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Benefits and drawbacks of determining reproductive histories for black bears (*Ursus americanus*) from cementum annuli techniques

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Abstract

Recruitment is difficult to estimate but is essential for determining population trend. Recruitment in bears can be estimated from patterns in width of cementum annuli that indicate years with cubs. We evaluated reproductive history estimates from cementum annuli of 19,101 black bears (*Ursus americanus* Pallas, 1780) collected over 20 years to determine the benefits and drawbacks of this technique for management agencies. The technique only worked to estimate reproductive histories for 25% of submitted samples, and 49% of samples with estimates contained uncertain litters. Whether uncertain litters were counted or not caused significant variation in estimates of age at first litter, number of litters per female, and interbirth intervals. Hence, naive treatment of uncertain litters may bias analyses. A dataset we optimized to reduce bias showed that litters per female ranged from 0 to 12, mean interbirth interval was 2.07 years, and both increased as females aged. Large samples of teeth collected from harvested bears over multiple decades potentially provides a wealth of information on reproductive parameters at a minimal cost compared to intensive field studies, but until uncertain litters are understood mechanistically and can be better quantified, reproductive estimates from this technique should be interpreted with caution.

Keywords: black bear, cementum annuli, recruitment, reproductive history, *Ursus americanus*

Estimates of demographic parameters, such as productivity and reproduction, are essential in determining the viability and trends of wildlife populations (Skalski et al. 2005). Recruitment is a combination of birth rate and survival of young, which measures productivity for wildlife populations (Bolen and Robinson 1995). Precise estimates of reproduction parameters can be expensive and logistically difficult to collect. With black bears (*Ursus americanus* Pallas, 1780), the most effective means of directly determining productivity is to capture and place tracking-collars on adult females, perform visits to winter hibernation dens to count the number of cubs born, and then perform follow up visits to the dens the following winter to determine the survival of each cub. Results of these surveys can be extrapolated to the population, with precision of estimates dependent upon sufficient sample sizes (Skalski et al. 2005).

An alternative method for bear species is to use cementum annuli techniques to determine reproductive histories (e.g., Rogers 1975; Coy and Garshelis 1992; Medill et al. 2009a). Cementum annuli layers in teeth are created each year for many species, including bears (Rogers 1975; Harshyne et al. 1998). Slow-growing bone is denser and stains darker, hence dark bands are associated with fall and winter (Rogers 1975; Coy and Garshelis 1992; Medill et al. 2009a). Lighter cementum layers from spring and summer are thought to be notably thinner when a female bear has a litter of cubs potentially because of the physiological costs of pregnancy and lactation (Rogers 1975; Coy and Garshelis 1992; Medill et al. 2009a). It is currently unknown what stage of development cubs must reach to create a thinner cementum layer in their mother's teeth. This could potentially range from gestation to the end of lactation (Rogers 1975; Coy and Garshelis 1992; Medill et al. 2009a). Female black bears raise cubs for over a year before dispersal, hence individuals do not usually give birth every year causing development of alternating pattern thick and thin light (summer) cementum layers, wherein thin bands indicate cub events (Rogers 1975; Coy and Garshelis 1992; Medill et al. 2009a).

There are potential benefits and drawbacks for management agencies and researchers for using cementum annuli to estimate black bear reproductive histories. Many management agencies collect teeth to estimate ages of harvested bears, leading to large annual sample sizes in many states and provinces,

and estimates of cub events from cementum annuli techniques therefore potentially provide a wealth of demographic data. Drawbacks include potential inaccuracy, as this method does not work for grizzly bears (*Ursus arctos* Ord, 1815) (Matson et al. 1999), and occurrence of ‘uncertain litter’ observations where cementum patterns are inconclusive or unusable for an individual bear during a given year. Cementum techniques also require special expertise for sample preparation and interpretation (Medill et al. 2009b), and the accuracy of the criteria for classifying years with and without cubs are subject to variations that are poorly understood. The potential benefits and drawbacks necessitate further analyses to understand accuracy and applicability to large datasets.

We evaluated the utility of reproduction estimates from cementum annuli of black bears for use in demographic and population estimates to determine the benefits and drawbacks for management agencies. We collected teeth from female bears harvested in the state of Wisconsin over a 20 year period and analyzed their reproductive histories. An issue that may affect utility for management agencies is the effect of uncertain litters, where reproductive evidence is inconclusive for a possible litter or a section of cementum is unusable over a period of years, introducing potential inaccuracies in the reproductive histories. We first evaluated the number of teeth where reproductive histories were able to be obtained and those with uncertain litters to determine the number of teeth required to obtain sufficient sample sizes, and if there was a trend in the percent of uncertain litters over time that may coincide with changes in technique. Second, we evaluated differences in reproductive parameters (age at first litter, interbirth interval, and total number of litters produced) with and without uncertain litters being included. Third, we created an optimized dataset that minimized error from uncertain litters and determined the reproductive data of female black bears (age at first litter, interbirth interval, and total number of litters produced). We then consider their associations with bear age, to evaluate potential reproductive senescence, and trends in reproductive patterns that would indicate changes in the population dynamics. Overall these evaluations will help determine the potential benefits and drawbacks of including reproductive histories from cementum annuli in demographic studies.

