EVALUATION OF AGE DETERMINATION OF POLAR BEARS BY COUNTS OF CEMENTUM GROWTH LAYER GROUPS

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Abstract: The ages of polar bears (*Ursus maritimus*) of known age from 2 to 18 years were estimated through counts of cementum growth layer groups in 105 vestigial, first premolar teeth. Each tooth was read independently by both investigators. The reader with more experience achieved an accuracy of 75% and estimated 93% of the teeth to within 1 year of the correct age. The reader with less experience achieved 58% accuracy and estimated 85% to within 1 year. Accuracy was best in the middle age ranges (i.e., 7–15 years), which are the most critical from a demographic perspective. Our method of determining the age of polar bears represents an almost 2-fold increase in accuracy over methods reported previously. Although our method required more effort and time during processing than standard methods, the higher costs were small relative to the cost of obtaining the samples. Further improvements in accuracy may be possible through the use of standard body-size measurements to identify younger animals and through having the readers with the most experience review teeth that readers with less experience flag as difficult to read.

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Key words: age determination, cementum growth layer group, known age, polar bear, premolar tooth, technique, *Ursus maritimus*.

Accurately determining the ages of individuals is critical for many mammalian wildlife studies. Scheffer (1950) first reported the use of growth layer groups in the cementum of teeth for determining the age of mammals. Today it has become the most commonly used technique for determining ages of large and long-lived mammals. Several reviews of using cementum layers for age determination have been published (Klevezal and Kleinenberg 1967, Spinage 1973, Grue and Jensen 1979, Fancy 1980), and the methodology has potential to further biological inquiry beyond simply estimating age (Myrick 1991, Coy and Garshelis 1992). For some teeth, however, investigators encounter problems with determining the number of growth layer groups unambiguously. In bears (Ursidae) the ease of use and accuracy of tooth sections for age determination differs among species and also within populations of the same species (Rogers 1978, McLaughlin et al. 1990). Polar bears, in particular, have been characterized as having teeth with cementum lines that are especially difficult to count accurately (Hensel and Sorenson 1980).

The practical utility of using extracted teeth as age estimators for both live and dead animals, coupled with the potential for accurate results from standardized laboratory and analytical methods, have led many researchers to attempt technical improvements (Allen and Melfi 1985). Our objective was to test the accuracy of our method of age determination by counting growth layer groups in the cementum of tooth sections prepared in a standardized fashion from first premolar teeth of known-age polar bears ≥2 years old.

Teeth used in this study were collected by numerous people working on many different programs throughout northern Canada. We particularly acknowledge D. Andriashek, A. Derocher, J. Lee, N. Lunn, R. Schweinsburg, I. Stirling, A. Sutherland, and M. Taylor for their assistance. S. Amstrup, G. Matson, and L.Van Daele critically reviewed the manuscript and offered many helpful suggestions. Primary funding for the project came from the Canadian Wildlife Service of Environment Canada with logistical support from the Polar Continental Shelf Project and the Manitoba Department of Natural Resources.

METHODS

Teeth were obtained from polar bears that were either captured as part of ongoing ecological studies or killed by Inuk hunters as part of an annual hunting quota. There were 105 teeth in our sample from 71 individual polar bears known to be at least 2 years old. A known-age bear was one that, from body size information and tooth eruption patterns, was determined to be a cub-of-the-year with its mother when first captured and permanently identified. Older cubs up to 3 years of age may still accompany their mother, but their age is more difficult to estimate with certainty. We decided, therefore, to be conservative and use only bears for which there was no doubt of their age on first capture.

Processing of Teeth

A first premolar tooth was obtained from each bear, using dental elevators to loosen the tooth from the connective tissue and dental pliers to extract it. Live-captured bears were anaesthetized (Stirling et al. 1989)

prior to tooth extraction. For each tooth, the unique identification number assigned to the bear and the date of sampling was written in pencil on a tag of waterproof paper that accompanied the tooth through all subsequent processing steps. Most teeth were then fixed in 10% neutral buffered formalin (Humason 1972) within 12 hours of extraction and remained in preservative for at least 72 hours. The volume of preservative was ≥10 times the volume of the tooth. A small number of teeth from bears killed by Inuk hunters were air-dried for several weeks before they were placed in formalin. No obvious differences were observed in counting cementum layers from teeth placed into formalin while fresh or after air-drying.

