AGE DETERMINATION OF LIVE POLAR BEARS

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Abstract: The reliability of counting cementum annulations in premolar sections was evaluated for age determination in live polar bears (*Ursus maritimus*). Structural irregularities in cementum deposits decreased accuracy of age assignments. Displacements of the neonatal line toward the exterior margin of cementum growth resulted in erroneous aging for young animals. Striated, wavered, and doubled growth layers affected accuracy for older animals. Sixty-eight unlabeled tooth slides representing 57 known-age bears, examined by 3 independent investigators, revealed that only 32-45 percent were correctly aged. Analysis of age-related body measurements of 46 male and 63 female polar bears of known age showed that morphometric regression equations could be used as an age indicator. Reproductive status, general body size, and tooth replacement or wear used as criteria to tentatively age animals in the field, combined with subsequent cementum counts and growth regression analyses, provided reliable age determinations.

Differential growth in tooth cementum has been widely used to determine the age of individual animals (Klevezal and Kleinenberg 1969). Cementum annuli were confirmed in known-age brown (*Ursus arctos*) and black (*U. americanus*) bears (Rausch 1961, Sauer et al. 1966, Stoneberg and Jonkel 1966) and from teeth taken at different intervals from known-age grizzly bears (*U. arctos*) (Craighead et al. 1970). Legibility of cementum layers in brown and black bears appeared correlated to retarded growth zones formed during winter denning.

Chronological layers formed in cementum of polar bear teeth are much less consistent because activity patterns differ, particularly as related to winter denning. Lentfer (1976) noted that polar bears (except parturient females) were active, or at least intermittently so, throughout the entire year. Harington (1968) reported that the denning period of polar bears varied considerably by age, sex, and physiological state. Rausch (1969) observed structural irregularities in polar bear teeth and cited variation in annual activities as a plausible reason for cementum having little value for determining chronological age.

The Alaska Department of Fish and Game and the U.S. Fish and Wildlife Service conducted a polar bear mark-and-recovery program between 1967 and 1976. During this time, 809 polar bears including 286 known-age litter members were captured, marked, and released in the Alaska sector of the polar basin. Eighty-nine of these were recaptured 1 or more times. It became apparent early in this program that structural irregularities of cementum layering posed a serious problem in assigning ages to marked polar bears. The recovery of known-age polar bears led to a partial assessment of this problem.

This study was made between September and December 1977 to evaluate the reliability of counting cementum annulation and to ascertain whether this method, combined with age-related reproductive, growth, and tooth-wear characteristics, would improve the accuracy of age determination in marked polar bears.

METHODS

A vestigial premolar (Pm_1, Pm_2, or Pm_3) was extracted from captured animals, including those of known age. Known-age animals, first captured and marked as cubs, yearlings, or 2-year-olds, were aged accurately from postnatal features, especially the developmental stage of permanent teeth.

Lentfer (1968) described techniques to capture and immobilize polar bears. Experienced field crews tentatively estimated the ages of captured bears by tooth wear, relative body size, and reproductive status. Tooth wear was the most useful indication of age until about 12 years; annual changes then became imperceptible. Reproductive status as an indication of age was based on litter age, condition of the vulva (infantile, turgid, open) and mammaries (niple size and color, lactation in past or at present), and whether a mature female was accompanied by a mature male. Male characteristics considered were size and condition of genitalia. January was arbitrarily chosen as the birth month for all bears.

Teeth were prepared for examination by decalcifying for 24 hours in Decal® (Scientific Products D1208 or D1210) and then rinsing in running tap water for 1.5 hours. Longitudinal and some cross sections were cut at 24 microns in a cryostat at -20 to -30 C. Sections were stained by 3 methods during the ensuing years. The first method involved a dilute multiple stain for frozen sections (Steen and McIlroy 1971). The second was essentially the same except that Harris hematoxy-

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lin replaced the multiple stain. The third method was a modification of Johnson and Lucier's (1975) technique. With this technique, sections were overstained for 15 minutes in heated Harris hematoxylin for Papanicolaou staining. After rinsing 4 minutes in running tap water, sections were destained to desired contrast by dipping for approximately 4-6 seconds in an acid-alcohol solution (10 ml concentrated hydrochloric acid and 1,000 ml 70 percent ethyl alcohol). After an immediate rinse in tap water for 10-15 seconds, sections were immersed in lithium carbonate and water (20 grams lithium carbonate and 2,000 ml water) for 5-10 minutes to neutralize the acid. Rinsed sections floated on to glass slides were blotted and air-dried for 12-14 hours before affixing cover slips with Permount. In sectioning polar bear premolars, it was especially important to align the narrow root tip before mounting since it often curved laterally.

