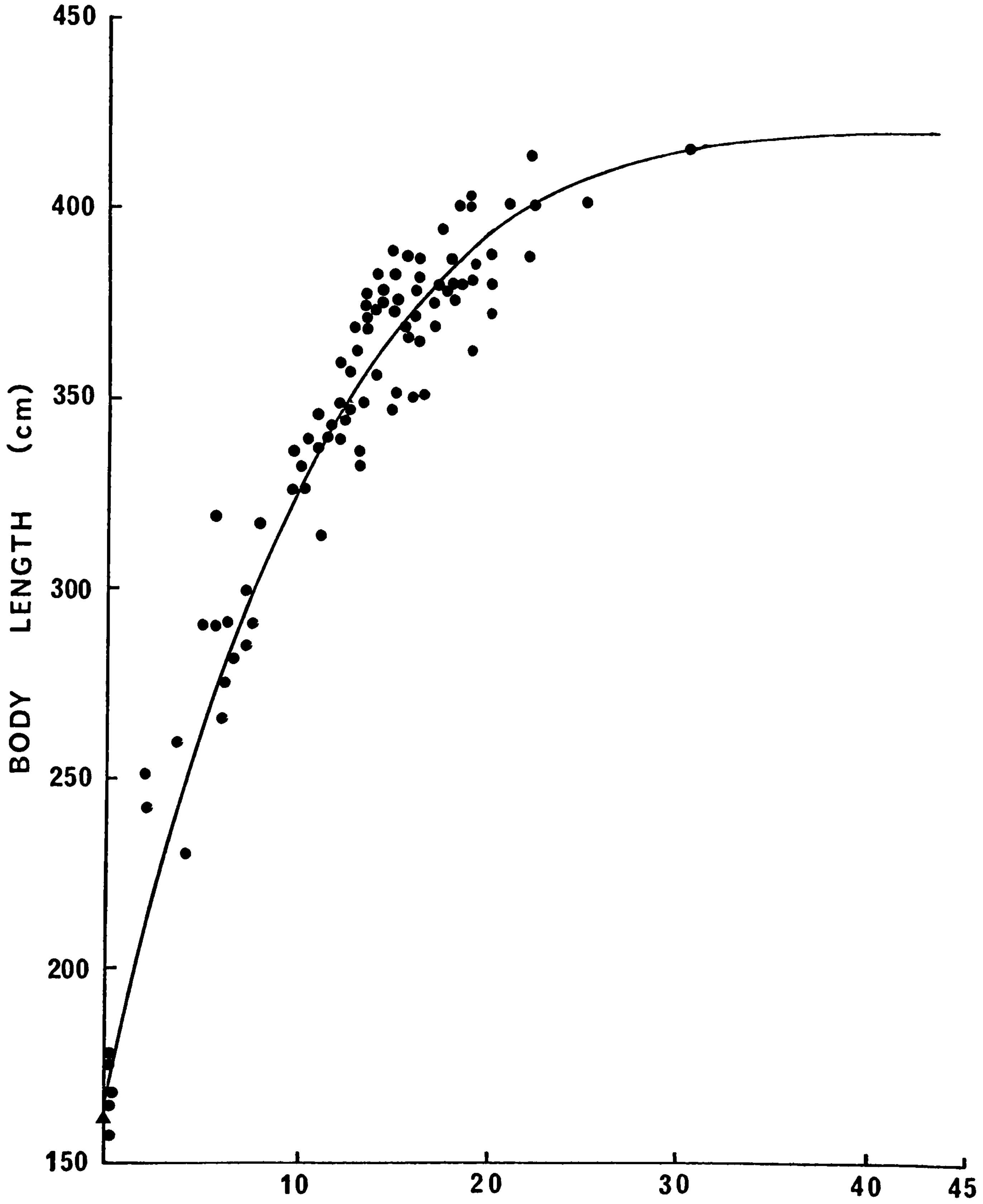

Fig. 9. Body length and age of 90 postnatal female narwhals. The triangle indicates the mean body length at birth. The curve is fitted by eye.

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Developmental Patterns in Osteology and External Morphology in *Phocoena phocoena*

Laurie J. Stuart and G. Victor Morejohn

Moss Landing Marine Laboratories of the California State University and Colleges
P.O. Box 223, Moss Landing, California

ABSTRACT

Dentinal growth layer groups (GLGs) in teeth were counted for 84 beach-cast specimens of *Phocoena phocoena* from the northeastern Pacific (mostly California). Measurements of external, cranial, and postcranial traits were found to be related to growth layer groups in most cases. Of the traits related to dentinal layering 70% were found to be sexually different in the slope of the relationship. Growth of the body posterior to the dorsal fin was more rapid and extensive in females as compared to males, especially during and after the attainment of sexual maturity. The growth curves of total length and length of longest rib showed a retardation in growth earlier in males (about three GLGs) than in females (about five GLGs). The total length growth curve for females closely resembled the juvenile growth phase for both sexes of *Stenella attenuata*.

INTRODUCTION

There is a paucity of information relating measurements of external morphology, cranial, and postcranial skeleton to dentinal layering in the common harbour porpoise, *Phocoena phocoena* (Linnaeus, 1758). Previous studies on this species related dentinal layering to age and total length (Nielsen, 1972; Gaskin and Blair, 1977). In the present study, we relate dentinal layering to several external morphological, cranial, and postcranial measured traits. The purpose of our study was to determine which measurable traits could be significantly related to age as estimated by counts of dentinal growth layer groups (GLGs) in teeth and to determine whether sexual differences in these relationships exist.

MATERIALS AND METHODS

We studied 105 specimens which had been beach-cast dead or stranded. Eighty-four of these had teeth for which the number of dentinal GLGs was determined; for 57 of these, the sex had been recorded.

Our major sources of specimens were from institutions in California: The California Academy of Sciences (CAS), San Francisco; Museum of Vertebrate Zoology (MVZ), University of California, Berkeley; and Museum of Birds and Mammals (MBM), San Jose State University, San Jose. Specimens in the private collection of Mr Ray Bandar, San Francisco, California, were also used. The geographical distribution of specimens studied is shown in Table 1.

Harbour porpoise teeth are very small, about 1 cm long and 3 mm wide. Teeth from the middle of the tooth row of either mandibular ramus were taken whenever available. *Lakeside 70* thermoplastic cement was used to mount the teeth on petrographic slides, which are somewhat shorter and wider than biological slides and are therefore easier to handle. Teeth were hand-ground to the longitudinal center with a circular motion using 600 grit silicon dioxide powder and water on a glass plate and then polished with 302.5 abrasive.

A film of water on the surface of the half-ground tooth facilitated reading of dentinal GLGs with a binocular dissecting microscope at 50 to 100X magnification. Reflected light was more useful than transmitted light for reading GLGs. By the use of fiber optical lighting with dissecting microscope of high optical quality, clarity of dentinal structures was increased and variability of counts was minimized. Thin sections of about 200 μm thickness were prepared by hand grinding, but the visibility of the layers was not enhanced using thin sections and teeth were prone to break, so the method was no longer used.

Measurements were made of 25 external, 43 cranial, and 29 post cranial traits using dial calipers and rules. Three external traits, 12 cranial traits, and 5 post cranial traits were selected for analysis on the basis of sample size and probable relationship to age.

In order to utilize many specimens of which anterior tips of rostra and mandibles were abraded due to tossing in the surf, the measurements cranial length, rostral length, mandibular length, and length of mandibular symphysis were made to the posterior margin of the anteriormost alveolus instead of the more conventional 'tip of rostrum' or 'tip of mandible'.

The selected measurements are as follows:

(TL)	Total length; tip of snout to notch in flukes (Fig. 1).
(STDF)	Snout to tip of dorsal fin (Fig. 1).
(FL)	Flipper length; anterior insertion to tip of flipper (Fig. 1).
(CL)	Cranial length; distance in the midline of the skull from a line connecting the posterior margins of the most anterior upper alveoli to a line connecting the posteriormost margins of the condyles (Fig. 2).
(RL)	Rostral length; distance in the midline of the skull from a line across the hindmost limits of the antorbital notches to the posterior margins of the most anterior alveoli (Fig. 2).

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Table 1

List of specimens of *Phocoena phocoena* from the northeastern Pacific (mostly California) with measurements taken thereof

Institutional number	County	Date	Dentinal layers	Log dentinal layers	Total length	Snout to tip of dorsal fin	Flipper length
Females							
CAS13923	San Francisco	29 July 1966	0.0	−1.0	780	–	–
CAS16341	San Mateo	6 July 1971	0.5	−0.3	955	585	125
CAS15949	San Francisco	23 Sep. 1973	0.5	−0.3	990	–	–
C29	Monterey	30 July 1968	1.0	0.0	1252	792	202
CAS15671	San Francisco	15 Nov. 1969	1.0	0.0	–	–	–
CAS7572	San Mateo	8 Mar. 1937	1.0	0.0	1180	–	–
C24	Monterey	14 May 1968	1.5	0.176	1262	735	204
C138	Monterey	8 Mar. 1971	1.5	0.176	1200	690	183
C199	Santa Cruz	25 Aug. 1977	2.0	0.301	1274	765	202
C103	Monterey	25 Aug. 1969	2.5	0.398	1371	–	–
CAS16109	Marin	22 Sep. 1971	2.5	0.398	1286	830	197
C190	Santa Cruz	31 May 1976	3.0	0.477	1580	895	244
CAS16749	San Mateo	7 Apr. 1976	3.0	0.477	1208	709	230
CAS15992	San Mateo	19 July 1971	3.0	0.477	1430	879	203
C169	Santa Cruz	7 Nov. 1968	3.5	0.544	1320	–	–
UCB90696	Contra Costa	19 June 1939	4.0	0.602	–	–	–
CAS16572	San Francisco	20 Aug. 1973	4.0	0.602	1370	790	205
C186	Santa Cruz	20 July 1975	5.0	0.699	1490	777	220
C3	Monterey	15 May 1967	5.0	0.699	1584	884	245
CAS12769	San Francisco	18 Feb. 1963	5.0	0.699	1630	970	240
RB2336	San Francisco	Sep. 1973	5.0	0.699	1750	–	–
CAS15281	San Mateo	20 Aug. 1969	5.5	0.740	1640	925	–
RB1843	Marin	23 Apr. 1971	5.5	0.740	1575	–	–
C7	Monterey	Oct. 1967	6.0	0.778	–	–	–
CAS16609	San Mateo	13 July 1973	6.0	0.778	1590	–	240
CAS16630	San Mateo	9 Apr. 1975	6.0	0.778	1460	–	–
RB2229	San Mateo	May 1974	6.0	0.778	1750	–	–
C33b	San Mateo	10 Feb. 1969	7.0	0.845	1760	952	274
CAS15987	Marin	18 Nov. 1970	7.0	0.845	1575	–	–
CAS16634	San Mateo	15 July 1974	7.0	0.845	1675	945	245
UCB135247	Monterey	28 Mar. 1967	7.0	0.845	–	–	–
Males							
C9	Monterey	5 Nov. 1967	0.5	−0.300	1045	560	172
CAS15948	Marin	23 Jan. 1971	1.0	0.000	1110	–	190
CAS16112	San Mateo	20 Nov. 1971	1.0	0.000	1165	632	195
UCB135248	Sonoma	10 June 1967	1.5	0.176	1230	–	200
C35	Monterey	18 Mar. 1969	2.0	0.300	1360	767	205
C127	Santa Cruz	26 Sep. 1970	2.5	0.398	1350	730	220
CAS5526	San Francisco	18 Oct. 1926	3.0	0.477	–	–	–
CAS13931	San Mateo	11 June 1967	3.0	0.477	1320	–	–
SJSU1906	Santa Cruz	6 Oct. 1961	3.0	0.477	1310	–	–
C155	Monterey	2 May 1972	3.5	0.544	1362	755	225
C28	Monterey	30 July 1968	3.5	0.544	1320	790	210
C4	Monterey	Aug. 1967	4.0	0.602	–	–	–
CAS16603	Marin	1 June 1973	4.0	0.602	1460	780	220
CAS15944	San Mateo	26 Mar. 1971	4.0	0.602	1370	820	200
CAS11037	Monterey	1 May 1956	4.0	0.602	1330	–	235
UCB90341	Contra Costa	2 Feb. 1940	5.0	0.699	1370	–	–
CAS15253	San Mateo	30 Oct. 1968	5.0	0.699	1412	–	–
CAS16602	Marin	1 June 1973	5.0	0.699	1380	780	205
C187	Monterey	12 Aug. 1975	5.0	0.699	1480	–	–
UCB96089	Contra Costa	19 July 1941	5.5	0.740	–	–	–
CAS13930	Santa Cruz	24 Mar. 1967	5.5	0.740	1410	720	210
CAS16629	San Francisco	26 May 1973	6.0	0.778	1400	844	242
C206	Santa Cruz	3 May 1978	6.5	0.813	1448	785	206
C166	Santa Cruz	18 May 1973	7.0	0.845	1610	895	255
UCB83459	Alameda	24 Mar. 1938	7.5	0.875	1470	850	–
UCB86878	Pacific*	28 Sep. 1938	9.0	0.954	–	–	–

*State of Washington

Table 1 (continued)

Institutional number	Skull length	Rostral length	Mandibular length	Mandibular height	Length of mandibular symphysis	Length of dental foramen	Skull width	Cranial width
Females								
CAS13923	179	76.26	135	32.16	14.79	53.36	95.22	102.39
CAS16341	212	90.46	160	41.35	20.19	61.68	111.26	113.91
CAS15949	195	80.24	141	39.12	14.24	56.35	108.41	116.60
C29	256	112.20	203	53.40	22.88	77.86	151.80	129.89
CAS15671	244	109.24	188	49.04	22.96	70.48	136.87	129.05
CAS7572	–	–	186	46.41	20.46	69.74	132.77	123.81
C24	251	105.80	195	51.30	26.81	74.36	135.75	123.85
C138	245	105.00	–	–	19.66	–	129.00	119.68
C199	262	118.50	198	55.27	28.00	79.30	146.69	136.06
C103	261	–	–	–	–	–	148.80	127.74
CAS16109	260	114.90	199	53.50	23.66	84.89	150.29	136.27
C190	295	131.00	232	57.14	27.35	82.22	159.00	133.22
CAS16749	270	120.13	210	53.68	26.16	78.04	154.00	133.68
CAS15992	262	120.00	202	51.46	27.61	–	145.09	128.94
C169	255	102.80	–	–	–	–	143.04	133.45
UCB90696	280	127.92	213	56.11	28.91	78.43	155.27	137.74
CAS16572	263	118.10	207	50.67	26.12	78.40	144.92	125.14
C186	273	122.48	210	52.47	26.67	86.28	147.00	124.16
C3	283	127.11	221	59.98	25.01	88.86	164.00	133.30
CAS12769	277	131.30	225	60.00	24.06	92.71	166.00	133.55
RB2336	289	134.66	227	59.84	24.62	95.90	174.00	132.91
CAS15281	287	133.07	219	56.43	27.19	86.60	166.00	133.24
RB1843	293	131.53	225	57.38	29.66	88.31	165.00	135.27
C7	259	110.20	202	54.70	27.17	79.21	155.00	132.88
CAS16609	278	130.40	215	54.85	26.01	79.79	159.00	135.29
CAS16630	289	128.78	226	54.55	31.37	78.10	158.00	133.36
RB2229	299	131.17	233	63.16	32.10	89.56	178.00	138.44
C33b	294	133.07	234	59.35	25.60	93.90	165.00	135.34
CAS15987	283	129.41	167	59.42	26.83	92.41	167.00	132.75
CAS16634	311	141.34	243	61.56	29.64	90.06	170.00	136.39
UCB135247	303	137.98	239	58.80	30.04	95.79	169.00	141.81
Males								
C9	–	–	–	–	–	–	–	–
CAS15948	234	102.98	175	45.79	18.20	66.79	129.39	119.68
CAS16112	241	102.79	181	46.00	18.80	72.95	131.41	125.17
UCB135248	256	108.62	193	49.05	22.08	73.00	139.73	128.08
C35	269	120.92	211	58.01	27.00	82.70	154.70	139.85
C127	261	115.10	205	52.51	24.70	72.89	152.00	128.06
CAS5526	242	102.79	187	50.80	23.62	77.14	141.22	129.97
CAS13931	255	116.89	195	55.61	22.79	77.80	151.42	127.18
SJSU1906	248	108.90	190	48.58	23.69	75.87	136.84	126.56
C155	270	120.80	214	54.96	28.92	86.39	154.00	125.64
C28	255	108.20	200	55.86	23.64	80.79	147.80	128.35
C4	278	117.20	210	52.55	23.66	78.84	149.00	130.60
CAS16603	276	115.45	208	54.55	24.86	78.10	157.00	135.27
CAS15944	263	116.81	205	52.43	23.35	80.89	155.00	127.96
CAS11037	259	121.24	203	56.59	28.68	74.42	145.63	127.61
UCB90341	260	117.50	199	53.35	22.61	80.00	151.46	131.51
CAS15253	271	120.08	210	53.82	24.14	79.76	151.80	127.74
CAS16602	263	121.21	204	57.20	23.80	80.15	147.50	133.24
C187	272	120.76	207	52.76	26.85	86.28	150.78	128.86
UCB96089	276	126.75	210	54.20	21.04	85.41	148.04	131.84
CAS13930	256	117.78	200	56.90	24.88	79.51	148.00	130.26
CAS16629	259	114.56	203	52.58	22.66	77.91	152.82	131.06
C206	256	113.91	200	55.70	25.50	74.90	152.03	129.33
C166	270	114.20	200	–	16.90	–	150.00	130.45
UCB83459	271	127.99	211	55.94	25.09	82.75	154.05	131.14
UCB86878	258	115.96	201	49.11	25.71	77.13	146.20	127.58

Table 1 (concluded)

Institutional number	Rostral width	Pterygoid length	Frontal crest to occipital condyle base	Height of basioccipital processes	Length of first rib	Length of longest rib	Scapular height	Greatest width of basi-thyrohyal complex	Greatest width of 23rd vertebra
Females									
CAS13923	42.21	21.34	88.76	16	–	–	–	–	–
CAS16341	49.55	24.91	99.45	18	70.42	146	59.84	61.44	83
CAS15949	50.45	23.98	92.50	20	66.01	125	51.79	–	27
C29	71.84	37.58	118.51	28	92.96	212	–	101.00	141
CAS15671	58.70	29.36	121.16	27	–	–	–	–	–
CAS7572	64.33	38.01	114.21	25	–	–	–	–	–
C24	66.50	–	117.60	25	100.00	198	–	91.02	133
C138	60.00	–	111.88	29	84.76	179	77.70	78.18	130
C199	71.39	36.32	124.49	26	–	–	–	86.70	–
C103	71.50	36.64	124.05	29	93.80	197	82.81	88.54	148
CAS16109	70.70	34.27	121.47	31	–	–	–	–	–
C190	77.12	44.65	128.21	32	123.49	239	109.60	–	181
CAS16749	72.64	39.70	124.64	30	108.51	220	–	–	158
CAS15992	71.20	34.63	124.79	27	108.44	220	91.97	102.25	161
C169	67.98	37.04	122.86	28	106.62	212	93.26	94.60	163
UCB90696	80.45	42.60	127.05	31	117.95	227	99.37	107.46	162
CAS16572	71.70	42.06	122.63	30	–	–	–	–	–
C186	73.40	41.05	122.25	27	107.21	219	91.86	92.12	167
C3	77.50	39.50	129.70	31	–	–	–	101.15	–
CAS12769	74.91	40.17	129.24	31	131.01	262	122.15	116.11	192
RB2336	83.15	41.65	129.00	32	–	–	–	–	–
CAS15281	79.63	37.16	131.22	31	132.63	250	111.27	115.29	202
RB1843	77.79	36.22	127.80	33	–	–	–	–	–
C7	67.34	–	126.89	–	–	–	–	107.27	–
CAS16609	71.85	44.56	128.67	33	124.30	265	115.96	108.15	196
CAS16630	78.80	38.55	130.37	31	124.55	249	114.70	109.56	–
RB2229	89.71	42.44	135.23	33	–	–	–	–	–
C33b	82.06	41.97	–	33	135.18	276	126.76	110.66	192
CAS15987	87.24	40.24	127.64	33	135.29	266	118.36	112.22	197
CAS16634	81.76	41.44	135.35	34	136.52	267	126.10	116.07	208
UCB135247	87.14	39.65	136.20	31	119.41	276	121.74	119.62	201
Males									
C9	–	–	–	–	71.59	159	–	–	92
CAS15948	57.11	–	110.05	22	86.41	–	76.45	–	121
CAS16112	56.09	31.63	118.02	23	86.26	168	–	81.75	116
UCB135248	61.88	31.60	119.18	27	88.42	188	81.39	83.14	144
C35	71.67	36.31	119.86	30	94.08	188	85.91	–	149
C127	69.75	39.20	126.25	30	95.45	205	–	96.08	153
CAS5526	66.40	34.74	114.45	28	95.15	178	80.05	87.64	121
CAS13931	73.78	33.56	115.92	31	–	–	–	–	–
SJSU1906	67.12	33.28	119.99	27	97.59	193	83.23	–	140
C155	74.75	40.21	121.71	30	98.28	213	91.24	87.86	156
C28	68.12	34.48	120.66	28	111.81	205	87.54	–	161
C4	67.55	42.80	132.21	24	–	–	–	102.06	–
CAS16603	73.69	38.60	130.37	–	111.80	216	89.39	115.71	156
CAS15944	74.08	38.45	126.98	27	115.68	221	96.40	–	164
CAS11037	66.72	39.75	122.96	27	101.35	203	86.20	91.25	155
UCB90341	65.44	39.25	123.20	25	110.76	224	102.16	103.53	164
CAS15253	71.15	42.59	124.67	26	–	–	–	–	–
CAS16602	71.46	33.68	123.53	27	106.66	224	95.23	97.72	160
C187	76.45	39.63	128.00	24	110.55	228	97.68	95.85	166
UCB96089	74.42	41.04	127.24	24	107.49	220	102.57	89.37	165
CAS13930	70.34	37.95	124.05	28	–	–	–	–	–
CAS16629	72.42	40.70	121.91	25	110.87	231	104.21	–	167
C206	72.94	35.00	126.25	24	–	–	–	–	–
C166	73.35	–	121.02	27	112.92	225	109.96	92.05	173
UCB83459	80.83	38.71	119.28	30	102.50	227	106.70	–	–
UCB86878	74.31	34.16	123.21	27	–	–	–	–	–

(ML)	Mandibular length (left); distance from the posteriormost tip of the left mandible to the posterior margin of the anteriormost alveolus taken parallel to the long axis of the mandible (Fig. 2).
(MH)	Mandibular height; greatest height of the left mandible taken as a perpendicular to the ventral margin of the mandible at the coronoid process (Fig. 2).
(LMS)	Length of mandibular symphysis; distance parallel to the long axis of the mandible from the posterior margin of the anteriormost alveolus to the most posterior edge of the mandibular symphysis (Fig. 2).
(LDF)	Length of dental foramen; distance from the most anterior edge of the left dental foramen to the mesial rim of the internal surface of the condyle (Fig. 2).
(SW)	Squamosal width; greatest width of the skull across the zygomatic processes of the squamosals (Fig. 2).
(IW)	Interparietal width; greatest width of parietals within postemporal fossae (Fig. 2).
(RW)	Rostral width; width of the rostrum along a line connecting the hindmost limits of the antorbital notches (Fig. 2).
(PTL)	Pterygoid length; greatest length of left pterygoid (Fig. 3).
(FCO)	Frontal crest to occipital condyle base; distance from the uppermost point of frontal crest to the farthest point on the left occipital condyle (Fig. 3). (After D.B. Yurick, M.Sc thesis, 1977.)
(HBP)	Height of basioccipital processes; from a midpoint between the most anterior edge of the basioccipital along the midline to the posterior ventral lip of the foramen magnum, a perpendicular is made ventrally from the basioccipital to a transverse line between the most ventrolateral margins of the basioccipital (Fig. 3). This region of the basioccipital is usually slightly posterior to the pituitary depression or *sella tursica*.
(L1R)	Length of first rib; greatest length of first left vertebral rib (Fig. 4).
(LLR)	Length of longest rib; greatest length of longest left vertebral rib (Fig. 4).
(SH)	Scapular height; from posterior margin of glenoid fossa to coracovertebral angle (Fig. 4).
(GWBTH)	Greatest width of basi-thyrohyal complex (Fig. 4).
(GW23V)	Greatest width of twenty-third vertebra from tip to tip of the transverse processes (Fig. 4).

A tabulation of these measurements is given in Table 1.

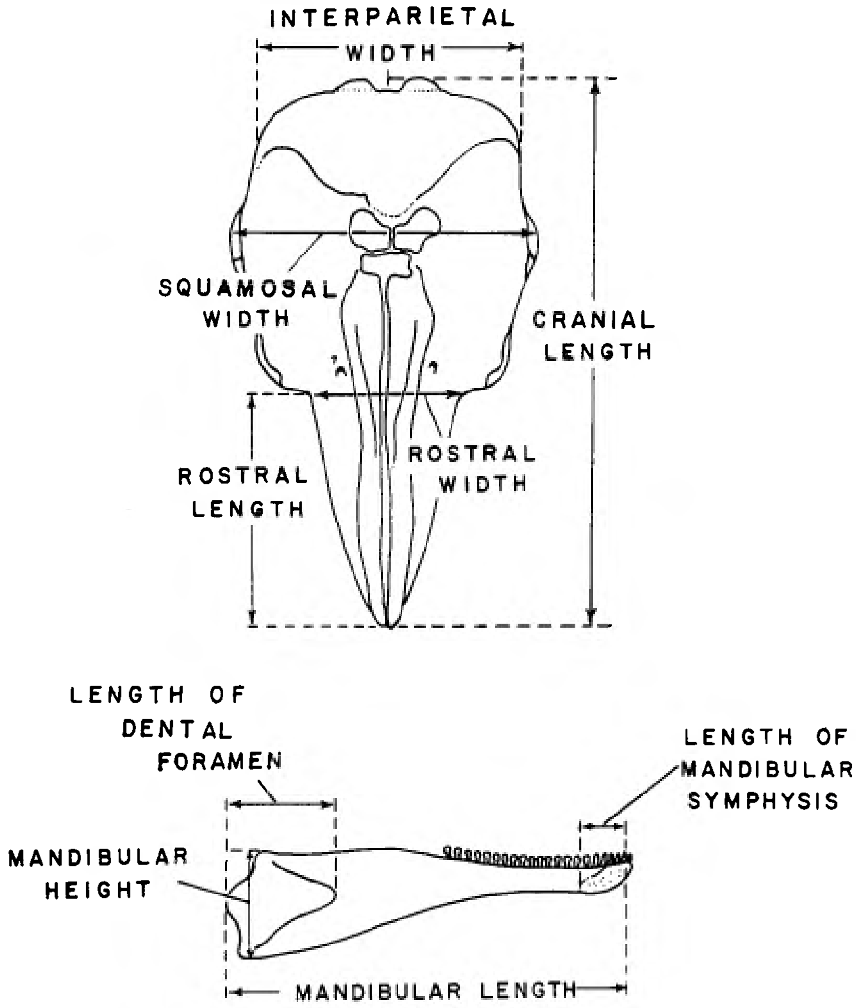

Fig. 2. Cranial measurements of *Phocoena phocoena* specimens from the northeastern Pacific. See text for descriptions.

Average growth rates of most traits (see Table 2 for exceptions, LMS and HBP) were estimated from the slopes of linearly regressed scatter plots of each trait versus growth

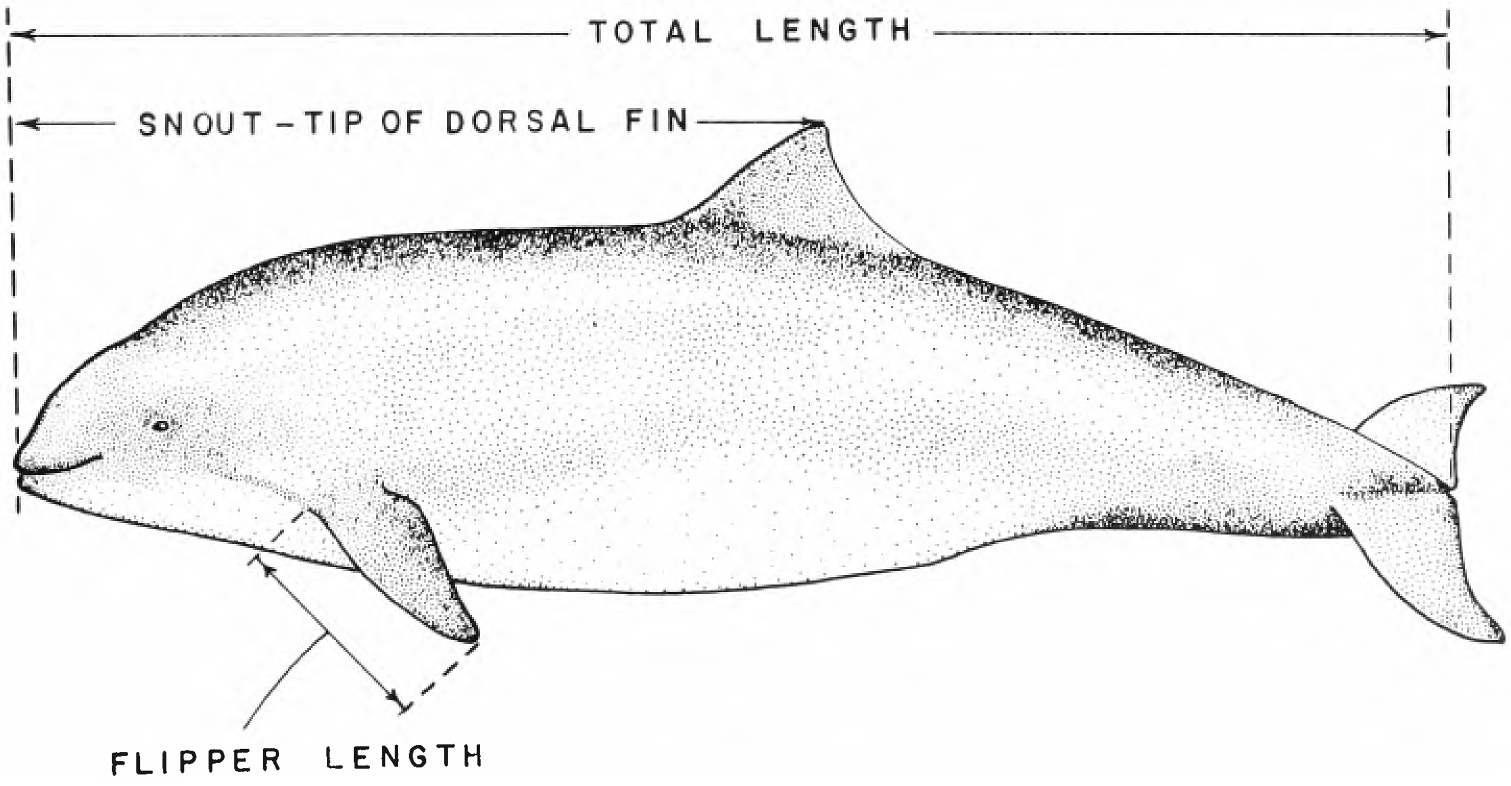

Fig. 1. Measurements of external traits in *Phocoena phocoena* from the northeastern Pacific. See text for descriptions.

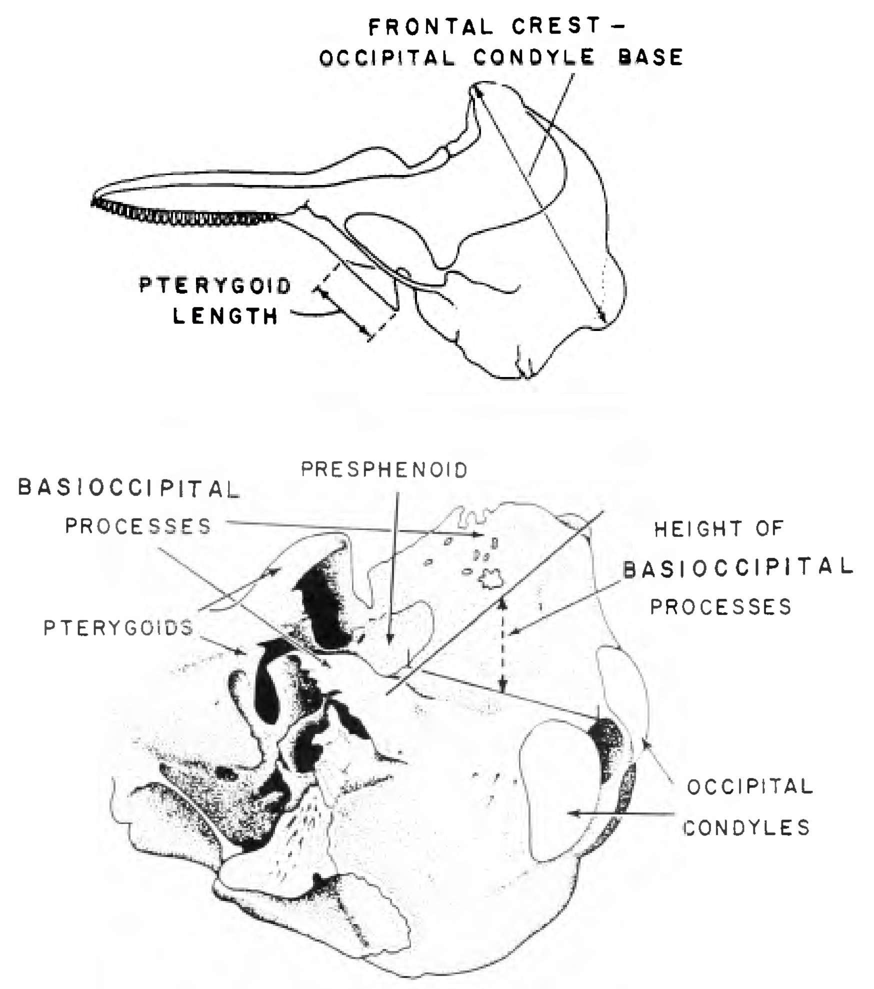

Fig. 3. Other cranial measurements of *Phocoena phocoena* specimens from the northeastern Pacific. See text for descriptions.

layer groups. Better fitted linear regressions of the traits were then made using semi-logarithmic transformations (Figs. 5–11). The common logarithm (base ten) was substituted for each dentinal GLG count. Specimen CAS 13923, which was scored as having zero dentinal GLGs, was assigned the value of one-tenth (0.1) of a GLG so that a logarithmic transformation could be made. A relationship of each trait to logarithms of dentinal GLGs (log GLG) was tested for at several significance levels by using a one-tailed test (Zar, 1974) for significance of correlation coefficients, r (Table 2).

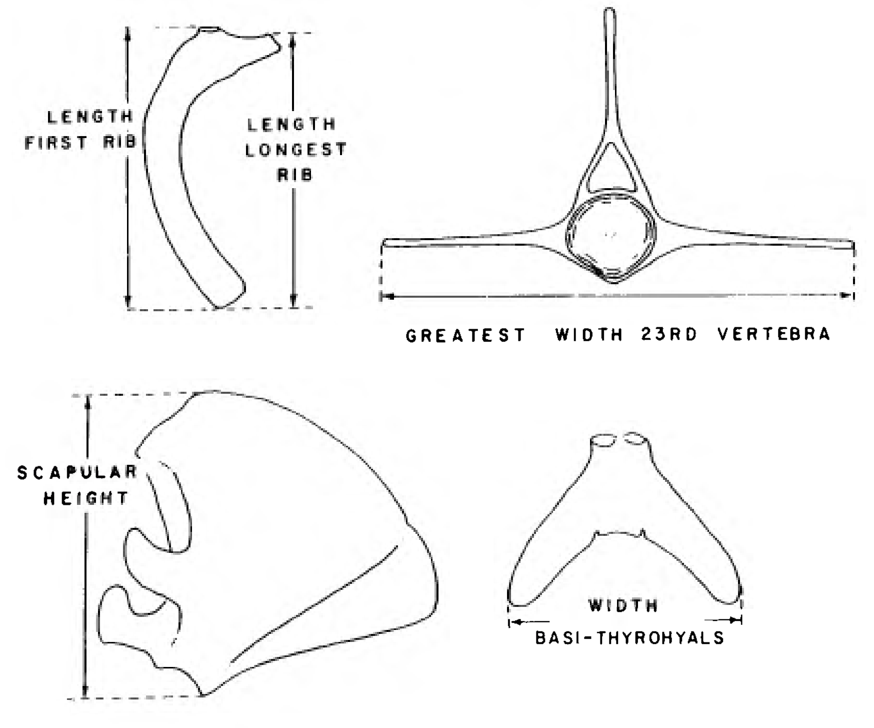

Fig. 4. Measurements of post-cranial traits of *Phocoena phocoena* specimens from the northeastern Pacific. See text for descriptions.

Differences between males and females were found in some traits by testing for significant differences in regression line slopes using a two-tailed t-test with the standard error and degrees of freedom equalling N-2 (Table 2).

Curves in both sexes were fitted to the relationship of total length to dentinal GLG by John Henderson (National Marine Fisheries Service, La Jolla, California) using Laird's single phase growth model (Laird, 1969) (Fig. 12). The total length growth curves given by Perrin, Coe and Zweifel (1976) for males and females of *Stenella attenuata* which were fitted to Laird's two-phase growth model are also shown in Figure 12 for comparison with the *P. phocoena* curves. The two-phase model was unstable when attempted for total length of *P. phocoena.*

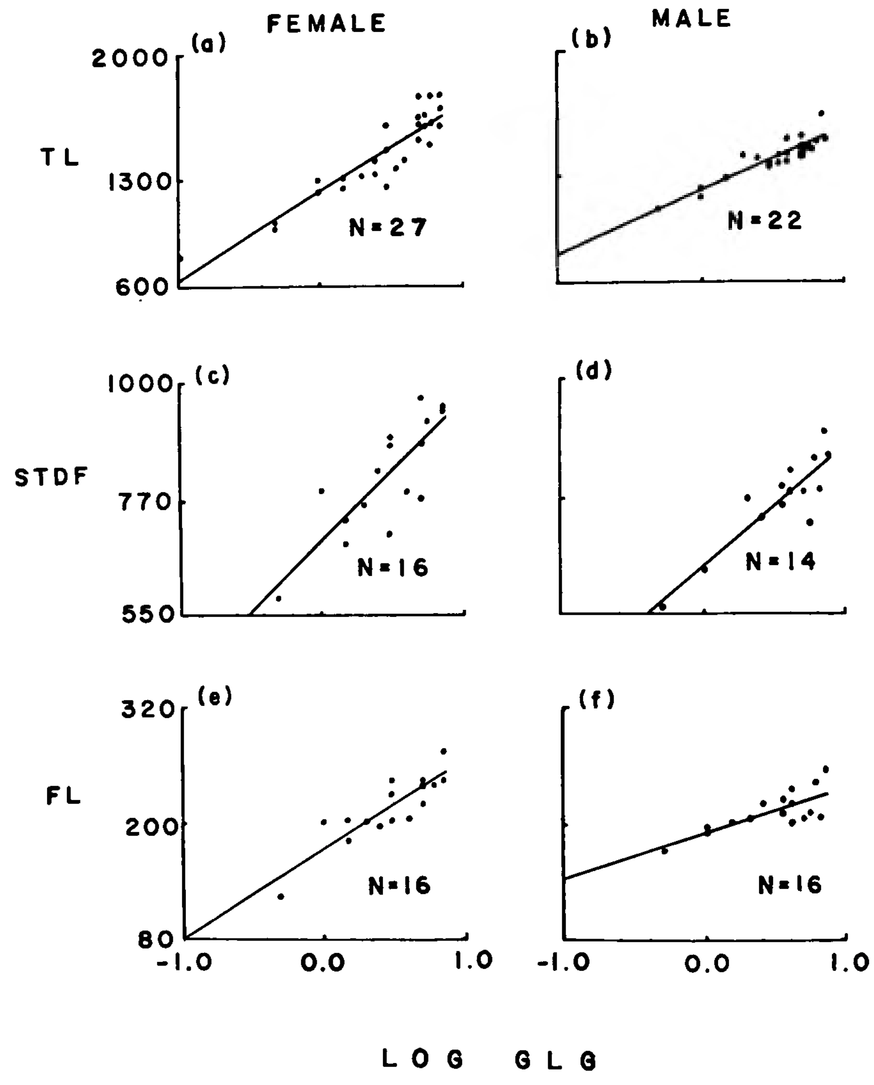

Fig. 5. Relationships of log GLG to total length (TL), snout to tip of dorsal fin (STDF), and flipper length (FL) from females (left) and males (right) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Equations of lines are: TL females, y = 528.9x + 1179.1; TL males, y = 377.5x + 1157.2; STDF females, y = 275.4x + 694.3; STDF males, y = 228.3x + 643.5; FL females, y = 92.7, + 173.6; FL males, y = 45.9 + 190.8.

Laird's model is

$$L(t) = L_0 \exp\left\{\frac{a}{\alpha}[1 - \exp(-\alpha t)]\right\},$$

where

$L(t)$ = length at time *t*
L_0 = length at birth (82.5 cm for *S. attenuata* and 75.0 cm for *P. phocoena*)
t = time (number of growth layer groups)
a = specific rate of exponential growth
α = rate of decay of exponential growth.

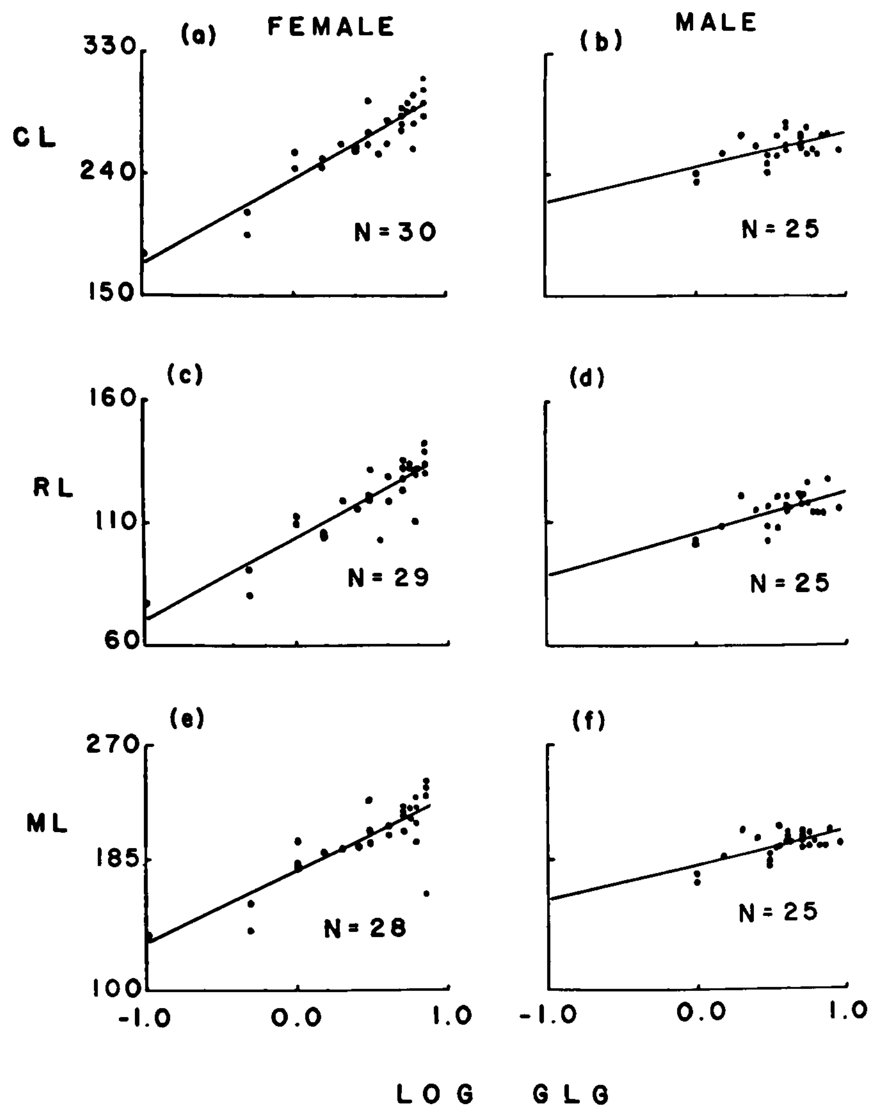

Fig. 6. Relationships of log GLG to cranial length (CL), rostral length (RL), and mandibular length (ML) from females (left) and males (right) of *Phocoena phocoena* from the northeastern Pacific. Measurement in mm. Equations of lines are: CL females, y = 64.7x + 237.2; CL males, y = 25.9x + 245.9; RL females, y = 33.6x + 103.6; RL males, y = 17.1x + 105.8; ML females, y = 50.4x + 182.7; ML males, y = 24.1x + 187.1.

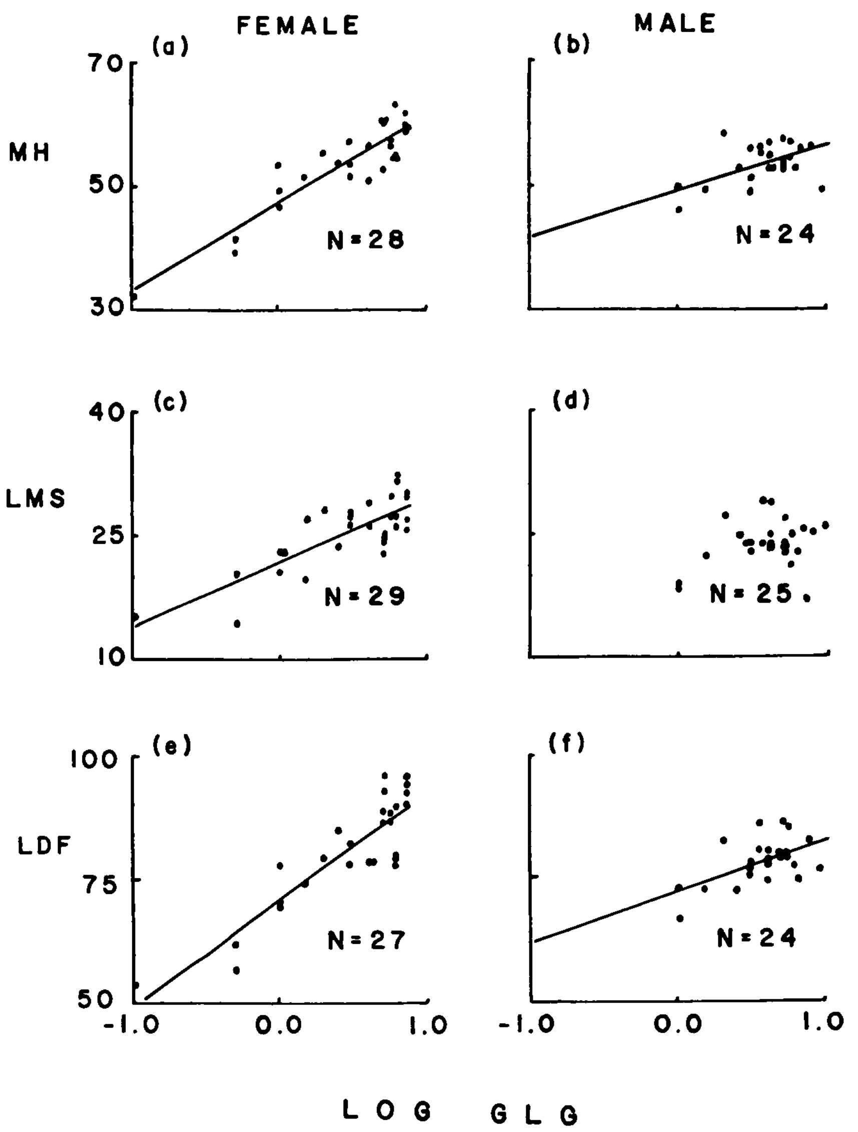

Fig. 7. Relationships of log GLG to mandibular height (MH), length of mandibular symphysis (LMS), and length of dental foramen (LDF) from females (left) and males (right) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Equations of lines are: MH females, y = 14.2x + 47.3; MH males, y = 7.3x + 49.0; LMS females, y = 7.8x + 21.8; LMS males, y = 3.6x + 21.7; LDF females, y = 21.8x + 71.0; LDF males, y = 10.4x + 72.6.

Table 2

List of growth statistics for each measurement in *Phocoena phocoena* from the northeastern Pacific
For *, P = 0.05; **, P = 0.01; ***, P = 0.001; ns = not significant

Measurement	Average growth rate (mm/yr) Female	Male	Correlation coefficient, r for measurement vs log GLG Female	Male	Level of significant difference (P) in growth between females and males
TL	95	55	0.914***	0.917***	<0.10
STDF	42	32	0.812***	0.889***	>0.40
FL	13	7	0.872***	0.748***	<0.10
CL	11	2.5	0.918***	0.565**	<0.05
RL	6	1.8	0.887***	0.555**	<0.05
ML	8.4	2.3	0.820***	0.618***	<0.10
MH	2.4	0.6	0.907***	0.531**	<0.10
LMS	1.3	–	0.804***	0.307 ns	–
LDF	4	1	0.887***	0.555**	<0.10
SW	7.2	1.7	0.912***	0.644***	<0.01
IW	2.6	0.5	0.851***	0.365*	<0.10
RW	4	2	0.890***	0.787***	<0.40
PTL	2	0.6	0.851***	0.455*	<0.40
FCO	4.2	1	0.925***	0.520**	<0.05
HBP	1.6	–	0.910***	0.126 ns	–
L1R	8	5.6	0.928***	0.897***	<0.01
LLR	16	10	0.928***	0.916***	<0.05
SH	9	5	0.935***	0.876***	<0.10
GWBTH	5.5	2.2	0.843***	0.504***	<0.40
GW23V	16	9	0.933***	0.906***	<0.05

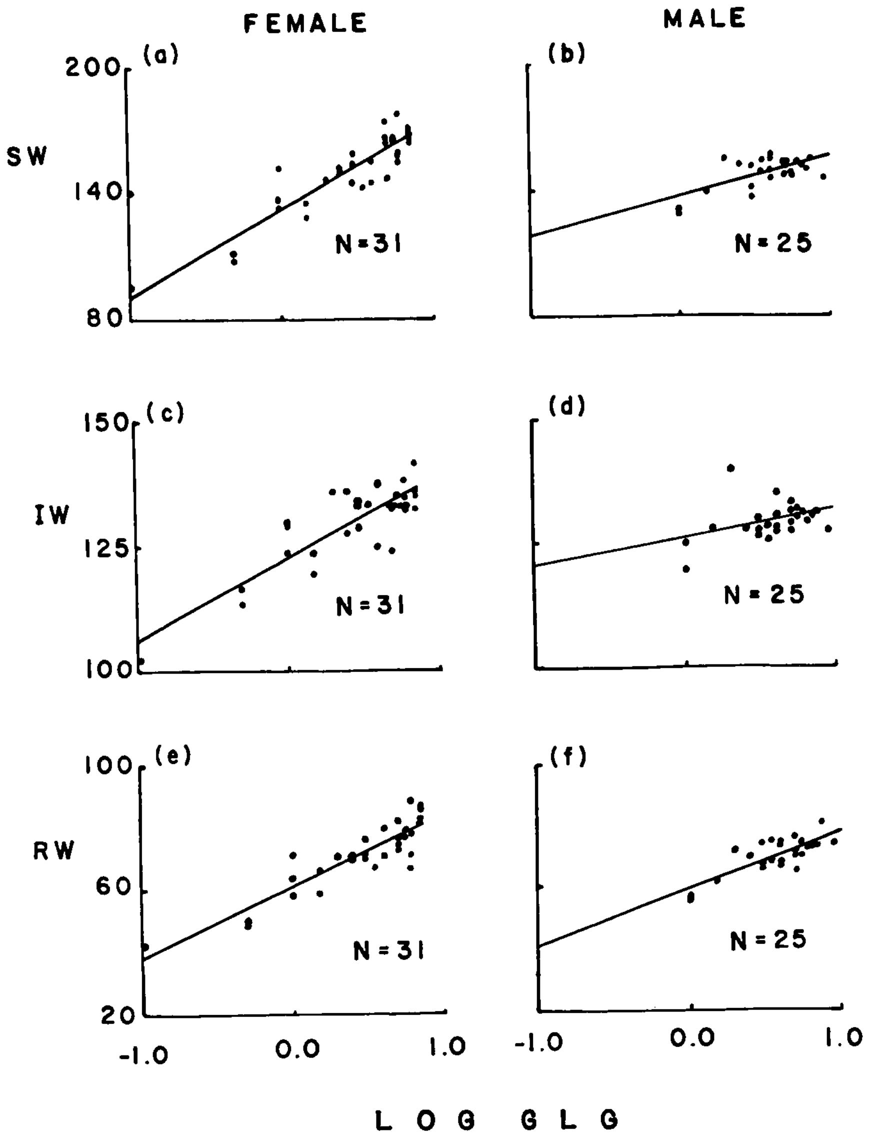

Fig. 8. Relationships of log GLG to squamosal width (SW), interparietal width (IW), and rostral width (RW) from females (left) and males (right) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Equations of lines are: SW females, $y = 41.2x + 131.7$; SW males, $y = 18.5x + 137.3$; IW females, $y = 16.7x + 122.8$; IW males, $y = 5.4x + 126.2$; RW females, $y = 22.9x + 61.7$; RW males, $y = 18.1x + 59.7$.

