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Individual variation in dental characteristics for estimating age of African lions

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Countries that allow sport hunting of African lions, *Panthera leo*, increasingly are mandating minimum age regulations for harvested individuals that require accurate aging techniques. However, individual variation in dental characteristics used for aging could result in errors and has not been quantified. We assessed individual variation in three dental methods for estimating age of lions using left and right paired PM²s from 31 free-ranging lions of unknown age from Zambia. Assignment of individuals to age class based on crown wear was largely consistent (87%) between paired PM²s; measures of pulp chamber closure also were highly consistent (< 4% variation) between pairs. Though pulp chamber closure varied little between pairs, calibrating pulp closure with known-aged lions is needed to fully develop this method. In contrast, cementum line counts differed between paired PM²s in 94% of lions, ranging from 1 to 7 cementum lines (i.e. 0.5–3.5 years). Of lions with cementum line counts that differed between teeth, 45% were estimated as meeting the 5- or 6-year-old minimum age used in trophy lion monitoring programs with one PM², but not the paired PM². Though individual variation in cementum line counts may render this technique unsuitable for aging lions, using the maximum or average count from both PM²s warrants further investigation. Individual variation in dental characteristics should be considered when developing lion aging techniques based on dentition.

The African lion, *Panthera leo*, is a species for which reliable age assessments are urgently needed. Sport hunting of African lions is important to the species' conservation in many countries (Baldus and Cauldwell 2004, Lindsey et al. 2012). However, concerns over the sustainability of lion hunting exist (Lindsey et al. 2013), with several studies reporting that overharvest of lions, in particular young or prime-aged breeding males, can lead to female-biased sex ratios, reduced cub survival and population decline (Yamazaki 1996, Loveridge et al. 2007, Packer et al. 2010). Conversely, age-based trophy selection models based on long-term demographic data indicate that removal of older (≥ 5 year-old) male lions has minimal adverse effects on populations, including cub survival (Whitman et al. 2004). Estimating lion age is therefore essential to management programs that allow harvest while promoting conservation and long-term population viability.

Morphological features including body size, mane size, nose pigmentation, and tooth wear have been used to assign lions to age classes (e.g. juvenile, subadult, adult (Schaller 1972, Smuts et al. 1978, Whitman et al. 2004, Whitman and Packer 2007, Ferreira and Funston 2010), however, many morphologic features vary across the species' range

(Patterson 2007). Countries that allow sport hunting of lions increasingly are adopting regulations that require harvested animals meet or exceed a minimum age standard (i.e. ≥ 5 - or ≥ 6 -years-old) (Lindsey et al. 2013, Tanzania Ministry of Natural Resources and Tourism 2013, Niassa Carnivore Project 2014, Zimbabwe Parks and Wildlife Management Authority and Panthera 2014). International permitting authorities including the U.S. Fish and Wildlife Service and European Union are considering age as part of proposed and recently adopted regulations, respectively, on trophy lion importation (USFWS 2014, European Commission 2015). Thus, for monitoring populations and managing lion hunting, improved post-mortem methods for reliable aging of lions are needed.

Mammalian teeth undergo predictable age-related changes (Bodecker 1925) and dentition is routinely used to estimate age in mammals (Klevezal and Kleinenberg 1967, Morris 1972, Spinage 1973). Common methods for estimating age of carnivores include tooth eruption (Slaughter et al. 1974), crown wear (Harris 1978, Stander 1997, Gipson et al. 2000), x-rays of pulp chamber closure (Marks and Erickson 1966, Zapata et al. 1997, Binder and Van Valkenburgh 2010), and cementum line counts obtained through tooth sectioning (Klevezal and Kleinenberg 1967, Matson 1981). Eruption of permanent teeth in African lions is usually completed by two years of age (Schaller 1972, Smuts et al. 1978), thus,

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other characteristics are necessary to age older individuals. Tooth crown wear increases with age (Schaller 1972, Smuts et al. 1978, Whitman and Packer 2007) and, although crown wear can vary regionally (Smuts et al. 1978), within a population is useful in age class assessments (Whitman and Packer 2007). Tooth pulp chambers acquire secondary dentine over time, causing pulp chamber closure to increase with age (Smuts et al. 1978). Cementum lines accumulate in annual or multi-annual increments such that the number of lines increases with age (Spinage 1976, Smuts et al. 1978, Cheater 2006). Assessments of lion tooth wear among tooth types (Schaller 1972, Smuts et al. 1978, Whitman and Packer 2007) and a comparison of cementum lines and pulp chamber closure in canines (Smuts et al. 1978) have been conducted, however, we found no study that assessed variation in dental characteristics at the individual level as applied to age estimation.

