Note

Estimating the Age of Male Gray Wolves (*Canis lupus*) Using Baculum Measurements

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Morphological characteristics of the bacula of 62 Gray Wolves (*Canis lupus*) harvested in Wisconsin were related to age estimates generated from cementum annuli analyses. Baculum analysis suggested that 47 of 62 wolves (75.8%) were correctly classified as the appropriate age category (pup, yearling, adult) assessed by cementum analyses; however, this success was limited for yearlings (53.5%) and adults (38.5%). Results could not corroborate future use of this approach for rapid aging of dead wolves. There remains a need for a wolf-aging technique that can be broadly implemented in a timely and cost-effective manner, while also preserving the inherent trophy value of an intact skull.

Key Words: Gray Wolf; age determination; baculum; canid; *Canis lupus*; cementum annuli; Wisconsin

Introduction

Understanding the age structure of wildlife populations is central to many monitoring and management programs (Mills 2013). Information on the age or age structure of a population can be used to assess numerous factors, such as population growth rates and trajectories (Skalski *et al.* 2011), harvest dynamics (Jensen 2000), and survival rates (Udevitz and Ballachey 1998). Numerous techniques exist for estimating the age of animals including patterns of tooth wear (Gipson *et al.* 2000), tooth replacement (Severinghaus 1949), and morphometrics (Brooks *et al.* 1998).

For harvested species, age-structure information is most commonly estimated by laboratory analysis of cementum annuli rings (Ballard *et al.* 1995). Although considered one of the most reliable methods available to age wolves (Landon *et al.* 1998; Gipson *et al.* 2000), accuracy is limited to 80–90% because of irregular and indistinct annuli (Matson's Laboratory 2016). In addition, its application may be hindered by the expensive and time-consuming process of exporting samples for professional laboratory analysis. Wildlife managers could greatly benefit from approaches that allow rapid age assessment in a more cost-effective and timely manner.

Thus, our objective was to determine whether we could accurately estimate the age of male Gray Wolves (*Canis lupus*) in Wisconsin based on baculum morphometrics. We were particularly interested in developing a statistical model of wolf age that would not rely on the subjective nature of age determination based on inspection of tooth wear and staining patterns (e.g., Landon *et al.* 1998). The baculum is a bone found in the penis of canids and many other mammals that, for example in marine mammals, has been shown to exhibit distinct growth patterns that can serve as a useful predictor of age (Stewardson *et al.* 2010).

Methods

We collected samples from 59 male wolves harvested by trapping or hunting in Wisconsin during the 2012–2013 and 2013–2014 seasons between 15 October and 23 December. Three wolves incidentally collected by the Wisconsin Department of Natural Resources (one roadkill collected in November 2013 and two illegally killed wolves seized in May and November 2012) were also included in the analysis.

We extracted a premolar tooth and removed the baculum from each wolf ($n = 62$). Teeth were aged by cementum annuli analysis at a commercial laboratory (Matson's Laboratory, Manhattan, Montana, USA). Bacula were processed by simmering for 12–24 h in water to remove fur and flesh. They were subsequently soaked in soapy water for approximately seven days, placed in a drying oven for 2 h, soaked again in soapy water for 2–3 days, and air dried at room temperature for 24 h. They were then soaked in hydrogen peroxide for 2–3 days and air dried for 24 h. Bacula were weighed on an Ohaus Explorer digital scale (± 0.01 g precision; Ohaus Corporation, Parsippany, New Jersey, USA), and length and width were measured with calipers (± 0.01 cm precision).

