CHAPTER EIGHT

SOME NEW AND POTENTIAL USES OF DENTAL LAYERS IN STUDYING DELPHINID POPULATIONS

Albert C. Myrick, Jr.

INTRODUCTION

Ever since the discovery by Nishiwaki and Yagi (1953) that it is possible to estimate the age of a dolphin by analyzing the layers in a single tooth, scientists have used tooth ages in conjunction with other data to conduct their biological investigations of dolphins (see Scheffer and Myrick 1980). By studying enough tooth samples from animals taken in the field, it is possible to identify important age-related characteristics of whole dolphin populations, such as the age structure of the population, the average age at sexual maturity (determined from reproductive organs and tooth layer counts), and the average reproductive longevity. This information can then be used, along with data derived from pregnancy and mortality rates, to assess the current condition of a given population and predict its future growth rates. Such assessments become particularly valuable for populations jeopardized by direct hunting, by alteration of their habitat, or by inadvertent kill through entanglement in or entrapment by fishing gear.

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. Thousands of dolphins are killed incidentally each year in the eastern tropical Pacific (ETP) when purse seines are set on dolphin schools to capture yellowfin tuna that associate with them (Perrin 1969; Smith 1983; Hammond and Tsai 1983). In 1972, concern over the biological consequences of this considerable annual mortality prompted the U.S. National Marine Fisheries Service to mount a long-term monitoring and research program to study the problem.

In 1978, I began work on the problem of trying to estimate ages from burgeoning samples of teeth collected from the kill by federal technicians aboard selected ETP seiners. To extract age or other biological information from layers in dolphin teeth requires a certain basic understanding of the teeth, such as which tooth to use, when the tooth tissues are formed, how and where layers are accumulated, what factors may affect layer deposition, how they appear when viewed microscopically, where in the layered tissue to begin making a tooth age reading, and what the layers represent in terms of time.

THE UNIQUE TEETH OF DOLPHINS

Teeth of dolphins are different from those in any other mammal group in a number of important respects. First, except for the rodents and lagomorphs (pikas, rabbits, and hares), in which teeth are highly modified for gnawing and grinding, and the edentates and allies (pangolins, some anteaters, sloths, and armadillos), in which teeth are strongly reduced or absent, almost all toothed mammals produce baby or milk teeth and adult or permanent teeth (Scott and Symons 1964; Peyer 1968; Walker 1968). Dolphins produce only a permanent set of teeth: in a dolphin, the teeth form before birth, erupt within a few months after birth, and remain in place throughout its life.

Second, with the exception of the Amazon River dolphin (*Inia geoffrensis*), whose rear teeth are almost molarlike, and a few species in which teeth have undergone retrogression (*Phocoenoides* sp.) or reduction (narwhals and beaked whales), virtually all dolphins have homodont dentition, that is, all teeth of an individual have one shape—usually simple cones or pegs. Homodonty is common in fish, amphibians, and reptiles, but among the mammals, it is limited only to toothed whales and dolphins. Most mammals have heterodont dentition, with teeth differentiated into incisors, canines, premolars, and molars, or some combination of two or more types (Peyer 1968).

Another feature peculiar to dolphin teeth is that they are far more numerous in most delphinid species than in any other mammals with nor-
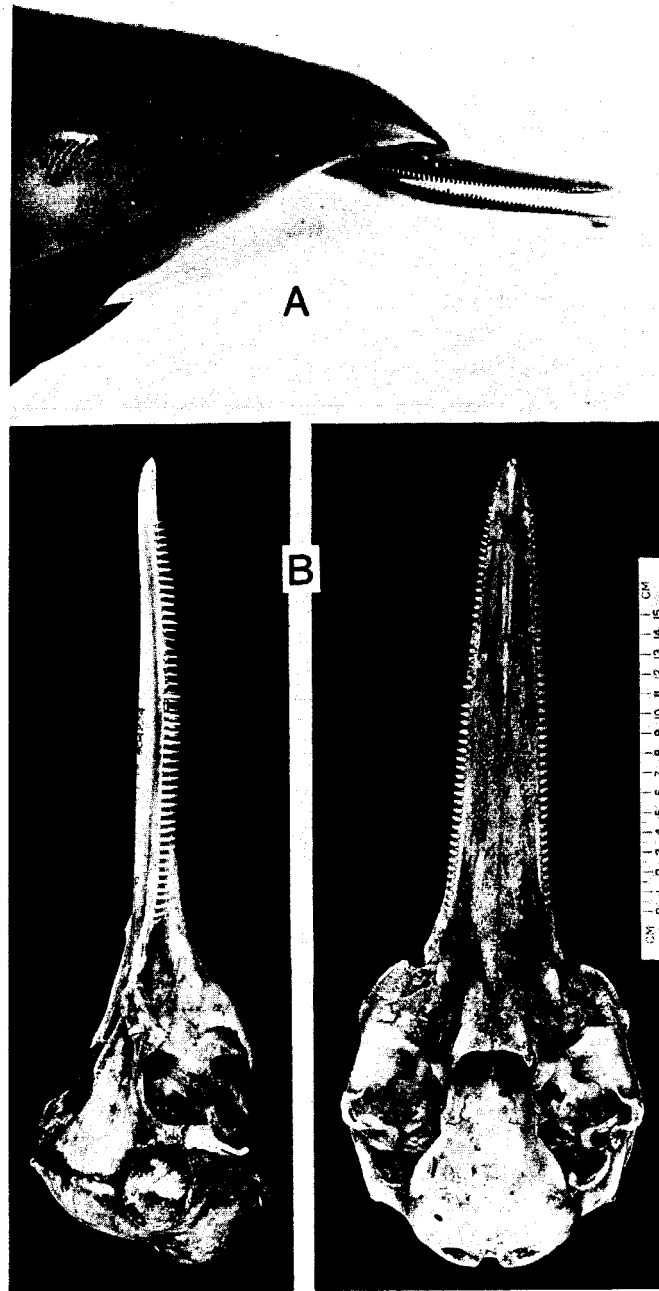
Fig. 8.1. Reiterative homodont teeth in dolphins. A. Head of spinner dolphin (*Stenella longirostris*) showing numerous teeth in upper and lower jaws. B. Right lateral and ventral aspects of a spinner dolphin skull, showing teeth. (Photo from Perrin 1972.)

mal teeth. Humans have 7 or 8 teeth on each side of both upper and lower jaws. Primitive placental mammals have 11 on a side, with a total of 44. But (to take one of the extreme examples) among spinner dolphins (*Stenella longirostris*), individuals may have a total of between 180 and 220 teeth, arranged in rows of at least 45 teeth on each side of each jaw (fig. 8.1). Among other living mammals, only the African dog (*Otocyon megalotis*), with a heterodont series of up to 50 rather weak teeth (Peyer 1968), and the giant armadillo (*Priodontes giganteus*), with up to 100 intermittently shed teeth (Scott and Symons 1964, Walker 1968), show any trend toward this kind of dental reiteration.

SOME DENTAL ANATOMY

A dolphin tooth is a natural recording device, somewhat like a trunk of a tree that registers changes through its own life by the characteristics of its accumulating rings. The tooth differs from trees, however, in having three layering tissues instead of one. Each tissue, enamel, dentine, and cementum, is deposited in incremental layers, apparently with clockworklike regularity (fig. 8.2). In small delphinid species, deposition of enamel, which forms the apical mantle of the tooth, probably commences in the fetus three or four months after conception and is completed shortly before the dolphin's birth (Myrick 1980). The layers of enamel appear to represent daily increments (ibid., Boyde 1980; fig. 8.3).