Decalcification, sectioning, and staining followed the methods of Stirling et al. (1977) and Stewart et al. (1996). Longitudinal sections from the center of the root canal of polar bear teeth were not necessarily clearest in all areas of the cementum; sometimes lines were clearer in a section slightly offset from the center. Therefore, a total of 60 sequential 10-μm-thick longitudinal sections, divided into 4 groups of 15 sections each, were taken from across the root canal then floated onto slightly basic water (pH = 8.5) for 20 minutes. Two sections were randomly selected from each group of 15, for a total of 8 sections from each tooth, then affixed to an albuminated glass slide.

Each tooth was aged independently by both authors. One of us (WC) had extensive experience (>15 years) in reading polar bear teeth, while the other (MAR) had less experience (<7 years) and had read considerably fewer teeth. Each reader examined the slides independently and did not refer to the known ages until after recording the results. Thus, the tooth section analysis procedure of each reader was the same as that routinely employed by us for teeth from bears of unknown age.

We counted the number of growth layer groups by examining each of 5 regions of the root on each of the 8 tooth sections, for a maximum of 40 counts/bear. Some teeth were damaged in 1 or more regions, resulting in fewer total counts for those teeth. We assumed that 1 dark-staining growth layer was laid down in the cementum region each year (Fig. 1). Each reader subjectively determined which tooth regions and sections showed growth layers most clearly and weighted the counts at those sites when assigning an age to the bear. A distal dark-staining line, contiguous with the outside surface of the root, was not counted if the date of collection was in autumn or early winter prior to 1 January (the arbitrary birth-date), but counted if after 1 January. In addition to an age estimate, we recorded the difficulty of reading and the degree of malformation or damage for each specimen. In cases where we were unsure of an age, we recorded a second possible age, almost always within 1 year of the preferred age, on the data form.

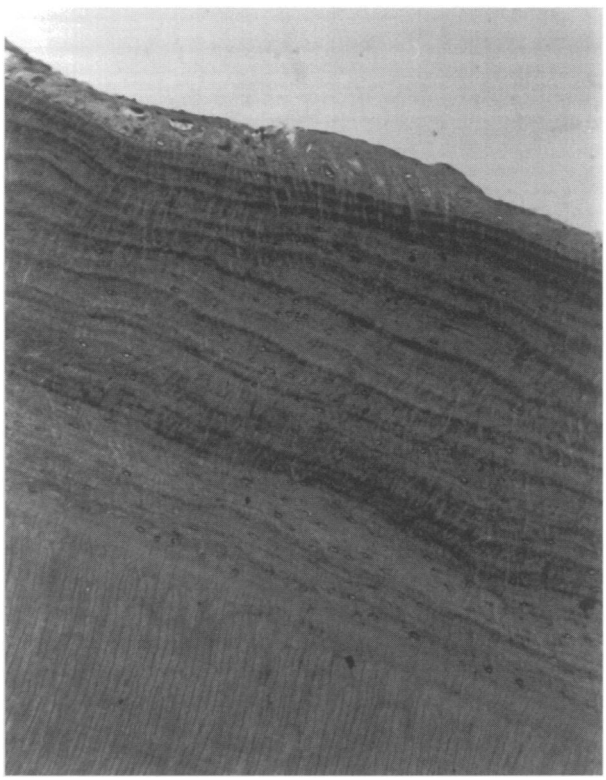

Fig. 1. Photo-micrograph of the cementum in the root of a premolar tooth taken from a polar bear known to be 11 years old, clearly showing the lines. This tooth was extracted in October, and a 12th dark-staining line had begun developing at the outer edge of the cementum.

