

REPRODUCTIVE HISTORY OF FEMALE BLACK BEARS FROM DENTAL CEMENTUM

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Abstract: Dental cellular cementum deposition (DCD) was studied in black bears (*Ursus americanus*) in Arizona between 1973 and 1990. Cub production and cub survival were monitored for 17 adult (>4 years) females. First premolars were later collected from these females; 11 had received intramuscular oxytetracycline injections to label cementum. Annual DCD layer measurements were made from transverse sections of first premolars. Dental cellular cementum deposition measurements were standardized by calculating a proportional width for each cementum layer to compensate for individual differences in cementum growth rate. Age effects on cementum growth rate were removed prior to analyses. Pairwise comparisons, after individual and age differences were removed, revealed that female DCD was reduced ($P = 0.003$) during years when cubs were successfully raised compared to years when the same females produced no cubs. Female DCD was also reduced ($P = 0.018$) during years of successful cub rearing compared to years when cubs were produced but lost. A predictive model with confidence intervals was developed to allow estimation of reproductive history for female black bears from dental cementum deposition.

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Reliable age and reproductive data are essential for understanding population dynamics of black bears. Use of dental cementum for mammalian age determination was first developed in marine studies (Scheffer 1950, Laws 1952). This technique has been verified for use on many mammals and is widely used (Fancy 1980). Its use for aging black bears was first reported in 1966 (Marks and Erickson 1966, Sauer et al 1966, and Stoneberg and Jonkel 1966). The first premolar is the preferred tooth for aging bears (Fancy 1980).

Reproductive data generally are obtained by intensive research of individual females within black bear populations. Cyclical changes in cementum or dentine deposition rate in females of some mammalian species has indicated a possible correlation with reproduction. For example, in female dolphins (*Stenella attenuata*) dark staining layers in dentine correlated with parturition (Klevezal and Myrick 1984). Reduced deposition of cellular (i.e., light staining) cementum during lactation years was noted in female black bears from northern Minnesota (Rogers 1975) and central Arizona (Carrel 1980).

Because of these observations, this study was undertaken to examine relationships between cementum deposition and female black bear reproduction in Arizona. If reproductive events are correlated with observable cementum characteristics, then a reliable method could be developed to determine female black bear reproductive history by analyzing cementum.

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METHODS

Tooth Extraction

Black bears were captured, eartagged, and radiocollared in 3 studies from 1973 to 1989: (1) in central Arizona chaparral and oak (*Quercus* spp.) woodland habitat (LeCount 1982); (2) in north-central Arizona ponderosa pine (*Pinus ponderosa*) and mixed conifer habitat (LeCount 1987a); and (3) in east-central Arizona mixed conifer and spruce-fir (*Picea-Abies* spp.) habitat (LeCount 1990). Bears were trapped during summer utilizing Aldrich foot snares at bait sites and immobilized with phencyclidine hydrochloride (Sernylan). Premolar teeth were extracted for aging. Beginning in 1980, age-related oxytetracycline doses were given by intramuscular injection to label dental cementum (Crier 1970, Johnston 1972, Day and Carrel 1986). Bears were recaptured in winter dens or during summer trapping. Tetracycline-labeled premolar teeth were extracted 1-4 years later and stored in the dark at -20°C .

Cub Production

Black bear cub production and cub survival were determined from 1974 until 1990 (LeCount 1984, 1987a, 1990). Dens of females were visited during February-March to record presence of cubs or yearlings. During these visits 23 cubs on the north-central Arizona study (LeCount 1987b) and 26 cubs on the east-central Arizona study (LeCount 1990) were equipped with mortality sensor break-away radio collars. Radio signals were monitored weekly from aircraft until cubs left dens, then monitored daily from

the ground. Time and cause of death were recorded for confirmed cub mortalities. If a collar broke away and no evidence of mortality was found, the female's den was checked the following winter to observe if cub(s) survived.

