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Morphometric characteristics of free-ranging Eurasian lynx *Lynx lynx* in Switzerland and their suitability for age estimation

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Knowledge of the age of individual animals is crucial to assess population dynamics, disease epidemiology and to successfully implement conservation strategies. Morphometric data reflect complex interactions of factors such as age and sex, and may also depend on genetics, population density, food availability, pathogen load and climate. The aims of this study were to assess the suitability of morphometric characteristics as an ageing tool for lynx during their growth period and to provide baseline data for the Eurasian lynx populations in Switzerland. Seventeen body measurements of 180 free-ranging Eurasian lynx *Lynx lynx* of known age, captured or found dead in Switzerland between 1981–2017 were compiled by sex and age class (juveniles, subadults, adults) and tested for significant differences between males and females, age classes, and populations (Jura Mountains, Alps). Classification tree analysis (CART) was performed to create an ageing tool based on physical characteristics. Generalised linear models revealed a significant effect of age and sex on measurements but no differences were found between populations. The growth pattern was characterised by a rapid increase of all parameters in the first year of life, followed by a slowdown in the subadult age class; the adult class corresponded to the post-growth period. Sex differences became apparent at the age of 9–11 months and were most pronounced in adults. The developed classification trees allowed us to correctly categorise 93% of the females and 92% of the males as juvenile, subadult or adult. In conclusion, classification trees based on body measurements can be used to place lynx into broad age categories and represent a standardised, non-invasive, fast, cost-free and very user-friendly tool. These trees can be successfully combined with tooth wear evaluation and deliver age information with an accuracy acceptable in the context of various epidemiological investigations and of the selection of individuals for translocation.

The Eurasian lynx *Lynx lynx* vanished from most European countries in the late 19th century (Breitenmoser et al. 1998, Hellborg et al. 2002) but subsequent conservation efforts have contributed to a partial recovery of lynx populations (Chapron et al. 2014). Among others, the release of lynx originating from the Carpathian Mountains resulted in two genetically distinct populations in Switzerland, one in the Alps and the other in the Jura Mountains, which together now consist of almost 200 independent individuals (Chapron et al. 2014, Breitenmoser et al. 2016). In parallel to conservation measures, numerous studies have been carried out on the lynx life history in various areas in Europe, and health surveillance programmes have been implemented (Wölfl et al. 2001, Ryser-Degiorgis et al. 2005, Molinari-Jobin et al. 2012, Breitenmoser et al. 2016). Knowledge of the age of individual animals is crucial to perform ecological studies, investigate disease epidemiology and successfully implement conservation strategies (Stander 1997, Gipson et al. 2000, Karels et al. 2004, Ryser-Degiorgis 2013, Chevallier et al. 2017). So far, the age of Eurasian lynx has been either roughly estimated or determined by counting cementum annuli, which is an invasive and costly method that cannot provide immediate results. Alternative tools would be valuable to both research and practical conservation work.

We previously showed that tooth wear scoring is a promising method to estimate the age of Eurasian lynx. However, tooth evaluation is limited in animals with jaws severely damaged by traumatic events (e.g. fatal traffic accidents), and the discrimination of subadults from old juveniles and from young adults remains challenging (Marti and Ryser-Degiorgis 2018). Considering that lynx continue to grow after their first year of life (Andersen and Wiig 1984, Yom-Tov et al. 2010, 2011), selected morphological measurements may contribute to a more accurate estimation of the age of young animals. Data on morphological characteristics of lynx may also be useful for other purposes since morphology may reflect the complex interactions of various external and internal factors including not only age and sex but also food availability, genetics, and climate (Naidenko 2006, Yom-Tov et al. 2011). In other species, morphological data were even shown to be indicators for population density, pathogen load, immune competence, survival, fertility and breeding success (Garshelis 1984, Gaillard et al. 2000, Christe et al. 2000, Mysterud et al. 2001, Soler et al. 2003, Moretti 2014).

At present, available morphometric data for Carpathian lynx are very limited regarding the sample size studied, age groups analysed and sample statistics provided. Furthermore, the existing documentation is only available in local language (Matjuškin 1978, Garcia-Perea 1990, Breitenmoser and Breitenmoser-Würsten 2008). The aim of this study was to assess the suitability of morphometric characteristics as an ageing tool for lynx during their growth period and to provide baseline data for the Eurasian lynx populations in Switzerland.

Material and methods

Animals

Data were collected from 180 free-ranging Eurasian lynx between 1981–2017 in Switzerland. The sample included 131 dead lynx submitted for pathological examination and 49 lynx that were examined alive (anesthetised with medetomidine/ketamine hydrochloride; Ryser et al. 2005). Animals originated from the Swiss Alps (n = 101), from the Jura Mountains (n = 65) and from a recently reintroduced population nucleus in northeastern Switzerland (n = 14). Age was determined using the following methods (Marti and Ryser-Degiorgis 2018): age of lynx clearly recognised as juveniles (small body size and milk dentation) was calculated in months based on the known narrow birth period in May–early June (Schmidt 1998, Henriksen et al. 2005, Breitenmoser-Würsten et al. 2007) given that they were found either alive or as fresh carcasses; age determination for all other individuals was performed by counting tooth cementum annuli in canines or incisors (Matson's Laboratory, Manhattan, MT, USA), unless the exact age was known thanks to marking procedures of lynx at the kitten age. Animals known to be older than 2 or 3 years (for females and males, respectively) thanks to repeated captures or detections by phototrapping were categorised as adults (without data about their exact age in years). Lynx age classes were defined as previously described (Marti and Ryser-Degiorgis 2018). Juveniles are lynx in their first year of life (i.e. from birth in May to April of the following year); subadults are females in their second and males in their second and third years; adults are all older lynx (≥2 years for females and ≥3 years for males). The sample included 103 (57%) juveniles, 30 (17%) subadults and 47 (26%) adults. The age ranged from 6 weeks to 18 years. The overall sex ratio was 85 males versus 95 females.

