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ORIGINAL ARTICLE

A tooth wear scoring scheme for age estimation of the Eurasian lynx (*Lynx lynx*) under field conditions

Iris Marti[1] · Marie-Pierre Ryser-Degiorgis[1]

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Abstract
Within the framework of conservation actions for the Eurasian lynx (*Lynx lynx*), ageing of individuals is required to assess suitability for translocation and to investigate population dynamics and disease epidemiology. We aimed to develop a standardised ageing tool for free-ranging Eurasian lynx, which would be non-invasive, time- and cost-effective, and applicable under field conditions. We used tooth pictures of 140 free-ranging lynx of known age from Switzerland. Tooth colour, calculus, number of incisor teeth and canine, premolar and molar tooth wear were recorded according to pre-defined criteria. Statistical comparisons among the categories of each criterion revealed significant differences for all criteria. Tooth colour and canine tooth morphology showed obvious and consistent alterations with age. Together with the molar tooth shape, premolar tooth tips, incisor teeth and amount of calculus, they pictured the age-related changes in lynx dentition. Crown fractures, enamel flaking and open pulp cavities were observed with an increasing prevalence over age but were also sporadically seen in juveniles. Based on the obtained results, we developed a classification scheme distinguishing six age classes: < 1 year, 1–2 years, 3–6 years, 7–9 years, 10–13 years, ≥ 14 years. The scheme was subsequently tested with the same lynx. Classification success rates of different readers ranged from 69 to 88% but errors did not exceed one age class. The homogenous tooth replacement pattern observed in lynx < 1 year allowed us to develop a separate ageing chart to age juveniles in months. The proposed scheme is a promising tool to objectively assign lynx to meaningful age categories.

Keywords Age determination · Teeth · Wild felid · Classification criteria · Tooth replacement · Method harmonisation

Introduction

The Eurasian lynx (*Lynx lynx*), once widely distributed in Europe, was reduced to a few remnant populations in Scandinavia and Eastern Europe by 1900 due to habitat loss, prey loss and persecution by humans (Chapron et al. 2014). In the second half of the twentieth century, protection measures and initiatives to restore lynx populations were taken in several countries (Breitenmoser 1998). Among others, the lynx was reintroduced to Switzerland in the 1970s, with individuals originating from the Carpathian Mountains (Breitenmoser et al. 1998). These reintroductions resulted in two reproducing lynx populations in the Swiss Alps and Jura Mountains, respectively (Chapron et al. 2014), which now serve as sources for reintroduction and restocking in other areas of the former lynx range. Within the framework of these conservation actions, population monitoring, health surveillance and translocation programmes have been implemented for the Eurasian lynx in several countries (Schmidt-Posthaus et al. 2002; Ryser-Degiorgis et al. 2005; Molinari-Jobin et al. 2012; Breitenmoser et al. 2016). For all of these programmes, data on age of individuals—whether captured or found dead—are required to assess population dynamics, study disease epidemiology and select suitable candidates for translocation (Stander 1997; Gipson et al. 2000; Ryser-Degiorgis 2013). Furthermore, parallel activities and collaboration of multiple teams from different disciplines and several countries call for a practical tool ensuring repeatability and data harmonisation.

Methods used for age determination in wild animals vary depending on the concerned taxon and differ in accuracy. Counting seasonal annuli in tooth cementum has been shown to be a reliable technique in various mammal species including all lynx species (*L. lynx*, *L. pardinus*, *L. canadensis*, *L. rufus*; Crowe 1972; Brand and Keith 1979; Kvam 1984;

✉ Marie-Pierre Ryser-Degiorgis
marie-pierre.ryser@vetsuisse.unibe.ch

[1] Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Postfach, Länggass-Strasse 122, 3001 Bern, Switzerland

Zapata et al. 1997). The method consists in counting the dark stained cementum lines formed annually on the outer tooth root, and adding 1 year to the counted number of cementum lines to obtain the age of the individual (Kvam 1984; Zapata et al. 1997). However, the application of this method is limited by ethical considerations regarding the required tooth extraction in live animals, by the costs and logistics associated with the shipment to a specialised laboratory and with the analyses, and by the time elapsed until results are available.

Age calculation based on coat pictures is possible in lynx and other felid species with a distinct individual fur pattern allowing individual identification (Trolle and Kéry 2003; Silver et al. 2004; Jackson et al. 2006; Karanth et al. 2006; Weingarth et al. 2012; Pesenti and Zimmermann 2013). Yet, this method requires a tight monitoring programme by phototrapping and the existence of pictures taken at the kitten age. Even if such data collection is carried out, an exact age can only be calculated for a few individuals.

An old and widely used ageing method in humans, domestic and wild animals is the evaluation of tooth wear (Morris 1972; Kim et al. 2000; d'Incau et al. 2012). Its applicability is non-invasive, time- and cost-effective, requires no specialised equipment and is suitable for live and dead individuals. Variations among individuals and populations as well as the subjectivity of the assessment reduce its reliability (Morris 1972; Stander 1997; Landon et al. 1998; Gipson et al. 2000; d'Incau et al. 2012) but various tooth wear scoring systems and ageing charts have been developed for wild carnivores to standardise the procedure and increase reproducibility (Stander 1997; Gipson et al. 2000; Van Horn et al. 2003; Smuts et al. 2009; Nakanishi et al. 2009; Olifiers et al. 2010).

Unless tooth extraction is performed, at present, the age of Eurasian lynx is roughly estimated based on morphological characteristics such as body size, reproductive organs and tooth condition, and animals are tentatively classified into three age categories (juvenile, subadult, adult; Slough 1996; Jedrzejewski et al. 1996; Tryland et al. 2011) but definitions are not consistent across all studies. Furthermore, the distinction between subadults and old juveniles or subadults and young adults is challenging, and the adult class comprises animals within an age range of more than a dozen years. For kittens, a more accurate age estimation can be done based on the calculation of the time elapsed between the assumed birth period in May–June (Kvam 1984; Breitenmoser-Würsten et al. 2007) and the month when the animal was found, but this does not work for occasional late births (Fritts and Sealander 1978; Mowat and Slough 1998; Breitenmoser-Würsten et al. 2007) or carcasses found long after death.

The aim of this study was to develop a non-invasive, economical ageing technique for the Eurasian lynx, which would be applicable under field conditions and would deliver immediate results. Considering that teeth, once changed, undergo permanent wear increasing throughout a lynx's life, we hypothesised that it is possible to age lynx during but also after their growth period by using a systematic dentition evaluation based on precise criteria.