A variable-power miscroscope (14-60X) with a flourescent illuminator was used to view tooth sections. Structural characteristics were recorded by the tooth areas depicted in Fig. 1 so that investigators could trace annuli more accurately and compare teeth easily. Additional use of an overhead variable-power projector enabled 2 investigators to view sections simultaneously and discuss different interpretations. Cementum in C areas and the anterior A area was often damaged during tooth extraction and therefore did not provide discernible layering for counting.

Sixty-eight slides of teeth from known-age bears were analyzed. These teeth included 57 premolars from animals 1-10 years old and 11 lower third molars from hunter-killed animals 2-5 years old. To determine the magnitude of error and variation in reading tooth sections, slides were independently examined twice by each of 3 investigators. Investigator I had considerable experience in preparing and reading tooth sections of various mammals including brown, black, and grizzly bears. Investigators II and III were well versed in this technique but lacked recent experience. Slides were rearranged prior to a second examination; assigned ages were then compared with correct ages for analysis of error.

A series of body measurements of captured animals provided morphometric data. The series included total zoological length from nose tip, following dorsal curvature, to tail tip; total length in a straight line from nose to tail tip; body circumference directly behind forelegs; neck circumference; and hind-foot length from tip of mid-claw to heel. Linear and curvilinear multivariate regressions of these data were calculated for 46 known-age males 1-6 years old and 63 known-age females 1-10 years old.

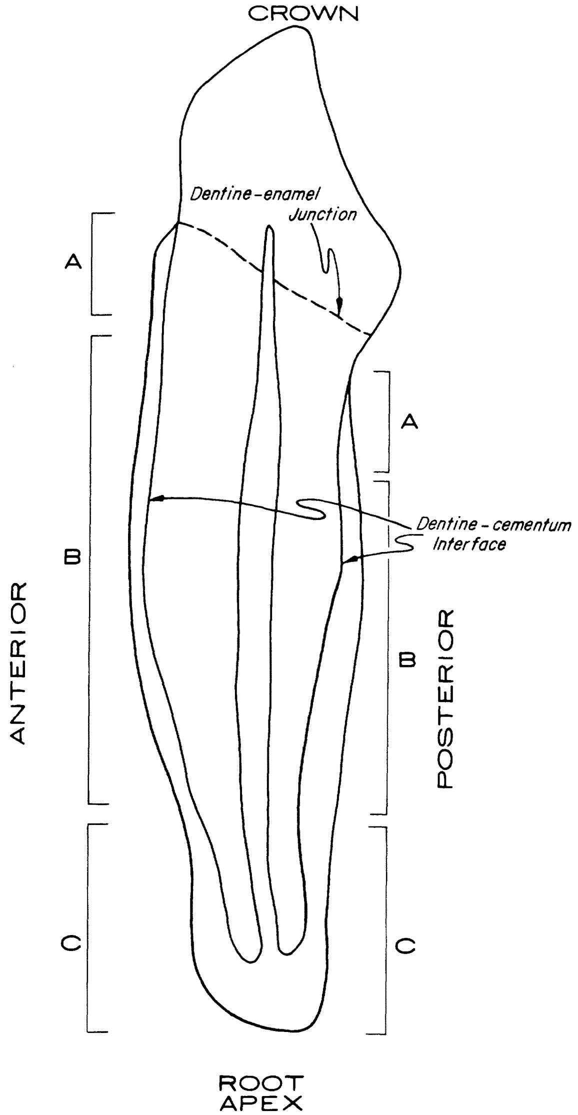

Fig. 1. Longitudinal section of polar bear Pm_1, showing cementum areas (A, B, C).

RESULTS AN DISCUSSION

Cementum Layering

Vestigial premolars apparently erupt as permanent teeth in polar bears as early as 2-3 months after birth. A fully erupted premolar of a 4-month-old cub disclosed a root length comparable to that of older bears. The root canal was open. Rausch (1961, 1969) found that because black and brown bear canines erupt between 9 and 12 months, there is 1 less cementum layer than the actual age. Similarly, the lower third molar was found in this study to erupt 9-12 months after birth and have 1 less annulation than the actual age.