The body of the tooth is composed of layers of dentine formed by continuous application of the tissue to the inner surface of the hollow dental cone. Dentine deposition begins concurrently with the onset of enamel formation, and thus, like enamel, prenatal dentine represents a record of a large part of the fetal life. However, unlike enamel, which forms prominent daily layers, prenatal dentinal layers show what seems to be a monthly depositional pattern (Myrick 1984*b*, Myrick et al. 1984).

Dentine deposited after birth, that is, postnatal dentine, is more conspicuously layered than prenatal dentine. As in most other mammals, postnatal dentinal deposition in dolphins begins at birth with the formation of a distinctive hypomineralized *neonatal layer* on the inner surface of the prenatal dentine (fig. 8.2). Formation of dentine usually slows down and gradually stops in most mammals but probably not in most dolphins. Postnatal dentinal layers in dolphins continue to accumulate internally until death or until the pulp cavity fills with dentine sufficiently to close off the supply of nutrients to the dentine-producing cells. Unless factors alter a dolphin's calcium or protein physiology sufficiently to interfere with the layering process, dentinal layers, from the neonatal layer to the pulp cavity margin, represent the dolphin's complete postnatal depo-
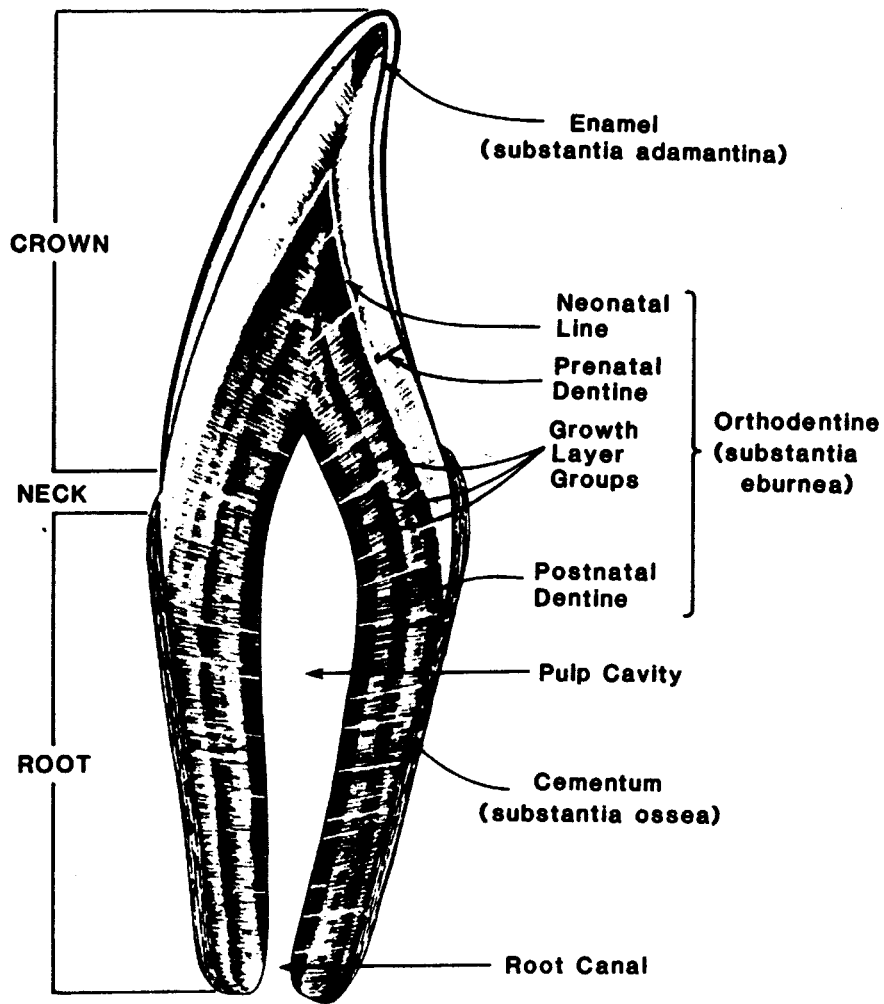
CROWN

NECK

ROOT

Enamel
(substantia adamantina)

Neonatal
Line

Prenatal
Dentine

Growth
Layer
Groups

Postnatal
Dentine

Orthodentine
(substantia
eburnea)

Pulp Cavity

Cementum
(substantia ossea)

Root Canal

Fig. 8.2.   Sketch of typical dolphin tooth in thin section showing tissues
and general anatomy (from Perrin and Myrick 1980).

sitional record. Of course, the dentinal record would be truncated if the
dolphin lived beyond the time of pulp-cavity occlusion.

Formation of cemental layers begins shortly after birth. Succeedingly
younger layers of this tissue are applied to the external surface of the
tooth's basal half. Because deposition occurs externally in the relatively
unconfined space of the tooth socket, or alveolus, accumulation of ce-
mental layers is thought to continue until death. This tissue should repre-
sent an uninterrupted layered recording of the entire postnatal life, pre-
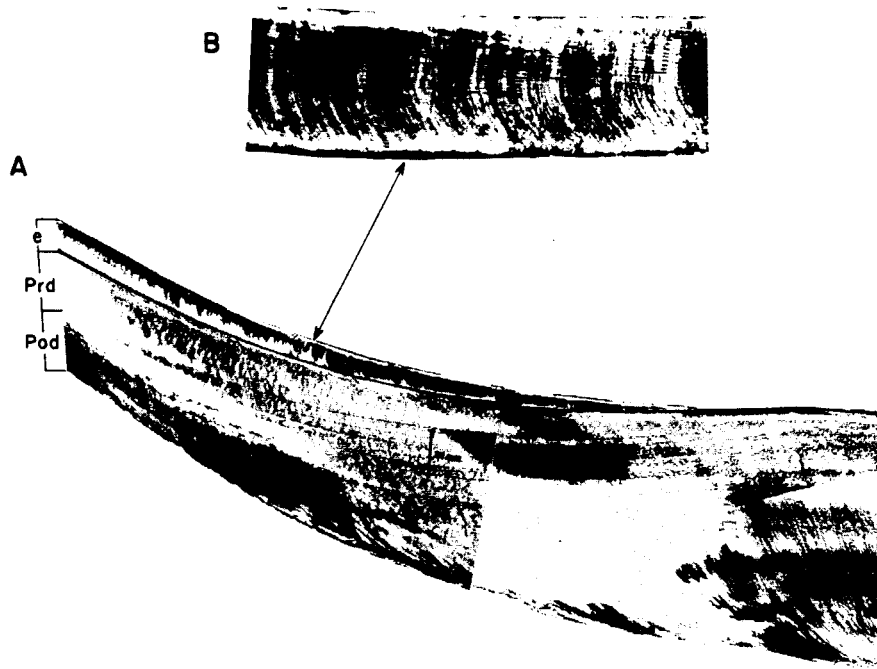suming again that there is no sustained physiological interference.

Fig. 8.3.   Daily (?) layering in enamel in a tooth of a spotted dolphin (*Stenella attenuata*). A. Half of a tooth section viewed with polarized light and showing layering in the enamel (arrow). B. Highly magnified section of enamel as shown in A showing layers as indicated by marks. Because of the oblique (off-lapping) arrangement 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 tooth depicted. Abbreviations: e, enamel; Prd, prenatal dentine; Pod, postnatal dentine. (From Myrick 1980.)

