Precision and Accuracy of Estimating Age of Maine Black Bears by Cementum Annuli

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Abstract: We investigated the precision and accuracy of age estimation by cementum annuli counts for Maine black bears (Ursus americanus). Precision of age estimation was assessed by: 1) a repeated measures analysis of variance design to evaluate effects of reader, reader experience, bear sex, bear age class, and trial; and 2) pair-wise comparisons of estimated years of birth (YOB) from series of premolars removed from bears over periods ranging from 2 days to 12 years. Age estimation accuracy was assessed through pair-wise comparisons of cementum-assigned YOB to known YOB for known-age bears. Experienced readers assigned significantly (P = 0.0001) lower age estimates than inexperienced readers; greater differences in age estimates occurred in old bears. Least variation in age estimates occurred in experienced readers’ estimates for young bears (SD = 1.08 yr). Experienced readers estimated age more precisely over 3 trials than inexperienced readers (P = 0.0051). YOB estimates from multiple teeth removed from individual bears showed decreasing agreement with increasing time between tooth removal (P = 0.002), and decreasing agreement with increasing age of bears (P < 0.001). Teeth removed later in life yielded later YOB estimates than teeth removed earlier. Cementum age estimates are accurate for bears <6 years of age, but may underestimate age in older bears. Managers using cementum age estimation should recognize the technique’s limitations in precision and accuracy, and minimize changes in personnel and methodology to reduce variation in estimates over time.

Cementum annuli are considered the most accurate indicators of age for black bears (Marks and Erickson 1966, Stoneburg and Jonkel 1966, Hildebrandt 1976, Rogers 1978, Matson’s Lab 1988). However, published reports on the reliability of this age estimation technique are supported by limited data. We found no published tests of the precision of cementum age estimates for bears, and tests of accuracy were based on small sample sizes containing few old animals (Marks and Erickson 1966, Sauer et al. 1966, Stoneburg and Jonkel 1966, Matson’s Lab 1988). This paper presents results of our investigations of precision and accuracy of cementum age estimates for Maine black bears.

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METHODS

Premolars (PM12, PM2) were extracted from hunter-killed bears from 1 September - 30 November 1984, and from live bears captured throughout the year during 1975-1988. Premolars were stored in collection envelopes ≤6 months before being decalcified in a 20% formic acid solution for 44 hours, then immersed in buffered water for a minimum of 18 hours. Buffered water was prepared by mixing 3,500 ml sodium phosphate dibasic solution (9.20 g NaH2PO4·H2O/L distilled H2O). Four central sagittal sections (8 μm) were removed from each premolar, using a freezing cryostat/microtome. Sections were mounted on microscopic slides, air-dried for ≥20 min, and immersed in a solution of 550 ml buffered water and 200 ml Giemsa stain for 20 min. Sections were viewed through binocular microscopes using 100X magnification and transmitted light. Age estimation was based on techniques reported by Willey (1974). Each age estimate was obtained as the sum of all complete annuli identified on the cross-section. Effects of “double” annuli (Hildebrandt 1976) were not considered in assigning estimates; incomplete annuli or accessory lines (Sauer et al. 1966, Rogers 1978) were discounted.

Precision of Age Estimation

Variation Within and Among Readers. — A 100-slide sample was chosen from 1,309 slides prepared from premolars collected from hunter-killed bears. Slides were chosen on the basis of age estimates that had been previously assigned by our most experienced reader (>1,600 readings). They were randomly selected without replacement until each of the following categories contained 25 slides: young males (0-4 yr), old males (>8 yr), young females (0-4 yr), and old females (>8 yr). Sex-age categories were selected to ensure sufficient separation for investigating the effects of sex and age, but the distribution of bear ages within each category was not controlled. Sex of bears was obtained from hunters,
through mandatory registration reports. All slides in the sample were readable, and represented the normal range of processing variation experienced in our laboratory.