Assessing Our Method of Age Estimation

The data were tested for normality and heteroscedasticity (Sokal and Rohlf 1995:396). For each reader, known ages were regressed on estimated ages using simple linear regression (Dapson 1980, Snedecor and Cochran 1980:153). To facilitate comparison with an earlier report (Hensel and Sorenson 1980), a mean percent error for each age group was calculated as follows:

$$\frac{\frac{\Sigma A}{N} - K}{K} \quad (100)$$

where A = estimated age for bear known to be in the age group; K = known age of group; and N = sample size in

the age group. A paired *t*-test was also used to determine between-reader precision (Sokal and Rohlf 1995:356).

We tested for differences in accuracy of age estimation between sexes using the chi-square test (Snedecor and Cochran 1980:124). This test was also used to determine whether the readers' estimates were less accurate with teeth acknowledged at the time of reading as difficult.

RESULTS

The mean age of the 105 known-age teeth sampled was 7.1 years (SD = 3.5), the median age was 6.0 years, and the range was 17 years. Reader 1 estimated the ages of 58% of the specimens correctly and 85% to within 1 year of the true age, while reader 2 scored 75% correctly and 93% to within 1 year (Table 1). The investigator with less experience (Reader 1) made an error of 2 years in assigning the ages of 12 specimens, and an error of >2 years for 3 other specimens. Reader 2 made an error of 2 years for 7 of the specimens and did not err by >2 years on any tooth. The 2 readers differed significantly in their age estimates (t = 2.1, 104 df, P = 0.04). For both readers the relationship between independently estimated age and the known age were highly significant (Reader 1, r^2 = 0.92; Reader 2, r^2 = 0.96, Fig. 2).

The mean percent error of estimated ages ranged from a maximum of 25% for 2-year-old bears aged by Reader 1, to <15% for all other age classes (Fig. 3). Both readers, but especially Reader 1, tended to overestimate the ages of younger animals and underestimate those of older ones. The greatest percent error made by Reader 1 was for bears 2, 3, and 5 years old, while for Reader 2 it was bears aged 16 and 17 years.

Sixty-six of the teeth were from females and 39 from males. Neither reader showed a significant tendency to assign an incorrect age to either sex (Reader 1, χ^2 = 0.78, P = 0.4; Reader 2, χ^2 = 0.74, P = 0.4).

Teeth that were judged easy to read showed well-defined incremental lines that were consistent in number in each tooth region (Fig. 1). Teeth that were judged problematic showed faint or ill-defined lines, lines that split or fused in different regions, or had damage to the cementum. Teeth that Reader 1 recorded as difficult to read were more likely to have been assigned an incorrect age (χ^2 = 6.8, P = 0.001) than were teeth judged not to be difficult. In contrast, Reader 2 showed no significant difference in accuracy of age determination (χ^2 = 2.6, P = 0.11) for either group of teeth (Table 2). Reader 1 flagged more teeth as difficult to read (n = 43) than did Reader 2 (n = 33) and included all those noted by Reader 2.

Considerable variability was noted in the visual definition of the incremental lines within the teeth. Typically, the first dark-staining line was spaced about as far from the dentin–cementum boundary as from the second dark-staining line. The spacing of growth layer groups then decreased with age, especially after the fourth year. The first line was frequently the least stained and most diffi-

Table 1. Number of known-age polar bear teeth assigned correct ages and the number misassigned by each reader.