Material and methods

Animals

We used tooth pictures of 140 free-ranging Eurasian lynx of known age from Switzerland (45° 49′ N to 47° 49′ N, 5° 58′ E to 10° 29′ E, including both the Alps and the Jura Mountains), taken from 2002 to 2016. Our material included 33 live lynx, which were either handled without anaesthesia (14 newborns) or were anaesthetised for marking procedures (using medetomidine/ketamine hydrochloride; Ryser et al. 2005) and 107 dead lynx sent to the Centre for Fish and Wildlife Health (FIWI, Bern) for pathological examination. For dead lynx, tooth pictures were taken either of fresh carcasses during necropsy ($n = 45$), or of skinned carcasses after storage in the deep freezer ($n = 13$), or of prepared skulls archived at the Museum of Natural History of Bern ($n = 49$). Overall, our sample included 65 females, 67 males and 8 lynx of undetermined sex.

Age determination

The starting point for a lynx year was placed in May because in Switzerland, lynx kittens are born in May and June with a peak in the second half of May (Breitenmoser-Würsten et al. 2007). Lynx clearly recognised as juveniles (body size, weight, milk dentation) were manipulated alive or found dead as fresh carcasses and their age was calculated in months based on the known narrow birth period (Breitenmoser-Würsten et al. 2007). Age determination for all other individuals was either calculated using monitoring data for individuals already recorded at the kitten age ($n = 20$), or it was performed by counting cementum annuli of canines or incisors ($n = 60$; Matson's Laboratory, Manhattan, MT, USA). The genus *Lynx* has very distinct cementum annuli arranged in a regular pattern, and age determination by cementum analysis is characterised by a 95% accuracy (Matson's Laboratory 1969).

The three main age classes were defined considering both sexual maturity and social behavior, as these two factors are relevant to the reproductive biology of the lynx and closely linked to the occurrence of intraspecies contacts potentially influencing pathogen transmission (Tryland et al. 2011). Lynx in their first year of life (i.e. from May to April of the following year: family life and early dispersal) were defined as juveniles. Female lynx were defined as subadult during their second year of life, and males during the second and third year of life (dispersal followed by the establishment of a territory of their own). Older individuals (≥ 2 years for females and ≥ 3 years for males) were classified as adults (i.e. animals taking part in reproduction; Zimmermann et al. 2005; Tryland et al. 2011).

Our sample included 60 juvenile, 28 subadult and 52 adult lynx. The age ranged from 1 month to 18 years.

Lynx dentition and tooth wear scoring

Like other felids, the lynx shows a phylogenetic reduction of premolar and molar teeth compared to primitive placental mammals, which have a total of 44 teeth (Stander 1997). Furthermore, while most felids possess 30 permanent teeth, lynx have lost the second upper premolar teeth (Matjuškin 1978). Thus, the genus *Lynx* is characterised by a dentition consisting of only 28 permanent teeth: I3/3, C1/1, P2/2 M1/1 = 28. The dental formula for deciduous dentition is dI3/3, dC1/1, dP2/2 = 24 (Garcia-Perea 1996). However, variations in tooth number are regularly observed in the Eurasian lynx. They occur at different frequencies in different populations, with supernumerary maxillary second premolar teeth and mandibular second molar teeth being the most common aberrations of the dental formula (Kvam 1985; Gomercic et al. 2009).

We evaluated the following criteria before obtaining exact information on the animal's age (i.e. blind evaluation): tooth colour, amount of dental calculus, number of incisor teeth, canine tooth morphology (wear on tips and ridges, presence of crown fractures, open pulp cavities and enamel flaking), extent of premolar tooth wear and molar tooth shape. Not all criteria could be applied to all lynx when picture quality was insufficient or only prepared skulls were available.

Tooth colour ($n = 85$ lynx) was recorded using four categories ranging from white to a distinct brown-yellow (Fig. 1). Discolouration of a single tooth was assumed to result from a pathological process (canine teeth with open pulp cavity in two adult males of 7 and 9 years old, respectively) and therefore not considered in the colour evaluation of the whole dentition.

We evaluated dental calculus ($n = 71$ lynx) using criteria inspired by dental calculus scoring systems developed for the domestic cat *Felis silvestris catus* (Clarke 2001, 2006) but adapted to describe the more discrete amounts of calculus in our material (Fig. 2). Museum skulls were excluded from the tooth colour and calculus evaluation because of skull preparation-related colour loss and calculus reduction.

Two prominent ridges (buccal and distal) can be observed on permanent lynx canine teeth. The buccal ridge runs longitudinally on the buccal side of both the maxillary and mandibular canine teeth and is flanked by two grooves, also referred to as bleeding grooves (Niemiec 2011). Appearance of the buccal ridge ($n = 106$ lynx) was categorised in two groups: present versus absent (Fig. 3). Lynx with absent buccal ridge but visible traces of the flanking grooves were included in the "absent" category. The distal ridge is visible on the maxillary canine teeth only. Appearance of the distal ridge ($n = 67$ lynx) was categorised into three groups: intact ridge, wear present and ridge worn down (Fig. 4). The presence of open pulp cavities, crown fractures and enamel fractures (also referred to as enamel flaking; Stander 1997; Bastone et al. 2000) on canine teeth was noted as present/absent for each individual. For the purpose of this study, flaking or chipping of a canine tooth without reduction of its length was called "enamel flaking" while damages resulting in a reduced length were categorised as "crown fractures" (Fig. 5).

