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MARKS IN TOOTH DENTINE OF FEMALE DOLPHINS (GENUS *STENELLA*) AS INDICATORS OF PARTURITION

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ABSTRACT.—In decalcified and hematoxylin-stained thin section, teeth of sexually mature female spotted dolphins (*Stenella attenuata*) often exhibit a deeply dark-stained layer (DSL) at the boundary of one or more of their annual growth layer groups. We compared DSL counts, made without reproductive information, to the corresponding reproductive condition in 75 females to test five hypotheses concerning the possible significance of DSLs. We found an exclusive association between DSLs and calving events. The teeth of an Hawaiian spinner dolphin, *Stenella longirostris*, labeled with tetracycline one month after giving birth to a calf in captivity, contained a dentinal DSL near the label. The number of DSLs in the dentine of a female probably indicates the minimum number of calves born to the female. In future studies DSL counts may be useful in estimating birth frequencies, year-specific birth rates, and calf mortality in odontocetes.

In the teeth of pinnipeds certain peculiar dentinal layers form concurrently with periods of active breeding, fasting, weaning, migration, and molting (Hamilton, 1934; Laws, 1952, 1953, 1962; Laws and Purves, 1956; Fay, 1953; Fisher, 1954; Fisher and MacKenzie, 1954; Kenyon and Fiscus, 1963; Carrick and Ingham, 1962; Scheffer and Peterson, 1967; Scheffer, 1975), although no causal relationship has been demonstrated. Klevezal' and Tormosov (1971) investigated unusually contrasting layers in the dentine of female sperm whales in an attempt to find an association between the layers and reproductive cycles, but failed to obtain clear correlations. Nevertheless, because such a connection, if established, would provide new avenues for the biological study of commercial and protected cetacean species, the search for reproductive marks in the layered hard parts of cetaceans has continued.

Annual layers or growth layer groups (GLGs, terminology of Perrin and Myrick, 1980) in dentine of toothed whales and dolphins consist of numerous layers having different optical densities, the number and contrast of which vary by age, individual, and species. Variations within a GLG are of at least two types: 1) differences in number and width of the fine layers; and 2) differences in optical contrast between the major zones. Probably, formation of the fine layers is influenced by major differences in growth patterns of the dentine, and differences in optical contrast of the major zones are due to differences in mineral content (Suchovskaya and Klevezal', 1982). With this distinction as a basis, we have assumed that the formation of fine layers, connected with lunar monthly periodicity by Laws (1962), Kasuya (1977), Myrick (1979. 1980, in press), and Hohn (1980) is associated, perhaps genetically, with the growth process, rather than with calcification. Conversely, the optical densities of broad zones within GLGs relate to changes in the mineralization process.

In some species of terrestrial mammals, formation of accessory layers in bone is caused by temporary cessation of bone growth, not by reproductive cycles (Klevezal' and Mina, 1973). Neither pregnancy nor lactation appears to alter the growth process of the skeleton or the teeth (Klevezal' and Mina, in press), but mineral content should be affected considerably during such peak periods of calcium demand.

If this is true for toothed cetaceans, then any marks connected with reproduction should be found in the major zones of dentinal GLGs in teeth of sexually mature females. The present paper reports our efforts to locate reproduction marks in the dentine of teeth of female spotted and Hawaiian spinner dolphins (*Stenella attenuata* and *Stenella longirostris*).

Materials and Methods

Spinner dolphin.—We examined thin sections of teeth from a female spinner dolphin from Hawaii, that had been labeled with tetracycline (given as a medical treatment) one month after her calf was born. We compared the position of the label, located in the dentinal pattern in an undecalcified thin section by ultraviolet light microscopy, with its estimated position in the pattern of decalcified and stained thin sections of her teeth.

Spotted dolphins.—We examined the dentine of teeth from spotted dolphins killed incidentally in the purse-seine fishery for yellowfin tuna in the eastern tropical Pacific (Perrin et al., 1976). The teeth were prepared as decalcified and stained mid-longitudinal thin sections, 25 μm thick, and we examined them at 39 and 150× in plain transmitted light with a compound microscope. The dentine in stained thin sections shows well-developed zones of different stain intensities, which correspond to the broad zones of different optical densities in undecalcified thin sections.