Data collection

Twelve physical parameters (Table 1, Fig. 1) were recorded and bilateral data collection for five parameters resulted in a total of 17 measurements per animal. All measurements were recorded to one decimal place using a flexible measuring tape. All lynx were measured in lateral recumbency. Dead lynx were placed on a table indoor while live lynx were mostly handled on the ground under field conditions. A number of collaborators (approximately 40) were involved in data collection over nearly four decades but all of them were instructed and trained to work according to the same standardised protocols. These protocols based on procedures applied at the Museum of Natural History of Bern and used in carnivore ecology (Table 1, Fig. 1; Boitani and Powell 2012). All measurements were taken once, originally for documentation purposes only.

Data analyses and statistics

We differentiated between juveniles < 9 months and 9–11 months old based on their dentition (Marti and Ryser-Degiorgis 2018) to test for differences between 'old juveniles' and subadults. We differentiated between three arbitrarily chosen adult categories to assess potential

Table 1. Overview of body measurements recorded from captured and dead Eurasian lynx *Lynx lynx* from Switzerland. Weight is measured within 0.1 kg all lengths within 0.1 cm.

Parameter	Description
Body weight	Total body weight
Body length (stretched)	Projection from the tip of the nose to the sacrococcygeal joint, with the lynx placed on a table in a stretched position
Body length (physiological)	From the tip of the nose to the sacrococcygeal joint, following the animal's contours, with the lynx in a physiological recumbent position
Neck circumference	Measured not too tightly around the caudal portion of the neck
Shoulder height	From the foot pad of the front paws to the top of the shoulder blade, in a straight line as if the lynx was walking (although the lynx is measured in recumbent position)
Tail length	From the sacrococcygeal joint to the tip of the last caudal vertebra
Hind foot length	Measured from the tip of the calcaneus to the distal end of the foot pad on the longest digit (both feet)
Inter-canine distance	Distance between canine tooth tips (maxilla and mandibula)
Ear length (notch–apex)	From the bottom of the lateral ear notch (intertragic incisure) to the apex of the pinna (left and right)
Ear length (anthelix–apex)	From the bottom of the fold behind the anthelix to the apex of the pinna (left and right)
Ear tuft length	Hair shaft length of the longest tuft hairs (left and right), measured from the basis to the tip of the hair shaft
Anogenital distance	Distance between the centre of the anus and the centre of the vulva/opening of preputium

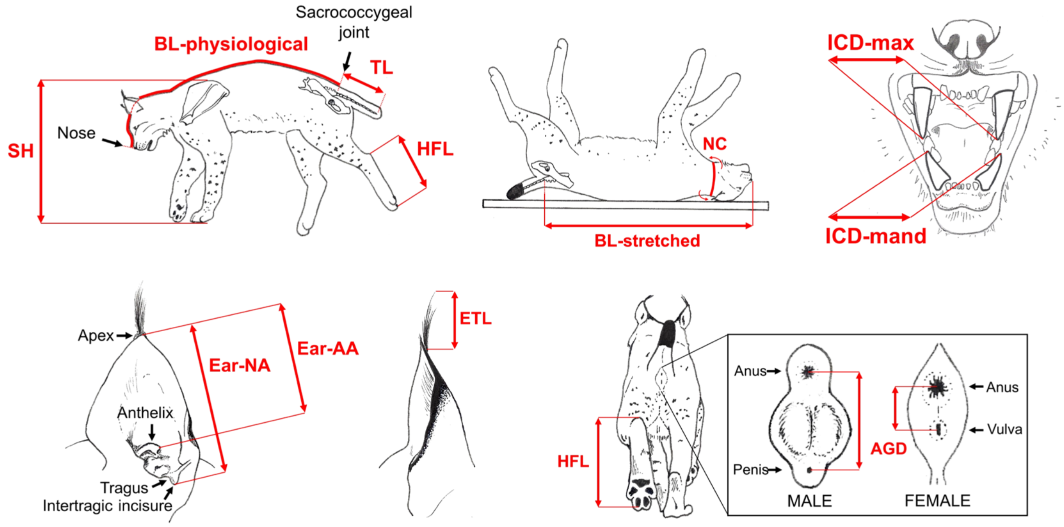

Figure 1. Physical parameters measured on Eurasian lynx *Lynx lynx* from Switzerland: BL-physiological = Body length measured with the animal placed in a physiological position, SH = Shoulder height, TL = Tail length, HFL = Hind foot length, BL-stretched = Body length measured in a stretched position, NC = Neck circumference, ICD-max = Inter-canine distance of the maxillary canine teeth, ICD-mand = Inter-canine distance of the mandibular canine teeth, Ear-NA = Ear length measured from the bottom of the lateral ear notch (intertragic incisure) to the apex of the pinna, Ear-AA = Ear length measured from the bottom of the fold behind the anthelix to the apex of the pinna, ETL = Ear tuft length, AGD = Anogenital distance.

differences within the adult age class: young adults (up to 5 years; n = 10), intermediate (>5–10 years; n = 15) and old (>10 years; n = 7) adults. Only lynx in physiological body condition (good muscle mass, no prominent bone processes on the back, shoulder, pelvis and hip joints) were included in body weight evaluation. Datasets of 89/180 individuals were incomplete due to damaged or missing body parts (advanced decay, scavenging, trauma), resulting in sample size variation among parameters.

Statistical analyses and figures were done using R (<www.r-project.org>). Level of significance was set at 0.05. In a first step, we performed univariate comparisons between each parameter and the factors sex, age and population by applying the two-sample Wilcoxon test (Wilcoxon rank sum test with continuity correction) when the explanatory variable included only two categories or the Kruskal–Wallis rank sum test with post hoc Mann–Whitney–Wilcoxon test followed by Holm–Bonferroni correction for three categories. Subsequently, we examined the effects of age, sex, population and their interactions on each physical parameter by fitting general linear models (glm). Best model selection was done using Akaike's information criterion (AIC) and considering that models with a ΔAIC of 0–2 provide similar support (Burnham and Anderson 2001).