As a corollary of the dolphin's homodont and reiterative dentition, deposition of the layered record in one tooth occurs at the same time, in the same sequence, and with virtually identical patterns as in almost every other tooth in a dolphin's jaws (Myrick et al. 1984). This means that the number and distinctness of dentinal layers identified in one tooth will be the same as those in adjacent teeth. More important, it indicates that all of the teeth are under complete systemic control. The subtle uniqueness of the layering pattern is so well replicated in each tooth of an individual that by comparing the layering patterns of two teeth, even if they have the same number of layers, I have usually been able to determine whether or not they were taken from the same specimen (fig. 8.4).
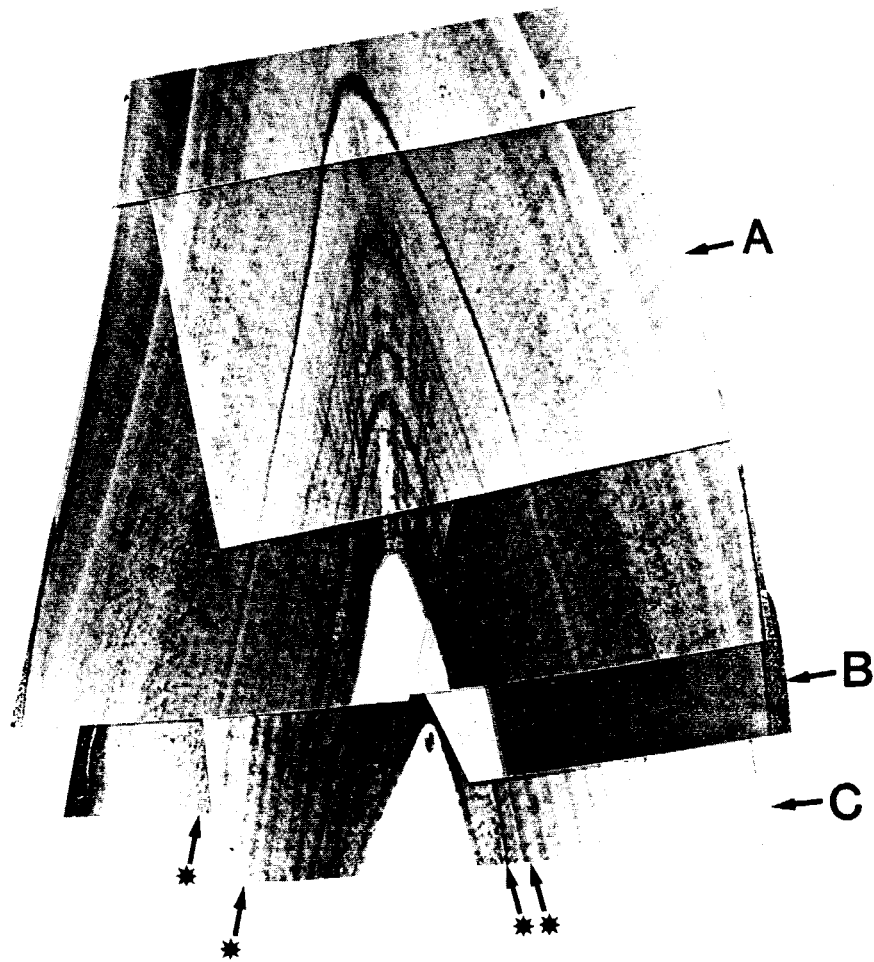
Fig. 8.4. Dentinal layering patterns of three individual spotted dolphins compared. Photographs of teeth from three specimens (A, B, C) are superimposed to show that a pattern common to all exists. B is the youngest, A the next youngest, and C the oldest. The three specimens were collected in different areas of the eastern central Pacific in different years. Although the patterns resemble one another closely, it is not difficult to locate individual differences (probably reflecting physiological differences), some of which have to do with intensity of some of the incremental layers (starred).

CALIBRATION

In early studies of the teeth of wild dolphins, there was no way to know for sure how long it took to lay down each similarly layered group of tissues, more recently termed growth layer groups, or GLGs (Perrin and Myrick 1980). Because of this initial uncertainty, tooth ages of dolphins were often stated in layer or GLG units, not in months or years. Before the full importance of thoroughly calibrating dental layers was realized, however, more than a few studies treated layer units as though layering rates had been measured. The lack of thorough calibration, as well as the subjectivity involved in selecting an "annual" layer or GLG, may account for some of the disparity in age-specific parameters given by different authors for different dolphin populations (Perrin and Reilly 1984).

One method used to calibrate the GLG deposition rate is to study the teeth of captive-born dolphins, because the ages are already known exactly. A count of GLGs in a single tooth from a dolphin of known age helps to verify the elapsed postnatal time they represent. Another method in use compares GLGs in teeth pulled from an animal in different seasons or years. A third approach is to mark the teeth of captive dolphins with tetracycline. Later, after the animals eventually die or when a tooth is removed from each live animal, elapsed time is compared with the number of GLGs formed after the tetracycline mark to estimate deposition rate.

A tooth from a captive-born dolphin of known age may show how many GLGs have been deposited since birth, but it gives no clue as to when a given GLG was formed or how long the process took: a deposition rate cannot be firmly established. A series of teeth extracted from an animal over time should provide a number of time periods within which the deposition of specific annual GLGs is defined with greater precision. But many teeth would have to be pulled over extended periods to determine deposition rates—a drawback to subject and experimenter alike.

Tetracycline marking, however, can be carried out repeatedly on a single animal to produce labels that are deliberately introduced weeks, months, or years apart. This permits examination and verification of deposition rates at almost any scale of detail.

Four features make tetracycline the GLG calibration tool of choice: (1) it combines immediately with the calcium incorporated into a newly forming incremental layer of dentine or cementum, (2) unabsorbed amounts of the drug are excreted within a few days after entry into the circulatory system, (3) when combined with the calcium in a layer viewed microscopically in ultraviolet light, tetracycline shows up as a fluorescent label among the layers in a tooth thin section, and (4) because it is an antibiotic, tetracycline causes no ill effects when introduced intra-

muscularly into a dolphin in low or moderate concentrations (30 to 75 mg/kg body weight, scaled to resting metabolic rate).

Of the three methods in use, multiple introductions of tetracycline over several years would seem most useful for determining deposition rates. Of course, the use of all methods together would be superior to any one of the three alone.

In 1979, I began a three-and-a-half-year experiment, assisted by staff members of Sea World, Inc., and Hubbs Marine Research Institute, to monitor the dentinal and cemental deposition rates in dolphin teeth. We used twelve bottlenose dolphins (*Tursiops truncatus*), including three captive-born animals and two control animals, maintained for public display by Sea World, Inc. Two of the wild-captured dolphins were from the California Pacific Coast and the remainder were from a population occupying coastal waters near Florida.

At the beginning of the experiment, we injected each animal with tetracycline and we extracted a tooth from each. (We used an extractor and an elevator after temporarily deadening the interalveolar nerve with a local anesthetic. The procedure is simple and apparently causes the animal little discomfort, and dolphins have plenty of teeth to spare.) About every three months thereafter we gave tetracycline to all but the control animals, and at approximately six-month intervals, we removed a tooth from all but the controls. In addition, sham events, in which animals were handled but did not undergo treatment or tooth removal, were conducted on the project animals at arbitrary intervals. At the end of the project (fall 1982), a tooth was taken from all animals (Myrick and Cornell 1990).