For trophy lion monitoring, understanding individual variation is critical because often only a single tooth is sampled from each lion under the assumption that results are consistent between an individual's left and right paired teeth. We assessed individual variation in three dental methods used to estimate age of African lions: 1) crown wear, 2) pulp chamber closure, and 3) cementum line counts by comparing paired left and right PM²s. We then considered individual variation in age estimates obtained from paired PM²s in the context of assigning lions to age classes as currently used in some lion harvest management programs.

Methods

We obtained paired left and right PM²s from 31 skulls of free-ranging male lions taken as trophies from 16 hunting concessions in Zambia during the 2007 hunting season. Mature lions vary in physical size and size data were not available, however each skull had fully erupted permanent dentition and therefore was considered ≥ 2 -years old (Smuts et al. 1978). We selected PM²s as they are the typical tooth being used in African lion aging programs (Tanzania Wildlife Division 2012, Zambia Lion Project 2007, Zimbabwe Parks and Wildlife Management Authority and Panthera 2014), are less likely to have suffered damage than other tooth types (Van Valkenburgh 1988, Patterson et al. 2003) and are more desirable than canines to remove from trophy lion skulls. We removed teeth from skulls, then air dried and placed them in paper envelopes labelled with the individual lion's ID number. We restricted samples to pairs where both teeth were single rooted. We assigned a second number to each envelope at random so that left and right PM²s could be scored separately to avoid assessor bias when measuring x-ray images and for cementum line counts conducted by an independent laboratory. Matched pairs were not disclosed until all measurements were completed.

We estimated age of each tooth separately using three methods before comparing age estimates between tooth pairs. We visually scored crown wear of each PM² as no wear/sharp, moderate wear/rounded, or heavy wear/flat (Schaller 1972, Smuts et al. 1978, Whitman and Packer 2007), and assigned each tooth to a corresponding age class and range: young adult, ≤ 4 years; adult, $> 4-6 <$ years; and old adult,

≥ 6 years. Crowns of two teeth were damaged during extraction (although roots were intact) leaving 60 individual teeth representing 29 tooth pairs. We then compared age estimates based on crown wear from paired left and right PM²s.

We obtained digital images of each tooth using a portable handheld dental x-ray device (Nomad Pro, Aribex Inc., East Orem, Utah). We quantified pulp chamber areas from digital images using ImageJ software (<http://rsb.info.nih.gov/ij/>). Radiographic visualization of the pulp chamber within the tooth crown is difficult (Paula A. White unpubl.) and the tooth crown in carnivores is most susceptible to age-related wear and post-mortem damage (Van Valkenburgh 2009). Therefore, we delineated the total root outline and open pulp chamber beginning below the crown perpendicular to the enamel-cementum junction and extending to the apical root tip (Fig. 1). The roots of a tooth from each of two lions were damaged during extraction and pulp chamber measurements were not possible, although crowns and root tips were intact. For the remaining 29 tooth pairs, we measured the total root area and open pulp chamber area then calculated the pulp to root area ratio. We also measured root and pulp chamber widths at the midpoint of the area measures along the horizontal plane (Fig. 1) to calculate the pulp to root width ratio for each tooth.