Materials and Methods

We used data from female black bears harvested in Wisconsin (1995-2014). Since 1986, the Wisconsin Department of Natural Resources (WDNR) has required bear hunters to extract and submit an upper premolar tooth from each black bear harvested. These teeth were aged, via cementum annuli (Matson's Lab, Manhattan, MT, USA) and these data were used by WDNR to determine bear ages for population estimation. Beginning in 1995, teeth from female bears were also analyzed to determine reproductive histories for females.

Matson's Lab compares width of alternating cementum layers to estimate years when bears had a cub event. This includes the presence of a thin summer cementum layer followed by a "rebound" to thicker cementum layer the following summer. However, these indications are not always present, and the characteristics of cementum also exhibit variation among individuals and populations (Matson 2017), and it is therefore not possible to estimate reproductive histories for all bears. The cementum indicators also vary with age and other variables. For example, the first year of cub rearing is often most clear, and becomes less clear with subsequent litters, particularly for older bears (Matson 2017), while information for litters during the year of harvest is often incomplete because of the lack of a confirming thicker cementum layer (Matson 2017). Therefore, Matson's lab notes 'uncertain litters' when evidence is inconclusive for a possible litter or a section of cementum is unusable over a period of years.

Observation of uncertain litters create a dilemma for population analysts. The most conservative approach would be to assume that uncertain litters represent loss of or failure to produce a litter for that individual during that year. This is probably untenable since the expectation is that bears tend to produce a litter every other year and would introduce a large negative bias. Alternatively, to be conservative an analyst could remove all individuals that had an uncertain litter, but this could substantially reduce sample sizes. If not removing samples, an analyst could 1) count all uncertain litters as evidence of cub events which likely introduces small positive bias, 2) remove counts of uncertain litters which may introduce an unknown bias, or 3) develop an intermediate strategy guided by natural history and additional information.

Statistical Analyses

We used program *R* version 3.3.1 (R Core Team 2016) for our statistical analyses. We considered $p < 0.05$ to be statistically significant for hypothesis tests.

We first determined the annual number and percent of bears that had uncertain litters in their reproductive histories (where reproductive evidence is inconclusive for a possible litter or a section of cementum is unusable over a period of years). Our analyses then considered three datasets representing the three strategies above. First we compared datasets where “all” and “none” of the uncertain litters were counted as cub events. We then created a third “optimized” dataset where we tried to conservatively minimize the bias of uncertain litters. We did this by only including uncertain litters when the litter occurred within 3 years of previous litters or were the first litter for a bear, because these are the litters most difficult to determine (Matson 2017).

After testing for normality, we used Student’s *t*-test to determine if age at first cub event differed when estimated with our ‘all’ and ‘none’ datasets. We then created summary statistics and an age distribution for our optimized dataset.

We used a Generalized Linear Model (GLM) with a Poisson link to determine if number of cub events in a female’s lifetime differed when estimated with our datasets using uncertain litters and not. We then created summary statistics using our optimized dataset, and tested whether total number of litters per female significantly correlated with their age at harvest. We used a GLM with a Poisson link with number of litters as our dependent variable and age as our independent variable.

We used GLMs with a Poisson link to test for variation in interbirth interval. First, we tested if our estimates of interbirth interval differed when estimated with our ‘all’ and ‘none’ datasets. We then created summary statistics using our optimized dataset, and used this dataset for three additional tests. First we tested for potential senescence by testing if interbirth interval differed by age at harvest. We then tested if interbirth interval indicated a yearly trend.

Results

From 1995-2014, teeth from 24,309 female black bears harvested in Wisconsin were submitted to Matson's Lab, of which 19,101 were ≥ 2 years old. The lab obtained reproductive data from the cementum annuli of 4,688 of the bears ≥ 2 years old (24.5%). Of the 4,688 bears with reproductive histories, an average of 49.2% ($\pm 4.4\%$ SE) annually had uncertain litters, but varied each year from a low of 12.4% in 1996 to 83.6% in 2003 (Figure 1).

Age at first cub event differed when uncertain litters were included or not ($t_{8,819} = 8.80$, $p < 0.0001$), with the age distribution being older when uncertain litters were not included ($\bar{x}_{\text{none}} = 4.14 \pm 0.01$ SE, $\bar{x}_{\text{all}} = 3.99 \pm 0.01$ SE). When using our optimized data, most (52.3%) first cub events occurred at 4 years of age, and 96.9% occurred between ages 3 and 5 (Figure 2).