Cementum deposited about the time premolar teeth erupt begins near the dentine-enamel junction and ends as a narrowing layer near the root apex. A thin, dark line extends the full length of the dentine-cementum interface, a structure described by Stoneberg and Jonkel (1966) as the neonatal line (Fig. 2).

Premolars from 14 polar bears 15-18 months old had completely or nearly closed root apices. The lightly stained growth layer was usually homogeneous except for C areas, where intense cellular activity adjacent to the dentine-cementum interface was believed to have displaced the neonatal line toward the exterior margin of the growth layer. This area stained lightly except for prominent dark spots probably comprised of interconnecting canaliculi and nuclei (Fullmer 1967). The active cellular area enlarged or invaded the root canal to thus obscure the first dark annulation. This obscurity persisted at all age levels in at least 90 percent of the teeth examined. Willey (1974) noted that the neonatal line became increasingly vague among older black bears.

Because cementum was deposited unevenly, the usual procedure was to select a counting area where annuli appeared as distinct layers. After identifying the neonatal line and the heavier band delineating the first growth layer, succeeding growth layers were counted from inside to outside. This procedure provided age estimates for premolars and for molars after adding another year to the total annuli.

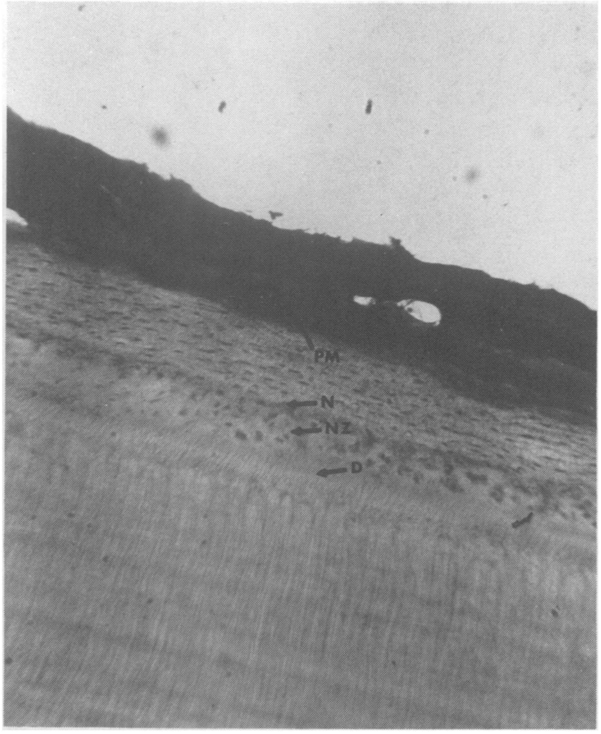

Fig. 2. Lower third posterior surface of a known-age yearling polar bear premolar showing expanded neonatal zone (NZ) and single cementum layer. Neonatal line (N), dentine (D), periodontal membrane (PM), 100X.

The single growth layer typifying 14 yearling premolars was distinct except for distortion toward the root apex. Double lines, striations, and band displacement in the C area hindered correct interpretation.

Premolar sections of 13 2-year-olds exhibited the reverse; C and B areas, particularly along the posterior surface, provided the best place for interpretation. The line denoting the first year was indistinct and sometimes absent from the A area. Cementum layers were often moderately striated along the entire root surface. Less than 31 percent of the 13 2-year-old premolars had well-defined lines.

The 5 premolars from 3-year-olds also contained indistinct lines in the A area; however, their intensity increased just distal to the enamel junction. Legibility improved in the anterior C area despite supplemental streaks and striations along lower root surfaces. Less than 20 percent had well-defined lines, and all but the anterior A area contained multilayered lines (Fig. 3A).

Cementum characteristics differed markedly for premolars of 11 4-year-olds because annual layers were less distinct and uniform than in younger animals. This lack of clarity was most evident along the lower posterior surface, where bands frequently wavered, converged, and diverged. The A and B areas of the anterior surface were legible, although less than 25 percent had well-defined growth layers.