During the experiment, we kept weekly records of water temperature and salinity measurements, and we recorded weekly averages of types and amounts of food consumed. We also noted any changes in behavior, activity, and apparent health and any episode that might be stressful, such as unusual amounts of handling of an animal or transporting of any animals between Sea World parks (in Cleveland, Ohio; Orlando, Florida; and San Diego, California). In addition, we recorded body weight and length measurements at each labeling or extraction session.

In 1980, with the cooperation of colleagues at Sea Life Park, Waimanalo, Hawaii, I also conducted a GLG calibration study of captive Hawaiian spinner dolphins (*Stenella longirostris*), in two stages: (1) a retrospective phase, and (2) a direct monitoring phase. For the retrospective phase, we used teeth of four carcasses (including a captive-born specimen) and three live animals. All seven specimens had received numerous therapeutic doses of tetracycline at various times over periods of years during their captivity, and I thought it highly likely that each treatment had inadvertently labeled teeth of the recipient. (Fortunately, the labels

Fig. 8.5. Dental labeling and tooth extraction. A. Hawaiian spinner dolphin (*Stenella longirostris*) receiving tetracycline injection to introduce a label into the dental tissues for layer calibration studies. B. Removing a tooth from the anesthetized lower jaw.

had been introduced.) With labels identified to dates of past therapeutic treatments taken from records provided by Sea Life Park, we were able to document depositional rates from many years earlier.

In the direct monitoring phase of the Sea Life Park study, we extracted three teeth and gave three additional tetracycline injections, over a one-year period, to each of the three live captive animals to monitor dental tissue accumulation directly (Myrick et al. 1984; fig. 8.5). During the year, no environmental or behavioral records were systematically made.

Appropriately prepared, multiply labeled tooth thin sections from both the Sea World and Sea Life Park experiments were examined separately under plain and ultraviolet light. By tracing labels and layers on plastic overlays of ultraviolet- and plain-light photographs of each specimen, I was able to determine the position of each label within the layering pattern of each tooth. I matched almost every label to a treatment date by comparing the spacing, brightness, and thickness of each label in a tissue with the relative elapsed time between treatments and the length and dose strength of each treatment. With labels identified and series of units of layered tissue bracketed by dates, it was a simple step to identify, measure (in μm), and characterize repeating annual and subannual depositional patterns (fig. 8.6).

What made the two studies successful was the scrupulous manner in which the extensive medical records were maintained for each animal by Sea Life Park and Sea World staff members. Although the records were complete and virtually error-free, a certain amount of detective work had to be done to try to identify labels in a few specimens for which no corresponding dates of treatment were found. In the case of one of the captive-born bottlenose dolphins in the Sea World project, a narrow label occurred in the postnatal dentine a scant distance from the neonatal layer, but the animal had no corresponding treatment record. Since it was not usual for very young calves to be treated with antibiotics or to take solid food, I guessed that the dam who suckled the calf had been given tetracycline, which combined with her blood calcium and was imparted to the calf through her milk. An examination of the dam's records revealed that about one month after the calf was born, the dam was being treated with tetracycline (Myrick and Cornell 1990).

A more complicated mystery was encountered in the Hawaiian spinner study (Myrick et al. 1984). Teeth of the (deceased) captive-born dolphin contained numerous labels, but its records indicated that it had been treated with tetracycline on only three occasions during the three and three-fourths years that it lived (fig. 8.7). I found two faint closely spaced labels within the dentine formed in the first year of postnatal life (fig. 8.7A), which corresponded to records showing that the calf's dam was
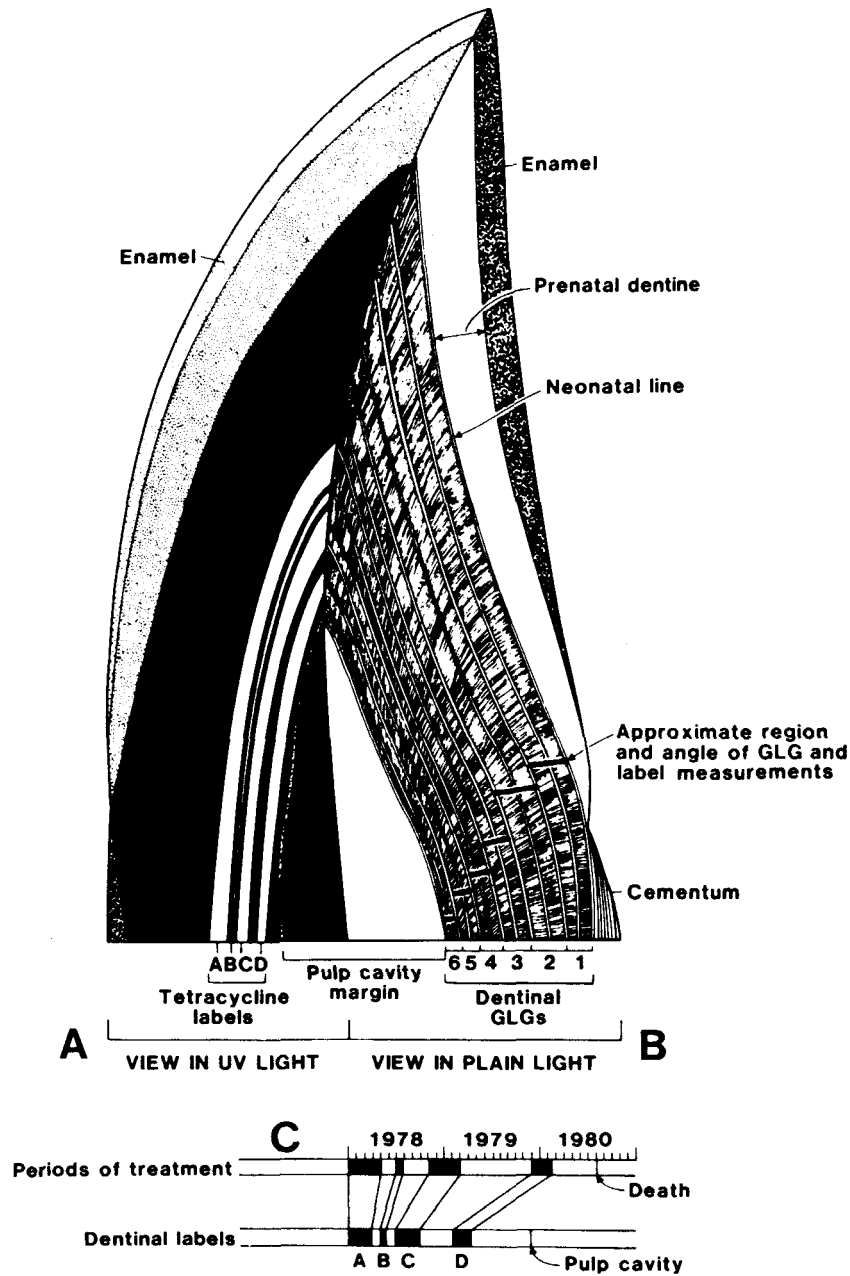
Fig. 8.6.   Line drawing of hypothetical dolphin tooth in thin section show-
ing appearance of tetracycline labels, A, B, C, D, under UV light (A, left-hand
side) and dentinal growth layer group (GLG) layering patterns under plain
transmitted light (B, right-hand side). C illustrates method of identifying labels
by comparing relative thickness and spacing of labels to duration of and elapsed
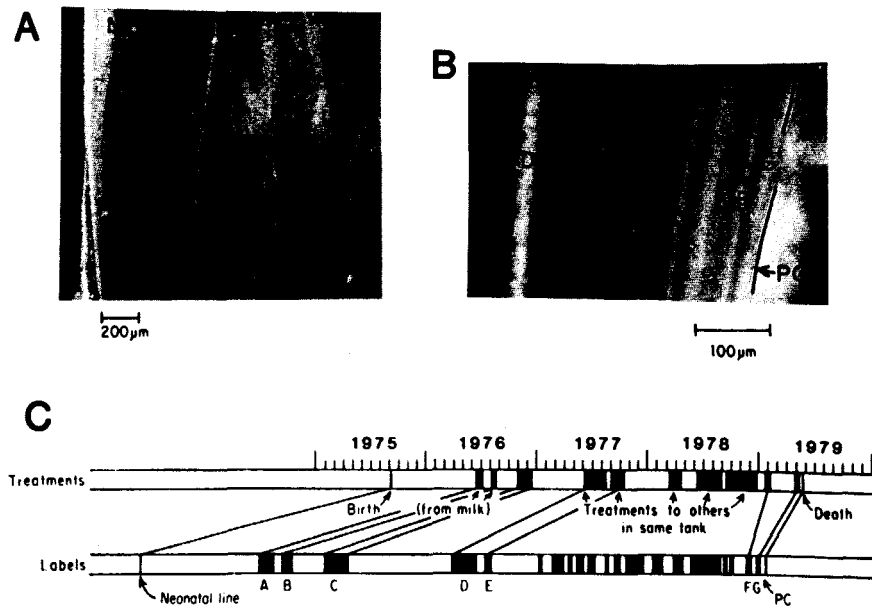time between treatments. (From Myrick et al. 1984.)