Number of cub events per female differed when uncertain litters were included or not ($z_{9,374} = -17.68$, $p < 0.0001$), with over 0.57 more cub events estimated when uncertain litters were included ($\bar{x}_{\text{none}} = 2.74 \pm 0.02$ SE, $\bar{x}_{\text{all}} = 2.17 \pm 0.02$ SE). When using our optimized data, the number of cub events a female had in her lifetime ranged from 0 to 12 ($\bar{x} = 2.56 \pm 0.02$ SE, Table 1), and the number of cub events had a positive relationship with age ($z_{4,686} = 56.41$, $p < 0.0001$, Figure 3). In our optimized dataset the 31 bears that had ≥ 9 litters were all ≥ 20 years old, the 6 bears that had ≥ 11 litters were all ≥ 24 years old, and the 2 bears with 12 litters were 25 and 28 years old respectively.

Mean interbirth interval differed when uncertain litters were included or not ($z_{14,181} = 2.24$, $p = 0.0254$), with our mean estimates larger when all uncertain litters were included ($\bar{x}_{\text{none}} = 2.061 \pm 0.004$ SE, $\bar{x}_{\text{all}} = 2.116 \pm 0.007$ SE). When using our optimized data, mean interbirth interval was 2.068 (± 0.004 SE) years. The interbirth interval significantly increased as females grew older ($z_{7,290} = 2.76$, $p = 0.0059$) (Table 2), but did not differ annually over the study ($z_{7,290} = 0.06$, $p = 0.96$).

Discussion

Our study suggests caution is needed when assessing reproductive data from cementum annuli layers of black bear teeth. The main drawback of the method is the potential bias from uncertain litters, and the imprecision these may introduce into the reproductive estimates. This may be an artifact of the commercial service used rather than having researchers develop a population-specific model of the relationship between cub events and cementum width using empirical local data (Medill et al. 2009b). However, estimates of recruitment and reproduction using this technique will depend greatly on how analysts treat uncertain litters. Including uncertain litters likely introduces positive bias, while removing uncertain litters may introduce a negative bias. We created an optimized dataset to conservatively limit errors, but without comparing estimates from cementum annuli to known reproductive histories we do not know how effective this solution is. The most conservative approach would be to remove all individuals which had an uncertain litter, but this would substantially reduce sample sizes. Despite these concerns, large collections of teeth from harvested bears over multiple decades provide information on demographic parameters at a minimal cost compared to intensive field studies.

The largest challenge of using cementum annuli techniques to estimate black bear litters is the potential sources of bias in the data, and how inference changes based on whether uncertain litters are included or not. In our case only 25% of the teeth from reproductive age female black bears that we submitted were able to be assessed for reproductive data, and an average of 49% of estimates each year contained uncertain litters. This may be partially because we used a commercial lab rather than a locally derived model (Medill et al. 2009b), but future study should focus on determining why uncertain litters occur. Mechanisms that determine the width of cementum bands in female bears remain uncertain, with potential reasons including nutritional stress and loss of calcium (Rogers 1975; Coy and Garshelis 1992; Medill et al. 2009a). Cementum width appeared tied to the duration of lactation in polar bears (*Ursus maritimus* Phipps, 1774) (Medill et al. 2009a), and if true in black bears, cementum width may only reflect survival of a litter through weaning. Until uncertain litters are understood mechanistically and can be better quantified, litter estimates from cementum annuli techniques should be interpreted with caution.

Understanding productivity, the number of young added to a population annually (Bolen and Robinson 1995), is essential for determining population trend and viability (Skalski et al. 2005). For long-lived species such as black bears, analysts must know how females at each age are contributing reproductively to populations. We found that bears most frequently had their first litter at 4 years of age, with most occurring between 3 to 5 years of age. First litters tended to be smaller than subsequent litters (McDonald and Fuller 2001), consequently changes in a bear population that shift the age at first litter from 4 years to 3 years may lead to increases in the population (e.g., Carel 1994). We found that the number of cub events was predictably strongly correlated with age, with confirmed cub events in bears as old as 27, but interbirth interval also increased with age.

A drawback of estimating reproductive histories through cementum techniques for demographic studies is that although the technique may give us a measure of when a cub event occurs, the technique does not give us any information on the size of the litter. The size of black bear litters generally varies from 1 to 4 (McDonald and Fuller 2001), but this number may vary with age (McDonald and Fuller 2001) and food availability (Rogers 1976; Noyce and Garshelis 1994). For example, individuals in artificially food-rich areas have larger litters, with an individual from farmland in Wisconsin having consecutive litters of 5 cubs (Malcolm et al. 2008). Further, if cementum patterns stem from nutritional restrictions associated with provisioning cubs, uncertainty may vary based on both habitat and litter size because large litters in marginal habitat could leave obvious patterns in the cementum whereas small litters in good habitat may leave an ambiguous pattern or none at all. Without knowing the number of cubs, estimates of recruitment are uncertain, and limit the use of estimating reproductive histories from cementum annuli for demographic or population studies. Additional research should focus on determining if there is a relationship between cementum width and litter size, food availability, and habitat quality. Cementum width did not correlate with litter size in polar bears (Medill et al. 2009a), but may vary in black bears, and if so could provide valuable demographic data.