First, we studied thin sections of teeth from 40 adult males and 40 adult females (ages 10–44 years) chosen arbitrarily from a random sample of 1600 specimens, the only requirement being that they were of high quality in general appearance. We found that in females the GLG boundary layers were distinctly thinner more often and more intensely stained than in males. Second, we examined 75 adult female specimens (ages 10–28 years) for which data were available regarding reproductive condition at death, to determine a possible connection between the presence of a deep stained layer (DSL) and reproductive state.

Working independently, we each determined the number and positions of DSLs, without access to the biological information of the specimens except for sex and estimated age. We resolved discrepancies in our independent results on some specimens by studying those specimens together, before accessing the reproductive data.

We formulated the following five hypotheses to test DSL relationships to reproductive condition. 1) DSLs relate to all ovulations, whether or not they result in pregnancies, or to all but the most recently fertilized ovulation. We tested this by assuming that each ovulation results in a corpus luteum (CL) that ultimately regresses to a corpus albicans (CA) and that each CA persists for life; therefore the DSL count should be equal to the total count of the CAs plus CLs. Additionally, the DSL count should be equal to CAs plus CLs excluding CLs associated with a fetus. 2) DSLs relate to pregnancy. If the gestation period for spotted dolphins is approximately one year (Perrin et al., 1976), then a DSL should occur within the last annual GLG in all pregnant females. 3) DSLs relate either to parturition or to lactation, or to both. The DSL count should be equal to or less than the count of CAs excluding any CL of an existing pregnancy, given that not all ovulations result in pregnancy and birth. 4) DSLs relate to lactation only. If lactation in non-pregnant females indicates the presence of a nursing calf, then in lactating non-pregnant females a DSL should occur in the last GLG with no space between the DSL and the pulp cavity. 5) DSLs relate to parturition only. In lactating females, a DSL should occur within the last two GLGs, given that nursing may last for one year or more in spotted dolphins (Perrin et al., 1976; Kasuya, 1976).

All 75 females used in the tests had at least one CA (range 1–13, mean 3.92). Thirty were pregnant. Forty were lactating. Three of those pregnant were lactating.

Results

Definition, occurrence, and spacing of DSLs.—A DSL differed from other GLG boundary zones by being distinctly more darkly stained and thinner. It stood out from other layers along its entire longitudinal extent from apex to base (Fig. 1). Only one DSL occurred within one GLG, but one often occurred in the next adjacent GLG, or in each of the adjacent two GLGs (rarely). Often DSLs were separated by two or more GLGs. Up to five DSLs were found in a single female specimen.

Despite the identification of DSLs in males, we independently arrived at the conclusion that DSLs in males were subtly different in character from those observed in females. Nevertheless, clear distinctions between DSLs in males and in females were difficult to describe, and we have here used the same definition for both sexes.

Doubtful DSLs.—The detection of DSLs depended on the quality of resolution of layers in a thin section. We observed other layers in the adult dentine of both sexes that resembled, but were rather fainter or thicker than DSLs and were more intensely stained than the other GLG boundary layers. These we have called "doubtful" DSLs. The difference between "definite" and "doubtful" DSLs remains subjective. "Doubtful" DSLs were not included in our final counts.

FIG. 1.—Tooth thin section from female spotted dolphin, DRD 0042, with three DSLs in dentine (arrows) (N = neonatal line).

Maturation layers.—A dark-stained boundary layer resembling a DSL, but less intensely stained and less distinct, occurred in the eight or ninth GLG in almost all males and in the sixth or seventh GLG of almost all females studied. Because they were deposited at least three years before females are known to ovulate (at 10–11 years) and before the testes of the males suddenly increase in weight (at 12 years), they were ignored in our DSL counts. We consider them to represent an attainment of some important plateau in the maturational process and we have called them maturation layers (see Fig. 2).

Comparison between DSL counts for males and females—The range of DSL totals by each of us for the 40 females was one to five. We observed DSLs in all specimens. For the 40 males, Klevezal' determined that the range was zero to two and found DSLs in only 10 specimens. The counts made by Myrick for the males ranged from 0 to 3 (3 in only one specimen) and he found DSLs in 14 specimens.