Six readers (3 experienced, 3 inexperienced) each assigned an age estimate to each slide on 3 separate occasions, yielding 1,800 age estimates for analysis. Experienced readers had read >600 slides of black bear premolar sections, including about 12 slides from known-age bears, prior to this study. Inexperienced readers were instructed in the age estimation process, but had viewed <24 slides prior to reading the sample. Individual readers viewed the entire sample in a session; 1-3 days elapsed between sessions.

Between- and within-tooth comparisons of age estimates were made using repeated measures analysis of variance. Precision was further quantified using confidence interval analysis.

**Variation Within Bears.** A sample of 115 bears, each with 2-5 premolars removed over intervals of 2 days to 12 years, provided 185 within-bear comparisons of YOB estimates. Premolars were removed from bears estimated at 1-18 years of age. Collection date for each premolar was referenced during age assignment. Although teeth were processed by different laboratory personnel during the collection period, all within-bear comparisons were based on age estimates assigned by one experienced reader. The initial premolar removed from each bear provided a YOB used as a base for pairwise comparisons with YOB estimates from subsequent premolars. Agreement of YOB estimates, and trends in differences between YOB estimates, were examined relative to bear sex, time between removal of teeth, and bear age at the time of tooth extraction.

**Accuracy of Age Estimation**

Fifty-six premolars were collected from 41 known-age bears ≤6 years of age, and assigned age estimates by one experienced reader. Premolar collection dates were referenced during age assignments. Accuracy of the resulting cementum age estimates was assessed through comparisons with actual ages.

**RESULTS**

**Precision of Age Estimation**

**Variation Within and Among Readers.** Experienced readers assigned significantly lower age estimates than inexperienced readers in both young and old age classes \((P = 0.0001)\) (Table 1); greatest differences occurred in old bears. Mean age estimates for young bears differed by 0.29 year between experience levels; mean age assigned to old bears differed by 2.11 years. Least variation in mean age occurred within experienced readers’ estimates for young bears \((SD = 1.08)\) (Table 1). Greatest variation of age estimates occurred in old bears, where variance in estimates from both experienced and inexperienced readers was about equal \((SD[exp] = 3.36, CV = 29.7\%; SD[inexp] = 4.07, CV = 30.4\%)\).

Experienced readers estimated age of individual teeth more consistently between trials than inexperienced readers \((P = 0.0051)\). Between-trial variation in age estimates increased with bear age, and decreased with reader experience (Figs. 1 and 2, Table 2). Approximate 95% confidence intervals \((\bar{x} \pm 2 SD)\) on between-trial differences in individual age estimates indicated experienced readers’ estimates for young bears varied ±0.4 year; their age estimates for teeth from old bears varied ±3 years between trials. Inexperienced readers’ age estimates showed greater variation; their age estimates for young bears varied ±1.5 years \((\bar{x} \pm 2 SD)\) between trials, and their estimates for old bears varied ±5 years (Table 2).

Because individual differences in age estimates were uniformly distributed and centered near 0, mean differences in age estimates were small. Between-trial variation in mean age estimates was assessed using approximate 95% confidence intervals for means \((\bar{x} \pm 2 SE)\) (Table 2). Experienced readers’ estimates for young bears varied about 0.07 year; their age estimates for old bears varied about 0.48 year between trials. Confidence intervals on between-trial mean differences in age assignments showed considerably larger variation in inexperienced readers’ estimates: 0.26 year for young bears, and 0.81 year for old bears. Mean age estimates differed significantly between trial 1 and trial 2 \((P = 0.025)\), but not between trial 2 and trial 3 (Fig. 3).

Experienced readers assigned identical age estimates to 59% of the teeth during all 3 trials. Ninety-two percent of their age estimates for young bears and 26% of their age estimates for old bears were identical in all trials.