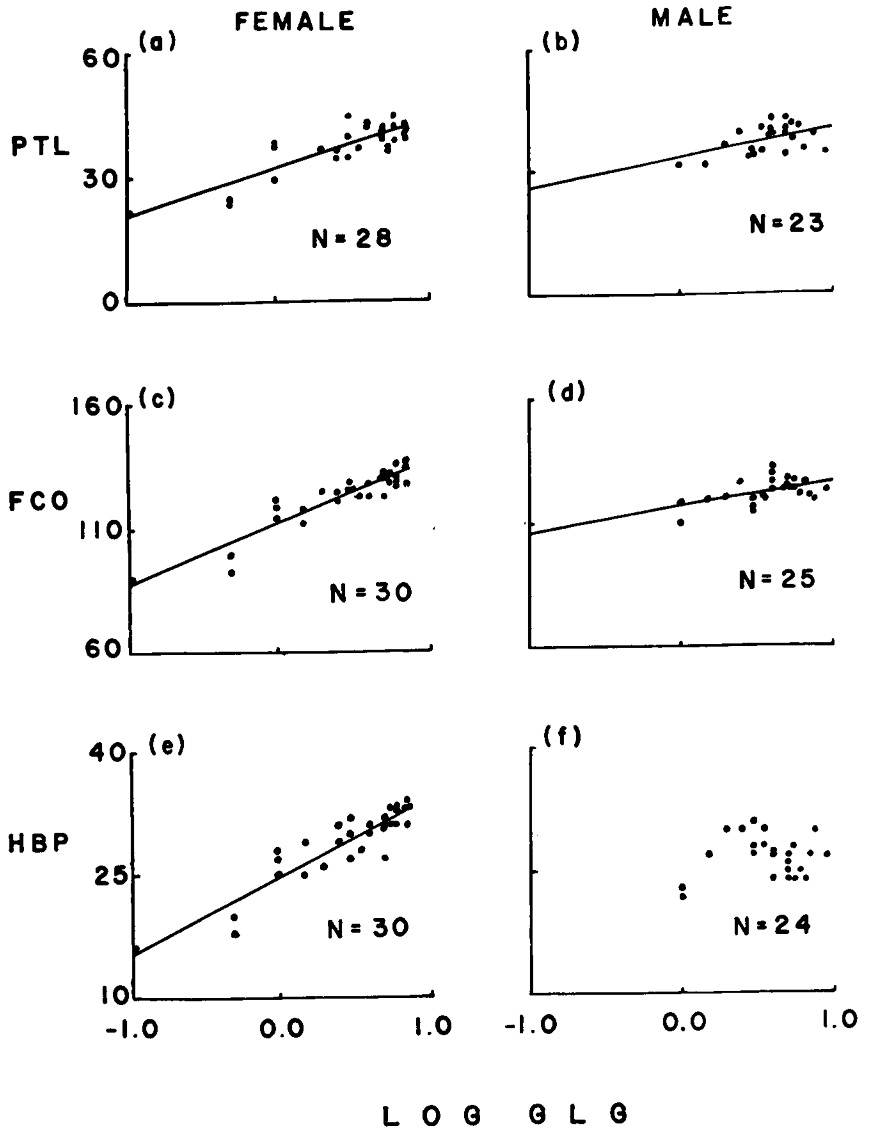

Fig. 9. Relationships of log GLG to pterygoid length (PTL), frontal crest to occipital condyle base (FCO), and height of basioccipital processes (HBP) from females (left) and males (right) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Equations of lines are: PTL females, $y = 11.5x + 32.2$; PTL males, $y = 7.0x + 33.2$; FCO females, $y = 24.5x + 111.9$; FCO males, $y = 10.4x + 116.5$; HBP females, $y = 9.4x + 24.8$; HBP males, $y = 1.2x + 26.0$.

For another representative characteristic showing sexual difference in growth pattern, the character length of longest rib was fitted to the von Bertalanffy growth model according to Gulland (1969) (Fig. 13). The von Bertalanffy model is

$$l_t = L_\propto [1 - e^{-K(t-t_o)}]$$

where

l_t = length at time t
$L_\propto$ = asymptotic length
K = growth constant
t = age (number of growth layer groups)
t_o = theoretical age at which $l = 0$.

RESULTS

All measurements of traits were significantly related to log GLG except for two (LMS, HBP), which were related to log GLG in females but not in males. Relationships of log GLG to external, cranial, and post cranial traits were shown in Figure 5, Figures 6–9, and Figures 10 and 11, respectively.

Seven traits (CL, RL, SW, FCO, LIR, LLR, GW23V) showed sexual differences in the slope of the relationship to log GLG at the 0.05 significance level or better. Seven measurements (TL, FL, ML, MH, LDF, IW, SH) showed a sexual difference in slope at the 0.10 level indicating a probable difference between sexes (see significance level discussion in Gilbert, 1973). Three characters (RW, PTL, GWBTH) showed a difference between males and females only at the 0.40 level (not significant), and one trait (STDF) showed no sexual difference even at the 0.40 level.

The average growth rates of characteristics for both sexes are shown in Table 2. All sexually dimorphic traits studied showed faster growth rates among females. Among the external traits, total length (TL) increased the fastest (95 mm/year for females and 55 mm/year for males); among the cranial traits cranial length increased at the fastest rate (11 mm/year for females and 2.5 mm/year for males); and of the postcranial traits length of longest rib (LLR) and greatest width of twenty-third vertebra (GW23V) grew fastest (16 mm/year for females and 9 and 10 mm/year for males). Two traits (LMS, HBP) grew at less than 2 mm/year in females and were not significantly related to estimated age in males. Arranged in order of decreasing rate of growth, six of the traits with the fastest growth rate were TL, STDF, LLR, GW23V, FL and SL.

Our single-phase fit of total length with dentinal GLGs was compared with the two-phase pattern of growth in total length as seen for *S. attenuata* (Perrin *et al*, 1976) (Fig. 12). The female *P. phocoena* total length curve approximates the *S. attenuata* curve for both sexes during the juvenile growth phase (before the convergence point of the two growth phases in *S. attenuata* is reached) beyond which few *P. phocoena* data points exist (Fig. 1). It is of course possible that a larger sample size would reflect an adolescent growth spurt (second phase of two-phase model), at an earlier age than in *S. attenuata*.

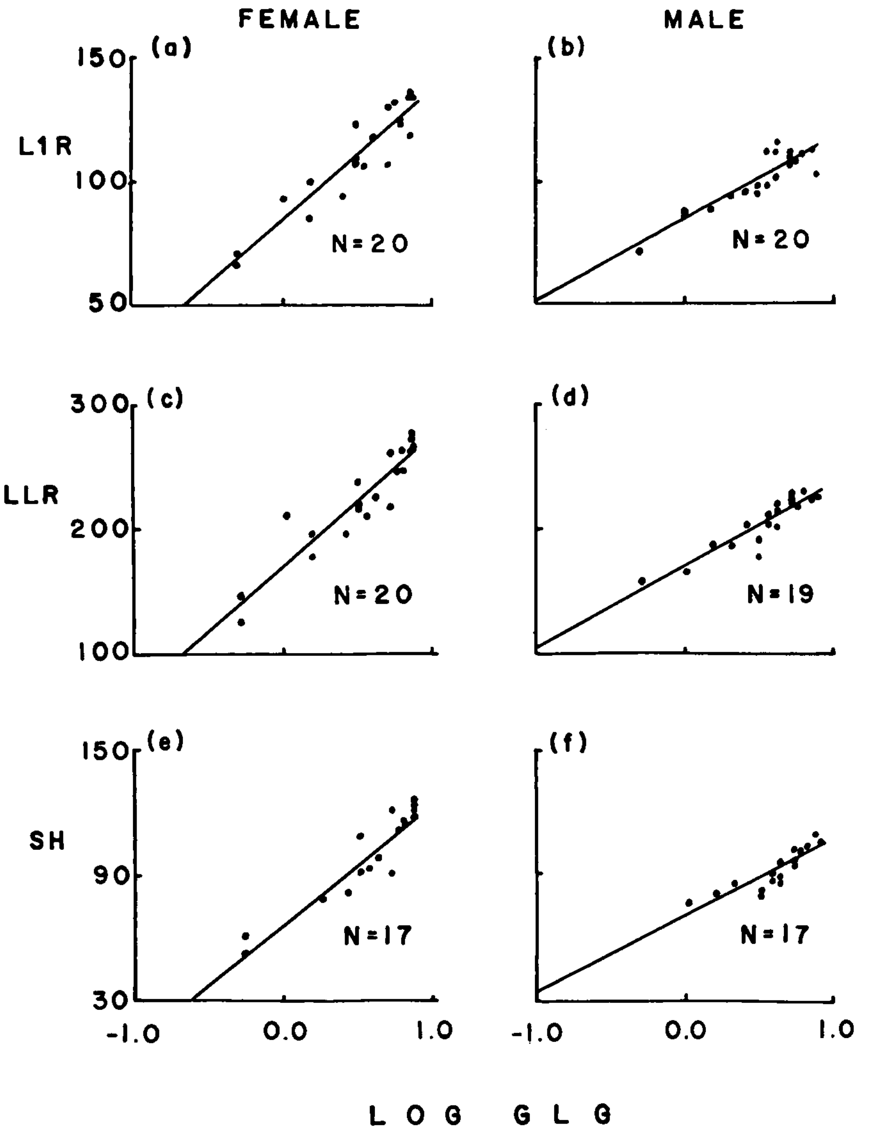

Fig. 10. Relationships of log GLG to length of first rib (LlR), length of longest rib (LLR), and scapular height (SH) from females (left) and males (right) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Equations of lines are: LlR females, y = 53.7x + 84.6; LlR males, y = 33.3x + 84.5; LLR females, y = 106.0x + 172.4; LLR males, y = 66.5x + 171.9; SH females, y = 58.0x + 69.7; SH males, y = 38.0x + 71.1.

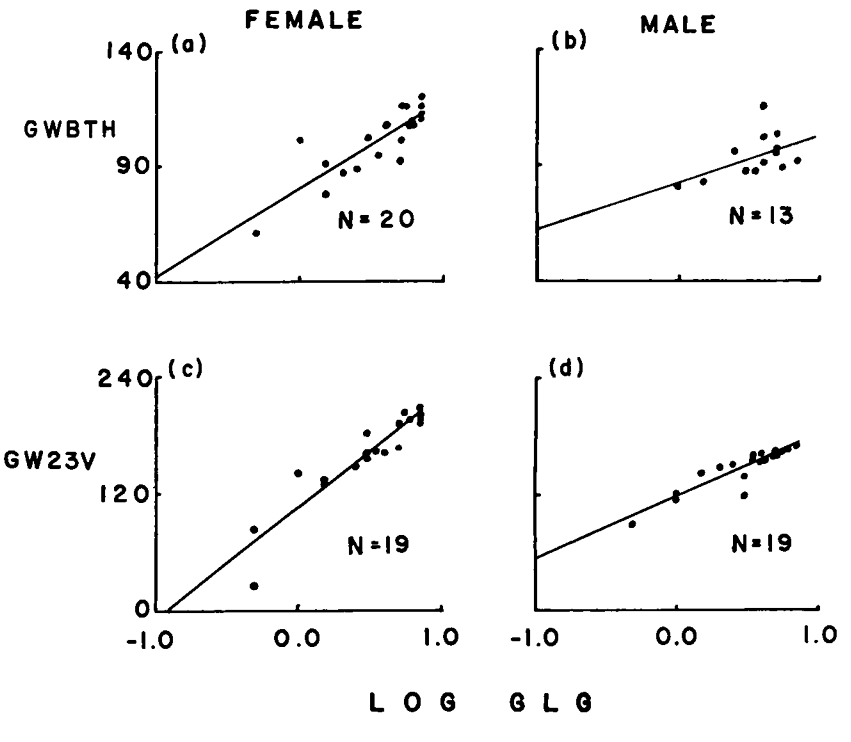

Fig. 11. Relationships of log GLG to greatest width of basi-thyrohyal complex (GWBTH) and greatest width of 23rd vertebra (GW23V) from females (left) and males (right) of *Phocoena phocoena* of the northeastern Pacific. Measurements in mm. Equations of lines are: GWBTH females, y = 38.1x + 80.1; GWBTH males, y = 19.7x + 83.4; GW23V females, y = 114.7x + 105.6; GW23V males, y = 64.0x + 118.6.

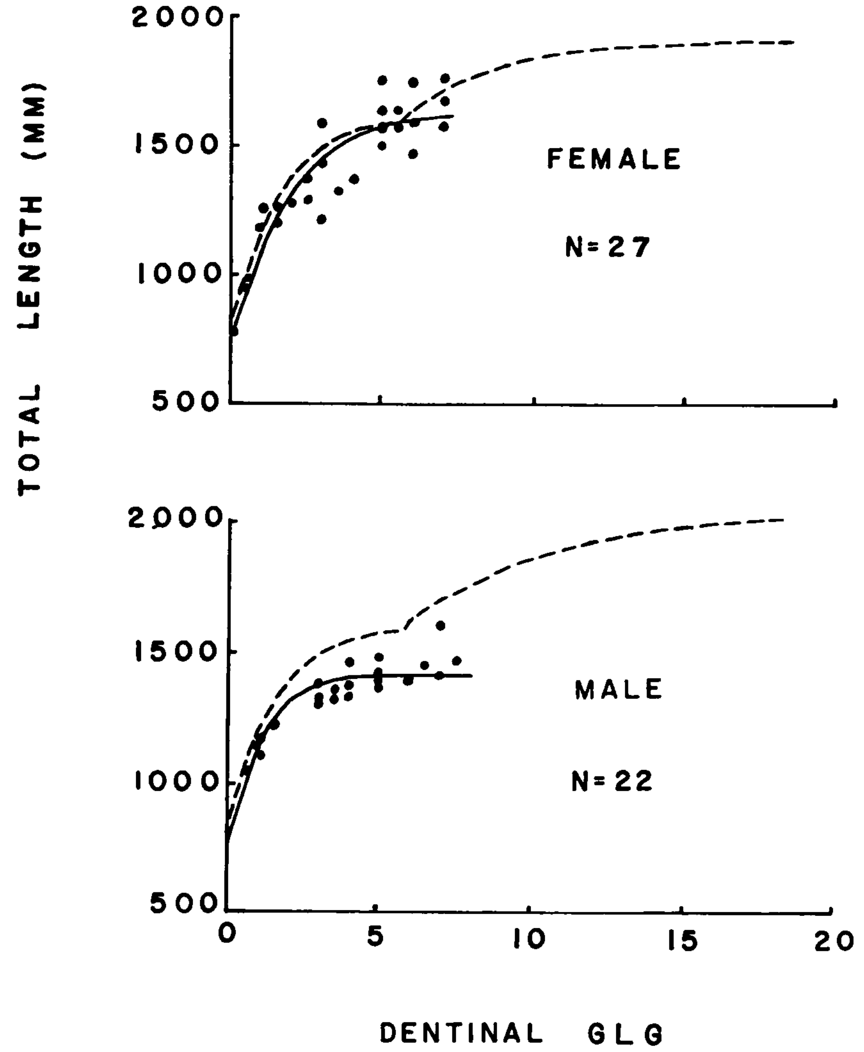

Fig. 12. Scatter plots of total length on number of dentinal GLGs for females (top) and males (bottom) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Solid line curves are fit using Laird's one-cycle growth model. Total length curves of *Stenella attenuata* which were fitted using Laird's two-cycle growth model are indicated by a dashed line (taken from Perrin, Coe, and Zweifel, 1976).

In sexually dimorphic characteristics, females tended to attain larger sizes than males. An example of this is our fit of the von Bertalanffy growth model to the character, length of longest rib (LLR) (Fig. 13). Females grow slower at first, but their rate of growth decreases much later than in males. This results in a longer growth period and the attainment of larger sizes by females of *P. phocoena* of the eastern Pacific.

DISCUSSION

Female harbour porpoises have been known to attain greater lengths than males (Mohl-Hansen, 1954; van Bree, 1973). Fisher and Harrison (1970, p. 482) indicated that their data were '. . . insufficient to state whether there is definite sexual dimorphism in size at sexual maturity or whether the male grows more slowly.' Our results suggest that males are smaller at sexual maturity and that their growth is slower and of shorter duration than that of females. Our correlations of total length with dentinal GLGs, as well as those of Gaskin and Blair (1977), suggest that the sizes at sexual maturity predicted by Fisher and Harrison (1970) (males 133 cm; females 145 cm) correspond to two to three and four to five dentinal GLGs for males and females, respectively. We have established that females have a greater total length at three GLGs and thereafter than do males of the same age, suggesting that sexual dimorphism in total length does exist at sexual maturity. This is also supported by the findings of Gaskin and Blair (1977) that both males and females are mature at four growth layer groups or older, at an average total length of 140 cm for males and 150 cm for females.

Many external, cranial, and post cranial traits are shown here to be sexually dimorphic in development. Measurement STDF (snout to tip of dorsal fin) shows no sexual dimorphism as such. Apparently, attainment of larger body size in females (Table 2) is due to more prolonged and rapid growth in the region posterior to the dorsal fin, perhaps to accommodate the fetus during pregnancy.

The absence of sexual dimorphism in PTL, RW, and GWBTH is common to *P. phocoena* and *S. attenuata*; however, fusion of the basihyal to the thyrohyals occurs much earlier in *P. phocoena* (at the latest, two growth layer groups) than in *S. attenuata* in which Perrin (1975, p. 52) states it '... occurs quite late in development' (six growth layer groups at the earliest).

We compared total length curves for *P. phocoena* with those constructed by Perrin *et al* (1976) for *S. attenuata*. In *S. attenuata*, body lengths are greater and sexual maturity is reached later (at about five GLGs) than in *P. phocoena*. In *P. phocoena*, sexual maturity is reached at three or four years (Fisher and Harrison, 1970) or four GLGs (Gaskin and Blair, 1977). In *S. attenuata*, males attain greater body lengths than females (Perrin *et al*, 1976) and individuals of both sexes are longer lived than those of *P. phocoena*. Of the 84 specimens of *P. phocoena* for which we counted GLGs, the greatest number of GLGs was ten (one specimen). This compares with a maximum of eight GLGs found by Nielsen (1972) and 13 GLGs found by Gaskin and Blair (1977). Although occlusion of the pulp cavity has not been observed, GLGs become relatively thinner with increasing age and are thereby difficult to count in older animals.

Our total length growth curve (Fig. 12) for *P. phocoena* females is based on a smaller sample size, but there is a general similarity to the juvenile growth phase in both sexes of *S. attenuata* (Perrin *et al*, 1976). The curve for *P. phocoena* males, however, shows more retardation in growth in comparison to the females after two growth layer groups. According to the one-cycle Laird growth curve (Fig. 12) males appear to cease growth after three growth layer groups, whereas females continue to grow until approximately five dentinal growth layer groups.

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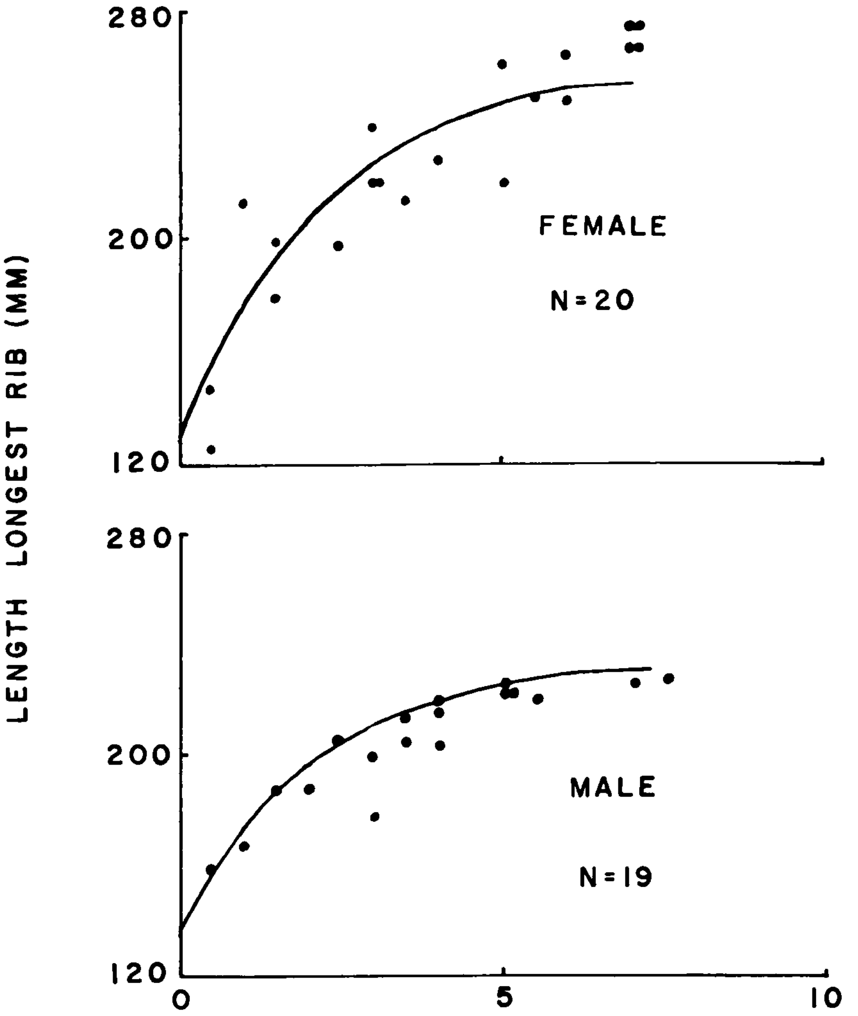

Fig. 13. Scatter plots of length of longest rib (LLR) vs dentinal GLGs for females (top) and males (bottom) of *Phocoena phocoena* from the northeastern Pacific. Measurements in mm. Curves are fitted using the von Bertalanffy growth model (Gulland, 1969).

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Age of Reputedly Old Killer Whale, *Orcinus orca*, 'Old Tom' from Eden, Twofold Bay, Australia

Edward Mitchell
Arctic Biological Station, Ste. Anne de Bellevue, Province of Quebec H9X 3R4, Canada
and
Alan N. Baker
National Museum, Wellington, New Zealand

ABSTRACT

The socially mature killer whale *'Old Tom'* described in the literature as a participant with whalers in symbiotic herding and killing of humpback and right whales off Twofold Bay, eastern Australia, has been referred to as the oldest killer whale in the world. A whale identified as *'Old Tom'* died in 1930, and the skeleton was preserved in the museum in Eden, Australia. We have been unable to locate any photographs of *'Old Tom'* prior to one taken from a movie film *circa* 1910–1912; the documented life span would then have been a minimum of approximately 19 years. Adding to this an estimate of 16 years for the age of social maturity, the estimated life span from the historical data would be approximately 35 years.

We examined a right lower tooth from *'Old Tom,'* and counted up to 25 dentinal layers, and approximately 10 additional cemental layers deposited after pump cavity closure. Estimates of age based on a count from dentinal and part of the cemental layers is therefore 35 Growth Layer Groups, to be taken in the present state of knowledge as 35 years. We conclude that *'Old Tom'* at death was closer to 35 years old, not the 50 – 90 years previously reported.

INTRODUCTION

A remarkable 'symbiotic-like' relationship between herds of killer whales and shore whalers at Eden on the southeast coast of Australia, has been the subject of numerous stories since the late 1800s. The killer whales were reputed to have alerted whalemen to the approach of humpback and right whales, and to have harassed and herded the prey into positions for convenient attack by the whalers.

Many of the killer whales were individually recognized, and given nicknames by the whalers. One such killer was *'Old Tom,'* a socially mature bull with a slight but noticeable deformation on the posterior margin of its tall dorsal fin. Published stories, and local folklore gave *'Old Tom'* an age of between 50 and 90 years at his death in 1930, thus making it supposedly the oldest killer whale in the world.

In June 1977, we visited Eden and examined a complete skeleton, reputedly of *'Old Tom,'* now preserved in the Eden Museum, at Twofold Bay, New South Wales, Australia. With the help of Mr Alan Whiter, we measured and photographed the skeleton labelled *'Old Tom,'* and extracted one tooth. We talked briefly with Mrs Mary Mitchell, the daughter of C.E. Wellings, one of the historians of the killer whales of Eden. We report our observations here.

PUBLISHED ACCOUNTS

Shore whaling at Twofold Bay

Some accounts indicate *'Old Tom's'* activities date from the earliest days of Eden whaling, but this is not borne out by a review of the literature. The town of Eden, in Twofold Bay, New South Wales, Australia, was established due to activities of early whalers in the 1830s and 1840s (*cf* Dakin, 1934, p. 94; Dunbabin, 1925, p. 31). Continued reports of whales at the Bay, and of natives utilizing them led Capt. Thomas Raine of Sydney to initiate shore whaling there in 1818 (Goddard, 1940) or in 1828 (Dakin, 1934, p. 43; Wellings, n.d., *a* (1970?), p. 37). There is little information about subsequent shore whaling at Twofold Bay (e.g. Dunbabin, 1925, p. 30) until the activities of the three Imlay brothers in the 1830s (*cf* Wellings, 1931; Dakin, 1934, p. 44; Wellings, 1964, p. 2). Benjamin Boyd began both deep sea sperm whaling, and shore whaling for right whales and humpbacks there, and by 1843 (Watson, 1907; Jauncey, 1918) he was competing with the Imlay brothers (Dakin, 1934; Wellings, n.d., *b*, p. 42; King, 1925, 1929; Anon, 1948). Shore whaling continued under various adventurers. The Alexander Davidson family (Mead, 1962) commenced whaling with Boyd in 1846 (Wellings, 1964, p. 2), and independently in 1866 (Dakin, 1934, p. 135). George Davidson, the third generation of Davidson whalers and the last of the shore whalers, ceased whaling in 1929 (Wellings, 1964, p. 2) or 1932 (Mead, 1962, p. vii).

Killer whales at Twofold Bay

The published stories about the killers of Eden range from laymen's accounts of behaviour known from other regions and species (standing by, succorant, attack, feeding, lobtailing and breaching, etc.), through fanciful stories in which the killers, having failed to raise the shore whalers by lobtailing or breaching signals, placed oyster shells in their blow holes to make a siren-like whistling sound (Stead, 1933, p. 44), to the more critical, objective writings of Dakin (1934). Mead's account (1962) is presented in part in fictionalized form, and sources are not well cited.

Killer whales are apparently not common along the South Australian coast, as judged from strandings and museum records (e.g. Jones, 1925; Cotton, 1943; Aitken, 1971). They are sighted irregularly off the Queensland coast (Bryden, 1978), but are seasonal migrants along the southeast and east coasts. They have been associated with shore whaling efforts at Twofold Bay since the inception of bay whaling there. Killer whales migrated into the area before the northward migration of right and humpback whales from southern waters. The killers remained throughout the whaling season, June to November (Mead, 1962, p. 5). Dakin (1934, p. 145) found diaries (in the Mitchell Library, Sydney) written by the artist Oswald Walters Brierly who spent five years at Twofold Bay, which describe killer activity there in 1843.

On one whale hunt which Benjamin Boyd accompanied, one of the eight whaleboats engaged in towing was stationed behind the dead whale 'to keep the "killer-whales" from taking too great a toll of the carcass as payment for their services in the chase' (Wellings, n.d., *a*, p. 47; n.d., *b*, p. 47). None of these accounts mentions individual killer whales.

Little was published about the killer whales in the 19th century, and there are few records purporting to identify individuals among them. According to C.E. Wellings (1944, p. 291) 27 'killers lived as three mobs or families, known then [1878] as "Hookey's," "Stranger's," and "Cooper's" mobs.' But stories of their behaviour circulated and became incorporated into contemporary published accounts, one of the best of which is 'Whaling at Eden, with some "Killer" yarns' by Hawkins and Cook (1908). These authors described in detail the attack and feeding behaviour of the killers on right whales. The whalers also apparently disentangled killer whales fouled in lines (Hawkins and Cook, 1908, p. 270).

The cooperation between the human whalers and the killers was mentioned in a zoological work (Lucas and Le Souëf, 1909, p. 58). A film was made of the shore whaling at Twofold Bay, by C. E. Wellings, during his 'residence at Eden and during the years 1910 and 1912,' but the film 'never reached publicity and enquiries in present years have resulted in no trace being found of the film' (H. P. Wellings, 1964, p. 14). H. P. Wellings saw the film at a private showing in a Sydney cinema theatre in 1912 (H. P. Wellings, 1964, p. 14), and apparently it is from this film that some published photographs of the Eden killers have been printed (see below).

From this time, 'about 1912, the school of killers did not re-appear, only occasionally were one or two seen again; . .' (C. E. Wellings, 1944, p. 292). There are few published accounts of the killers' activities during the next few years. Then:

> In 1928 only two Killer whales, 'Old Tom' and 'Hookey,' were seen at Twofold Bay. In the two following years only 'Old Tom' appeared, and in September of the latter year (1930) he was seen one afternoon under conditions which seemed to indicate that things were far from well with him. The next day his carcass was discovered washed up on the beach. (Dakin, 1934, p. 146.)

The whaling activities of the Davidson family have been recounted in anecdotal form by Mead (1962; 1975), who stated that George Davidson 'thought Tom was about ninety years old,' because 'Tom had apparently been about since' Ben Boyd's time in the 1840s (1962, p. 2). In perhaps the most critical account, Dakin (1934, pp. 138–139; and see Dakin, 1938; 1963; 1977) concluded that the Twofold Bay killers provided evidence for the homing instinct in killers, 'an intelligent co-operation' between the killers and the whale hunters, and the first direct observation of the length of life of any cetacean to that date. Dakin interviewed some of the old whalers, was on the scene less than three years after *'Old Tom's'* death, and concluded that 'for over fifty years he has been watched at such close quarters as would make mistake impossible,' and that *'Old Tom'* might even have 'been recognized year after year for possibly eighty-odd years' (Dakin, 1934, p. 139).

These stories of the killer whales' behaviour, and statements of *'Old Tom's'* great age, have passed into the literature of cetological mythology. Sanderson (1956, pp. 329–330) accepted Dakin's estimate of more than 80 years age for *'Old Tom,'* as did Colwell (1964, pp. 77–81; 1970, pp. 86–89). Alpers (1961, pp. 162–169) and Gaskin (1972, pp. 119, 120–121) accept Dakin's estimate of age, but temper the accounts of behaviour with interpretations based on the known habits of odontocetes elsewhere. The famous symbiotic relationship between the killers and whale hunters has been cited (Busnel, 1973, p. 130n; Mitchell, 1975, p. 68) based on Dakin's and Mead's accounts.

In order to assess information on the historically documented occurrence of *'Old Tom,'* we attempted to determine: whether the killers were referred to individually in the early bay fishery, when *'Tom'* or *'Old Tom'* was first mentioned in contemporary descriptions of the fishery, and the specific evidence used in identifying the carcase found on 17 September 1930 as that of *'Old Tom.'* Our chronological survey of the literature follows.

Fig. 1. – 'A Whale Killer *"Tom"* at Eden, N.S.W.,' Australia, photograph by or from C. E. Wellings, from a 'Kodak Austral' postcard, undated.

(Reproduced as: Pl. 33 in Dakin, 1934 (and see p. 140), where projection is referred to as on 'front edge (sic) of dorsal fin;' and carried through with same caption in Dakin, 1938, 1963, 1977; on pl. 15 in Wellings, 1944; and pl. oppos. p. 6 in Mead, 1962.) In all these, the photograph is somewhat cropped, and in the Mead plate the posterior 'tubercle' on the dorsal fin does not show (perhaps the result of retouching?).

Identity of Twofold Bay killers

We suspect that there may be a wealth of information, including photographs, in local newspaper and magazine accounts, but we have been unable to locate readily more than those enumerated below chronologically. Some dates (e.g. for George Davidson) are given to aid in identifying the date of other events in Mead (1961, 1962, 1975) which is a reconstruction of the fishery based on interviews with Davidson. This interesting episode of southern Bay Whaling clearly merits more attention by historians and cetologists.

1828 – Thomas Raine of Sydney initiated Twofold Bay shore whaling (Dakin, 1934, p. 43; Wellings, n.d.; 'John' Raine, *fide* Mead, 1962, p. vii; and see Goddard, 1940, p. 282, '1818').

1831–1838 – Imlay brothers were shore whaling at Twofold Bay (Dakin, 1934, p. 44; Wellings, 1931, 1945, p. 12, 1964; and see Dunbabin, 1925, p. 30).

1843 – Benjamin Boyd engaged in shore whaling at Twofold Bay in competition with Imlay brothers' operation (Dakin, 1934; Wellings, 1936, n.d. (2nd ed.), n.d., *b*, pp. 42–47; see Jauncey, 1918, and Watson, 1907). Oswald Brierly observed killer activity associated with shore whaling (diary quoted above from Dakin, 1934, p. 145), and painted the scene (Dakin, 1934, *frontis*; Anon, 1948).

1846 – Alexander Davidson began whaling for Boyd (Dakin, 1934, p. 135; Mead, 1962, p. vii).

1864 – George Davidson born (d. 1952; Mead, 1962, p. vii, photo at age 86, oppos. p. 23; the son of John Davidson, grandson of Alexander Davidson; information useful in interpreting Mead's account).

1866 – Alexander Davidson began whaling independently, with son John as a crewman (Dakin, 1934, p. 135; Mead, 1962, p. vii).

1871 – Contemporary, brief mention of Twofold Bay whaling did not refer to killers (Anon, 1871).

1870s – Reference to *Tom, Jackson, Humpy, Ben, Hookey, Stranger* as aiding John Davidson when 'George Davidson, a sturdy little figure in short pants' was a school boy (Mead, 1962, pp. 5–7). (The aboriginal crewmen believed that any of their fellow crewmen killed during whaling would reappear as a killer whale. Newly appearing killers were thus christened with the name of the last killed whaler, 'thus "Tom" after a deceased crew member of their boats. "Ben" and "Old Ben" both recalled other crewmen' (H.P. Wellings, 1964, p. 12).)

1878 – George Davidson started whaling at age 14 (Mead, 1962, pp. 11, 15, 18). The killers were then segregated into three pods, known as *Hookey's, Stranger's* and *Cooper's* 'mobs,' totalling 27 animals altogether (C. E. Wellings, 1944, p. 291).

1879 – *Tom* and *Old Tom* used interchangeably in Mead's account (1962, pp. 62, 66). *Tom* and *Hookey* aided in George Davidson's second whaling season (Mead, 1962, p. 61).

1881 – Reference in Mead (1962, pp. 61, 66) to *Tom* and *Hookey*, with distinctive fins, 'one sharp-pointed with a little round knob and the other just an ordinary fin dropping sideways.'

1895 – Cooperation or use of killers in fishery cited but no mention of individual whales (Anon, 1946, 'From Bega Standard 20/8/1895').

1901 – *Typee* and a grampus it had attacked with the killer pack stranded on shore, and were killed by a local resident (C. E. Wellings, 1944, p. 291). (A photograph of *Typee*, Wellings, 1944, pl. 15, if contemporary with the 1910–1912 film, raises a discrepancy here.)

1904 or earlier – *Old Tom* cited as leader of pack including *Humpy, Hookey, Stranger, Jackson* and *Old Ben* (Mead, 1962, p. 2).

1904 – Killers involved in whale hunt included: *Tom, Stranger, Humpy, Hookey, Cooper, Charlie, Kinscher* (Mead, 1962, p. 126); a poor year, so *Tom* and *Hookey* were fed fish from rocks (Mead, 1962, pp. 113, 121); standing-by behaviour on part of *Tom* when George Davidson was dragged overboard by harpoon line (Mead, 1962, pp. 126–128); and *Jackson* killed on shore by Harry Silks (Mead, 1962, pp. 123–125). The killers chased and beached a fin whale in the fall of 1904 season (Mead, 1962, pp. 136–140). (A photograph of *Jackson* in Wellings, 1944, pl. 15, is undated – if it is from the 1910–1912 movie, this raises a discrepancy; see below.)

1905 – Author Mead has character George Davidson saying, 'All I know is that old Tom has been seen here every season for the last fifty years at least' (1962, p. 151; although contemporary accounts cited above do not mention *Tom* by name).

1906 – *Tom, Stranger* and *Humpy* mentioned as working a whale, 'hanging to its lips' (Mead, 1962, p. 188). First reported season *Tom* grabbed harpoon line with teeth (Mead, 1962, pp. 183–184; both *Tom* and *Stranger* grabbed whale boat painter, earlier?, Mead, 1962, p. 147).

1907 – The *Stranger* was killed by a Botany fisherman in August (Stead, 1933, p. 40).

1908 – A contemporary account for 1908 (or the 1907 season?) did not cite *Tom*:

> Nearly all the killers are known by name; there is 'Hooky,' 'Walker,' 'Big Jack' and 'Little Jack,' 'Skinner,' 'Humpy,' and many others. About thirty visit Eden yearly and have been known individually to the whalers for over 25 years. (Hawkins and Cook, 1908, p. 270.)

This and earlier accounts mentioning *Stranger* are apparently the basis of Dakin's remarks:

> There is 'Old Tom' once again – you can spot him by that peculiar mark on the front of his fin – and there is 'Hookey' – no mistaking 'Hookey,' his high dorsal fin has got a bend in it where probably it was 'belted' by a whale in some great fight. Then 'Humpy' is recognized; and 'Stranger.' But these are only the best known, there used to be twenty-five or more. (Dakin, 1934, p. 140.)

1909 – Contemporary account mentioned *Old Humpy, Stranger* (pack leader), *Jackson, Kinchen, Tom, Cooper* and *Hookey*, catching right and humpback but seldom finback whales, and reference to *Old Humpy* freed from entanglement in line by George Davidson (Brady, 1909, *cf.* 1910).

1910–1912 – C. E. Wellings shot movie film of killers in 1910 and in 1912; movie shown privately, subsequently lost or destroyed (H. P. Wellings, 1964, p. 14). Photographs of *Old Tom* may be from this film (see below).

1912 – 'When the whaling industry languished, about 1912, the school of killers did not re-appear, only occasionally were one or two seen again; . .' (C. E. Wellings, 1944, p. 292; but see Mead, 1962, pp. 191–193).

1919 – In July, *Tom* snatched the harpoon line in his mouth and pulled it out of the boat (Stead, 1933, p. 42).

1926 – The pack consisted of *Old Tom, Humpy, Hookey*, the *Kinscher, Charlie* and young *Ben*. 'Humpy and Hooky were showing signs of age, though strangely enough Tom seemed to retain perpetual youth' (Mead, 1962, pp. 196–197, and see pp. 205–206). (Photographs of *Hookey* include: Wellings, 1944, pl. 15; Mead, 1962, oppos. p. 6; no dates given.) Davidson whaling sporadically (Dunbabin, 1926).

1928 – No whales captured at Twofold Bay subsequent to 1928; only three killers frequented whaling area during preceding 4–5 years; *Old Tom* recognizable 'possibly owing to a broad greenish white band which encircled his body behind the long dorsal fin' (Wellings, 1945, p. [11]).

1929 – Description of 'Night whaling at Twofold Bay' (Morgan, 1929) in which 'Tom, the largest, and one of the most active of the killers, seized the now loose whale line in his teeth,' attempted to restrain a harpooned whale. *Humpy* very feeble this season, and did not come back the next year (Mead, 1962, p. 208). ('Humpy . . . is believed to have been known for about seventy years, but I have no proof of this,' Stead, 1933, p. 43 and p. 40; see photographs of *Humpy*: Wellings, 1944, pl. 15; Mead, 1962, oppos. p. 22.)

1930 – *'Tom'* came back alone and moped for days, 'apparently no longer interested in how many whales were passing along the coast' (Mead, 1962, p. 208). 'On Wednesday, September 17th, 1930, a "killer" was seen floating dead in the bay, and upon investigation it was found to be "Tom," who evidently had died a day or two earlier' (C. E. Wellings, 1944, p. 292; Mead, 1962, p. 4, pl. oppos. p. 22).

> Whether this Killer washed ashore was really Tom or not, the local people at Eden seemed to have been quite sure of it, when they actually held a public meeting, soon after the Killer's death, to decide what should be done with his remains. It was then decided to erect a special building for the skeleton, . . (Stead, 1933, p. 39).

The photograph of the carcase (Mead, 1962, oppos. p. 22) shows the dorsal fin partly underwater, hence the 'scallop' and 'tubercle' if they were present on the fin are not visible, and no other published information is available to substantiate the identification. 'According to Logan and the whaler, Davidson (both most familiar with the killers at close quarters), there was no doubt about its being "Old Tom"' (Dakin, 1934, p. 146). George Davidson assisted in skeletonizing *'Tom.'* Measurements were: total length 22 ft, fluke width, tip to tip, 5 ft, dorsal fin height 5 ft 8 in, and flippers 4 ft long by 2 ft 10 in wide (Wellings, 1944, pp. 292–293; Mead, 1962, p. 208). The stomach was empty, and the carcase appeared emaciated (Mead, 1962, p. 208).

1932 – Shore whaling at Twofold Bay ceased (Mead, 1962, p. vii).

1933 and subsequently – Various accounts list the names of some of the killers known individually to the whalers. David G. Stead collected Eden killer stories for 30 years and reported the following individuals:

> Some of the names by which the principals have been well known, were as follows: Tom, Cooper, Humpy, Hookie, Walker, Skinner, Big Jack, Little Jack, Charlie Adgery, Kinchie (or the Kinchen), Big Ben, Stranger, Montague, Typee and Jimmy. In Humpy the great dorsal fin, instead of standing up vertically on the back, lies right over to one side, while Hookie has the same fin bent over into the form of a hook. It is affirmed that Humpy had been seen at Twofold Bay for over sixty years. Charlie Adgery was named after a well-known local black.
>
> *** While Tom and Humpy seem to have been the outstanding attackers, Charlie Adgery was also known for the great impetuosity of his movements, . . (Stead, 1933, pp. 40, 43).

Wellings (1944, p. 291) contributed information on the fate of some individuals:

> Several of them were easily recognised, chiefly by their fins, and were accordingly named. 'Tom' had a particularly high fin with an indentation near the top. 'Humpy's' fin was bent round almost touching his side. 'Hookey's' fin was bent to the right at an angle of about 45°. These fins, consisting of cartilage and not bone, probably were damaged by a whale's tail during an attack in early life. The following are names by which some were recognised in the early days of whaling, as far back as the year 1800. 'Hookey,' 'Humpy,' 'Stranger,' 'Cooper,' 'Tom,' 'Jackson,' 'Typee,' 'Kincher,' 'Jimmy', 'Albert,' 'Old Ben,' 'Young Ben.'
>
> Of these, several disappeared and young ones arrived. 'Old Ben' died off Mowara Point, 'Young Ben' later disappeared, 'Jimmy' fouled the buoy-line fast to a dead whale and was found drowned when the line was taken in next morning, 'Jackson' also fouled a buoy-line some years later, but being noticed in difficulty the whaling crew rescued him. The line was unwound by Geo. Davidson, the killer remaining quiet and then it 'floptailed' before making off.

Conclusion on identity – The published literature is sparse in contemporary documentation of individual animals. The first contemporary mention of *Tom* is in 1909. (*'Old Tom'* was the term used apparently for the same whale by Mead, 1962, pp. 62, 66; and was possibly first used by Dakin, 1934. There is no direct evidence of two whales, one a descendant of the other, although this remains a possibility.)

Identity of skeleton labelled *'Old Tom'* (tooth wear and behaviour)

One contemporary account mentioned the habit of some of the killers of Twofold Bay (see '1906', above) of pulling on harpoon lines, whale boat painters, and the anchor ropes of moored fishermen:

> It is not always safe to fish in the locality; several parties have found themselves being towed to sea at a rate not at all acceptable. The killers had caught hold of the kellick-rope and amused themselves, or showed resentment for intrusion in their domain. When a whale is caught and sunk, the schnapper are very thick round the carcass. Some very large hauls have been made with handlines, but one must not attempt to kellick – the killers will soon shift you . . . (Hawkins and Cook, 1908, p. 271).

Subsequent accounts, e.g. Wellings, summarized this and related it to *Old Tom's* dentition:

> 'Tom' developed a bad habit of getting across the slack line between the whale and boat, holding it under a fin and towing the boat in any direction. This habit was deliberate, and appeared to be mischief, though a nuisance. Even small boats whilst fishing have had the kellick line taken and the boat dangerously towed off. It was suggested that he at times took the line in his teeth and that the missing teeth in the top jaw were broken out in this way. However, upon examination of the skeleton, a well-known dental surgeon positively attributed their loss as the result of a severe abscess (Wellings, 1944, p. 292).

Dakin also commented on this:

> A great piece has been knocked out of one side of his jaw, he must have lost one or two teeth at the time of this accident, but the most striking feature is that all the teeth are worn down to rounded bosses, especially the front ones (Dakin, 1934, p. 146).

Few other wild odontocetes have been observed to pull consistently on lines – one is a wild bottlenosed dolphin, *Tursiops truncatus* seen to tug on mooring lines and tow small craft in Great Britain (Lockyer, 1978, p. 518; Webb, 1978).

In the skeleton labelled *'Old Tom,'* we observed evidence of an abscess anteriorly in the upper right tooth row, and found that one, possibly two teeth were missing from that area, and had been probably dropped before death. Such abscesses are known from other killer whales (e.g. Harmer, 1927, p. 83; Colyer, 1938; Fraser, 1946, p. 38; Yablokov, *et al.*, 1972, 1974, Fig. 50). The teeth on the remainder of the upper right tooth row, the lower right, most of the upper left and the posterior half of the lower right tooth rows were in relatively good condition. There was some evidence of wear facets on the anterior and posterior surfaces of tooth crowns caused by occlusion or by wear (see Eschricht, 1866; Caldwell and Brown, 1964). However, the most striking wear occurred on the upper left (?fourth), the lower left series 1–6, and the lower right 1–2, where the crowns were completely worn off, and the remaining pegs were worn flat, down to near the gum line (Fig. 3). The lower left seventh tooth has a large groove cut across the anterior surface at the base of the crown (Fig. 3).

In those specimens known where the crowns have worn off teeth, resulting in short, flat-tipped teeth, most or all of the teeth are so affected beginning anteriorly, and this usually but not always occurs after the body of the tooth has filled with dentine and the root canal is closed or barely patent (e.g. Eschricht, 1862, 1866, p. 172; Tomes, 1873, 1898, p. 377; Caldwell and Brown, 1964; Harmer, 1927, p. 83; Fraser, 1953, p. 27; Beltrami, 1943; Lilljeborg, 1861–1862, 1866, pp. 234–235; Gray, 1870, Fig. 1, same as Fig. 7 of Gray, 1871; Owen, 1840–1845, Vol. 1, p. 351; Tomilin, 1957, Fig. 126, 1967, p. 615; Carl, 1946; Dieren, 1931). Ulmer (1941) noted somewhat similar asymmetrical wear in one presumed male, in which all anterior teeth were much worn and flattened, and rear teeth of the right upper and lower jaws were extremely worn while those on the left side were not. However, he correlated this lateral asymmetrical wear with the right mandible being badly out of alignment.

Ulmer (1941), Caldwell *et al* (1956) and others have noted horizontal grooves on killer whale teeth, but have correlated such grooving with wear resulting from the tips of occluding teeth.

To the best of our knowledge, no killer whale has heretofore been reported with both the asymmetrical wear pattern, and with the single grooved tooth posterior to the worn stubs, as in the skeleton labelled *'Old Tom.'* Whether these features are associated with boat towing and line grabbing or not, no contemporary account singles out *'Old Tom'* as the sole practitioner of this behaviour, and the peculiar dental wear pattern cannot be used to identify certainly the skeleton as that of *Old Tom.*

EXAMINATION OF A TOOTH

Materials and methods

We examined the skeleton labelled *'Old Tom'* in the Eden museum for a short period on 18 June 1977, shot photographs, took measurements assisted by Jennie Whiter, and extracted one lower mandibular tooth (front, right side).

In Montreal, V. M. Kozicki undertook preparation of the tooth. It was covered with a layer of *DAW* silicone grease, embedded in *Ward's Bioplastic* (Ward's Natural Science Est., Inc., Rochester), and cut longitudinally on a band saw. A longitudinal section 0.5–2.0 mm in thickness (due to uneven surface of cut following midline of curved tooth) was cut from one of the halves on an *Isomet* Low Speed Saw (Buehler Ltd). The section was thinned down and polished by hand on a variety of wet stones to a uniform thickness of 200 μ. The resulting thin section was embedded in *Bioplastic* between two sheets of *ca.* 500 μ clear plastic. The other side of the tooth was decalcified and etched in 10% formic acid (in La Jolla) for 20 hours, washed for five hours in running water, and air dried.

Both halves of the bisected tooth and the thin section have been returned to the Eden Killer Whale Museum and Historical Society (Box 304, Imlay Street, Eden 2551).

Results

Measurements and meristics of the skull and skeleton are presented in Table 1. Photographs include a series taken at close range but in sequence to construct a lateral view of the skeleton (Fig. 2), and details of the skull and dentition (Figs. 3–4). The tooth was also drawn (Fig. 5) before sectioning (Figs. 6–7).

Efforts to read the dentinal layers in the etched half section of the tooth at the La Jolla Workshop on Age Determination (Perrin and Myrick, eds., this volume, MS Table 12) resulted in the following readings of Growth Layer Groups (GLGs) by seven observers (approximations in parentheses): '15+, 24+, (35), (18), 33, 14+(EDM, 17(VMK), range 17–35, average 25.8.' Other readings led to the conclusion of the Workshop that decalcified and stained thin sections of cementum hold more promise.

Accordingly, in Montreal we cut a thin section (but did not decalcify or stain it). Counts by two observers (VMK and EDM) of all the dentine, and the cementum deposited after pulp cavity closure, in the 200 μ section (Fig. 7) are: dentine, 26, 25; cementum, 11, 10.

The estimate of the total age of the animal is based upon a combination of these as mentioned in the discussion, below.

DISCUSSION

Historical evidence of *'Old Tom's'* longevity

The use of nicknames for individual cetaceans seen regularly in the field, and identifiable through a combination of physical and behavioural characteristics, is common practice for those who work with live cetaceans, and has been responsible for giving world-wide fame to animals observed by the general public, e.g. *'Pelorus Jack' (Grampus griseus), 'Opo George' (Tursiops truncatus)* (Alpers 1961; Baker, 1974). In the case of *'Pelorus Jack,'* regular and well documented observations were made by many people over a 24-year period from 1888 to 1912.

We are thus prepared to accept the use of nicknames in the Twofold Bay fishery on face value as evidence of repetitive recognition of individuals over periods of time.

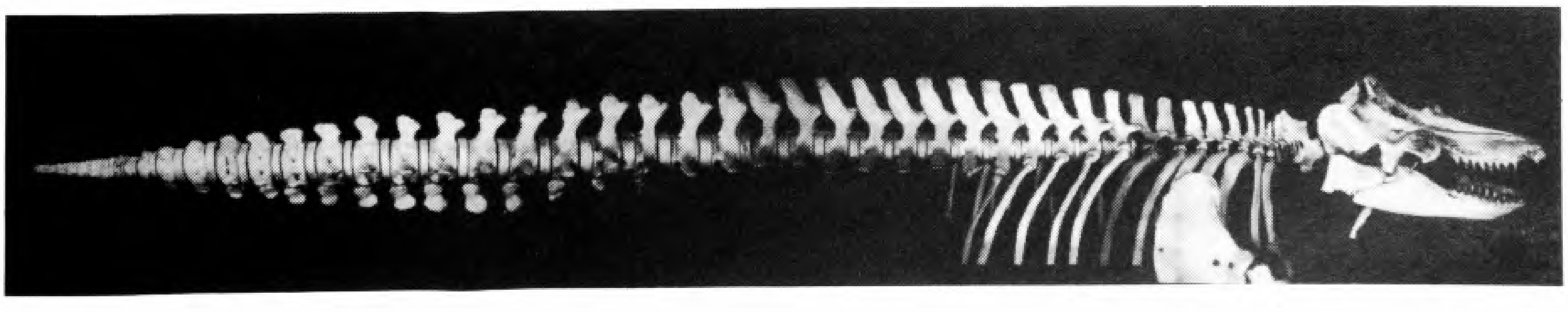

Fig. 2. – Skeleton of *Orcinus orca*, labelled *'Old Tom'* in the Eden museum, Eden, N.S.W., Australia.