Fig. 8.7. A, B. Ultraviolet-light view of tetracycline labels in dentine of known-age Hawaiian spinner dolphin. C. Illustration of how labels were identified with intentional and unintentional introductions of tetracycline fluorophores. Labels A and B apparently were introduced when calf's dam was treated with tetracycline for two periods while the calf nursed. Labels received in 1977 and 1978 were introduced when the youngster stole and ate tetracycline-dosed smelt intended as treatment for other dolphins in the tank. (From Myrick et al. 1984.)

treated with tetracycline during two periods while she suckled the calf. (We proposed that a method of treatment might be developed using nursing as a vehicle to transmit certain medicines to sick calves in captivity without disturbing the calves or separating them from their dams (Myrick et al. 1984).

The mystery of the other undocumented labels in the teeth of the captive-born Hawaiian spinner dolphin solved itself when I matched the records of other dolphins that, at various times, had shared tanks with this young animal. Dolphins are usually treated with tetracycline by slipping the pills inside fish that are then given as food to the patients. As I discovered, many of this young animal's tank companions were being treated with tetracycline-dosed smelt. Interviews with a staff member confirmed that the young dolphin was very adept at stealing and eating fish intended for others and had been observed doing so with some frequency (fig. 8.7B,C).

MONTHLY LAYERS

Multiple labels introduced into the dentine only weeks or months apart showed repeatedly, in various specimens, that annual GLGs consist of thin layers that are deposited with lunar monthly regularity (Myrick et al. 1984). These results support observations made earlier suggesting that incremental layers of annual dentinal GLGs represent monthly records (Laws 1962; Kasuya 1977; Myrick 1979, 1980; Hohn 1980; fig. 8.8).

With the assumption that the dentinal recording device is keeping some sort of lunar monthly time, we may be able to develop a new tool that could furnish us with information about the dolphins in which lunar monthly layers are distinctly visible. A back-count of monthly layers might yield the year and month of birth of an individual if the date of its death is known.

Yearly peaks or other patterns in reproduction, including year-to-year changes in reproductive activity, should be detectable by monthly layer determinations of birth dates of specimens from a population sample
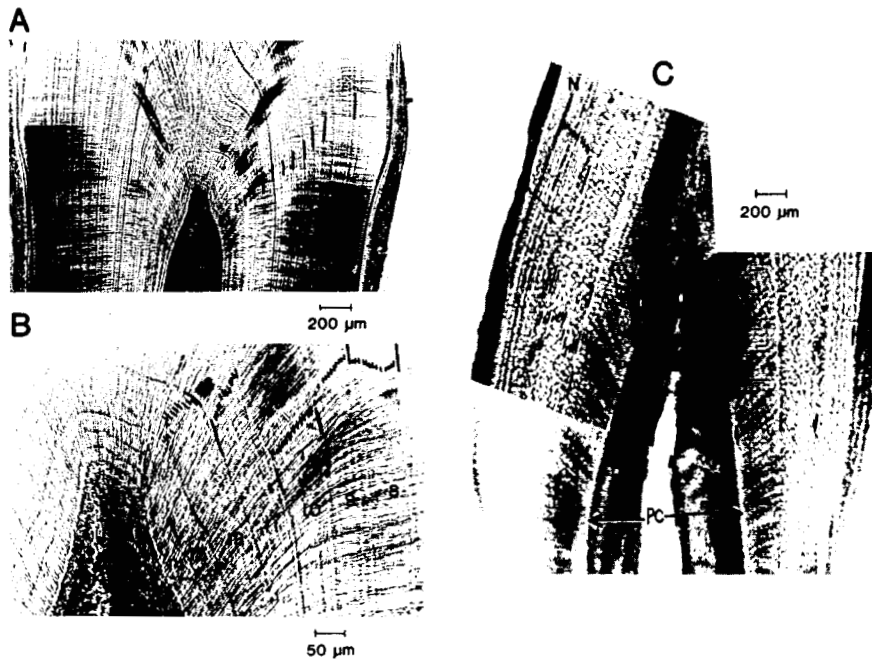

Fig. 8.8.   Dentine of Hawaiian spinner dolphins showing apparent lunar monthly layers (fine marks) within the GLGs (heavy marks). A, B. Two magnifications of same specimen. C. Tooth of known-age dolphin. (From Myrick et al. 1984.)

(Myrick 1980, 1984*a*). If, for example, monthly layer counts for most of the specimens sampled from one population produced a significant birth peak in March and the gestation period was twelve months long, then serious perturbations of the population in the early spring might interfere with courting, breeding, and calving sufficiently to alter reproductive rates. Suppose another sample of the same hypothetical population is taken ten years after the initial sample and seven years after three successive years of strong El Niño conditions in the spring. Monthly layer counts from the new sample produce a broad birth peak at May-June-July. From this, a hypothesis might be formulated that the protracted change in the environment in the spring was connected with the shift in and broadening of the reproductive season.

Inferences might be made of the reproductive compatibility or isolation of two or more geographically contiguous populations from reproduction patterns generated from the counts (Myrick 1984*a*). If counts for the sample from one population produce a significant peak for spring and counts for the sample from another population produce a peak only for fall, a case for limited interbreeding between the two populations might be considered.

If the reproductive season of a population is limited to a short period, monthly layer counts might be used to estimate the approximate months of death in analyses of "die-offs" suspected of being connected with environmental changes (Myrick 1984*b*). Consider a hypothetical case in which several months after a huge oil spill, remains of many dolphins become washed up on nearby beaches. Morphometrics indicate that the specimens are from an offshore population whose calving season is in October and November. The oil spill is suspected of being connected with the large die-off of dolphins. Counts of monthly layers (from neonatal layer to pulp cavity) in teeth of the beach-cast specimens would give the number of lunar months from the beginning of November (the middle of the two-month peak calving season) until they died. In other words, counts would indicate the approximate month in which the animals died and whether deaths occurred at about the same time as the oil spill.