In a second step, we conducted a classification tree analysis (CART analysis) to produce an ageing tool. CART analysis was performed for males and females separately. Advantages of this method are that it does not assume normally distributed data, is not influenced by outliers and that variables can be selected multiple times at each stage unlike in parametric stepwise procedures (Karels et al. 2004). Recursive partitioning uses a series of dichotomous splits (e.g. longer or shorter than 85 cm) to produce decision trees that are easy to interpret (Kim et al. 2010, Zimmerman et al. 2016). We set age category (juvenile, subadult or adult) as a response variable and included all body measurements in model construction. Our sample size did not allow splitting the data into train and test sets, thus all available data were included in model construction. To avoid overfitting the data, trees were pruned using the built-in function (prune.tree) with which the optimal number of terminal nodes was defined by determining a nested sequence of subtrees of the original tree by recursively snipping off the least important splits, based upon the cost-complexity measure (<www.r-project.org>). Classification accuracy of the trees was assessed with the same lynx included in the model construction (n = 159).

Testing the accuracy of classification trees and their combination with our tooth wear scheme

We used a subset of 24 lynx, for which not only exact age and body measurements but also good quality tooth pictures were available, to compare the performance of the classification trees and of our tooth wear scheme (Marti and Ryser-Degiorgis 2018). The test set included eight juveniles between 9–11 months, eight subadults and eight adults, each group with four males and four females. The first author aged all lynx by applying first the tooth wear scheme, followed by the classification trees. This order was chosen because the age class selection with the classification trees cannot be influenced by the results of the tooth wear scheme, whereas the contrary cannot be completely ruled out.

Results

Median, interquartile range, minimum and maximum values of the 17 recorded body measurements are indicated by sex and age class in Table 2 and 3. Generalised linear models and the univariate comparisons revealed significant effects of age and sex but not of population on body measurements

Table 2. Median followed by the interquartile range (separated by a comma) and minimum and maximum values (in parentheses) for 17 body measurements for female Eurasian lynx *Lynx lynx* from Switzerland. Values for body weight are given in kg and for all other parameters in cm.

Parameter	Juvenile (all)	Juvenile 9–11 months	Subadult	Adult	Sample size
Body weight	6.8, 3.7 (1.5–14.2)	11.5, 3.0 (6.1–14.2)	16.0, 2.9 (9.8–18.5)	18.0, 2.2 (15.0–21.0)	89
Body length (stretched)	59.0, 10.0 (34.5–85.2)	67.5, 10.8 (58.0–75.0)	81.0, 6.7 (66.0–88.0)	85.0, 3.5 (80.0–93.0)	76
Body length (physiological)	70.7, 11.7 (37.0–93.0)	82.0, 7.8 (67.0–93.0)	96.5, 10.4 (77.0–103.0)	98.0, 5.5 (92.0–105.0)	98
Neck circumference	23.0, 5.1 (15.0–29.0)	26.8, 2.9 (22.0–29.0)	28.6, 2.8 (23.0–37.0)	29.8, 4.8 (23.0–35.5)	103
Shoulder height	35.5, 8.4 (20.0–47.0)	41.5, 7.5 (33.0–48.0)	48.0, 4.7 (35.5–54.0)	50.7, 4.1 (46.0–56.0)	102
Tail length	14.0, 4.0 (9.2–20.0)	17.8, 4.0 (12.5–20.0)	18.2, 3.5 (13.5–21.0)	20.0, 2.0 (16.8–23.5)	105
Hind foot length, right	19.0, 3.0 (10.9–23.0)	20.5, 1.4 (19.0–23.0)	22.0, 1.6 (19.0–24.0)	22.9, 0.8 (21.5–24.0)	104
Hind foot length, left	18.5, 3.4 (10.8–22.0)	20.5, 1.5 (16.5–22.0)	22.0, 1.8 (19.0–24.0)	22.5, 1.0 (19.5–24.0)	108
Inter-canine distance, maxillary canine teeth	2.5, 0.4 (1.8–3.1)	2.7, 0.2 (2.5–3.0)	2.9, 0.2 (2.4–3.3)	3.0, 0.2 (2.7–3.2)	104
Inter-canine distance, mandibular canine teeth	2.0, 0.6 (1.2–2.8)	2.4, 0.1 (2.0–2.6)	2.6, 0.2 (2.2–3.0)	2.6, 0.2 (2.3–2.9)	104
Right ear (notch–apex)	7.5, 0.9 (5.7–8.8)	7.8, 0.7 (7.0–8.2)	8.0, 0.4 (7.6–9.5)	8.6, 0.3 (8.2–9.0)	70
Left ear (notch–apex)	7.5, 0.7 (6.0–8.9)	8.1, 0.4 (7.5–8.4)	8.1, 0.6 (7.5–8.6)	8.6, 0.4 (8.2–9.0)	69
Right ear (anthelix–apex)	6.5, 0.7 (4.5–7.4)	7.1, 0.2 (6.0–7.3)	7.5, 0.2 (6.9–8.0)	7.5, 0.4 (7.1–8.0)	93
Left ear (anthelix–apex)	6.6, 0.7 (4.5–7.5)	7.1, 0.3 (6.5–7.5)	7.5, 0.15 (6.7–8.0)	7.6, 0.4 (7.2–9.0)	98
Right ear tuft	2.2, 1.1 (0.9–4.0)	3.0, 0.5 (2.3–4.0)	3.3, 0.5 (2.0–4.0)	3.5, 0.4 (2.8–4.5)	103
Left ear tuft	2.0, 1.2 (1.0–3.8)	3.2, 0.5 (2.4–3.6)	3.3, 0.5 (2.0–4.0)	3.5, 0.8 (2.8–4.4)	102
Anogenital distance	2.0, 0.4 (1.0–3.0)	2.4, 0.5 (1.2–3.0)	2.5, 0.7 (1.8–3.4)	2.6, 0.5 (2.1–3.5)	99

(Table 4–6), consequently lynx from different geographical origins were pooled.