Tooth Sectioning

Three transverse 50 μ sections were cut from the lower one-third of the tooth root (Carrel 1980, Johnston and Watt 1980). Sections were sanded to 40 μ , cleaned in an ultrasonic cleaner, decalcified with 10% formic acid, stained with Paragon multiple stain, and mounted on slides. Sections were viewed microscopically (16-80x) and age estimated by counting annuli.

From tetracycline-labeled teeth, a second set of 3 sections was cut, sanded, cleaned, and mounted on slides in a 3:1 water:glycerine solution with cover glass. Slides were stored in the dark at -20°C. Sections were examined for fluorescent cementum labels using a fluorescence microscope with blue light excitation. Color photographs were taken as a permanent record. I used tetracycline cementum labels as time-specific reference lines based on injection dates. This allowed accurate determination of calendar years when cementum layers were deposited. Otherwise, deposition year for each layer was backdated from root surface based on tooth extraction date. Known reproductive condition (i.e., no cubs, cubs produced but lost, or cubs survived) by year was cross-referenced with cementum layers.

Cementum Layer Measurements

Annual dental cellular cementum deposition (DCD) layers were measured in first premolar teeth. I defined annual DCD as light staining, cellular cementum occurring between, but not including, successive cementum annuli (i.e., dark staining layers of acellular cementum). Measurements were made by projecting transverse tooth section microscope images through a video camera to a computer monitor. Measurements were taken along 2 straight lines, perpendicular to cementum layers, and at each side of the thickest cementum region of each projected image (Fig. 1). Each projected annual DCD layer was measured to the nearest 0.1 mm.

Cementum Layer Analysis

I calculated a mean for the 2 measurements of each annual DCD layer for all 17 premolars selected for analysis because I observed thickness variation of these layers within a tooth section. Annual DCD measurements could not be directly compared between

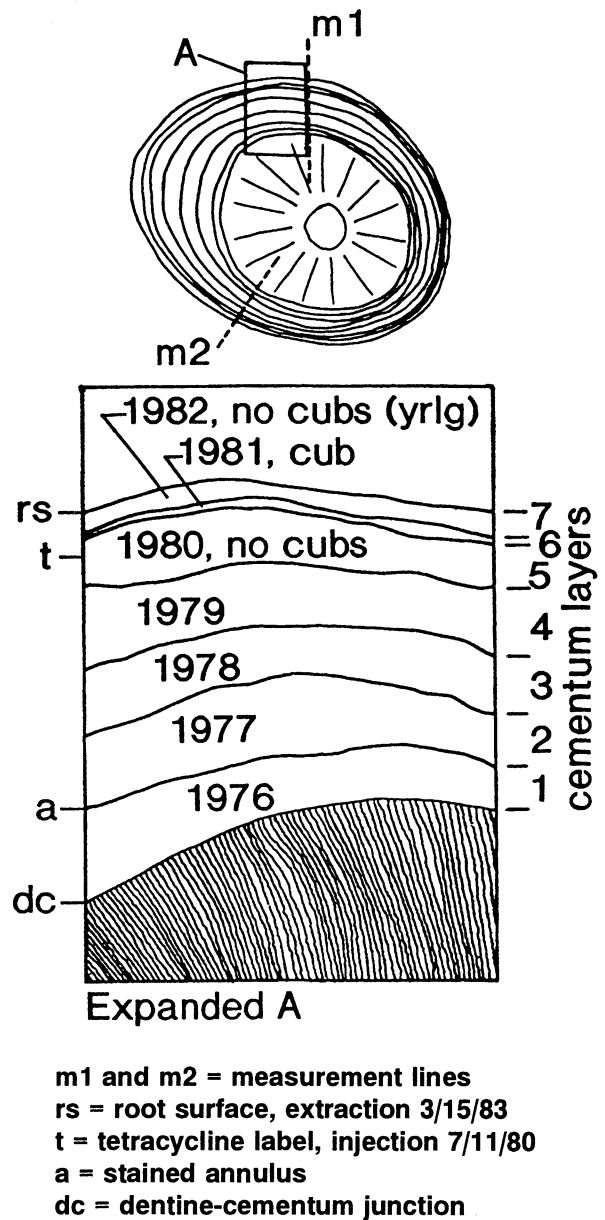


Fig. 1. Expanded microscope image of first premolar tooth section (Bear #71) for cementum layer measurements and reproductive condition cross-reference. Layer 6 was when successful cub rearing occurred.