GENETICALLY BASED PATTERNS

Our calibration experiment with the bottlenose dolphins included detailed monitoring of food consumption, weekly average water temperature and salinity, and notation of handling, activity, and behavior. I examined dentinal and cemental layers that were deposited during periods of sudden fluctuations in some of these monitored conditions, for ex-

ample, abrupt drops in water temperature, expecting to find correspond-
ing alterations or interruptions in the regular layering patterns. Instead,
comparisons showed that monitored exogenous changes had no percep-
tible effect on dentine deposition or layering pattern.

When I compared patterns of all the Sea World project specimens cap-
tured from the Florida coast, I found them to be very similar, especially in
the annual thickness of dentine deposited at a specific age. I then com-
pared the Florida pattern with the pattern exhibited in common by the
two Pacific Coast captives in the project sample. In this comparison, I
noted a sharp difference in age-specific GLG thicknesses between Florida
and Pacific Coast specimens, even though they belonged to the same
nominal species (*T. truncatus*). For example, Florida coast captives de-
posited dentinal GLGs in the first, second, and third years of life which
were at least half again the thickness of those deposited by project speci-
mens representing the Pacific Coast population. Such results are sug-
gestive of a genetically determined dentinal depositional pattern, but the
possibility exists that patterns and their differences could be an artifact,
somehow, of the captive situation.

To consider the idea of genetically based patterns in greater detail,
some colleagues and I compared annual GLG depositional patterns of
Sea World captives from Florida waters with patterns in teeth taken from
thirteen wild Florida coastal bottlenose dolphins captured and quickly re-
leased back into the wild (Myrick et al. 1985). Because they were from a
population that had been the subject of extensive field studies for many
years, the wild animals were individually identifiable and had been moni-
tored from year to year, so that they were either of known age or known
minimum age.

In the captive/wild pattern study, we used the age-specific dentinal
GLG pattern (developed from studies of Sea World Florida coast cap-
tives) to estimate the tooth ages of the wild dolphins. The ages of the wild
dolphins were already documented by the field studies, but they were un-
known to me at the time I made the tooth age estimates. We wanted to
determine whether wild and captive animals from the same population
had similar depositional rates and patterns. An answer to this question
would also resolve whether or not the wild pattern was measurably al-
tered by conditions of captivity. Results showed that the age estimates
made with the captive pattern of GLG thicknesses as a guide were very
close to the known ages of the wild dolphins (Myrick et al. 1985). These
results and the results of an earlier study in which I found layering and
thickness patterns to be nearly identical in spotted dolphins and Hawai-
ian spinner dolphins (*Stenella attenuata* and *S. longirostris;* fig. 8.9)
are evidence that at least some of the pattern is genetically determined
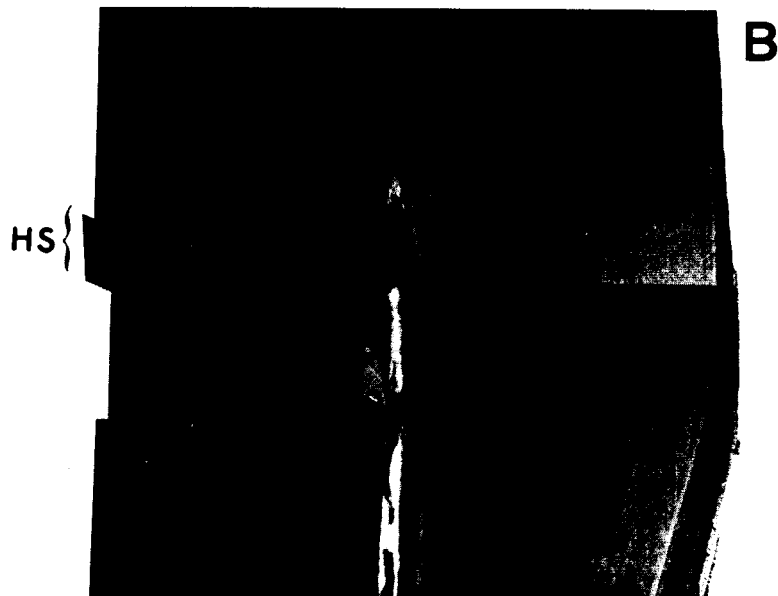(Myrick et al. 1983).

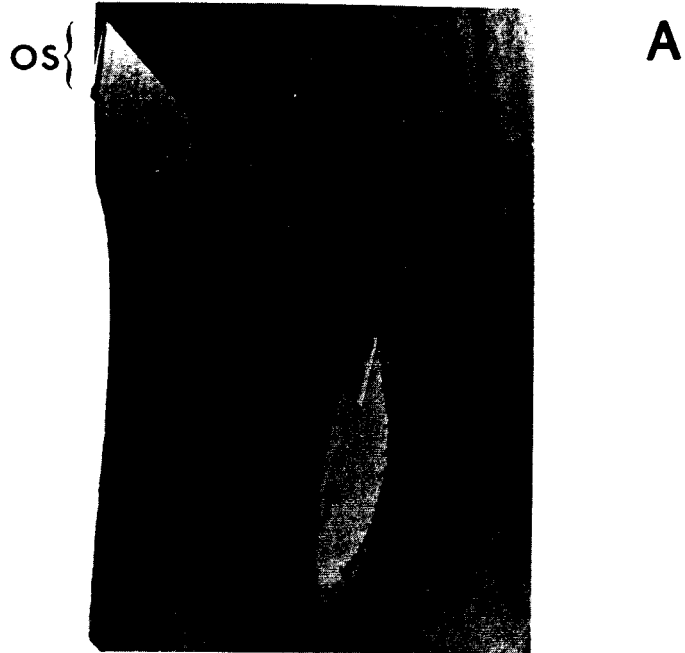*Fig. 8.9.* Swatches of dentine from a specimen of one species of dolphin imposed on the dentinal pattern of a specimen of a different species to show pattern similarities and dissimilarities. A. Offshore spotted dolphin (OS), *Stenella attenuata*, swatch on Hawaiian spinner dolphin, *Stenella longirostris*, tooth. B. Hawaiian spinner dolphin swatch (HS) on offshore spotted dolphin tooth. (From Myrick et al. 1983.)

If adequate criteria can be established to define unique genus-, species-, or subspecies-specific patterns, future studies might use patterns to determine genetic closeness (fig. 8.4), perhaps even the degree of interbreeding, of contiguous or adjacent dolphin populations. The degree of physical and genetic separation of stocks belonging to the same species is of vital interest in fisheries management. In the case of the ETP purse seine fishery, for example, it is sometimes difficult to distinguish between two stocks of spinner dolphins (*Stenella longirostris*) affected by fishing operations because of the rather high overlap in external identifying characteristics (Perrin et al. 1985, Anon. 1986).

INDIVIDUAL VARIABILITY

It is not difficult to detect individual variability within what I suspect are species-specific patterns (fig. 8.4). However, based on calibration studies previously described, it must be concluded that exogenous factors that we monitored probably do not cause measurable pattern variability. I found no pattern variation associated with removing an animal from the water, changing the diet from pure fish to mixed fish and squid, or environmental changes, such as seasonal water temperature fluctuations or transporting animals to locations differing in latitude by up to 13 degrees (Orlando to Cleveland) (Myrick and Cornell 1990). An animal in one of the calibration studies accidentally leaped out of its tank. It remained out of the water, on the ground, perhaps for hours before it was discovered and returned to the tank, apparently uninjured. This unpredictable accident provided us with an excellent opportunity to test whether an "instantaneous," presumably stressful situation causes dentinal pattern variability. Later, when I examined the dentine formed during the period encompassing the accident, I could discern no changes in the normal pattern.