We submitted teeth for cementum line counts (Matson's Lab, Milltown, Montana, USA) where teeth were decalcified, laterally sectioned, stained, and mounted on slides following standard protocols (<http://matsonslab.com/cementum-aging.html>). All cementum line counts were performed by Matson's certified agers. Results consisted of the number of lines for each tooth and a letter code of A or B indicating the degree of certainty of the line count. When lines were indistinct, a count range was provided. The highest reliability code A indicated that the cementum characteristics of the tooth section very nearly matched those of a standardized aging model (e.g. one line per year). Code B indicated that

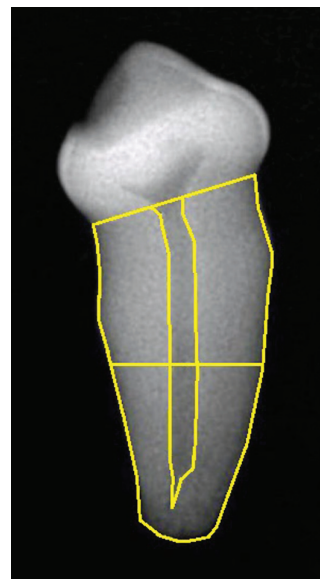


Figure 1. Digitized x-ray image of African lion PM² showing pulp and root area and width measures obtained using Image J software. Measures have been traced to aid visualization.

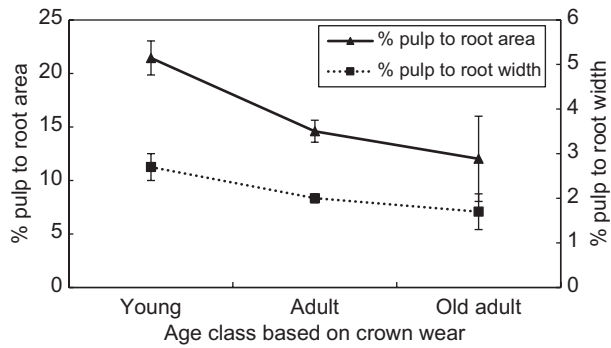


Figure 2. Comparison of percentage pulp to root area ($n = 59$) (triangles, solid line) and percentage pulp to root width ($n = 60$) (squares, dotted line) by age class based on crown wear of 60 PM²s from 31 free-ranging lions from Zambia. Values shown are means \pm 95% CI.

the correct age was expected to be within the count range provided. We estimated ages using two formulas for lions: 1) one cementum line per year, plus one for the first year of life before eruption of the permanent teeth (Smuts et al. 1978, Cheater 2006), and 2) two cementum lines per year plus one year, as deposition of secondary dentine in mammals south of the equator may occur at a rate of two lines per year (Spinage 1976).

We summarized pulp chamber measures data by crown wear categories and calculated 95% confidence intervals. We used Pearson's correlation coefficients to assess the association between paired pulp chamber measures and used lines with a slope of 1 forced through the origin for visualization.

To assess potential differences in dental characteristics between left and right teeth, we used generalized linear mixed-effects models (GLMM) in program R (ver. 3.0.2; <www.r-project.org>) to determine whether the number of cementum lines detected, crown wear category, or pulp root area was affected by tooth sidedness or the interaction of lion age and tooth sidedness. For lion age, we used the calculated mean age from cementum line counts in left and right teeth. We controlled for lion age and, for analyses of cementum lines, also controlled for certainty code of cementum line counts. We used individual as a random effect in all models. We report means with \pm 1 standard error (SE) and set statistical significance at $\alpha = 0.05$. Because of small sample sizes,

we used power analyses to estimate the probability of committing a type II error for each of the response variables.

Results

Of the 60 PM²s suitable for crown wear assessment, 7 (12%) were categorized as no wear (≤ 4 years old, young adult), 48 (80%) as moderate wear ($> 4-6 <$ years old, adult), and 5 (8%) as heavy wear (≥ 6 years old, old adult; Fig. 2, Table 1). Age class assignment based on crown wear was consistent between paired PM²s in 25 of 29 lions (86%). The remaining four pairs differed by one age class, three involved categories of moderate and heavy wear and one involved categories of no wear and moderate wear.

From power analyses, the probability of committing a type II error for any response variable was low ($b < 0.05$). There was no difference in assigned crown wear category between left and right teeth ($t_{26} = 0.072$, $p = 0.943$) and no interaction of estimated age and left or right tooth on crown wear category assigned ($t_{26} = 0.103$, $p = 0.918$).