Table 1

Measurements and meristics for skeleton of *Orcinus orca* in Eden museum, Eden, NSW, labelled *'Old Tom,'* parameters from Table 2 of Perrin, 1975, all measurements in centimetres.

1.	Condylobasal length – from tip of rostrum to posteriormost margin of occipital condyles	102.1
2.	Length of rostrum – from tip to line across posteriormost limits of antorbital notches	50.8
3.	Width of rostrum at base – along line across posteriormost limits of antorbital notches	32.5
4.	Width of rostrum at 60 mm anterior to line across posteriormost limits of antorbital notches	36.1
5.	Width of rostrum at midlength	29.4
6.	Width of premaxillaries at midlength of rostrum	12.0
7.	Width of rostrum at ¾ length, measured from posterior end	22.5
8.	Distance from tip of rostrum to external nares (to mesial end of anterior transverse margin of right naris)	68.9
9.	Distance from tip of rostrum to internal nares (to mesial end of posterior margin of right pterygoid)	64.7
10.	Greatest preorbital width	57.3
11.	Greatest postorbital width	68.7
12.	Least supraorbital width	56.4
13.	Greatest width of external nares	14.5
14.	Greatest width across zygomatic processes of squamosal	64.9
15.	Greatest width of premaxillaries	23.0
16.	Greatest parietal width, within post-temporal fossae	39.1
17.	Vertical external height of braincase from midline of basisphenoid to summit of supraoccipital, but not including supraoccipital crest	48.1
18.	Internal length of braincase from posteriormost limit of occipital condyles to anteriormost limit of cranial cavity along midline	30.7
19.	Greatest length of left post-temporal fossa, measured to external margin of raised suture	26.0
20.	Greatest width of left post-temporal fossa at right angles to greatest length	15.7
21.	Major diameter of left temporal fossa proper	14.3
22.	Minor diameter of left temporal fossa proper	14.0
23.	Projection of premaxillaries beyond maxillaries measured from tip of rostrum to line across anteriormost tips of maxillaries visible in dorsal view	0.2
24.	Distance from anteriormost end of junction between nasals to posteriormost point of margin of supraoccipital crest	5.4
25.	Length of left orbit – from apex of preorbital process of frontal to apex of postorbital process	13.1
26.	Length of antorbital process of left lacrimal	10.0
27.	Greatest width of internal nares	17.5
28.	Greatest length of left pterygoid	20.0
29.	Greatest width of anterior overhang of supraoccipital crest	None
30.	Greatest length of bulla of left tympanoperiotic	7.7
31.	Greatest length of periotic of left tympanoperiotic	7.6
32.	Length of upper left toothrow – from posteriormost margin of posteriormost alveolus to tip of rostrum	42.0
33.	Number of teeth – upper left	13
34.	Number of teeth – upper right (+ 3 missing, 2 abscessed)	10
35.	Number of teeth – lower left	12
36.	Number of teeth – lower right	12
37.	Length of lower left tooth row – from posteriormost margin of posteriormost alveolus to tip of mandible	39.5
38.	Greatest length of left ramus	79.2
39.	Greatest height of left ramus at right angles to greatest length	25.4
40.	Length of left mandibular fossa, measured to mesial rim of internal surface of condyle	34.0
41.	Deviation of skull from symmetry in dorsal view, in degrees	4–5°
48.	Number of thoracic vertebrae, defined as equal to number of ribs on side with greatest number	13
49.	Number of lumbar vertebrae	9
50.	Number of caudal vertebrae (column complete)	29
51.	Total number of vertebrae	54
52.	Number of fused cervical vertebrae	3
66.	First vertebra with unfused epiphysis	12
67.	Last vertebra with unfused epiphysis	36
78.	Number of vertebral ribs – left	13

79.	Number of vertebral ribs – right	13
80–81.	Number of two-headed ribs – left or right	7
82–83.	Number of floating ribs – left or right	1
84–85.	Number of sternal ribs – left or right	5
94.	Number of mesosternal elements	5
95.	Number of mesosternal elements fused	5
96.	Number of chevron bones	14
97.	Number in anteriormost series of unfused chevron bones	2
98.	Vertebra bearing first chevron bone	30th
99.	Vertebra bearing last chevron bone	43rd
114–17.	Number of phalanges in first through fourth digits	3, 5, 4, 2
118.	Number of phalanges in fifth digit [absent in this preparation]	–

And, data on pigmentation variation in *Orcinus orca* (e.g. Carl, 1946; Ivanova, 1961; Evans and Yablokov, 1978) has been used as part of the basis of a study of killer whale individual and pod movements off British Columbia and Washington (Bigg *et al*, 1976 Ms; Chandler *et al*, 1977; Balcomb, 1978).

Conclusive evidence for longevity of any Twofold Bay individual is lacking. There are some discrepancies between the stated times of death of some individuals, and possible dates of published photographs (e.g. *Jackson, Typee*). The first appearance of *'Tom,'* or the first use of the name, is not related to unique physical characters. One account (Wellings, 1945, p. [11]) mentions the presence of the postdorsal saddle (Mitchell, 1970) as distinctive, and indeed it is individually variable and might serve to identify individuals in combination with other pigmentation and scarring features (e.g. Evans and Yablokov, 1978), but all published photographs of the Eden Killers that we have examined (e.g. Wellings, 1944, pl. 15) show a strongly accentuated postdorsal saddle in each individual. The earliest photograph of *'Tom'* is apparently that published first by Dakin (1934; see our Fig. 1). In our conversation with Mrs Mary Mitchell (daughter of Charles E. Wellings who was the elder brother of H. P. Wellings) on 18 June 1977 in Eden,

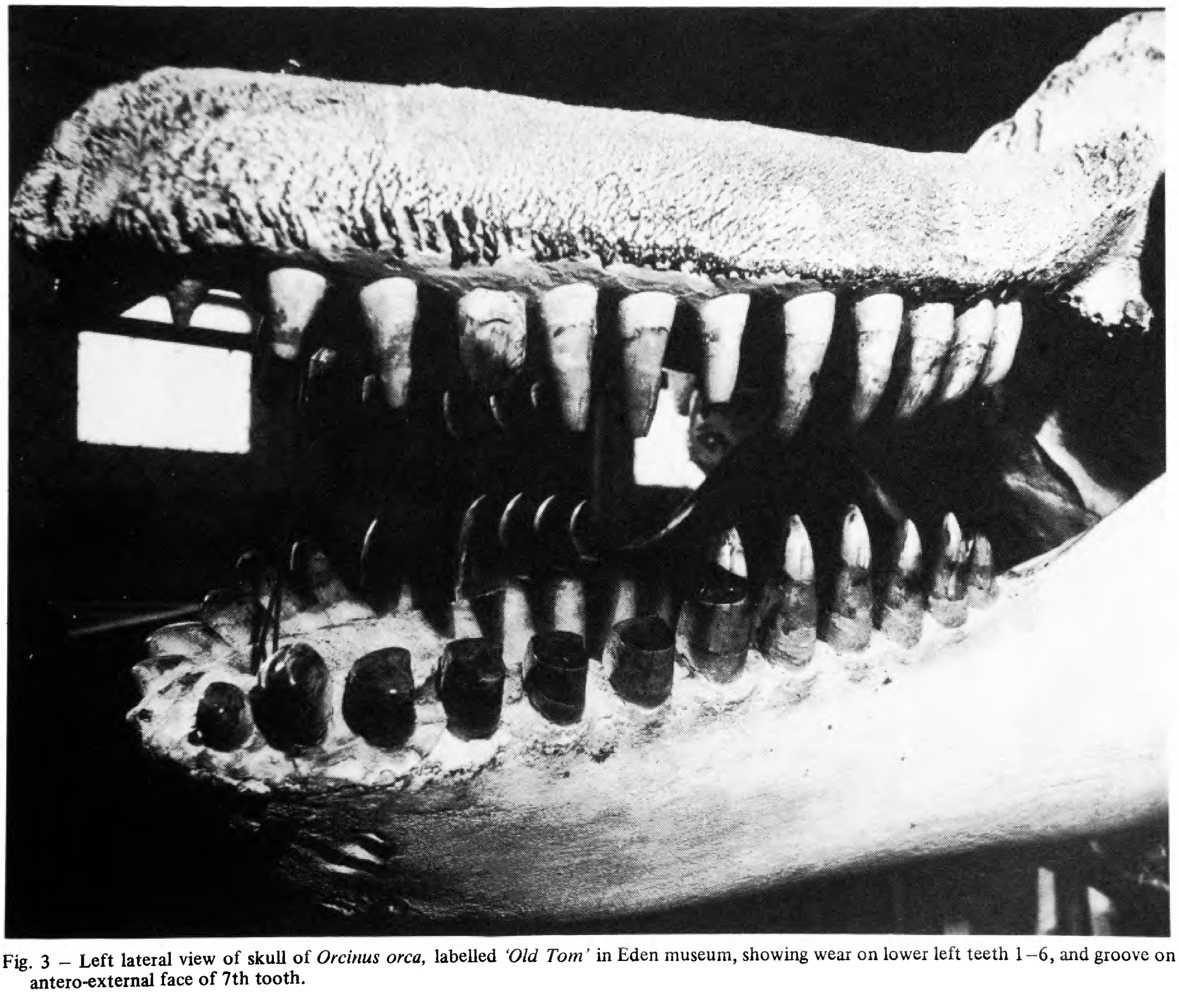

Fig. 3 – Left lateral view of skull of *Orcinus orca*, labelled *'Old Tom'* in Eden museum, showing wear on lower left teeth 1–6, and groove on antero-external face of 7th tooth.

Mrs Mitchell was unsure if the photograph (which we reproduced from a postcard that she gave us at that time) was from the 1910–1912 film made by C. E. Wellings or not. Another photograph, of which we do not have a copy, shows *'Old Tom'* involved in a chase in the same frame with a whaleboat and a whale, and is definitely from the film according to Mrs Mitchell. We examined a print in her possession, and agree that the killer appears to be *'Old Tom.'* (This small photograph is published on p. 2 of Wellings, 1964, where the 'scallop' on the dorsal half of the trailing edge of the dorsal fin is barely visible.) This may represent the earliest direct evidence of *'Old Tom,'* and places him as a fully grown and probably socially mature killer in 1910–1912.

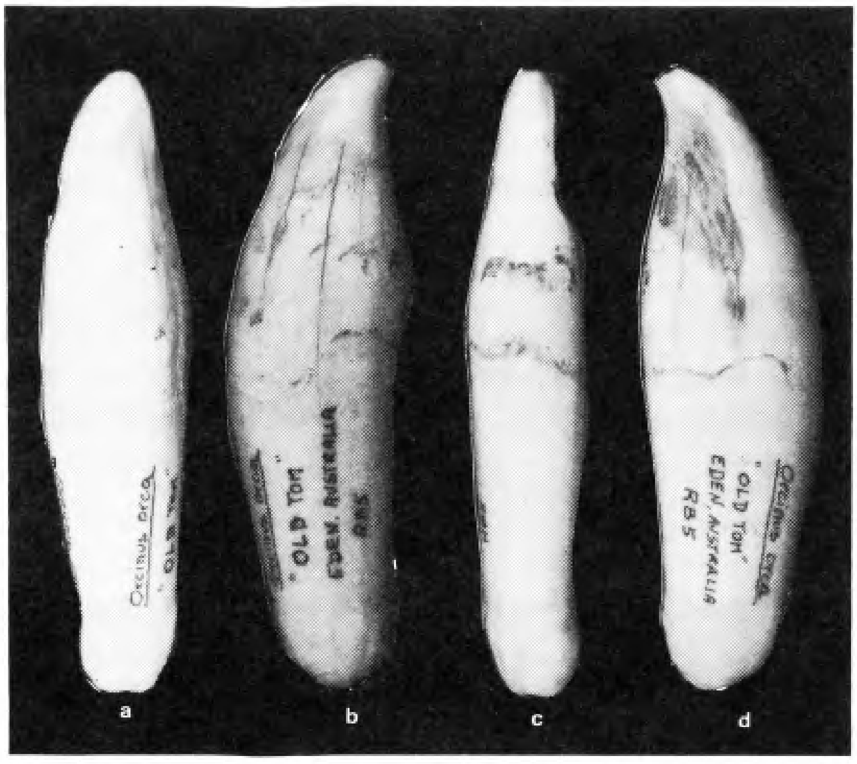

Fig. 4. – Right lower tooth from jaw of *Orcinus orca* in Eden museum labelled *'Old Tom.'*
a. anterior, *b.* internal, *c.* posterior and *d.* external views.

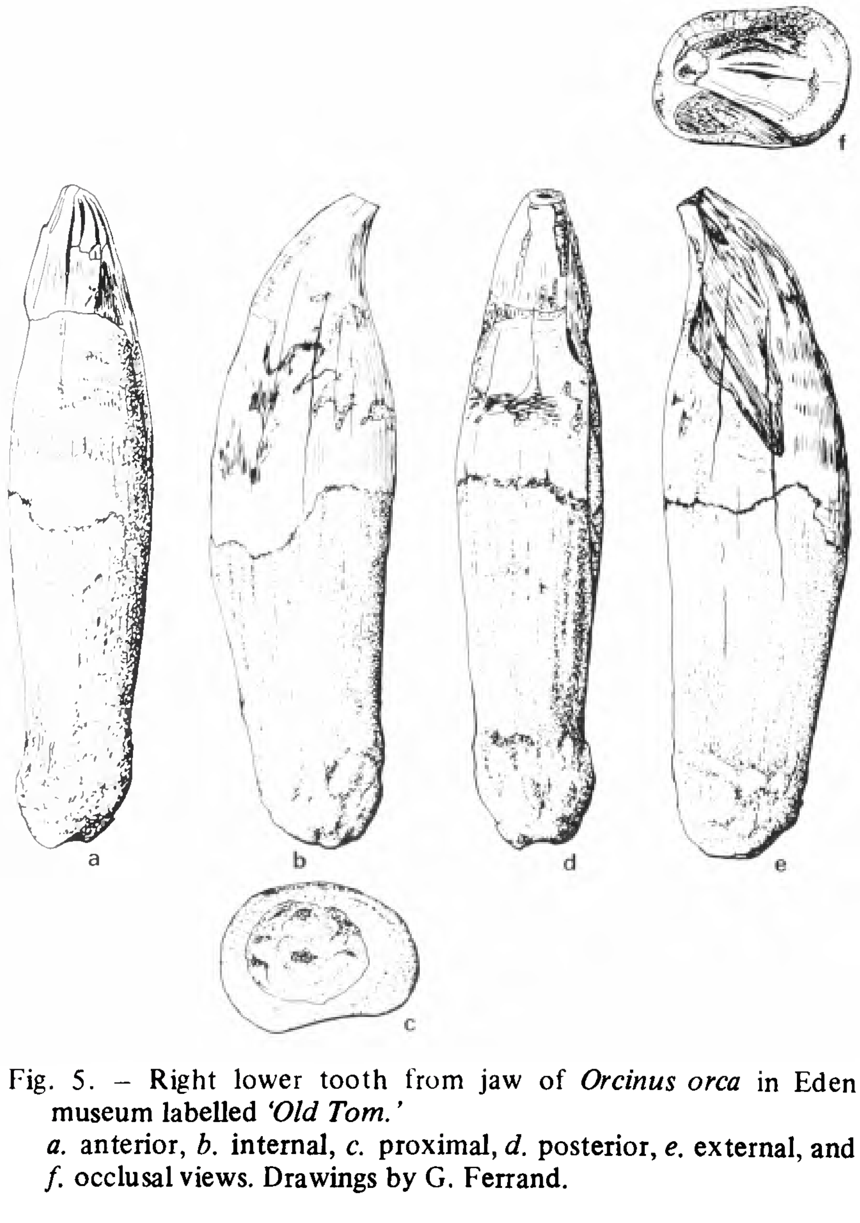

Fig. 5. – Right lower tooth from jaw of *Orcinus orca* in Eden museum labelled *'Old Tom.'*
a. anterior, *b.* internal, *c.* proximal, *d.* posterior, *e.* external, and *f.* occlusal views. Drawings by G. Ferrand.

The age at sexual maturity is not documented for killer whales, nor is the age at social maturity for males (*cf* references in Mitchell, ed., 1975, pp. 914–916). In the best studied, bulbous-headed odontocete most similar to the killer, the long-finned pilot whale, *Globicephala melaena*, the males become sexually mature at age 12 and over as judged from dentinal layer counts (Sergeant, 1962, pp. 43–45). We will take this by analogy as the minimum age for sexual maturity for our estimate of *'Old Tom's'* lifespan from historical data. The age at sexual maturity in the sperm whale, *Physeter macrocephalus*, another adononocete in which there is strong sexual dimorphism with large dominant bulls, is at 19 years, 73% of the age at social maturity (Lockyer, 1976). Taking this value, by analogy social maturity would have been 16 years of age, or older for a socially mature bull like *'Tom.'*

Then, picking the mid-point of the movie making sessions, 1911, we estimate that *'Tom'* would have been 16 years or older at that time. (There are citations to the reputed use of the name *'Tom'* or *'Old Tom,'* previous to 1895, back to the 1870s but the earliest contemporary citation we could locate was in 1909; see above.) The carcase of the killer identified as *'Old Tom'* by George Davidson and others was found dead on 17 September 1930. For this estimate, accepting Davidson's identification (but in the absence of actual photographic evidence showing the distinctive features of the dorsal fin), this would make *'Old Tom's'* age: 16 + 19 = 35 years (+) by our estimate based on historical data.

(The identity of the carcase called *'Old Tom'* remains in question. The 1930 photograph (Mead, 1961, oppos. p. 22) shows a dorsal fin tip markedly convex on the trailing edge, different from the concave trailing edge on *Tom* in Fig. 1.)

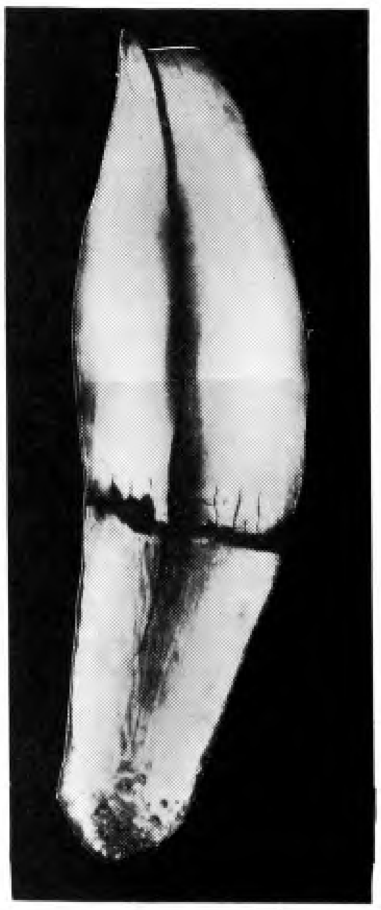

Fig. 6. Thin section of right lower tooth of *Orcinus orca* in Eden museum labelled *'Old Tom.'* Section 200 μ thick, cut slightly left (internally) of axis of tooth.

Biological evidence of *'Old Tom's'* longevity

Our readings of the tooth are not very satisfactory. All observers who studied the bisected tooth, and the thin section made subsequent to the Workshop meeting, agreed that it is difficult to read, and that much subjectivity and interpretation is involved.

The dentinal readings are relatively straightforward – we counted 22–25 GLGs. The counting of cemental layers is more difficult, and choosing the correlation between the two is very subjective. Our method was to count down on one side to the last major dentinal GLGs, about GLG 22–25, then decide which adjacent cemental layer was deposited concurrently with that particular dentinal layer. We continued the layer count proximally, rootwards using the cemental layers only with a count of 10.

This method is subjective, and the layers do not show well on a photograph of the base of the tooth (Fig. 7). After closure of the pulp cavity, cementum was deposited across the end of the tooth. Thus, the complete dentinal count, plus the layers deposited in cementum at the base of the tooth subsequently, should represent total age.

We conclude that on the basis of dentinal and cemental Growth Layer Group counts, the skeleton labelled *'Old Tom'* had approximately 35 GLGs, and presumably was as many years old.

Comparisons based on age

There are few killer whales from which the teeth have been sectioned and aged, by any method. Various investigators have examined small numbers, but the results are not published in detail (e.g. Sergeant, 1959, p. 285). Of these the oldest was a female with at least 25 Growth Layer Groups (Mitchell, 1975, ed., p. 914). This female was a 5.21 m, 4,094 lb whale from Newport, California captured alive on 18 November 1961 (EDM F-321; LACM M-1790). D. E. Sergeant examined one tooth and concluded that in spite of dentine layers having been lost from the worn tooth tip, '25 such layers and root-ridges (between dentine and cement)' were visible (Caldwell and Brown, 1964, p. 136; also cited by Scheffer, 1967). We can make no other comparisons of *'Old Tom'* with age-length keys, or with data on age at sexual, physical and social maturity.

Comparisons based on length

At death, the carcase identified as *'Old Tom'* by George Davidson was 22 ft long. Based upon size, *'Old Tom'* was relatively small for an aged, socially mature male *Orcinus orca.* Data for the North Atlantic show male sexual maturity attained at a minimum of 22 ft (6.7 m), maximum lengths

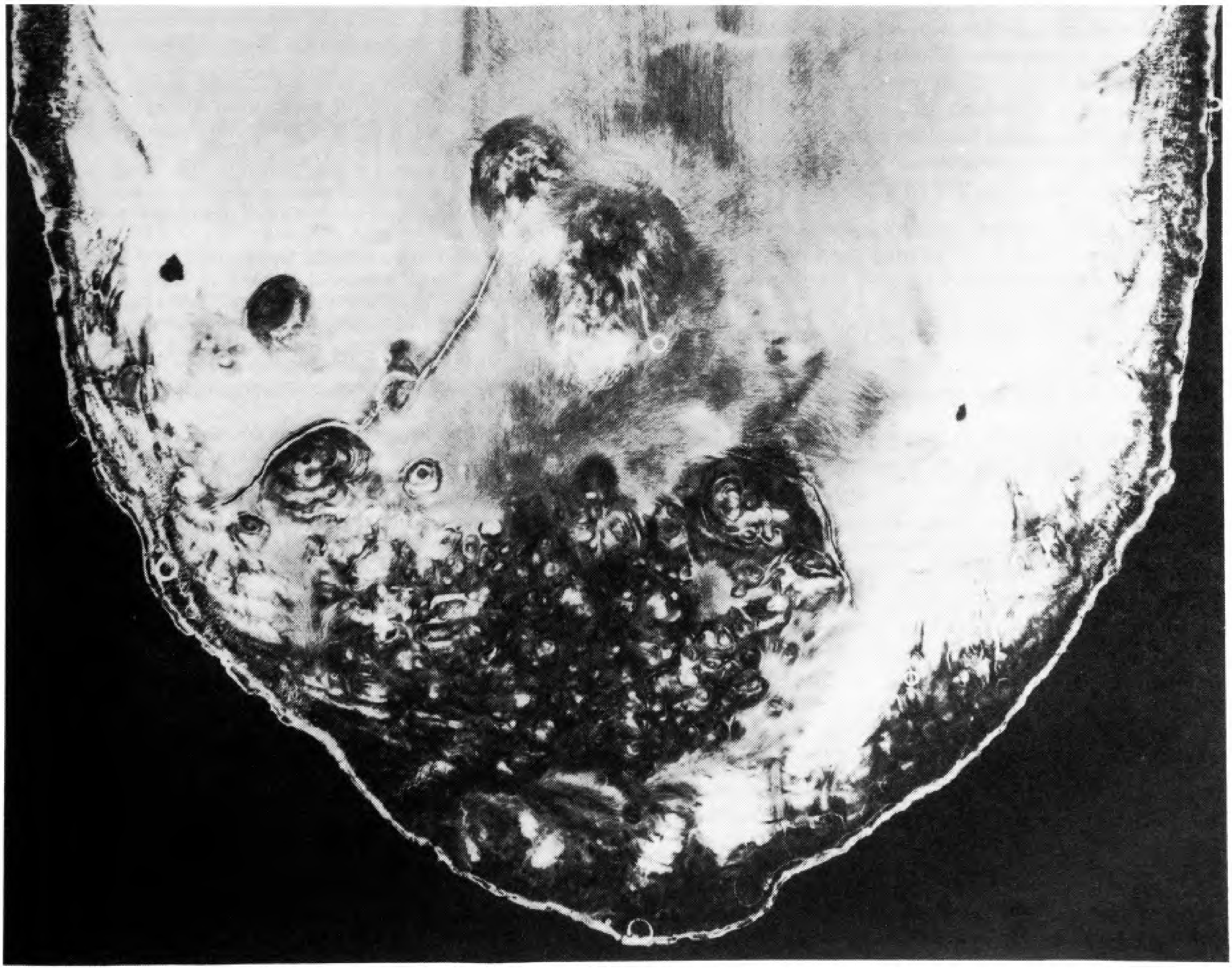

Fig. 7. – Portion of thin section of right lower tooth of *Orcinus orca* in Eden museum labelled *'Old Tom.'* Section 200 µ thick, detail of base of tooth in Fig. 6. Photograph shows interrelation between last few dentinal layers, and subsequent cemental layers.

of males to 31 ft (9.4 m), and the dorsal fin height up to 6 ft (1.8 m) (see Jonsgård and Øynes, 1952; Jonsgård and Lyshoel, 1970; Scheffer, 1967; summary in Mitchell, ed., 1975, p. 914). North Pacific males also grow to 31 ft (Nishiwaki and Handa, 1958). By these criteria, *'Old Tom'* was a very small male. At an adult, socially mature stage, *'Old Tom'* barely attained the length of the smallest sexually mature males in the Northern Hemisphere, but had a 5 ft 8 in dorsal fin, nearly as high as the maximum for any adult bull.

If not due to individual variation, these differences may indicate that separate populations of *Orcinus orca* differ in length, if not also in age, at sexual and social maturity and asymptotic size.

CONCLUSIONS

1. Sufficient published evidence exists to document part of the life span of the Twofold Bay killer whale *'Old Tom'* for at least 19 years. We believe that at the time of the earliest published photograph of *'Old Tom,'* he was already a dominant bull 16 years old or older. Thus we estimate *'Old Tom's'* age based on the historical data as 35 years.

2. Readings of both the dentinal and the cemental layers in a thin section of a right lower jaw tooth from the skeleton labelled *'Old Tom'* in the Eden museum result in estimates of the age as approximately 35 Growth Layer Groups, i.e. 35 years.

3. There is no published evidence available to show that the *Orcinus orca* carcase found dead at Eden on 17 September 1930 is the Eden killer *'Tom'* or *'Old Tom.'* The sole available information is Dakin's statement, published in 1934 (p. 146) and apparently based on interviews with Davidson, Logan and others in 1932, that they were sure 'there was no doubt about its being *'Old Tom'*.'

4. If the Eden museum skeleton represents *'Old Tom,'* then two approaches to estimating age indicate that *'Old Tom'* was approximately 35 years of age when he died on 17 September 1930, not 50–90 years of age as generally reported in the literature about the 'killers of Eden.'

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Analysis of Growth Layers in the Teeth of *Tursiops truncatus* using Light Microscopy, Microradiography, and SEM

Aleta A. Hohn

Department of Zoology, University of Maryland, College Park, Maryland
and
Division of Mammals, Smithsonian Institution, Washington, D.C.

ABSTRACT

Preliminary results show microradiography and scanning electron microscopy (SEM) to be more accurate methods of accessing growth layer groups (GLGs) in the teeth of *Tursiops truncatus* than transmitted light microscopy. Microradiography shows the rhythmic deposition of mineral as alternating radiopaque and radiolucent layers. It improves the resolution of GLGs near the pulp cavity in older individuals, better than either SEM or light microscopy. SEM of etched sections show GLGs as ridges and grooves which are easily counted from the micrograph. SEM also shows GLGs to be composed of fine incremental layers of uniform size and number which may allow for more precise age determination. Accessory layers are usually hypomineralized layers within the hypermineralized layer of the GLG and are more readily distinguishable as such in SEM of etched sections and microradiographs than in thin sections viewed under transmitted light. The neonatal line is hypomineralized, appearing translucent under transmitted light, radiolucent in a microradiograph, and as a ridge in SEM.

INTRODUCTION

Conventional methods of counting growth layer groups (GLGs) in odontocete teeth, by use of undecalcified or decalcified and stained thin sections, have been used extensively in age determination (for example, Nishiwaki and Yagi, 1953; Sergeant, 1959, 1962, 1973; Klevezal' and Kleinenberg, 1967; Best, 1970, 1976; Christensen, 1973; Kasuya, 1972, 1976, 1977; Sergeant, Caldwell, and Caldwell, 1973) but do not always permit definition of all GLGs. Repeated counts on the same tooth by one person or several people sometimes give different results with the average or median count used as the age indicator (this workshop; Kasuya, Miyazaki, and Dawbin, 1974; Perrin, 1975; Kasuya, 1977). The development of a technique that will lead to better resolution of GLGs, and therefore more accurate age determination, would be useful. Preliminary work with microradiography and scanning electron microscopy (SEM) indicates their potential value in clarifying GLGs in the teeth of odontocetes.

Microradiography provides a non-destructive means of determining mineral density differences within hard tissues. It is particularly useful in resolving GLGs since GLGs consist of alternating poorly mineralized and more highly mineralized layers. It involves the use of very concentrated, low-voltage x-rays to expose a high resolution photographic plate, which increases sensitivity over standard x-rays, and gives sharply defined images. This permits the detection of local variation in mineral content in the tooth sections, since the x-rays are absorbed chiefly by the calcium and phosphate in the tooth (Trautz, 1967). Areas of greater mineral density are radiopaque and appear as light zones. Radiolucent or dark zones are the poorly mineralized areas that allow more x-rays to expose the plate.

An advantage of microradiography is that mineral density can be compared to optical density on the same thin section. This technique has been used by Sergeant (1962) and Nielsen (1972) to study odontocete teeth. Sergeant mentions that radiography confirmed his hypothesis that the opaque zones of teeth of *Globicephala melaena* were the better calcified dentine while the translucent zones were poorly calcified. In the teeth of *Phocoena phocoena*, Nielsen found that the radiopaque layers are equivalent to the light layers on thin sections when viewed with transmitted light. Because this result is contrary to Sergeant's (1962) findings, Nielsen cautions that optical density may not be dependent on mineral density.

A less ambiguous method of resolving GLGs may be by the removal of superficial calcium from a half-section of tooth, a procedure referred to as etching. The mineral is dissolved in an acid, usually formic, nitric, or hydrochloric, or removed by a chelating agent such as EDTA (see Boyde and Jones, 1974). After drying, the surface of the section has a topography of alternating ridges and grooves, each pair of which corresponds to a GLG, that results from the greater collapse of dentinal tissue in those layers which were initially hypercalcified.

Although etching seems to be a good technique for enhancing GLGs in sperm whale teeth (Bow and Purday, 1966), it has not frequently been applied to small odontocetes, probably because of difficulty in counting individual ridges and grooves. One method to facilitate counting is by use of the SEM. The advantage of the SEM is that it produces a three dimensional image of the surface of the tooth, allowing for more accurate interpretation of the GLGs.

The purpose of this study is to compare the techniques of transmitted light microscopy, microradiography and SEM for examination of *Tursiops* teeth for the clear resolution of GLGs, identification of accessory layers, and correlation of mineral density with optical density for the layers.

METHODS

Two teeth were taken from each of 15 *Tursiops truncatus* females which had stranded on the east coast of the United States, and which are in the collection of the National Museum of Natural History. Generally the teeth were removed directly from the skulls, most of which had been boiled clean before addition to the collection. When possible, central mandibular teeth were extracted. If the teeth had already been removed from the jaws, large relatively straight teeth were chosen.

All teeth were sectioned with a *Buehler Isomet* 11-1180 low speed saw. Longitudinal half-sections were cut by gluing the tooth on a microscope slide with a cellulose nitrate base adhesive and cutting medially. For thin sections, the cut surface of one half-section was glued down on a slide and all but 150 μm cut off, leaving the thin section adhering to the slide. Sections thicker than 150 μm were sanded down using 320 and 400 grit sandpaper. To prepare thick cross sections the tooth was placed in a clamp-like chuck accessory of the saw and cut in the zone of most recent growth as determined from the longitudinal thin section. Another cut was made farther down the tooth to make the sections about 0.25 cm thick for use in the SEM.

Preparation for microradiography involved removal of the longitudinal thin sections from the slide and then cleaning it in an ultrasonic cleaner to remove loose surface particles. One section from a tooth of each animal was microradiographed, following the method of Ortner and Yong (1975), for one and one-half hours at 11 kv. GLGs were counted from the microradiograph using a dissecting microscope.

The cross sections prepared for SEM were soaked in 5% formic acid for three hours, rinsed in water, then sonically cleaned. Each section was air dried, mounted on an SEM stub, plated with gold-palladium, and viewed with a *Cambridge Stereoscan* Mk IIA or S4-10 or AMR 1000A SEM. Micrographs were taken at a 45° tilt-angle under low magnification (15–21X) at 10 kv for large sections and 20 kv for smaller sections, including at least one-half of the surface of the tooth from the center to the periphery. Details were viewed under higher magnification at 20 kv.

RESULTS

GLGs

GLGs are the most prominent layered components within the tooth (see workshop glossary for definitions), within which there are finer layers. Using light microscopy with undecalcified thin sections, each GLG is composed of a translucent and an opaque layer (Fig. 1). In etched sections, each GLG consists of a ridge and an adjacent groove (Fig. 2). In microradiographs, each GLG consists of one radiopaque and one radiolucent layer, indicating that each GLG comprises one hypermineralized layer and one hypomineralized layer (Fig. 3). Cross counts of GLGs using the three methods gave the same whole-year counts for most of the specimens. Discrepancies occurred in some cases where the microradiograph resolved the beginning of a new layer, resulting in a slightly higher count (Table 1), and where it made visible last-formed layers in older animals in which the pulp cavity had been occluded (Figs. 1, 2, and 3). In the latter case, the additional GLGs followed the periodicity and intensity of the preceding GLGs.

Figs. 1–3. Comparison of GLGs in the same tooth by each of the techniques used.

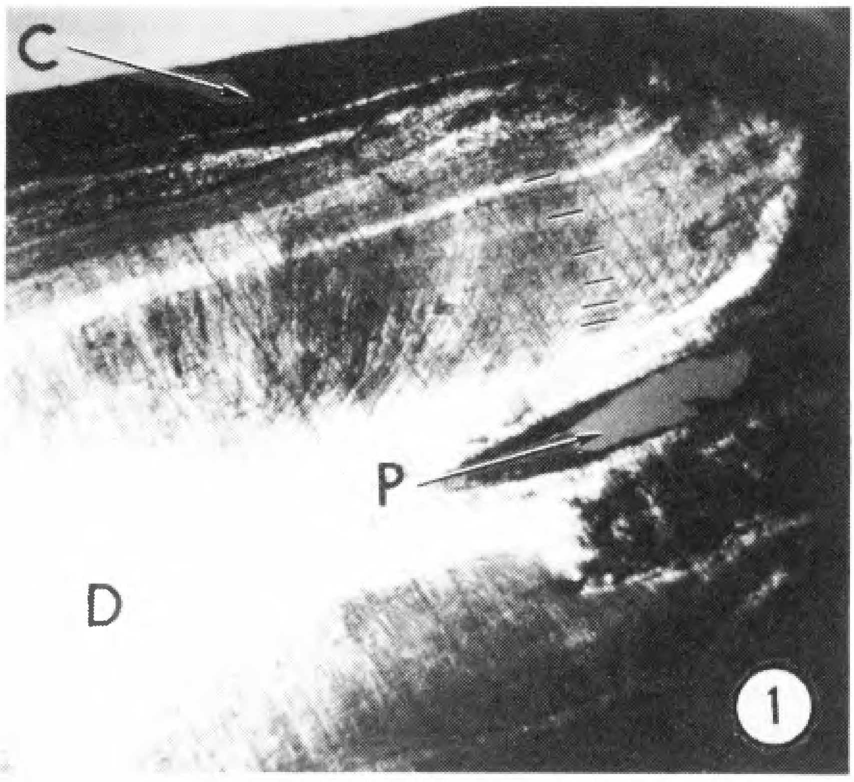

Fig. 1. Ground longitudinal section (150 μm) under polarized transmitted light. GLGs visible only on the sides of the tooth and near the base close to the pulp cavity. Bars demarcate GLGs. Original magnification: x17. P – pulp cavity, C – cement, D – dentine.

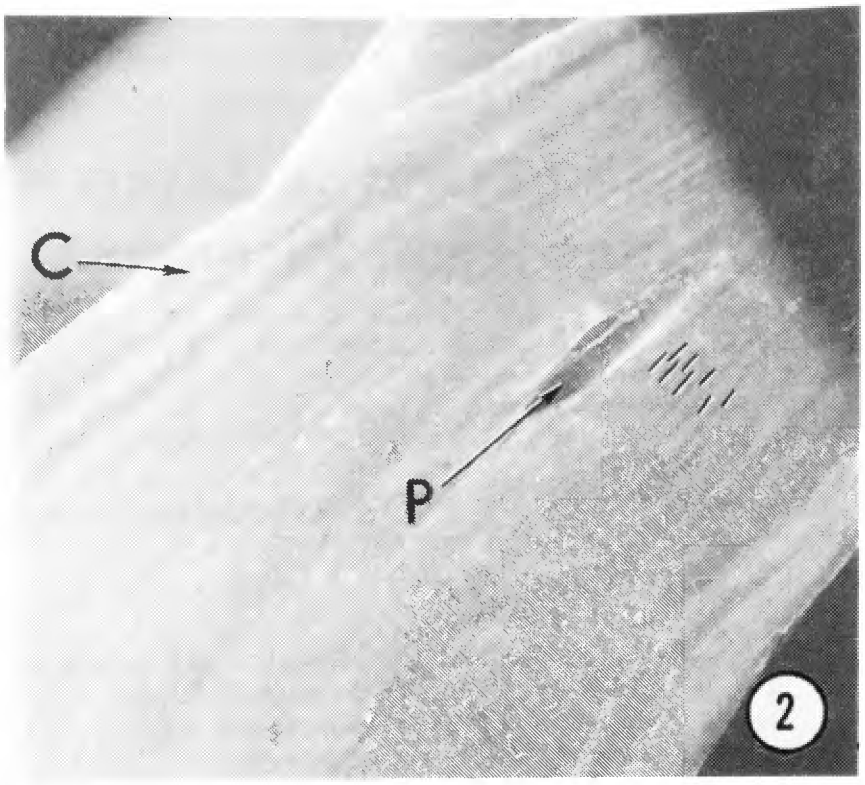

Fig. 2. Scanning electron micrograph of etched longitudinal half-section. GLGs more clearly resolved than in thin section. Original mag.: x15.

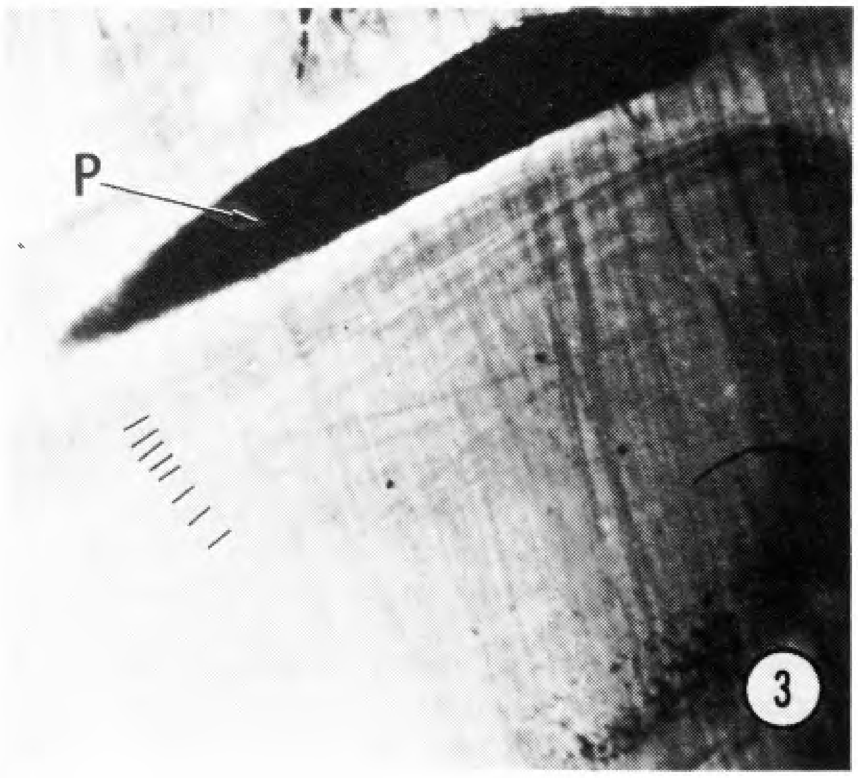

Fig. 3. Microradiograph of ground thin section. More GLGs appear surrounding the pulp cavity than are resolved either by light microscopy or SEM. Original mag.: x25.

Table 1
Examples of discrepancies in dentinal GLG counts in teeth of *Tursiops* compared by method of examination. Light microscopy counts were made using transmitted polarized light.
TL – total length of the animal

		Number of GLGs counted		
USNM No.	TL (cm)	Light microscopy	SEM	Micro-radiography
504590	155	1	1	1¼
504583	185	3	3	3¼
395670	–	12	12½	12½
504559*	252	14	14	18

*Pulp cavity occluded.

Incremental growth layers

Incremental growth layers are layers which occur parallel to the formative surface of the dentine (see workshop glossary) and compose GLGs. In cross sections of teeth, incremental growth layers are arranged concentrically around the pulp cavity (Figs. 4 and 7). They are most prominent in SEM micrographs where they appear as fine ridges and grooves within the ridges and grooves of the GLGs. In microradiographs and thin sections viewed with light microscopy, incremental growth layers appear as adjacent fine radiolucent and radiopaque layers or translucent and opaque layers, respectively, but are not as clear as in the SEM micrographs. The incremental growth layers averaged 10 to 13 per GLG and 2.0 μm to 3.5 μm in width measured from SEM micrographs, with those in the most recently formed GLGs sometimes smaller than those in the first formed GLGs. Although they were in all of the teeth, they were often not apparent in old animals in the first-formed GLGs nor in the compressed, last-formed GLGs. In sections which showed incremental growth layers in the last-formed GLG, there was a constant number in the teeth of animals that died during the same time of year.

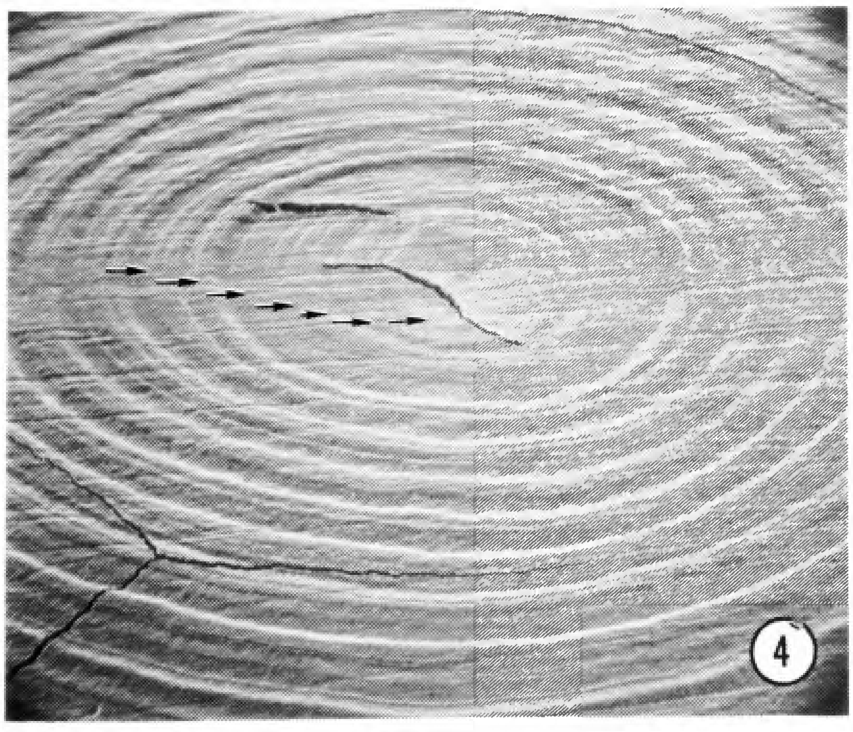

Fig. 4. SEM of etched cross section showing the alternating ridges and grooves. Also faintly visible are some incremental growth layers which compose the GLGs. Arrows indicate GLGs. Original mag.: ×50.

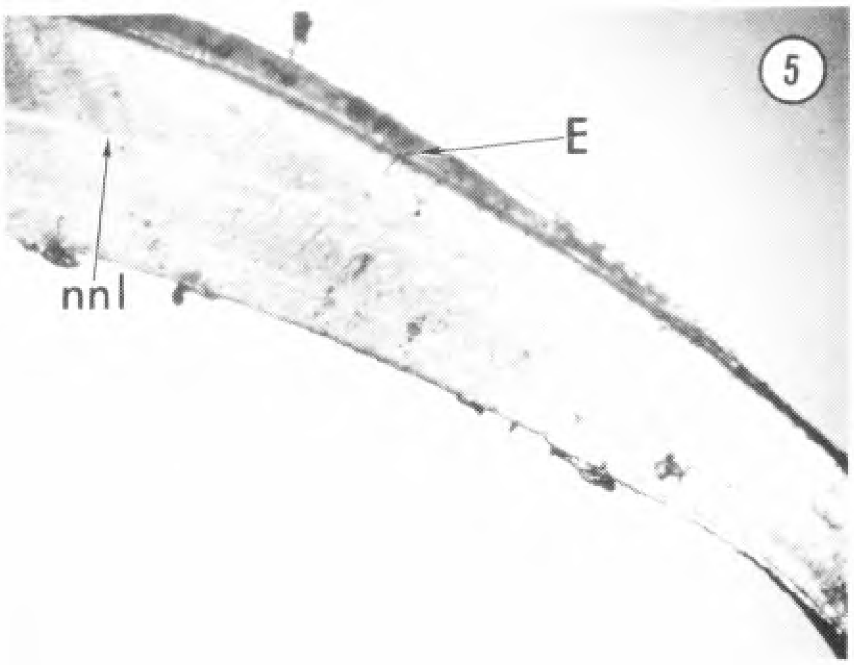

Fig. 5. Ground thin section from the tooth of a young animal viewed under polarized transmitted light. The many accessory layers prevent accurate distinction of the beginning of a new GLG. The neonatal line appears translucent. Original mag.: ×30. E – enamel, nnl – neonatal line.

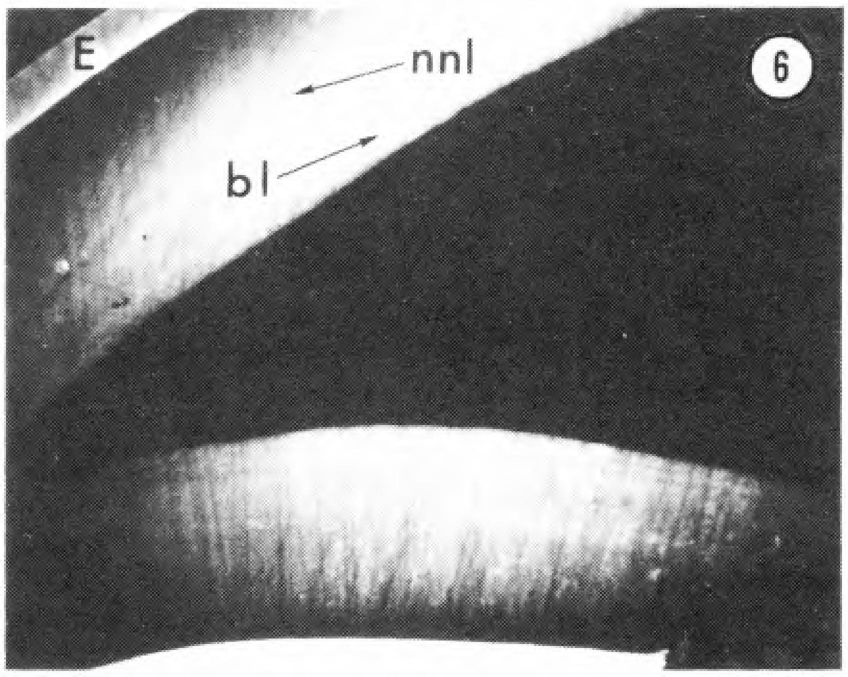

Fig. 6. Microradiograph of the ground thin section in Fig. 5. One radiolucent line stands out and is considered the GLG boundary layer. Original mag.: ×30. bl – boundary layer.

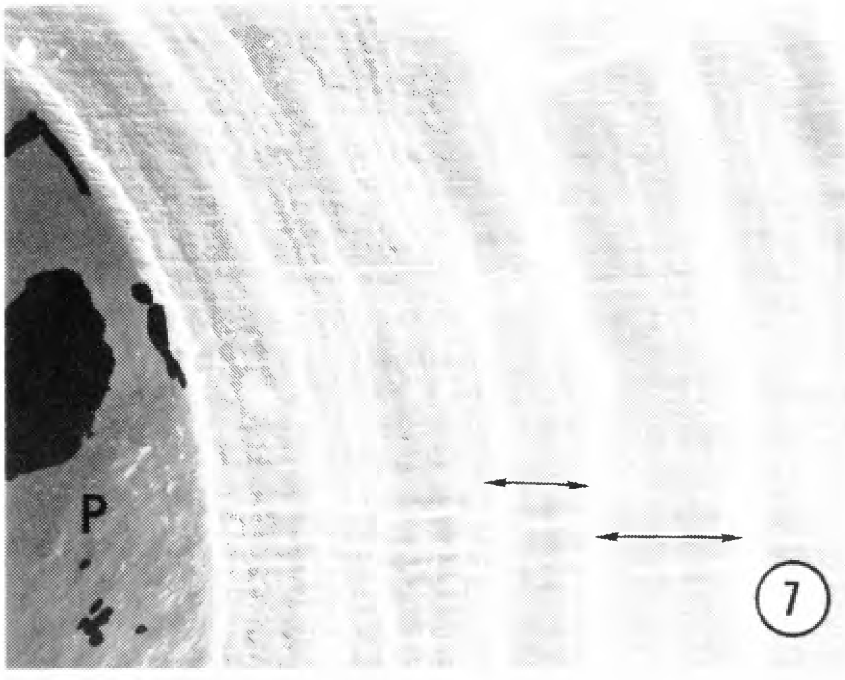

Fig. 7. SEM micrograph of an etched cross-section showing the incremental layers, arranged concentrically around the pulp cavity, which compose the GLGs. The lines running from left to right are saw marks; dentinal tubules are not visible. The arrows denote GLGs. Original mag.: ×180.

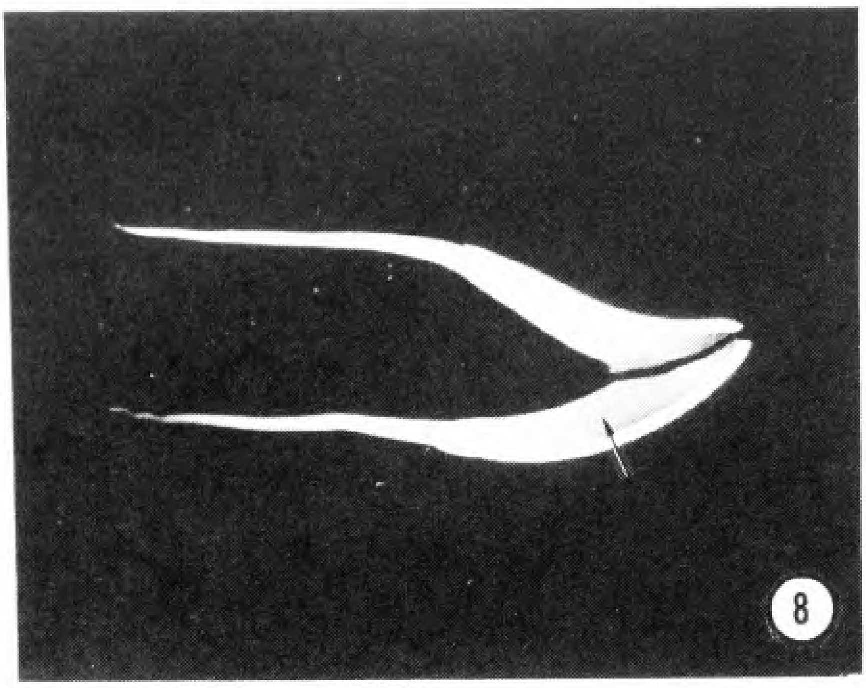

Fig. 8. Microradiograph of ground thin section. The enamel is strongly radiopaque (light), the neonatal line radiolucent. Slight differences in thickness of the section prevented the x-rays from exposing the plate at the root end of the tooth, so it appears highly mineralized (radiopaque). The arrow indicates the neonatal line. Original mag.: x7.