Canine tooth tips ($n = 107$ lynx) were characterised as sharp, rounded or worn down to the same level as premolar teeth. We assumed that wear affects all canine teeth equally, and therefore all individuals with at least one sharp canine tooth tip were placed in the sharp-category. To differentiate between canine

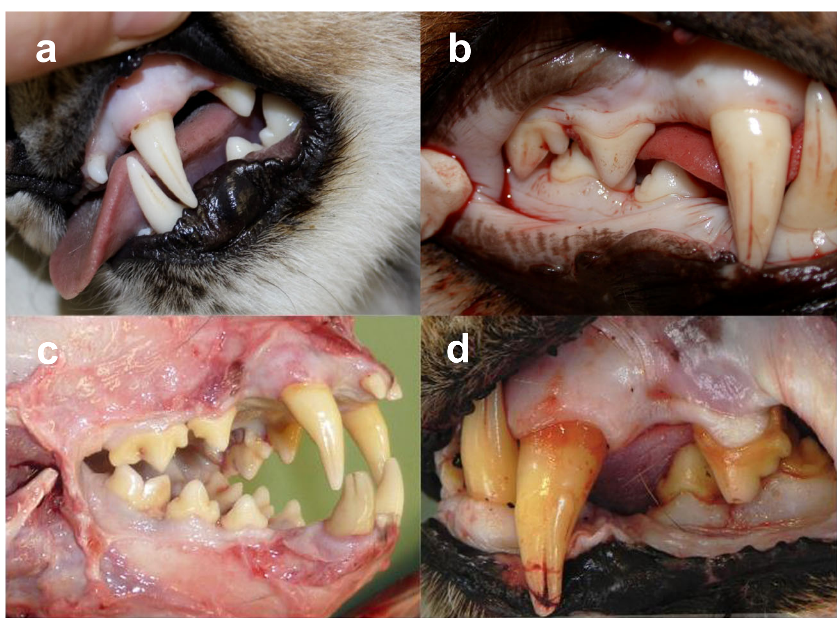


Fig. 1 Tooth colour graduation. **a** White: teeth show a clear white colour after cleaning. **b** Beige: colour stays beige all over the teeth even when they are cleaned. **c** Slight brown-yellow: teeth have a yellowish or slightly brownish appearance. **d** Distinct brown-yellow: teeth show an intense yellow, orange or brown colour

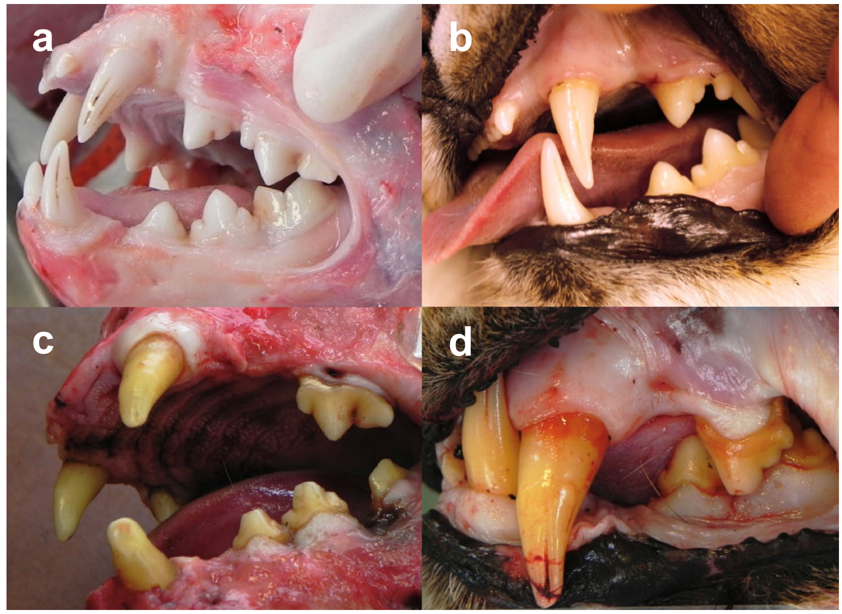


Fig. 2 Amount of dental calculus. **a** None: no signs of dental calculus, possible debris on teeth feels soft and can be easily removed by rubbing the teeth. **b** Slight: thin margins or focal flecks of dental calculus, typically on third premolar and maxillary canine teeth. **c** Moderate: clearly visible margins or extended flecks of dental calculus, typically on premolar, molar and maxillary canine teeth. **d** Severe: distinct deposition of dental calculus encasing the cervical area of teeth which leads to changes in shape of the affected teeth

crown fractures and wear on tips, canine teeth with reduced length but sharp edges and those with an obvious difference in length compared to the contralateral side were classified as fractured canine teeth whereas those with rounded edges and similar length on both sides were considered as worn.

We differentiated between premolar teeth with sharp and premolar teeth with rounded tips (considering the major tip of each premolar tooth; $n = 89$ lynx). For the evaluation of molar teeth, the shape of the notch between para- and protoconids of the mandibular molar teeth was classified as v- or u-shaped ($n = 74$ lynx; Fig. 6).

Data analyses

Statistical analyses were conducted using the program R (R Development Core Team 2015). The level of significance was set at $p < 0.05$. Age distribution (in years) was compared among categories of each criterion, using Kruskal-Wallis test and post hoc Mann-Whitney-Wilcoxon test followed by Holm-Bonferroni correction. Linear regression with adjusted R^2 and subsequent F test was performed to assess the correlation between age and incisor teeth loss, prevalence of open pulp cavities, crown fractures and enamel flaking on canine teeth. The ageing scheme was constructed by compiling the results obtained for all tooth criteria.

Scheme evaluation

The validity (accuracy) and reliability of the developed ageing scheme were evaluated running blind tests on the same lynx. The first author classified all lynx of the study with complete tooth pictures ($n = 131$) into one of the defined age categories. Subsequently, a subset of 16 lynx from different age categories for which high-quality pictures were available was selected by the first author and presented for evaluation to the second author and 12 other independent readers, including veterinarians and biologists with various experience in lynx examination as well as undergraduate students. The scheme accuracy was assessed by calculating the success rate (i.e. percentage of correctly classified lynx) for each reader. The inter-reader agreement was assessed by computing Fleiss's kappa. Finally, the two authors repeated the classification a year later, and the intra-reader agreement was assessed using Cohen's kappa (Hallgren 2012).