Variation within dentinal layering patterns is more conspicuous and more frequent in female dolphins than in males. In decalcified and stained thin sections of teeth, much of the variability exists as thin, intensely stained layers that are intermittently distributed in the dentine and that stand out rather starkly against the comparatively muted normal pattern of differentially stained layers of the GLGs.

In a study of the dentinal patterns in *Stenella* spp. (Klevezal' and Myrick 1984), we found that most of these strongly contrasting layers (SCLs) occur in females in dentine formed after attainment of sexual maturity. It seemed likely to us that SCLs could represent dentinal calcium fluctuations connected with reproductive cycles of the females—possibly calving events. To test this idea, Klevezal' and I first looked at thin sec-

tions of tetracycline-labeled teeth from a captive female Hawaiian spinner dolphin that had given birth to a calf while in captivity. This animal was one of the seven specimens used in the study to calibrate dental layers in teeth of Hawaiian spinner dolphins (Myrick et al. 1984). We then studied tooth thin sections of seventy-five sexually mature female spotted dolphins selected from a larger, random sample used for age-specific reproduction studies (Myrick et al. 1986). Sexual maturity of a female was determined by the presence of at least one ruptured Graafian follicle (corpus luteum) or a scar from such a rupture (corpus albicans) on either ovary, indicating that the animal had ovulated at least once.

The Hawaiian spinner dolphin had been in captivity for about ten months when she delivered a calf; the calf survived for only three days. One month after parturition, the dam was started on a therapeutic treatment of tetracycline that lasted more than one month. This treatment produced a dentinal label identified as Label B in the teeth of this animal (Myrick et al. 1984; fig. 8.10). To locate Label B for the SCL study, we had to use untreated thin sections (because treating teeth involves decalcification, which destroys the labels). To look for any SCLs near the position of Label B, we had to use decalcified and stained thin sections (because SCLs are narrow, dark-stained layers). After only a little searching of the decalcified and stained dentine, we identified an SCL in about the same position as Label B in the untreated thin sections (fig. 8.11). This reinforced the idea that SCLs were connected with some part of the female reproduction cycle. But, what part? Did SCLs represent pregnancy, parturition, early lactation, or all or none of the above?

To limit the number of possibilities, we began the other part of the study using teeth from the seventy-five female spotted dolphins. This exercise was different from the search for the SCL in the Hawaiian spinner dolphin tooth, in that we independently identified, counted, and noted the position of SCLs for each specimen without knowing anything about the reproductive condition of the specimen in advance (except that each female was sexually mature).

We erected five hypotheses directed at the question of which condition (if any)—pregnancy, ovulation, parturition, lactation, or parturition and lactation—was most frequently associated with the presence of an SCL. The following reasoning was used in reaching our conclusions:

1. If the layers have no specific connection with reproduction, then they are just as likely to occur in reproductively inactive females as in reproductively active females. [They did not.]
2. If the layers represent ovulations, then they should be exactly equal in number to the total count of ovarian corpora (because corpora, once formed, probably never disappear) (Perrin and Donovan 1984).
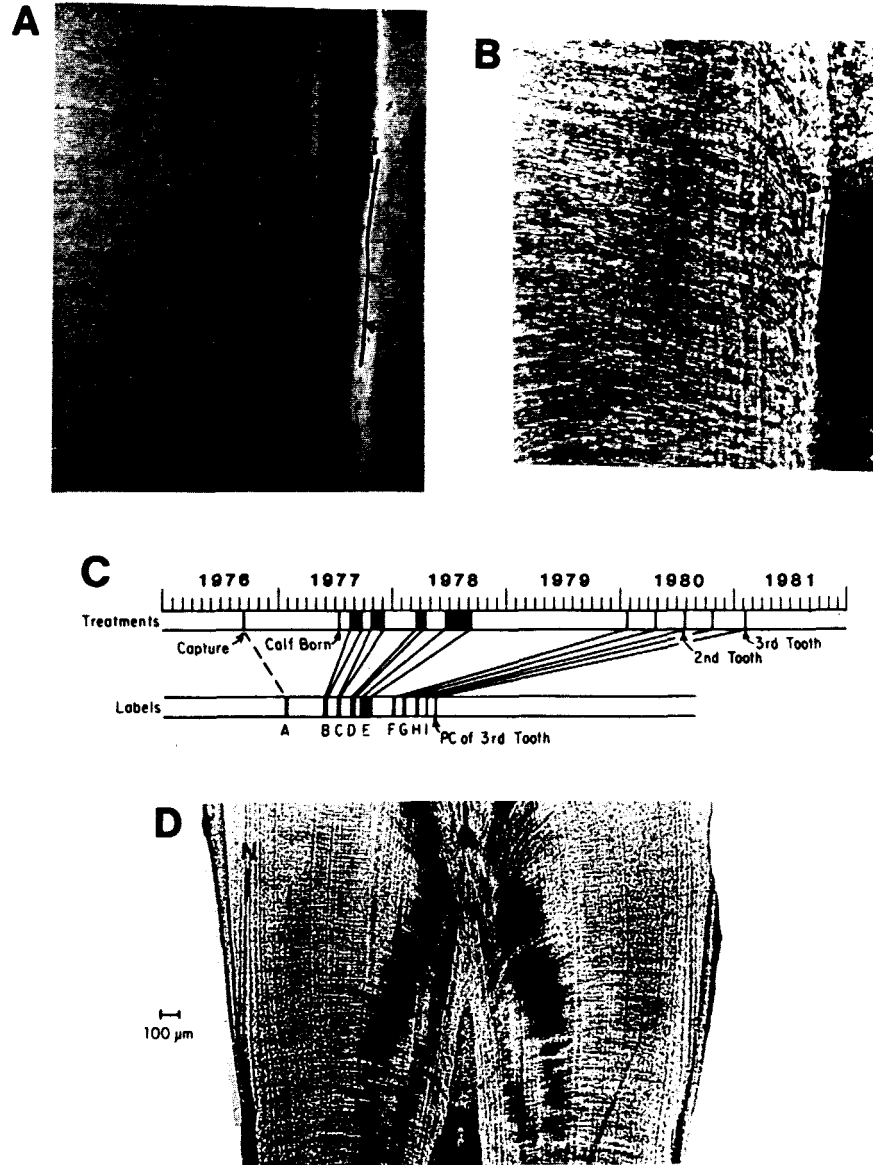
Fig. 8.10. Tooth labels, layers, and matching of labels with treatment dates of female Hawaiian spinner dolphin that gave birth to a calf in captivity. A. Labels are lettered A-I. Label B was introduced one month after the female gave birth. PC = pulp-cavity margin. B. Labels are located in layered tissues by marking plastic overlays of UV photos and plain-light photos. C. Labels matched to treatment dates using label spacing and treatment duration. D. Gross view of layering pattern across entire tooth. (From Myrick et al. 1984.)

A

200μm

B

Fig. 8.11. A, B. Photographs of two decalcified and stained thin-sectioned teeth from female Hawaiian spinner dolphin that gave birth in captivity. A strongly contrasting layer (SCL), indicated by arrow, is near the position of a tetracycline label introduced one month after the female's calf was born. (From Klevezal' and Myrick 1984.)