We used two approaches to relate baculum morphometrics to wolf age determined by cementum annuli. First, we developed a multiple regression model of age that included both baculum length and weight as covariates. We evaluated the support of alternative models that included only one of the two covariates using the Akaike information criterion (Burnham and Anderson 2002). Second, we collapsed estimated ages into three developmental age classes (pup < 1 year, yearling ≥ 1 year to < 2 years, and adult ≥ 2 years) that are often used in demographic modeling of canids (Webb *et al.* 2011). We then developed a multinomial model of age classes, again using both baculum length and weight as covariates and a model selection framework for determining the support of reduced models. We evaluated model predictions of absolute ages and age classes based on analyses of cementum annuli. Statistical analyses were conducted using R version 0.97.551 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Samples were assessed from 62 wolves ranging in age from zero to five years; they included 34 pups, 15 yearlings, and 13 adults. Mean age based on cementum aging was 0.90 (standard error [SE] 0.17) years. Mean baculum length and weight was 8.98 (SE 0.16) cm and 1.88 (SE 0.16) g, respectively (Table 1).

Our best multiple regression model, according to our model selection criteria, had moderate predictive power ($R^2 = 0.57$) and included only weight as a covariate ($\beta_w = 0.83$, $t = 9.10$, $P < 0.01$). This model generally under-predicted the age of wolves, particularly older wolves (Figure 1). Post-hoc visual inspection of diagnostic plots for this model suggested minimal departures from constant variance, with no points exhibiting excessive leverage based on Cook's distance.

As with our linear regression model, our best multinomial model also included only baculum weight (g) as a covariate (Table 2). This model assigned the correct age class to five of 13 adults, eight of 15 juveniles, and 33 of 34 pups, a total success rate of 75.8% among all wolves in our sample (Figure 2).

TABLE 1. Weight and length of harvested Gray Wolf (*Canis lupus*) bacula collected in 2012–2014 in Wisconsin. Age class determination was based on a multinomial model.

	Baculum weight, g			Baculum length, cm		
Age class	Minimum	Maximum	Mean (SE)	Minimum	Maximum	Mean (SE)
All	0.48	5.55	1.88 (0.16)	6.25	12.83	8.98 (0.16)
Pup	0.48	2.10	0.95 (0.08)	6.25	9.99	7.80 (0.17)
Yearling	1.62	3.91	2.73 (0.18)	9.14	11.48	10.24 (0.17)
Adult	2.30	5.55	3.33 (0.27)	9.58	12.83	10.62 (0.27)

Note: SE = standard error of the mean.

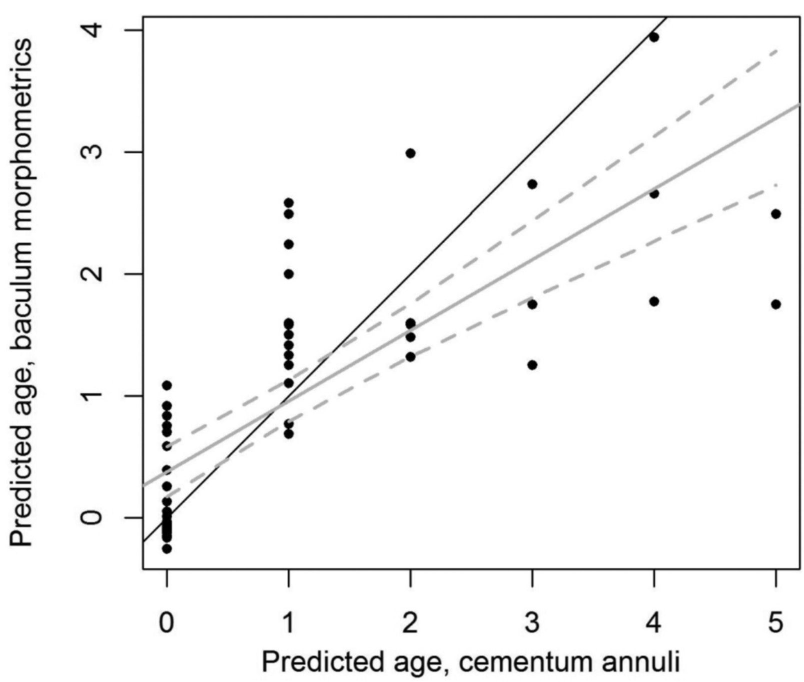

FIGURE 1. Scatterplot of estimated Gray Wolf (*Canis lupus*) ages, in years, based on cementum annuli (x axis) and baculum length and weight (y axis) of samples collected in Wisconsin, 2012–2014. The black line indicates concordance between the two approaches. The gray line indicates the predicted trend relation between the two approaches based on a linear model, while the dashed lines indicate 95% prediction intervals.