Overall mean root area was 18.99 ± 0.22 mm ($n = 60$). Mean difference in pulp to root area ratios between matched pairs was $1.39 \pm 0.17\%$, ($n = 29$ pairs), with differences in root area ratio measures $< 2\%$ for 21 of 29 pairs. There was a strong positive correlation between pulp to root area ratios of paired PM²s ($r = 0.951$, $p < 0.05$; Fig. 3a). Mean difference in pulp to root width ratios between matched pairs was $0.02 \pm 0.003\%$, ($n = 29$ pairs), with a strong positive correlation between the root width ratios of paired PM²s ($r = 0.893$, $p < 0.05$; Fig. 3b). There was no difference in pulp root area measures between left and right teeth ($t_{27} = 0.416$, $p = 0.681$) and no interactive effect of estimated age and left or right tooth on pulp root area measures ($t_{26} = 0.134$, $p = 0.895$).

Cementum line counts were obtained from all 62 PM²s. Line counts between left and right PM² pairs differed in 29 of 31 (94%) lions. Mean difference in line counts between pairs was 2.2 ± 1.8 (SD) lines ($t_{30} = 2.73$, $p = 0.010$). Most (19 of 31 pairs) differed by 1–2 lines, although differences of up to 7 lines were observed (Fig. 4). Differences in paired age estimates based on line counts (2 lines per year + 1 year) ranged from 0.5 (42%) to 3.5 years and produced conflicting age class assignments in 48% of the lions sampled (Fig. 4).

Table 1. Crown wear scores and estimated ages of free-ranging lions from Zambia using two formulas based on cementum line counts of PM²s and frequency of cementum line count certainty codes (A = higher certainty).

	Crown wear			Cementum line count		Certainty code		Estimated age (years)	
	None	Moderate	Heavy	No. lines	n	A	B	1 line per year + 1 year	2 lines per year + 1 year
1	0	0	0	3	1	1	0	4	2.5
1	0	0	0	4	1	1	0	5	3.0
1	2	0	0	5	3	0	3	6	3.5
1	3	1	1	6	5	0	5	7	4.0
2	10	1	1	7	13	2	11	8	4.5
0	6	0	0	8	6	3	3	9	5.0
0	9	3	0	9	13	12	1	10	5.5
1	5	0	0	10	7	5	2	11	6.0
0	5	0	0	11	5	5	0	12	6.5
0	7	0	0	12	7	6	1	13	7.0
0	0	0	0	13	0	0	0	14	7.5
0	1	0	0	14	1	1	0	15	8.0

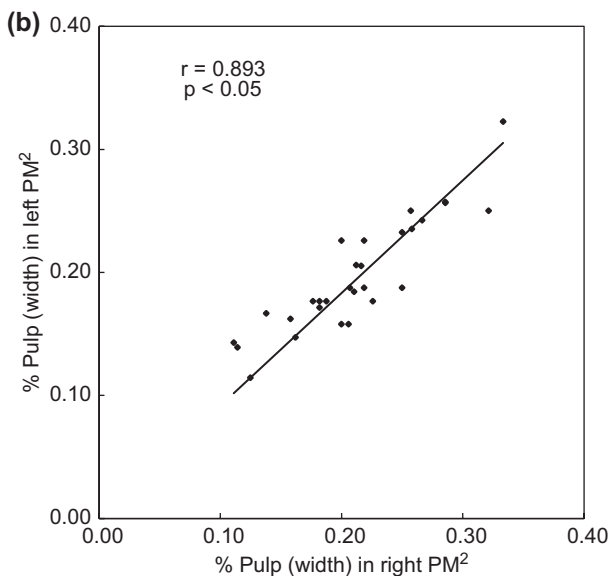
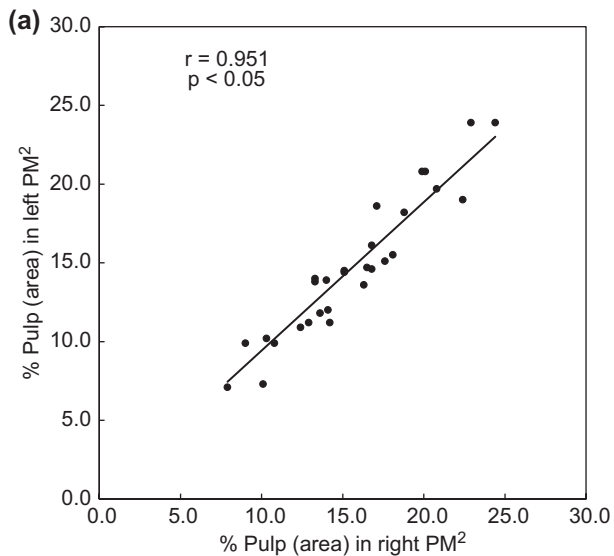


Figure 3. Pearson's correlation of pulp to root area ratios (a) and pulp to root width ratios (b) of paired left and right PM²s from 29 free-ranging lions from Zambia.