Cementum deposition in older animals appeared to have progressed from the dentine-enamel junction to the root apex. Teeth without new deposits over the entire root surface had more cementum in A areas than in C areas. New growth is apparent later in March and during April as evidenced by 80 percent of the teeth collected during this period. Dark-stained bands denoting periods of reduced growth appeared in December specimens, and a new, well-defined line appeared in specimens taken during February and later.

Anterior cementum on the first lower premolar was deposited nearer the dentine-enamel junction than on the posterior surface because this tooth erupts at an angle, causing the anterior protion to be embedded deeper in the mandible. Other vestigial premolars developed perpendicular to the mandible and contained equal cementum deposits on both surfaces.

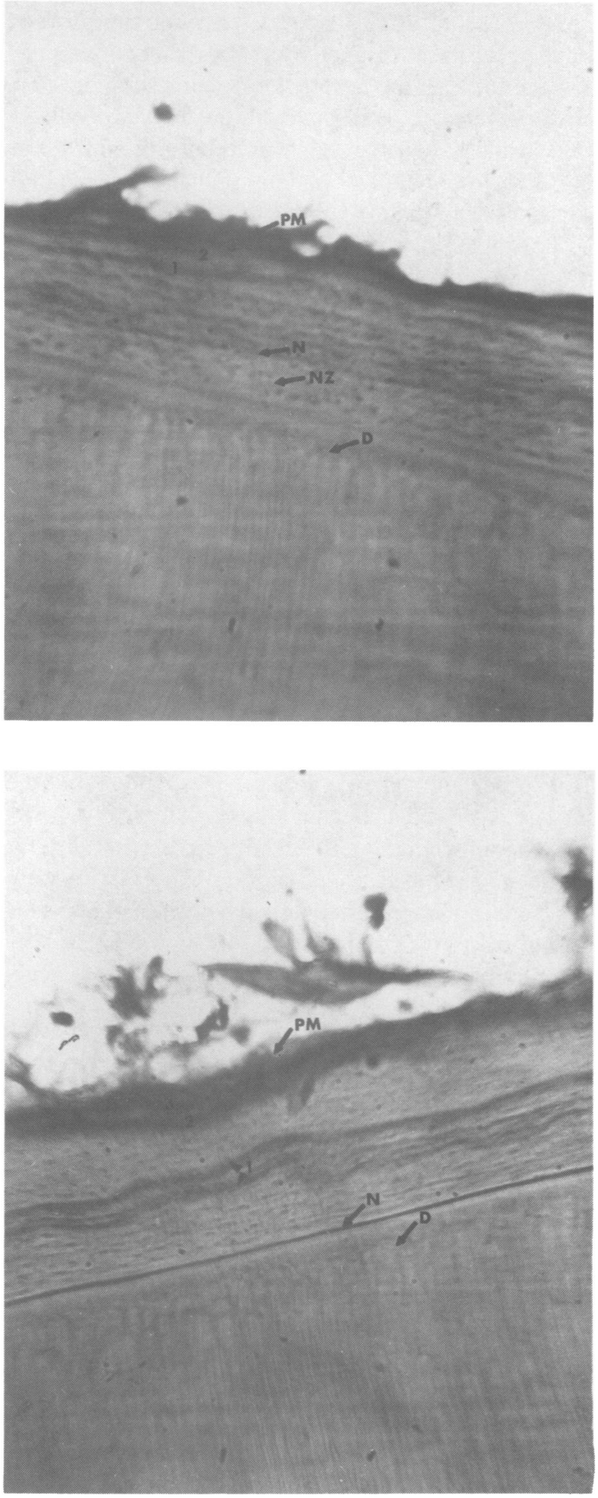

Fig. 3. Posterior surface of a known-age 3-year-old polar bear premolar showing (A) the neonatal zone (NZ) and multiple banding in Area C, and (B) double band of the first annual layer in Area B. Annual lines are numbered (A). Neonatal line (N), dentine (D), periodontal membrane (PM), 100X.

The first 2 growth layers were usually twice as wide as succeeding layers, with very narrow layers occurring after 5 or 6 years. Cementum deposits were widest along the posterior surface, particularly along the inward curved segment of B area. After the third or fourth layer had been deposited, succeeding annuli had uniform widths except near the exterior margin, where recent growth was sometimes narrowed. Double banding was prevalent, which may be described as the division of a single line into 2 dark lines bordering a lightly stained area (Fig. 3B). Because of double banding, dark-staining annuli often apeared as wide or wider than lightly stained growth layers; this occurred regularly in B and C areas of premolar sections.