		Reader 1 error (yr)							Reader 2 error (yr)						
Age	*n*	>-2	-2	-1	0	1	2	>2	>-2	-2	-1	0	1	2	>2
2	4				2	2						4			
3	8				5	3						8			
4	9				6	3						8	1		
5	16				10	2	3	1				12	3	1	
6	18		1		8	7	2				2	10	5	1	
7	13				11	1	1					12	1		
8	11		1	1	6	1	1	1		1	2	7	1		
9	8	1	1	2	2	1	1			1		6		1	
10	4				3	1						3	1		
11	4			2	2							4			
12	2				2							1	1		
14	2				2							2			
15	1				1							1			
16	1				1					1					
17	1			1						1					
18	3		1	2							2	1			
Total	105	1	4	8	61	21	8	2	0	4	6	79	13	3	0

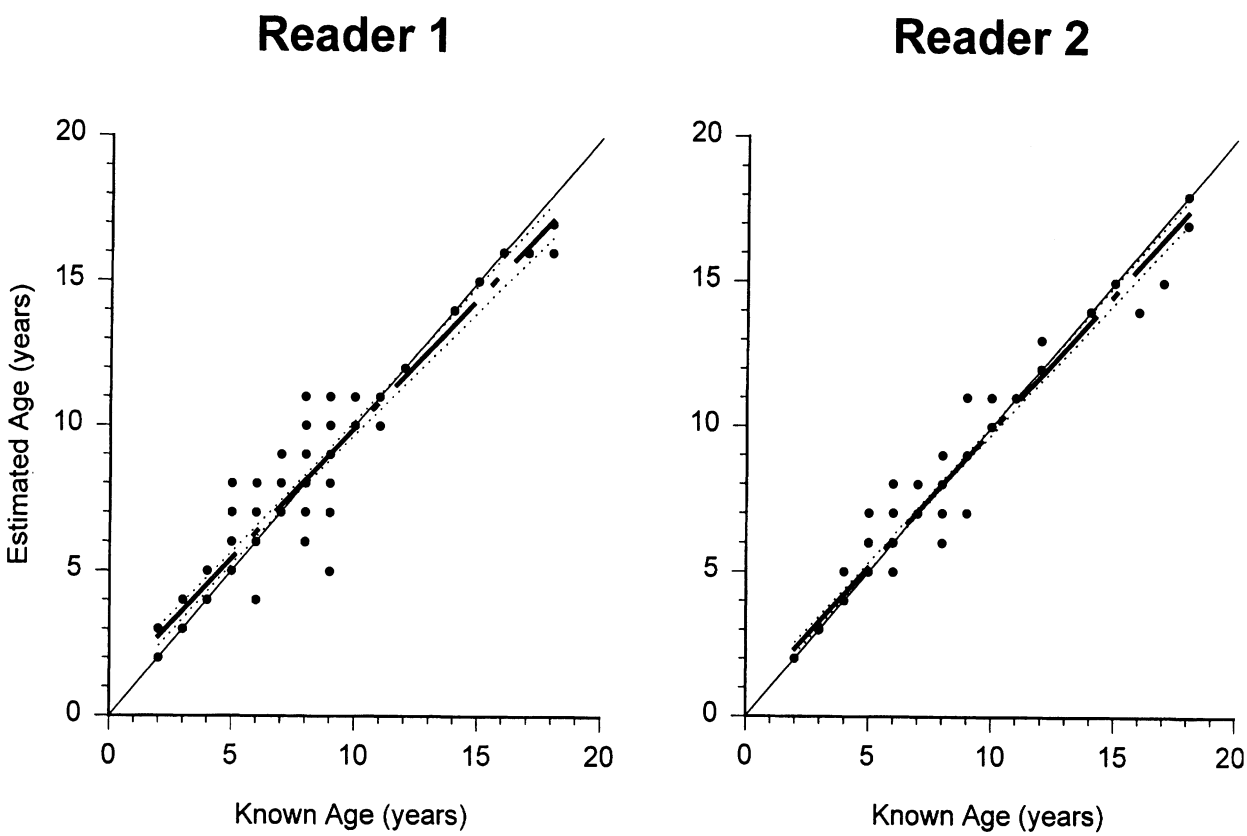

Fig. 2. Relationship between known ages and the estimated ages assigned to polar bear teeth by each reader. Hatched lines are the best-fit regression to the data; solid lines have slope = 1 (i.e., if the estimates were perfectly accurate). Note that many data are superimposed. For Reader 1, $y = 0.94 + 0.90x$ ($F = 1156$, $P < 0.0001$; SE estimate = 0.21; $r^2 = 0.92$), and for Reader 2, $y = 0.45 + 0.94x$ ($F = 2773$; $P < 0.0001$; SE estimate = 0.14; $r^2 = 0.96$).

cult to discern. With older bears it was usually possible to estimate, from the relative spacing, where the first line should lie.