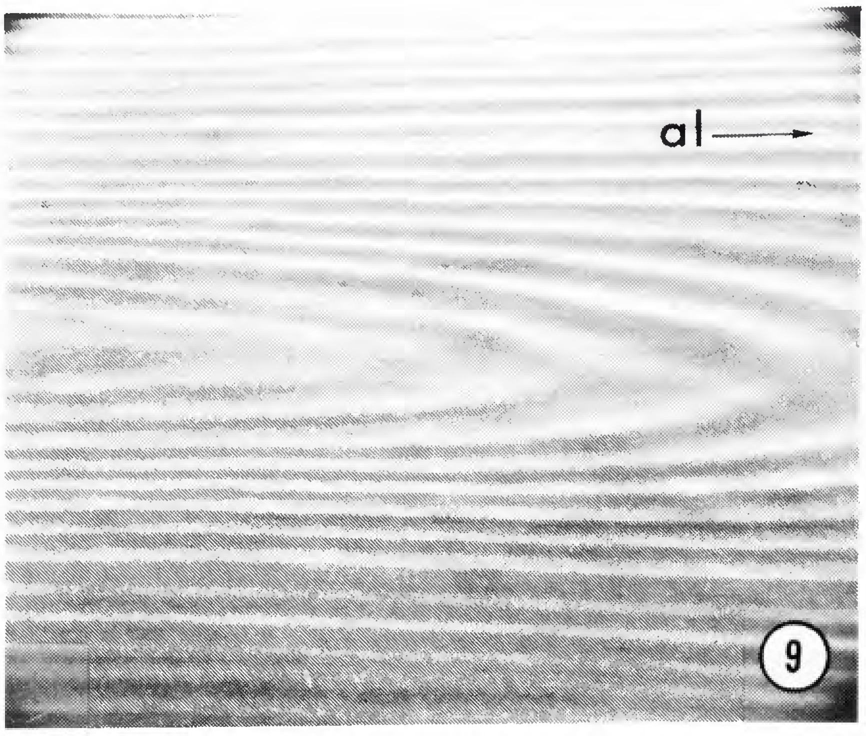

Fig. 9. SEM micrograph showing an accessory layer as an additional ridge between two GLG ridges.

Accessory layers
Sometimes an incremental layer appears particularly prominent within the GLG because it is relatively more hypo- or hypermineralized than the GLG layer in which it is located (Fig. 9). For purposes of this study, these prominent layers are referred to as accessory layers, defined as irregularly occurring, nonrhythmic layers that disrupt the expected mineralization pattern within a GLG and complicate the problem of counting GLGs. They seem to occupy the same relative position in GLGs in the teeth of a given individual, but vary in number and position among teeth of various specimens. Accessory layers occur most commonly near boundaries of GLG layers.

Comparison of techniques for counting GLGs
Scanning electron micrographs of etched sections is the easiest method by which to count GLGs, because the contrast in topographic relief between the layers of a GLG is generally great enough to easily distinguish adjacent GLGs (Fig. 4). Although incremental layers and accessory layers are apparent using SEM, their effect in confusing the counting of GLGs is diminished by etching. In microradiographs, the first-formed GLGs are not as clearly delineated, because the contrast between adjacent layers of the GLG is less than in etched sections. In undecalcified thin sections examined with light microscopy, the accessory layers were sometimes so conspicuous near the boundary layer of GLGs that divisions between GLGs were obscured. This problem occurred most often within the first four or five GLGs. When the boundaries of the GLGs were not definable, the number of GLGs could only be estimated.

Correlation between optical and mineral density
Optical density corresponds to mineral density for each layer of the GLG when comparing the three methods (Table 2). Sections viewed with transmitted light show the neonatal line as translucent with the prenatal zone and the immediately postnatal incremental layer of the first GLG appearing opaque, and the second layer of the GLG appearing translucent. In etched sections, the neonatal line appears as a ridge, and the prenatal and immediately postnatal incremental layer are grooves. In the microradiographs, the neonatal line is radiolucent (relatively hypomineralized), the prenatal and postnatal incremental layers are radiopaque (relatively hypermineralized), with the second incremental layer of the GLG radiolucent. This cyclic deposition of mineral, i.e. adjacent hypermineralized and hypomineralized layers, continues throughout the postnatal dentine.

Table 2
Correlation of GLG components with relative mineral density in the teeth of 15 specimens of *Tursiops* using transmitted light microscopy, SEM, and microradiography

Method of examination			Relative mineral density
Transmitted light	Micro-radiography	SEM	
opaque	radiopaque	groove	greater
translucent	radiolucent	ridge	less

DISCUSSION

GLGs
Each of the preparations should permit adequate age estimates to the nearest whole GLG, but microradiography seems to be the best of the three techniques for accurately identifying the nature, i.e. mineral density, and extent of growth of the newly forming GLG in teeth which were still accumulating dentine when the animals died. This information, collected over at least a year for a species, will show the cycle of mineralization, helping to explain the mechanisms influencing mineralization and its relationship to life history attributes of the animals. Previous work, e.g. on *Tursiops truncatus* (Sergeant, 1959), *Globicephala melaena* (Sergeant, 1962), *Hyperoodon ampullatus* (Christensen, 1973), *Delphinapterus leucas* (Sergeant, 1973), and *Berardius bairdii* (Kasuya, 1977), has shown the consistency of the stainability or optical density of the last GLG component with the season of death for each species.

In some cases, more GLGs may be deposited, in terms of mineralization cycles, than may be determined using conventional light microscopy. Because of its sensitivity to differential mineral density, microradiography may be used to detect these GLGs and extend the maximum GLG count.

Incremental growth layers

Early work in dental histology demonstrated the existence of incremental lines in dentine that express the constant rhythmic changes in the degree of mineralization of the matrix (Schour and Steadman, 1935; Schour and Hoffman, 1939a; Schour and Massler, 1940). According to Schour and Hoffman (1939a), two adjacent lines, one more and one less mineralized, measured 16 µm in all species examined. These lines were later interpreted as incremental lines of von Ebner. More recently, smaller incremental lines (von Ebner lines), e.g. 5 µm, have been described that represent daily increments of growth of the tooth (Krauss and Jordan, 1965; Miani and Miani, 1972; Newman and Poole, 1974; and Yilmaz, Newman, and Poole, 1977). However, neither incremental growth of teeth on other than a daily rhythm nor the annual mineralization pattern of mammal teeth have been well demonstrated.

Sub-annual incremental layers, where GLGs in at least *Tursiops* teeth are considered to be annual (see Sergeant, 1959; Klevezal' and Kleinenberg, 1967; Sergeant *et al*, 1973; this workshop–known-age dolphins), have been described in some marine mammals. Kasuya (1977) found the 'long cycles' (GLGs) in *Berardius* teeth to contain many (11.0–13.4) 'short cycles', and Kasuya and Nishiwaki (1978) found that coarse layers (GLGs) in *Dugong* tusks contain 10 to 15 fine growth layers (see also Scheffer, 1970). They interpreted the fine growth layers or short cycles as representing the lunar cycle or an endogenous rhythm of about one month manifested in the growth of the tooth. Myrick (this volume) noted incremental layers (his accessory lines) of the same periodicity in the teeth of different species of dolphin, as well as von Ebner lines which are interpreted as representing a daily growth pattern. The rhythmicity and periodicity of the incremental lines or layers, i.e. 10 to 13 per GLG, in this study, also suggests the incremental layers in the North Atlantic Ocean *Tursiops* to be incremental growth of the tooth similar to that described by Kasuya (1977), Kasuya and Nishiwaki (1978) and Myrick (this volume). In this case, the incremental layers appear to be the result of a finer mineralization pattern within the more gross annual mineralization cycle of the GLGs. This is evident in the three-dimensional pattern of the incremental layers in etched sections, where calcium has been removed, and in microradiographs.

Accessory layers

If the mineralization cycles, both annual and sub-annual, seen in dolphin teeth are the result of extrinsic environmental factors, then irregular changes in the expected deposition pattern may be a visible manifestation of a change in environmental parameters. Similarly, if mineralization is intrinsically controlled, appropriate changes within the animal's system should appear in an actively growing tooth. Accessory layers may be a result of either of these types of fluctuations. If so, they may be useful in distinguishing stocks of animals, and careful notation of these changes may aid in explaining mechanisms responsible for the mineralization patterns seen in dolphin teeth; therefore, it is important to distinguish accessory layers as such. But, in regards to a practical application of odontocete teeth for age determination, a method is needed that permits clear resolution of individual GLGs without the sometimes overwhelming interference of accessory layers. Of the three methods in this study, SEM best serves this purpose.

Correlation between optical and mineral density

The determination of the mineral density of each layer of a GLG, especially the last-formed layer, may be important for resolving the basis of formation of GLGs in odontocete teeth. However, there has been disagreement about the interpretation of optical density and mineral density in some of the species examined (Sergeant, 1962; Klevezal' and Kleinenberg, 1976; Nielsen, 1972; and Kasuya, 1976). In response to these discrepancies, Nielsen (1972; this volume) feels that no species-wide generalization can be made to correlate optical to mineral density.

In this study, the correlation of mineral density to optical density in the teeth of *Tursiops* agrees with Sergeant's (1962) results with *G. melaena.* In agreement with many previous studies (*Stenella coeruleoalba,* Nishiwaki and Yagi, 1953; *T. truncatus,* Sergeant, 1959; *G. melaena,* Sergeant, 1962; *B. bairdii,* Kasuya, 1977; and *Phocoena phocoena,* Gaskin and Blair, 1977) the neonatal line is translucent (or unstainable) and the immediately postnatal layer is opaque (transmitted light). Although the event causing the formation of the neonatal line does not occur again, the neonatal line is hypomineralized (Irving and Weinmann, 1948) and is generally easily identifiable. Therefore, it can be used as a landmark for identification of mineral densities of the following layers. In other words, if the neonatal line appears translucent, and we know it to be hypomineralized by microradiography, then each translucent layer within subsequent GLGs should be hypomineralized. This is the case in all of the *Tursiops* teeth examined.

Evaluation of techniques

Of the three techniques, SEM is the easiest method by which to count GLGs. While the method is procedurally more complicated than preparation of thin sections, the results justify the slight additional preparation time, especially for a large number of specimens. Microradiography, valuable as a source for information on mineral density, requires exceptional precision in thickness of sections and timing of the x-ray to achieve worthwhile results and would be too demanding for age determination of a large number of specimens. The conventional method of light microscopy, although less complicated in terms of tooth preparation, seems to be least reliable for accurate counting of GLGs.

Although each of the methods permits adequate estimates of GLGs for age determination, additional information about the deposition and mineralization of dentine is available from microradiography and SEM. Such information helps to better define GLGs, as well as to ascertain what processes influence layering in odontocete teeth.

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

Makoto Kimura

National Oceanic and Atmospheric Administration, National Marine Fisheries Service
Southwest Fisheries Center, La Jolla, California 92038

ABSTRACT

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

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

INTRODUCTION

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

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

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

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

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

MATERIALS AND METHODS

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

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

RESULTS

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

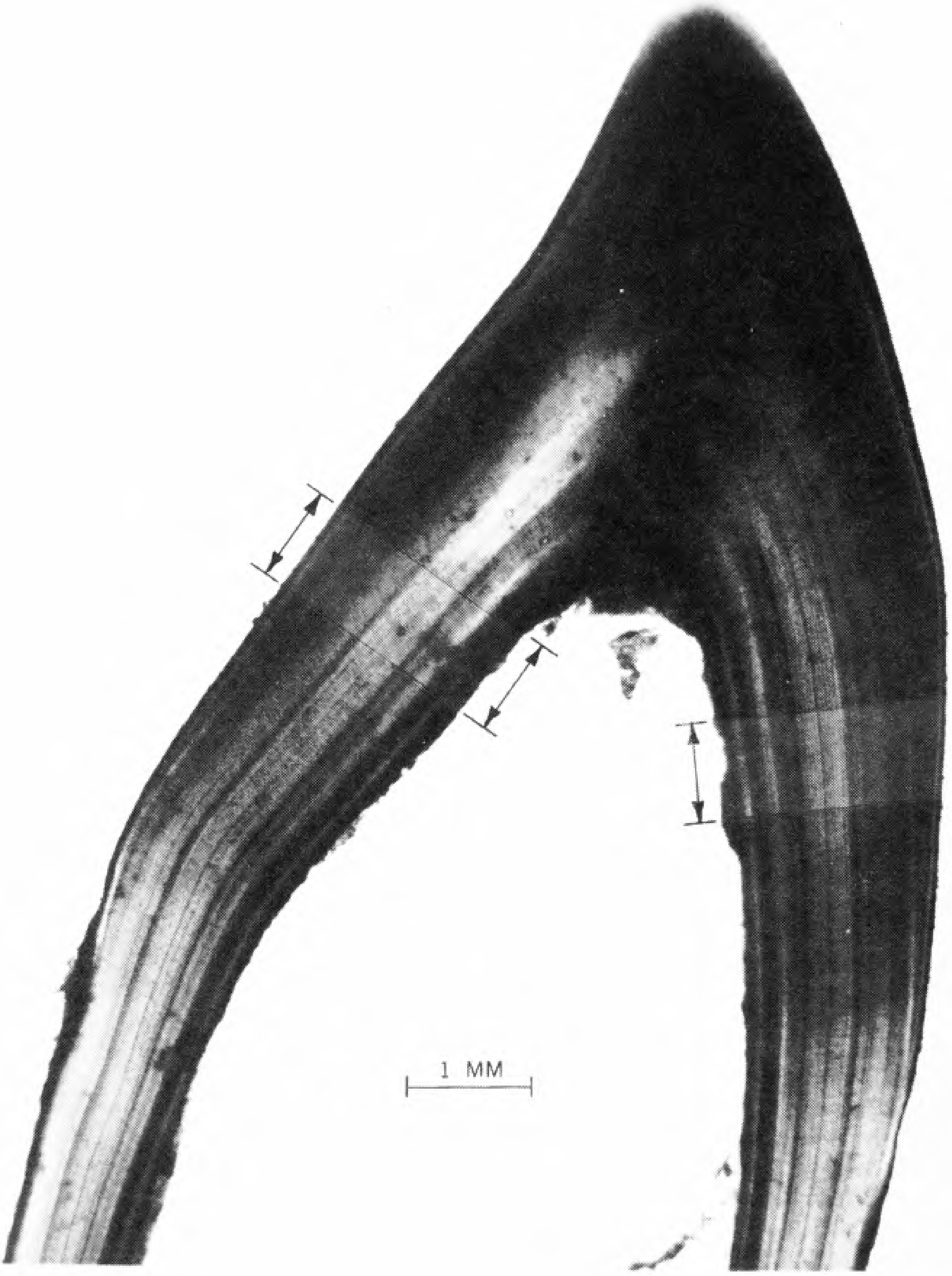

Fig. 1. Photomicrograph of longitudinal tooth section of the 3.3-year-old specimen of *Tursiops truncatus*.

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

DISCUSSION

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

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

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

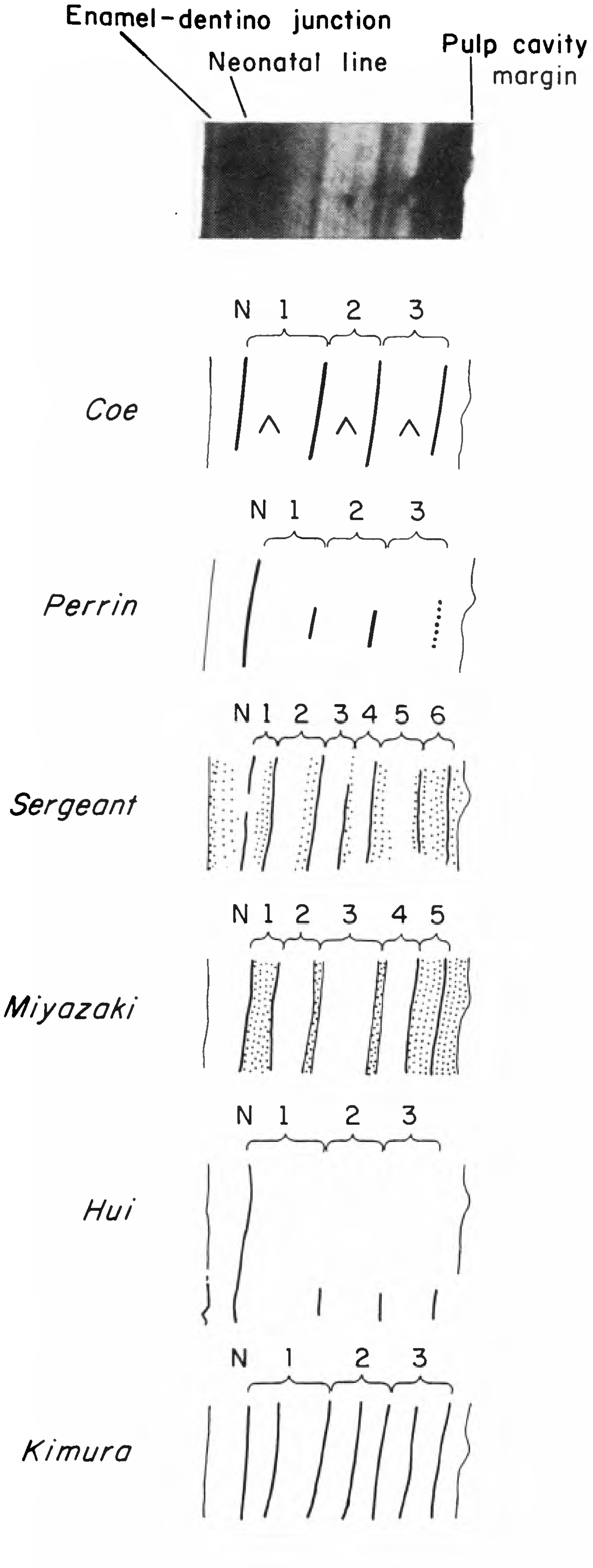

Fig. 2. GLG counts for the 3.3-year-old specimen of *Tursiops truncatus* as estimated by various readers. Inverted v's indicate accessory layers. The photograph of the field in the tooth section where the GLG counts were made is shown on the top.

ACKNOWLEDGEMENTS

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The Use of Tetracycline in Age Determination of Common Dolphins, *Delphinus delphis*

V. S. Gurevich and B. S. Stewart
Hubbs/Sea World Research Institute, 1700 S. Shores Road, San Diego, California
and
L. H. Cornell
Sea World Inc., 1720 S. Shores Road, San Diego, California

ABSTRACT

Tetracycline combines with dental and bone calcium during deposition and can be detected in dental tissue using ultraviolet illumination. We tested the applicability of this knowledge in examining growth rates and interpreting the age of some common dolphins, *Delphinus delphis*.

Four adult females, maintained at Sea World since 1975 were given intramuscular injections (3 gm) of oxytetracycline in February 1977. All teeth from two of these animals, which died approximately one year later, were retained for analysis. On 28 April 1978, several teeth were extracted from each of the two surviving animals and they received another 3 gm injection of oxytetracycline. Teeth were longitudinally sectioned and examined microscopically to determine tetracycline incorporation and extent of growth since marking. Bone sections were also taken from the thin pan region of the left mandibles of the two expired animals.

We found that tetracycline dosages of 39 to 54 mg/kg administered intramuscularly are effective in marking dental and bone tissue. Dentinal layering in the teeth of the upper jaw appears to cease before that in the lower jaw. Apparently only one dentinal growth layer group is deposited annually.

INTRODUCTION

Much of the recent literature dealing with life history of odontocetes has emphasized the need for age determination for analysis of population dynamics and stock assessment. The most commonly used method of age determination for toothed whales is based on counts of dentinal layers in the teeth. Many authors have indicated that a definite number of layers are deposited in hard tissue (dentine, cementum, and bone) each year (Table 1). This assumption has not been tested and the precise rate of layer formation in all species is unknown.

Most authors agree that one postnatal growth layer group (GLG, see terminology of workshop report, this volume) consists of one opaque subunit and one translucent subunit. However, much of the difficulty in defining layers (resulting in opinion differences as seen in Table 1) seems to have arisen from varying interpretation of the finer laminal structures within GLGs. As Klevezal' and Kleinenberg (1967) have noted, minute bands occur within the opaque bands and when especially distinct, may be confused for an opaque band of the GLG.

Another source of confusion in the literature is the substitution of the terms light and dark for transparent and opaque without specifying method of illumination. The terms transparent and opaque refer to the differential optical density of incremental growth layers within a GLG, and Klevezal' and Kleinenberg (1967) noted that with the light microscope, translucent zones appear 'dark' under reflected light and 'light' under transmitted light while opaque zones appear 'light' under reflected light and 'dark' in transmitted light. Nielsen (1972) further indicated the potential confusion when terms for optical density are used to describe mineral content of dentinal zones.

Table 1

Estimated rates of dentinal-GLG deposition in some odontocete cetaceans

Species	Number of GLGs formed annually in dentine	Authors
Tursiops truncatus	1	Sergeant (1959), Hui (1978), Ross (1973)
Stenella coeruleoalba	1	Kasuya (1972a), Miyazaki (1977)
Stenella attenuata	1, 1 or 2	Kasuya *et al* (1974), Perrin *et al* (1976)
Stenella longirostris	1 or 1½	Perrin *et al* (1977)
Globicephala melaena	1	Sergeant (1959, 1962)
Delphinapterus leucas	2, 1	Khuzin (1961), Sergeant (1962), Brodie (1969, 1971)
Physeter macrocephalus	1, 2	Ohsumi *et al* (1963), Best (1970), Berzin (1964), Gambell and Grzegorzewska (1967)
Delphinus delphis	2 (stainable), 1	Kleinenberg and Klevezal' (1962), Hui (1973)
Lagenorhynchus obscurus	1	Best (1976)
Phocoena phocoena	1	Nielsen (1972), Gaskin and Blair (1977)
Hyperoodon ampullatus	1	Christiansen (1973, 1975), Mitchell and Kozicki (1975)
Inia geoffrensis	1	Kasuya (1972b)
Berardius bairdii	1	Kasuya (1977), Omura *et al* (1955)
Ziphius cavirostris	1	Kenyon (1961), Omura *et al* (1955)
Hyperoodon planifrons	1	Tomilin and Latyshev (1974)

Most age determination studies have generally suffered from two basic difficulties: (1) incomplete knowledge of rates of deposition and (2) inadequate known-age samples for calibration. Sergeant (1962), Nielsen (1972) and Hui (1978), using small samples of known-age specimens, have addressed the problem of GLG depositional rates in a limited number of species. An additional method has utilized tetracycline or lead acetate to 'mark' layers in dental tissue. Bevelander *et al* (1961) demonstrated that administration of tetracycline results in its incorporation into hard tissue (e.g. dentin, bone) by forming several metallic ion complexes; this is detectable as fluorescence under ultraviolet illumination. By analyzing laminae accumulated subsequent to the mark, average depositional rates may be estimated.

Using fluorescent techniques, Best (1976) showed that an oral dose of tetracycline as low as 58 mg/kg produced a detectable mark in teeth of *Lagenorhynchus obscurus.* Nielsen (1972) found that an oral dose of 65 to 90 mg/kg was sufficient to mark dental tissue of the harbour porpoise *Phocoena phocoena.* Continuing investigations on *Phocoena* are employing oral doses of 25 to 30 mg tetracycline per kg (Nielsen, 1972).

Intramuscular injections may be the most reliable method for tetracycline administration to captive animals and are practical for field use (Best, 1976). Effective intramuscular dosage, however, has not yet been reported in the literature for odontocetes.

The common dolphin, *Delphinus delphis,* is one of the three major species involved in the yellowfin tuna purse seine fishery in the eastern tropical Pacific (Evans, 1975). Data on population dynamics are needed to assess the fishery impact on this species. Knowledge of dentinal layer deposition rates would allow greater accuracy in age determinations of individuals, thus allowing estimation of age structure of the natural populations.

The main aim of our study was to investigate rates of deposition of dentinal layers in the common dolphin by employing intramuscular injections of tetracycline as an intravitam marking method and to determine the intramuscular dosage required to produce a detectable fluorescent label.

METHODS AND MATERIALS

Each of four female common dolphins (Dd 502, Dd 504, Dd 505, Dd 506) maintained at Sea World Inc., San Diego, subsequent to their capture nine miles west of Point Loma, California in 1975, were given total doses of 3 gms *oxytetracycline 100* via three intramuscular injections, administered in one treatment session in February of 1977 (Table 2). Each injection contained one gram of *oxytetracycline 100* (50 cc) to which was added approximately 2 cc of 2% *lidocaine* solution. Injections were administered in the dorsal musculature, 5 to 10 cm anterior to the base of the dorsal fin just off the midline.

Dd 502 and Dd 506 died approximately one year later; the other two remain in captivity. All teeth from Dd 502 and Dd 506 were removed, sectioned, and examined microscopically under transmitted plain light and reflected ultraviolet light. On 28 April 1978, two teeth were extracted from the left mandible of each surviving animal (Dd 504 and Dd 505). All teeth were sectioned longitudinally through the pulp cavity using a *Buehler Isomet* (11-1180) low speed, double-bladed diamond saw to produce sections 250 to 300 microns thick. The undecalcified sections were examined for tetracycline-marked dentinal lamina with an *Olympus Vanox* microscope equipped with an *Olympus* U.V. system and *Zeiss* fluorescent vertical illuminator with a filter reflector No. 48-77-05 combination. Pictures were taken at 125 X with an *Olympus* PM 10-M photomicrographic system camera and at 200 X with a similar *Zeiss* system. Thin sections of bone were cut from the thin pan region (Fig. 1) of the right mandibles of Dd 502 and Dd 506 and examined microscopically with ultraviolet light for tetracycline fluorescence using the same equipment.

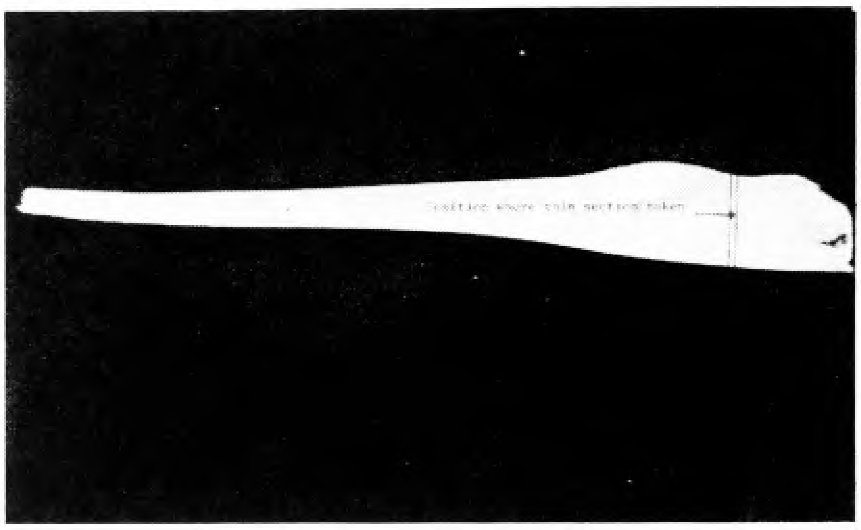

Fig. 1. Right mandible of Dd 502 indication position from which thin sections were taken.

TOOTH SECTIONS

We counted GLGs in the teeth of all four specimens. A dentinal GLG as we define it for counting purposes consists of two opaque and two translucent zones.

Eleven complete dentinal GLGs were counted in tooth sections from Dd 502 (Fig. 2a). An additional zone of dentine lying between the last complete layer and the pulp cavity was considered an incomplete GLG. Under ultraviolet illumination, a green-yellow tetracycline-associated fluorescent band was discernable in the dentine (Fig. 2b). Microstructure of the dentine showed two opaque and two translucent

Table 2

Experimental animals treated with tetracycline

Specimen	Sex	Length	Weight	Date treated	Total dosage	Date of death[1] or tooth extraction[2]	Post-treatment growth period (days)	Number of dentinal GLGs after treatment	Total number of complete GLGs observed
Dd 502	F	195 cm	77 kg	2/7/77	3000 mg	12/29/77 (1)	328	1	11
Dd 504	F	191 cm	68 kg	2/7/77	3000 mg	4/28/78 (2)	441	--	13
Dd 505	F	191 cm	56 kg	2/7/77	3000 mg	4/28/78 (2)	441	1 plus one translucent zone	9
Dd 506	F	188 cm	74 kg	2/7/77	3000 mg	1/14/78 (1)	354	1	9

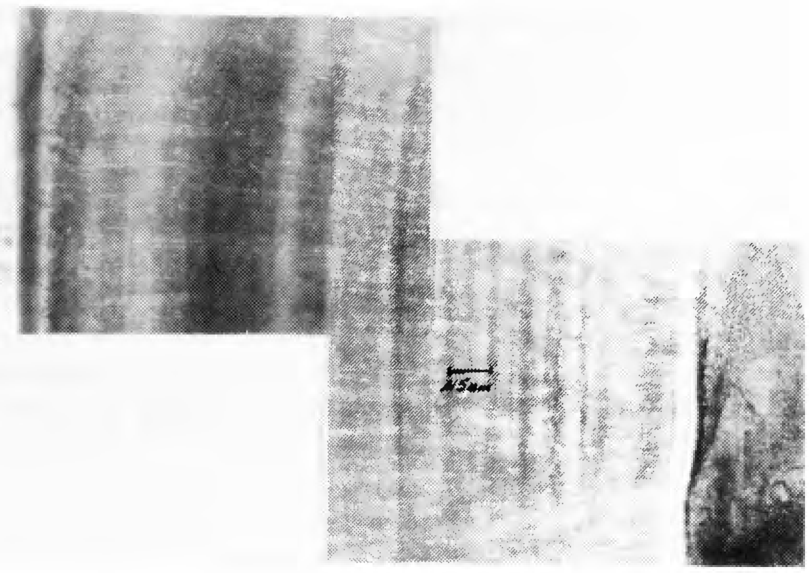

Fig. 2a. Thin section from left mandibular tooth of Dd 502 showing dentinal layering structures and pulp cavity.

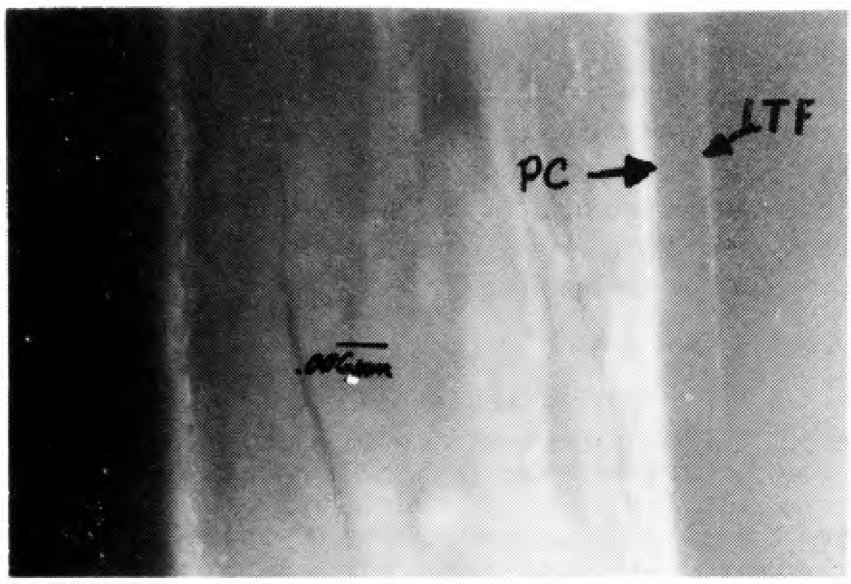

Fig. 2b. Thin section from left mandibular tooth of Dd 502 showing line of tetracycline fluorescence and pulp cavity.

bands (Fig. 2a). Tetracycline marks were most easily observed in teeth from the middle regions of the lower jaw. Teeth from the extreme front and rear regions of the lower jaw showed only faint or no fluorescence. No tetracycline marks were detected in teeth of the upper jaw.

Thirteen complete GLGs plus an additional dentinal zone (an incomplete GLG) were observed in teeth of Dd 504. The pulp cavities were noticeably occluded in teeth from this animal. Tetracycline marks were not observed.

Nine complete GLGs were observed in Dd 505 with an additional zone (incomplete GLG) present between the last complete GLG and the pulp cavity. A green-yellow fluorescent line in the dentine was located in the third opaque layer external to the pulp cavity. Three translucent layers alternated with the opaque layers between the mark and the pulp cavity. The elapsed time between marking and tooth extraction was 441 days (Table 2).

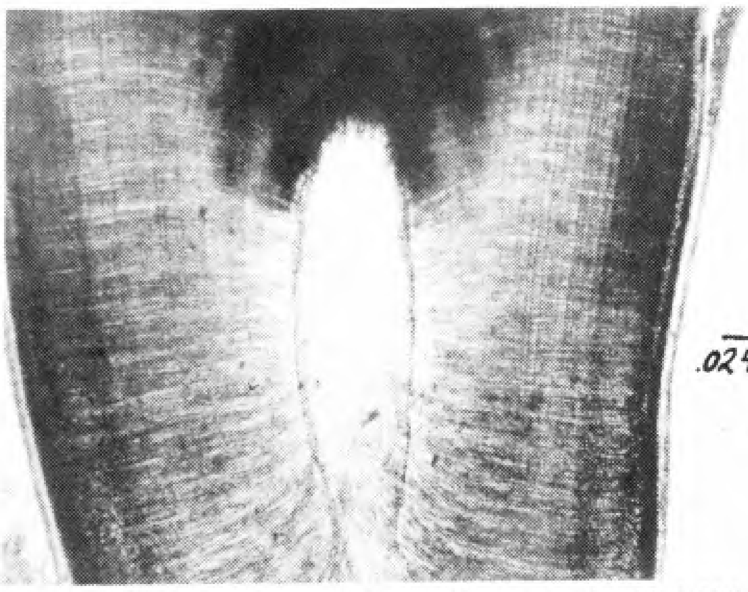

Fig. 3a. Thin section from left mandibular tooth of Dd 506 showing dentinal layering structures and pulp cavity.

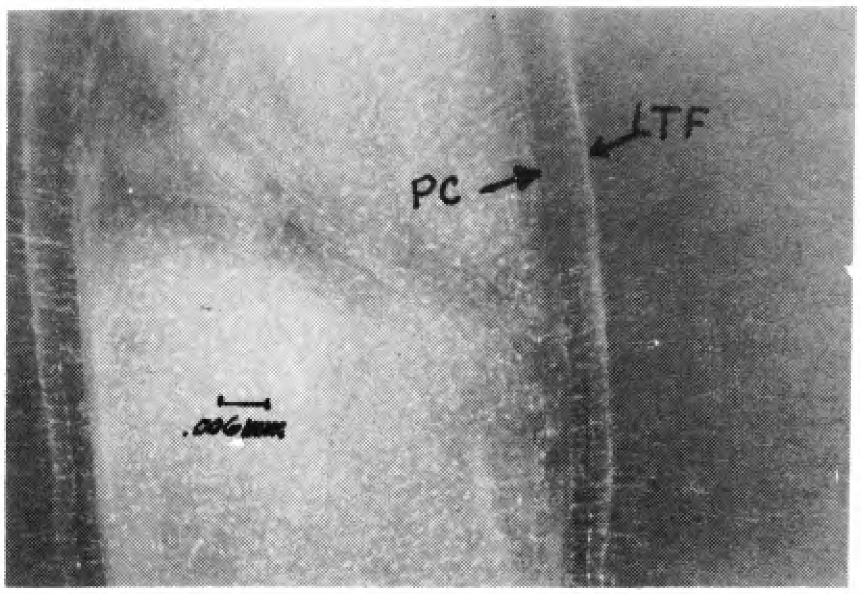

Fig. 3b and 3c. Thin section from the left mandibular teeth of Dd 506 showing line of tetracycline fluorescence (LTF), pulp cavity (PC) and microstructure of dentine (MD) deposited subsequent to mark.

Nine complete GLGs and one incomplete GLG were observed in Dd 506 (Fig. 3a). Under ultraviolet illumination a green-yellow tetracycline mark was observed in the dentine of several tooth sections; tetracycline marks were also most easily observed in teeth from the middle regions of the mandibles. Very faint fluorescent marks were discernable in a few teeth from the upper jaw. In the teeth in which marks were detectable, the tetracycline mark occurred in the third translucent layer external to the pulp cavity (Fig. 3b, c). This layer was separated from the pulp cavity by two opaque layers and two translucent layers (Fig. 3a). The dentine lying between the marked band and the pulp cavity presumably represents 354 days of deposition.

In teeth from Dd 502, Dd 505, and Dd 506, which exhibited tetracycline fluorescence, marks did not appear to be continuous around the pulp cavity in the marked layer.

BONE

Thin pan bone sections from Dd 502 and Dd 506 were examined for periosteal layering. One periosteal GLG consists of one opaque and one translucent zone.

Tetracycline localization was evident in Haversian systems of the spongiosum, and in the periosteal bone of thin sections from lower jaws of Dd 502 and Dd 506. Bone resorption was evident in sections from Dd 506 and GLG counts in the bone were not considered to be productive or useful to this study.

Bone resorption was not apparent, however, in sections from Dd 502 and counts revealed 12 periosteal GLGs.

DISCUSSION

Klevezal' and Kleinenberg (1967) remarked that annual layers of dentine are deposited in odontocete teeth until the entire pulp cavity is filled. They further noted that the time it takes to fill the pulp cavity differs not only among species, but also between individuals and among various teeth of a single animal.

The four animals examined in this study exhibited advanced stages of pulp cavity occlusion. Teeth from Dd 504 (with pulp cavities filled and 13 complete GLGs) showed no evidence of tetracycline, while teeth from Dd 505 (9 complete GLGs), Dd 506 (9 complete GLGs) and Dd 502 (11 complete GLGs) all exhibited tetracycline marks. These data suggest that dentinal deposition may have ceased in Dd 504 prior to injection of tetracycline, and further, that dentine deposition ceases and/or pulp cavity occlusion occurs at approximately 10 to 12 layers. If this is the case, then ages of older individuals arrived at by dentinal GLG counts may be inaccurate.

The appearance of tetracycline in mandibular teeth of Dd 502 but not in maxillary teeth of this animal can be compared with the situation for Dd 506, which exhibited tetracycline fluorescence in mandibular teeth as well as faint tetracycline fluorescence in some maxillary teeth. In both these animals, differences were noted in the fluorescence in various mandibular teeth. The strongest fluorescence (and presumably greatest uptake of tetracycline) was evident in teeth from the middle tooth positions of the mandibles. These data suggest temporal differences in cessation of dentine deposition in teeth from various positions within and between upper and lower jaws, that are in agreement with similar observations made by Klevezal' and Kleinenberg (1967).

The teeth of Dd 502 and Dd 506 accumulated two opaque and two translucent layers in approximately one year. Klevezal' (personal communication) has indicated that when decalcified tooth sections are stained with *Ehrlich's haematoxylin*, each GLG contains two stainable layers in this species.

A comparison of periosteal GLG counts and dentinal GLG counts from Dd 502 suggests that the number of dentinal GLGs correspond to the number of periosteal GLGs; one periosteal GLG was accumulated in bone of both specimens after the treatment. It should be emphasized, however, that while bone resorption was evident in Dd 506 (9 dentinal GLGs), resorption was not observed in the older Dd 502 (11 dentinal GLGs). Such variability may place limitations on the calibration of periosteal GLG counts with dentinal GLGs. Examinations of bone sections should be conducted with a larger sample to clarify the problems of resorption affecting early periosteal layers.

We suggest that future studies using tetracycline administration be conducted using a larger sample consisting of, but not necessarily limited to, younger animals in which layered structure might be more reliably analyzed. It is also suggested that GLGs be studied using periosteal and dentinal tissues simultaneously to examine correlations between the two.

We have determined that an intramuscular tetracycline dosage adequate to mark hard tissues of *D. delphis* is 39 to 54 mg/kg of body weight. Additional experimentation with dosage concentrations should be carried out. Bevelander *et al* (1961) have indicated that high dosages of some tetracyclines interfere with and inhibit calcium deposition.

SUMMARY

(1) Our studies have shown that tetracycline dosages of 39 to 54 mg/kg, administered intramuscularly, are effective in marking hard tissue in *Delphinus delphis*.

(2) Cessation of dentine deposition in *Delphinus delphis* appears to occur after 10 to 12 dentinal GLGs have been deposited. This suggests that age estimates of older specimens may be inaccurate.

(3) Dentinal layering in maxillary teeth appears to cease before layering in teeth of the mandible, suggesting that middle mandibular teeth should be used for GLG counts.

(4) One dentinal GLG, composed of two opaque and two translucent zones, and one periosteal GLG, composed of one opaque and one translucent zone, represent one year in the specimens examined by us.

(5) Bone resorption was observed in mandibular bone sections, and future investigations are required to determine resorption characteristics in *Delphinus delphis* if periosteal layers are to be used in age correlations.

(6) Future investigations of tetracycline marking should involve larger samples composed of several age classes, especially younger animals.

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AOSI/2

Preliminary Note on Age Determination and Growth of the Rough-Toothed Dolphin, *Steno bredanensis*, off the Pacific Coast of Japan

Nobuyuki Miyazaki

Department of Zoology, National Science Museum, Tokyo

ABSTRACT

Age determinations were made for 39 rough-toothed dolphins, *Steno bredanensis* (Lesson, 1828), collected on Izu Peninsula in 1972. There is no significant variation in the readability of the growth layers with positions of the teeth in the jaws. The stainable layer in the decalcified dentine corresponds approximately with the opaque layer in the undecalcified dentine. All the animals killed in June were forming the unstainable layer, while about half of the animals killed in October were forming the stainable layer. The stainable layer may form in winter and the unstainable in summer. Based on examinations of decalcified and stained tooth sections, the specimens had 14 to 32 layers in the dentine and from 15 to 36 in the cementum. Adult males attain a greater size than adult females. The male at 225 cm in body length, or with 14 dentinal layers, has already reached sexual maturity, and the female has already reached sexual maturity at 225 cm, or at 17 layers. The following relationship of body length (L, in cm) to body weight (W, in kg) is derived from ten specimens: Log L = 1.8253 + 0.2641 Log W. Characteristic coloration of adult animals is noted.

INTRODUCTION

The rough-toothed dolphin, *Steno bredanensis* (Lesson, 1828), occurs in tropical and subtropical waters. Occurrence of the species has been reported from the Atlantic coast of the United States (Richardson, 1973), from the eastern tropical Pacific (Orr, 1965; Perrin and Walker, 1975) and from Hawaiian waters (Nishiwaki, 1972). In the Japanese coastal waters this species is very occasionally caught by the drive-in (Oikomi) fisheries at Kawana, Futo, and Arari in Shizuoka Prefecture, and at Taiji in Wakayama Prefecture. Very little published information is available on the biology of the species. The purpose of this note is to evaluate the age and growth of rough-toothed dolphins caught in Japanese coastal waters.

MATERIALS AND METHODS

The available life history information for 104 animals from four schools includes at least, but is not restricted to, sex and body length (Table 1). Data of body length and weight for 10 captive rough-toothed dolphins were provided by Dr Tobayama.

Several teeth were collected from the center of the lower tooth row of each dolphin with a hammer and chisel, and fixed in 10% formalin. Transverse and longitudinal half-sections of teeth of 39 dolphins were prepared using a grinder. The ground surface of the tooth was glued to a plastic board with synthetic resin, and then the other side was ground in the same manner to produce a thin section about 50 μm thick. Dentinal layers in the undecalcified section were counted under transmitted light with a stereoscopic microscope (x80 to x60). The section was then ground to a thickness of between 10 and 20μm, and cemental layers were counted under a compound microscope (x100 to x400). Afterwards, the thinner-ground section was decalcified in 5% formic acid for several hours and stained with Mayer's haematoxylin solution for about 30 minutes. The number of opaque or stained layers in the dentine and cementum was counted with a stereoscopic and a compound microscope respectively.

Some teeth were prepared in both longitudinal and transverse sections. Both sections were found to give similar readings of number of dentinal and cemental layers. In older animals, however, transverse cuts which pass through all growth layers and enter the pulp cavity are inadequate for age determination because of proceeding deposition of osteodentine. Since all specimens used in this study are older animals, longitudinal sections were employed for ageing (Table 2).

Table 1

Materials used in this study of *Steno bredanensis*

School No.	Date of Catch	Date of Kill	No. of dolphins Caught	No. of dolphins Studied	Locality	Researcher
1	26 September 1965	26 September 1965	27	17	Arari	Tobayama
2	18 October 1968	18 October 1968	50	3	Futo	Tobayama
3	17 June 1972	21 June 1972 22 June 1972 3 July 1972	53	42	Arari	Miyazaki
4	12 October 1972	18 October 1972	47	45	Kawana	Miyazaki

Table 2

List of specimens of *S. bredanensis* used in this study

School No.	Specimen No.	Date of catch	Body length (cm)	Sex	Longitudinal section				Last dentinal layer on stained teeth
					No. of dentinal layers		No. of cemental layers		
					Undecalcifcation	Staining	Undecalcification	Staining	
3	S1	27 June 1972	225	F	16	17	28	28 (18,28,32)	Unstainable(*)
3	S2	27 June 1972	228	F	20	20	28	–	Unstainable(*)
3	S3	27 June 1972	235	F	17+	13+	28	–	–
3	S4	27 June 1972	237	F	23	20	36	26 (20,26,32)	Unstainable(†)
3	S5	27 June 1972	225	F	22	22	28	21 (20,21,26)	Unstainable(*)
3	S6	27 June 1972	–	M	12	14	29	15 (14,15,16)	Unstainable(†)
3	S7	27 June 1972	231	M	20	21	28	21 (20,21,28)	Unstainable(*)
4	M2	12 Oct. 1972	–	–	21	22	22	24 (22,24,48)	–
4	M3	12 Oct. 1972	240	F	22	26	29	18 (17,18,19)	Stainable(†)
4	M4	12 Oct. 1972	–	–	20	19	19	25 (24,25,28)	Unstainable(†)
4	M5	12 Oct. 1972	–	–	22	20	20	–	–
4	M10	12 Oct. 1972	232	M	12+	18	30	26 (24,26,28)	Stainable(†)
4	M11	12 Oct. 1972	225	M	23	21	29	–	Stainable(†)
4	M13	12 Oct. 1972	228	M	19	22	25	15 (14,15,15)	Stainable(†)
4	M14	12 Oct. 1972	227	F	25	24	28	17 (16,17,17)	Unstainable(†)
4	M19	12 Oct. 1972	232	M	18	19	34	20 (14,20,27)	Unstainable(*)
4	M22	12 Oct. 1972	235	M	19	18	25	24 (24,24,26)	Stainable(*)
4	M23	12 Oct. 1972	235	M	16	17	26	22 (21,22,22)	Stainable(†)
4	M25	12 Oct. 1972	225	M	30	27	44	32 (31,32,36)	Unstainable(†)
4	A1	12 Oct. 1972	235	M	22	17	26	18 (18,18,24)	Unstainable(*)
4	A2	12 Oct. 1972	223	M	20	19	23	21 (16,21,22)	Stainable(†)
4	A3	12 Oct. 1972	238	M	19	–	26	–	–
4	A4	12 Oct. 1972	230	M	18	20	24	22 (21,22,24)	Unstainable(†)
4	A6	12 Oct. 1972	228	M	22	20	42	34 (28,34,43)	Unstainable(*)
4	A7	12 Oct. 1972	236	M	19	18	24	25 (20,25,26)	Unstainable(†)
4	A8	12 Oct. 1972	230	M	19	18	24	19 (18,19,23)	Unstainable(*)
4	A9	12 Oct. 1972	234	F	21	20	31	36 (35,36,36)	Unstainable(*)
4	A10	12 Oct. 1972	225	F	17	15	22	17 (16,17,23)	Unstainable(†)
4	A11	12 Oct. 1972	222	M	21	18	28	22 (18,22,23)	Unstainable(*)
4	A12	12 Oct. 1972	253	M	20	19	29	20 (18,20,20)	Stainable(†)
4	A13	12 Oct. 1972	232	M	26	28	43	32 (28,32,44)	Stainable(†)
4	A14	12 Oct. 1972	225	M	17	19	26	20 (19,20,20)	Stainable(†)
4	A15	12 Oct. 1972	235	M	22	22	33	24 (23,24,30)	Stainable(†)
4	A16	12 Oct. 1972	228	M	17+	32	26	20 (17,20,26)	Stainable(†)
4	A17	12 Oct. 1972	240	M	13	15	25	15 (15,15,17)	Unstainable(*)
4	A18	12 Oct. 1972	239	M	18	20	27	19 (18,19,23)	Unstainable(†)
4	A19	12 Oct. 1972	228	M	18	19	30	20 (18,20,20)	Stainable(†)
4	A20	12 Oct. 1972	240	F	29	30	36	33 (29,33,35)	Unstainable(†)
4	A21	12 Oct. 1972	228	F	17+	20	27	21 (17,21,22)	–

*Thin. †Thick. The figures in parentheses indicate the repeated count.

The ovaries were collected from 8 females and the number of corpora albicantia and corpora lutea were counted.

The testes were collected from 9 males; two of the samples were examined histologically.

A comparison of teeth from different positions of the same animal showed no significant difference in the number of dentinal layers (Table 3). In practice, teeth were collected from the middle part of the lower jaw because they were more easily removed.

Table 3

Age determinations using stained sections of teeth collected from various positions of jaws in one rough-toothed dolphin (Specimen No. A19)

	Tooth No. from tip of jaw			
Jaw	10	11	13	20
Left upper jaw	–	–	17 (19)	17 (17)
Left lower jaw	17 (18)	–	–	17 (19)
Right upper jaw	–	19 (17)	–	17 (18)
Right lower jaw	18 (19)	–	–	19 (19)

The figures in parentheses indicate the age determined in undecalcified specimens.

AGE DETERMINATION

In order to find the annual accumulation rate of dentinal growth layers, the appearance of the last forming layer was checked in teeth of animals killed on both 27 June 1972 and 10 October 1972. Table 4 shows that all of the animals killed in June were forming the translucent or unstainable layer, while about half of the animals in October were forming the opaque or stainable layer. If each opaque and translucent layer represents about 6 months, respectively, the above result could indicate that the translucent layer may form in summer and the opaque in winter. This is the reverse of the annual accumulation patterns obtained for *Tursiops truncatus* (Sergeant, 1959), *Globicephala melaena* (Sergeant, 1962), *Physeter macrocephalus* (Ohsumi, Kasuya and Nishiwaki, 1963), *Stenella attenuata* (Kasuya, Miyazaki and Dawbin, 1974) and *Stenella coeruleoalba* (Kasuya, 1972; Miyazaki, 1977). However, this result was obtained by the analysis of a small number of older animals; the problem requires further study.

A comparison of dentine deposition of undecalcified sections with decalcified and stained sections shows that the stainable dentinal layer seems to correspond approximately with the opaque layer of undecalcified dentine (Table 4). This result coincides with those for *Callorhinus ursinus* and *Dugong dugon* (T. Kasuya, personal communication), and is in disagreement with those for *S. attenuata* and *S. coeruleoalba* (Kasuya, 1976).

Figure 1 shows a comparison of counts of dentinal layers in undecalcified sections with those in decalcified and stained sections. No significant differences between techniques are indicated; the two methods give almost the same result. Because counts of cemental growth layers in decalcified and stained sections agree only about 10% to 30% of the time in repeated counts, repeated counts for the same tooth section are indicated in parentheses (Table 2).