The two individuals with identical line counts had 9 lines in each PM² (i.e. 5.5-year-olds).

Cementum line counts in 58% of teeth were of high certainty (code A) and more often had a greater number of lines (i.e. older lions) than teeth of lower certainty code B (Table 1). Certainty in cementum line counts was greater ($\chi_1^2 = 6.62$, $p = 0.012$) for right PM²s (74% code A) than for left PM²s (42%). Of the ten matched pairs where both left and right PM²s were assigned certainty code A, eight pairs differed in cementum line counts. However, there was no difference in cementum line counts between left and right teeth ($t_{27} = 0.159$, $p = 0.875$) and no interaction of estimated age and left or right tooth on number of cementum lines counted ($t_{27} = 0.266$, $p = 0.792$).

Crown wear scores suggested that most (80%) lions sampled were > 4–6-years-old (Table 1). Pulp to root area and pulp to root width ratios generally declined with increasing

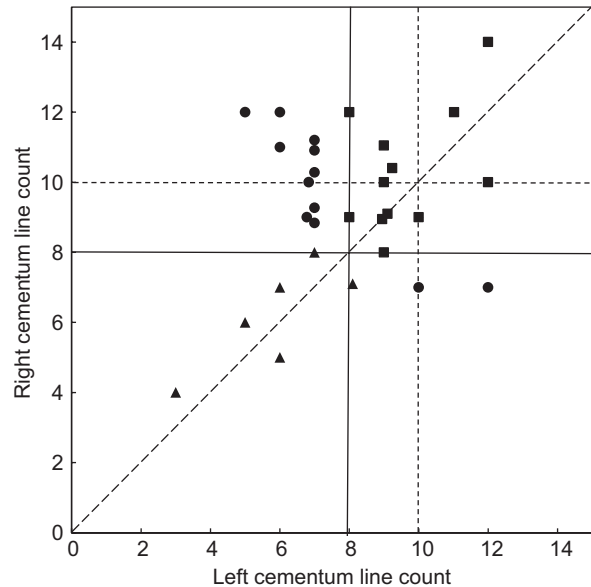


Figure 4. Bi-plot of cementum line counts of paired PM²s from 31 free-ranging lions from Zambia. Symbols deviating from the diagonal dashed line represent individuals with cementum line counts that differed between paired PM²s. Solid horizontal and vertical lines coincide with an age of 5 years and dashed horizontal and vertical lines coincide with an age of 6 years using the cementum line formula of 2 lines per year + 1 year. Triangles depict lions assigned by one or both PM²s as < 5 years old, circles represent mismatched age assignments with one PM² as > 5 years old and one PM² as < 5 years old, and squares represent both PM²s as ≥ 5 years old. Overlapping points were offset to display all pairs.

crown wear categories (Fig. 2). Using 1 cementum line per year + 1 year, mean age of PM²s was 9.6 ± 2.3 (SD) years (median = 10.0; Table 1). Using 2 lines per year + 1 year, mean age was 5.3 ± 1.1 (SD) years, (median = 5.5). Due to the variation in cementum line counts between matched pairs, in 15 of 31 (48%) cases, age class assignment based on cementum lines from a single PM² would have produced conflicting results as to whether the lion was considered ≥ 5 years old, and in 14 of 31 (45%) cases when using a minimum age standard of ≥ 6 years old (Fig. 4).