TABLE 2. Multinomial model results of baculum morphometrics based on baculum weight (g) collected from Gray Wolves (*Canis lupus*) harvested in 2012–2014 in Wisconsin.

	Intercept		Weight	
Class	β (SE)	*P*	β (SE)	*P*
Juvenile	2.94 (1.66)	0.040	−0.93 (0.54)	0.040
Pup	14.21 (4.29)	> 0.001	−6.68 (2.14)	0.001

Note: SE = standard error of the mean.

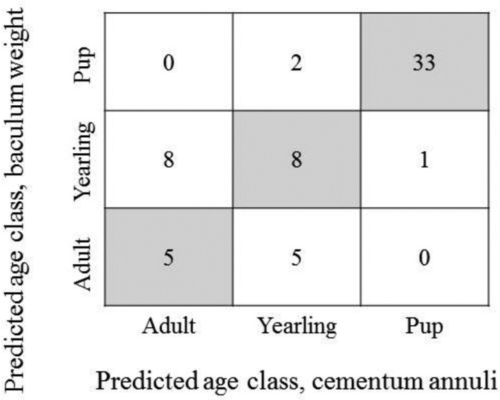

FIGURE 2. Predicted age class of Gray Wolf (*Canis lupus*) samples collected in Wisconsin, 2012–2014, based on baculum weight (g) versus predicted age class based on cementum annuli analysis. Gray boxes indicate concordance between the two approaches. Sample sizes were 34 pups, 15 yearlings, and 13 adults.

Discussion

We found that we could fairly accurately classify wolf pups using baculum length and weight, but we were unable to assign correct ages to adults and yearlings. Our more accurate classification of pups was similar to the finding of a previous study of pinniped ages and baculum development, where classification of individuals that had not yet reached breeding status was more accurate than for individuals that had already reached breeding status (Stewardson *et al.* 2010). This is likely because baculum development occurs rapidly during the period in which an animal matures sexually, but is relatively limited during earlier developmental stages, making those early developmental stages easier to identify. Although for some species the binary distinction between sexually mature and immature may be sufficient for population modeling efforts (Skalski *et al.* 2005), we consider this to be a drawback of using baculum morphometrics to estimate age structure in wolves. However, it should be noted that the cementum annuli approach, to which we were comparing our results, is known to have somewhat limited accuracy for classification of wolves as well, particularly older age classes (Gipson *et al*. 2000). Thus, our measures of accuracy should be interpreted with caution, as the true ages of our samples were unknown.

Several methods exist for estimating the age of wolves, with varying levels of accuracy and precision (Gipson *et al.* 2000; Mech 2006). The use of baculum to age wolves appears to be sufficient for pups; however, the precision of our approach is limited for yearlings and adults. Perhaps the predictive ability for older wolves could be improved with a larger sample size or by including additional baculum measurements (e.g., diameter and mass) that have shown strong relation to animal age in other species (Miller *et al.* 1999; Dyck *et al.* 2004). However, at present we cannot recommend this baculum aging technique as an improvement over other established aging methods.

The proposed benefits of baculum aging are that measuring and weighing bacula can be completed in a more timely and cost-effective manner than sending samples for cementum analyses at commercial laboratories, and it requires minimal training. Although these benefits could assist management agencies that use age-structure information in monitoring wildlife populations (Skalski *et al.* 2005), the underwhelming accuracy of this approach precludes our recommendation for future use. Further, it is limited to dead male wolves. There remains a need for a wolf-aging technique that is both quantitative and easy to use by various personnel (unlike tooth-wear analysis) and can be applied to either sex and to live or dead wolves. For harvested animals, such an approach should also preserve the inherent trophy value of the skull, which is compromised by the removal of a tooth for cementum analysis.

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