Table 4

Appearance of the last-forming dentinal layer in 34 specimens of *Steno bredanensis*

Appearance of dentinal layer	No. of animals killed	
	On 27 June 1972	On 10 Oct. 1972
(1) Opaque layer		
– in early deposition	0	0
– nearly completed	0	14
Translucent layer		
– in early deposition	1	0
– nearly completed	5	14
(2) Stainable layer		
– in early deposition	0	1
– nearly completed	0	12
Unstainable layer		
– in early deposition	4	7
– nearly completed	2	8

The number of dentinal layers was almost the same as cemental layers in teeth of the animals of 20 dentinal layers or less, but in older animals the number of cemental layers exceeds in number the dentinal layers in nearly all specimens (Fig.2). In the case of undecalcified specimens, the number of cemental layers greatly exceeds that of dentinal layers (Fig.3). In the old animals the pulp cavities are not completely filled with osteodentine. The discrepancy of readings between the dentinal and cemental layers may have been caused by difficulty in discerning the thin dentinal layers of older animals, or some teeth may have been sectioned in the wrong plane.

GROWTH

Length-frequency

Length-frequency distributions by 5 cm increments for males and females, combining dolphins from four schools, are presented in Figure 4. This figure shows that males of *S. bredanensis* reach a larger maximum size than females. Length frequencies decrease at 240 cm to 250 cm in the

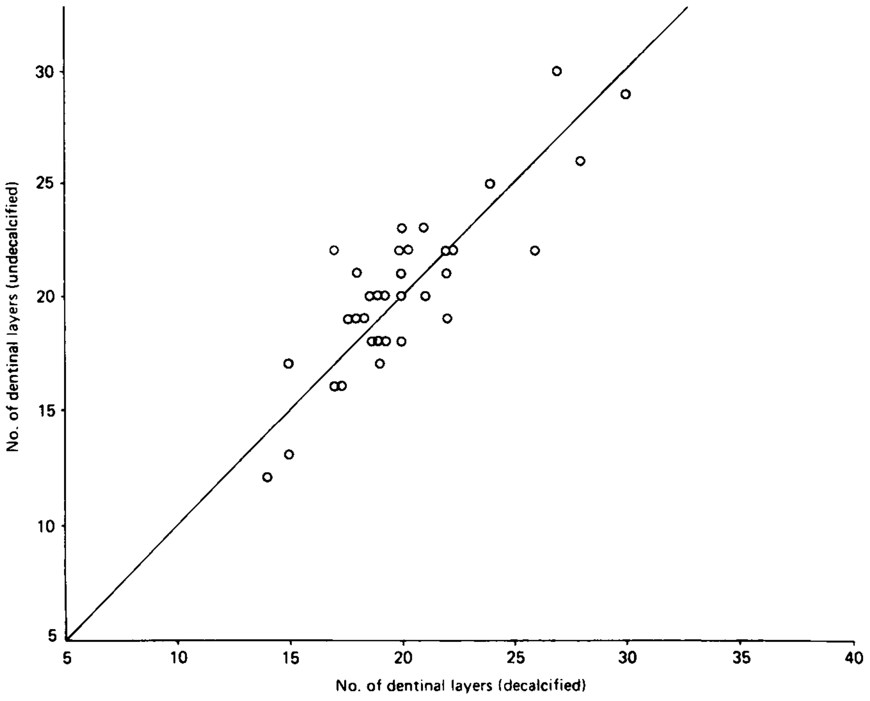

Fig. 1. Relationship of the number of layers in undecalcified, unstained dentine to that in decalcified and stained dentine.

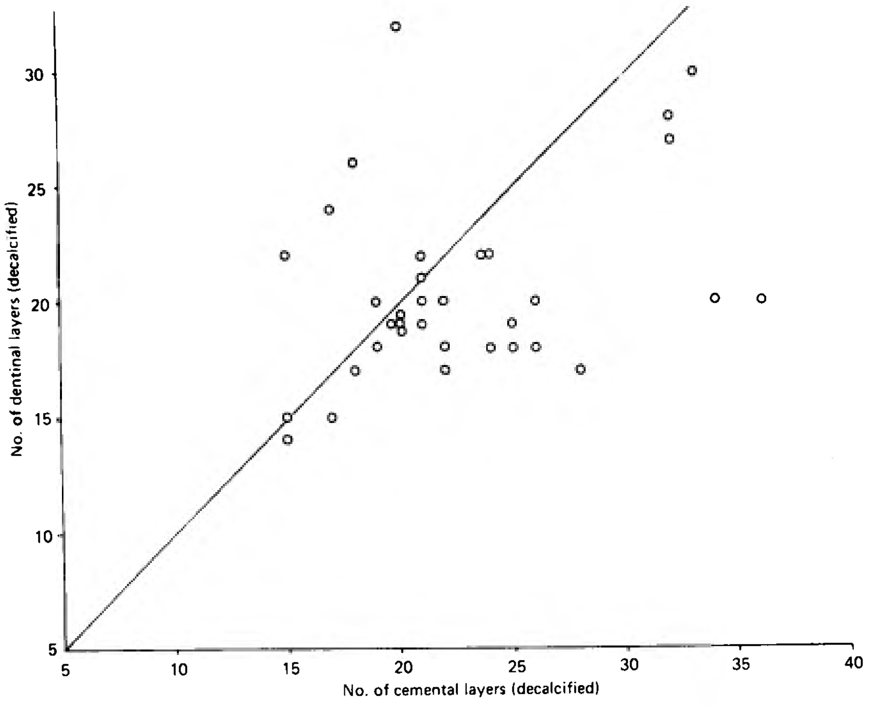

Fig. 2. Relationship of the number of dentinal layers (decalcified and stained) to the number of cemental layers (decalcified and stained).

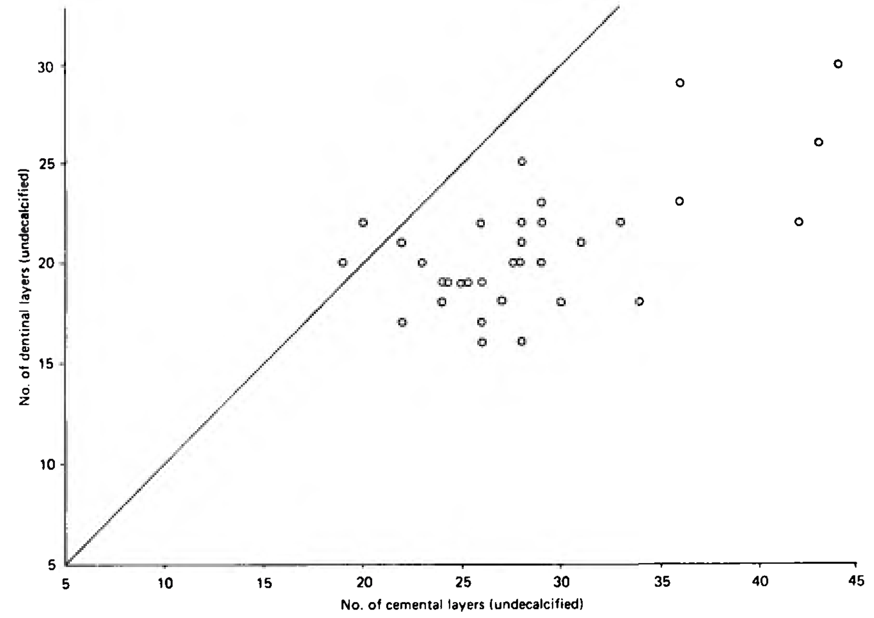

Fig. 3. Relationship of the number of dentinal layers (undecalcified) to the number of cemental layers (undecalcified).

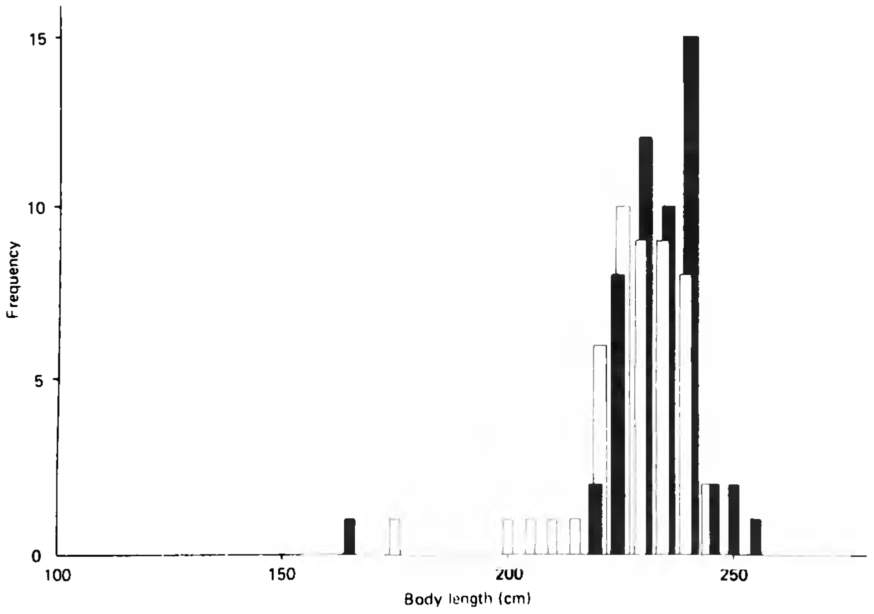

Fig. 4. Body length frequency of specimens of *S. bredanensis.* Black bar indicates males, white bar indicates females.

males and at 230 cm to 240 cm in the females. The sharp decline in relative numbers occurred around the lengths at which the animals are sexually mature; the smallest mature animal was 225 cm long in both sexes. The maximum frequency was reached at a length of 240 cm for males and 225 cm for females, and maximum lengths attained were 253 cm for males and 247 cm for females.

Postnatal growth

Since at least one lactating female was found in school No. 4, in which the body lengths of all animals were measured, it would be safe to say that the smallest animal of the school, a male 167 cm long, may have been nursing.

As indicated in Table 5 the male having a left testis weight of 175 g has already attained sexual maturity. As indicated in Tables 5 and 6, sexual maturity is already reached by the male at 231 cm in body length, or 14 dentinal layers, and by the female at 225 cm, or 17 layers.

In the females with 17 to 26 dentinal layers, the number of ovulations ranged from 5 to 14 (Table 6). Two animals (specimen Nos. S1 and S2) had two corpora albicantia each. From these data it is impossible to expect a linear relationship between the numbers of corpora and dentinal growth layers. The rough-toothed dolphin may exhibit several almost simultaneous ovulations, as does *Globicephala melaena* (Sergeant, 1962) and *Delphinapterus leucas* (Brodie, 1972). This problem invites further study.

The plot of growth layers in decalcified and stained dentine against body length was constructed for males and females separately (Fig.5). All the animals plotted are adults, and a growth curve for *S. bredanensis* was not obtainable.

Relationship between body length and weight

Table 7 shows the relationship of body length to weight in 10 specimens collected on 17 June 1972. As the number of specimens is low, this relationship was calculated by combining data for both sexes. Calculation by the least squares method gives the formula, $\text{Log L} = 1.8253 + 0.2641 \text{ Log W}$ ($r = 0.7128$); where L = body length in cm and W = body weight in kg.

Coloration

The coloration of the adult rough-toothed dolphin is remarkably distinctive. Especially noticeable are the white tips of both upper and lower jaws and the white patch on the ventrum (Figs. 10 and 11). Coloration varies individually among adults. Two young animals (a male 167 cm long and a female 175 cm long) did not exhibit the adult coloration. Similar white jaw tips in the adult animals have been observed in *S. attenuata* (Kasuya *et al.*, 1974).

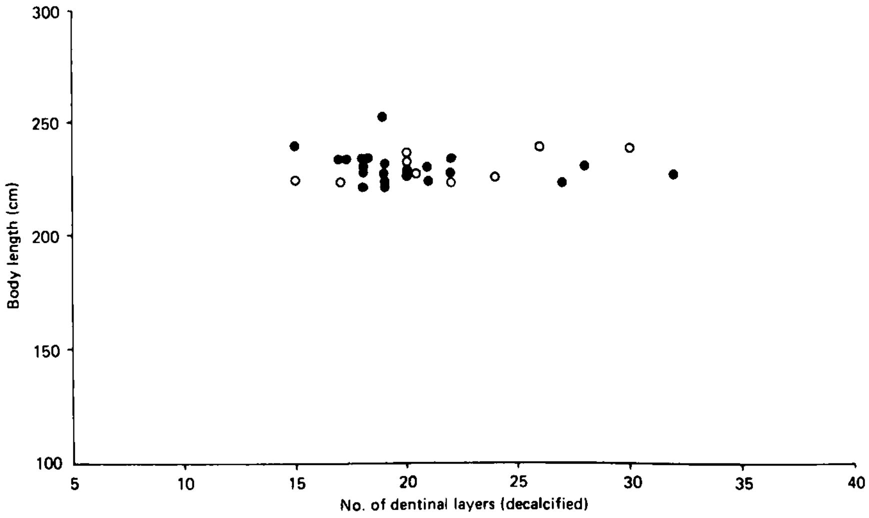

Fig. 5. Age plotted against body length for specimens of *S. bredanensis.* Closed circle indicates males, open circle indicates females.

Table 5

Age, length, and reproductive condition of males of *Steno bredanensis*

School No.	Specimen No.	No. of* dentinal layers	Length (cm)	Testis weight (g) L	R	Comments
3	S6	14	–	290	330	Sperm present
3	S7	21	231	175	1030	Sperm present
4	M11	21	225	1050	–	
4	M13	22	228	250	–	
4	M22	18	235	1050	–	
4	M25	27	225	1150	–	
4	A3	19†	238	920	–	
4	A4	20	230	680	–	
4	A6	20	228	630	–	

*Tooth decalcified and stained.
†Tooth undecalcified.

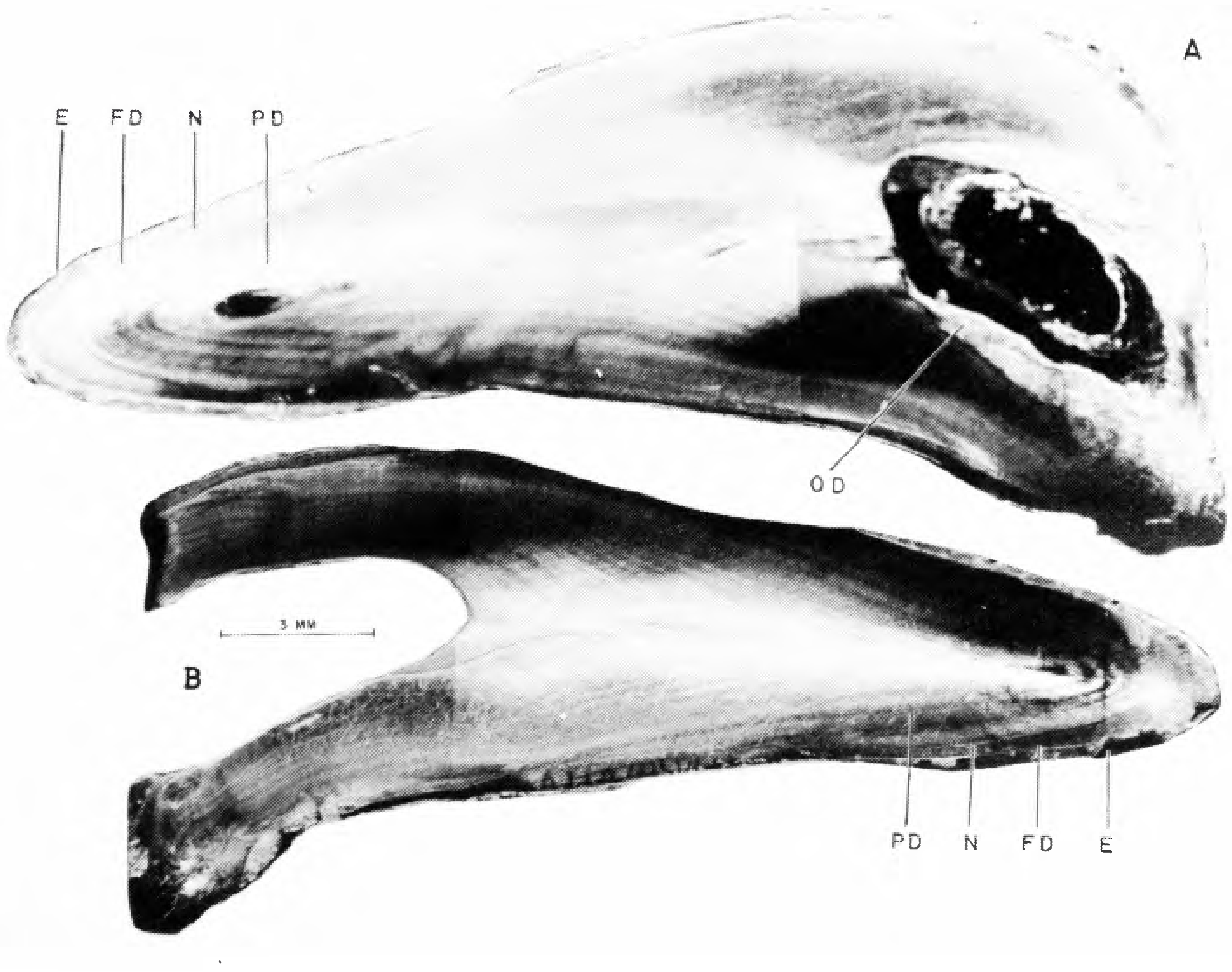

Fig. 6. Dentinal layers in undecalcified ground thin sections of the teeth of *S. bredanensis* under transmitted light.
A – Tooth of an adult female (specimen no. M3), 240 cm.
B – Tooth of an adult male (specimen no. A11), 222 cm.
E. Enamel, FD. Fetal dentine, N. Neonatal line, PD. Postnatal dentine, OD. Osteodentine.

Table 6

Age, length, and reproductive condition of females of *Steno bredanensis*

School No.	Specimen No.	No. of* dentinal layers	Length (cm)	Ovary (g)		No. of corpora lutea		No. of corpora albicantia		Comments
				Left	Right	Left	Right	Left	Right	
3	S1	17	225	2.9	11.0	1	1	0	8	Pregnant: no fetus
3	S2	20	228	2.4	9.9	0	2	0	8	Pregnant: no fetus
3	S3	13+	235	5.1	12.3	0	1	0	9	Pregnant: no fetus
3	S4	20	237	11.2	5.4	1	0	5	2	Pregnant: no fetus
3	S5	22	225	6.0	6.0	0	1	13	0	Pregnant: no fetus
4	M3	26	240	14.9	3.4	1	0	4	0	Pregnant: fetus length 38.0 cm(♀)
4	M14	24	227	11.9	4.4	1	0	4	0	Pregnant: no fetus
4	A9	20	234	16.1	5.7	1	0	4	0	Pregnant: fetus length 9.0 cm(♀)
4	A20	30	240	–	–	–	–	–	–	Lactating

*Tooth decalcified and stained.

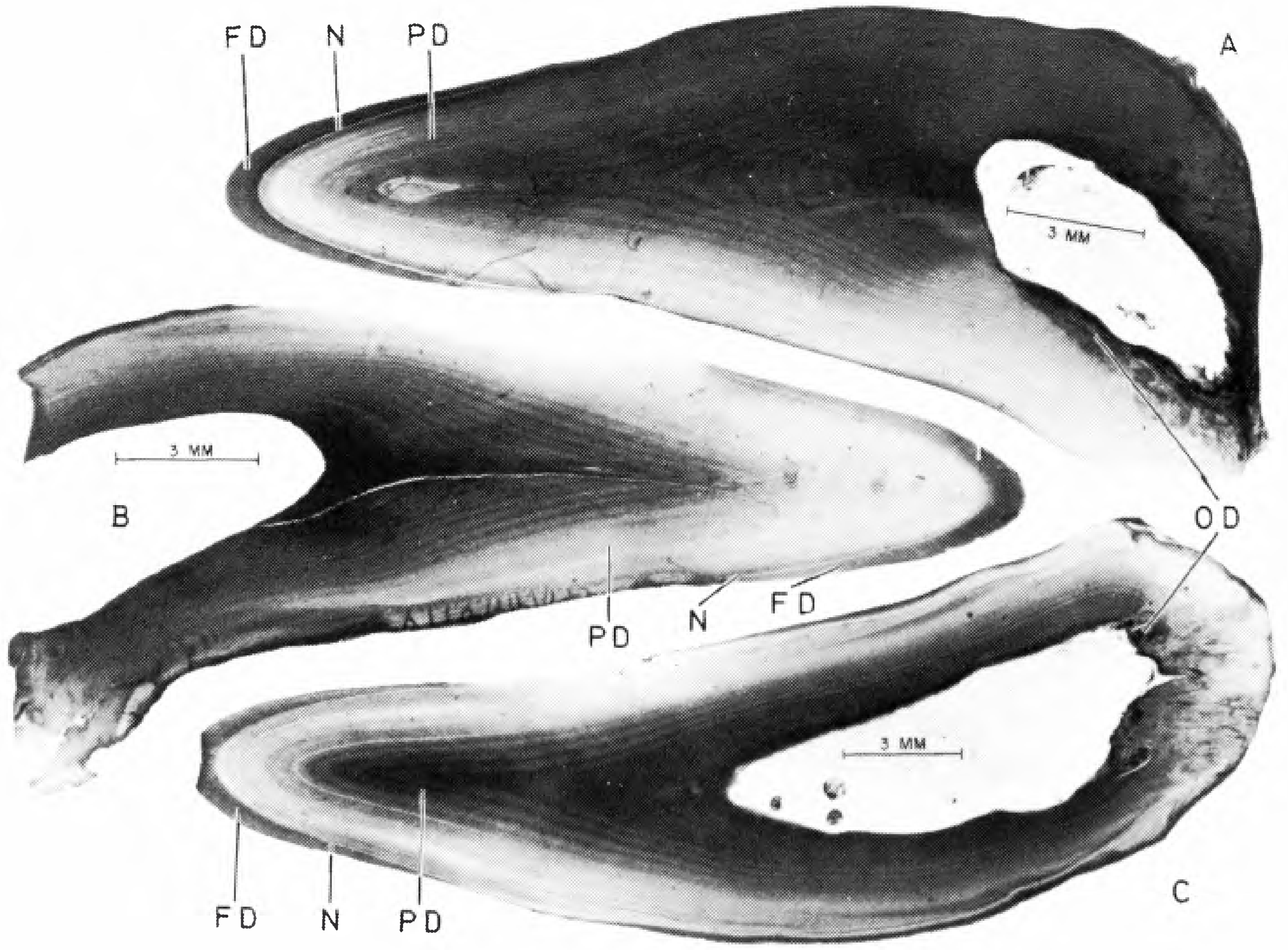

Fig. 7. Dentinal layers in decalcified and stained ground thin sections of teeth of *S. bredanensis*.
A – Tooth of an adult female (specimen no. M3), 240 cm.
B – Tooth of an adult male (specimen no. A11), 222 cm.
C – Tooth of an adult female (specimen no. A10), 225 cm.
FD. Fetal dentine, N. Neonatal line, PD. Postnatal dentine, OD. Osteodentine.

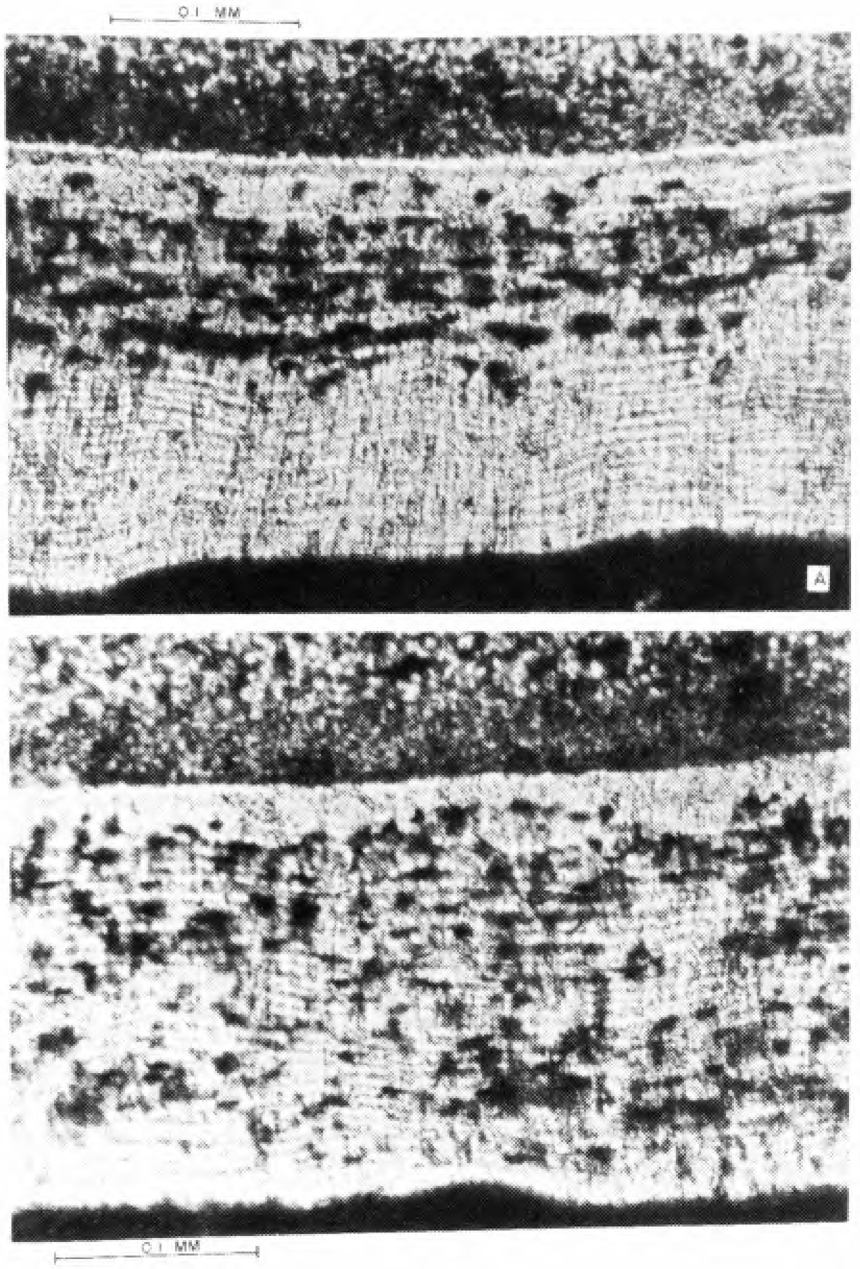

Fig. 8. Cementum in undecalcified ground thin sections of teeth of *S. bredanensis*.
A – Photomicrograph of cementum of an adult female (specimen no. A20), 240 cm.
B – Photomicrograph of cementum of an adult male (specimen no. A 19), 228 cm.

Table 7
Body length and weight of ten captive rough-toothed dolphins

Sex	Body length (cm)	Body weight (kg)
Female	221	115
Female	224	108
Male	241	124
Male	241	126
Female	227	100
Male	250	145
Female	242	115
Male	233	105
Female	230	115
Female	238	110

ACKNOWLEDGEMENTS

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Fig. 9. A school of rough-toothed dolphins driven into the port at Kawana on October 12, 1972.

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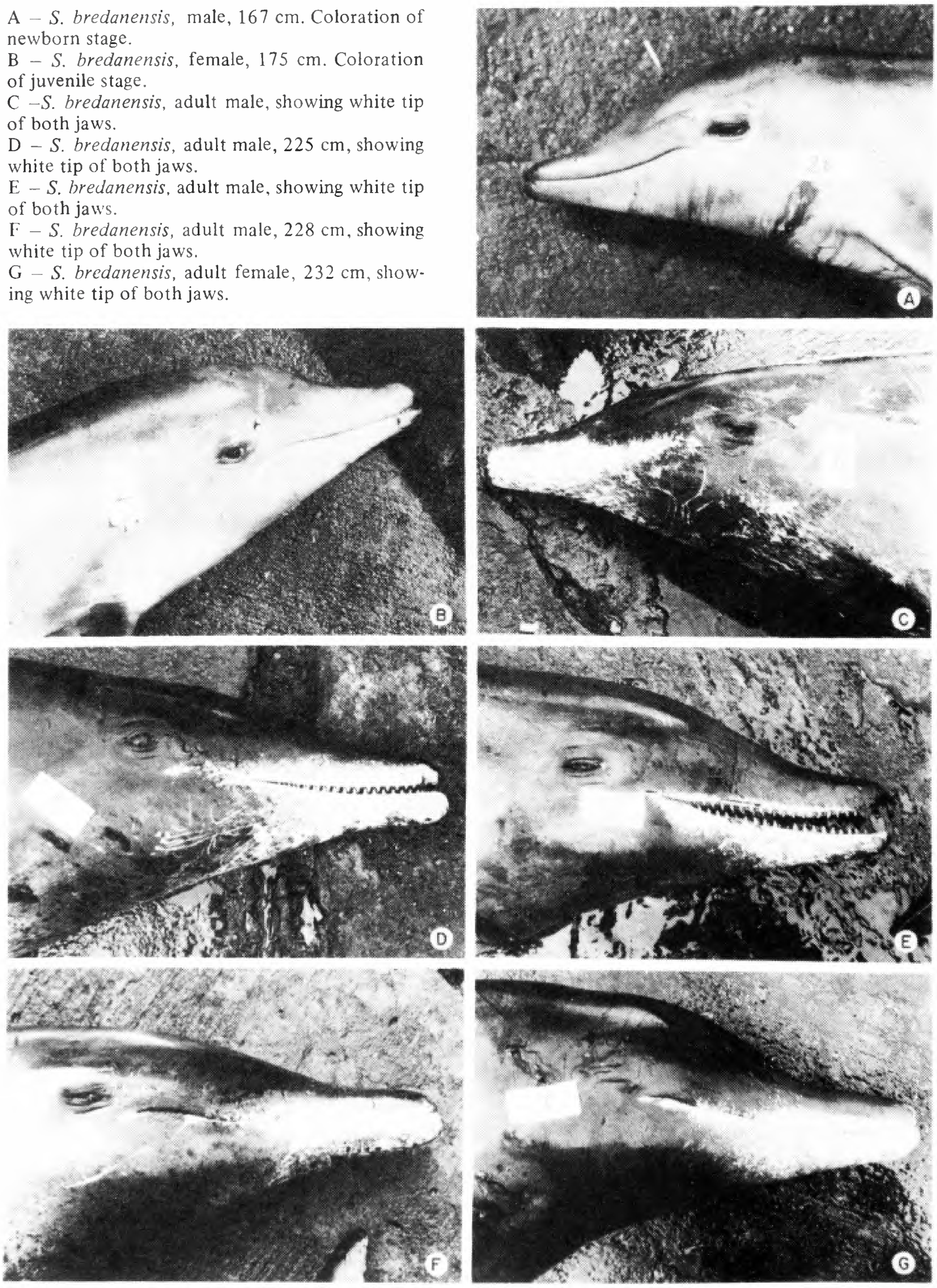

A – *S. bredanensis*, male, 167 cm. Coloration of newborn stage.

B – *S. bredanensis*, female, 175 cm. Coloration of juvenile stage.

C – *S. bredanensis*, adult male, showing white tip of both jaws.

D – *S. bredanensis*, adult male, 225 cm, showing white tip of both jaws.

E – *S. bredanensis*, adult male, showing white tip of both jaws.

F – *S. bredanensis*, adult male, 228 cm, showing white tip of both jaws.

G – *S. bredanensis*, adult female, 232 cm, showing white tip of both jaws.

Fig. 10. Comparison of coloration on jaws in young and adult specimens of *S. bredanensis*.

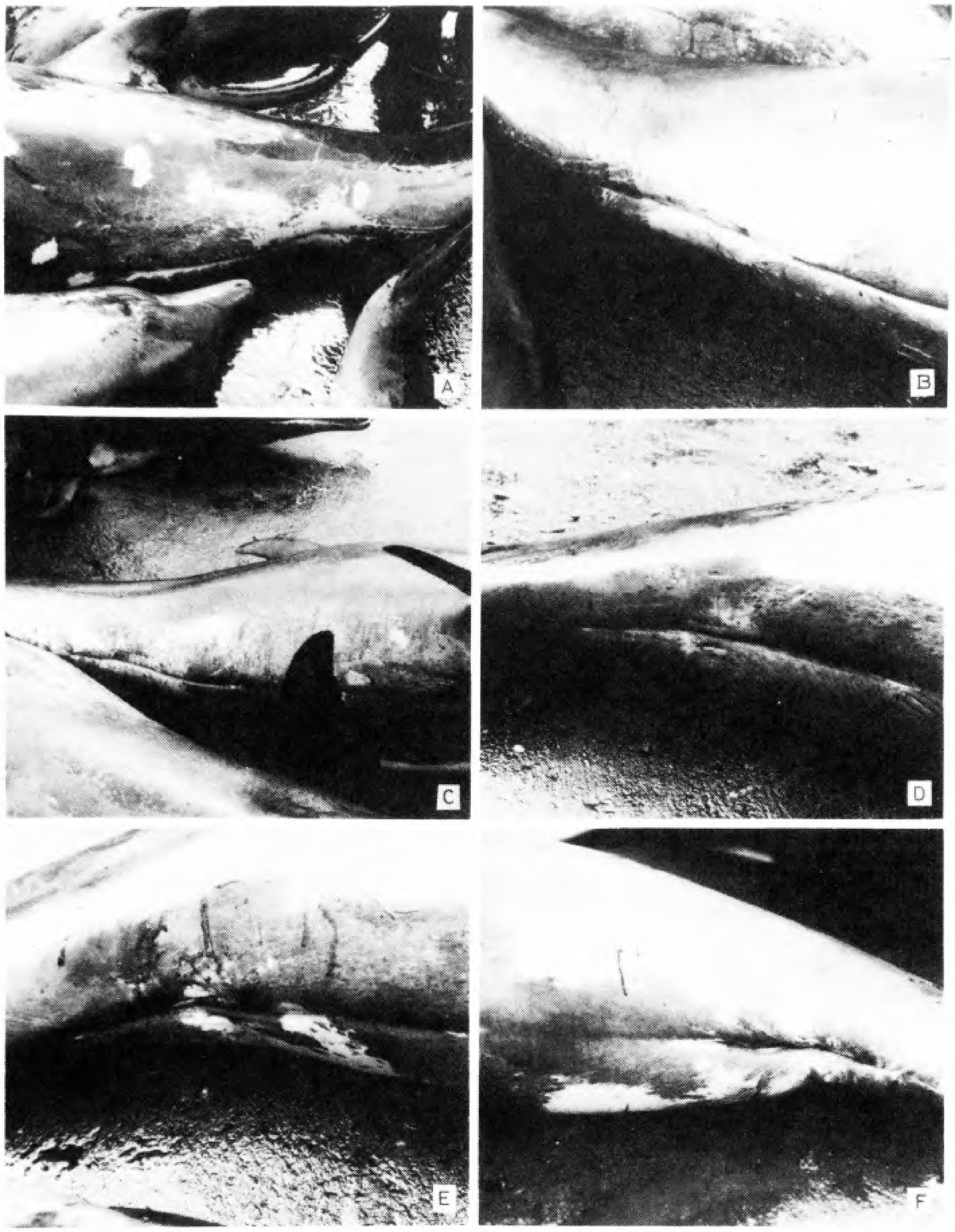

Fig. 11. Individual variation of the ventral white patch in adult specimens of *S. bredanensis*.
A – *S. bredanensis*, adult male, showing white patch on the ventral.
B – *S. bredanensis*, adult female, showing white patch on the ventral.
C – *S. bredanensis*, adult male, 228 cm, showing white patch on the ventral.
D – *S. bredanensis*, adult female, 227 cm, showing white patch on the ventral.
E – *S. bredanensis*, adult female, 232 cm, showing white patch on the ventral.
F – *S. bredanensis*, adult female, showing white patch on the ventral.

Age Determination of the Dugong (*Dugong dugon* (Müller)) in Northern Australia and its Biological Implications

H. Marsh

Zoology Department, James Cook University of North Queensland
Townsville 4811, Australia

ABSTRACT

In the absence of known age material, incisor dentinal growth layer groups (GLGs) have been examined from 129 dugongs from northern Australia with a view to age estimation. Material was available from animals which died in all months of the year.

Each GLG consists of a thick zone of intermediate optical density followed by a thin zone consisting of one of three pairs of contiguous layers. One layer in each pair is opaque, the other translucent. The intensity of haematoxylin staining, the susceptibility to acid etching, and the radio-opacity of each layer mirror its degree of optical density.

Examination of the most recently deposited zone in teeth from 106 dugongs whose date of death was known suggests that one GLG is deposited each year, formation of the narrow zone generally occurring from July to October inclusive.

The permanent incisors erupt and become worn in adult males and occasionally in females, making a total GLG count impossible for these animals. Total GLG counts in unworn tusks of females suggest a life span of fifty years or more. The maximum number of GLGs in a worn tusk of a male was 34.5. Puberty in both males and females is estimated to occur at a minimum of about nine years of age in the Townsville population, but probably not until several years later in the Mornington Island population. The tusks erupt in male dugongs after puberty at about 12 years (Townsville population) or 14 years or later (Mornington Island population). A growth curve describing the age-length relationship, based on number of dentinal GLGs, has been developed for both males and females.

The ontogeny of the cheek teeth is described relative to the age estimates. The use of the pattern of cheek-tooth development as a means of estimating absolute age is described for animals estimated to be up to nine years old. Equations have been developed for the prediction of age from the size of molars 2 and 3 in animals from ten to thirty years of age.

INTRODUCTION

The dugong, *Dugong dugon* (Müller), is one of only four extant species of Sirenia or seacows. It is of particular interest as the only existing herbivorous mammal which is strictly marine.

Historically, the range of the dugong was broadly coincident with the tropical and subtropical Indo-Pacific distribution of the sea-grasses on which it feeds, but it is presently considered rare over most of its range (Bertram and Bertram, 1973) and is listed as a vulnerable species (IUCN Red Data Book, 1976).

Aerial surveys over the last four years have, however, established that sizable populations of dugongs still exist in the shallow seas around northern Australia (Heinsohn, *et al*, 1976; Ligon, 1976; Heinsohn *et al*, 1978; Marsh *et al*, in press) and a broadly-based programme of research has been established at James Cook University which aims to develop effective conservation and management programmes for dugongs in this region.

A major problem is that no definitive life history information is available for the dugong. There have been no successful field studies of specific individuals over any time span. Dugongs have rarely been maintained in captivity and have never bred in captivity. Eleven-year growth records are, however, available for two captive male dugongs in India (Jones, 1967, 1976). Heinsohn (1972) used the body lengths of 68 sexed dugongs accidentally drowned in nets throughout the year to distinguish four age classes. He estimated sexual maturity at approximately two years and constructed an hypothetical growth curve which described the increase in body length with age of northern Australian dugongs in their natural environment. His growth estimates suggest that dugongs grow much faster in the wild than Jones's observations on captive animals indicate.

Pocock (1940) used closure of cranial sutures and Mitchell (1973) a series of skulls and teeth to develop criteria for the determination of relative age in the dugong.

Several workers have counted dentinal growth layer groups (GLGs) in the tusks as an indicator of age (Scheffer, 1970; Mitchell, 1976, 1978; Kasuya and Nishiwaki, 1978). These studies were hampered by lack of biological information about all but a few of the dugongs from which the tusks were obtained, and it was not possible to establish the rate of deposition of the GLGs, especially as there is controversy over the deposition rate in tropical mammals (Klevezal′ and Kleinenberg, 1967; Spinnage, 1973, 1976).

The present study is based on data records, skulls and reproductive tracts from 129 dugongs, the majority of which came from two locations in Queensland, northern Australia, and aims to estimate dugong life history parameters, such as maximum longevity and age at reproductive maturity, which are essential to the development of effective conservation strategies.

THE DENTITION OF THE DUGONG

Heuvelmans' (1941) classification of dugong teeth is followed in this paper.

Two pairs of upper incisors, neither of which is erupted, are present in all young and some older dugongs (Fig. 1). The anterior pair is referred to as the deciduous incisors. The posterior incisors, which are permanent, form the tusks and later erupt in males (Fig. 2) and occasionally in females.

The tusks are the only teeth present throughout life in all dugongs. Up to three pairs of vestigial incisors and one pair of canines may occur occasionally in the rudimentary sockets under the horny plate which covers the downturned symphysial portion of the lower jaw.

Apart from the erupted tusks, the only functional teeth are the cheek teeth. These teeth are difficult to classify since *in situ* replacement of the milk dentition does not occur. During the life of the animal there is a total of six pairs of cheek teeth in both jaws. These teeth are referred to as premolars 2, 3, 4 and molars 1, 2, 3.

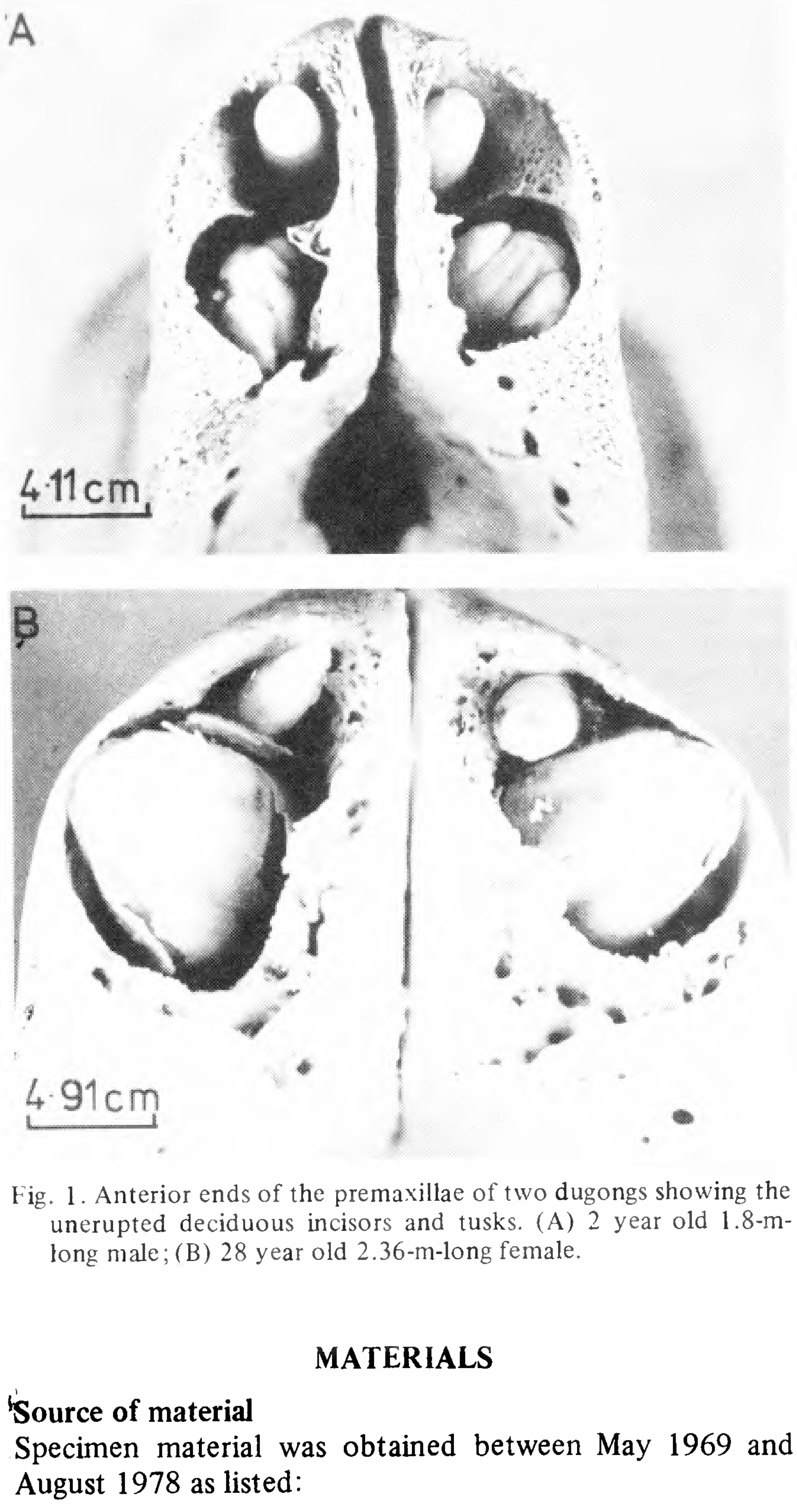

Fig. 1. Anterior ends of the premaxillae of two dugongs showing the unerupted deciduous incisors and tusks. (A) 2 year old 1.8-m-long male; (B) 28 year old 2.36-m-long female.

MATERIALS

Source of material

Specimen material was obtained between May 1969 and August 1978 as listed:

from 70 dugongs accidentally drowned in nets (usually shark nets set for swimmer protection) near Townsville (19°15′S; 146°15′E) within 2 h and 2.5 days of death (Heinsohn, 1972; Heinsohn and Spain, 1974);

from 39 dugongs speared and drowned for food by aboriginals in the Wellesley Islands (16°30′S; 139°30′E) within 0.5 h and 12 h of death (Marsh *et al,* in press);

from two dugongs similarly killed by Torres Strait islanders at Thursday Island (10°30′S; 142°3′E) within 0.5 h and 12 h of death; and

from one dugong caught off Cairns (16°55′S; 145°46′E) and held in captivity in the Cairns Oceanarium for several months prior to its death.

Four isolated dugong skulls, eleven isolated tusks and three isolated ribs collected from various northern Australian beaches are also included in this study.

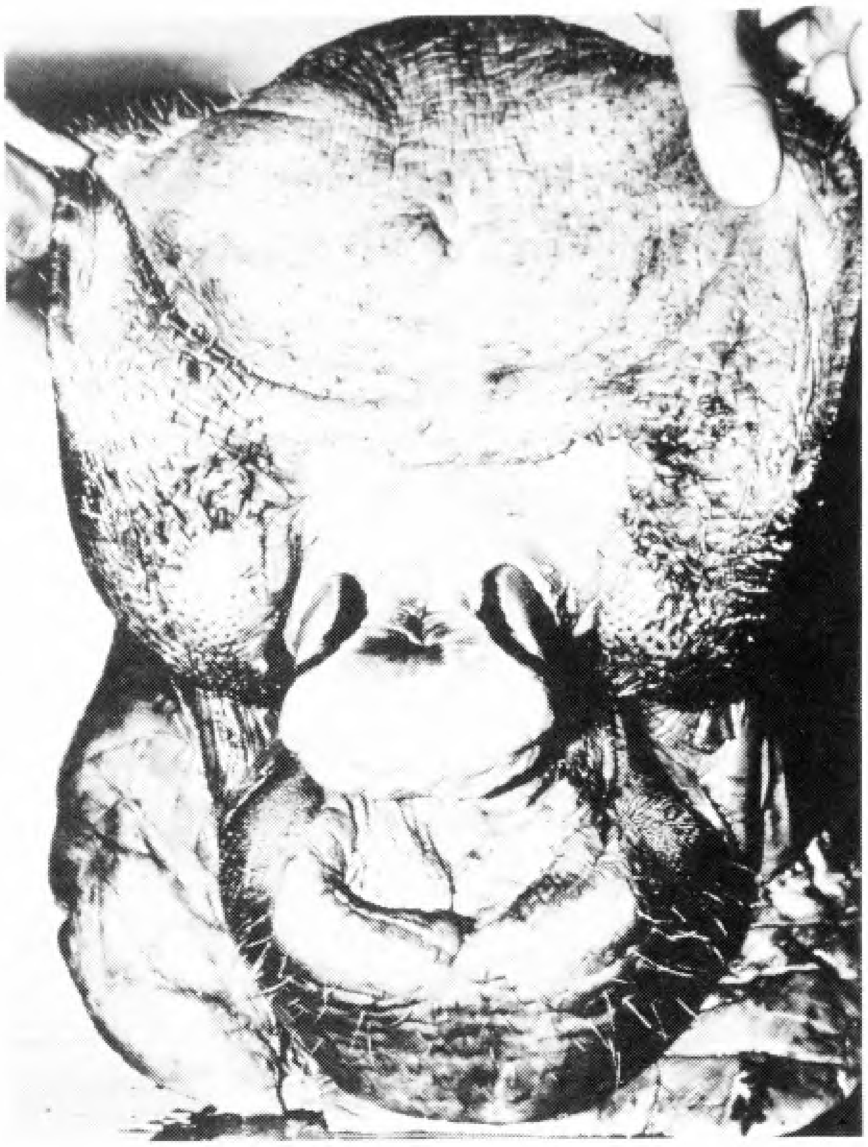

Fig. 2. The head of a 2.7-m-long adult male dugong. There were 22.5 GLGs in each of the erupted and worn tusks.

Collection procedure

Unless very badly decomposed, each dugong carcass was sexed, measured and dissected to collect the stomach contents, skull and reproductive tracts. The entire reproductive tract was preserved by injection with, and immersion in, 10% sea water formalin (SWF). Small fetuses were preserved in the uterus. Larger fetuses were removed, injected, and fixed in SWF. If time permitted, details of the ovarian follicles and corpora lutea of each female were recorded before fixation, and a sample of mammary gland was fixed in SWF. In the case of each male, a 1-cm cube from the centre of the right testis was removed and fixed in Cleland's solution (Rowley and Heller, 1966). The skull of each animal was collected and cleaned by boiling (Heinsohn, 1972; Heinshohn and Spain, 1974).

Description of material

Body-length frequencies for the dugongs from Townsville and Mornington Island are illustrated (Fig. 3), arranged by sex and in 20-cm body-length classes for all animals for which these data were available.

The age-frequency distribution of the samples (Fig. 4) is reflected in these length-frequency histograms and indicates a paucity of young animals in the Mornington Island sample. No confirmed neonates were available, and only two females (both from Townsville) carrying near-term fetuses are presented.

The Townsville sample indicates that similar numbers of male and female dugongs drown in the shark nets. The total Mornington Island sample consisted of 23 males and 27 females and reflects the practice, adopted by at least some of the aboriginal hunters, of avoiding the capture of pregnant females as a conservation measure (Marsh *et al,* in press).

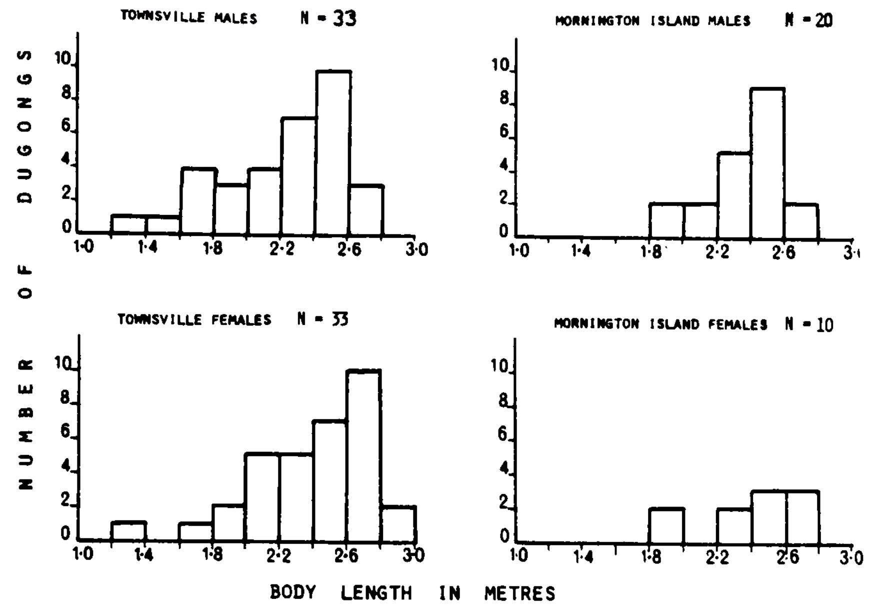

Fig. 3. Body-length frequency diagrams (by 0.2m classes) of the male and female dugongs collected from near Townsville and Mornington Island, 1969–1978.