bears because of individual differences in cementum growth rate (Klevezal 1970). Additionally, DCD thickness varied according to section location within the tooth root. Thickness tended to decrease from root tip to tooth crown. To correct for these problems, I calculated a proportional width (PW) of each annual DCD layer. I divided annual DCD width by

cumulative width of DCD layers up to and including that width. This procedure expressed a given DCD layer as a proportion of total DCD that occurred up to that time.

Direct comparison of annual DCD widths between years was further confounded by visual observations that DCD rate decreased with increasing age. This same phenomenon occurs in tree rings (Fritts 1976). Age, therefore, was a biological growth trend that affected annual DCD width. I had to compensate for age prior to analyses. To eliminate age-related factors, PWs were plotted against age and a seventh-order polynomial regression was fitted to the data (Fig. 2). This regression represented "expected" values relative to a best fit relationship for the data. Effect of age was factored out by generating relative width indices (RWIs) for all DCD layers as:

$$RWI = \text{Actual PW} \div \text{Expected PW.} \quad (1)$$

Relative width indices increase homogeneity of variance, reduce auto correlation, and scale the mean to approximate one (Fritts 1976:266). Relative width indices were then plotted against age to verify that age-related factors had been removed (Fig. 3). Conversion of DCD layer measurements to PWs and then to RWIs for 1 bear is illustrated in Table 1.

Known reproductive conditions and RWI values for DCD layers were cross-referenced by calendar year. Relative width indices were assigned 1 of 3 reproductive conditions: (1) no cubs produced; (2) cubs produced but lost during the first year; and (3) cubs produced and successfully reared (cubs survived first year). Mean RWI values were calculated for each

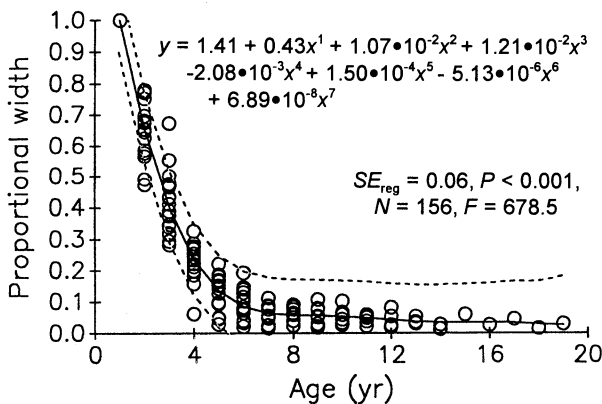


Fig. 2. Relationship between proportional widths of dental cementum layers and ages at the time of deposition for female black bears in Arizona, 1992.

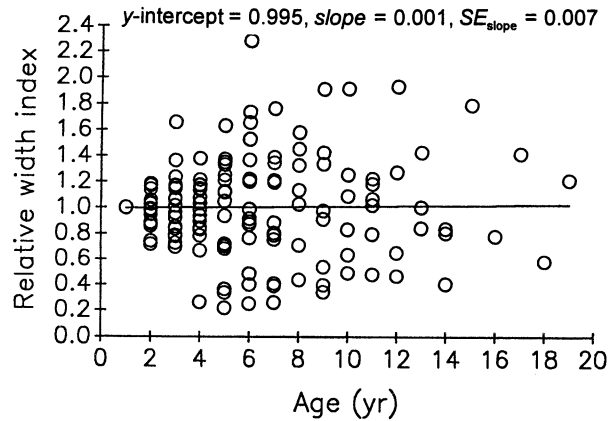


Fig. 3. Relationship between relative width index values and ages at time of deposition for female black bears in Arizona, 1992.

reproductive condition within each bear. The initial 4 years of the bears' lives were excluded because Arizona bears do not normally reproduce before the age of 4 (LeCount 1990). Therefore, these years did not have an equal opportunity of being represented in any reproductive condition. Reproductive conditions (represented by \bar{x} RWIs) were compared using Wilcoxon matched-pairs signed-ranks tests (Snedecor and Cochran 1989:140). Each comparison only included those females that exhibited both known reproductive conditions.