In contrast to premolars of other bears as well as sea otters (*Enhydra lutris*) (Schneider 1973), harbor seals (*Phoca vitulina*), and sea lions (*Eumetopias jubatus*) (personal observation), polar bear premolars were without bulbous cementum deposits at the apex.

Growth layers were usually absent from the apex but, if present, were compacted toward the exterior margin. As well-defined lines approached the apex, they became discontinuous and illegible. Lines in the posterior segment of the C area were displaced and illegible. Lines in the posterior segment of the C area were displaced to the exterior margin in 20 percent of the premolars. Cellular activity associated with the neonatal line is believed to have caused the initial band to waver toward the exterior margin and to have compressed succeeding bands in these instances.

Lines along the posterior surface wavered along or distal to the inward curved segment of B area. This area is usually much wider than other areas, and wavering was more pronounced among premolars of immature polar bears but decreased in older specimens, particularly those with compact lines. As growth diminshed in older animals, cementum layers became more compact toward the outside surface. Lightly stained lines or striations appeared frequently among older specimens (Fig. 4). Irregularities such as swirls and indentions occurred among all ages.

Premolar specimens from 11 animals 5-10 years old exhibited characteristics similar to those of 4-year-old animals. These similarities included about the same legibility and better band definition in A and B areas of the posterior surface. Annuli compactness and a reduced incidence of multilayering constituted major differences.

Difficulties encountered in assigning ages to polar bears arise from indistinct annuli, double bands, multiple streaks, and striations. These irregularities are

Fig. 4. Area C, anterior surface of a known-age 4-year-old polar bear premolar showing multiple striations. First growth layer obscured by neonatal zone (NZ). Dentine (D), periodontal membrane (PM), 100X.

probably associated with differences in food availability and other environmental conditions such as weather and breeding season. Protracted environmental conditions may cause intermittent denning throughout the long arctic winter and consequently disrupt the retarded growth layers and alter the legibility of cementum layers.

At present, the only way to determine whether lines are annual or not is to compare the relative width and numbers of growth layers. Although double lines occur frequently, they relate to a single annulation or to retarded growth layers. Double lines usually develop midway in root sections and converge or disappear toward the root apex. Interpretation can therefore be made near the dentine-enamel junction and above the root apex. Multiple streaks and striations often appeared in sections moderately stained with hematoxylin. With a certain amount of skill these structures can be distinguished from principal bands from which cementum layers are counted.

Age determination can be further complicated by compacted, uneven layers, particularly in outer growth layers. Under these circumstances any narrow, dark-staining lines can be equated with lighter or unstained growth layers to ascertain age. Low magnification often shows individual patterns that can be interpreted more easily than with greater magnifications. In teeth of older bears, annual layers may be more discernible if lightly stained. Future improvements in staining might resolve some of these problems.

Accuracy of Assigning Ages

A Student's t-test revealed no significant difference in accuracy of assigning ages to males and females ($t=0.86$, $P<0.1$), so data were combined to determine accuracy of assigning ages. Results of independent age assignments made by 3 persons for 57 unlabeled slides from known-age bears are diagramed in Fig. 5. An error of 2 years was considered to be unduly large.

The most experienced investigator (I) assigned correct ages to 45 percent of the 57 slides and made errors of 2 or more years for 2-, 4-, and 9-year-olds. For the less experienced investigators, only 32 and 37 percent were correct, with errors of 2 or more years made in all age-classes. All 3 investigators erred most frequently at the 3- and 4-year age levels. Repeating the examination for the second time, accuracy at the younger age level