The relative number of DSLs defined as "doubtful" was considerably higher in males than in females. For males, one of us noted 12 "doubtful" with 14 "definite," the other noted 23 "doubtful" with 10 "definite." For females, one of us noted 12 "doubtful" in addition to 96 "definite" DSLs; the other noted 32 "doubtful" compared to 96 "definite."

Test of hypothesis 1: DSLs reflect ovulations.—We studied this in 75 females (Table 1, 1).

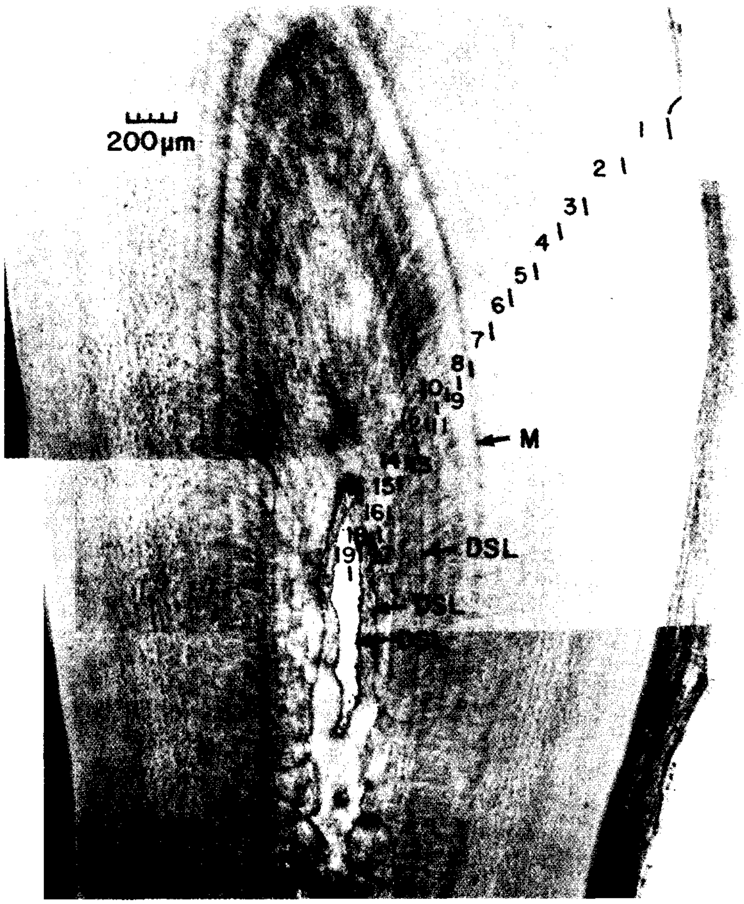

FIG. 2.—Tooth thin section from 19-year-old female spotted dolphin, FBG 0030. Arrows indicate maturational layer (M) at end of seventh year. DSLs at end of 12th, 17th, and 18th dentinal GLGs.

In 65 cases, the DSL count was less than the number of CAs plus CLs. In 10 females the DSL count was equal to the CA plus CL count (Table 1, 1a). When CLs associated with fetuses (30) were excluded from each count, 58 of the females had a DSL count less than the CA count and 17 had equal numbers of DSLs and CAs (Table 1, 1b). If DSLs reflect all ovulations, or if DSLs reflect all ovulations except those represented by the last formed pregnancy, the DSL counts should have been equal to the CA and/or CA + CL counts. The hypothesis was not sustained.

Test of hypothesis 2: DSLs reflect pregnancies.—This was studied in the 30 pregnant females (Tables 1, 2). A DSL was detected in the last GLG in only one individual. She was pregnant and lactating at the time of death. If DSLs reflect pregnancies, all 30 pregnant females should have had a DSL in the last GLG. The coincidence test was negative (Table 1, 2a).