<table>
<thead>
<tr>
<th>Reader experience</th>
<th>Young ((n = 50))</th>
<th>Old ((n = 50))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>SD</td>
</tr>
<tr>
<td>Experienced</td>
<td>1.87</td>
<td>1.08</td>
</tr>
<tr>
<td>Inexperienced</td>
<td>2.15</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Table 1. Mean age estimates assigned to young (<4 yr) and old (>8 yr) Maine black bears by reader experience level.
Inexperienced readers assigned identical age estimates to 31% of all teeth during the 3 trials, including 53% from young bears, and 9% from old bears.

**Variation Within Bears.** — Fifty-seven percent of 185 pair-wise YOB comparisons showed exact agreement. Mean difference in YOB estimates was -0.23 year (SD = 1.34)(Table 3). No difference in agreement (P = 0.218) was observed between the sexes. Fifty-two percent of 97 pair-wise comparisons from female bears agreed on YOB; 64% of 88 pair-wise comparisons from males agreed.

As time between tooth removal increased, agreement in estimated YOB declined (P = 0.002). Eighty percent of YOB estimates agreed when the second tooth was

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**Table 2.** Within-tooth differences in cementum age estimates assigned to premolars from young (<4 yr) (n = 50) and old (>8 yr) (n = 50) Maine black bears between 3 trials.

<table>
<thead>
<tr>
<th>Reader experience</th>
<th>Trial 1 - Trial 2</th>
<th>Trial 2 - Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age class</td>
<td>Diff. (yr)</td>
</tr>
<tr>
<td>Experienced</td>
<td>Young</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>0.020</td>
</tr>
<tr>
<td>Inexperienced</td>
<td>Young</td>
<td>-0.193</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>-0.367</td>
</tr>
</tbody>
</table>

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**Table 3.** Mean pair-wise difference in estimated year of birth based on cementum annuli counts from multiple teeth removed from Maine black bears.

<table>
<thead>
<tr>
<th>Bear Sex</th>
<th>No. agree on YOB</th>
<th>Diff. (Yr)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>97 50</td>
<td>-0.33</td>
<td>1.52</td>
</tr>
<tr>
<td>Male</td>
<td>88 56</td>
<td>-0.13</td>
<td>1.12</td>
</tr>
</tbody>
</table>

**Time Between Tooth Removal**

| <1 yr | 35 28 | 0.06  | 1.37|
| 1-4 yr| 120 67| -0.09  | 1.02|
| 5 yr  | 30 11 | -1.13  | 2.00|

**Estimated Age of Bear**

<table>
<thead>
<tr>
<th>1st Premolar 2nd Premolar</th>
<th>&lt;5 &lt;5</th>
<th>&lt;5 ≥5</th>
<th>≥5 ≥5</th>
</tr>
</thead>
<tbody>
<tr>
<td>54 45</td>
<td>58 30</td>
<td>73 31</td>
<td>0.02</td>
</tr>
<tr>
<td>58 30</td>
<td>73 31</td>
<td>-0.22</td>
<td>1.06</td>
</tr>
<tr>
<td>73 31</td>
<td>-0.43</td>
<td>1.86</td>
<td></td>
</tr>
</tbody>
</table>

**Total**

| 185 106                     | -0.23| 1.34|

* Based on initial premolar removed from bear.
removed <1 year after the first (n = 35). Teeth removed 1-4 years after the initial premolar produced the same YOB estimate in 56% of 120 cases, and 37% of 30 YOB estimates from teeth removed ≥5 years after the first premolar agreed with the initial premolar's YOB estimate.

Agreement among YOB estimates decreased as age of the bear (at the time the first premolar was removed) increased (P < 0.001). Eighty-three percent of 54 pairwise comparisons where both teeth were removed before bears reached 5 years of age agreed on YOB. Fifty-two percent of cases where the first premolar was removed when the bear was <5 years of age, and the second tooth was removed when it was ≥5 years of age agreed on YOB (n = 58). Only 42% of 73 pair-wise comparisons between teeth removed after bears reached 5 years of age agreed on YOB.