DISCUSSION

Using a larger sample of teeth from known-age polar bears (n = 105 vs. 57) and with a wider range of ages (2–18 years vs. 1–10 years), our method of preparing tooth sections and counting cementum growth layer groups was more accurate than that reported by Hensel and Sorenson (1980). Whereas their 3 readers aged 32%–45% of the bears correctly, our methods allowed us to age 58%–75% correctly.

A possible complication in comparing the2 studies may be that Hensel and Sorenson (1980), by including specimens from bears that were thought to be yearlings or 2-year-old cubs when first handled, may have inadvertently introduced errors in their known-age assignments. Our more conservative definition of a known-age bear would remove any possibility of error in assigning the known age.

Like us, Hensel and Sorenson's (1980) readers tended to overestimate the age of young bears and underestimate the age of older ones. This is understandable, because teeth from older bears have many closely spaced lines in their cementum while in younger bears the cementum is relatively wide with few and poorly stained lines.

Our reader with less experience was able to evaluate which teeth were most likely to be assigned an incorrect age. Improvement in the accuracy of age estimates might result if such teeth were flagged and reassessed by a reader with more experience.

For the youngest age classes of polar bears that are independent of their mothers (i.e., 2–4 years old), standard body-size measurements might allow an independent verification of the age estimate. Full adult body length and weight are not achieved until polar bears are at least 5 years old (Kingsley 1979). Regressions of age determinations generated from known-age teeth could be used to

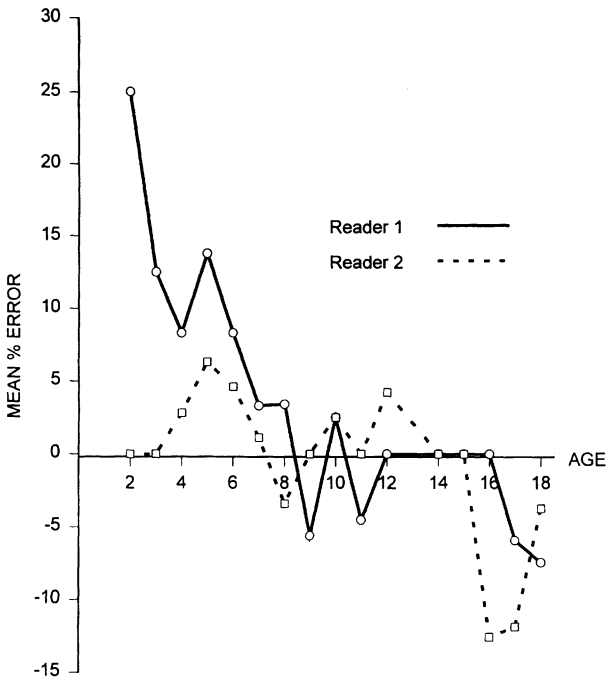

Fig. 3. The mean percent error of ages assigned independently by each reader from counts of cementum growth layers in polar bear teeth.

correct estimates of ages from bears of unknown age. However, readers probably will differ in the nature of their biases. We recommend, therefore, that all laboratories involved in estimating the ages of polar bears by counting cementum growth layers maintain a collection of known-age teeth. These can be used to evaluate the accuracy of readers in estimating ages and to train new readers.

Table 2. Number of polar bear teeth aged correctly or incorrectly and difficulty noted by each reader.

	Difficulty in reading tooth			
Tooth aged correctly	Reader 1		Reader 2	
	Y	N	Y	N
Y	18	43	21	58
N	25	19	12	14
Total	43	62	33	72
Proportion Incorrect	(0.58)	(0.31)	(0.36)	(0.19)

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