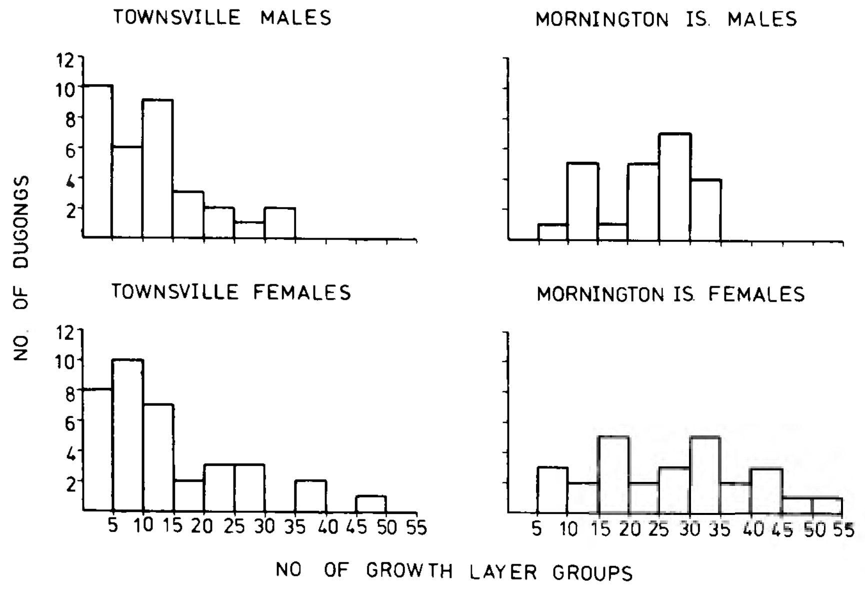

Fig. 4. Frequency diagrams of 5-year age classes (determined by number of dentinal GLGs in the tusks) of dugongs and isolated dugong skulls collected from near Townsville and Mornington Island 1968–78. Males with erupted tusks have been assigned to the age class which corresponds to the number of GLGs present in their erupted tusks.

Seasonality of collection

The monthly distribution of the dates of death of the specimens is shown in Figure 5. Specimens from Townsville were obtained in all months of the year; the small number of animals caught in June-July is a result of the removal of the shark nets at that time (Heinsohn, 1972; Heinsohn and Spain, 1974). The peak period of dugong hunting activity at Mornington Island is in July-August, the major period when an observer was on hand to collect specimens (Marsh *et al*, in press).

METHODS

Tooth growth and succession

Deciduous incisors. If present, one deciduous incisor was removed from each skull with forceps. The pulp cavity was recorded as open or closed and any tooth resorption or external growth layers were noted. The length was measured to the nearest 0.1 mm using vernier calipers.

Tusks. Unerupted tusks were extracted from the skulls by removing a window from the premaxilla above the tusk using a small abrasive disc in a hand-held flexible drive. The length of each tusk was measured to the nearest millimetre along the curvature in line with the centre of the tooth, using a flexible tape. The depth of the socket was measured using a set of vernier calipers as a probe. All tusks were examined for external evidence of growth layers.

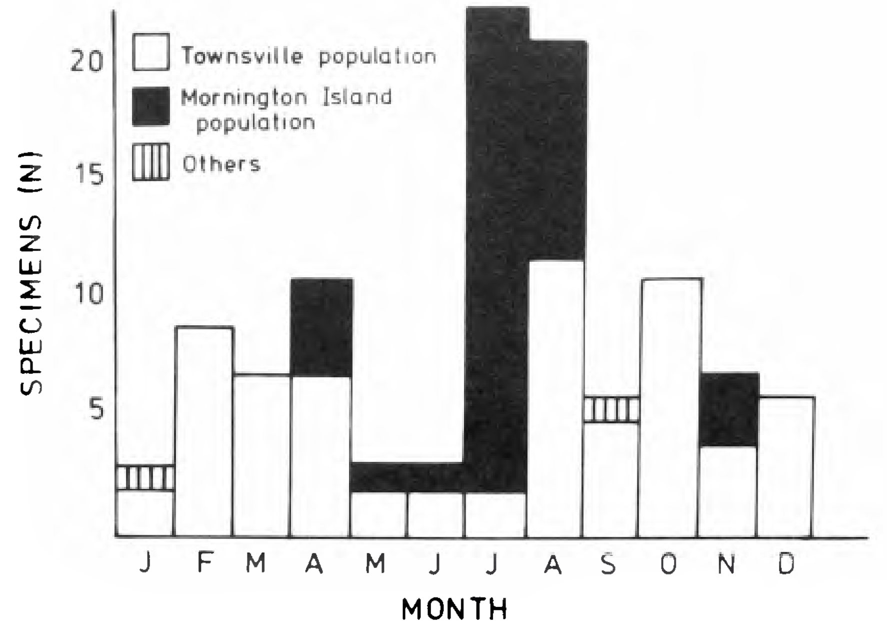

Fig. 5. Frequency distribution of the number of dugongs caught in each calendar month. All animals collected from 1969 to 1978 are included.

Cheek teeth. All skulls in which molar 3 had not erupted were x-rayed using a *Toshiba* 4-valve, single-phase, x-ray generator with rotating anode tube with *Ilfex* 90, 18 x 24 cm, non-screen film. The mandibles were x-rayed from the right buccal surface at 42–60 kVP and 200 mAS; the crania were x-rayed obliquely from the ventral aspect at 65–77 kVP and 200 mAS.

The cheek dentition of each quadrant of each skull in the collection was recorded. Each tooth was listed as either: (1) unerupted; (2) partly erupted; (3) erupted and unworn; (4) erupted and in wear; (5) wizened (extensively resorbed); (6) broken (stump only in alveolus); (7) absent, alveolus empty; (8) absent, alveolus partly filled with spongy bone, or (9) absent, alveolus not visible.

A cheek-tooth index was calculated for each skull as a quantitative measure of the number and state of all the cheek teeth. A tooth in state (8) or (9) was scored 0; state (1), (2), (6) or (7) as 0.25; state (3) or (5) as 0.5 and state (4) as 1. The pulp cavity of each tooth was recorded as open, tapered at the base, or closed.

Estimates of the occlusal areas of molars 2 and 3 which were in wear in the lower right hand quadrant of each skull were obtained by tracing the outline of the crown surface of each molar. The tracing was then cut out and weighed correct to 1×10^{-5} g on a *Sauter* balance. Calibration was performed by weighing 1-cm squares of the same paper.

Estimates of the central cross-sectional areas of the same teeth, both including and excluding the pulp cavity, were obtained by placing each tooth buccal surface uppermost on the x-ray plate and x-raying at 60 kVP and 200 mAS. Two contact prints were made of each x-ray and the areas calculated by cutting out and weighing as above. The central cross-sectional area of the pulp cavity of each tooth was obtained by subtraction.

Preparation of teeth and bones to demonstrate growth layer groups

The tusk was the main tooth selected for this part of the age determination study. It is present throughout life, has a permanently open pulp cavity, and is unerupted and unworn in most females and juvenile males. One tusk was prepared from each animal unless there was a large difference in the size of the two tusks in which case both were prepared. One deciduous incisor was also examined provided that resorption had not commenced.

In the sections that follow, both 5% HCl in 70% ethanol, and 10% formic acid were used to etch or decalcify the teeth with equivalent results. There was also no substantial difference in the results obtained with the different haematoxylins used.

Etched half teeth. Each tusk was bisected longitudinally in the mesiodistal plane using a 10-inch diameter saw as follows: the cutting line was marked before each tusk was embedded in ice in the desired orientation prior to cutting; the optimum plane of section was maintained by holding the broad flat surface of the ice in contact with the movable carriage of the saw. Unresorbed deciduous incisors and tusks less than 4 cm long were mounted on chucks with dental impression compound and bisected using a 4-inch diamond saw. After the teeth were cut, the depth of the apex of the pulp cavity was measured along the curvature of the tooth using a flexible tape.

The cut surface of one half of each tooth was polished using progressively finer grades (150–600) of wet and dry sandpaper. This half tooth was then etched by immersing it in 5% HCl in 70% ethanol for 3 h at room temperature (25°C), washed and allowed to dry thoroughly with the cut surface uppermost. The resultant relief was emphasized by rubbing the surface with pencil. The tusks were usually examined without magnification.

Small tusks (less than 6 cm long) and deciduous incisors were stained with Mayer's haemalum after etching as above and examined under a binocular microscope (x 8) using oblique light.

Thick sections. The unetched half of each tusk was cut transversely about 1 cm distal to the apex of the pulp cavity; tusks less than 6 cm long were processed without further cutting. The longitudinal cut face of the part of the tooth which included the pulp cavity was then polished using progressively finer grades of wet and dry sandpaper (150–1200) and glued on to a *perspex* slide with cyano-acrilate monomer glue. The tooth was then cut longitudinally on a diamond saw to produce a mounted section approximately 1 mm thick which was ground to a thickness of approximately 500 μm, etched in 5% HCl in 70% ethanol for 1 h, washed, stained with Mayer's haemalum, washed and mounted in glycerol. For two dugongs thick sections were also prepared of lower left molars 2 and 3.

Thin sections. One deciduous incisor and one tusk from each dugong less than 2 m long were mounted on chucks with dental impression compound and bisected longitudinally using a 4-inch diamond saw. The cut face of each half tooth was polished using whetstones (1200 and 4000 grit) and glued to a *perspex* slide. Sections 30 to 80 μm thick were prepared by grinding as above. One section from each tooth was then mounted in glycerol. The other section was decalcified in 10% formic acid for 2 h to 3 h, washed and stained for 3 min with Gill's haematoxylin (Lillie and Fullmer, 1976, p. 207), washed and mounted in glycerol.

Longitudinal and transverse sections from adult tusks and transverse sections from three ribs from uncatalogued dugongs, one tympanic bone and one malleus from each of two catalogued dugongs, one humerus from a catalogued dugong and one rib from another catalogued dugong were also prepared and stained as above.

Microradiographs. Four transverse sections (280–600 μm thick) were cut from one tusk, polished with whetstones without gluing to a slide, cleaned ultrasonically and microradiographed using a *Picker Mini Shot* (Model 1) x-ray unit for 3 h at 13 kv and 1.5 mA. The source-to-film (*Kodak*, high resolution) distance was 20 cm. The microradiographs were examined with a compound microscope using transmitted light and photographed.

Determination of the pattern of the growth layer groups

An ontogenetic series was prepared of thin sections of one deciduous incisor and one tusk from one fetal (body length approximately 1 m) and 15 juvenile dugongs (body lengths 1.25 to 1.98 m).

The sections in this series were ranked in ascending order of body length and examined with transmitted light using both a binocular (x 8) and a compound microscope (x 63) to determine the nature and thickness of the prenatal dentine, the neonatal line and the pattern of GLG deposition.

Intercalibration of techniques

Polished and polished and etched half teeth. The dentinal layering seen in polished half teeth was intercalibrated with that seen in polished, etched and pencil-rubbed half teeth by masking a portion of the polished surface of one-half tooth with *Paraplast* before etching and pencil rubbing. The wax was removed with xylene prior to examination.

The effect of etching on the layers visible on the surface of the worn tip of an erupted male tusk was examined by masking half the worn area with wax before etching and pencil rubbing, etc., as above.

Untreated and decalcified and stained thin sections. The structures seen in undecalcified and unstained and in decalcified and stained sections were intercalibrated by masking part of several thin sections with dental wax prior to decalcification and staining. The sections were dehydrated in graded ethanols and the wax removed with xylene. The sections were then rehydrated, mounted in glycerol and examined as above.

Inter-calibration by performing several treatments successively on the same section. One face of the 600-μm section that was microradiographed (see above) was subsequently masked with dental wax before etching by immersion in 5% formic acid for 1.5 h. The wax was removed and the resulting relief on the face which had not been masked was then emphasized by rubbing with pencil. The section was then examined using both transmitted and reflected light. The pencil markings were removed and a 200Å-thick layer of carbon was evaporated onto the etched surface of the specimen, which was then photographed with reflected light. The section was then ground down to 90 μm by grinding both faces, mounted in glycerol, examined under a compound microscope (x 63) and photographed with transmitted light. The section was then decalcified, stained with haematoxylin and examined and photographed with transmitted light. The inter-calibration was performed by comparing the photographs of each stage.

Scoring the tooth and bone preparations

Counting the layers. Each preparation was scored independently several times without reference to the biological information concerning the dugong from which it came. The GLGs in the etched tusks were counted without magnification, using oblique light. For old animals, scoring was facilitated by photographing the etched tusk and counting along the convex edge of the tusk in the photograph.

Determination of the rate of deposition of growth layer groups. The nature and zone thickness of the dentine being laid down at the time of death (i.e. adjacent to the pulp cavity) was examined and plotted against date of death as indirect evidence of the depositional rate of the GLGs. The thick sections of tusks were all masked with random numbers and examined under a binocular microscope (x 8) with reflected light without knowledge of the date of death. Each section was examined independently by two people a total of six times before the slides were unmasked. For two dugongs, thick sections of molars 2 and 3 were also examined.

Indications of cranial growth

For each skull the condylo-premaxillary length and the zygomatic width (Spain and Heinsohn, 1974) were measured correct to 1 mm with vernier calipers. The basioccipital suture (between the basioccipital and the basisphenoid), the condyle sutures (between the basioccipital and the exoccipitals) and the basisphenoid suture (between the basisphenoid and the presphenoid) were examined and scored as open, partly fused (bony bridges present, suture line visible), or fused.

Measurement and histological preparation of reproductive materials

Each fixed testis was trimmed of epididymis and fat and weighed correct to 0.1 g. The Cleland's-fixed specimen obtained at autopsy was included with the remainder of the right testis. The length, width at mid-length and dorso-ventral thickness at mid-length were measured with vernier calipers. Tissue specimens were taken as follows: three from the centre along the longitudinal mid-line and two from along the transverse mid-line of each testis and one from each corpus and each cauda epididymis. These specimens were post-fixed in Bouin's fixative (Lillie and Fullmer, 1976, p. 61) and along with the Cleland's-fixed specimen dehydrated in ethanol, cleared in xylene, embedded in *Paraplast,* sectioned at 5 or 6 μm and stained with Mayer's haemalum and *Young's Eosin-Erythrosin.*

The preserved female material has not yet been measured and sectioned.

Data analysis

Computation was carried out either on the CSIRO *Cyber* 76 in Canberra or on the *PDP 10* computer at James Cook University of North Queensland. The Genstat System of programs was used for the multiple regression analyses. The Maximum Likelihood Program from Rothamsted and the Non-Linear Regression Program from IMSL (Anon, 1975) wêre used for the growth curves and Tustat II (Koh, 1973) for the t-tests.

RESULTS

Description of the structural components of the teeth used for age determination

Deciduous incisor. In the fetus and the juveniles with less than three GLGs the tip of the deciduous incisor (Fig. 6A) is covered with a layer of *enamel* approximately 100 μm to 130 μm thick; in older animals the tip is resorbed (Fig. 6C). The *prenatal dentine* appears reasonably homogeneous and is of intermediate optical density but generally less dense

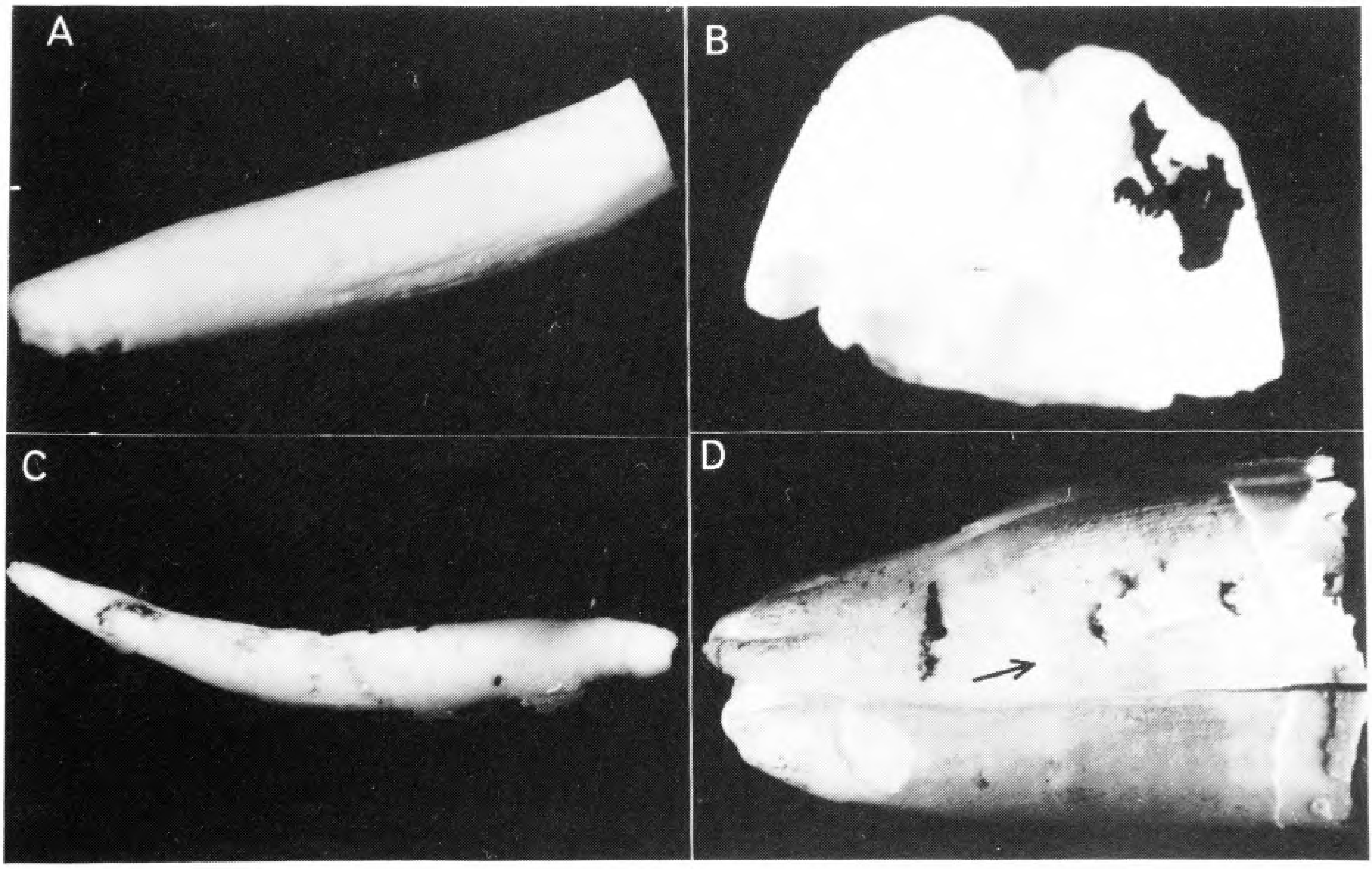

Fig. 6. The deciduous incisors and tusks from young dugongs. (A) 2.26-cm-long deciduous incisor and (B) 0.55-cm-long tusk from a 1.3-m-long male dugong less than three months old. (C) 4.18-cm-long deciduous incisor and (D) 2.4-cm-long tusk from a 1.8-m-long dugong with two GLGs in the tusk. An external growth layer is (D) arrowed.

than the postnatal dentine. It is about 5 mm thick at the tooth apex. The last-formed prenatal dentine is generally slightly more opaque than that formed earlier and stains more intensely with haematoxylin. The *neonatal line* (Fig. 7A) is a fine translucent unstainable layer about 40 µm thick. It lies between the opaque, intensely-stained edge of the prenatal dentine and another opaque (intensely stainable) line about 30 µm thick. This tripartite structure forms a groove in etched specimens, the stainable layers being at the sides of the groove.

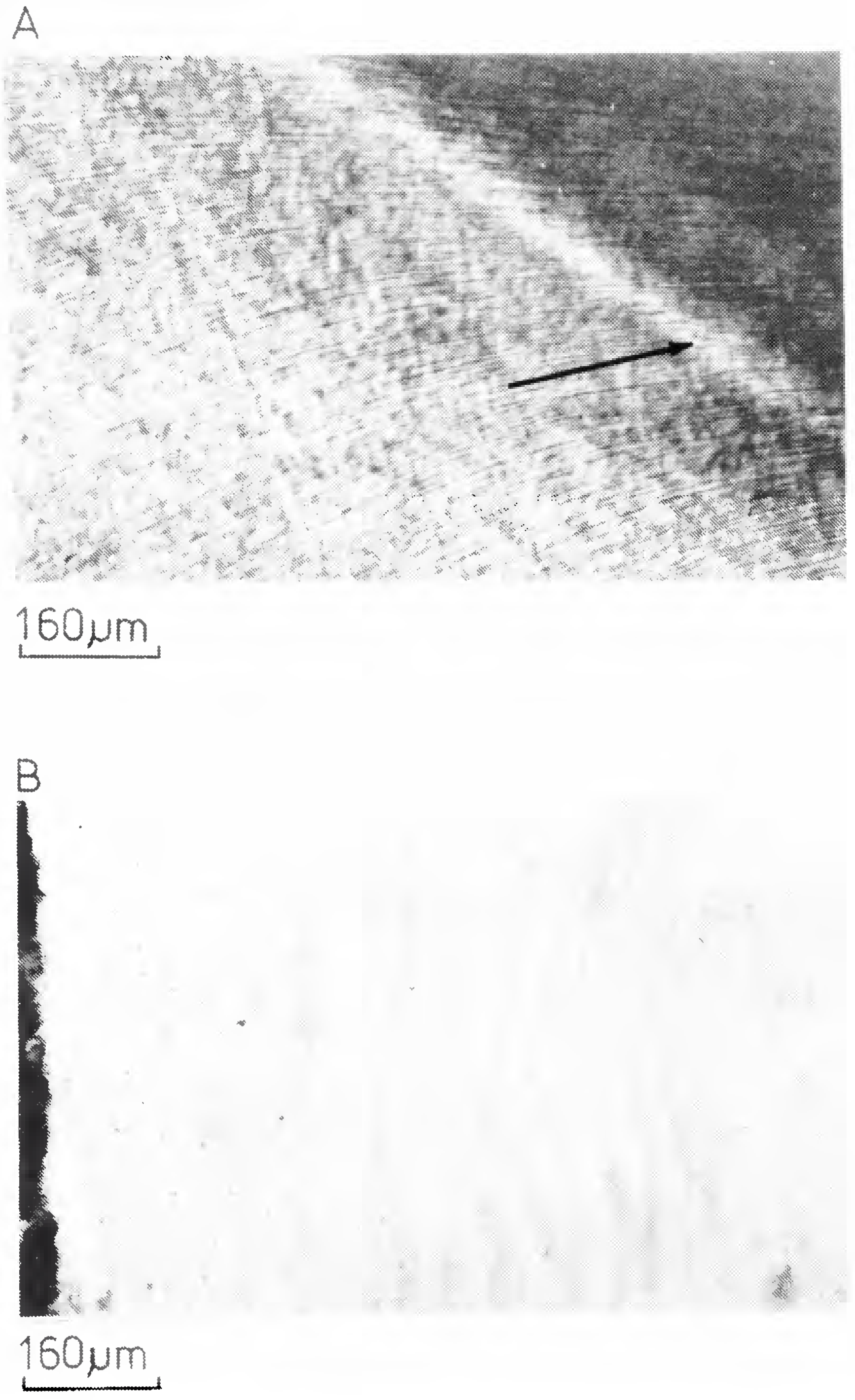

Fig. 7. (A) Decalcified and stained section of a deciduous incisor from a 1.5m-long male dugong estimated to be one year old showing the unstained neonatal line (arrowed). (B) Cementum layers in a decalcified and stained transverse section from the midlength of an erupted and worn tusk from a 2.65-m-long adult male dugong. Twenty layers can be seen in the cementum. 34 GLGs were counted in the tusk.

A GLG in the *postnatal dentine* consists of both fine and thicker accessory layers. The fine layers are opaque, stain strongly with haematoxylin, and are about 20 µm wide and separated by about 50 µm of intermediately staining dentine. About 12 fine layers are followed by one or more opaque (stainable) and thicker layers each about 60 µm wide and followed by translucent dentine. The combination of opaque and translucent thicker layers forms a groove in etched teeth. Because of the prominence of the fine accessory layering, the boundaries of GLGs may be difficult to distinguish in etched deciduous incisors.

The base of each deciduous incisor is coated with cementum 80 to 130 µm thick. In older animals, cementum covers almost the entire incisor except where resorption has taken place.

Tusk. The tip of the tusk consists of several cusps (Fig. 6B) which may be variably developed. Covering the tip is a layer of *enamel* up to about 330 µm thick. The enamel becomes thinner on the sides of the tusk and disappears altogether after the first four or five GLGs except on the ventromesial side, where a layer of enamel about 330 µm thick continues beneath the cementum to the base of the tusk in both males and females (Fig. 8A).

The thickness of the *prenatal dentine* varies according to the development of the cusps and may be up to 500 µm thick. It is generally less dense than the postnatal dentine both in stained and unstained preparations.

The *neonatal line* is a fine translucent unstainable layer about 30 µm thick. It is often difficult to see at the center of the tooth but is more obvious along the sides.

The *postnatal dentine* is deposited in a prolonged series of coaxial cone-shaped increments. Each GLG (Fig. 8) consists of a wide zone of intermediate optical density, referred to as Zone A, containing numerous faint accessory layers, followed by a relatively narrow zone (Zone B) in which the accessory layering is enhanced. Typically, Zone B consists of one to three opaque, intensely stainable layers, each followed by a translucent unstainable layer. Usually the opaque layers are more obvious and are more readily identified as the boundary of the GLG, but in some teeth and in some GLGs the translucent component(s) predominate, particularly near the central axis of the tooth. Thus in most polished half teeth, Zone B appears light, but in some, often particularly clearly marked teeth, it appears dark. Zone A forms the ridge in etched teeth and Zone B the groove (Figs 8C, 9A). The accessory layers in Zone A were not studied.

The groove marking the boundary of the first GLG tended to etch very strongly and often appeared as a crack. Both in untreated and decalcified and stained sections, the boundaries of the first two or three GLGs were more difficult to see than those formed subsequently. The mean thickness of the GLGs in dentine of various ages is given in Table 1.

Table 1

Change in thickness of growth layer groups with age
Measurements were made along the central axis
of etched tusks using vernier calipers

GLG No.	No. of teeth measured	Mean (mm)	SD (mm)
Unerupted tusks			
0–5	9	42.4	12.4
6–10	9	56.0	10.2
11–15	8	46.5	10.5
16–20	5	43.0	10.5
21–25	5	37.3	10.1
25–30	4	28.0	8.4
31–35	3	24.5	4.9
35	3	16.9	4.2
Erupted tusks			
*(X+0) – (X+5)	4	46.5	3.7
(X+5) – (X+10)	4	48.8	2.9
(X+10) – (X+15)	4	49.8	5.5
(X+15) – (X+20)	3	40.3	5.1

*X is the unknown number of layers which have been worn away.

Cementum covers almost the entire surface of the tusk of older animals but is absent from the tip of the tooth in young animals. The layering in the cementum (Fig. 7B) was not used to determine age. In many of the specimens studied, the cementum had been damaged, presumably by prolonged boiling.

External growth layers, where present, formed ridges on the surfaces of the tooth (Fig. 9B). They were found to correspond to Zone B.

Intercalibration of structures revealed by different techniques

Study of the masked teeth showed that the degree of optical density was mirrored by the degree of stainability (Fig. 8D). Examination of the tusk section which was subjected to a variety of treatments to demonstrate the GLGs (Figs. 8A to 8D) suggested that, for this animal at least, the degree of optical density corresponded to both the degree of radio-opacity and of staining capacity. However, this relationship may not hold for all dugong teeth. When the section was etched, pencil-rubbed, and examined under transmitted light the translucent component of Zone B was found to lie on the side of the groove. The opaque component was at the deepest part of the groove.

Growth layers are visible in the worn area at the tip of erupted tusks as poorly developed concentric ridges and grooves which presumably result from the differential abrasion of GLG components by the substrate as the dugong feeds. When half the worn tip was masked by wax before etching, it was evident that the more readily-abraded layer corresponded to the more readily-etched layer, i.e. Zone B. However, it was not possible to determine whether the opaque or translucent component of Zone B was the more abraded.

Scoring the number of dentinal growth layer groups

The first three or four dentinal layers were much more difficult to score than the layers formed subsequently. Hence, for young animals it was found to be necessary to use a range of preparations to determine age, i.e. both untreated and decalcified and stained sections of the deciduous incisors and the tusks; the external growth layers (Fig. 6D); and the half teeth.

The etched and pencil-rubbed half teeth (Fig. 9A) were used for counting GLGs in older animals. One ridge plus one groove was scored as one GLG; the ridge only, as half of a GLG. The layering in most teeth could be counted very easily, and there was usually complete agreement between repeat counts. Dentine deposition appeared to be occurring even in teeth with small pulp cavities. It was possible to count the layers in all teeth that were examined, although the nature of the zone next to the pulp cavity could not be ascertained with certainty in the teeth of six very old animals.

Counts ranged from zero to 51 in unerupted tusks. A maximum count of 34.5 was recorded for an erupted tusk from a male. The two erupted tusks from females had GLG counts of 37.5 and 45.5. When both tusks from the same animal were examined they were found to have the same number of GLGs despite any difference in size.

The external growth layers of older animals (Fig. 9B) were often difficult to see.

Rate of deposition of the growth layer groups

Uniformity of layer deposition in different teeth from the same animal. The zones being laid down at the time of death in the deciduous incisor, tusk, molar 2 and molar 3 in two animals and in the deciduous incisor and tusk of 13 animals were compared. They were found to be the same for all teeth examined from the same animal. The pulp cavity of each of the deciduous incisors and molars examined was open.

Seasonal pattern of layer deposition. When the nature of the zone being laid down at the time of death (Fig. 10) was tabulated against the date of death (Table 2), Zone B was seen to be almost always formed in the latter half of the year. These data were obtained from 106 animals, 57 of

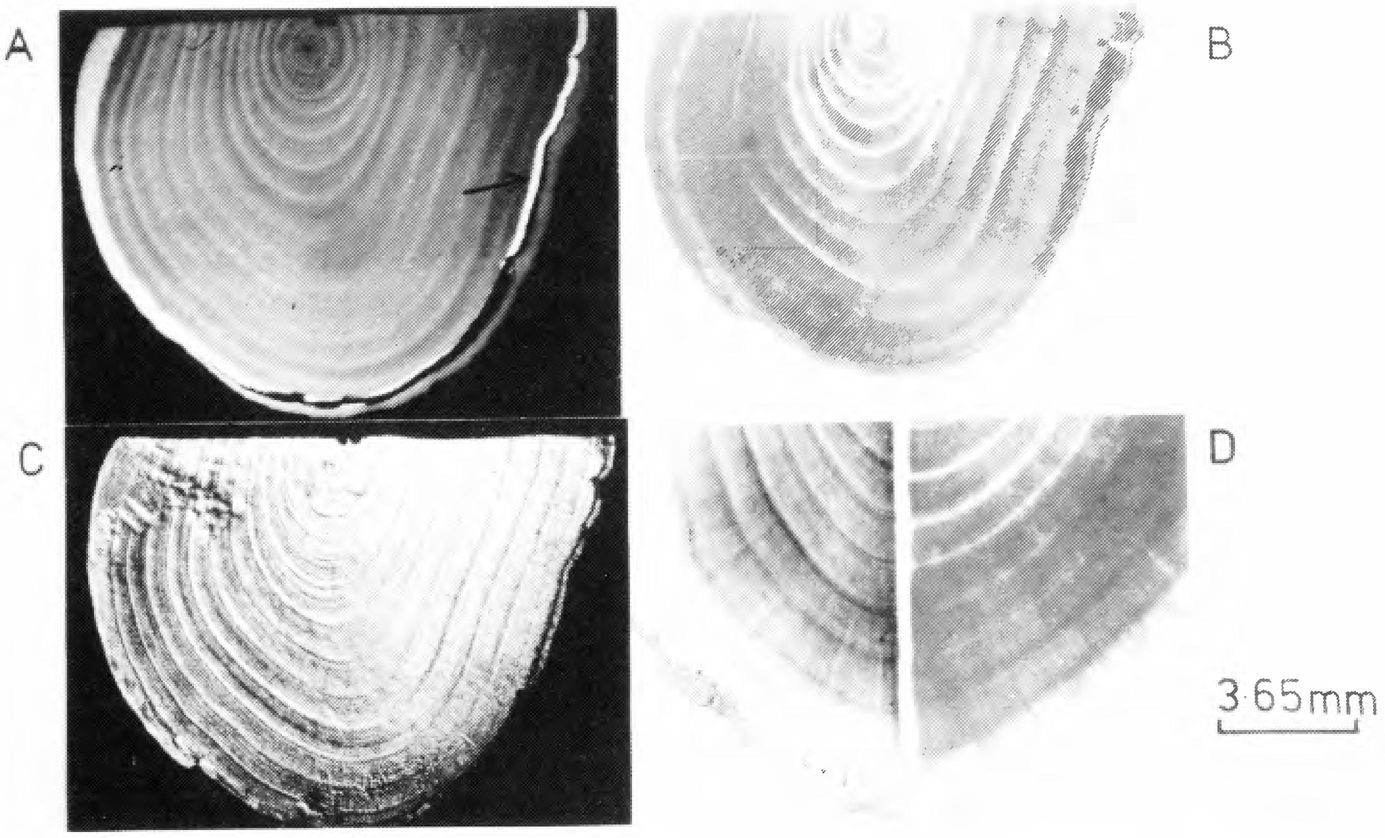

Fig. 8. Transverse sections from the midlength of the same tusk as the section in 7(B). (A) microradiograph of the ground section that is etched and coated with carbon and viewed with oblique light in (C), and that is viewed with transmitted light in (B). The section was 2 cm wide at the top. The enamel layer is arrowed in (A). (D) Adjacent section viewed with transmitted light. The right-hand portion of the section is untreated. The left-hand portion is decalcified and stained.

Table 2

Dugongs from northern Australia (1969–1978)
Distribution by month of the dentinal GLG zone in process of formation
Data are numbers of animals recorded

Type of Zone being formed	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Zone A (Haematoxylin +)	3	9	7	10	2	1	7	4	1	3	6	4
Zone B (Haematoxylin ++)	0	0	0	2	0	1	16	14	4	8	2	2

which died in the process of depositing Zone A and 49, Zone B. Only 15 of the 57 animals (26%) depositing Zone A died between July and October inclusive, while 42 of the 49 (86%) animals depositing Zone B died within this period ($P < 0.001$, χ^2 Heterogeneity Test). These data suggest that one GLG is usually deposited each year.

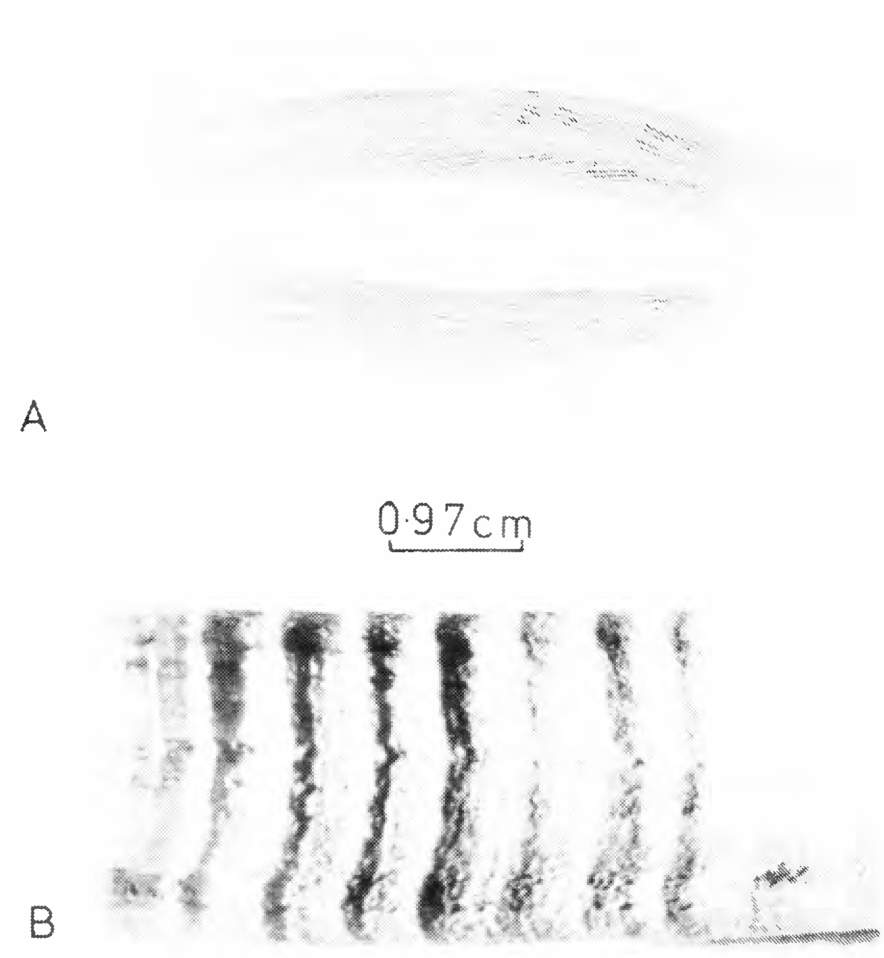

Fig. 9. (A) The etched and pencil-rubbed longitudinal surfaces of two dugong tusks. The top tusk is the erupted and worn 14.5-cm-long tusk from a 2.72-m-long male dugong showing 29.5 GLGs in the dentine. The 11.5-cm-long lower tusk is from a 2.5-m-long female dugong and has 17.5 GLGs. (B) The unusually clear external growth layers in the tusk of a 2.39-m-long adult male dugong.

The thickness of the Zone A next to the pulp cavity was recorded relative to that of the previous Zone A for all animals laying down Zone A at the time of death (Table 3 and Fig. 10). The zone was less than a third as wide as the penultimate Zone A in 11 animals, between one-third and two-thirds of the penultimate Zone A in 14 animals, and more than two-thirds as wide as the penultimate Zone A in 32 animals. The results indicated that the first third of Zone A was laid down between October and January, the second third between December and April and the last third between February and September, further supporting the thesis of a one-per-year GLG deposition rate.

In Tables 2 and 3, data from both sexes, all areas, and all years are combined. The data were also considered separately, but no differences from the general pattern were found. However, data from 31 dugongs killed in Townsville shark nets during 1972 considered separately are even more striking evidence of an annual deposition rate (Table 4). The only exception in this group was one animal, which appeared to be laying down a Zone B in April and had a score of two GLGs.

Layer deposition in sick or pregnant dugongs. Four animals collected from Mornington Island in July-August were described by the aboriginals as 'sick' and unfit to eat. These animals were observed to tire quickly, were easy to catch, and had an abnormally high parasite load (B. R. Gardner, pers. comm.). On the basis of pathological examination, R.S.F. Campbell (pers. comm.) reported that they had oedematous fat and muscle. The layer deposition pattern of these animals did not differ from that of the 12 apparently healthy dugongs caught at Mornington Island during the same period. The two females with large fetuses collected from Townsville, one in December, the other in July, were both laying down Zone B.

Relationship between changes in body length and the number of growth layer groups in the tusk

On the assumption that one GLG is deposited each year (see

Table 3

Dugongs with Zone A being formed at time of death
Distribution by month of thickness of Zone A relative to thickness of penultimate Zone A
Data are numbers of animals recorded

Thickness of Zone A being formed relative to thickness of penultimate Zone A	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Less than one-third	1									3	6	1
One-third to two-thirds	2	3	3	3								3
Greater than two-thirds		6	4	7	2	1	7	4	1			

Table 4

Dugongs killed in Townsville shark nets in 1972

Distribution by month of nature and thickness of dentinal GLG zone being formed at time of death

	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Zone A : less than one-third												
Zone A : one-third to two-thirds	1	2	2	1								
Zone A : greater than two-thirds		6	3	3	1			2				
Zone B				1				1	3	3	1	1

section on GLG deposition rate), the relationship between body length (L) and age (A) (number of dentinal GLGs) was calculated using a standard single-phase asymptotic growth curve of the form

$$L_A = L_\infty + \beta\gamma^A$$

where L_A = length at age A

L_∞ = asymptotic length

and β and γ are constants.

This is algebraically equivalent to the von Bertalanffy Growth Equation (von Bertalanffy, 1938, 1957).

For the calculation of growth curves, dugongs were assigned ages that represented a mid-year point within their respective year classes. Thus, dugongs with less than one GLG were aged as 0.5 years, dugongs with one but less than two GLGs as 1.5 years, etc. This process takes account of the range of sizes within a year rather than at the beginning of a year, which was particularly important for dugongs with less than one GLG as the length at birth was not known with certainty.

Five growth curves were calculated for the Townsville population as follows: females; males (animals with unworn tusks only); all males (number of GLGs in worn tusks used as an estimate of age); males and females (unworn tusks only) (Fig. 11); all animals. As all but two of the females from Mornington Island were almost old enough for the asymptotic length to have been reached, separate analyses were not performed for males and females from this population but were restricted to age estimates based on unworn tusks and all tusks as above.

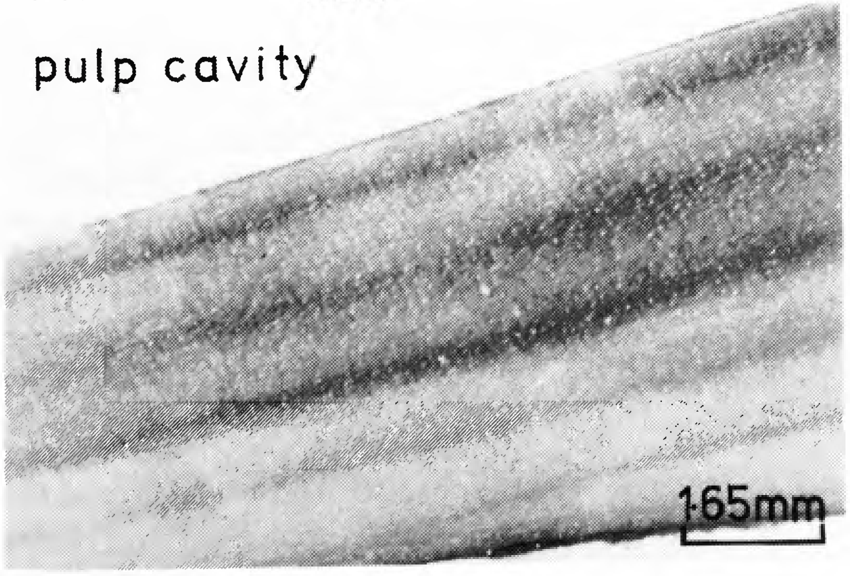

Fig. 10. Longitudinal tusk section used for determining the thickness and nature of the GLG adjacent to the pulp cavity.

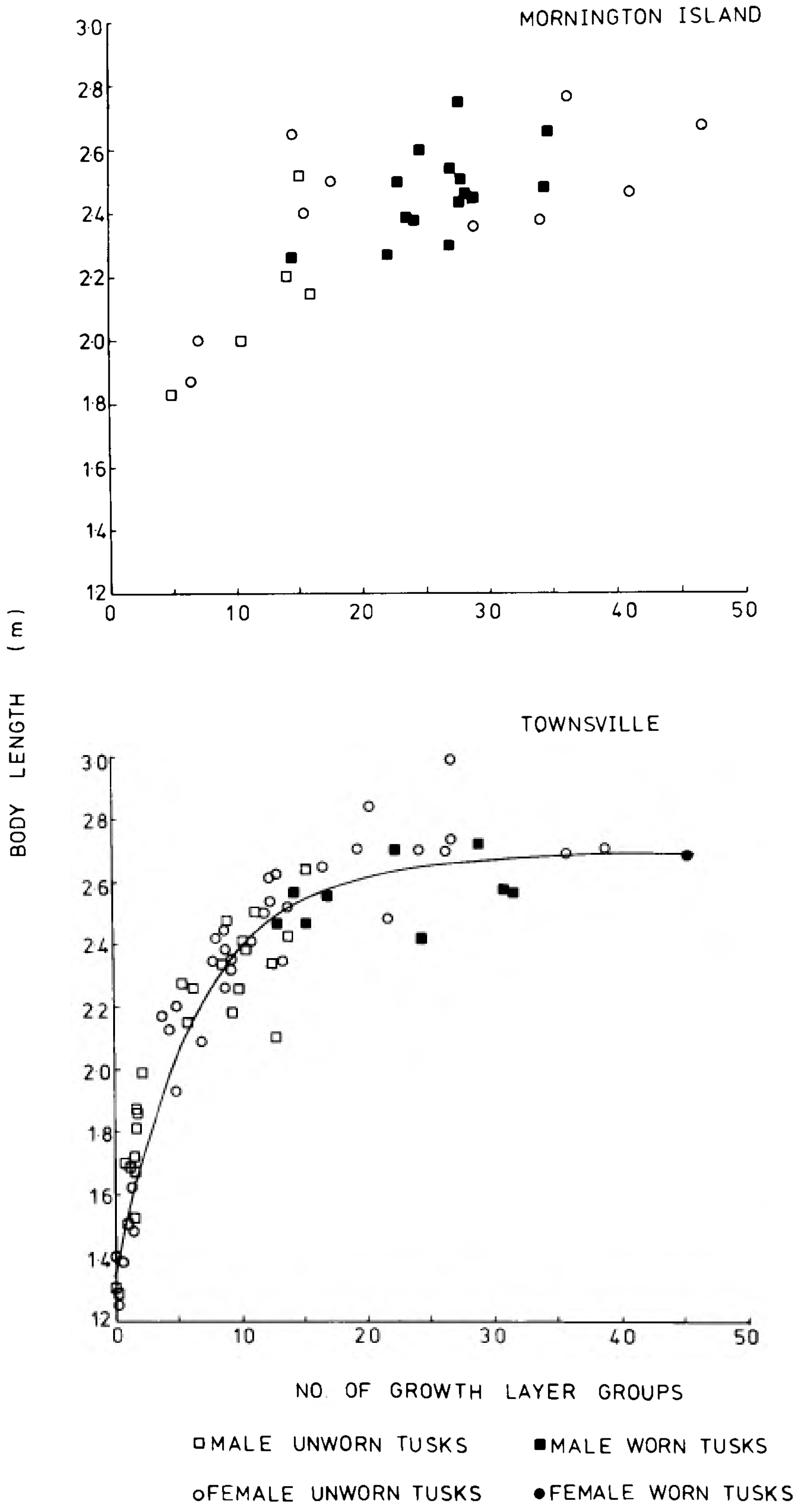

Fig. 11. Relationship between body length (L_A) in metres and age (A) in years (expressed by number of dentinal GLGs) for dugongs from Mornington Island and Townsville. The regression fitted to the Townsville data (unworn tusks only) is $L_A = 2.69 - 1.35\ (0.86)^A$.

Table 5

Estimates, including 95% confidence limits, of the asymptotic length (L_∞), birth length (L_0), and growth curve constants for dugong growth curves. The coefficient of determination (r^2) is also included

Sex	Condition of tusks	N	L_∞ (95% CL)	L_0 (95% CL)	β (95% CL)	γ (95% CL)	r^2
Townsville population							
Female	Unworn	34	2.72 (2.63, 2.82)	1.31 (1.16, 1.45)	−1.41 (−1.57, −1.27)	0.86 (0.83, 0.89)	0.92
Male	Unworn	25	2.39 (2.30, 2.48)	1.10 (0.86, 1.35)	−1.29 (−1.51, −1.09)	0.70 (0.61, 0.80)	0.91
Male	Unworn + worn	34	2.53 (2.44, 2.62)	1.27 (1.10, 1.44)	−1.26 (−1.42, −1.10)	0.80 (0.74, 0.87)	0.91
Female + male	Unworn	59	2.69 (2.59, 2.79)	1.34 (1.24, 1.45)	−1.35 (−1.46, −1.23)	0.86 (0.83, 0.89)	0.91
Female + male	Unworn + worn	69	2.67 (2.60, 2.74)	1.35 (1.25, 1.45)	−1.32 (−1.43, −1.22)	0.86 (0.83, 0.88)	0.91
Mornington Island							
Female + male	Unworn	16	2.56 (2.37, 2.74)	0.93 (−0.33, 2.19)	−1.62 (−2.81, −0.44)	0.87 (0.77, 0.97)	0.71
Female + male	Unworn + worn	30	2.55 (2.43, 2.66)	1.05 (0.14, 1.96)	−1.49 (−2.34, −0.65)	0.88 (0.81, 0.95)	0.67

Estimates and 95% confidence intervals of the asymptotic lengths, birth lengths, and constants based on the above analyses are given in Table 5 with the coefficient of determination (r^2) for each curve. The estimates of L_∞ and γ were all significantly different from zero at the 0.001 level. Those for β were significant at the 0.05 level or less.

The growth curves for Mornington Island dugongs (Table 5) are unsatisfactory as the confidence intervals for the birth lengths are too large to support the estimates of this parameter. This is not surprising as the youngest animal in the Mornington Island sample was estimated as five years old.

At a value of approximately 20 GLGs, all the curves approach an asymptote. The confidence intervals for the asymptotic lengths of the Townsville females and males, based on age estimates from both unworn tusks only and all tusks, do not overlap (Table 5), suggesting that the asymptotic length of the females is greater than that for the males. When the mean lengths of all Townsville females and males with 20 or more GLGs are compared using a t-test, the mean length for females is also found to be significantly larger ($0.025 < P < 0.05$).

A similar comparison between males and females from Mornington Island does not demonstrate any sex difference in asymptotic length at the $P = 0.05$ level, perhaps because the sample size is so small.

When the confidence intervals of the asymptotic lengths (both sexes combined) of the Townsville and Mornington Island populations are compared (Table 5), they overlap, possibly because of the comparatively small size of the Mornington Island sample. However, when the mean body lengths of all animals from the two populations with over 20 GLGs are compared using t-tests, the asymptotic length of the Townsville animals is found to be significantly greater ($P < 0.0005$; both sexes together). When the sexes are considered separately, the differences between the two populations are not significant. This may be related to the small sample sizes or may reflect the differences in the sex composition of the two samples.

The asymptotic length estimates of the Townsville dugongs produced by all the curves may be too low. In the case of the curve for males with unworn tusks only, this is obviously because animals with over 20 GLGs were not included in the analyses. Two females out of nine and two males out of five with more than 20 GLGs had body lengths greater than the upper limit of the 95% confidence interval for their respective asymptotic lengths.

Many mammals exhibit a 'growth spurt' at or around puberty (von Bertalanffy, 1957) and fitting a single curve obscures this feature and probably leads to the underestimation of asymptotic length. A two-stage growth curve could be fitted, but this would involve making an arbitrary decision concerning the starting point of the second section of the curve. In view of the limitations of the present data, a single curve seems to adequately describe the increase in body length relative to dentinal GLGs throughout life. The values of r^2 obtained for the curves fitted to the Townsville data were high (> 0.91) and the residuals from the fitted curves showed no systematic bias.

Relationship between puberty and the number of growth layer groups in the tusk

Males. Reproductive material from 52 male dugongs has been examined histologically. None of the 20 animals (13 of which had GLG counts over 10, 3 over 20) from Townsville and Mornington Island that died between November and April inclusive and only ten of the 32 animals from the same areas that died in the remainder of the year had sperm in their testes or epididymides. Included in these 32 animals were 12 with more than 20 GLGs which were collected from Mornington Island in July; only four of these had sperm in their testes and epididymides. These results suggest a seasonal cycle of reproductive activity in the male dugong in which not all males are in rut simultaneously.

Male dugongs have been classified as immature, mature active, or mature quiescent on the basis of testes weight, tubule diameter, state of tubule lumina, and the presence or absence of mature sperm. The oldest animal from Townsville classified as immature had six dentinal GLGs and the youngest with mature sperm in the testes and epididymides had nine. Corresponding figures for Mornington Island dugongs were 14.5 (oldest immature), 14 (youngest mature quiescent), 22.5 (youngest mature active). Although only six animals with between 10 and 20 GLGs have been examined from this population, these preliminary results suggest that male dugongs may be maturing later at Mornington Island than in the Townsville region.

Two 'mature active' males, one with nine and the other with 10.5 dentinal GLGs, had unerupted tusks. The tusks were just erupting in another with 12 GLGs. A 'mature quiescent' animal from Mornington Island had unerupted tusks and 14 GLGs. These results indicate that the tusks erupt after puberty.

Females. Examination of the female reproductive tracts has not proceeded sufficiently to enable any firm statements about the age of female reproductive maturity in the dugong. The reproductive tracts of 20 female dugongs from Townsville have, however, been examined at autopsy. The oldest which had no follicles visible on the surface of the ovaries had nine GLGs; the youngest with follicles had eight GLGs. The youngest dugong with corpora lutea had 13 GLGs and the youngest pregnant dugong had 16.5 Only seven female dugongs from Mornington Island have been examined and of these only three had less than 20 GLGs. The youngest with follicles had 14.5 dentinal GLGs. These results suggest that puberty does not occur before at least eight or nine GLGs are deposited.

Relationship between incisor growth and the number of growth layer groups in the tusk

Deciduous incisors. In the fetus (body length 1 m) 1.6 cm-long deciduous incisors were present. The deciduous incisors were 2.26 cm long (Fig. 6A) in the youngest juvenile, with a body length of 1.3 m and estimated to be less than three months old.