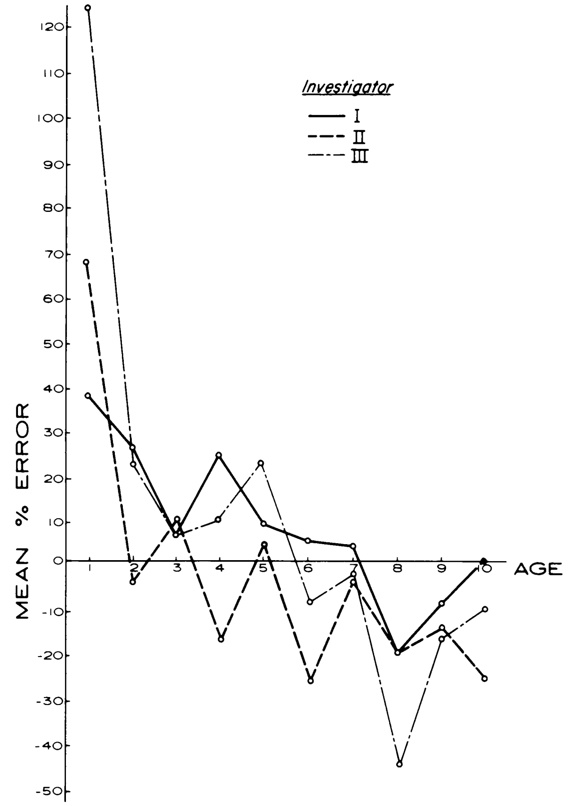

Fig. 5. Mean percentage error of ages assigned by 3 investigators from cementum annuli counts of polar bears.

improved for Investigator III but slightly decreased for the other investigators. These percentages are less meaningful because of the few specimens 5 or more years old.

The results of both examinations were combined to calculate a mean percentage error using the following equation:

$$\text{Mean \% error} = \frac{\Sigma \frac{A}{N} - K\ (100)}{K},$$

where A=assigned age, K=known age, and N=sample size.

Each investigator consistently overestimated ages of younger bears and underestimated ages of older bears (Fig. 5). Age assignments made by Investigator II varied from low to high until the 5-year level. The same individual tended to underestimate even ages and overestimated odd ages; the others began at 6 and 7 years to underestimate all ages. Similar inaccuracies were realized with molar sections.

Although differences in staining techniques have biased the results, this accuracy examination illustrated the magnitude of error inherent in counting cementum annulations in polar bear teeth. Double lines were a primary source of error, a situation that evidently persists throughout the life-span of most polar bears. Errors of 2+ years increased during even years as shown in Fig. 5. This increase may be partly attributed to a higher probability of misinterpreting an even number rather than an odd number of lines. The frequency and the magnitude of error present a serious problem, particularly among younger animals, the largest segment of the marked populations. For example, a 2-year error for a actual 4-year-old bear constitutes a 50 percent error. The width of growth layers in younger animals, however, reduces the probability of a miscount attributed to double lines. Compressed lines increased the probability of errors of 2+ years in older animals.

Age-related Morphometrics

Linear and curvilinear regression analyses of age-related body measurements were calculated for 46 known-age males, ages 1-6, and 63 known-age females, ages 1-10. Because growth diminishes after sexual maturity, at approximately 5 years for females especially (Manning 1964), 2 regressions were calculated using females 1-4 and 1-10 years old. The female sample contained all ages except 5- and 6-year-olds and the mark-recapture program has yet to provide known-age animals older than 10 years for study. Curvilinear regression provided higher correlation coefficients than linear regression. Table 1 presents curvilinear correlation coefficients. The highest age correlations in males and females were neck circumference, total length, and straight-line length. Correlation between age and size was greater for males than females. The lowest correlation in both sexes was between age and hind-foot length. Females had a greater correlation for ages 1-4 than when all ages were combined.

A standard forward stepwise regression was applied to determine the best combination of measurements. Equations for ages 1-4 are more meaningful than for ages 1-10 because of the small sample beyond 5 years (2 males, 5 females). These equations are:

$$\text{Males age 1-6} = 9.25 - 0.558(N) + 0.015(N^2) + 0.00165(SL^2) - 0.163(SL) + 0.00024(TL^2)$$
$$r^2 = 0.93$$

$$\text{Females ages 1-10} = 31.92 - 3.446(N) + 0.091(N^2) + 0.0007(SL^2)$$
$$r = 0.70$$

$$\text{Females age 1-4} = 11.58 - 0.819(N) + 0.0237(N^2) + 0.0022(SL^2) - 0.1799(SL)$$
$$r^2 = 0.78,$$

where N=neck circumference, SL=straight-line length, TL=total length (spinal curve), and r_2=coefficient of determination.

The coefficient of determination (r_3) identified only 78 percent of the variable for females ages 1-4 but 93 percent of the variability for males ages 1-6. The data for each known-age bear sampled were used in the corresponding equation to compare these results with the known age. Mean errors for ages calculated by regression equations are given in Table 2. Assuming a January birth date, most bears captured between March and May would have grown for 0.25-0.42 year, so errors less than 0.50 are acceptable.