Test of hypotheses 3, 4, and 5: DSLs reflect parturition and/or lactation.—We tested hypothesis 3 in 75 specimens. DSL counts were equal to or less than the number of CAs plus CLs, excluding CLs of pregnancy, for corresponding specimens in 70 cases (Table 1, 3a). In 25 of the 30 pregnant females DSL counts were less than the CAs plus CLs of pregnancy (Table 1,

TABLE 1.—*Percent coincidence tests of five hypotheses of relationships between DSLs and reproductive condition in 75 female spotted dolphins,* Stenella attenuata. *DSL, deep stained layer; GLG, growth layer group.*

Hypotheses: formation of DSL associated with		Tests of coincidence	Total number of specimens	Coincidence: Number of specimens	Coincidence: Percent	Noncoincidence: Number of specimens	Noncoincidence: Percent	Result of test
1. Ovulation								
a. all ovulations	1a.	DSL count = CAs + CLs	75	10	13	65	87	Negative
b. all but most recent fertilized ovulation	1b.	DSL count = CAs + CLs excluding CLs of pregnancy	75	17	22	58	78	Negative
2. Pregnancy	2a.	In all pregnant females, DSL in last GLG	30	1	3	28	97	Negative
3. Parturition or lactation	3a.	DSL count ≤ CAs + CLs excluding any CLs of pregnancy	75	70	93	5	7	Positive
	3b.	In all pregnant females, DSL count < CAs + CLs of pregnancy	30	25	83	5	7	Positive
4. Lactation only	4a.	In all lactating females, DSL in last GLG without a space between DSL and pulp cavity	40	0	0	40	100	Negative
5. Parturition only	5a.	In all lactating females, at least one DSL within the last two OLGs.	40	35	88	5	12	Positive