The growth of the deciduous incisors is summarized in Figure 12. The maximum length recorded for the deciduous incisors from a male dugong was 4.25 cm (6.5 GLGs) and for a female 5.72 cm (13 GLGs). After about 2.5 to 3 GLGs the pulp cavity of the incisor closes and the tooth starts to be resorbed (Fig. 6C). In males, the deciduous incisors are lost around the time of tusk eruption. The sockets of the deciduous incisors are lost as the tusks expand. In the female, small partially-resorbed incisors may persist until the tusk has laid down up to 30 GLGs (Fig. 1B).

Tusks. The tusks exhibit dramatic axial growth in both sexes (Fig. 13), making transverse sections useless for age determination based on dentinal layering (Fig. 9A). The 1.3 m juvenile had tusks 0.55 cm long (Fig. 6B). A maximum tusk length of 17.8 cm was recorded for two females, one with 36 GLGs (tusk unworn); the other 37.5 GLGs (tusk erupted and worn). The maximum tusk length recorded for a male was 15.8 cm. The tusk had 26.5 GLGs and was erupted and worn.

The growth of the tusks is similar in both sexes until about ten GLGs are laid down and the tusk is about 10 cm long (Fig. 12). The tusk grows posteriorly through the premaxilla, and, after the deposition of one or two GLGs, a hole appears in the lateral side of the premaxilla at the root of the tusk, enabling its state of growth to be seen. The increase in length of the tusk is generally accompanied by an increase in length in the pulp cavity, although the rate of increase in the size of the pulp cavity is less after the deposition of about five GLGs (Fig. 12).

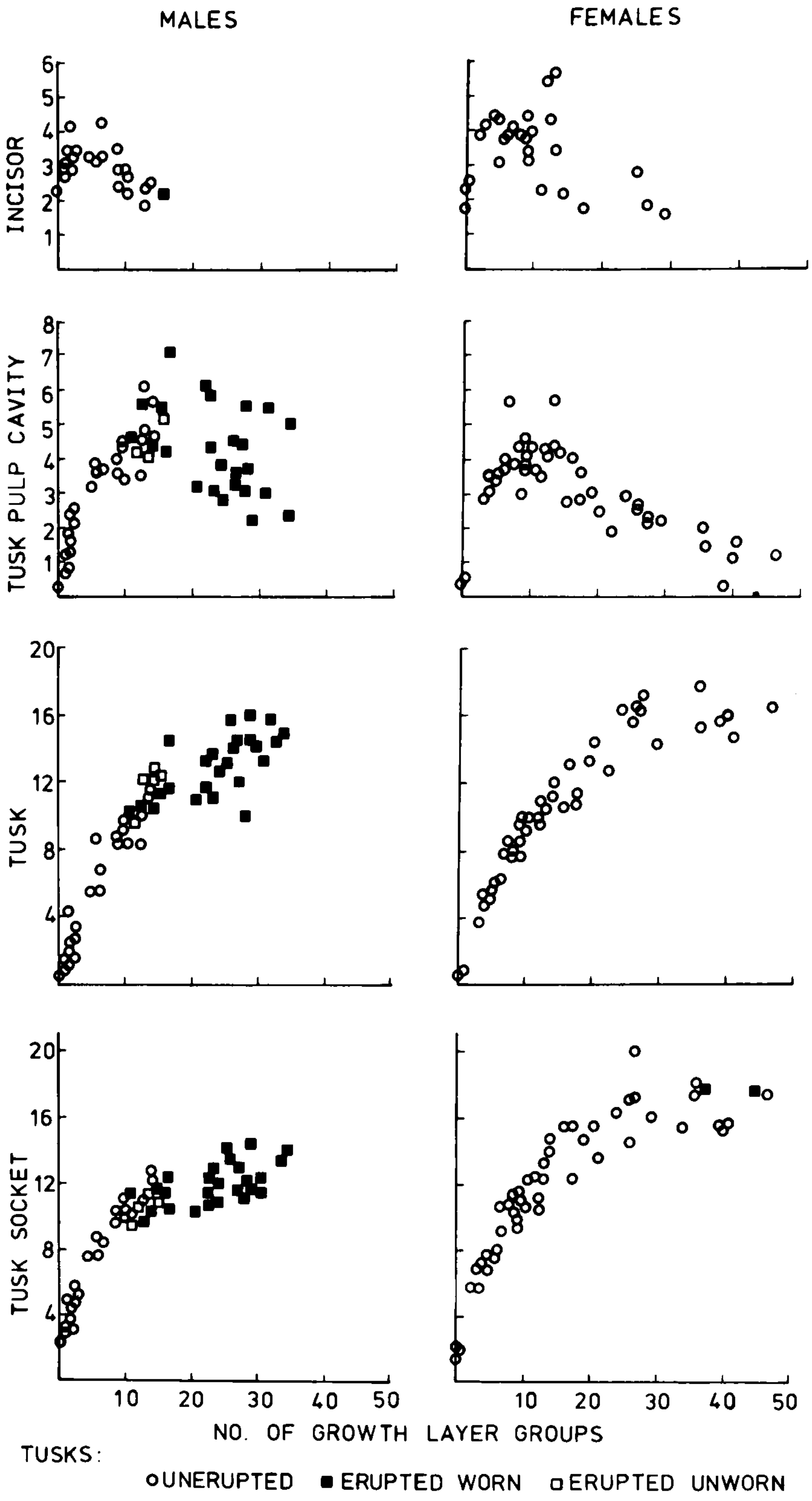

Fig. 12. Diagram showing the length (in cm) of the deciduous incisor, tusk pulp cavity, tusk, and the tusk socket at various ages (expressed as number of dentinal GLGs for male and female dugongs).

The female tusk continues to grow posteriorly through the premaxilla. The increase in length of the tusk is accompanied by a corresponding increase in the length of the alveolus (Fig. 12) which continues to extend up the premaxilla, the hole in the premaxilla marking the base of the alveolus. In older animals, the width of the GLGs decreases (Table 1) and the pulp cavity decreases in length (Fig. 12). The inclination of the GLGs from the long axis of the tooth also increases with time, resulting in the later layers in very old teeth being almost perpendicular to the long axis of the tooth. In two females, with 45.5 and 38.5 GLGs, the tusks had erupted and worn, presumably because they had reached the base of the premaxilla and could not grow posteriorly any farther. Tusk eruption cannot therefore be considered to be diagnostic of male dugongs.

In the male, the tusks erupt (Fig. 2) after 12 to 15 GLGs have been laid down. The length of the tusk alveolus remains relatively constant thereafter and never reaches the base of the premaxilla. The premaxillary bones are much thicker than in the female and the hole in each premaxilla at the base of each tusk usually disappears around the time that the tusks erupt. The decrease in the width of the GLGs is

Fig. 13. The 0.55-cm-long tusk from a 1.28-m-long dugong and the 13.7-cm-long tusk from a 2.39-m-long male dugong.

not as great as in the female (Table 1). The inclination of the GLGs to the long axis of the tooth decreases in old animals. This causes the tusks of old males to taper at the base. However, the length of the pulp cavity decreases appreciably less than in females (Fig. 12).

The anterior erupted end of the tusk is worn into a chisel shape (Figs. 2, 9A), the cutting edge of which is reinforced by the enamel layer below the cementum (Fig. 8A) in this region.

Relationship between the ontogeny of the cheek teeth and the number of growth layer groups in the tusk

There was no instance of all six cheek teeth having been erupted and in wear at once in any one of the 107 skulls examined. Only one skull had all six cheek teeth present in each quadrant, molar 3 being unerupted. Any unerupted teeth always occurred at the posterior end of the tooth row. Teeth in which the roots were resorbed and which were wizened or broken always occurred at the anterior end. X-rays of the crania and mandibles did not reveal any unerupted teeth that were not visible from superficial examination. The enamel of all three premolars was usually present only in very young dugongs. Molars appeared to lose most of their enamel before or soon after eruption, as reported by Fernand (1953).

Usually the development state of the cheek teeth was similar in both jaws of any one skull. When differences existed, development in the upper jaw was found to be lagging behind that in the lower.

The development of each cheek tooth is considered separately below in relation to the number of GLGs in the tusk.

Premolar 2 (PM2): This tooth (Figs. 14A, B) is cylindrical and considerably smaller than the other teeth. It seems to be variably present, at least in the upper jaw, being absent both from the 1-m fetus and from another animal estimated to have been less than six months old. It was slightly worn in the youngest of the juvenile animals, and in wear in all other animals with less than two GLGs, except for two animals with one GLG, in which it was broken. The pulp cavity is open at birth but is occluded by the time the first GLG is deposited. The tooth is usually lost by the time the animal has laid down about five GLGs and all trace of the alveolus has disappeared by the time the animal has laid down eight or nine GLGs.

Premolar 3 (PM3): PM3 (Figs. 14A, B, C) was present and erupted in the 1-m fetus, erupted, but only slightly worn, in the youngest juvenile (1.3 m body length) and worn in all other animals in which it was present. As the tooth wears, it loses its original bilophodont shape and becomes peg-like. The pulp cavity is open at birth, starts to taper at the base by the time one GLG has been laid down and occludes by the time two GLGs have been laid down. After this, the tooth begins to erode. It is generally lost by the time six to ten GLGs have been laid down, all trace of the alveolus being lost by the time the animal has 14 GLGs in the tusk.

Premolar 4 (PM4): PM4 (Figs. 14A, B, C, D) was present and erupted in the fetus and in wear in all others except the youngest juvenile dugong, with resultant rapid loss of its originally bilophodont shape. The pulp cavity tapers at the base after about one GLG has been laid down and is generally closed by the time four GLGs have been laid down. The tooth is usually severely eroded (Fig. 14D) by the time the animal has eight GLGs in the tusk and may even be lost at this stage. In some animals it remains as a wizened stump for much longer (up to 16 GLGs). The alveolus is usually filled by the time the animal has 20 or 30 GLGs in the tusk.

Molar 1 (M1): M1 (Figs. 14A, B, C, D, E) is present but unerupted at birth. It erupts and comes into wear during the laying down of the second or third GLG. Its pulp cavity starts to taper at the base after about five GLGs have been deposited in the tusk and it closes at about 12 to 14 GLGs. M1 loses its bilophodont shape as it wears and becomes peg-like. It can persist for 45 or more GLGs but is usually lost by the time 25 GLGs have been deposited in the tusk.

Molar 2 (M2): The unerupted M2 was first seen in skulls from dugongs with one tusk GLG. It erupts and comes into wear during the time that the fourth GLG is laid down. The pulp cavity remains open throughout life although it is very reduced in old animals (Fig. 15A). M2 loses its initial bilophodont shape and wears down to a simple peg shape (Figs. 14C, D, E, F). It shows pronounced axial growth throughout life. In old animals M2 may be markedly curved to the front (Fig. 15A).

Molar 3 (M3): M3 (Figs. 14C, D, E, F) is first apparent as an unerupted tooth when the dugong has three to four tusk GLGs. It erupts and comes into wear at between seven and nine GLGs. Unlike the other teeth it retains its bilophodont shape, the crown having a characteristic keyhole outline (Figs. 14D, E, F). However, the occlusal surface wears flat as in the other teeth. The pulp cavity of M3 remains open throughout the life of the animal but becomes relatively smaller with age (Fig. 15B). M3 increases dramatically in size throughout life (Fig. 15B) as a result of prolonged axial and radial growth.

The ontogeny of each cheek tooth in relation to the number of GLGs in the tusk is summarized in Figure 16.

Relationship between relative growth criteria and the number of growth layer groups in the tusk

The age of young animals can be estimated from relative growth criteria, which become less reliable indicators of age as the animals get older.

The following age classes have been recognized by correlating changes in dentition characters, cranial sutures, body length, and cranial measurements with age, estimated as the number of dentinal GLGs in the tusks.

> ***0–1 year:*** PM2, PM3, PM4 erupted and come into wear soon after birth; deciduous incisor, tusk, M1 unerupted; pulp cavities of all teeth open; basisphenoid suture open; body length less than 1.5 m; zygomatic width less than 15 cm.

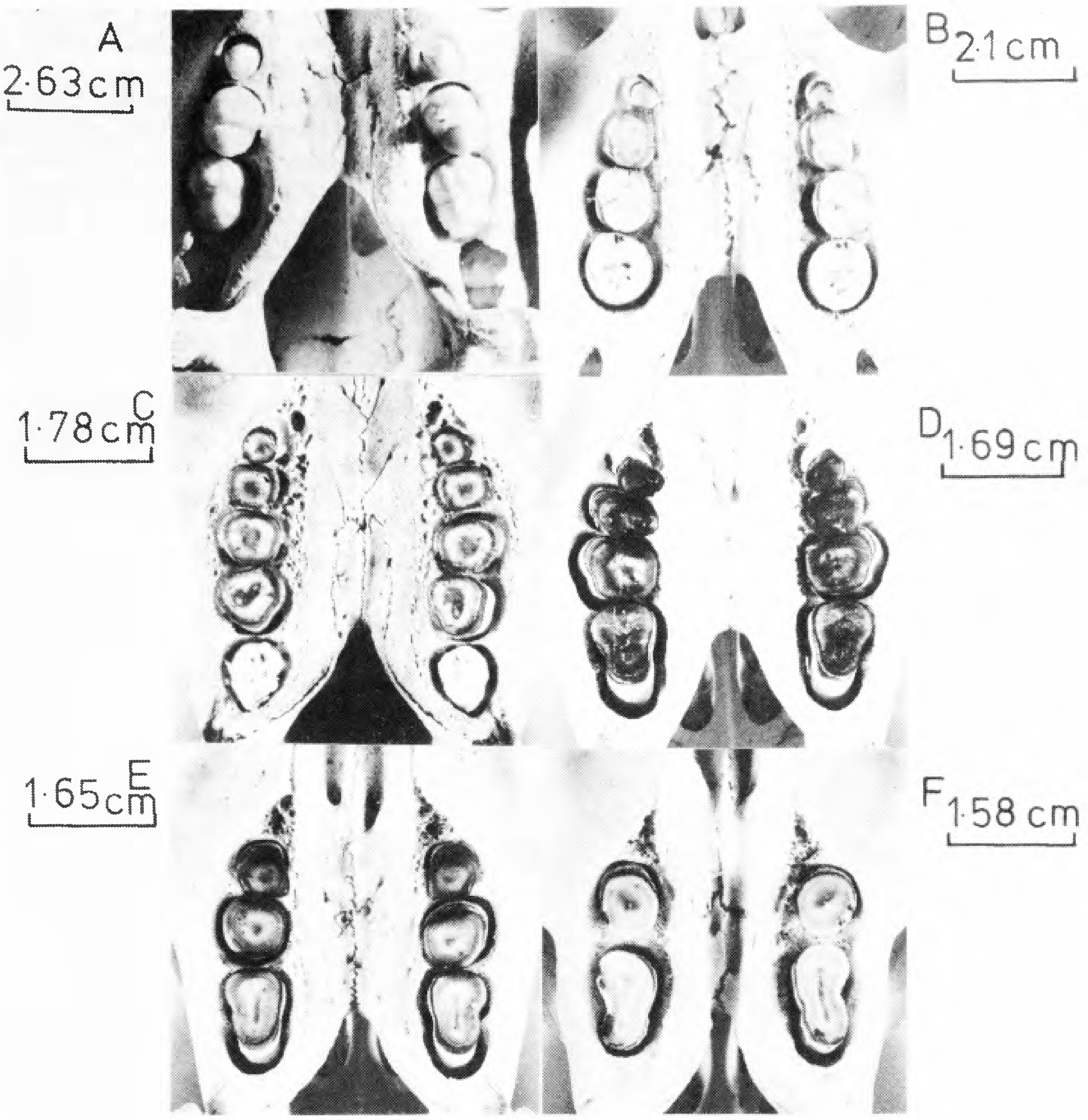

Fig. 14. The upper jaw cheek tooth dentition of dugongs of various ages. (A) presumed neonate; PM2, PM3, PM4 erupted, M1 unerupted. Wear can be seen on the crown surface of PM2. (B) 1.5 GLGs PM2, PM3, PM4, M1 erupted and in wear. (C) 6 GLGs; socket of PM2; PM3, PM4, M1, M2 in wear; M3 erupted and unworn. (D) 13 GLGs; socket of PM3; PM4 eroded; M1, M2, M3 in wear. (E) 16 GLGs (tusk worn); socket PM4, M1, M2, M3 in wear. (F) 29 GLGs (tusk worn); socket M1, M2, M3 in wear.

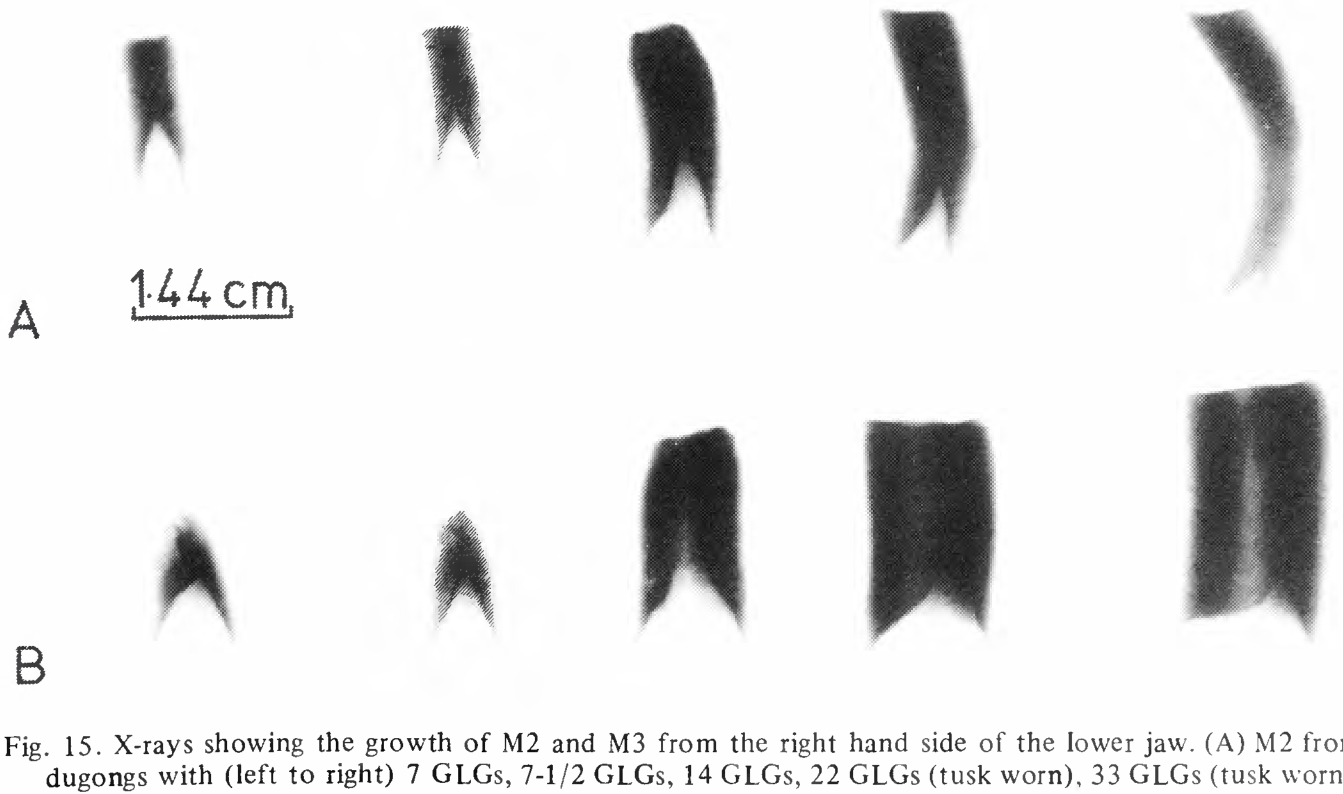

Fig. 15. X-rays showing the growth of M2 and M3 from the right hand side of the lower jaw. (A) M2 from dugongs with (left to right) 7 GLGs, 7-1/2 GLGs, 14 GLGs, 22 GLGs (tusk worn), 33 GLGs (tusk worn). (B) M3 from the same dugongs.

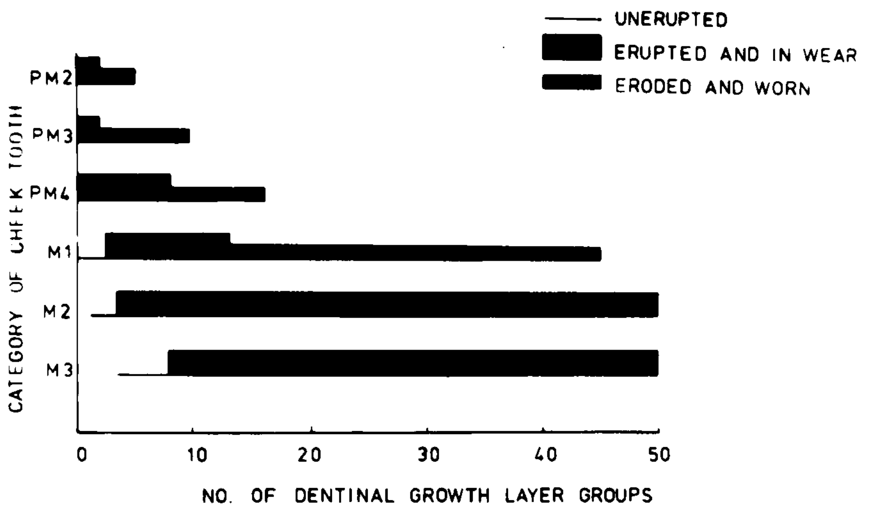

Fig. 16. Summary of the pattern of eruption and wear in dugong cheek teeth as related to age (number of dentinal GLGs in the tusks). The ages given for the persistence or eroded and worn teeth are the maximum observed. In many animals the teeth are lost before this.

1–2 years: M1 may be erupted; M2 unerupted; pulp cavity of PM2 closed; pulp cavities of PM3 and sometimes of PM4 tapered at base; basisphenoid suture closed; body length 1.5 to 1.75 m; zygomatic width 15 to 15.5 cm.

2–3 years: PM2 eroded, may be lost; M1 erupts and comes into wear; M2 unerupted; pulp cavity of deciduous incisor may be occluded; pulp cavity of PM3 occludes; zygomatic width 15.5 to 17.5 cm; body length 1.75 to 2 m.

3–4 years: PM2 usually lost; PM3 eroded; M2 erupts and comes into wear; M3 unerupted; pulp cavity of PM4 usually occluded.

4–6 years: PM3 eroded and may fall out; pulp cavity of PM4 occluded; pulp cavity of M1 tapered at base.

6–9 years: PM3 usually lost; PM4 eroded, may be lost; M3 erupts and comes into wear; condylar sutures may close.

After the eruption of M3, relative growth criteria become much less reliable. PM4 is variably lost between 8 and 16 years. Although the pulp cavity of M1 tapers and closes at 12 to 14 years, M1 may persist throughout life; however it is usually lost by 25 years. In males, the tusks erupt between 12 and 15 years and the deciduous incisors are usually lost around this stage. In females, the deciduous incisors may persist for 30 years. The condylar sutures close between 8 and 13 years, and the basioccipital usually, but not invariably, closes by 20 years.

Body length is a poor indicator of age except for young animals as the range of size at a given age is considerable (Fig. 11). The best non-linear regression that could be fitted between body length and age for animals in which M3 was erupted explained only 28% of the variance in body length with age.

Relationship between cheek-tooth parameters and the number of growth layer groups in the tusk

An attempt was made to describe the relationship between age and selected cheek-tooth parameters to see if they could be used as predictors of age.

Bivariate scatter diagrams were drawn on linear and logarithmic scales for all dugongs with molar 3 erupted and in wear to compare the number of GLGs used as an estimate of age (A) and the following cheek-tooth parameters: cheek-tooth index (T); crown area molar 2 (C_2); central cross-sectional area molar 2 (X_2); central cross-sectional area pulp cavity molar 2 (P_2); crown area molar 3 (C_3); central cross-sectional area molar 3 (X_3); central cross-sectional pulp cavity molar 3 (P_3).

Initially, data for males and for females and for the Townsville and Mornington Island populations were each analyzed separately, but as the analyses showed that there were no significant ($P < 0.05$) differences between the groups, the data were not separated for the subsequent analyses.

Scatter diagrams (Figs. 17A to D) are presented for the relationships between A and C_2, C_3, X_2, X_3 (Fig. 15) on linear scales. Each of the variables increases with age, particularly up to about 30 GLGs.

A similar plot of the relationship between T and A showed that T decreases with age (Fig. 18). Logarithmic transformations of both A and the independent variables were used to rectify the data and stabilize the residual variance in each case.

Least squares univariate linear regressions were computed for the logarithm of A and the logarithms of all the other variables. Data from animals with more than 30 GLGs were omitted from the analyses because the variance of the cheek-tooth parameters is so great after 30 GLGs have been reached. Two separate sets of analyses were performed. The first set included data from animals with unworn tusks only; the second set included data from all animals.

The resulting regression lines all explained a significant proportion of the variance in A ($P < 0.01$), except those for $\log_e P_2$, and $\log_e P_3$. Table 6 lists the estimates of the intercept a, the slope b and the coefficient of determination (r^2) for all significant regression lines. The estimate of the intercept a was significantly different from zero ($P < 0.001$) as was the estimated slope b ($P < 0.01$) (Table 6) of each of the significant regression lines.

A step-up multiple regression technique was used to compute the best relationship between the logarithm of age and the logarithms of the various cheek-tooth parameters.

The best relationship for the animals with unworn tusks for which estimates of the intercept and all the partial regression coefficients were significantly different from zero ($P < 0.05$) was:

$$\begin{aligned}\log_e A &= 3.65 + 0.34 \log_e C_3 - 0.62 \log_e T + \\ &\quad 0.38 \log_e X_3 - 0.32 \log_e C_2, \qquad R^2 = 0.83 \\ \text{or } A &= 38.47\, C_3^{0.34}\, T^{-0.62}\, X_3^{0.38}\, C_2^{-0.32}\end{aligned}$$

and similarly for the animals with worn plus those with unworn tusks

$$\begin{aligned}\log_e A &= 3.50 + 0.27 \log_e C_3 - 0.54 \log_e T + \\ &\quad 0.57 \log_e X_3 - 0.34 \log_e X_2, \qquad R^2 = 0.89 \\ \text{or } A &= 33.12\, C_3^{0.27}\, T^{-0.54}\, X_3^{0.57}\, X_2^{-0.34}.\end{aligned}$$

The same technique was used to compute the best significant relationship between $\log_e A$ and the $\log_e$ of those cheek-tooth parameters that could be obtained by x-raying a live animal, i.e. parameters excluding C_2 and C_3.

The best relationship for animals with unworn tusks was

$$\begin{aligned}\log_e A &= 4.35 + 0.78 \log_e X_3 - 0.90 \log_e T - \\ &\quad 0.44 \log_e X_2, \qquad R^2 = 0.82 \\ \text{or } A &= 77.48\, X_3^{0.78}\, T^{-0.90}\, X_2^{-0.44}\end{aligned}$$

and for the animals with worn plus those with unworn tusks

$$\begin{aligned}\log_e A &= 3.94 + 0.82 \log_e X_3 - 0.76 \log_e T - \\ &\quad 0.43 \log_e X_2, \qquad R^2 = 0.87 \\ \text{or } A &= 51.42\, X_3^{0.82}\, T^{-0.76}\, X_2^{-0.43}.\end{aligned}$$

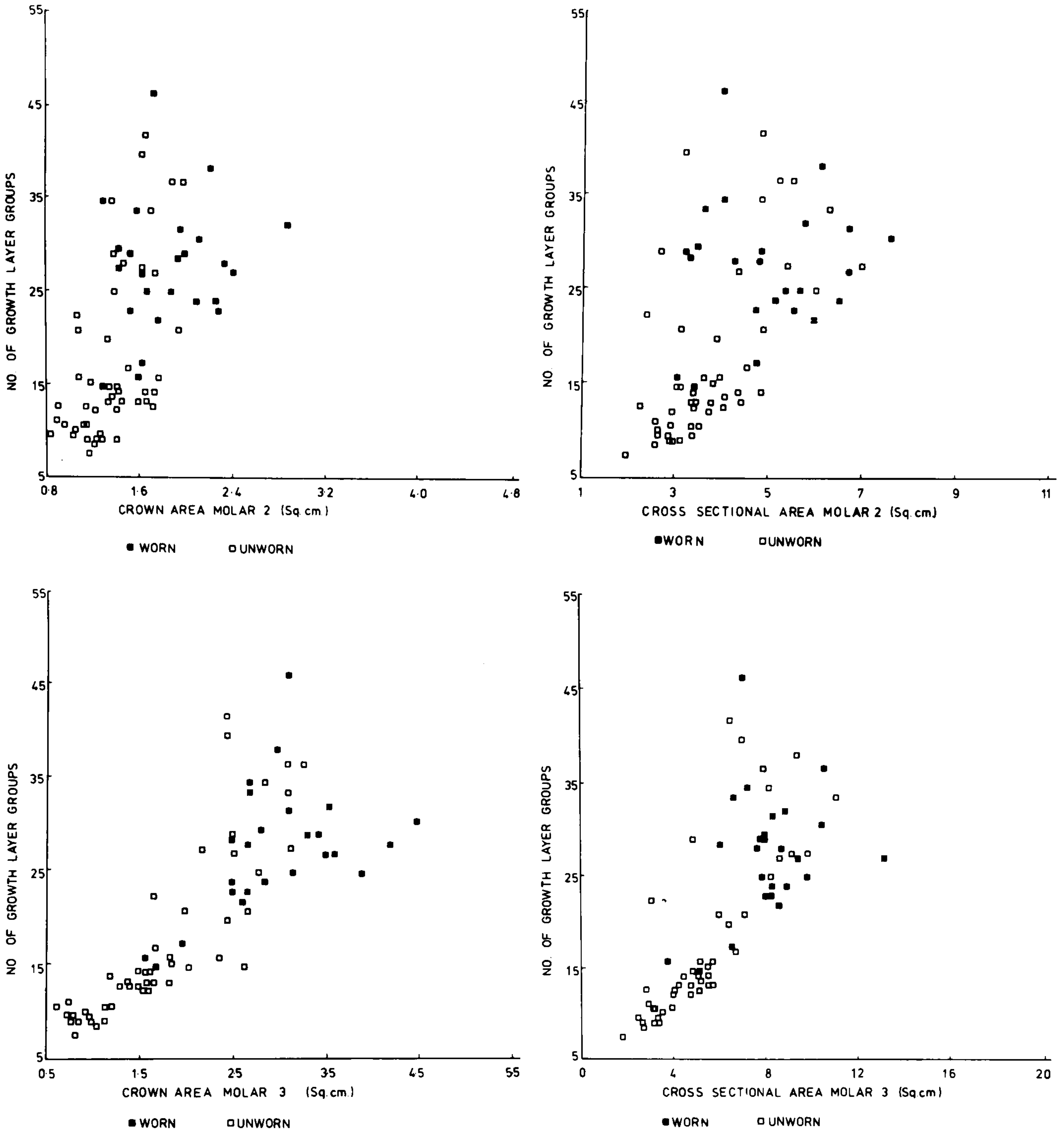

Fig. 17. Bivariate scatter plots showing the relationships between age (A) (the number of dentinal GLGs in the tusk) and the crown area (C) and central cross sectional area (X) of both M2 and M3 from the right hand lower jaw. Measurements are included from all animals in which M3 is erupted and in wear. Unworn and worn refer to the tusks.

F tests for all the above regression lines were significant ($P < 0.001$) as were all partial regression coefficients ($P < 0.05$).

Examination of other hard tissues

No GLGs were observed in the humerus or malleus. Layers were, however, clearly discernible in the tympanic bones and ribs. Twelve layers were counted in the tympanic bone of a dugong with 17 layers in its unworn tusk; 23 in a dugong with 33 layers in its erupted and unworn tusks. Eight layers were counted in the cross section of rib from a dugong with nine layers in its erupted and unworn tusk. The ribs from uncatalogued dugongs had six, seven, and at least 11 layers, respectively. The counting of layers in dugong ribs is difficult because of accessory layering and the orientation of the Haversian systems.

DISCUSSION

The sample

The collection of measurements, skulls and reproductive tracts from shark-netted dugongs from the Townsville area, acquired since 1969 by Heinsohn and his co-workers and used in this study, is the first substantial series of well-documented dugong material. In the absence of field studies, it is impossible to gauge how representative this sample of the Townsville dugong population is. When the distributions of sexes, sizes and estimated ages are considered (Figs. 3 and 4) there appear to be no major gaps. From the age determination viewpoint, it is fortunate that carcass material was collected in every month of the year. It would, however, have been desirable to have more individuals from certain age groups, particularly late fetal and neonatal animals.

Most of the material from Mornington Island dugongs was collected in July-August by somewhat selective hunting and no really young animals were obtained from the aboriginal hunters. This sample cannot, therefore, be considered representative of the Mornington Island dugong population.

Age determination using growth layer groups in the tusk

The tusks are ideal for age determination. They are present throughout life and do not erupt and wear except in post-pubertal males and occasionally in old females. Except in the case of some old females, the pulp cavity remains open and dentine appears to be deposited throughout life. The GLGs in the tusks of dugongs from the areas studied are

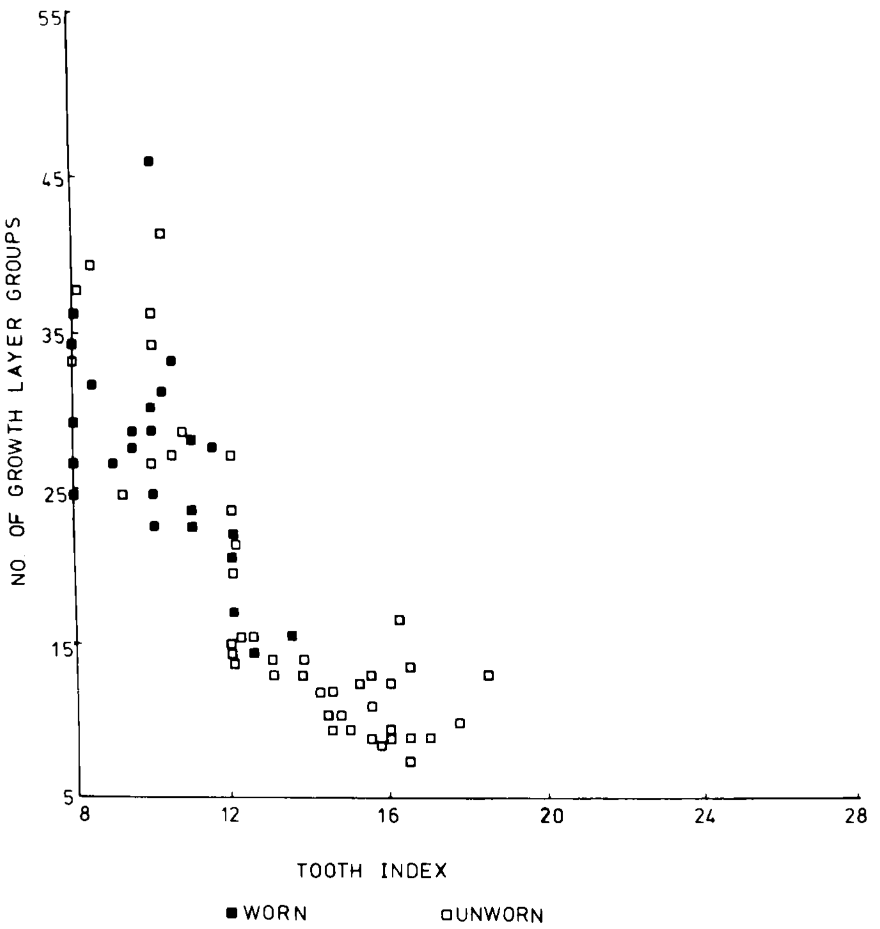

Fig. 18. Bivariate scatter plot of the relationship between age (A) (number of dentinal GLGs in the tusk) and the cheek tooth index (T) of all animals in which M3 is erupted and in wear. Unworn and worn refer to the tusks.

extremely clear and are usually easy to count, yielding highly repeatable results. Etched half teeth are ideal for this purpose (Fig. 9A), except in the case of young animals, where thin sections of both a deciduous incisor and a tusk should be used.

The description of the dentinal GLG in the tusk of the dugong is rather more complex in this paper than those of Kasuya and Nishiwaki (1978) and Mitchell (1978).

The arrangement of a broad zone of intermediate optical density followed by a narrow zone consisting of contiguous opaque and translucent layers is very similar to that described in the Ziphiidae (Report of the Ziphiid Group, published in this volume).

Intercalibration of different techniques used to demonstrate growth layer groups

In the masked specimens examined, the degree of optical opacity was mirrored by the intensity of haematoxylin staining (Fig. 8D). This has also been observed in dugongs by Kasuya and Nishiwaki (1978 and pers. comm. 1978). However, this relationship does not hold for all species examined (Kasuya, pers. comm. 1978). The neonatal line (Fig. 7A) seems to be always translucent and unstainable in all marine mammal species so far examined by Kasuya (pers. comm. 1978), including the dugong.

In the GLGs of the single specimen of dugong tusk studied in detail (Figs. 8A to D), the degree of optical density of the layers not only appeared to correspond to the degree of stainability, but also to the degree of radio-opacity (Fig. 8A) and etchability (Fig. 8C). This observation must be viewed with caution as the most and least optically opaque layers are both narrow and contiguous. It is similar to the observations by Hohn (published in this volume) of the properties of the components of the GLGs of *Tursiops truncatus* except that Hohn did not use stain. In contrast, in *Phocoena phocoena* the optically translucent zone was found to be more radio-opaque (Nielsen, 1972). However, Grue-Nielsen (pers. comm.) considers that this relationship may not remain constant with increasing age even within one specimen of this species.

Although the relationships between the layering demonstrated by various techniques are of interest, they are not very important to the practical problem of absolute age determination as the numbers of GLGs revealed by each method are clearly similar (Fig. 8).

Time scale represented by the layers

Zone B of the GLG was almost always formed between July and October (Table 2) and the thickness of Zone A increased from October to September (Table 3). Both these results suggest that one GLG is deposited per year.

Kasuya and Nishiwaki (1978) also reached this conclusion. Mitchell (1976 and 1978) suggested that the deposition rate was annual or biannual. The patterns of layer deposition observed by Mitchell (1978) in 11 dugongs from northern Australia and by Kasuya and Nishiwaki (1978) in two dug-

Table 6

Estimates of the intercept (a), slope (b), result of the significance test for b, and value of the coefficient of determination (r^2) for the significant univariate linear regression relationships between $\log_e$ age (A) and $\log_e$ of various cheek tooth parameters.

Separate analyses are presented for dugongs with unworn tusks only (U) and for those with unworn and worn tusks (U + W)

		a	b	Pb	r^2
Tooth index (T)	U	7.22	−1.76	≤ 0.001	0.65
	U + W	7.05	−1.68	≤ 0.001	0.75
Crown Area 2 (C_2)	U	2.41	0.76	≤ 0.01	0.16
	U + W	2.40	1.08	≤ 0.001	0.40
Central X-sectional Area 2 (X_2)	U	1.63	0.80	≤ 0.001	0.33
	U + W	1.68	0.83	≤ 0.001	0.42
Crown Area 3 (C_3)	U	2.35	0.71	≤ 0.001	0.73
	U + W	2.36	0.75	≤ 0.001	0.83
Central X-sectional Area 3 (X_3)	U	1.48	0.76	≤ 0.001	0.66
	U + W	1.43	0.82	≤ 0.001	0.76

ongs from this area, one dugong from Luzone (16°30'N) and two dugongs from the Celebes (2°S), are the same as that observed in this study. One dugong from northern Australia observed by Mitchell (1978) was exceptional in that it appeared to have completed Zone A in November. However, the date of death of this animal is questionable. It thus seems reasonable to assume a deposition rate of one GLG per year.

Factors which may influence layer deposition

Attempts to show seasonality of layer deposition in other marine mammals in the tropics have so far been unsuccessful; e.g. Perrin *et al* (1976), who studied offshore tropical populations of *Stenella attenuata.* Spinnage (1976) studied several species of terrestrial mammals in tropical Africa. He observed one annual layer in the cementum of animals living in unimodal rainfall areas and two layers per year in animals living in areas of bimodal rainfall.

There is a unimodal drop in sea water temperature in the Townsville area which reaches a minimum in July (Fig. 19), the month in which the deposition of Zone B is thought to commence. Both Townsville and Mornington Island have one wet season per year, typically between December and April when Zone A is being deposited.

Not all the dugongs that died between July and October in any one year had commenced laying down Zone B. This suggests that layer deposition may not be cued by an external stimulus but may be controlled by an endogenous rhythm of growth starting from birth. The asynchrony may thus reflect the extended calving season thought to last from August to January.

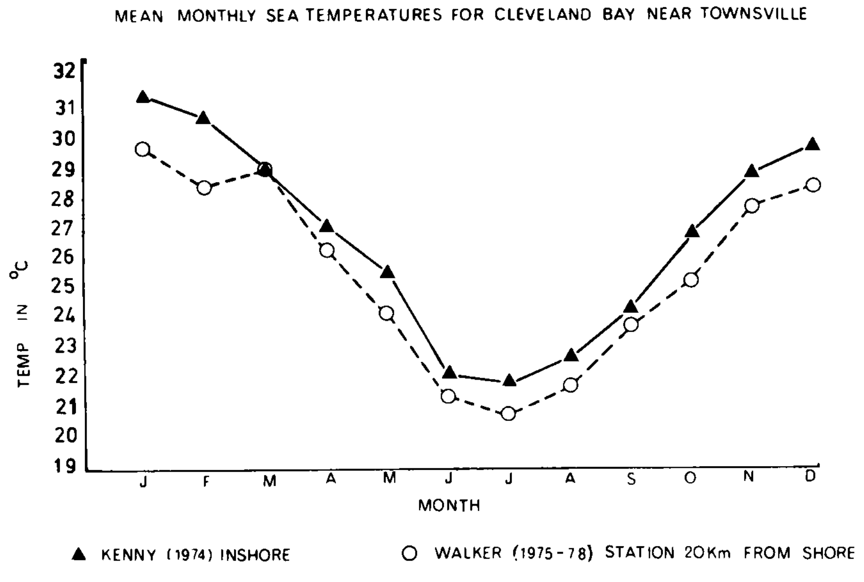

Fig. 19. Annual variation in surface sea temperatures for Cleveland Bay near Townsville.

About 12 fine accessory layers per GLG have been observed in the deciduous incisor in this study. Kasuya and Nishiwaki (1978) observed 10 to 15 fine layers present per GLG in the dentine of the tusk. They consider that they represent lunar months or an endogenous cycle of about 30 days as observed in *Berardius bairdii.* Tidal cycles which are determined by the phases of the moon are probably very important regulators of dugong feeding activities. Scheffer (1970) considered that the fine layers present in the tusk may represent days and that the coarse layers may represent months. The seasonality of GLG (= coarse layer) deposition suggested in this paper weakens his argument. If the fine accessory layers can be shown to represent months, they would be very useful in age determination, particularly of young animals.

Mitchell (1978) observed that tusks from the Torres Strait area (10°S) were difficult to read as they had more marked accessory layering than teeth from Townsville (19°S). This difference was not observed in the two specimens from Torres Strait included in this study, but the one specimen etched and examined from the oceanic island of Palau (7°30'N) had so many accessory layers that it was almost impossible to read using the standards and methods applied for the major part of this study. Kasuya and Nishiwaki (1978), however, successfully counted layers in the tusks of two dugongs from the Celebes (2°S). It seems likely that marked accessory layering may be a feature of the teeth of individual dugongs rather than of all dugongs living close to the equator.

Age determination in adult males

Although not critical to the conservation and management of the Australian dugong populations, the accurate estimation of the age of males with erupted and worn tusks is an unsolved problem as an unknown number of dentinal GLGs are lost from the anterior end of the tusk in these animals. Counts of GLGs ranged from 13 to 17 in newly-worn tusks and from 21.5 to 34.5 in tusks which had been substantially worn.

Cemental counts from near the tip of an unerupted tusk may be useful in dugong age determination (Kasuya and Nishiwaki, 1978), but the cementum from this region will, of course, be worn away as the tusk erupts and wears. Cementum counts are therefore unlikely to be useful for aging males with worn tusks (Fig. 7B).

Results obtained from counting GLGs in other hard tissues in the dugong do not appear particularly promising. No distinct layering was seen in sections of humerus or malleus. Mitchell (1978) found four layers in the periosteal zone of the ventral edge of the mandible of a dugong with 10.5 GLGs in the tusks. I counted 12 layers in the tympanic bone of one dugong which had 17 GLGs in the tusk; 23 GLGs in another which had 33 GLGs in the tusk which was worn. Eight layers were counted in the rib of a dugong which had nine layers in the tusk. Rib layering is, however, difficult to count. Kasuya has observed 11 to 14 layers in an uncatalogued dugong rib (pers. comm. 1979). Thus the number of layers seen in the mandible, rib and tympanic bone are lower than those in the tusk.

The number of layers in the ribs, tympanic bones and the tusks should be compared in a larger sample of dugongs from a range of ages. If the number of layers in the tympanic bones is found to be predictably lower than that in the tusk in dugongs, tympanic bone layer counts may also be useful in determining age in the manatee. Rib-layer counts may also prove to be useful in this regard (Domning and Myrick, 1980), although the layering was very indistinct in the one *Trichechus manatus* rib I have examined. Useful age estimates cannot be obtained from layer counts in manatee teeth as they are replaced at frequent intervals (Domning and Magor, 1977).

Age determination using cheek teeth

Attempts to obtain age estimates more accurate than those from erupted tusks, by using cheek teeth, have not been very successful. The equations that have been computed to describe the relationships between age and the cheek-tooth parameters of dugongs with unworn tusks only are not substantially different from those which include data from dugongs with worn tusks. Thus the use of cheek-tooth parameters for age determination would seem to be most valuable when it is not possible to count the dentinal GLGs in the tusk. The method should be applicable to captive dugongs, the teeth of which could be x-rayed.

The relative aging of young dugongs based on cheek-tooth succession that is reported in this paper, does not differ greatly from that of Mitchell (1973, 1978). Some of the differences may be attributable to the problems associated with counting the dentinal layers in the tusks of young animals, where, of course, accuracy is critical.

Growth of young dugongs

Kasuya and Nishiwaki (1978) and Mitchell (1978) appear to have had some uncertainties about the ages of the few young specimens that they studied. I share this uncertainty and regard the growth curves for young dugongs proposed from the Townsville data (Fig. 11B and Table 5) as tentative. They are comparable with the observed growth of a manatee born in captivity (Odell, in press).

The upper and lower limits of the 95% confidence intervals for the birth lengths calculated for the Townsville population ranged from 1.10 m to 1.45 m (excluding the curve based on unworn tusks only in which the confidence limits are very wide). Heinsohn (1972) recorded a near-term fetus that was 1.14 m long and a calf 1.09 m long, both from the Townsville population. Unfortunately, material from these animals was not available for this study. One female in the Townsville sample had the dentition of a neonate and a body length of 1.39 m, and the stump of the umbilical cord was still evident in a 1.23 m male examined recently. Thus the confidence intervals for the birth length estimated by the curves appear to be valid.

Attempts have been made to estimate dugong growth rates by observing captive animals. These estimates are probably less reliable than those obtained for manatees because of the considerable problems associated with feeding captive dugongs (personal observation) which eat sea-grasses, the collection of which is extremely labour-intensive.

A 1.4-m-long dugong was captured near Cairns, northern Australia, in March 1978 and maintained at the Cairns Oceanarium until it died in July 1978. It did not increase in body length during this period and it failed to regain the 10% loss in body weight that occurred in the first few weeks following its capture, despite its being bottle-fed milk and provided with more sea-grass than it could eat.

One of the two male dugongs, maintained in captivity at Mandapam Camp, India and fed sea-grass, increased in body length from 1.60 m to 2.05 m in nearly seven years and to 2.07 m after a further four years (Jones, 1967; 1976). This dugong may have been suckling at the time of capture and it is possible that the rapid and untimely weaning may have resulted in growth retardation.

In contrast, the body length of the other dugong (1.96 m long at capture) after 11 years in captivity was 2.26 m, which is comparable with that recorded for several dugongs from Mornington and Thursday Islands estimated to be more than 20 years old.

In view of the uncertainty of extrapolating the results of growth studies on captive dugongs to field situations, efforts should be made to monitor the growth of young marked dugongs in the wild over several years.

Life history parameters

Maximum longevity. The maximum number of GLGs counted in an unerupted female dugong tusk was 51. Mitchell (1978) scored four such tusks as over 50, the maximum being 57.5. Assuming that one GLG is laid down per year, I estimate the maximum longevity of female dugongs as 50 to 60 years. In view of the maximum male GLG counts, it seems likely that the figure for males is similar to that of females.

Puberty. On the same basis, puberty is estimated to occur at around nine years for Townsville males and possibly not until four or five years later at Mornington Island.

The fact that the tusks do not erupt until several years after mature sperm first appears in the testes and epididymides of Townsville dugongs, suggests that there may be a difference between puberty and sexual maturity in male dugongs parallel to that observed for sperm whales by Best (1969). The function of the erupted tusks has not been confirmed by direct observation, but parallel scars are often observed on the dorsal surface of both male and female dugongs. The distance between these scars corresponds with the distance between the tusks and it seems likely that the tusks are used to roll the female over for mating (Anderson and Birtles, 1978) and in fighting.

Information is insufficient to make a firm estimate of the age of sexual maturity in the female. Evidence so far accumulated in this study suggests that it is unlikely to be earlier than nine years. In addition an immature female with seven GLGs from the Celebes has been reported by Kasuya and Nishiwaki (1978). Mitchell (1978) reported that an immature female from north-eastern Australia had 9.5 GLGs in its tusk.

Heinsohn (1972), on the basis of examination of reproductive tracts of 14 dugongs drowned in Townsville shark nets from 1969 to 1971, suggested that sexual maturity in both males and females occurred at a body length of approximately 2.4 m. He constructed an hypothetical growth curve based on the body lengths and dates of death of 73 dugongs and concluded that a body length of 2.4 m could be reached at about two years of age. Spain and Heinsohn (1974) showed that a body length of 2.4 m corresponded to a condylo-premaxillary length of about 34 cm. Mitchell (1978) showed that this corresponded to a dentinal GLG count of nine to ten and concluded that sexual maturity occurred at an age of about ten years providing that one GLG is deposited annually.

The above estimates of sexual maturity are all based directly or indirectly on the results of the examination of reproductive tracts. There is other evidence that sexual maturity occurs at about ten GLGs. Kasuya and Nishiwaki (1978) reported that after the deposition of an average of ten GLGs, the layering in dugong tusks becomes irregular with conspicuous accessory layers. This change in layering is similar to that seen in the maxillary teeth of many sperm whales after sexual maturity (Ohsumi *et al,* 1963).

The changes in the pattern of tusk growth (Mitchell, 1978) which lead to sexual dimorphism in tooth form also begin to become obvious at about ten GLGs (Fig. 12). This could be expected to occur near puberty if the erupted tusk is a secondary sexual characteristic of males, as seems likely.

Using body length as an indicator of age is obviously a poor method except in very young animals. The range in body lengths for a particular age group is considerable (Fig. 11). A sexually mature, non-captive dugong has been recorded with a body length of 2.2 m and 33 dentinal GLGs. A female dugong 2.59 m long was considered to be immature on gross examination (Mitchell, 1978).

Possible sex and population differences in asymptotic length and/or age at sexual maturity

The sex difference in estimated asymptotic length observed within the Townsville population of dugongs (Table 5) has not been noted previously (Heinsohn, 1972) and warrants further investigation with a larger sample size. The possibility that dugongs may be growing longer and maturing faster near Townsville as compared to Mornington Island should also be examined. These differences, if they indeed occur, may be in response to differences in population density. The age of reproductive maturity is known to be density dependent for at least 15 other species of large mammals, eight of these being marine (Fowler *et al*, 1978).

Density-dependent differences in asymptotic body length have also been observed. For example, the asymptotic lengths of both male and female southern elephant seals *(Mirounga leonina)* from the exploited population at South Georgia were larger than those from the unexploited Macquarie Island population (Carrick *et al*, 1962). Gambell (1976) states, however, that there is little evidence for a change in the size of whales at physical maturity since the 1920s despite increased levels of exploitation.