Table 8.1. Tooth Layering and Reproductive Condition in Female Spotted Dolphins

Hypotheses—Formation of SCL Associated with:	Tests of Coincidence	Total Number of Specimens	Coincidence		Noncoincidence		Result of Test
			NUMBER OF SPECIMENS	PERCENT	NUMBER OF SPECIMENS	PERCENT	
1. Ovulation							
a. all ovulations	1a. SCL count = CAs + CLs	75	10	13	65	87	Negative
b. all but most recent fertilized ovulation	1b. SCL count = CAs + CLs excluding CLs of pregnancy	75	17	22	58	78	Negative
2. Pregnancy	2a. In all pregnant females, SCL in last GLG	30	1	3	28	97	Negative
3. Parturition or lactation	3a. SCL count ≤ CAs + CLs excluding any CLs of pregnancy	75	70	93	5	7	Positive
	3b. In all pregnant females, SCL count < CAs + CLs of pregnancy	75	70	93	5	7	Positive
4. Lactation only	4a. In all lactating females, SCL in last GLG without a space between SCL and pulp cavity	40	0	0	40	100	Negative
5. Parturition only	5a. In all lactating females, at least one SCL within the last two GLGs	40	35	88	5	12	Positive

Note: This table compares the percent coincidence tests of five hypotheses of relationships between SCLs and reproductive condition in female spotted dolphins (*Stenella attenuata*). SCL: strongly contrasting layers; GLG: growth layer group; CA: corpus albicans; CL: corpus lutem. (Modified from Kevezal' and Myrick 1984.)

3. If the layers represent pregnancies, then in nonpregnant females, they should be equal to or less than the total count of ovarian corpora (because not all ovulations may result in pregnancy). Furthermore, in all pregnant females, an SCL should be present within the last-formed dentinal GLG (because the female was pregnant at death).

4. If the layers represent lactation, then SCLs should be equal to or less than the total count of ovarian corpora (because lactation is preceded by a pregnancy and not all ovulations may result in pregnancies). Furthermore, in all lactating females, an SCL should be present within the last-formed dentinal GLG without a space between it and the edge of the pulp cavity (because the female was lactating when killed).

5. If the layers represent parturition only, then the first point in #4 should be true. Furthermore, in all lactating females, an SCL should be present within the two last-formed GLGs (because nursing may continue for more than a year after parturition) (Perrin and Reilly 1984).

The percent coincidence tests we used showed a consistently strong (83–93%) association of layers with parturition exclusive of lactation (table 8.1). The tests excluded the high likelihood of SCLs as indicators of any other condition that we considered.

Since that study, I have had the opportunity to examine the teeth of three long-lived, captive female bottlenose dolphins with extensive reproductive and calving histories. Two had each given birth to three calves; the other, to one calf. I found a parturition layer in the dentine formed during the date recorded for each calving event (fig. 8.12). I also noted similar spacing of anomalous layers in the cementum of these specimens.

Reliable criteria for detecting SCLs that indicate parturition would be extremely useful in managing dolphin populations. In the case of the dolphins associated with the yellowfin tuna purse seine fishery, evaluation of the fishery's historical impact on the size and health of the dolphin populations has been a major problem. The dolphins became directly affected by the fishery in 1959 when the fishing method changed from bait fishing to purse seining. From that time through the 1960s and into the early 1970s, dolphin fishing mortality is estimated to have reached between 100,000 to 400,000 animals annually (Smith 1983). The impact assessment problem exists because (1) there are no firm estimates of pre-1959 population levels of dolphins (numbers of individuals), (2) the dolphin populations are presumed to have undergone changes in reproductive rates as a density-dependent response to the substantial fishing mortalities, and (3) almost no mortality samples were collected before 1972 (long after the populations are presumed to have responded to the effects

Fig. 8.12.   Highly magnified section of decalcified and stained tooth from a female bottlenose dolphin that gave birth to three calves in captivity. Two strongly contrasting layers, SCLs (arrows), occur in the regularly layered dentine, a third may be next to the pulp-cavity margin (the female died not long after giving birth to the third calf).

of the high kills). In short, virtually no information exists concerning the dolphins before the 1970s.

If SCL definitions can be refined for ready identification, possibly they could be used to interpret the reproductive histories, including per-capita calf production and calving frequency, of sexually mature females from

their dentinal and cemental patterns. The years of heavy kill did not take place before 1959 (13 years before sampling started), and the average age at sexual maturity for females is between 6 and 11 years old (depending on the population). These two facts indicate that tooth samples of females between 20 and 35 years old collected in 1973, 1974, and 1975 should contain SCLs that were formed well before the introduction of purse seining. If such specimens exist in sufficient number, they could be useful in the estimation of reproductive rates that occurred before the impact of purse seine fishing. In addition, counts and spacing of SCLs in those and younger specimens might help fill the data gap concerning any changing rates during the heavy-kill period (1960–1975). This possibility has not yet been examined in any detail.

SUMMARY

Results from research that I initially conducted to develop more accurate age determination methods show that layers in dental tissues have a much greater potential as tools for biological inquiry than merely servicing the need for age estimates. Now that layering patterns in various species are being calibrated with time, it soon may be possible to use monthly layers to estimate peak reproductive seasons for a population and, thereby, to measure the degree of reproductive compatibility between adjacent populations.

Several of our studies have shown that local exogenous fluctuations do not perceptibly alter the genetically based dentinal layering patterns. This permits consideration of the use of dentinal patterns to estimate genetic closeness of sampled populations at the genus, species, and even perhaps at the subspecies level.

Recognition of the existence of genetic patterns has facilitated the identification of individual variation within the patterns. Because external factors do not seem to affect the patterns, variation must reflect, to a large extent, certain individual physiological changes. Parturition layers in sexually mature females seem to be a likely example of this. These layers are a prime candidate for development as a tool for population management because they may provide a record of the complete calving history of a female. In addition, other variations in the dental patterns are known to exist in both male and female delphinids. There is little doubt that they reflect other physiological events or biological cycles yet to be identified. Extensive study and experimentation will be needed to determine if such potential tools can be fully developed for practical use.

Despite the very difficult task of trying to study such secretive animals as dolphins, techniques are being developed and improved to address

some of our most important questions concerning delphinid biology. In the 1950s, researchers were developing a tool for estimating a dolphin's age. Since then, the method has been used to help provide answers to questions about the reproductive health and future growth of dolphin populations. Results of the calibration studies that I have described should add greater firmness to some of those answers, but it is becoming increasingly clear that dental recording devices of dolphins have been underutilized as potential sources of biological information.

When we realize that the recordings themselves are chronological histories written in the biochemical language of the animals' own physiology, we are prompted to search for a Rosetta stone that will allow us to translate these histories. By learning what physiological conditions cause the recorded features, we can then use the features to interpret past conditions, as we are beginning to do with parturition layers. Perhaps, eventual interpretation of large parts of an individual's physiological history may be possible (see, for example, Myrick 1988).

After decades of work to understand the natural and human-caused environmental effects on tree growth, dendrologists are now able to determine much of the historical growth physiology of trees from details of their annuli. With dolphins, the problem differs only in our limited ability to monitor their wild environment and their physiological responses to it closely enough. Until that ability is achieved, we must settle for remote and retrospective monitoring to answer our questions about the dolphin populations. No doubt, some of these interim answers will come from layers in the teeth.

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