Age

All parameters including lengths and weight were characterised by a rapid increase during the first year of life, a slowdown during the following 1–2 years, and finally a plateau from 2 and 3 years old in females and males, respectively (Fig. 2, Table 4, Supplementary material Appendix 1 Fig. A1, Fig. A2). Differences between 9–11 months old juveniles and subadults were significant for body weight and for 6 length parameters (body length, shoulder height, neck circumference, maxillary and mandibular inter-canine distance) for both females and males. Significant differences were additionally found for tail length and ear tuft lengths in males, and ear lengths (measured from anthelix to apex) in females only. Hind foot lengths were longer in subadults compared to juveniles but differences were significant only on the right and left side in males and females, respectively (Table 5).

No significant differences were detected among the three adult subcategories (young, intermediate and old adults) and all adults were subsequently treated as a single group. The comparison of subadults with adults revealed significant differences for body weight and body length in both males and females. Additionally, adult females had significantly longer tails, inter-canine distances, ear lengths (notch-apex) and right hind feet, and adult males had longer hind feet, anogenital distances and left ear tufts (Table 5).

A juvenile male hit by a train in January 2006 was identified as an outlier regarding all body measurements. The stage of tooth replacement corresponded to an age of 5 months (Marti and Ryser-Degiorgis 2018) and body measurements were in the same range as those of other 5-month-old lynx. Consequently, this animal was considered as a kitten from a replacement litter (born in August). It was kept in the dataset as 'juvenile' but excluded from all data analyses which considered the exact age.

Sex

There were no significant differences between males and females in their first year of life for any of the body measurements considered, except for the anogenital distance (wider in males, p-value < 0.001) and for the maxillary inter-canine distance in 9–11 months old lynx (wider in males; p-value $= 0.010$; Table 6). Among subadults, males had significantly higher values for all parameters except for the physiological body length and ear lengths. Among adults, differences between males and females

Table 3. Median followed by the interquartile range (separated by a comma) and minimum and maximum values (in parentheses) for 17 body measurements for male Eurasian lynx *Lynx lynx* from Switzerland. Values for body weight are given in kg and for all other parameters in cm.

Parameter	Juvenile (all)	Juvenile 9–11 months	Subadult	Adult	Sample size
Body weight	6.0, 2.6 (1.6–14.2)	12.3, 1.7 (8.9–14.2)	19.5, 4.0 (16.0–23.5)	22.4, 3.2 (19.0–26.8)	68
Body length (stretched)	60.0, 3.6 (37.0–76.5)	74.3, 2.0 (70.0–76.5)	89.5, 7.0 (85.0–102.0)	91.0, 3.6 (87.0–109.0)	64
Body length (physiological)	68.9, 12.0 (40.5–91.0)	83.0, 4.7 (77.0–91.0)	98.5, 3.8 (40.5–101.0)	103.0, 4.5 (94.0–108.0)	81
Neck circumference	23.0, 4.0 (13.2–33.5)	25.2, 1.5 (23.0–27.5)	32.3, 3.7 (27.5–40.0)	33.0, 3.8 (27.0–43.0)	87
Shoulder height	37.0, 7.0 (20.0–48.0)	44.3, 2.4 (41.0–48.0)	52.6, 5.3 (46.0–60.5)	53.6, 5.5 (49.0–61.0)	86
Tail length	14.0, 3.1 (4.0–18.9)	15.8, 1.6 (14.0–18.9)	21.0, 2.8 (13.2–24.0)	21.5, 2.0 (17.6–27.0)	91
Hind foot length, right	18.2, 2.7 (11.0–23.0)	21.0, 0.7 (20.5–23.0)	22.9, 1.0 (19.2–24.2)	23.8, 1.5 (21.9–25.5)	90
Hind foot length, left	18.1, 2.6 (11.0–23.4)	21.2, 1.0 (20.5–23.4)	23.0, 1.0 (19.1–24.5)	24.0, 1.0 (21.2–26.5)	90
Inter-canine distance, maxillary canine teeth	2.5, 0.8 (1.2–3.7)	2.9, 0.2 (2.8–3.7)	3.1, 0.4 (2.8–3.5)	3.2, 0.2 (2.8–3.9)	89
Inter-canine distance, mandibular canine teeth	2.0, 0.7 (1.4–3.1)	2.5, 0.0 (2.3–3.1)	2.8, 0.4 (2.3–3.3)	2.8, 0.2 (2.0–3.4)	82
Right ear (notch–apex)	7.6, 1.0 (5.7–8.8)	8.5, 0.4 (8.1–8.8)	8.8, 0.8 (8.0–9.5)	9.0, 0.9 (8.3–9.7)	64
Left ear (notch–apex)	7.6, 0.6 (5.5–8.6)	8.2, 0.2 (8.0–8.3)	8.5, 1.0 (6.8–9.6)	9.0, 0.4 (8.4–9.8)	66
Right ear (anthelix–apex)	6.7, 0.9 (5.0–8.0)	7.4, 0.3 (7.2–8.0)	7.9, 0.6 (7.0–8.5)	8.0, 0.4 (7.0–8.6)	80
Left ear (anthelix–apex)	6.8, 0.8 (4.8–8.0)	7.5, 0.2 (7.2–8.0)	7.5, 0.8 (5.4–8.6)	8.0, 0.7 (7.0–8.5)	82
Right ear tuft	2.0, 0.7 (1.0–3.6)	3.1, 0.3 (2.5–3.6)	4.5, 0.6 (2.8–4.9)	4.5, 0.6 (3.5–5.5)	82
Left ear tuft	2.0, 0.8 (1.0–3.8)	3.4, 0.4 (2.8–3.8)	4.0, 0.9 (2.8–5.0)	4.5, 0.8 (3.5–5.7)	86
Anogenital distance	4.8, 0.8 (2.5–7.0)	5.5, 1.3 (4.0–7.0)	6.1, 0.7 (4.8–8.0)	6.5, 1.3 (5.5–8.8)	78

were most pronounced, with males having significantly higher values for all parameters except for the ear lengths (Table 6).

