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Age estimation of live arctic foxes *Vulpes lagopus* based on teeth condition

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Aging individuals is a prerequisite to many studies in wildlife biology. In mammals, the most accurate methods are based on cement lines analysis and require tooth extraction. Here, we adapted a method based on tooth wear assessment in live individuals and calibrated against cement lines counts on dead individuals. We developed and tested this less invasive method in arctic foxes *Vulpes lagopus*. We photographed dentition of live individuals captured in 2006–2013 and of skulls collected opportunistically in 2003–2013. Five observers assessed twice the tooth wear of 245 sets of pictures (left, frontal, and right views of the dentition for 179 captures and 66 skulls, with a mean of 7.2 pictures per set) to obtain a teeth condition index (TCI) for each set. TCI was highly repeatable, with an intraclass correlation coefficient averaging 0.89 and 0.83 within and between observers, respectively. We then used 44 known-aged individuals to predict fox age from TCI through linear regression (\( \text{age} = 0.62 \times \text{TCI} + 1.04, r^2 = 0.64 \)). We tested through jackknife validation the ability of this model to accurately age foxes. The rate of correct classification of exact age was 39%, while 80% and 100% of predicted ages were within one and two years of the true age, respectively. The model predicted correctly the age class (young, prime-age adult, old) of individuals 75% of the time. We conclude that our proposed method, which is much less invasive than those based on tooth extraction, can allow progress in the study of demography of arctic foxes, and potentially other mammal species.

Age is a key parameter in wildlife biology as it has a strong impact on the behaviour and ecology of individuals, and thus on the demography and dynamics of populations. For example, reproductive and survival rates are strongly age-dependent in mammals. The growth rate of a population is thus intimately linked to the proportion of individuals in particular age classes, called the population age structure. Yet, researchers often lack such information, even when individuals can be captured (Delahay et al. 2011).

More than 10 methods have been reported for aging wild mammals (Morris 1972, Spinage 1973, Harris 1978). Among these, the most effective approach consists in counting incremental lines in tooth cement (Harris 1978). The cement, a calcified tissue surrounding the dentine and deposited seasonally (Santymire et al. 2012), is made of light and dark layers formed respectively during periods of rapid and restricted growth. Each layer pair represents one year in most cases (Pekelharing 1970, Coy and Garshelis 1992, Lohr et al. 2011). Cement lines count is a common technique for aging mammals in general (Grue and Jensen 1979, Gipson et al. 2000) and carnivores in particular polar bears *Ursus maritimus* (Christensen-Dalsgaard et al. 2009), coyotes *Canis latrans* (Linhart and Knowlton 1967), red fox *Vulpes vulpes* (Harris 1978, Cavallini and Santini 1995), arctic fox *Vulpes lagopus* (Grue and Jensen 1976), gray wolf *Canis lupus* (Landon et al. 1998). Age estimates obtained by counting the number of growth layer groups deposited in teeth cement proved to be accurate for many species (King 1991, Hamlin et al. 2000). However, this method requires a tooth extraction and subsequent laboratory analyses, which generate potential ethical problems as well as delays in age determination of individuals. The need for a faster and less invasive method for age estimation of wild animals is clear.

Harris et al. 1992, Gay and Best 1996, Hewison 1999, Gipson et al. 2000, Rosatte and Silver 2007), anesthetized (Stand 1997, Van Horn et al. 2003, Olifiers et al. 2010, Delahay et al. 2011) or captive (Grau et al. 1970) animals. To our knowledge, only Lohr et al. (2011) used tooth wear to age non-anesthetized wild mammals, in their case, the brushtail possum Trichosurus vulpecula. Moreover, many studies cited above required a detailed expertise in dental morphology, which might necessitate some specific training at costs and constraints not affordable to many projects. An aging method available to non-experts and applicable to live and non-anesthetized animals captured in the wild would represent substantial progress in wildlife biology.

We used the arctic fox as study model to validate a minimally invasive method to age wild carnivores from their teeth condition. This work was conducted in the context of a detailed study on the behaviour and ecology of the species in the Canadian High Arctic. Our studied population is monitored since 1982 through diver surveys and since 2003 through capture–mark–recapture, but age of individuals captured as adults is unknown. Given that cement lines count can accurately determine age of arctic foxes due to large differences between winter and summer diet (Grue and Jensen 1976), we combined cement lines count on skulls of dead foxes found opportunistically in the field and analysis of tooth wear on live foxes to test a teeth condition index for arctic foxes. This method was modified from Olifiers et al. (2010) and adapted for our model species. We have three specific objectives: 1) to propose a visual teeth condition index (TCI) for an arctic predator, 2) to determine its repeatability within and across observers and 3) to build and test a predictive model with data from known-age individuals.