Predictive Model

To build a predictive model I assumed data from 17 females truly characterized the female black bear population in Arizona (Table 2). The probability that a given cementum layer (i.e., RWI) represented a given reproductive condition could then be calculated. This was done with a modification of the *t*-test (Sokal and Rolf 1981:229). Critical *t*-values for a reference sample size of 17 were 2.16, 1.77, and 1.35 for *P* = 0.05, 0.10, and 0.20, respectively.

RESULTS

Between 1980 and 1990, teeth were collected from and oxytetracycline injections given to 50 females. Tetracycline-labeled teeth (*n* = 41) were collected during recaptures. Fifteen labeled teeth were collected within 1 year and showed little or no cementum beyond the tetracycline label.

Tetracycline labels defined known periods of DCD between injection dates or from injection date to tooth

Table 1. Comparison of dental cellular cementum deposition during years of different reproductive condition for a female black bear (Bear #71) in Arizona.

Cementum layer	Measurements (mm) ^a			Proportional widths (PW)		Relative width indices (RWI) ^d	Known reproductive history
	Line 1	Line 2	\bar{x}	Actual ^b	Expected ^c		
1	17.3	18.7	17.98	1.0000	0.9986	1.0014	
2	26.2	20.8	23.48	0.5663	0.6585	0.8599	
3	24.0	18.9	21.40	0.3405	0.4058	0.8391	
4	22.3	20.8	21.50	0.2549	0.2366	1.0773	
5	15.2	12.9	14.05	0.1428	0.1360	1.0499	No cubs
6	2.7	4.2	3.45	0.0339	0.0846	0.4006	Cubs reared
7	7.9	9.1	8.45	0.0766	0.0635	1.2071	No cubs

^a Refer to Fig. 1.

^b Based on actual measurements of cementum layers (e.g., $PW_c = C \div A + B + C$).

^c Predicted values from regression curve of proportional widths by age.

^d Values generated by $RWI = \text{Actual PW} \div \text{Expected PW}$ to remove age-related factors from all cementum layers.

extraction date. These labels showed that double lines often seen in mature female cementum represented 2 annular lines, and thus 2 years of DCD. By comparing reproductive condition by year with DCD layers in both stained tooth sections and in photographs of undecalcified tetracycline-labeled tooth sections visual observations revealed a relationship between narrow DCD layers and years of successful cub rearing (Carrel 1990).

Dental cellular cementum deposition measurements were made from 17 female black bear stained-tooth sections. Fourteen bears were included because they met all following criteria: (1) known reproductive condition by year; (2) clearly defined and dated DCD layers for the same years; and (3) at least 1 known event of successful cub rearing during those years. Three additional females were included to increase sample size of the other 2 reproductive conditions; no cubs produced and cubs produced but lost. Eleven females had tetracycline labels in their cementum.

Total age, added together for all 17 bears, was

Table 2. Mean relative width index for reproductive conditions of female black bears, Arizona, 1992.

Reproductive condition	<i>n</i>	$\bar{x}RWI^a$	SE
No cub	14	1.243	0.082
Cubs survived	14	0.537	0.062
Cubs died	9	1.216	0.152

^a RWIs for age ≤ 4 excluded.

determined by DCD count to be 156 years. A total of 68 nonreproductive years occurred before sexual maturity (< 4 years). Of 88 years of possible cub production, reproductive conditions were unknown for 37 years of life prior to capture. Thus, reproductive conditions were known for only the remaining 51 years (Table 3). From observations during capture-recapture and monitoring of radio-collared cubs, it was determined that 23 were years of no cub production, 10 were years of cub(s) produced but lost, and 18 were years of cub(s) successfully reared (LeCount 1984, 1987a, 1990).