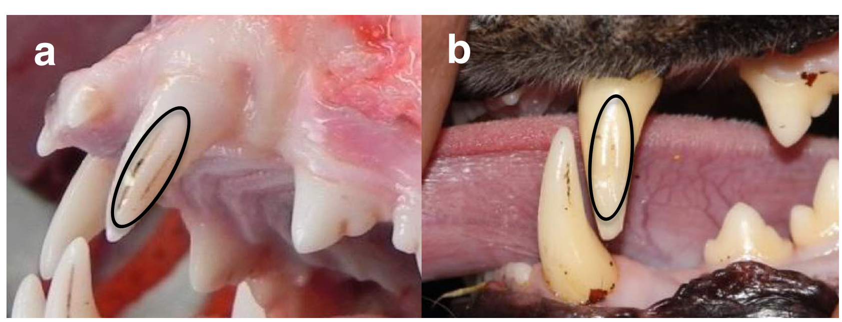


Fig. 3 Status of the buccal ridge. **a** Present: prominent projection of the buccal ridge (black circle), the ridge is flanked by one groove on each side. **b** Absent: the buccal ridge (black circle) is worn down, the grooves or traces of them might still be visible

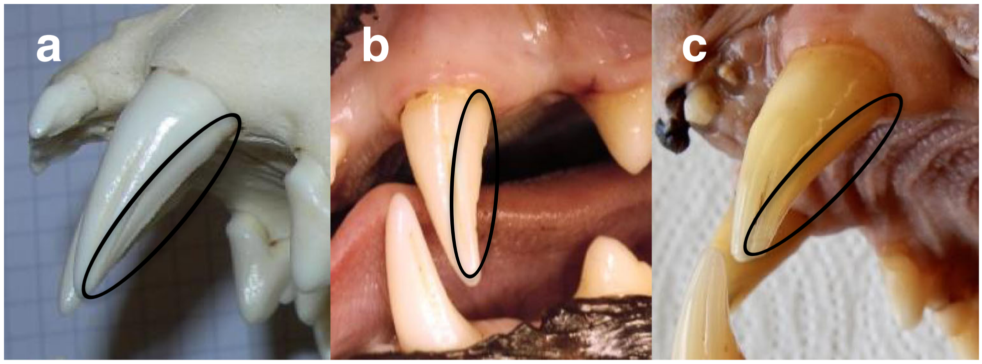


Fig. 4 Status of the distal ridge. **a** Intact: the distal ridge runs along the distal surface of the maxillary canine teeth (black circle). **b** Wear present: parts of the distal ridge are missing (black circle), usually wear is more advanced on the apical part. **c** Worn down: the ridge is completely worn down (black circle), a superficial enamel defect might be left

Results

Tooth replacement

A total of 47 out of 60 juvenile lynx showed either deciduous dentition or signs of tooth replacement. Their age ranged from 1 to 9 months. Eruption and replacement sequences followed a homogenous pattern in our material (Table 1, Fig. 7). Permanent canine teeth became apparent at the age of 5 months but deciduous canine teeth remained until 6 months, which led to a double dentition during that time (6/6 of the 5-month-old animals). Differently from the permanent ones, deciduous canine teeth do not have buccal or distal ridges. At the age of 9 months, tooth replacement was completed but permanent canine teeth and the third mandibular premolar teeth had not yet fully emerged. All juveniles aged 10 and 11 months ($n = 10$) showed a permanent dentition with completely erupted teeth.

Tooth colour

The four colour categories (white: $n = 48$, beige: $n = 14$, slight brown-yellow: $n = 18$, distinct brown-yellow: $n = 5$) showed only a minimal overlapping age pattern (Fig. 8) and age differences among them were highly significant (white versus beige: $p < 0.001$, beige versus slight brown-yellow: $p < 0.001$, slight brown-yellow versus distinct brown-yellow: $p = 0.002$). Juveniles had mostly white teeth (44/48, 92%). The beige category included seven subadults, four juveniles and three adults ≤ 3 years. All older lynx (≥ 4 years, $n = 21$) had a slight or distinct brown-yellow tooth colour. Four of five lynx over 12 years had distinctly brown-yellow coloured teeth. Tooth yellowing was less intense on incisor teeth, whereas canine teeth were the teeth most affected by discolouration.

Dental calculus

Age differences between the four calculus categories were significant except for the comparison between the groups with moderate and severe deposits (none versus slight: $p < 0.001$, slight versus moderate: $p = 0.003$, moderate versus severe: $p = 0.122$). Absence of dental calculus was noted in 37/41 (90%) juveniles and 4/5 subadults. Adults ≤ 6 years typically showed slight amounts of dental calculus (10/13, 77%). Between 7 and 11 years, slight ($n = 3$) to moderate ($n = 3$) amounts of calculus were noted. Moderate ($n = 3$) to severe ($n = 3$) deposits of dental calculus were observed in the six adults ≥ 12 years (Fig. 8).

Incisor teeth

The number of incisor teeth decreased significantly with increasing age (Fig. 8, linear regression: $y = -13.7254\ x + 186.1929$, adjusted $R^2 = 0.717$, F test: $p < 0.001$).

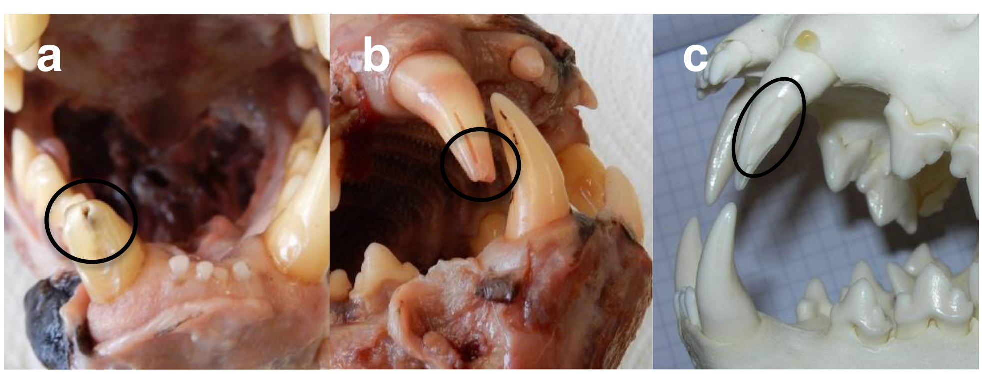
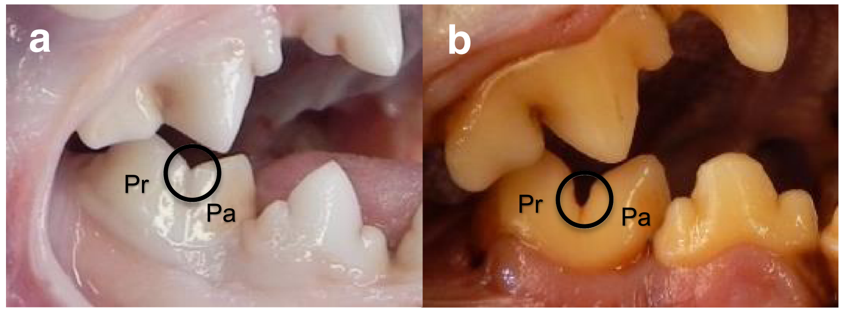