Material and methods

Study area

We worked in the south plain of Bylot Island (73° N, 80° W) which is part of Sirmilik National Park, Nunavut, Canada. Our study area increased from 425 km² in 2003 to 520 km² since 2008. The arctic fox is one of the main terrestrial predators of the local food web (Gauthier et al. 2004, Legagneux et al. 2012). Its diet is composed of lemmings, Lemmus trimucronatus and Dicrostonyx groenlandicus, and greater snow geese Chen caerulescens atlanticus, mostly eggs and goslings (Béty and Gauthier 2002, Careau et al. 2008, Tarroux et al. 2012, Giroux et al. 2012), as well as seal pups and seal carcasses found on the sea ice during winter and spring (Gagnon and Berteaux 2009).

Field protocol

We captured arctic foxes between mid-May and mid-August, from 2006 to 2013. We trapped cubs and adults using collapsible live traps (Tomahawk cage trap no. 205) or padded leghold traps (Softcatch no. 1) visited every six hours (Proulx et al. 1994). We sexed and ear-tagged each captured fox and photographed the dentition of ≥ 1 year-old individuals. One observer maintained the fox mouth closed with the lips pulled apart to expose dentition, while the other took the pictures. A minimum of three pictures (right lateral, left lateral and frontal views) were taken for each capture event. All pictures taken during a capture event constitute hereafter a ‘set of pictures’, with a mean of 5.5 pictures per set since some pictures were repeated when quality was uncertain.

We anesthetized 60 of 179 captured adults judged too agitated for safe manipulation, using an intramuscular injection of medetomidine (0.05 ml kg⁻¹) and ketamine (0.03 ml kg⁻¹). We used atipamezol (0.05 ml kg⁻¹) as antidote before releasing foxes. The remaining 119 adults were manipulated and photographed carefully with kevlar gloves only.

We collected adult-size skulls in the study area between 2003 and 2013. Some were found on the ground, often with little skin remains, after they had been exposed to scavengers and weathering, whereas others were collected from fresh carcasses of dead foxes. After cleaning skulls, we photographed their dentition as was done for live individuals, with a mean of 11.9 pictures per set. We then extracted a tooth, usually a canine or molar. All extracted teeth were sectioned and aged by the Matson’s Laboratory LLC (Milltown, MT, USA) following the methods described in Grue and Jensen (1976) and Matson and Matson (1981).

Teeth condition index (TCI)

Based on the work of Olifiers et al. (2010) on brown-nosed coati Nasua nasua and crab-eating fox Cerdocyon thous, we classified teeth condition by tooth type (incisor, canine or premolar/molar) with molar and premolar lumped together because wear appeared similar. Condition was based on tooth state (0 = intact, 1 = if one tooth or more is broken or absent) and wear (0 = sharp, 1 = worn but not flat, 2 = flat; Table 1, Fig. 1). Tooth wear is considered more indicative of age than any other criterion (Wood 1958) but tooth loss and tooth breakage is also age-dependent (Valkenburgh 1988). Tooth condition is not evaluated for each tooth, but rather evaluated across teeth within a given tooth category (e.g. two sharp and two flat canines resulted in a wear index for the tooth category canine of 1; Fig. 1). As shown in Table 1, the TCI is the sum of the various scores described above, and can vary from 0 (excellent teeth condition) to 9 (very bad teeth condition).

In order to evaluate the reliability of the visual assessment of teeth wear and state, we calculated the repeatability of these assessments within and between observers. Five

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incisor</td>
<td>state (IS) complete and intact (0),</td>
</tr>
<tr>
<td></td>
<td>incomplete or broken (1)</td>
</tr>
<tr>
<td></td>
<td>wear (IW) sharp (0), worn (1), flat (2)</td>
</tr>
<tr>
<td>Canine</td>
<td>state (CS) complete and intact (0),</td>
</tr>
<tr>
<td></td>
<td>incomplete or broken (1)</td>
</tr>
<tr>
<td></td>
<td>wear (CW) sharp (0), worn (1), flat (2)</td>
</tr>
<tr>
<td>Premolar/molar</td>
<td>state (MS) complete and intact (0),</td>
</tr>
<tr>
<td></td>
<td>incomplete or broken (1)</td>
</tr>
<tr>
<td></td>
<td>wear (MW) sharp (0), worn (1), flat (2)</td>
</tr>
<tr>
<td>Teeth condition index (TCI)</td>
<td>IS + IW + CS + CW + MS + MW</td>
</tr>
</tbody>
</table>
observers performed the visual assessment of teeth twice for each set of pictures. At least two days elapsed between the assessments of a given set of pictures by a given observer. Order of pictures was random and changed between notation sessions to decrease effects of observer fatigue or learning. Observers were professional biologists but not experts at evaluating teeth condition. All received the same protocol containing an annotated figure similar to Fig. 1. An assessment was considered complete when the state and wear of all tooth types was scored (this was sometimes impossible due to poor quality pictures or tooth loss).