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Table 1. The number of cub events for black bears (*Ursus americanus* Pallas, 1780) of each age in Wisconsin from 1995-2014 in our optimized dataset. We provide the sample size (n), mean, standard error, and range for bears at each age of harvest.

Age	n	mean	SE	range
2	1	1.00	0.00	1 to 1
3	50	1.00	0.00	1 to 1
4	365	1.00	0.00	1 to 1
5	763	1.11	0.01	1 to 2
6	612	1.57	0.02	1 to 2
7	640	1.92	0.02	1 to 3
8	458	2.42	0.03	1 to 3
9	414	2.75	0.04	1 to 4
10	306	3.17	0.05	1 to 4
11	240	3.74	0.05	1 to 5
12	188	4.09	0.07	0 to 5
13	162	4.54	0.06	2 to 6
14	110	4.89	0.10	1 to 6
15	93	5.39	0.11	2 to 7
16	70	5.51	0.17	1 to 7
17	59	5.83	0.17	2 to 8
18	49	6.49	0.19	3 to 8
19	30	7.00	0.28	3 to 8
20	23	7.39	0.36	1 to 9
21	13	7.23	0.57	3 to 9
22	11	6.27	0.70	2 to 10
23	13	8.08	0.55	5 to 10
24	6	8.17	0.95	5 to 11
25	3	10.33	0.88	9 to 12
26	0	-	-	0 to 0
27	3	10.00	0.58	9 to 11
28	3	10.00	1.15	8 to 12
29	2	9.00	2.00	7 to 11
30	0	-	-	0 to 0
31	1	11.00	0.00	11 to 11

Table 2. The interbirth interval for black bears (*Ursus americanus* Pallas, 1780) of each age in Wisconsin from 1995-2014 in our optimized dataset. We provide the sample size (*n*), mean, standard error, and range for bears at each age.

Age	<i>n</i>	mean	SE	range
4	10	2.00	0.00	2 to 2
5	827	2.00	0.00	1 to 3
6	1541	2.02	0.00	2 to 3
7	1115	2.08	0.01	1 to 4
8	995	2.07	0.01	2 to 5
9	663	2.08	0.01	2 to 6
10	585	2.09	0.02	2 to 6
11	394	2.14	0.03	2 to 6
12	332	2.07	0.02	2 to 6
13	214	2.06	0.02	1 to 4
14	180	2.14	0.04	2 to 5
15	121	2.09	0.03	2 to 4
16	97	2.16	0.06	2 to 6
17	63	2.29	0.13	2 to 9
18	63	2.22	0.09	2 to 6
19	23	2.57	0.41	2 to 11
20	27	2.22	0.08	2 to 3
21	11	2.27	0.14	2 to 3
22	13	2.15	0.10	2 to 3
23	4	2.25	0.25	2 to 3
24	5	2.20	0.20	2 to 3
25	3	2.33	0.33	2 to 3
26	2	3.00	1.00	2 to 4
27	2	2.50	0.50	2 to 3

Figure 1. Availability and accuracy of cementum annuli techniques to estimate reproductive histories for female black bears (*Ursus americanus* Pallas, 1780) >2 years old in Wisconsin (1995-2014). Bars indicate total number of harvested black bears for whom a tooth is submitted annually, representing the number for whom it was (darker) and was not possible (lighter) to estimate reproductive histories. The line represents the error rate, calculated as the annual percent of individuals with litters estimated that had at least one uncertain litter.

Figure 2. Ages of female black bears (*Ursus americanus* Pallas, 1780) associated with their first cub event as estimated from cementum annuli techniques in our optimized dataset.

Figure 3. Correlation between age of the female black bear (*Ursus americanus* Pallas, 1780) at time of harvest in Wisconsin (1995-2014) and total cub events estimated by cementum annuli techniques in our optimized dataset.

Figure 2. Ages of female black bears (*Ursus americanus* Pallas, 1780) associated with their first cub event as estimated from cementum annuli techniques in our optimized dataset.

254x144mm (300 x 300 DPI)

Figure 3. Correlation between age of the female black bear (*Ursus americanus* Pallas, 1780) at time of harvest in Wisconsin (1995-2014) and total cub events estimated by cementum annuli techniques in our optimized dataset.

228x172mm (300 x 300 DPI)