When teeth were removed >5 years apart, later teeth produced later YOB estimates than the first tooth. Mean difference in YOB was -1.13 years (SD = 1.99)(Table 3).

**Accuracy of Age Estimation**

Cementum age estimates agreed with the known age of bears in 88% of 56 comparisons (x̄ difference = +0.04 year, SD = 0.421). Cementum estimates agreed with true age in 93% of the 43 comparisons from female bears ranging from 1-6 years of age, and in 69% of 13 comparisons from males ranging from 1-5 years of age. Five disagreements involved cementum estimates 1 year older than the bear's true age, 1 bear's age was estimated 1 year younger, and 1 bear was estimated 2 years younger than its true age.

**DISCUSSION**

Most readers learn age estimation by cementum annuli through tutoring by an experienced reader, not from reading large numbers of slides from known-age bears. Age estimation criteria developed and shared in the absence of known-age reference slides may perpetuate biases and errors in age assignments. Our readers were trained “in-house”, and may have produced age estimates of greater precision than expected of estimates among experienced readers with independent training.

Several characteristics of age estimates suggest experienced readers used stronger criteria for identification of annuli than inexperienced readers. The age estimates they assigned to young bears had lower variation than inexperienced readers, and they assigned age estimates more consistently between trials. Experienced readers also assigned lower age estimates than inexperienced readers. Identification of the first-year annulus, which is closely associated with the dentine-cementum interface (Willey 1974), apparently improves with reading experience. The degree of separation of the first annulus from the interface may depend upon the bear’s nutritional condition and date of den entry during its first year of life (Rogers 1978). Annual fluctuations in fall food abundance, condition of bears, and denning dates are substantial in Maine, and may produce wide variation in distance between the dentine-cementum interface and first-year annulus. Consequently, difficulty in identification and separation of the dentine-cementum interface from the first-year annulus may have accounted for many differences in age estimates, particularly for inexperienced readers assigning ages to premolars of young bears. Experienced readers may also use stricter criteria in determining complete annuli, and consequently produce lower age estimates.

Closer spacing of annuli in older bears’ premolars probably caused the high variance (low precision) in age estimates for this age class. Within-bear comparisons of YOB showed increasing variance in age estimates with increasing time between removal of premolars, and with increasing bear age at premolar removal. In addition, within-bear YOB comparisons based on teeth removed...
several years apart suggested cementum annuli counts underestimate age of older bears. As annuli become compressed, they become increasingly difficult to separate and count. Visual separation of closely-spaced annuli was difficult for all readers, regardless of reading experience. This difficulty arose when bears reached about 8-10 years, as reported by Willey (1974).

Our sample of known-age bears demonstrated cementum age estimates were accurate for young bears, corroborating reports by Marks and Erickson (1966), Sauer et al. (1966), and Stoneburg and Jonkel (1966). Precision analysis indicated most teeth from young bears would be read within 1 year of true age by experienced readers.

MANAGEMENT IMPLICATIONS
Cementum annuli counts provide age estimates of varying precision for black bears, dependent on reader experience and bear age. Greatest precision can be expected with premolars from young (<4 yr) bears. Cementum annuli of old bears (≥8 yr) are difficult to identify and produce age estimates with greater variance.

Annuli counts appear accurate for bears ≤6 years of age, but may underestimate age of older bears. Underestimation of age in old bears may have little consequence in harvest age samples that typically contain few old bears, but could have substantial impact on age-specific parameter estimates for populations of adult animals, such as year-specific estimates of reproduction.

Valid comparisons between age distributions of samples collected over time will require standardization of laboratory and reading procedures, and use of experienced readers. Minor between-sample differences in age distributions should be interpreted cautiously, particularly if age estimates were generated using inexperienced readers or readers who have not compared age estimation criteria.

These considerations become increasingly important in small samples, where individual age estimation errors have greater influence on age distributions. Managers using cementum age estimates should minimize personnel changes and invest considerable effort in training new readers, including frequent review of known-age teeth.

LITERATURE CITED