Data analyses

Repeatability can be assessed by the intraclass correlation coefficient (ICC) (Wolak et al. 2012), with

$$ICC = \frac{S_d^2}{S_d^2 + S_w^2}$$

where $S_d^2$ is the variance among groups and $S_w^2$ is the variance within groups. To estimate within-observer repeatability, each group was a pair of assessment by a given observer. To estimate between-observer repeatability, each group was a set of five assessments for a given set of pictures. To estimate between-observer repeatability was calculated twice, once with the first assessment done by each observer on each set of pictures, and once with the second assessment. ICC varies between 0 (measurements not repeatable) and 1 (measurements fully repeatable). We estimated ICC using the R package ICC (Wolak et al. 2012), with an alpha level of 0.05 to assess the 95% confidence interval.

We used a linear regression to relate real fox age determined by cement lines count or first capture as a cub to our tooth condition index (TCI). We then tested the ability of the resulting model to accurately classify foxes within their respective age categories through jackknife validation. To do this, we built again our regression model while excluding one fox for which age was to be predicted, repeating this procedure for each fox. When several sets of pictures were available for a given individual due to multiple captures or live capture followed by skull recovery, we used only the first set obtained. Because known ages of foxes were integers, we rounded ages estimated from the model before comparing known and estimated ages. In addition, we tested the ability of the model to classify foxes in age categories, using the following age classes: yearling (1 year-old fox), prime-age adults (2, 3 and 4 years old) and old (≥5 years old).

All analyses were conducted with the statistical software R (<www.r-project.org>).

Results

Real ages of foxes (≥1 year-old) obtained from cement line counts on skulls ranged from 1 to 8 years (mean ± SD = 3.45 ± 2.04, n = 38). In addition, real ages were known for 6 adult-sized foxes that were captured as cubs in the study area (from 1 to 4 year, mean ± SD = 2.60 ± 1.03).

Repeatability of the tooth condition index

We succeeded to estimate the TCI of 234 sets of pictures out of 245 (57 out of 66 skulls and 177 out of 179 live captures; Table 2). TCI varied along all possible scores between 0 (excellent condition) and 9 (very bad condition) (median: 3). Within-observer repeatability ranged from 0.83 to 0.96 and averaged 0.89, while between-observer repeatability was 0.81 the first time observers assessed teeth condition and 0.84 the second time (average = 0.83). These repeatability values indicate an excellent precision of the TCI, both within and across observers, with an overall estimation error of 17%, of which 6% was due to differences between observers (0.89–0.83) and 11% to differences within observers (1–0.89).
Validation of the teeth condition index

The linear regression between TCI (x) and real fox age (y) yielded the following predictive equation:

\[ y = 0.062 \pm 0.07 \text{ SE} x + 1.04 \quad (r^2 = 0.64, n = 44) \]

Tooth wear increased linearly with fox age (Fig. 2). The jack-knife validation indicated that this model predicted correctly the age class (young, prime-age adult, old) of individuals 75% of the time (the age class of 33 out of 44 individuals was correctly assessed; Table 3).

The rate of correct classification decreased to 38.6% (17 out of 44 individuals) when exact age (rather than age class) was predicted. However, in 79.5% (35 of 44) of the cases, the model estimate was within one year of the true age, and in 100% of the cases it was within two years of true age.

Figure 2. Linear regression (with standard error, dotted lines) between the known age in years (y) and the mean of the teeth condition index (x) for 44 individuals in an arctic fox Vulpes lagopus population studied on Bylot Island, Nunavut, Canada. Regression equation: \( y = 0.62x + 1.04 \quad (r^2 = 0.64, p < 0.001) \). Size of data points in the plot reflects the number of observations (n = 1 or n = 2).

Table 2. Sample sizes used to build a teeth condition index (TCI) for arctic foxes Vulpes lagopus studied on Bylot Island (Nunavut, Canada) between 2006 and 2013.