Precise estimates of the sizes of the dugong populations in the Townsville and Mornington Island areas are not available. However, evidence from aerial surveys suggests that the population is now larger at Mornington Island than at Townsville. A maximum of 98 dugongs has been counted in 1977 in 25 aerial surveys of the Townsville area (Heinsohn, unpublished report). In contrast, a maximum of 374 dugongs has been counted per survey in the Wellesley Islands (of which Mornington Island is one) (Marsh *et al*, in press).

The mortality rate of Townsville dugongs has increased markedly in recent years with the introduction of shark-netting. More than 200 animals have been killed in shark nets since 1964, 82 in the first year of netting (Heinsohn, 1972; Heinsohn and Spain, 1974). At Mornington Island, the number of dugongs killed by aboriginal hunters appears to have remained fairly constant at 40 to 50 dugongs per year for many years (Anderson and Heinsohn, 1978; Marsh *et al*, in press). The habitats, as observed subjectively, seem similar in both areas and the dugongs are feeding on the same species of sea-grasses (Marsh, unpublished observation). It seems possible that the larger body size and shorter pre-reproductive period observed for the Townsville dugongs are density dependent. If so, reduction in the Townsville population caused by shark-netting could be expected to exacerbate such effects.

Tooth wear

Erupted tusks wear quickly on the outer surface (Fig. 9A). This wear presumably occurs when the dugong uses its snout to grub sea-grasses from the bottom. The cutting edge of the tusk is reinforced with enamel as in the order Rodentia (Peyer, 1968). This action forms a feeding trail as described by Anderson and Birtles (1978). However, erupted tusks cannot be essential equipment for feeding as they are absent in almost all females and in young males.

Tooth wear due to the abrasive action of food plants is a problem for most herbivorous mammals, including sirenians. Abrasive materials consumed by dugongs include a considerable amount of silt and sand (Spain and Heinsohn, 1973) and epiphytic siliceous diatoms which infest the leaves of most sea-grasses (Birch, 1975; Murray *et al*, 1977). The amount of abrasive materials in dugong food is reflected in the high ash content of dugong stomach contents, which has been measured at up to 19.9% (Spain and Heinsohn, 1973).

The dugong's cheek teeth have adapted to dietary wear quite differently from those of the manatee. As the manatee's cheek teeth wear, they are replaced horizontally throughout life by an apparently limitless supply of supernumerary molars (Domning and Magor, 1977). In contrast, no new teeth erupt in the dugong after seven to nine GLGs are deposited in the tusk, and tooth wear is countered principally by the continued axial growth of M2 and M3 and the radial growth of M3. The latter allows the total occlusal surface area of the cheek teeth to be maintained and increased even after the anterior cheek teeth are lost.

Implications for conservation

The results of this study have important implications for dugong conservation. They provide strong evidence that the dugong is a long-lived mammal which does not reach sexual maturity for at least nine years, probably later in some areas. This is comparable with a recent estimate of eight years as the age of sexual maturity in the manatee (Odell, 1977).

Definite observations on the gestation period of the dugong are lacking although it is believed to be about a year (Kingdon, 1971). In comparison, the gestation period of the Florida manatee is estimated to be 13 months. (Hartman, 1971). Single births are typical in the dugong, although twin fetuses have been reported (Troughton, 1928; Norris, 1960; Jarman, 1966; Thomas, 1966; Bertram and Bertram, 1968). The cow-calf relationship is believed to be well-developed and long-lasting (Banfield, 1968; MacMillan, 1955; Thomas, 1966) and calves estimated to be up to 1.5 years old have been caught in Townsville shark nets with their presumed mothers. The calving interval there is likely to be two or more years, a similar period to that estimated for the manatee by Hartman (1971).

Dugongs, and probably all sirenians, appear to be long-lived animals with a low reproductive rate, a long generation time and a large investment in each offspring. Incidental exploitation caused by net drownings has a deleterious effect on dugong populations in certain areas, as seen for the Townsville region (Heinsohn, 1977; Heinsohn and Spain, 1974; Heinsohn *et al*, 1977). The threats of direct exploitation such as hunting by natives may also be considerable in some areas. Past exploitation, mainly through hunting and netting by indigenous people, is thought to have reduced dugong populations to their present low levels over most of the species' range (Bertram and Bertram, 1973).

Dugongs are specialized, virtually obligate bottom-feeders (Domning, 1976) which feed principally on tropical and sub-tropical sea-grasses (Heinsohn and Birch, 1972; Lipkin, 1975; Heinsohn *et al*, 1977), although algae may be eaten when sea-grasses are scarce (Spain and Heinsohn, 1973). The inshore habitats, especially sea-grass communities, required by dugongs are particularly vulnerable to human disturbances (Heinsohn *et al*, 1977).

It is important that measures which are adequate to protect dugongs and their specialized habitats be implemented before dugong numbers are further substantially reduced.

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AOSI/8

Tetracycline Marking and the Possible Layering Rate of Bone in an Amazonian Manatee *(Trichechus inunguis)*[1]

Daryl P. Domning
Department of Anatomy, College of Medicine
Howard University, Washington, D.C.
and
Albert C. Myrick, Jr
National Oceanic and Atmospheric Administration, National Marine Fisheries Service
Southwest Fisheries Center, La Jolla, California

ABSTRACT

This paper reports the first successful use of tetracycline marking of layered bone in a manatee and suggests that this technique may be useful in estimating age for species of this family. A six-month-old male Amazonian manatee *(Trichechus inunguis)* was first injected with *Terramycin* on 23 August 1976 (2.2 mg/kg) and subsequently on 18 and 19 November 1977 (7.1 and 8.5 mg/kg, respectively). After the animal died, on 3 May 1978, a transverse thin section of one of its ribs was examined microscopically using plain and polarized transmitted light and ultraviolet reflected light. One fluorescent mark, probably representing the 1977 injections, was found in the periosteal bone between the first and second of three layers containing aligned Haversian systems. It is likely that, for this species, 2.2 mg/kg dosages are insufficient to mark bone, but dosages between 7.1 and 8.5 mg/kg are adequate. An average accumulation rate of one layer per year is suggested by our analysis of this specimen.

INTRODUCTION

In recent years the use of hard-tissue layering systems to determine age has become an important area of research in biological studies on sirenians. For dugongs the problem is simplified by the presence in these animals of tusk-like incisors that contain well-defined dentinal growth layer groups (Scheffer, 1970; Mitchell, 1976, 1978; Kasuya and Nishiwaki, 1978; Marsh, this volume). The manatees possess no incisors; their molariform teeth are rapidly and continuously replaced and growth layers do not accumulate (Odell, 1977). Age estimates for manatees have been based on body lengths (loc. cit.).

Since the histological study by Fawcett (1942) it has been known that certain bones of the manatee contain growth layers. His descriptive comments foreshadow the use of bone growth layers to determine age in manatees:

> The arrangement of periosteal bone [in the ribs of manatees], distinguished by slight differences of internal architecture, suggest that the osteogenic activity of the periosteum is periodic or seasonal. . . the most recently deposited bone is found. . .[on the outer curvature of the rib]. . . while older and older layers are encountered toward the inner edge. (p. 277)

Odell (1977) was unable to define growth lines in prepared histological sections of the manatee ribs that he examined. No other attempts at age estimation of manatees using growth layers have been reported.

Layering rates in hard tissues of sirenians are not known due to a lack of animals of known age (Marsh, this volume; Odell, 1977). Marsh (this volume) reports that

> There have been no successful field studies of specific individuals [of dugongs] over any time span. . . [and although they] have rarely been maintained in captivity [they] have never been bred in captivity.

Several manatees have been born in captivity (Odell, 1977). Apparently, none of these has been examined for growth layers.

Layering rates in marine mammals may be studied by use of *in vivo* administrations of tetracycline antibiotics which are incorporated into the hard tissue shortly after their introduction (Yagi, Nishiwaki and Nakajima, 1963; Best, 1976; Gurevich, Stewart and Cornell, this volume; Myrick, this volume). If given in dosages of sufficient strength, tetracycline labels the layer which is forming at the time the drug is introduced. The label may later be identified as a fluorescent band in the tissue when thin sections are viewed microscopically with reflected ultraviolet light.

The *average* layering rate may be calculated by dividing the number of layers that have accumulated since the marking event by the time elapsed between marking and death of the animal. By assuming that the layering rate is constant, one may estimate the animal's age by counting all layers and multiplying that value by the depositional rate.

This paper reports the first successful tetracycline-marking of skeletal tissues in an Amazonian manatee, indicates the adequate dosage required to produce a detectable mark in the tissues, and suggests the layering rate of periosteal bone. This technique should be of value in future studies of age and growth of sirenians.

MATERIALS AND METHODS

A male *Trichechus inunguis* calf, probably less than six months old and in severely malnourished condition (total curved-line length 93 cm, weight 9.1 kg), was given a single intramuscular injection of 20 mg of *Terramycin* (a tetra-

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cycline antibiotic) (dosage 2.2 mg/kg) on 23 August 1976. It was maintained thereafter on a diet of milk formula and aquatic plants. By 16 November 1977 it had reached a curved-line length of 125 cm and a weight of 35.2 kg. Single intramuscular injections of 250 and 300 mg of *Terramycin* were given on 18 and 19 November, respectively (dosages 7.1 and 8.5 mg/kg). The animal died on 3 May 1978, at a length of about 128 cm and still weighing about 35 kg; the lack of further growth was attributed to termination of the milk diet. The frozen carcass of the animal used in this study is preserved at the Instituto Nacional de Pesquisas da Amazônia (INPA), Departamento Peixe-Boi, Manaus, Brazil (catalog number INPA-PB 11).

A left rib from the middle thorax (chord length 15.5 cm) was cleaned by maceration and cut transversely at the midpoint of its length, and a thin section (anteroposterior diameter 15 mm, mediolateral diameter 10 mm, thickness ≅ 75 μm) was cut with a diamond saw (Fig. 1). The section was mounted in *Permount* and viewed at 50 to 200 X in reflected ultraviolet light, using a *Zeiss* fluorescent vertical illuminator with a filter reflector No. 48-77-05 combination and in plain and polarized transmitted light. Plain and polarized transmitted-light examinations of several thin sections taken from ribs and mandibles of adult and young adult manatees from Florida were made for histological comparison.

RESULTS

Histology

The histological arrangement of the bone in the thin section examined by one of us (Myrick) corresponds to that described by Fawcett (1942) for thin sections of manatee ribs. The section consists of a stratified series of concentric zones of densified bone that arch outwards from the pleural edge. According to Fawcett (1942) these zones result from different proportions and arrangements of primary (fetal) trabeculae that have become filled gradually by secondary bone, forming a dense central core. They are invested by abundant nutritive channels, but contain progressively fewer Haversian systems toward the outer curvature.

Toward the outer curvature of the section the original fetal trabecular architecture of primary spongiosum is easily recognizable (see Figs. 1 and 2); the inter-trabecular spaces are large and the walls have not been thickened appreciably by deposition of secondary bone. The more proximal trabeculae are thickened, with spaces that are

Fig. 1. Composite photograph of transverse thin section of rib from 2.2 year old male *Trichechus inunguis*, viewed in low-contrast crossed polarized light (x12). Abbreviations: N = probable location of neonatal line separating periosteal bone from spongiosum. T = approximate level of periosteal tissue in which fluorescent mark from *Terramycin* injection may be seen under ultraviolet light.

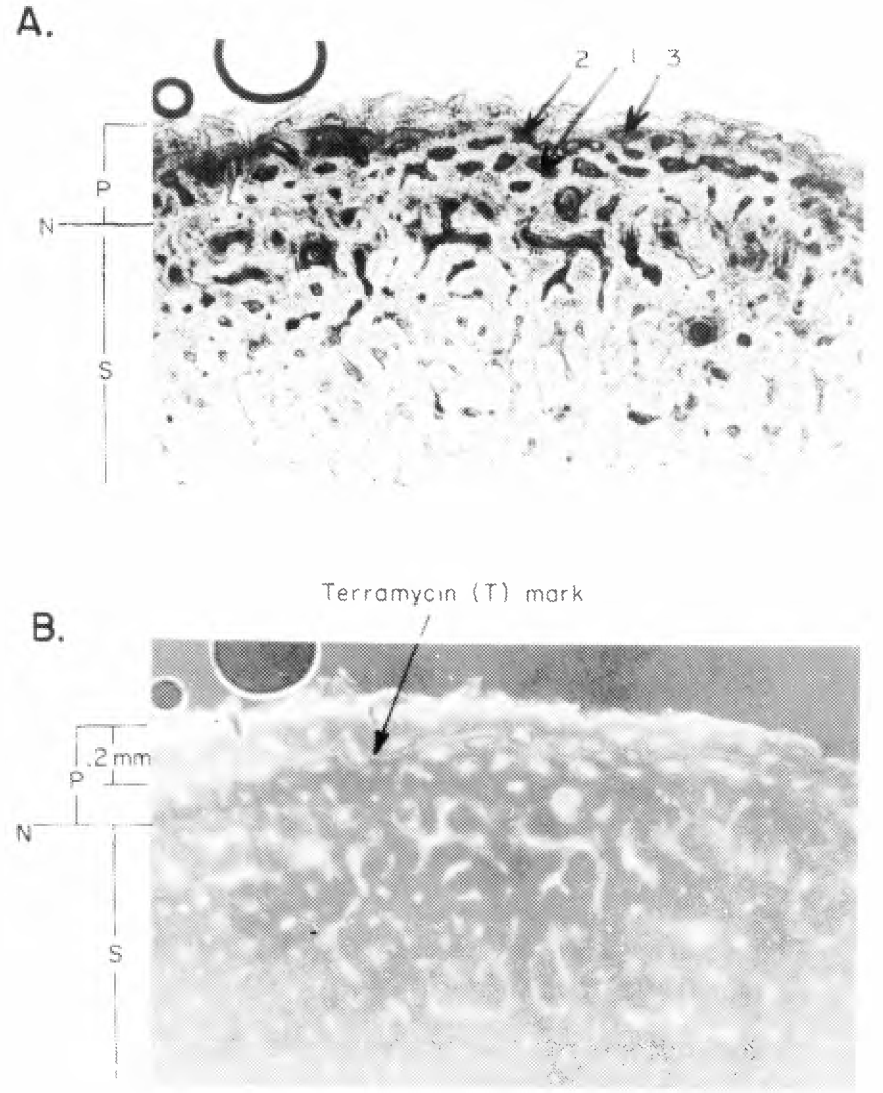

Fig. 2. Transverse thin section of rib as shown in Fig. 1, showing histology near the external margin (×50).

A. Low-contrast polarized light photograph showing three layers (numbered) of aligned Haversian systems in periosteal bone (P). N indicates neonatal line separating periosteal bone from spongiosum (S).

B. Section as shown in A using reflected ultraviolet light. *Terramycin* (T) is represented by fluorescent band (arrow). N indicates well-defined dark region representing neonatal line.

almost entirely filled. Apparently, the deposition of secondary bone commences in the pleural region and progresses outwardly toward the external surface (Fawcett, 1942).

The boundary between the periosteal bone and the fetal core of primary spongiosum is recognized by a sharp change in orientation and shape of the trabeculae. The trabeculae of the core tend to be columnar and radiate outwards from the pleural region in an irregular pattern. The neonatal boundary, i.e. where deposition of periosteal bone begins, truncates the fetal trabeculae with a thickened, continuous envelope of bone containing abundant, flattened osteocytes whose long axes lie parallel to the external curvature of the rib.

External to the neonatal boundary, the bone contains two, possibly three layers of Haversian systems that tend to be open and flattened somewhat, with long axes that are arranged end-to-end, parallel to the external curvature. The bone surrounding the Haversian systems forms a series of more or less continuous walls that accentuate the stratified pattern, but no cemental (adhesion) lines, as described in Fawcett's study, were found in the periosteal bone in the section.

Structures marked by tetracycline

Ultraviolet light produced fluorescence as follows (see Figs. 2 and 3).

(1) Fluorescent rings in the walls of open trabeculae internal to the periosteal (i.e. neonatal) boundary.

(2) Fluorescent rings in the walls and fluorescence of the canaliculi of the Haversian systems comprising the first and second layers, but not the third layer (Figs. 3A, B).

(3) A single, continuous, somewhat irregular fluorescent band situated between the first and second layers of Haversian systems, most closely associated with the second layer, approximately 0.2 mm internal to and parallel with the outer curvature of the bone (Figs. 2B, 3B).

DISCUSSION

Osteogenic activity indicated by fluorescent labels

(1) ***Deposition of secondary bone*** – As indicated by the fluorescent labels, the peripheral systems immediately internal to the periosteal boundary were active when the bone was labelled. This agrees with the interpretation (Fawcett, 1942) that the inter-trabecular spaces of the fetal spongiosum become filled with secondary bone after the fetal framework is formed.

(2) ***Activity and deposition of periosteal bone*** – Fluorescence in the walls and canaliculi of the Haversian systems in the first and second layers indicate that osteoblastic systems in both layers were active at the time tetracycline was introduced. Intimate association of the fluorescent band with the Haversian systems of the second layer suggests that this layer was forming at the time the bone was being labelled. The absence of fluorescence in Haversian systems comprising the third, outermost layer shows that this layer was formed after the tetracycline was incorporated into the system.

(3) ***Adequate dosage required to label bone*** – Three *in vivo* injections of *Terramycin* were given; one (2.2 mg/kg) on 23 August 1976 and the others (7.1 and 8.5 mg/kg) on 18 and 19 November 1977, respectively. Because only one fluorescent band was visible in the section and the 1977 dosages were stronger, we interpret the 18–19 November administration dates to be represented by the band. Apparently, the injection given in 1976 was of inadequate strength to produce a visible mark. We conclude that a dosage of between 7.1 and 8.5 mg/kg is sufficient to label the bone.

(4) ***Possible layering rate in periosteal bone*** – If, as we presume, the animal was (at most) six months old when the first injection was given (August 1976), it would have been about 2.2 years old when it died in May 1978. The total thickness of periosteal bone, containing two and part of a third layers of Haversian systems, would then represent 2.2 years of accumulation. Division of layers by that elapsed time gives an *average* layering rate of approximately one layer per year for the specimen (see Figs. 3B, 3C). However, the animal was young and in poor health and the layers in its rib used in the calculation of layering rate are not separated by adhesion lines observed in peripheral periosteal layers in ribs of adults. No ontogenetic series of rib sections of young and mature animals was available for detailed histological comparison of the early layers. This precludes a confident blanket statement regarding layering rates in periosteal bone of manatees in general.

(5) ***Periosteal layers in other specimens***
Ribs – Haversian systems were not discernible in large numbers in thin sections of periosteal bone from other

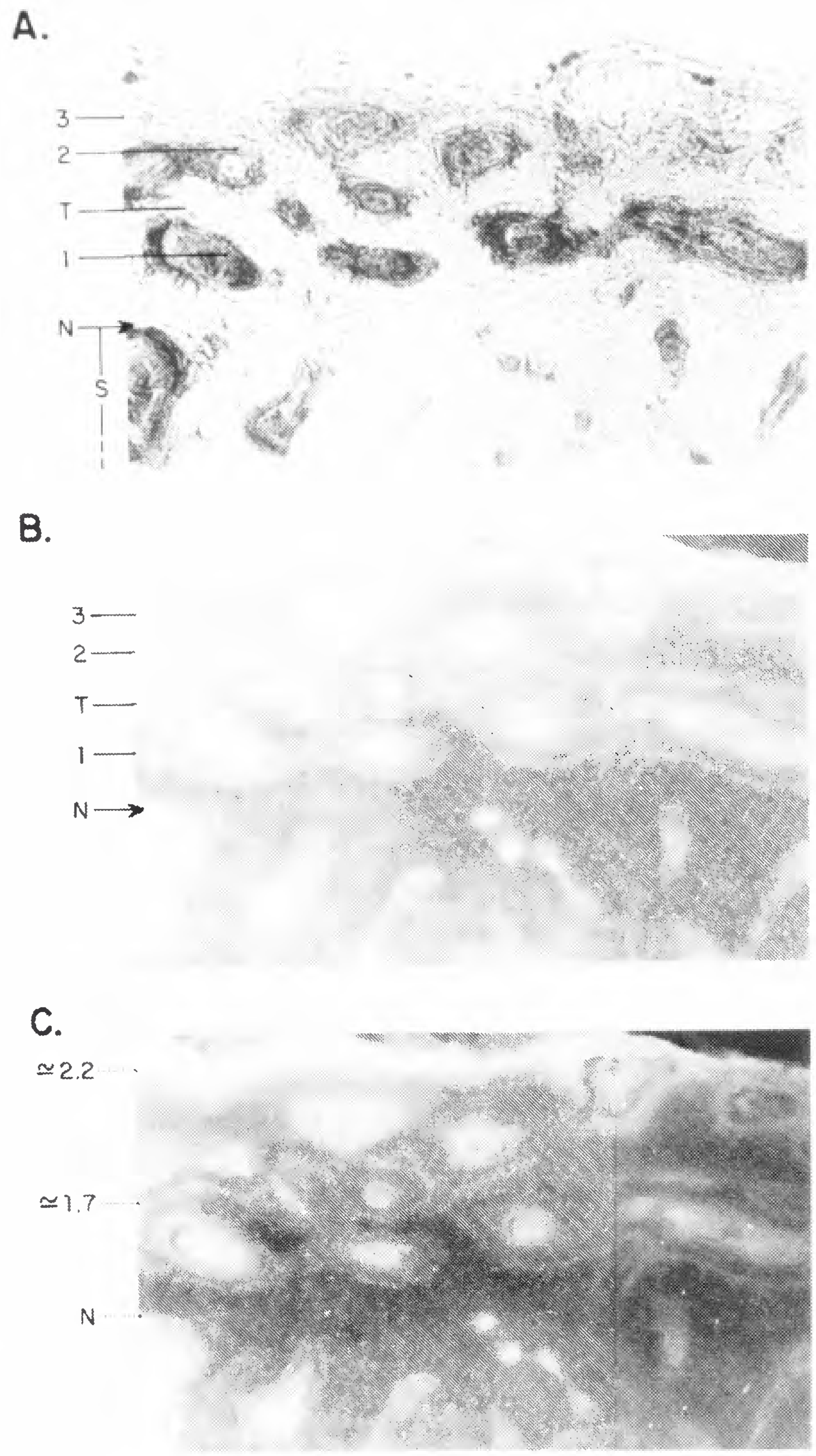

Fig. 3.A. Highly magnified (x200) view in low-contrast polarized light of section shown in Fig. 2. Two and part of a third layer(s) of aligned Haversian systems are shown (numbered). B. Section as in A viewed with reflected ultraviolet light showing fluorescent labels ringing Haversian systems in layers one and two but not in those of the third. Brightness at external margin (top) is autofluorescence which is not of same quality or colour as that produced by the *Terramycin.* (See Fig. 2 for abbreviations.)
C. Section as in B showing time brackets applied to layers used to obtain estimate of layering rates. N indicates time of birth. ≅1.7 indicates accumulation after 1.7 years when the second and third injections of *Terramycin* were administered. ≅2.2 indicates approximate age in years when animal died.

specimens *(T. manatus)* examined by one of us (Myrick). In these specimens the layers contained only flattened osteocytes aligned parallel to the bones' external curvatures. Nevertheless, the boundary (earliest) layer in each section truncated the columnar arrangement of the spongiosum as was observed in the section of marked manatee rib. An ontogenetic series of ribs from neonates through young adults (not available to us) might help in determining whether or not the presence of Haversian systems in the boundary layers is characteristic of the formation of early periosteal bone in manatees. It would also be useful in defining the absorption rate (if any) of periosteal layers in adult specimens.

Mandibles – Although layers were fairly well definable in some of the rib sections examined, they do not always occur with equal clarity or in adjacent areas of the same rib (see Fawcett, 1942; Marsh, this volume). On the other hand, transverse thin sections taken from the posterior regions of mandibular rami of two Florida manatees were found (by Myrick) to show layering very clearly and continuously. These layers appear to be more easily distinguishable than the periosteal layers in ribs. Odell (1977) suggested that investigation of the layers in flipper bones of the manatee might prove useful in making age estimates, but we now believe that periosteal layering in the mandible will give superior results (see also Mitchell, 1978).

SUMMARY

The study suggests the following:

(1) Tetracycline antibiotics may be used safely to label periosteal bone of manatees.

(2) The dosage sufficient to produce a detectable mark under ultraviolet light is between 7.1 and 8.5 mg/kg of body weight.

(3) Periosteal bone may be deposited at a rate of one layer per year in manatees, but data are inconclusive.

(4) In the rib of the manatee, the boundary (i.e. neonatal line) between the fetal spongiosum and the periosteal (postnatal) compactum is demarcated by a truncation of the columnar trabeculae by tissues arranged parallel to the external curvature of the bone.

We make the following recommendations:

(1) Age determination studies of manatees should include investigations of the layering patterns of periosteal bone in the posterior region of the mandible which seems to be superior to ribs in discernibility of layers.

(2) When possible, future studies should include construction of an ontogenetic series of bone samples ordered by carcass body length and separated by sex. Samples would provide information on relative age, ontogenetic development of layers, and deposition and resorption patterns.

(3) When feasible, live animals should be sexed, measured, tagged, and injected with tetracycline in anticipation of future recovery of their carcasses.

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The Constitutional Basis for Longevity in the Cetacea: Do the Whales and the Terrestrial Mammals Obey the Same Laws?*

George A. Sacher
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

ABSTRACT

The maximum lifespans in captivity for homeothermic mammalian species can be estimated by means of a multiple linear regression of logarithm of lifespan *(L)* on the logarithm of adult brain weight *(E)* and body weight *(S)*. This paper describes the application of regression formulas based on data from terrestrial mammals to the estimation of odontocete lifespans. The regression formulas predict lifespans that are in accord with the data on maximum odontocete lifespans obtained in recent years by objective age determination procedures. More remarkable is the correct prediction by the regression formulas that the odontocete species have nearly constant lifespans, almost independent of body weight over a 300:1 body weight range. This prediction is a consequence of the fact, remarkable in itself, that over this range of body weights, the odontocete brain weight is proportional to body weight to the 1/3 power, as compared to a 2/3 power relation for the Mammalia as a whole.

INTRODUCTION

The biology of longevity is a two-sided problem. On the one hand, there is the problem of determining the ages of animals in the wild, and of using such data to construct valid life tables and reproductive histories. This problem is being studied actively in the odontocete whales, as evidenced by the papers in this volume, and also in a number of other vertebrate species.

The other side of the biology of longevity is concerned with identifying the evolutionary, ecological, and molecular-cellular factors that contribute to the determination of animal lifespans and life tables. This too is an active field, especially in those aspects that are of interest in theoretical population biology. However, it has not received the attention that it merits in regard to the relationship of species longevity to constitutional factors such as body weight and length, organ weights, energy metabolism, and reproductive factors.

I shall briefly review the conclusions about the constitutional basis of longevity that have been educed from the study of longevity in the terrestrial mammals, and apply these to the estimation of the lifespans of cetacean species. The discussion will show that despite the great disparity in their environmental adaptations and body dimensions, the whales and the terrestrial mammals obey the same quantitative relation of longevity to brain size and body size.

CONSTITUTIONAL CORRELATES OF LIFESPAN IN TERRESTRIAL MAMMALS

The investigation of the relation of mammalian lifespan to constitutional characteristics goes back to the work of Rubner (1908) on the relation of lifespan to body weight and metabolic rate, and to the work of Friedenthal (1910) on the relation of lifespan to brain weight. I took up this problem in the 1950s, when it became evident that quantitative data about these relationships are essential for a correct understanding of the evolution of longevity in the vertebrates. In my previous work on lifespan in mammals, the longevity statistic used was the maximum lifespan recorded for the species under conditions of captivity. In view of the unique characteristics of data concerning longevity in zoos (Sacher, 1980), the maximum lifespan is a better estimate of longevity than is the life expectation, which is the average survival time in a defined sample, measured from a specified age. This is because the life expectation is more influenced by environmental factors than is the maximum lifespan, and because valid life expectations cannot be calculated from the heterogeneous and incompletely reported longevity data gathered from zoos throughout the world.

The first analysis of longevity in mammals was based on a sample of 67 species for which lifespan, brain weight, and body weight were tabulated (Sacher, 1959). A subsequent study used a series of 239 species for which these three variables were measured. A few of the pertinent results from this latter sample are discussed here (for more details see Sacher, 1976, 1978a). The relations of lifespan, *L*, to brain weight, *E*, and body weight, *S*, are allometric in that the pairwise relations of the logarithms of these variables are linear. Lifespan is correlated to brain weight and body weight, but these two anatomical variables are also correlated. Hence, it was necessary to calculate the multivariate regression of logarithm of lifespan on the logarithms of brain weight and body weight. The least-squares regression relation, from the sample of 239 species, was found to be

$$\log L = (0.519 \pm 0.036) \log E - (0.173 \pm 0.025) \log S + 0.982 \pm 0.044 \qquad (1)$$

The partial regression coefficients for brain weight, *E*, and body weight, *S*, are both highly significant, indicating that each of these variables influences lifespan independent of the other. The standard error of estimate, i.e. the standard error of the discrepancy between an observed value and the predicted value, is 0.138 in logarithmic units, corresponding to a percentage error of ± 37%. The 239 species in this analysis were drawn from 12 orders including 12 whale species, but excluding taxa, such as the bats, that utilize daily torpor or annual hibernation.

Several major taxa, including the orders Rodentia and Artiodactyla, and the suborder Anthropoidea, had a sufficiently large number of representatives in the sample so that the multivariate regressions could be calculated for

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these taxa individually. The relation for the Anthropoidea is particularly interesting here, because this group is comparable to the cetaceans in degree of cephalization and in lifespan. The regression for the Anthropoidea was found to be

$$\log L = (0.648 \pm 0.112) \log E - (0.202 \pm 0.079) \log S + 0.871 \pm 0.114 \quad (2)$$

This was based on a sample of 43 species, and the standard error of estimate was 0.111 in logarithmic units, corresponding to a percentage error of ± 29%. The relations for the orders Rodentia and Artiodactyla were not significantly different from the relation found for the Anthropoidea (Sacher, 1975).

Another way of examining the dependence of lifespan on brain and body weight for the mammals is to average the logarithms of these variables for each of the 12 orders and then to do a regression based on the averages for orders. This yielded the between-orders regression relation

$$\log L = (0.655 \pm 0.079) \log E - (0.244 \pm 0.052) \log S + 1.047 \pm 0.091 \quad (3)$$

This equation, based on a sample of 12 orders, including the Cetacea but excluding the Chiroptera, has a standard error of estimate of 0.060 in logarithmic units, corresponding to a percentage error of ± 15%, and does not differ significantly from the relation for the Anthropoidea (Eq. 2) (Sacher, unpublished).

These regression relations indicate that there is a single relation of longevity to brain and body weight between and within orders of the class Mammalia. However, the negative coefficients of partial regression of lifespan on body weight in Equations 1 to 3 were somewhat puzzling, for the simple correlation of $\log L$ with $\log S$ is positive, and the high negative correlation between body weight and metabolic rate would lead one to expect a positive dependence of lifespan on body weight (Rubner, 1908). To examine this question further I examined another sample of 85 homeothermic species for which, in addition to brain and body weight, there were also data on the resting specific metabolic rate, M, in calories per gram, and for the body temperature, T_b. The regression of lifespan on these four variables was found to be (Sacher, 1978a)

$$\log L = 0.62 \log E - 0.41 \log S - 0.52 \log M + 0.026\, T_b + 0.90 \quad (4)$$

Each of the four partial regression coefficients has a t value greater than 3.0, indicating that lifespan has a significant coefficient of partial regression on each variable, independent of the others. The partial regression on log body weight, S, in Equation 4 is more negative than in the previous three equations, but the negative dependence of $\log L$ on $\log M$ is in accord with the expectation from Rubner's theory. This analysis shows that the negative dependence of lifespan on body weight in Equations 1 to 4 arises from a contribution of body weight to longevity that is independent of the relation of body weight to metabolic rate. This body weight term is presumably due to a cephalization factor, i.e. to the relation of body weight to brain weight. These matters have been discussed elsewhere (Sacher, 1978a).

RESULTS

Table 1 gives data on adult brain weights and body weights of a number of odontocete and mysticete whales. Also given in Table 1 are the observed maximum lifespans, obtained by objective age determination procedures, such as counts of dentine or cementum growth layers in the odontocetes and ear plug layer counts in the mysticetes. The two right-hand columns of Table 1 give maximum lifespan estimates obtained from the regression of log lifespan on log brain weight and log body weight for the Mammalia. Two estimates are given, using Equations 1 and 3. Figure 1 is a plot of brain weight against body weight for 12 odontocete species, from Table 1, on logarithmic scales.

Data on metabolic rates and body temperatures of several cetacean species are reviewed by Lockyer (1976a, b), but such data are available only for *Physeter* and *Tursiops* among the odontocetes, and the additional information on metabolic rate and body temperature does not improve the lifespan estimates for these species. This is not an unexpected outcome, and the situation can be expected to improve as better data become available.

DISCUSSION

The two-variable relationship based on 239 mammalian species (Equation 1) yields predicted odontocete lifespans that are in good agreement with the values inferred from tooth layer counts for three of the four species for which comparison is possible. The lifespan estimates for the odontocete species given by Equation 3 are slightly higher than the estimates from Equation 1, whereas the estimates of mysticete lifespans by Equation 3 average about 4 years lower than the estimates in Equation 1. The two regression relations utilize the same data, but weight them in somewhat different ways.

It should be borne in mind that these equations predict the lifespans that would be expected for these species in captivity, if captive conditions were as benign for the whales as they are for most terrestrial species. Therefore we should expect the predicted maximum spans to be somewhat above the maximum lifespan values inferred for the free-ranging animals. This expectation is confirmed for the odontocetes, but not for the mysticetes. Some possible reasons for the low lifespan estimates for mysticetes are listed below.

The relation of lifespan to the constitutional variables for the odontocetes has some remarkable features. The first of these may be seen in Figure 1, in which brain weight is plotted against body weight on a log-log grid. The six data points above 100 kg body weight were fitted by least squares regression with the line

$$\log E = (0.33 \pm 0.04) \log S - 0.52 \pm 0.12 \quad (5)$$

The 1/3 slope does not apply to the odontocete species with body weights below 100 kg. The six points in this range follow a somewhat steeper slope, which is, however, not too well defined because the range of body weights is short relative to the scatter around the line.

The 1/3 slope for the odontocetes is markedly different from the slope of 0.665 ± 0.013 for the Mammalia as a whole, which is based on a sample of 239 species (Sacher, 1976). The least squares allometric relation between brain weight and body weight for all mammals,

$$\log E = 0.665 \log S - 0.923 \quad (6)$$

is also drawn in Figure 1.

Returning to Equation 1, if one sets lifespan, L, equal to some constant value, L^*, and solves for the relation of E to S when lifespan is held constant, one obtains

$$\log E = 0.33 \log S + 1.93 \log L^* - 1.89. \quad (7)$$

Table 1

Brain weights, body weights, and observed and predicted life-spans for cetaceans.
Inferred lifespans are obtained from layer counts. Predicted lifespans are from two-variable regression formulas based on terrestrial mammals, Equations 1 and 3 in text.
The literature references are for the inferred lifespans.

		Brain weight g E	Body weight g $\times 10^{-6}$ S	Reference	Lifespan, years		
						Predicted	
					Inferred	Eq. 1	Eq. 3
		ODONTOCETI					
Berardius bairdii	Baird's beaked whale			1	71		
Delphinapterus leucas	white whale	2350	0.45	2	40	66	75
Delphinus delphis	common dolphin	829	0.085			51	57
Globicephala melaena	long-finned pilot whale	2450	0.85	3		60	66
Globicephala macrorhynchus	short-finned pilot whale			4	56		
Hyperoodon ampullatus	northern bottlenose whale	2780		5	37		
Lagenorhynchus acutus	Atlantic white-sided dolphin	1200	0.120			58	67
Lagenorhynchus obliquidens	Pacific white-sided dolphin	1140	0.090			60	69
Orcinus orca	killer whale	4500	1.86			72	81
Phocoena phocoena	harbour porpoise	515	0.053			43	47
Phocoenoides dalli	Dall's porpoise	834	0.098			50	55
Physeter macrocephalus	sperm whale	7875	30	6	64	60	60
Stenella attenuata	spotted dolphin			7	46		
Stenella attenuata graffmanni	coastal spotted dolphin	780	0.077			50	56
Stenella coeruleoalba	striped dolphin	855	0.055	8	58	56	65
Tursiops truncatus	bottlenose dolphin	1690	0.15			67	79
		MYSTICETI					
Balaena mysticetus	bowhead whale	5900		9	>40		
Balaenoptera borealis	sei whale	4460	50	10	70	41	36
Balaenoptera physalus	fin whale	6820	59	11	>50	49	46
Balaenoptera musculus	blue whale	6250	40	9	>29	50	48
Megaptera novaeangliae	humpback whale	6610	75			47	42

1. Kasuya, 1977.
2. Khuzin, cited by Brodie, 1971.
3. Sergeant, 1962.
4. Kasuya, personal communication.
5. Christensen, 1973.
6. Ohsumi *et al*, 1963.
7. Kasuya, 1976.
8. Kasuya, 1978, and personal communication.
9. Slijper, 1961.
10. Lockyer, 1974.
11. Nishiwaki *et al*, 1958.

In other words, the data for terrestrial mammals contain the implication that all species with brain weights and body weights that lie along a straight line on a log-log plot with slope of 1/3 should have the same lifespan. Therefore, those odontocete species with body weights above 100 kg should be expected to have constant lifespans, independent of body weight. Table 1 shows that odontocete lifespans, as inferred from tooth layer counts, have only a narrow range of variation. Moreover, the regression of log lifespan on log body weight over the 300:1 range of body weights from 100 kg to 30,000 kg does not differ significantly from zero slope.

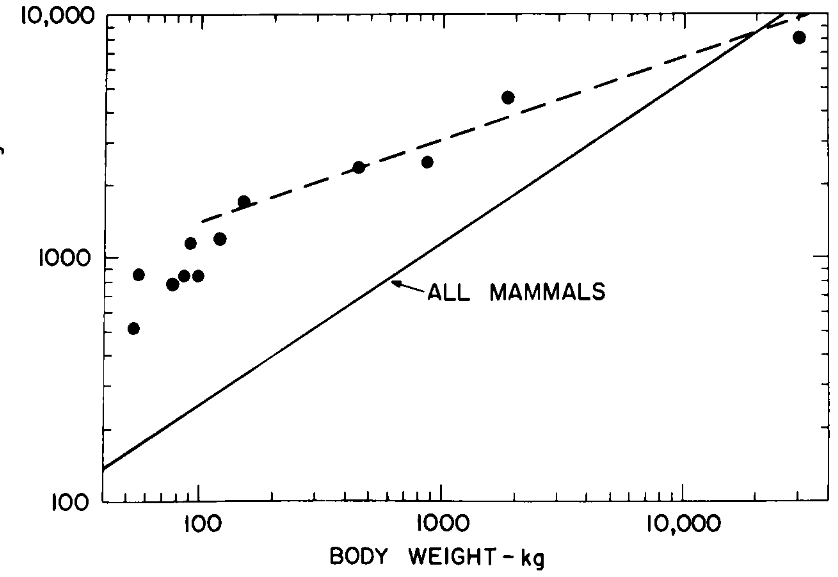

Fig. 1. Relation of brain weight to body weight for the odontocetes. Each point represents a different species. Data are plotted on logarithmic scales. The dashed line has least-squares slope of 0.33 and is based on the data for the species with body weight greater than 100 kg. The solid line is the allometric brain:body relation for all mammals, Equation 6 in the text.

The implication in Equation 7 that lifespan is constant along a 1/3 power brain:body trend line was established by data mostly from land mammals, yet there is no terrestrial taxon that exemplifies this relation as clearly as do the odontocetes. The suborder Pinnipedia (seals), the family Ursidae (bears), and the subfamily Mustelinae (weasels) are the terrestrial groups that most clearly display approximately 1/3 power brain:body allometry (Sacher, unpublished). Each of these groups is characterized by a narrow range of lifespan, but the body weight ranges are not great enough for a statistical test of the hypothesis that lifespan is independent of body weight. Seal longevities over a 50-fold body-weight range lie within the limits of 20 to 36 years for free-living populations, relatively independent of body weight. The lifespans of bears, with body weights of 50 kg to 300 kg, lie within the limits of 24 to 36 years, and members of the weasel family, from the least weasel to the mink, have lifespans of 6 to 10 years (Sacher and Jones, 1972, and unpublished). These examples are given for illustration and not as additional corroboration, since they were part of the data that yielded the regression relations in Equations 1, 3, and 4 above.

The next question is 'Why do the large odontocete species have a one-third power relation of brain weight to body weight and almost constant lifespans?' The definitive answer must wait on more extensive research, but the fact that this near-constancy was predicted by an allometric relation based on data from many orders of mammals means that the eventual explanation must be valid for the Mammalia as a whole, and not merely for the Odontoceti.

Some of the difficulties and uncertainties of the regression estimates of cetacean lifespans need to be considered. One question is how to take account of the differences in body composition between most terrestrial mammals and the larger cetaceans. One solution to this problem may be to use body length instead of, or in addition to, body weight. Body length is a better size variable than is body weight for some purposes, and there is the practical advantage that extensive data on body length are available for cetacean species but few good body weight determinations. However, body length and body weight also represent two independent size factors, and both may be necessary in the ultimate analysis.

Further evidence is needed on the question of whether the metabolic rates of the odontocetes and the mysticetes, respectively, are systematically higher or lower than those of the typical terrestrial mammals. The discrepancies between observed and estimated lifespans for the mysticetes, in which the predicted values are lower than those calculated from ear plug layer counts, may arise from any of several influences, including: the large blubber mass of the mysticetes; error in the ear plug layer counts, or in the formula relating number of layers to age; and the possibility that mysticete metabolic rates are lower than the expectations based on the Brody-Kleiber relation of metabolic rate to body weight for the terrestrial mammals (Kleiber, 1947). These factors need to be evaluated before it can be concluded that the mysticetes have a different relation of longevity to brain weight and body weight than do the odontocetes and the terrestrial mammals.

There are no useful longevity records for cetaceans in captivity, so it will be necessary to develop a new basis for cetacean longevity analysis. The choices are:

(a) Maximum age in harvested samples, determined by age-determination procedures such as tooth layer counts, or by marking and recapture;

(b) Mean annual natural mortality rate or its reciprocal, mean survival time.

Method (a) was used here because it is most compatible with the maximum longevity datum used for the analysis of data from captive mammals. However, maximum lifespan is not the preferred statistic for application to data from wild populations, because the mortality processes of free-living cetacean (and terrestrial mammal) populations are dominated by the age-independent mortality arising from environmental causes, and in these circumstances the oldest age in the sample is not always a good estimate of the effective upper longevity limit for the species that is set by its underlying, but unexpressed, Gompertzian mortality process (Sacher, 1959). Nevertheless, the maximum age does give information about the attainable species longevity, especially if, as in the present instance, the samples for all species are reasonably large and comparable in size, on the order of hundreds of animals.

In the long run, the phylogenetic analysis of mammalian longevity must be re-examined using the annual mortality rate or a comparable measure of the age-independent mortality term, not because it is a better statistic, but rather because it is a different statistic. It measures the ecologically imposed limits on survival, whereas maximum lifespan, when properly determined, estimates the intrinsic limits on longevity set by the senescence process. These two mortality terms are functionally related across species as a consequence of selection for fitness (Sacher, 1978b), so a major agenda item for the ecological gerontology of the future will be to work out the nature of that relationship.

Considerations such as these may contribute to our understanding of the remarkable uniformity of species lifespan over the great range of species body size in the toothed whales, and thereby lead to the identification of an important constraint on the evolution of constitution and longevity in the mammals.

CONCLUSION

Two inferences have emerged from this analysis of the constitutional basis of longevity in the Cetacea. First, the relation of lifespan to brain weight and body weight educed from data on terrestrial mammals is found to be in good agreement with the odontocete data. This supports the hypothesis that there is a single relation among these three variables for all homeothermic mammals.

The second finding is that the brain:body allometric relation for the odontocetes has a low slope of approximately 1/3 and that this relation, when inserted into the longevity regression relation for the terrestrial mammals, makes the correct prediction that odontocetes have nearly constant lifespans, independent of body weight.

The near-constancy of the maximum lifespans of odontocetes, coupled with the 1/3 slope of their brain:body allometry, are findings that need to be examined more thoroughly, for these relationships imply that there is an ecological or physiological constraint on the attainable longevity of the Odontoceti, and of the mammals in general.

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Readability of Growth Layers in Teeth of Beaked Whales, Ziphidae

E. D. Mitchell
Arctic Biological Station, Montreal, Canada
and
J. G. Mead
Smithsonian Institution, Washington, D.C.
and
V. M. Kozicki
Arctic Biological Station, Montreal, Canada

ABSTRACT

We examined teeth of more than 60 specimens, from museum and other sources, of the following nominal ziphiid species:

Hyperoodon ampullatus (10), *H. Planifrons* (2), *Mesoplodon stejnegeri* (1), *M. bidens* (17), *M. mirus* (1), *M. layardii* (1), *M. europeaus* (5), *M. grayi* (1), *M. densirostris* (1), *Berardius bairdii* (4), *B. arnuxii* (4), *Ziphius cavirostris* (12), *Tasmacetus shepherdi* (1).

Generally, we found that the dentine of *Berardius* spp. and *Mesoplodon* spp. become massive and difficult to interpret. Growth layers are more readily distinguishable in *Z. cavirostris* and *Hyperoodon* spp. Tooth size, external ruggae, possibly cemental layers and other features may afford a better alternative method of aging some ziphiid species than dentinal growth layers.

Narwhal with Lower Jaw Tusk; and Aspects of Hard Tissue Deposition in Narwhals

E. D. Mitchell
Arctic Biological Station, Montreal, Canada
and
J. B. Kemper
Department of Indian and Northern Affairs, Ottawa, Canada

ABSTRACT

During the summer 1978 ice edge hunt by Inuit for narwhals *(M. monoceras)* in Pond Inlet, Baffin Island, Northwest Territories, an adult male, 4.52 m in overall length, was taken with a normal 1.95 m-long, left maxillary tusk and a small, ventro-externally-curved, lower right, dentary tusk, 0.30 m in curved length. Both of these tusks, the upper right vestigial tusk, and some bony tissues from the upper and lower jaws of this male, as well as comparable materials from a normal male, 4.36 m in overall length, taken in the same hunt were examined for growth layers. These samples represent one of the first attempts to determine age of sexually mature male narwhals.

Age Determination Studies on *Physeter macrocephalus*

C. Lockyer
Sea Mammal Research Unit, Cambridge, England

ABSTRACT

A method of age determination for the sperm whale using teeth is described. The first mandibular tooth is collected for this purpose because it is usually least worn at the crown, straightest and easiest to extract. The tooth, after cleaning, is bisected through the apex of the crown and pulp cavity using a diamond saw, etched in 10% formic acid solution for thirty hours, washed in water, and finally dried. The acid-etched dentine and cement both display ridges and grooves corresponding with the dark and pale laminae respectively, observed in the unetched tooth in reflected light. The dentine is found to be most suitable for layer counting. In order to enhance the relief of the ridges and grooves, soft pencil is rubbed over the etched surface. An adjacent ridge and groove are considered as a basic unit growth layer, although not infrequently ridges appear double in structure, have accessory ridges, and spacing of growth layers is occasionally irregular, complicating age determination. The evidence for an annual or biannual formation rate of growth layers is reviewed and discussed. Most evidence is based on the type of lamina forming on the pulp cavity edge: whether a ridge or a groove occurs last. Results for teeth examined from the South African, Antarctic and Australian waters are compared. For females, the formation pattern is similar for both South African and Australian stocks (there are no females from the Antarctic), and indicates an annual growth layer formation rate. Results for males from the Antarctic appear six months out of phase with these females, and results for males from South African and Australian waters also appear different from males from the Antarctic, and from each other. Because evidence concerning tagged sperm whales of known minimum age indicates that an annual growth layer formation rate is more likely than a biannual one, it is concluded that on the basis of dentinal layer studies, less than two growth layers and most probably one growth layer forms annually. However, there are probably differences in the timing of lamina formation both between sexes and stocks.

Fitting Growth Curves to Odontocete Tooth Layer/Length Data

J. R. Zweifel and W. F. Perrin
Southwest Fisheries Center, La Jolla, California

ABSTRACT

Attempts in the past to fit single continuous curves to length/age data for odontocetes have not been successful. Length/age scatterplots for sperm whales, ziphiids and delphinids show a discontinuity at puberty. A similar pattern exists in the primates. For lower mammals, Gompertz-type growth curves fit the data well. Application of a two-phase Gompertz model to delphinid data produces better fits than previous models tried, and this approach has promise for analysis of growth of other odontocetes.

Review of the Formation of Incremental Lines in Tooth Cementum of Terrestrial Mammals*

H. Grue-Nielsen
Institute of Comparative Anatomy, Copenhagen, Denmark
and
B. Jensen
Game Biology Station, Rønde, Denmark

ABSTRACT

Stained sections of decalcified teeth have been prepared from about 2,500 specimens representing 32 species of terrestrial mammals –mostly carnivores and ungulates from the palaearctic region.

Intra- and interspecific variation in the character of incremental lines in tooth cementum and in the time of formation of these lines have been studied. Based on this material and on the literature, the influence of different factors on the formation of incremental lines in tooth cementum is discussed.

It seems to be an endogenous rhythm in growth which is the underlying cause, but environmental factors, especially those which affect condition of the animals, may have an influence on line formation.

It is concluded that only planned physiological experiments under well defined conditions can contribute substantially to a further understanding of the mechanisms regulating the formation of incremental lines in tooth cementum as well as in dentine and in bones. The knowledge of the formation of the first incremental line and of the time of year incremental lines are formed are important factors for correct age determinations.

*Submitted for publication in *Danish Review of Game Biology,* 1979.

Reliability of Using Dentinal Layers for Age Determination in *Tursiops truncatus**

C. A. Hui
Naval Ocean Systems Center, San Diego, California

ABSTRACT

Several aspects of dentinal cycles in *Tursiops truncatus* were examined.

Longitudinal sections 0.381 mm thick were etched in 1% to 2% formic acid. Teeth from animals 3.4 and 3.3 years of age show more than three but less than four complete dentinal layers. Another animal had a tooth extracted 2.5 years after it had deposited nine layers. It showed more than two but less than three additional dentinal layers. Layers of dentin apparently are deposited annually in *T. truncatus.*

Food consumption patterns were compared to dentinal layer patterns in nine animals with data for more than twelve months each. Neither the type of food consumed nor the rate of consumption was cyclic, although the dentin displayed normal cyclic deposition in all nine cases. Therefore, no relationship between food consumption pattern and dentinal layers is indicated.

Lunar cycles throughout the life of one individual were compared to its dentinal cycles. There was no systematic relationship.

Four teeth from each of four regions of the jaw of six animals were compared. There were no consistent differences in the dentinal layer count between the left and right sides nor between layers in the mandible and maxilla. The posterior teeth have more layers. The inter-tooth variability of the number of dentinal layers increases rapidly when there are more than fifteen layers in the posterior teeth.

Methods in addition to dentinal layer examination need to be developed for age determination of odontocetes.

*Complete paper comprised the final report to the Marine Mammal Commission, Report No. MMC-77/09. U.S. Department of Commerce, National Technical Information Service, PB 288444 (1978), 25 pp.

Interspecies Relationships among Some Biological Parameters Related to Natural Mortality Coefficient in the Cetacea*

S. Ohsumi

Far Seas Fisheries Research Laboratory, Shimizu, Japan

ABSTRACT

The natural mortality rate is difficult to estimate accurately, although it is one of the fundamental biological parameters for population studies of living resources. If interspecies relationships exist among biological parameters related to the natural mortality rate, this parameter may be estimated from other biological parameters.

The data which were reported previously on the body length at physical maturity in males (L, meters), the maximum life span (T, years) and the natural mortality coefficient in adult stage (M), were collected and reviewed for 31 cetacean species and subspecies. The interspecies relationships between T and M, between L and M and between L and T were examined and the following relationships were obtained:

$M \cdot T = 4.68 \pm 0.69$ $M = 0.159\,e^{-0.0600\,L}$ $T = 31.3\,e^{0.0547\,L}$

L is more easily estimated accurately than T and M in cetacean species, T and M can be estimated by use of these equations and L of the species. It is estimated that T of cetaceans is 30 years in the smallest species and 130 years in the largest species, and M is 0.15 in the smallest species and 0.035 in the largest species.

The accuracy of age determination for a cetacean species will be checked with the values of M and T estimated for the species.

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