Fig. 5 Canine tooth alterations. **a** Open pulp cavity: the pulp cavity is visible in the centre of the tooth (black circle). **b** Crown fracture: note the sharp edges (black circle). **c** Enamel flaking: flaking of the enamel without reduction of tooth length (black circle)

Fig. 6 Molar tooth shape. **a** V-shaped: the notch (black circle) between paraconid (Pa) and protoconid (Pr) of the mandibular molar tooth is v-shaped; its depth can vary. **b** U-shaped: the notch (black circle) between paraconid (Pa) and protoconid (Pr) of the mandibular molar tooth is u-shaped; its depth can vary



Canine teeth

Age differences between the three categories of canine tooth tip wear were significant (sharp versus rounded: $p < 0.001$, rounded versus premolar length: $p = 0.038$). Most lynx ≤ 6 years (84/87, 97%) had at least one canine tooth with a sharp tip. Individuals > 14 years had their canine teeth worn down to the same length as premolar teeth (4/5 cases). Between 7 and 13 years of age, canine tooth tips were found to be rounded (11/17, 65%), sharp (4/17, 23%) or worn down to premolar tooth length (2/17, 12%) (Fig. 8).

Regarding the buccal ridge, there was a significant difference between the ages of lynx in the "present" and "absent" categories ($p < 0.001$). The buccal ridge was present in nearly all lynx ≤ 5 years (82/83, 98.8%) and in 5/9 lynx from 6 to 7 years old. All 14 lynx ≥ 8 years had their buccal ridge worn down (Fig. 8). Traces of the two flanking grooves were regularly observed after the ridge was worn down.

Similarly, all three distal ridge categories (intact distal ridge, wear present, distal ridge worn down) significantly differed from each other (intact versus wear present: $p < 0.001$, wear present versus absent distal ridge: $p < 0.001$). An intact distal ridge was found in 13/15 (87%) juveniles. The proportion of lynx with worn ridge and the wear severity increased with age, and from ≥ 6 years old, almost all (14/15, 93%) had their distal ridge completely worn down (Fig. 8). There were no grooves visible after the distal ridge was worn down, but often a superficial enamel defect could be seen at the site of the worn ridge.

Open pulp cavities, crown fractures and enamel flaking were observed on canine teeth of lynx of all ages but the prevalence of these lesions increased with age. The coefficient of determination was highest for the prevalence of open pulp cavities (adjusted $R^2 = 0.798$, F test: $p < 0.001$), followed by that of enamel flaking (adjusted $R^2 = 0.784$, F test: $p < 0.001$) and of canine crown fractures (adjusted $R^2 = 0.650$, F test: $p < 0.001$). Open pulp cavities and enamel flaking were present in all lynx ≥ 10 years.

Premolar teeth

Five different premolar tooth wear patterns were observed: wear on mandibular p3 ($n = 8$), wear on mandibular p3 and maxillary P3 ($n = 5$), wear on mandibular p3 and p4 ($n = 3$), wear on mandibular p3, p4 and maxillary P3 ($n = 3$) and wear on all premolar teeth ($n = 6$). This suggested that the first premolar teeth losing their sharp tips were mandibular p3 followed by maxillary P3 and mandibular p4, and at last wear extended to maxillary P4. This sequence was very consistent in our material; however, the age distribution among the different wear patterns clearly overlapped. More distinct age differences were seen when premolar wear was summarised in three groups (sharp tips, wear on p3 and extended wear—i.e., wear on multiple premolar teeth; Fig. 8). These differences were

Table 1 Ageing scheme for juvenile Eurasian lynx based on tooth eruption, distinguishing eight different age classes

Age (months)	Deciduous teeth			Permanent teeth			
	Incisors	Canines	Premolars	Incisors	Canines	Premolars	Molars
1	Erupted	Erupted	Erupting (p3 & p4–P3 & P4)	–	–	–	–
2–3	Erupted	Erupted	Erupted	–	–	–	–
4	Displaced	Erupted	Erupted	Erupting	–	–	–
5	–	Still in place (double dentition)	Erupted	Erupted	Erupting	–	Erupting
6	–	Being displaced	Erupted	Erupted	Erupting	–	Erupting
7–8	–	–	Displaced	Erupted	Erupting	Erupting (P4–P3 & p4–p3)	Erupted
9	–	–	–	Erupted	Erupting	p3 still erupting	Erupted
10–11	–	–	–	Erupted	Erupted	Erupted	Erupted