Discussion

The extent of individual variation within and among dental methods assessed for age determination varied considerably for African lions. Crown wear scores were largely consistent between paired teeth, with most PM²s having smoothed edges and moderate wear. However, scoring of wear is qualitative and assignment of teeth to age classes (three in this study) as opposed to a specific age inherently reduces variability (Storm et al. 2014). Lion PM²s are relatively protected in the maxillary tooth row, wear gradually (Smuts et al. 1978, Whitman and Packer 2007), and exhibit the least amount of damage compared to other tooth types (Van Valkenburgh 1988, Patterson et al. 2003). Though tooth wear may vary regionally in relation to substrate (Smuts et al. 1978), within populations, relative tooth wear appears useful for assigning lions to broad age classes (Schaller 1972, Smuts

et al. 1978, Whitman and Packer 2007). Though one lion in this study was assigned to two different crown wear categories of no wear/sharp and moderate wear/smooth, young (≤ 4 years old) lions are easily recognizable by other features including incomplete coalescence of skull sutures and an intact or nearly intact enamel ridge along the distal side of the upper canines (Smuts et al. 1978).

Both pulp chamber metrics were highly correlated between matched pairs. Pulp width ratios differed less than pulp area ratios as a consequence of the single, straight-line measures. Because pulp chamber shape corresponds roughly with tooth shape (Scott and Symons 1982), the exact location at which width is measured may not be critical. Thus, width ratios may ultimately serve as a replacement for total root area ratios for estimating lion age. Though we were unable to assess measurements of known-aged individuals, it is unlikely that the small differences in pulp measures between pairs ($< 2\%$ in most cases) would affect assignment of individual lions to age class.

Smuts et al. (1978) examined the maximum pulp chamber width in canine teeth of African lions and developed nonlinear regression equations demonstrating a good fit for progressive closure of pulp chamber with increasing age. As rates of closure differed between mandibular and maxillary canines and between males and females, Smuts et al. (1978) recommended use of maxillary canines or averaging values of all canine teeth when available. Smuts et al. (1978) also cautioned that pulp chamber closure was most accurate for lions 2- to 7- or 8-years old after which secondary dentine formation slows or ceases.

In contrast, reported pulp chamber measurements in lion PM²s were not strongly correlated with age with the exception of the pulp to tooth (including crown) area ratio, but sample sizes were small and 95% confidence intervals in all age classes examined were ≥ 1 year (Cheater 2006). Cheater (2006) sampled lions of both sexes across a broad geographic range and results may have been affected by regional or population-level variability. Cheater (2006) also included captive-bred lions from South Africa. Although Smuts et al. (1978) found no differences in dentine formation or rates of pulp chamber closure of canine teeth between wild and captive lions, differences in skull morphology between captive bred and wild lions occur, including some dental features (Zuccarelli 2004).

Cementum line counts were highly inconsistent and differences between paired teeth resulted in conflicting age class assignments in about half the lions. Smuts et al. (1978) reported individual variation in the deposition of cementum lines in lions, but concluded that the pattern of deposition was one line per year for lions in South Africa. Spinage (1976) reported counting 20 cementum lines of an expected 22 lines for a captive lion from Kenya. Cheater (2006) used two formulas (1 line per year and 1 line per year + 1 year) to estimate age, reporting high congruence between cementum line counts from PM²s and 'determined ages' derived from natural log ratios of tooth measurements for lions in Tanzania and South Africa. However, the differences between line counts and determined ages ranged from -2.9 to 8.2 years for Tanzania and from -3.3 to 2.8 years in South Africa. In this study, using an acquisition rate of two cementum lines

annually for Zambian lions was more congruent with crown wear scores.

Differences between Cheater's (2006) line counts and determined ages may have been due in part to use of a single PM² per lion and variability in cementum line counts between PM²s. Cementum lines in lions from Serengeti National Park, Tanzania, were considered too indistinct for counting (tooth type not reported; Schaller 1972:183). Differences among studies suggest regional differences in cementum deposition for lions. Cementum lines are most apparent where there is strong seasonality in food resources and breeding seasons (Matson 1981), and species that range across broad geographic areas may exhibit regional variation in cementum deposition. Intra-specific differences in patterns of cementum line deposition have been found in other widely-distributed carnivore species including bobcats, *Lynx rufus* (Matson's Laboratory 2013), and black bears, *Ursus americanus* (McLaughlin et al. 1990, Costello et al. 2004). Because African lions occur across a wide range of latitudes and use diverse habitats and prey (Skinner and Smithers 1990), regional variation in cementum deposition and other dental characteristics may be expected.