A curvilinear relationship ($r^2 = 0.97$, $n = 156$, $F = 678.5$, $P < 0.001$) was found between actual PWs of cementum layers and age at time of DCD (Fig. 2). Relative width indices regressed on bear age, verified that no significant age effect ($r^2 = 0.0002$; $P = 0.857$) remained (Fig. 3). Female DCD was reduced ($Z = -2.981$, $n = 12$, $P = 0.003$) during years when cubs were successfully raised ($\bar{x}RWI = 0.55$, $SE = 0.07$) compared to years when the same females produced no cubs ($\bar{x}RWI = 1.25$, $SE = 0.09$). Female DCD was also reduced ($Z = -2.366$, $n = 7$, $P = 0.018$) during years of successful cub rearing ($\bar{x}RWI = 0.63$, $SE = 0.08$) compared to years when cubs were produced but lost ($\bar{x}RWI = 1.29$, $SE = 0.18$). Dental cellular cementum was not significantly different ($Z = -0.280$, $n = 8$, $P = 0.779$) between years when no cubs were produced and years when cubs were lost.

Predictive modeling resulted in a cub year RWI value of 0.559 that still permitted differentiation from a no

Table 3. Known reproductive histories of black bears in Arizona, 1975-90; A = central Arizona (LeCount 1984), B = north-central Arizona (LeCount 1987a), and C = east-central Arizona (LeCount 1990). Reproductive data: number = cubs produced, (number) = cubs successfully raised.

A.

Bear #	Year								Date ^a	Age ^b
	1975	1976	1977	1978	1979	1980	1981	1982		
25	0	0	0	2 (0)	2 (2)	0	2 (2)	-	2/03/82	9
27	0	2 (2)	2 (0)	0	0	3 (2)	0	-	2/20/82	13
37	-	0	1 (0)	0	0	-	-	-	2/25/80	7
47	-	-	2 (2)	0	2 (2)	0	2 (2)	-	2/17/82	14
49	-	-	0	0	2 (0)	2 (-) ^c	-	-	1/25/80	7
55	-	-	-	0	1 (1)	0	2 (0)	2 (0)	1/18/83	20

B.

Bear #	Year						Date ^a	Age ^b
	1980	1981	1982	1983	1984	1985		
71	0	1 (1)	0	-	-	-	3/15/83	7
73	0	2 (2)	-	-	-	-	3/04/82	7
83	2 (2)	0	2 (0)	-	-	-	3/12/83	9
94	-	0	2 (0)	1 (1)	-	-	3/13/84	14
100	-	1 (1)	-	-	-	-	4/23/82	5
118	-	-	0	0	0	0	3/04/86	5
128	-	-	-	0	2 (2)	-	11/10/84	5
133	-	-	0	2 (0)	2 (2)	0	3/08/86	12

C.

Bear #	Year			Date ^a	Age ^b
	1986	1987	1988		
191	? (2)	0	? (0)	3/23/89	11
197	-	2 (1)	0	3/21/89	6
200	-	2 (2)	-	9/02/89	6.7

^a Collection date of tooth used for measurements.

^b Cementum age when tooth (for measurements) extracted.

^c Final fate of cubs unknown.

cub or a year that cubs were lost ($t = -2.161$). This means, on average, RWIs ≤ 0.559 can be assigned as representing successful cub rearing years with 95% confidence. If one is willing to accept a 90% confidence, RWIs can be ≤ 0.682 ($t = -1.772$).

DISCUSSION

Two problems had to be overcome to compare DCD during successfully-reared cub(s) years and no cub years. First, morphology of bear teeth precluded "knowing" that tooth sections of different individuals were taken in the same position on a tooth. Because absolute DCD layer width may change depending upon where sectioning is done, it was necessary to calculate a growth ratio to make DCD widths comparable among individuals. The approach taken was to generate a proportional width, which resulted in a classical inverse growth curve. The assumption inherent in this approach was that relative width of a given DCD layer to adjacent layers is constant within a given tooth.