Age classification trees

The obtained classification trees to place lynx in one of the three age categories (juvenile, subadult, adult) differed between males and females (Fig. 3). The parameters included in the trees and the differences observed among juveniles, subadults and adults are illustrated in Fig. 4. The first prediction factor for both sexes was body length (measured in physiological position), which distinguishes juveniles from older lynx. Furthermore, shoulder height, maxillary inter-canine distance and ear length for females and body length and anogenital distance for males were used to differentiate between subadults and adults.

Overall, the classification trees placed 92.7% (76/82) of the females and 92.2% (71/77) of the males in the correct age category. Accuracy of the trees was highest in the juvenile and adult age class (94.3% for females: 54/56 juveniles and 12/14 adults, and 98.5% for males: 45/45 juveniles and 19/20 adults correctly classified) but acceptable in subadults (70.8% for females: 10/12 subadults, and 58.3% for males: 7/12 subadults correctly classified).

Classification trees and tooth wear scheme

Overall the tooth wear scheme resulted in a higher number of correctly classified animals in the test set than did the trees. In

Table 4. Parameters of the best glm obtained for body length (physiological) and anogenital distance of Eurasian lynx *Lynx lynx* from Switzerland.

Model	Term	Estimate	SE	t-value	p-value
Model: Body length (physiological) AGE + SEX:AGE	Intercept	72.555	1.335	54.340	<0.001 ***
	AGE	0.316	0.044	7.257	<0.001 ***
	SEX:AGE	–0.107	0.052	–2.040	0.043 *
Model: Anogenital distance AGE + SEX + SEX:AGE	Intercept	4.681	0.134	34.828	<0.001 ***
	AGE	0.021	0.003	7.866	<0.001 ***
	SEX	–2.575	0.175	–14.694	<0.001 ***
	SEX:AGE	–0.014	0.004	–3.525	<0.001 ***

Table 5. p-values of the Wilcoxon rank sum test with continuity correction applied to body measurements of Eurasian lynx *Lynx lynx* from Switzerland for both sexes to compare different age groups.

	Females		Males	
Parameter	Juveniles (9–11) versus subadults	Subadults versus adults	Juveniles (9–11) versus subadults	Subadults versus adults
Body weight	0.002 **	<0.001 ***	0.004 **	0.002 **
Body length (stretched)	0.014 *	0.014 *	0.006 **	0.303
Body length (physiological)	<0.001 ***	0.094	<0.001 ***	<0.001 ***
Neck circumference	0.031 *	0.343	<0.001 ***	0.304
Shoulder height	0.002 **	0.084	0.001 **	0.211
Tail length	0.465	0.006**	0.005 **	0.273
Hind foot length, right	0.056	0.027 *	0.034 *	0.024 *
Hind foot length, left	0.008 **	0.105	0.051	0.023*
Inter-canine distance, maxillary canine teeth	0.029 *	0.041 *	0.057	0.642
Inter-canine distance, mandibular canine teeth	0.049 *	0.028 *	0.110	0.502
Right ear (notch–apex)	0.264	0.024 *	0.599	0.071
Left ear (notch–apex)	0.384	0.017 *	0.146	0.067
Right ear (anthelix–apex)	0.008 **	0.153	0.105	0.728
Left ear (anthelix–apex)	0.004 **	0.125	0.340	0.483
Right ear tuft	0.216	0.199	0.005**	0.172
Left ear tuft	0.613	0.112	0.013 *	0.017 *
Anogenital distance	0.065	0.730	0.228	0.030 *

juveniles (n = 8) and adults (n = 8), the tooth scheme classified all lynx correctly while the classification trees overestimated one juvenile female and underestimated one adult male. In subadult males, the tooth scheme performed slightly better, as it underestimated one male while the trees wrongly classified two animals (over- and underestimation, respectively). In subadult females, the tooth scheme underestimated two lynx, whereas all were correctly classified by the trees. Regarding the separation of subadult from adult females, the performance of the tooth scheme and tree could not be directly compared as the tooth scheme does not allow a separation of subadults from 2 years old adult females. No individual was placed in the wrong category by either method.

Discussion

We provided baseline data on body measurements of Eurasian lynx from Switzerland for different sex and age categories. These data may be useful for future studies on various topics and can also contribute to determine the age of lynx for which the application of the classification trees is not possible (missing measurements).

Age

The observed growth patterns show that lynx not only increase in size during their first year of life but that they also continue their physical development up to their second and third year in females and males, respectively, in agreement with previous reports (Andersen and Wiig 1984, Yom-Tov et al. 2010, 2011). The age of skeletal maturity varies among felines. For example, the domestic cat *Felis silvestris catus* is fully grown at 20–24 months while lions *Panthera leo* grow up to the age of 4.5 years (Smith 1969, Kirberger et al. 2005). With a growth period lasting up to 24 (females) and 36 months (males), Eurasian lynx seem to take up an intermediate position.

Table 6. p-values of the Wilcoxon rank sum test with continuity correction applied to body measurements to compare male and female Eurasian lynx *Lynx lynx* from Switzerland within the same age group (all juveniles, 9–11 month-old juveniles, subadults, adults).