Cementum line acquisition also may be influenced by endogenous factors (Grue and Jensen 1979) including metabolic stressors (Asmus and Weckerly 2011) such as population density (McCullough 1996) and sex-specific stresses associated with reproduction or foraging (Weckerly 1993, Asmus and Weckerly 2011). Thus, cementum line acquisition can vary among individuals in the same population. Individual variation in cementum line counts between tooth types (Roberts 1978, Storm et al. 2014) and varying tooth locations (maxillary versus mandibular) (Knowlton and Whittemore 2001, Kershaw et al. 2005) also has been reported. Studies of cementum lines in black bear from Maine found 80% age agreement between paired PMs extracted ≤ 1 year apart (McLaughlin et al. 1990). However, a similar study found only 46% age agreement from black bear in New Mexico, with discrepancies of 1–12 years ($X = 2.8$ years) (Costello et al. 2004). Cementum age estimates from paired incisors of deer, *Odocoileus hemionus*, disagreed in 31% of animals sampled and while most (78%) pairs differed by only one year, differences up to six years were recorded (Asmus and Weckerly 2011). Paired left and right lower canines of feral cats, *Felis catus*, in Hawaii differed in cementum line counts in 36% cases (8 of 22) with a mean difference of 1.5 years and maximum difference of four years (Danner et al. 2010).

In this study, cementum line counts from matched tooth pairs disagreed in 94% of lions sampled, with a mean difference of 2.2 lines. The high percentage of mismatched ages between pairs suggests that not all age differences could be attributed to technician error because all cementum line counts were performed by Matson's trained and certified agers. Sidedness in humans during mastication can influence muscle morphology and result in associated skull pathologies (Santana-Mora et al. 2013). Hypothetically, the pressures associated with sidedness could influence pairwise asymmetry in cementum line deposition. However, the apparent sidedness seen in Fig. 4 was not supported. Further, because tooth wear is associated with mastication, sidedness great enough

to influence cementum line deposition would be expected to produce greater differences in crown wear between matched pairs than was observed. Smuts et al. (1978) noted little or no wear on the PM²s in lions 5–6-years-old, sometimes older. In coyotes, *Canis latrans*, tooth wear patterns were found to generally underestimate age of older animals (Linhart and Knowlton 1967, Bowen 1982).

Trophy lion monitoring programs typically employ multiple characteristics when assigning individuals to age class including overall tooth wear, tooth coloration, and coalescence of skull sutures in addition to other phenotypic traits such as scarring, mane size, and nose coloration (Tanzania Wildlife Division 2012, Niassa Carnivore Project 2014, White 2014, Zimbabwe Parks and Wildlife Management Authority and Panthera 2014). Crown wear was relatively consistent between pairs and appears useful in assigning lions to broad age classes. Because of limited individual variation and quantified measures, PM² pulp chamber measures obtained from x-rays hold promise for estimating age of lions especially in combination with other traits, though calibration with known-aged wild lions from different regions are needed. Cementum line counts remain an accurate method for determining age of many wild carnivore species (Matson's Laboratory 2013). However, until intra- and inter-individual variation in cementum line deposition is assessed with known-aged lions from different regions, reliance solely on PM² cementum line counts to age African lions is not recommended.

For almost half the lions in this study, comparison of age estimates based on cementum line counts from paired PM²s produced conflicting age class assignments in determining whether a lion was deemed to be ≥ 5 - or ≥ 6 -years-old. Five years of age is considered the minimum age for sustainable offtake (Whitman et al. 2004) and ≥ 5 - or ≥ 6 -years-old represents minimum legal trophy age in some countries (Tanzania Wildlife Division 2012, Niassa Carnivore Project 2014, Zimbabwe Parks and Wildlife Management Authority and Panthera 2014). Until such time as cementum line counts can be thoroughly assessed for African lions, considering the results from both PM²s as one index of age may be of value. Further understanding of intraspecific variability and continued refinements of aging techniques for lions will facilitate management and conservation programs.

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