The second problem was that rate of DCD was strongly, but nonlinearly, correlated with age. As a bear aged, rate of DCD underwent a relative but predictable decline. This decline was least during the first 4 years of age. This problem was solved by best-fitting a curve to a proportional width series and calculating relative width indices (RWIs). This procedure is routinely used in standardization and analysis of tree growth rings (Fritts 1976).

In analyzing DCD differences during various reproductive conditions, small sample size of females called for conservative statistical methods. A Wilcoxon match-pairs signed-ranks test was used to test RWI differences for reproductive conditions within each bear. This avoided variations that occurred within individual bears. Additionally, because the objective was to test DCD equality between control (no cub) years and treatment (successful cub rearing) years, inclusion of treatment years in producing the standard DCD curve from which RWIs were generated was taken as a conservative approach.

In some cases reduced DCD was very pronounced, resulting in appearance of double lines. Consequently alternating thick and thin annual DCD caused a repeated, paired pattern of annuli. In a few cases, no light staining cementum was visible between 2 annuli in very old females. Annuli appeared as a single extra darkly-stained annulus. This is a source of error in aging very old females.

Although cementum resorption is possible, calcified tissues of teeth, once laid down, are not normally

remodelled as in bone (Ten Cate 1980). Thus, I considered incremental cementum line patterns a fixed and permanent record. This allowed reproductive history interpretation of successful years of cub rearing for mature female black bears.

Annuli usually are most distinct in localized thickenings of cementum, and in these thickenings, annuli of older bears can best be counted (Sauer et al. 1966, Craighead et al. 1970). I observed that narrow spacings, delineating successful cub rearings, tended to increase to an average width in these local cementum thickenings. Therefore, these areas, although useful for counting annuli in aging, are not the best areas for defining reproductive history. Regions on either side of the widest layering of cementum in transverse sections showed the best differentiation between successful cub rearing layers and no cub or lost cub(s) layers. This study did not test for section plane preferability; thus longitudinal sections may or may not allow reproductive history analysis as easily as transverse sections.

It is critical to have teeth sections that are well defined to the outer edge so accurate back-dating of cementum layers can be made. Only then, without biomarkers present, can individual cementum layers be reliably compared with internal and external factors that may influence deposition. Boiling skulls or mandibles for tooth extraction, chemical use, and abrasive physical action may damage outermost cementum layers. Outer layers are extremely narrow in very old females. Therefore, extra care must be taken in extraction and handling.

Successful cub rearing is more valuable than cub production for modeling black bear populations. Cub production refers to birth of cubs and requires intensive fieldwork to obtain. Cub mortality can be high; 25-30% (Bunnell and Tate 1981) or 34-42% (LeCount 1984, 1987a, 1990). Cub production does not represent recruitment (i.e., addition of self-sufficient individuals into a population). Successful cub rearing accounts for cub mortality, provides an estimate of recruitment, and can be acquired without fieldwork. In many areas, teeth are routinely collected from harvested bears for aging. Such teeth from mature females are available for determining reproductive success.

Likewise, age of first successful cub rearing is more valuable than age of first litter. Age of first litter for female black bears has been correlated with forage base (i.e., habitat quality) and is often reported in bear studies (Bunnell and Tate 1981). It strictly refers to birth of cubs and denotes age at which females reach sexual maturity. Age of first successful cub rearing is more important because it denotes age females

successfully add new individuals to populations.

MANAGEMENT IMPLICATIONS

Although complete surveys of reproductive history of black bear populations would be beneficial for management, there is currently no economical or practical technique available for such intensive surveys. For many areas, hunter harvest information and tooth samples from harvested bears provide the only applicable statewide management data.