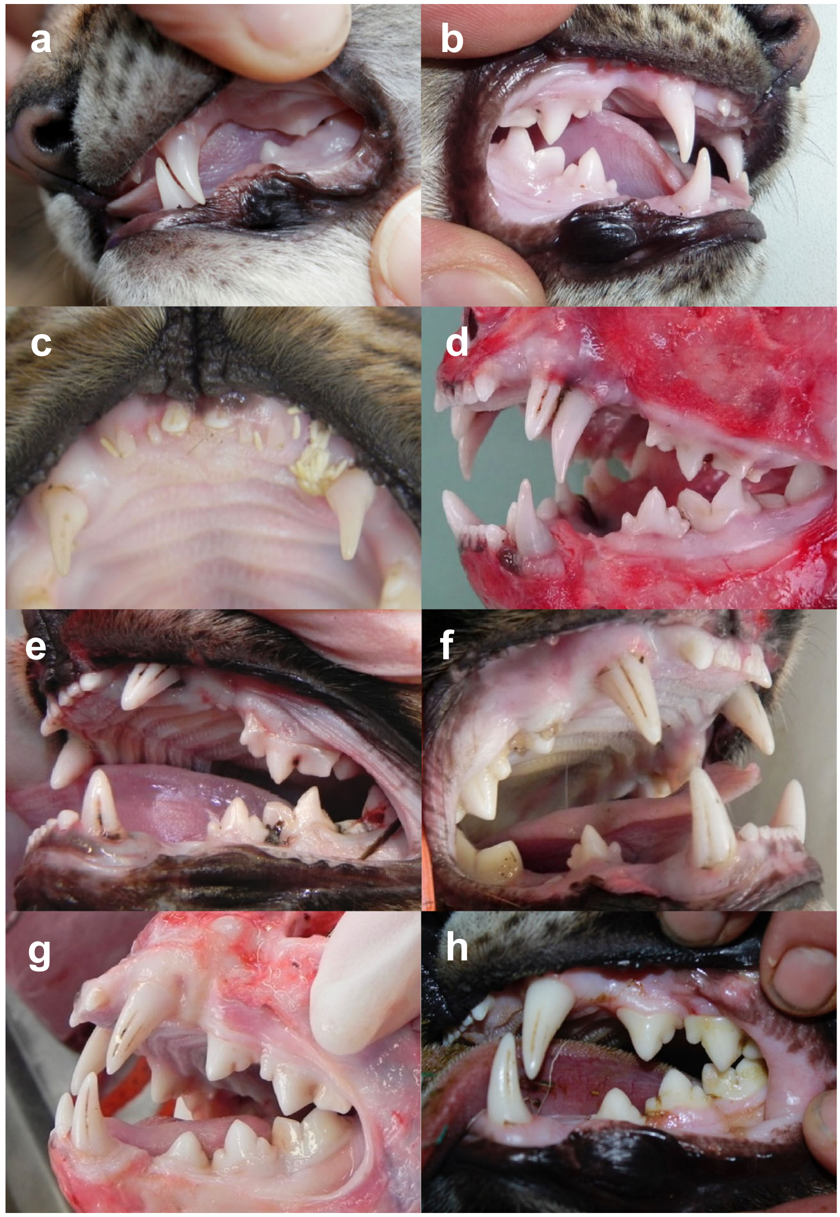


Fig. 7 Tooth replacement sequence. **a** Deciduous incisor and canine teeth erupted, premolar teeth erupting. **b** Complete deciduous dentition. **c** Permanent incisor teeth erupting, note the central concave groove present only on permanent incisor teeth (the white, rice grain-like structures are fly maggots). **d** Permanent canine and molar teeth erupting, deciduous canine teeth still in place, resulting in a "double dentition". **e** Deciduous canine teeth displaced, molar and canine teeth erupting. **f** Replacement of premolar teeth in the order maxillary P4–P3 and mandibular p4–p3. **g** All deciduous teeth replaced, mandibular p3 and canine teeth not yet fully erupted. **h** Complete permanent dentition

significant between the categories "sharp tips" and "wear on p3" ($p < 0.001$) but not between "wear on p3" and "extended wear" ($p = 0.094$). All juveniles, 20/21 subadults and 21/27 adults ≤ 6 years had sharp premolar tooth tips. Among adults ≥ 7 years, wear was observed on mandibular p3 in 5/20 (25%) and on multiple premolar teeth in 14/20 (70%) lynx. Missing premolar teeth with associated osteomyelitis of the alveolus were observed in 11 adult lynx ≥ 4 years, including 3/5 adults ≥ 14 years old.

Molar teeth

Molar teeth showed equally sharp tips in all individuals but there was a highly significant age difference between lynx with a v-shaped notch between the para- and protoconids of the mandibular molar teeth and lynx with a u-shaped notch ($p < 0.001$). The notch was v-shaped in 17/19 (89%) juveniles and 12/17 (71%) subadults. In adults ≤ 4 years, v-shaped (7/15, 47%) and u-shaped (8/15, 53%) notches were observed with a similar frequency. Molar notches were u-shaped in 23/25 (92%) lynx ≥ 5 years (Fig. 8).

Ageing scheme

The obtained data on the relationship between age and the selected tooth criteria were collated to propose an ageing scheme. Years with identical characteristics were pooled, resulting in a first general scheme differentiating six age categories (Table 2). An analogous, separate ageing scheme with