Parameter	Juveniles (all)	Juveniles (9–11)	Subadults	Adults
Body weight	0.117	0.128	<0.001 ***	<0.001 ***
Body length (stretched)	0.335	0.134	0.001 **	0.009 **
Body length (physiological)	0.647	0.384	0.068	<0.001 ***
Neck circumference	0.662	0.260	0.016 *	<0.001 ***
Shoulder height	0.704	0.324	0.010 *	0.002 **
Tail length	0.814	0.481	0.003 **	0.001 **
Hind foot length, right	0.238	0.182	0.013 *	<0.001 ***
Hind foot length, left	0.447	0.151	0.038 *	<0.001 ***
Inter-canine distance, maxillary canine teeth	0.865	0.010 *	<0.001 ***	<0.001 ***
Inter-canine distance, mandibular canine teeth	0.997	0.240	0.004 **	0.002 **
Right ear (notch–pex)	0.531	0.105	0.050	0.084
Left ear (notch–apex)	0.840	0.798	0.131	0.014 *
Right ear (anthelix–apex)	0.518	0.210	0.025 *	0.040 *
Left ear (anthelix–apex)	0.657	0.341	0.107	0.052
Right ear tuft	0.481	0.667	<0.001 ***	<0.001 ***
Left ear tuft	0.367	0.389	<0.001 ***	<0.001 ***
Anogenital distance	<0.001***	0.002 **	<0.001 ***	<0.001 ***

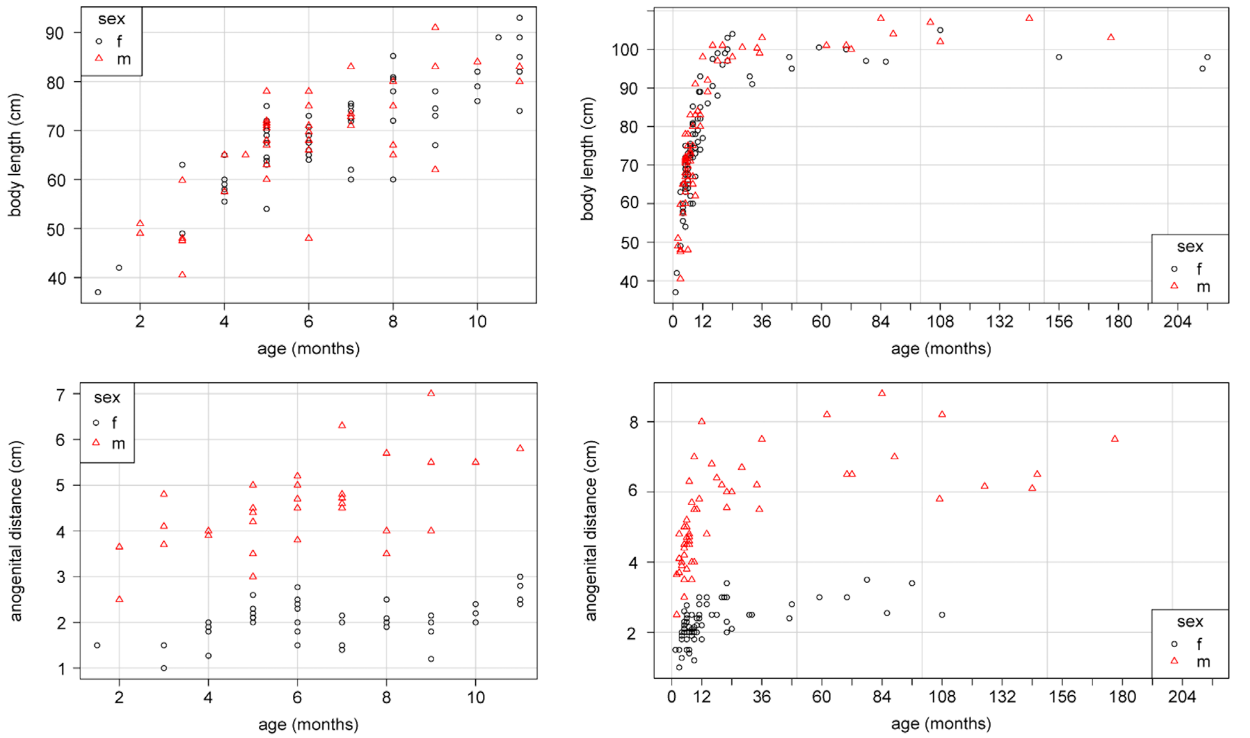

Figure 2. Growth curves of female (circle) and male (triangle) Eurasian lynx *Lynx lynx* from Switzerland, illustrated with the example of body length measured in a physiological position (top) and anogenital distance (bottom) during the first year of life (left) and for the entire life-span (right).

Our morphometric measurements indicate that the three lynx age classes correspond to the initial fast growth period (juveniles), the final slower growth period (subadults) and the post-growth period (adults). These classes were originally defined based on the typical social behaviour of lynx (family life, dispersal, establishment of a territory of their own and reproduction (Zimmermann et al. 2005, Tryland et al. 2011). In the Eurasian lynx approximately 50% of the females are fertile at < 1 year and about 50% of males reach a fertile stage during the mating season of their second year, although successful reproduction usually takes place only in subsequent years (Kvam 1991, Axnér et al. 2009). Sexual maturity indeed often precedes skeletal maturity in mammals (Kilborn et al. 2002). Thus, while the three age classes are largely in agreement with social and reproductive behaviour of lynx, they are most closely linked with their growth pattern, i.e. body measurements represent interesting parameters to discriminate the three age classes.