This study demonstrates a valuable technique for estimating female black bear reproductive history from dental cementum deposition using teeth from captured-harvested bears. Reading events of successful cub rearing can provide a means to estimate past reproductive success and recruitment rates. These estimates could be compared to current harvest rates in different areas to determine if over-harvesting might be occurring.

Reproductive histories from cementum would also provide valuable data on age of first successful cub rearing, frequency of successful cub rearing, and a trend of reproductive potential. These data may be correlated with habitat quality in studies to indicate habitat differences or changes.

Reproductive data from harvested females is cumulative. Each succeeding year of black bear harvest will provide a clearer picture of reproductive success for past years. Therefore, it becomes important to collect multiple years of data.

Further study is needed to determine error limits for the DCD method of reading reproductive history. This could be done by conducting "blind" tests, with known reproductive history material but without the technician's advance knowledge. Management biologists should know about expected error limits to establish a level of confidence in applying this technique.

APPENDIX A. ZEISS FLUORESCENCE MICROSCOPE SYSTEM

Universal microscope, Vertical Illuminator IV FL with high-pressure mercury lamp HBO 100 W/2, Vertical Illuminator III RS (nosepiece, filter set, and filter holder), Objectives: Plan 1.25/0.04, Plan 6.3/0.16, 10/0.22, MC35 camera, Winder M, M63 automatic camera control.

Transmittance spectra of filter set for incident-light fluorescence microscopy. Designation of filter sets according to type of excitation.

Filter set for incident-light fluorescence microscopy—Excitation: Blue, spectral band 450-490; Exciter filter: BP450-490; Dichromatic beam splitter: FT510; Barrier filter: LP520. This filter set is for intense excitation with high-performance wide-band pass filters (interference filters). The transmittance curve of the exciter filter exhibits sharp cut-off on both sides. The barrier filter transmittance starts near the exciter filter peak.

In incident-light fluorescence, the illuminating beam passes through an exciter filter before striking a dichromatic beam splitter which is oriented at 45° to the illuminating beam. Dichromatic beam splitters are interference mirrors which reflect a high percentage of the light striking the mirror below a certain wavelength, while longer wavelengths are almost entirely transmitted. The interference mirror reflects the excitation wavelength through the objective, which acts as a condenser, onto the specimen. The longer-wavelength fluorescence excitation from the specimen then travels through the objective and is transmitted by the interference mirror and barrier filter prior to observation.

APPENDIX B. RECOMMENDATION FOR LABELING TOOTH CEMENTUM AND DENTINE IN BLACK BEARS WITH TETRACYCLINE

11 mg/kg of body weight, for black bear (1 and 2 years old)

13.2 mg/kg of body weight, for black bear (3-5 years old)

15.4 mg/kg of body weight, for black bear (6+ years old)

Tetracycline sources:

Liquamycin LA-200 (oxytetracycline injection)
200 mg/ml concentration
Pfizer Inc., Agricultural Division
New York, NY 10017

Oxy-Tet 100 (Oxytetracycline HC1)
100 mg/ml concentration
Anchor Laboratories, Inc.
A Division of Philips Roxane, Inc.
St. Joseph, Missouri 64502

The 200 mg/ml concentration is preferable for adult bears because of the smaller drug volume required.

Oxytetracycline is a broad spectrum antibiotic. It is an antimicrobial agent that is effective in the treatment

of a wide range of diseases caused by susceptible gram-positive and gram-negative bacteria.

Since bacteriostatic drugs may interfere with the bactericidal action of penicillin, it is advisable to avoid giving oxytetracycline in conjunction with penicillin.

Oxytetracycline does not require refrigeration; however, it is recommended that it be stored at room temperature, 15-30°C.

Teeth and Sections

Recovered teeth with tetracycline labeling should be stored in a freezer in the dark. Tooth sections must be cut without decalcifying and also should be stored in a freezer. Teeth or sections should be frozen in water to prevent drying out and cracking during long-term storage. Tooth sections are mounted on a slide in a 1:3 glycerine-water solution for microscopic examination under blue light excitation.

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