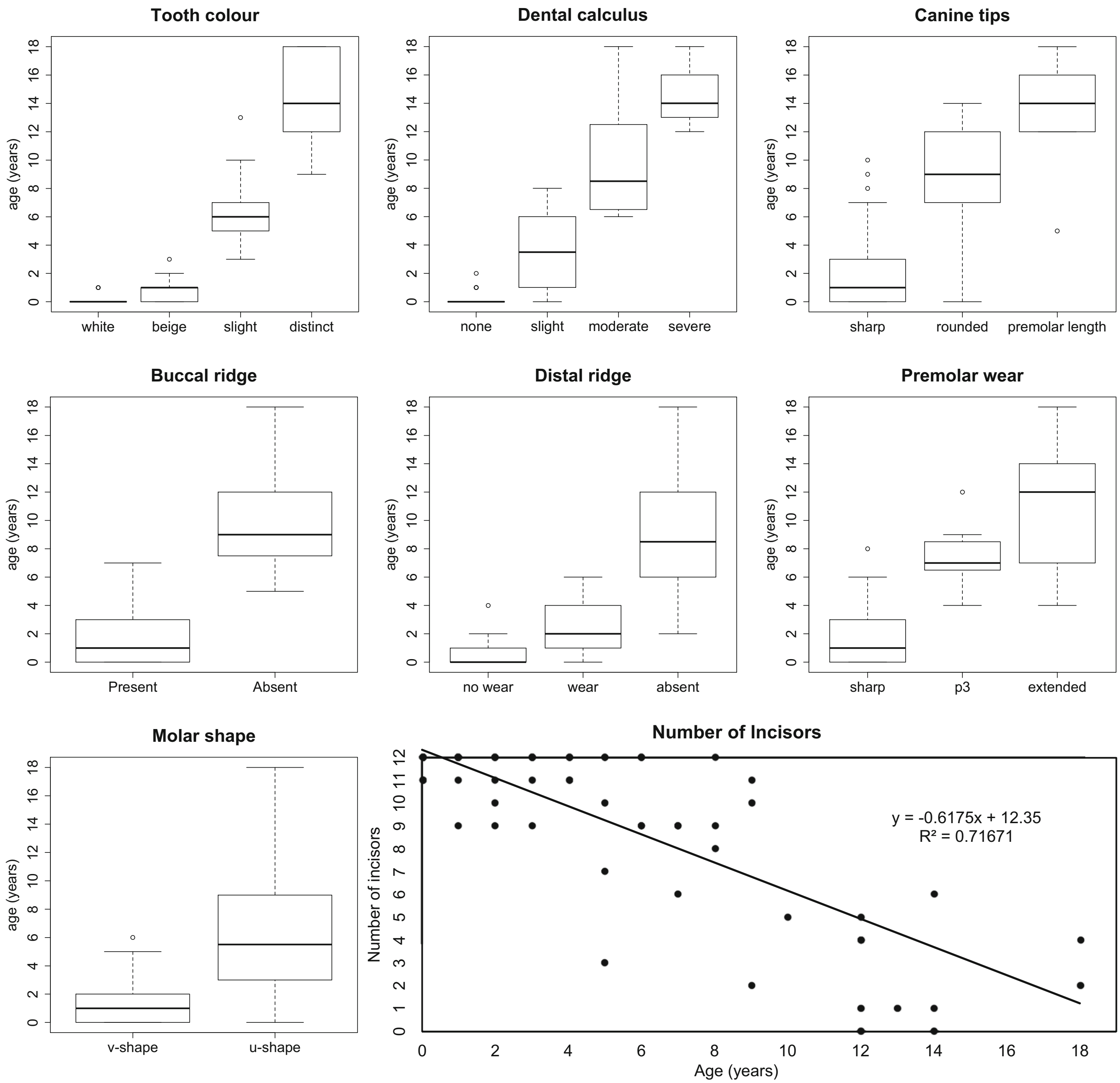


Fig. 8 Distribution among the different age categories for tooth colour, dental calculus, canine tooth tips, buccal ridge, distal ridge, premolar tooth wear, molar tooth shape and number of incisor teeth. The colour categories "slight" and "distinct" refer to the degree of brown-yellowish discolouration

eight categories was additionally developed for juveniles with deciduous dentition or signs of tooth replacement (Table 1).

Scheme evaluation

When the general scheme was tested on the same animals as those used to develop it, the first author correctly classified 113/131 (86%) of all evaluable lynx, with all lynx < 1 year ($n = 60$) and ≥ 14 years ($n = 4$) being properly categorised. Prepared skulls were most susceptible to wrong classification because tooth colour and dental calculus could not be considered. When these skulls were excluded, classification success increased to 94% (79/84 correctly classified). Success rates of the 13 independent readers when categorising the subset of 16 lynx ranged from 69 to 88%. No lynx was over- or underestimated by more than one age category, and experience did not seem to influence individual ageing success: Veterinarians who had formerly manipulated < 12 lynx and a student who had never seen lynx teeth before had high success rates (81–83%). A 7-year-old lynx was classified correctly by only two readers and an 11-month-old lynx by three readers; the other readers consistently under- and overestimated them, respectively, by one age category. The obtained Fleiss kappa value for the 13 readers was 0.7. Cohen's kappa was 0.92 for both authors.

Table 2 Ageing scheme for Eurasian lynx based on tooth wear, distinguishing six different age classes

Age (years)	Colour	Calculus	Buccal ridge	Distal ridge	Canine tips	Premolar tips	Molar shape (%)	Incisors	Open pulp cavity (%)	Enamel flaking (%)
<1	White	None	Present	Intact	Sharp	Sharp	V shape (89)	11–12	10	13
1–2	Beige	None	Present	Wear present	Sharp	Sharp	V shape (65)	9–12	14	45
3–6	Slight brown-yellow	Slight	Present	Wear present	Sharp	Sharp-extended wear	U shape (60)	3–12	40	50
7–9	Slight brown-yellow	Moderate	Absent (± traces of grooves)	Worn down	Rounded	Extended wear	U shape (100)	2–10	70	75
10–13	Distinct brown-yellow	Moderate-severe	Absent (± traces of grooves)	Worn down	Rounded	Extended wear	U shape (100)	0–6	100	100
≥14	Distinct brown-yellow	Moderate-severe	Absent (± traces of grooves)	Worn down	Premolar length	Extended wear	U shape (100)	0–4	100	100

The exact age was known from field monitoring and additionally determined by counting cementum annuli for three lynx. In an adult male, the age determined by cementum analysis (5 years old) was correct and it also matched with the age category obtained with our tooth wear scheme. In another adult male, the age determined by cementum analysis was underestimated by 1 year (13 instead of 14 years); this slight underestimation also occurred with the tooth wear scheme (one age category). An adult lynx known to be 17 years old was underestimated by 5 years (12 years old) with the cementum analysis, whereas it was correctly classified in the ≥ 14 years category with our tooth wear scheme.

Discussion

The scheme proposed here represents the first non-invasive, standardised and widely applicable method to age Eurasian lynx. We used criteria repeatedly described in the literature for the evaluation of tooth wear (colour, calculus, wear on canine and premolar teeth; Stander 1997; Gipson et al. 2000; Nakanishi et al. 2009) and combined them with new ones such as the molar tooth shape and number of incisor teeth. We found the previously described criteria to be well applicable to lynx but we obtained the highest ageing accuracy when all of our criteria were combined. An advantage of our scheme is the possibility to discriminate several, clearly defined adult age categories under field conditions, providing a practical tool relevant to epidemiological studies, population monitoring and conservation actions (Gipson et al. 2000; Olifiers et al. 2010; Ryser-Degiorgis 2013).

The tooth replacement sequence observed in our material corresponded well with the sequences described for all species of the *Lynx* genus (Saunders 1964; Jackson et al. 1988; Garcia-Perea 1996). The contemporaneous presence of deciduous and permanent canine teeth and the gradual replacement of premolar teeth in all evaluated juveniles ensure a functional dentition in all phases of the tooth replacement (Garcia-Perea 1996).

Tooth wear scoring criteria

Tooth colour evolved with age from white to a distinct brown-yellow, as described in other species (Stander 1997; Smuts et al. 2009; Olifiers et al. 2010). This age-related graduation was more pronounced for canine compared to incisor teeth, an observation also reported in leopards *Panthera pardus* (Stander 1997) and humans (Watts and Addy 2001; Wetter et al. 2009). However, different lighting conditions and individual variation in saliva staining represent limitations to the reliability of colour evaluation as an ageing criterion (Watts and Addy 2001).

Evaluation of dental calculus in our material was challenging because the extent of calculus was generally low. Only three animals showed relatively marked amounts of calculus but when applying the scoring system developed for domestic cats, we obtained a calculus score of only 3/6 (= calculus covers less than 25% of the buccal surface of the crown; Clarke 2001, 2006). This discrete amount of dental calculus even in older lynx is likely to be diet-related. Similarly, feral cats in Australia had significantly lower calculus scores compared to non-feral domestic cats (Clarke and Cameron 1998), and free-ranging Alaskan grizzlies (*Ursus arctos horribilis*) showed less extensive calculus formation than captive brown bears (*U. arctos*; Wenker et al. 1999). Main lynx prey in Switzerland are roe deer followed by chamois (> 80% of prey items; Molinari-Jobin et al. 2007). Predating and feeding on large ungulates is likely to provide regular teeth friction with a preventive effect on calculus formation. Thus, calculus can be a useful criterion for ageing free-ranging lynx but it needs to be scaled down to the deposit amounts expected for animals hunting natural prey.