Furthermore, our results show that it is worthwhile to measure not only the body length for ageing purposes but also additional body parts, as not all of them have the same growth pattern. Indeed, growth plates of different bones

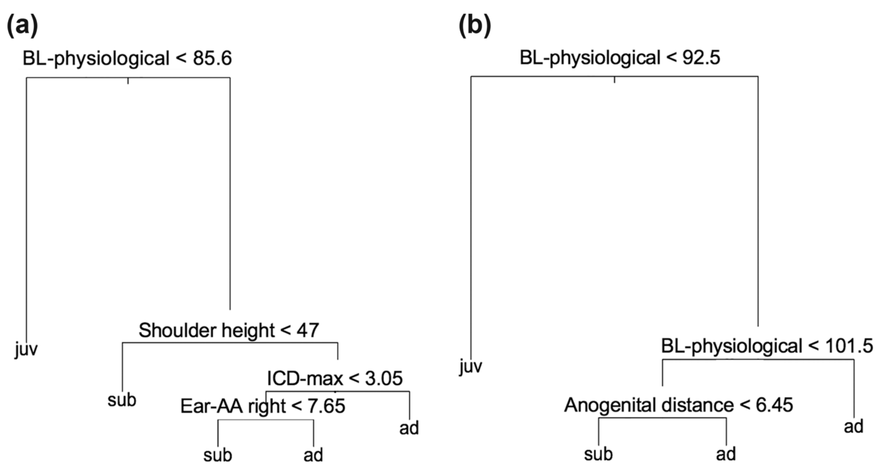

Figure 3. Age classification trees to categorise female (a) and male (b) Eurasian lynx *Lynx lynx* from Switzerland as juvenile (juv), subadult (sub) or adult (ad). Values are given in cm. BL-physiological = body length measured with the animal placed in a physiological position, ICD-max = inter-canine distance of the maxillary canine teeth, Ear-AA right = Ear length measured from the anthelix to the apex of the pinna on the right side.

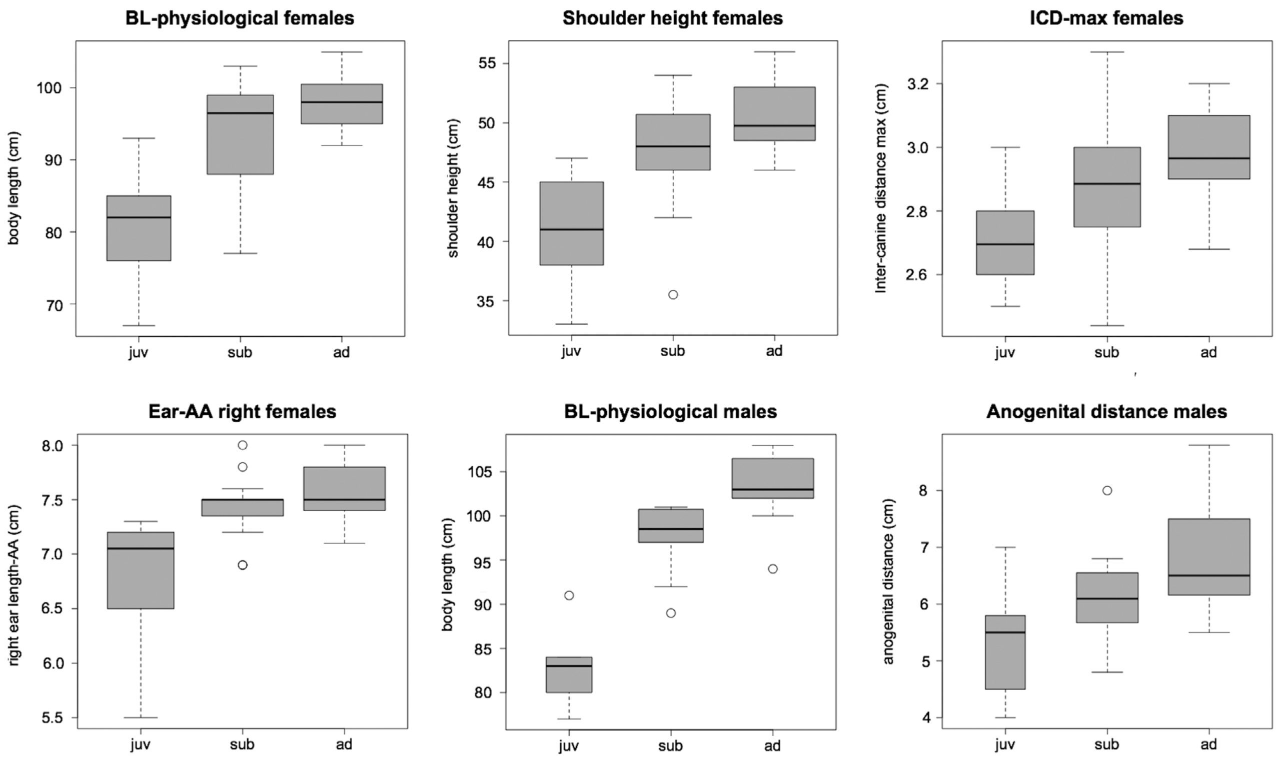

Figure 4. Overview of the measurements in the juvenile, subadult and adult age categories (juv: juvenile; sub: subadult; ad: adult) of the Eurasian lynx *Lynx lynx* in Switzerland, which were integrated into the classification trees.

close at different ages (Smith 1969, Kilborn et al. 2002). We detected significant differences between age classes for only one of the bilateral measurements for hind foot lengths and ear tufts. Asymmetric growth is typically associated with pathological processes (Gurney 2002, Samoy et al. 2006, Huynh et al. 2007, Miller et al. 2016), which we did not observe in our lynx. Although we cannot totally exclude asynchronous growth, these differences may have been due to measurement errors or to the limited sample size.

Evidence of replacement litters has rarely been documented in free-ranging Eurasian lynx (Breitenmoser-Würsten et al. 2007). The incidental detection of a juvenile with morphological characteristics indicating a birth in August illustrates the interest of morphological data to identify individuals born outside the regular birth period.