The mean error for all males is within 0.3 year except for age 6, which is underestimated. The males were aged accurately by the above equation (e.g., a 4-year-old bear in March would be 4.25 years, and 4.18 years by the equation). The age equation for 1-to 4-year-old females alternated between over- and under-estimation and, except for age 4, mean error for each group was within ±0.3 year.

Generally, equations became inaccurate at the upper limit as evidenced by the female equation for ages 1-10. For ages 8, 9, and 10, the equation underestimated the actual age by about 3 years. The small sample of older bears biased the equations toward younger animals and also slightly increased their ages.

The possibility that polar bears form discrete popu-

Table 1. Correlation coefficients for body measurements and ages of 109 known-age polar bears. *TL* = total length, *SL* = straight-line length, *G* = girth circumference, *N* = neck circumference, *HF* = hind-foot length.

Sex and age (years)	*TL*	TL^2	*SL*	SL^2	*G*	G^2	*N*	N^2	*HF*	HF^2
Males										
1-6	0.88	0.91	0.87	0.89	0.87	0.89	0.90	0.91	0.84	0.85
Females										
1-4	0.75	0.76	0.73	0.77	0.72	0.72	0.77	0.78	0.56	0.57
1-10	0.69	0.71	0.63	0.67	0.66	0.67	0.74	0.76	0.49	0.51

Table 2. Mean error in years calculated from regression equation. Sample sizes are in parentheses.

Regression equation	Known age									
	1	2	3	4	5	6	7	8	9	10
Males										
1-6	0.15(27)	0.14(8)	0.05(1)	0.18(7)	0.08(1)	−0.30(2)	-	-	-	-
Females										
1-4	0.04(29)	−0.10(24)	0.29(1)	−0.86(4)	-	-	-	-	-	-
1-10	0.12(29)	0.26(24)	1.68(1)	0.66(4)	-	-	1.45(1)	−3.38(2)	−3.31(1)	−2.83(1)

lations (Jonkel 1970; Larson 1971; Manning 1971; Lønø 1972; Lentfer 1974, 1975; Stirling 1974) would require separate regression equations for each population. At this time it would be difficult to obtain large enough samples, especially for older animals, to evaluate this relationship.

Evaluation of Aging Criteria

Age estimates based on cementum annuli combined with individual regressions of age-related body measurements and with teeth and reproductive characteristics were used to evaluate the individual ages tentatively assigned to 546 polar bears of unknown age. Ninety percent of the bears were believed to be less than 11 years old when first captured.

Of 546 animals, 70 percent of the assigned ages required no adjustment, whereas 20 and 10 percent had to be adjusted by 1 and 2 years, respectively. Bears older than 11 years exhibited less conformity; 17 percent required 1-year and 25 percent required 2-year adjustments. The same tendency to age younger animals as older animals and vice versa prevailed, however.

Tentative age estimates made in the field proved remarkably close to age estimates derived from cementum annuli. This close agreement reflected the quality of observations made on age-related tooth wear, reproductive, and growth characteristics of each immobilized bear. Age assignments were finalized if field estimates corresponded to those obtained from discernible tooth sections. Illegible or questionable tooth sections, on the other hand, prompted greater reliance upon age-related regression equations, reproductive parameters, and tentative age estimates.

A mean breeding interval of 3.4 years and a 3-year minimum breeding age (Lentfer et al. 1979) permitted age extrapolation for some females. Any disparity in litter age or frequency related to these parameters indicated the necessity to adjust ages for maternal females. Growth regression equations provided a reliable method for age determination of females up to 4 years and of males up to 6 years. As additional known-age material becomes available these processes will be refined and perhaps augmented by other criteria such as skull measurements following the technique outlined by Manning (1964).

An accurate method of age determination is prerequisite to understanding the periodicity of reproduction, age composition, and age-specific mortality in Alaska's polar bear populations. This preliminary study suggested that exclusive use of cementum annuli for age determination would adversely affect the credibility of population studies. As this age determination study continues, compensatory steps to alleviate this problem include (1) assuring that tentative age estimates of captured animals be carefully considered; (2) taking consistent and accurate body measurements; (3) improving techniques for preparing and analyzing tooth sections; and (4) combining all age-related data to arrive at the best possible age estimate.

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