Assessment of canine tooth wear revealed alterations of tips, distal and buccal ridges over time, whereas the distal ridge was more prone to wear than the buccal one. This wear difference between the two ridges was also described for leopards (Stander 1997). It was more challenging to evaluate wear on canine tooth tips in older individuals because it was difficult to differentiate between old crown fractures with rounded edges and advanced wear of tips. According to our definitions, symmetrical changes on either both maxillary or mandibular canine teeth were considered as extended wear but crown fractures could occur symmetrically on several canine teeth and result in an overestimation of wear. Changes on canine teeth such as crown fractures, enamel flaking and open pulp cavities were present in all age categories. Consequently, these criteria alone are not suitable for an estimation of the age of an individual. Nevertheless, considering their increasing prevalence over time, their absence/presence represents complementary information.

Other than in leopards (Stander 1997) and grey wolves (*Canis lupus*; Gipson et al. 2000), which showed flattening and progressive wear on incisor teeth, we did not detect such changes in our material. However, in lynx, the number of incisor teeth showed a strong, negative correlation with increasing age. The loss of incisor teeth was also noted in old grey wolves and leopards although their number has not been tested for correlation with age (Stander 1997; Gipson et al. 2000). Similarly to canine tooth damages, incisor tooth loss is likely to be due to traumatic events, which may induce errors when applying the developed scheme. Yet, the consistent number of incisor teeth observed for each age category (Table 2) makes it a useful ageing criterion.

Scheme evaluation

Material limitations hampered the development of a scheme with optimal accuracy. First, although the sample size was remarkable for a non-hunted, small population of an elusive species such as the Eurasian lynx, it remained low for powerful analyses. This particularly affected the older age categories. Second, most of the teeth pictures had originally been made merely for documentation purposes in the framework of routine health procedures, without awareness of the critical points we would later consider for a thorough age evaluation. Thus, on some pictures, saliva and blood deposits, foreign material in the oral cavity, poor light conditions and not fully retracted lips complicated the evaluation. Third, we increased our sample size by using prepared skulls, which had the advantage to allow a prospective, standardised data collection but prevented the evaluation of several valuable criteria such as tooth colour and calculus.

It was not possible to correctly classify all lynx with the ageing scheme, even when skulls were excluded. However, classification success ranged from 69 to 88% when a subset of lynx was aged by different readers, which is similar to the values obtained for tooth wear evaluation in wolves (Gipson et al. 2000) and corresponds to the level of accuracy reached by tooth cementum analysis in species not listed in the high accuracy group (Matson's Laboratory; Gipson et al. 2000). Experience in lynx examination did not seem to influence the success rates of the readers, which indicates that criteria standardisation and user instructions are adequate but also suggests that the success rate depends on the meticulousness of the person using the ageing scheme. The kappa values were within a good to excellent range (Hallgren 2012), suggesting a high degree of inter- and intra-reader agreement. The few animals that were consistently over- or underestimated by most of the readers point to individual variations among lynx, which may be the major source of wrong classifications. However, no lynx was over- or underestimated by more than one age category, revealing that individual factors (either on the reader or the lynx side) only minimally affects the results, and that overall the accuracy and reliability of the scheme are satisfactory. Another element in favour of our scheme was that it performed similarly to or even better than the cementum analysis in three lynx for which the exact age was known thanks to field monitoring. It has also been observed in wolves that tooth wear evaluation may be superior to cementum analysis when estimating the age of older animals (Gipson et al. 2000). Although anecdotal, this observation not only highlights the relevance of our scheme, it also indicates that using the cementum annuli method as a gold standard may be another source of errors.

Our sample size did not permit us to test for potential differences between lynx from the Alps and from the Jura Mountains, which are known to be genetically distinct (Breitenmoser et al. 2016). Interpopulation variations in tooth wear have been observed in several herbivorous species such as roe deer *Capreolus capreolus* (Hewison et al. 1999), Spanish ibex *Capra pyrenaica* (Fandos et al. 1993) and Yellowstone bison *Bison bison* (Christianson et al. 2005) but they were attributed to differences in diet, namely to the siliceous and fluoride content of plants and soil. In carnivores such as African lions or grey wolves, no differences were detected among populations (Gipson et al. 2000; Smuts et al. 2009). We suppose that the strictly carnivorous diet of the Eurasian lynx leads to a relatively uniform tooth wear, even though prey species distribution can vary among populations (Breitenmoser and Breitenmoser-Würsten 2008). Thus, we assume that our scheme is applicable to free-ranging lynx from other parts of Eurasia.

Finally, since we tested the scheme on the same lynx used to develop it, the obtained level of accuracy may be overestimated. Future experience with lynx from Switzerland and other countries is required to evaluate the wide applicability of this ageing method.

Conclusion

We showed that a standardised evaluation of tooth wear is a practical approach to estimating the age of Eurasian lynx. If we accept that tooth wear assessment is susceptible to errors caused by individual variation and keep in mind that a stay in captivity (such as rehabilitation or quarantine) may complicate the evaluation due to diet factors and repeated tooth trauma, the accuracy of our scheme is expected to be sufficient for a wide range of research, monitoring and conservation purposes. The scheme is a particularly promising tool in situations where the risk of errors is outbalanced by its non-invasive, immediate and economical applicability. Additionally, it is a step forward in the harmonisation of methods as the scheme does not require any specialised equipment and can be applied at captures as well as during necropsies. For successful application of the scheme, we recommend considering the totality of the proposed criteria and taking at least one frontal and two lateral (left and right side) photographs of the dentition. Special attention should be paid to the adequate retraction of the lips (visible premolar and molar teeth including notches), cleaning teeth of foreign material, and appropriate light (tooth colour, visibility of canine tooth ridges and calculus deposits). As it is particularly challenging to obtain a good view on the distal canine ridge, we recommend palpating it and recording its condition on paper documents in addition to the pictures.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and institutional guidelines for the care and use of animals were followed.

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