Sex

We noted the first difference between sexes at 9–11 months, when males had a significantly greater inter-canine distance. From that period onwards, sexual dimorphism was increasingly marked, with males continuing to grow for one year longer than females. The larger size of adult males has been described throughout the genus *Lynx* (Saunders 1964, Crowe 1972, Beltran and Delibes 1993, Yom-Tov et al. 2011). The age of 9–11 months corresponds to the period of separation of juveniles from the dam, which begins in February and reaches a peak in April (Zimmermann et al. 2005). Therefore, our findings indicate that male and female lynx kittens grow equally as long as they are together with their mother, in agreement with previous observations in captive lynx kittens (Naidenko 2006).

The anogenital distance was the single measurement significantly differing between sexes from the youngest age, reflecting the different anatomy of males and females (Salomon et al. 2018). Male lynx present the typical anatomical features of male domestic cats, which include a penis orientation towards caudal, a very small *glans penis*, and a very short but thick and hair-covered preputium which also opens caudally (Dyce et al. 1991). Visually, this results in a small opening just below the testicles that resembles the vulva of females. In lynx like in cats, it takes several weeks until the testicles have reached their final location in the preputium, and testicles are then located close to the perineum (i.e. surface between the pubic arch and tail bone) and the preputium is hidden by a thick hair layer (Dyce et al. 1991). In lynx, juvenile immature testicles are very small and remain invisible for several months. Thus, the anogenital distance may be useful for sex determination before testicles are apparent.

Population

We did not detect differences in lynx body mass or size between the two studied populations, similarly to a study on lynx pelvis measurements (Morend 2016). In Switzerland the two main lynx populations have developed from a small number of individuals captured in the Carpathian Mountains (Breitenmoser 1998). They have gone through a massive genetic bottleneck and are now genetically distinct (Breitenmoser et al. 2016) and characterised by different frequencies of specific coat patterns (Thüler 2002). Our data suggest that body size was not affected by the genetic drift and that our data may also be valid for Carpathian lynx in other European countries. However, a study in the Iberian lynx provided evidence for morphometric differentiation in skull measurements among three populations (Pertoldi et al. 2005). Detailed investigations including genetic data would be necessary to further elucidate possible consequences of population bottlenecks on morphometric traits in Carpathian lynx. Anyhow, our measurements and classification trees can probably not be applied to other Eurasian lynx subspecies due to known size differences (Matjuškin 1978, Naidenko 2006, Breitenmoser and Breitenmoser-Würsten 2008).

Classification trees

Classification tree analysis was successfully used to age hoary marmots *Marmota caligata* and Vancouver Island marmots *M. vancouverensis* (Karels et al. 2004). We have shown that this approach is well applicable to lynx, as we obtained a high classification accuracy for both sexes (93% in females, 92% in males); this was even higher than in marmots (81%; Karels et al. 2004). These proportions of animals correctly classified by CART analysis are among the highest compared to other non-invasive, standardised ageing methods across wild terrestrial mammal species (Garshelis 1984, Gipson et al. 2000, Høye 2006, Olifiers et al. 2010, Chevallier et al. 2017). However, since we tested the classification trees on the lynx that were included in the model construction, the accuracy of our trees might be overestimated. Furthermore, our dataset did not allow assessing any intra- or inter-observer agreement, and measurement errors could not be excluded. Nevertheless, considering that the trees are based on data collected over four decades by numerous persons in the field, i.e. under the same conditions as those for which the trees have been developed, we expect the trees to be a quite robust tool. Classification trees based on body measurements appear to be a promising method for ageing wild mammals. It is indeed a particularly user-friendly method: once the measurements are taken, no training, special equipment or specific knowledge is required to use the trees, and the data collected in the field do not need to be fitted into calculation-intensive models.

We compared the classification trees and the tooth wear evaluation with a small subset of lynx of known age and obtained a slightly higher overall accuracy with the tooth wear scheme (87.5 versus 83.4%). Another advantage of the tooth wear scheme is that it achieves a more precise ageing thanks to the possibility of dividing juveniles in eight subclasses (based on tooth replacement) and adults into four subclasses (based on tooth wear). However, only the trees permit a distinction between subadult and young adult females. Considering the different advantages and drawbacks of these two methods, we recommend using them as complementary ageing tools. Their combination is also expected to reduce the risk of misclassifications, as none of our lynx was misclassified by both methods. The results of our comparison test suggest relying on tooth wear evaluation when the two approaches deliver contradicting results, unless the tree classifies a female as a subadult. Ideally, the tooth wear scheme should be applied before the classification trees to maximize the objectivity of the assessment. Last but not least, the availability of two different methods provides alternatives when data collection is hampered by advanced decay, severe trauma or technical limitations.

Like any other method, the trees and the tooth wear scheme (Marti and Ryser-Degiorgis 2018) may be both influenced by errors during data collection. Therefore, to successfully apply the trees it is crucial that the measurements are taken consistently and according to a standardised protocol; for the application of the tooth scheme, the examination of tooth characteristics needs to be done carefully, by strictly following the provided instructions for the appreciation of tooth colour and scoring of wear. Once these data are collected, the final age class selection can be done in an absolutely (trees) or fairly (tooth scheme) objective way.

Conclusion

Body measurements can be used to classify Eurasian lynx in three main age classes (juveniles, subadults and adults). Fitted to classification trees (CART analysis), they provide a standardised, non-invasive, fast, cost-free and very user-friendly ageing tool applicable under field conditions. These trees can be successfully combined with tooth wear evaluation and deliver age information with an accurracy acceptable in the context of various ecological and epidemiological investigations and for the selection of individuals for translocation.

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Supplementary material (available online as Appendix wlb-00432 at <www.wildlifebiology.org/appendix/wlb-00432>). Appendix 1.