<table>
<thead>
<tr>
<th>Sets of pictures</th>
<th>Skulls</th>
<th>Captures</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCI calculated</td>
<td>66</td>
<td>179</td>
<td>245</td>
</tr>
<tr>
<td>Individuals</td>
<td>57</td>
<td>177</td>
<td>234</td>
</tr>
<tr>
<td>Individuals</td>
<td>66</td>
<td>149</td>
<td>205</td>
</tr>
<tr>
<td>Individuals of age (sets of pictures)</td>
<td>63 (63)</td>
<td>13 (19)</td>
<td>69 (82)</td>
</tr>
<tr>
<td>Individuals aged through cement lines count (sets of pictures)</td>
<td>63 (63)</td>
<td>7 (12)</td>
<td>63 (75)</td>
</tr>
<tr>
<td>Individuals aged through marking as cub (sets of pictures)</td>
<td>0 (0)</td>
<td>6 (7)</td>
<td>6 (7)</td>
</tr>
<tr>
<td>TCI with age associated (sets of pictures)</td>
<td>55 (55)</td>
<td>13 (18)</td>
<td>61 (73)</td>
</tr>
<tr>
<td>( \geq 1 ) year-old individuals with calculated TCI (sets of pictures)</td>
<td>38 (38)</td>
<td>13 (18)</td>
<td>44 (56)</td>
</tr>
</tbody>
</table>

Table 3. Comparison of known age with age estimated from the teeth condition index for 44 live arctic foxes Vulpes lagopus studied on Bylot Island (Nunavut, Canada). Perfect match between estimates and real ages appears in bold.

<table>
<thead>
<tr>
<th></th>
<th>Yearling</th>
<th>Prime-age adults</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>yearling</td>
<td>1</td>
<td>4</td>
<td>3 1</td>
</tr>
<tr>
<td>prime-age adults</td>
<td>2 2 5</td>
<td>3 3 1</td>
<td>0 0</td>
</tr>
<tr>
<td>old</td>
<td>4 0 1</td>
<td>2 4 3 1</td>
<td>0 1</td>
</tr>
<tr>
<td>Age predicted by the model</td>
<td>5 6 7 8 9</td>
<td>2 0 1 3 0</td>
<td>1 2</td>
</tr>
<tr>
<td>Skulls Captures Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Our proposed teeth condition index allowed an estimation of the age of individuals captured from a wild population of carnivores without relying on tooth extraction. Use of distinguishable wear categories following Olifiers et al. (2010) allowed us to limit subjectivity when evaluating tooth attrition, which likely improved the repeatability of our assessments. Moreover, the constant increase of tooth attrition with fox age indicated the relevance of a linear model to predict age of individuals, whereas the strong relation \( (r^2 = 0.64) \) between tooth wear notation and known age indicates that individuals could be reliably aged from tooth wear in our studied population. For comparison, the \( r^2 \) of the rank regression between tooth wear and cement lines count was only 0.10 for males and 0.35 for females in brushtail possums (Lohr et al. 2011).

The model predicted the exact age of 39% of foxes, an estimated age that was within one year of true age for 77.3% of foxes and the correct age category (yearling, prime-age adult, old adult) for 75% of individuals. Such a level of accuracy is higher than that achieved in other studies where individuals were aged from tooth wear by multiple observers. In roe deer, the age estimated by tooth wear of 50% of individuals was within one year of true age (Hewison 1999), and in gray wolf, the estimated age of 75% of individuals was within two years of true age (Gipson et al. 2000). The accuracy for age categories matches the results obtained for crab-eating foxes by Olifiers et al. (2010), where 67 to 100% of individuals were correctly classified according to definite age categories.

We observed a maximum age of 8 years in our population, while the oldest individuals were 7 or 8 years-old in other Canadian populations (Bradley et al. 1981, Hiruki and Stirling 1989), 8 years-old on Saint Lawrence Island in Alaska (Fay and Rausch 1992), and 10 years-old in Iceland (Hersteinsson 1992) and Svalbard (Eide et al. 2012). This indicates that maximum longevity seems to be a rather constant parameter across arctic fox populations. Published population age structures of arctic foxes were inferred from multiple aging techniques and presented using age classes defined in multiple ways (Macpherson 1969, Bradley et al. 1981, Hiruki and Stirling 1989, Fay and Rausch 1992, Hersteinsson 1992, Pagh and Hersteinsson 2008, Eide et al. 2012).
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