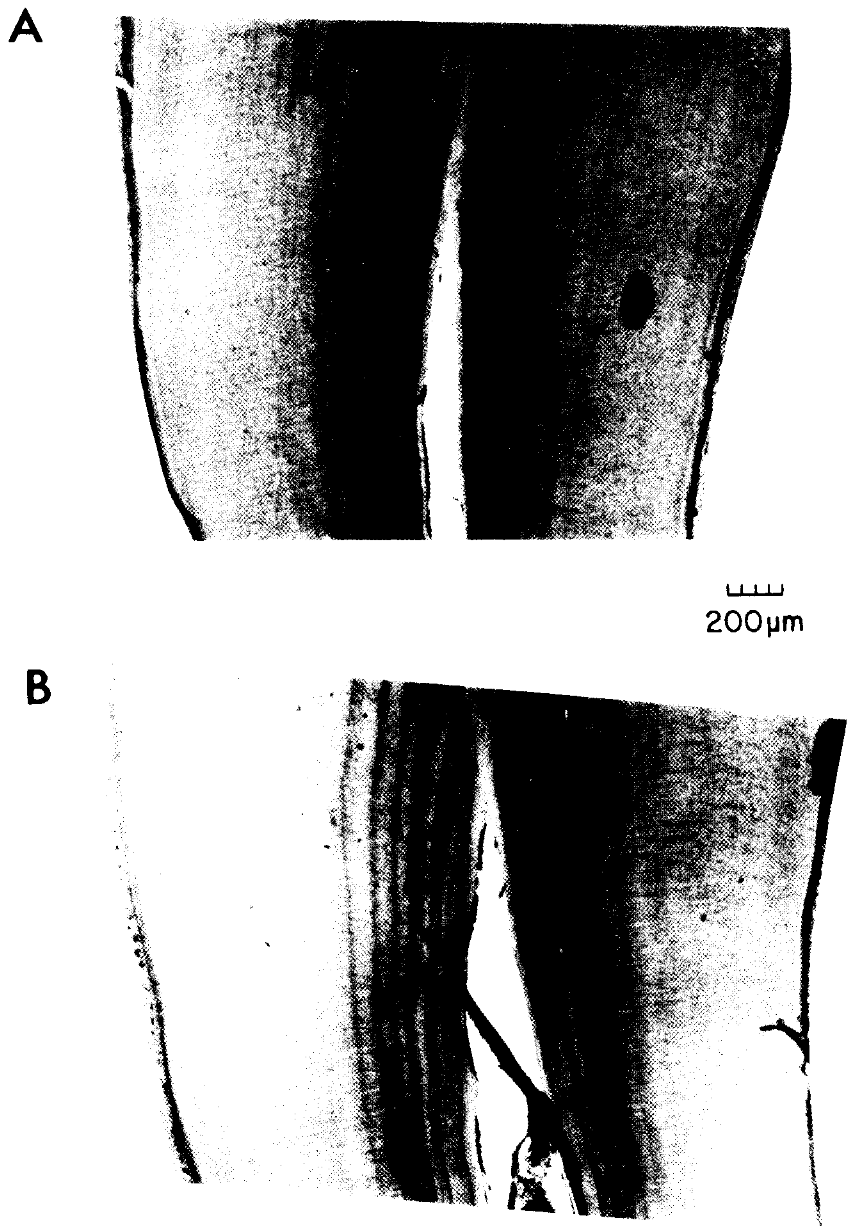

Fig. 3.—Thin sections of two teeth (A and B) from female Hawaiian spinner dolphin labeled with tetracycline one month after giving birth to calf in captivity. DSL (arrows) occurs in dentine at same position in both teeth, immediately external to label observed in untreated thin sections under ultraviolet light.

3b). The highly positive test results of this hypothesis permitted us to test hypotheses 4 and 5 separately.

We tested hypothesis 4, that DSLs reflect lactation exclusively (Table 1, 4) in 40 lactating specimens. In no case was a DSL observed in the last GLG without a space between the DSL and the pulp cavity (Table 1, 4a). Only seven of the 40 had a DSL in the last GLG. If DSLs are indicators of lactation, we should have found a DSL in each last-formed GLG with no separation from the plup cavity.

We tested hypothesis 5, that DSLs reflect parturition only (Table 1, 5) in 40 lactating females. Thirty-five had a DSL within the last two GLGs (Table 1, 5a). In seven cases a DSL was present in the last GLG and in 28 cases one was found in the next-to-last GLG. Because parturition occurs a year or more before nursing is thought to cease in spotted dolphins (Perrin et al., 1976; Kasuya, 1976), a DSL should be present in either the last or next-to-last GLG in lactating females if DSLs are indicators of parturition. A highly positive result was obtained in this test.

Captive female Hawaiian spinner dolphin.—In this individual's teeth, we found only one distinct DSL (Fig. 3). The DSL was situated adjacent to the position of the tetracycline label introduced soon after the calf was born.

Discussion

Assuming that DSLs reflect parturitions exclusively, exceptions to the positive results must be addressed. In 12% of the lactating females no DSLs occurred within the last two GLGs (Table 1, 5a). In identifying DSLs near the pulp cavity it was not always easy to determine precisely which GLG they occupied. GLGs near the pulp cavity in adult specimens are thin (40–50 μm thick) and commonly their complete patterns are not fully stained. In our opinion, much of the error in determining the precise positions of the DSLs stems from the problem of GLG resolution.

It is more difficult to explain why 17 out of 35 nonlactating females, pregnant and nonpregnant, had DSLs in the last two GLGs. Although some of the problem may have been due to imprecision in GLG delimitation, we think that many of these females may have lost their calves after birth and ceased lactating.

Eight of the 75 females had between 9 and 13 CAs, but the greatest number of DSLs counted was five (in a 17-year-old with seven CAs). We would have expected a proportional increase of DSLs with an increase in scars and we are unable to explain why such a result was not obtained. It is possible that CAs and parturitions may not be significantly correlated, i.e., the proportion of CAs representing eventual pregnancy and birth may be quite irregular (S. Reilly, NMFS, pers. comm., 1982). Perhaps DSLs are not always recorded with equal clarity in later-deposited dentine and we may have failed to detect some of the DSLs in the older specimens. We assume that the DSL counts represent a minimum of the actual calving episodes.

Proportionately fewer DSLs (mode = 0) were found in the random sample of adult males (17–25%, range 1 to 3) than females (100%, mode = 2, range 1 to 5). We are unable to explain, at this time, why DSLs occur in males, but note that "DSLs" in males appear to be of a different nature than those in females. In any case, their presence in males does not invalidate the highly consistent association of DSLs with births in females.

At present there is no basis for rejecting the hypothesis that DSLs reflect parturitions exclusively. Further work is required on larger samples in which the sex of the specimens is not known to the investigator during DSL identification. More precise distinctions of DSLs between males and females is necessary. A more objective description of DSLs in females is needed to minimize discrepancies in DSL counts. Similar marks should be sought in teeth of toothed cetaceans other than *Stenella* spp. With validation and refinement of the method, DSLs may prove to be a useful tool in future studies of the reproductive biology of toothed whales and dolphins. They may be used in estimating the frequency of births, extrapolating year-specific birth rates, and estimating calf mortality rates.

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