Validation of gum-line recession as a reliable technique to age tigers

María C. Fàbregas* · Carlos Garcés-Narro

Abstract Like other members of the Panthera genus, tigers are threatened throughout their range. Given their conservation status research focused on their population dynamics in the wild is needed, including population growth, lifespan, and breeding success, which requires reliable estimates of age. Current techniques to estimate age in tigers are either not reliable and/or difficult to apply in the field. Gum-line recession accurately estimates age for mountain lions, but has never been tested for tigers. The goal of this study was to determine whether gum-line recession of the upper canine teeth is a reliable indicator of age in tigers and if so, to define the equation that enables age estimation. We measured gum-line recession in 12 individuals of known ages and performed linear regression analysis to investigate the validity of this technique for tigers. We found a strong relationship between gum-line recession and age, where the model provided reliable age estimates for animals within 1-year age classes in 10 out of 12 tigers measured, providing increased accuracy over current methods.

Keywords Validation · Age estimation · Felids · Tigers · Gingival recession · Conservation

M. C. Fàbregas*

C. Garcés-Narro

Introduction

Like many large carnivores, tigers (*Panthera tigris*) are endangered throughout their range (Chundawat et al. 2011). Due to hunting and poaching (Karanth and Stith 1999; Jhala et al. 2008), habitat loss and fragmentation (Linkie et al. 2006), and depletion of prey species (Karanth and Stith 1999; Miquelle et al. 1999), tiger populations have declined from an estimated 100,000 in the early 1900s, to around 3,500 tigers at the beginning of this century (Dinerstein et al. 2007; Morell 2007). Given their conservation needs, field studies to monitor and understand population dynamics are crucial to tiger conservation (Smirnov and Miquelle 1999; Karanth 2003). Reliable estimates of age for individuals are needed to calculate growth rates, onset of sexual maturity, senescence, and life span to monitor populations and to inform conservation strategies (Karanth 2003; Kerley et al. 2003).

Methods to age carnivores either rely on skull measurements or fusion of cranial sutures (Schweikher 1930; Smuts et al. 1978; Gay and Best 1996), or analysis of dental characteristics, including tooth wear (e.g., Smuts et al. 1978; Ashman et al. 1983; Stander 1997), tooth color (Shaw 1986), tooth eruption (Linhart and Knowlton 1967; Smuts et al. 1978; Van Horn et al. 2003), cementum annuli (e.g., Laws 1952; Smuts et al. 1978; Roulichová and Anděra 2007), width of the pulp chamber (e.g., Smuts et al. 1978; Landon et al. 1998), and gum-line recession (Currier 1979; Laundré et al. 2000). However, the drawbacks of tooth wear and tooth color are that they might be dependent upon diet and degree of mastication (Currier 1979), and they are difficult to standardize and somewhat subjective (Linhart and Knowlton 1967; Laundré et al. 2000). Tooth eruption may be used to categorize into broad age classes (Smuts et al. 1978), and cementum annuli and the width of the pulp chamber require tooth extraction and analysis in a lab, which complicates its use under field conditions (Currier 1979) while presenting ethical concerns.

Department of Production Animals Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa e-mail: maria.fabregas@gmail.com

Department of Production and Animal Health, General Veterinary Health and the Science and Technology of Food Products, University CEU Cardenal Herrera, Valencia, Spain

Gum-line recession is widely used in wildlife studies as an indicator of age for several carnivore species, including tigers (Goodrich et al. 2011). However, to our knowledge, the validity of this technique has only been tested in female mountain lions (*Puma concolor*) (Laundré et al. 2000). The goal of this study is to validate the use of gum-line recession of the upper canine teeth as a technique to accurately estimate age of wild tigers and to develop an equation to allow age estimation.

Materials and methods

This study was conducted at Laohu Valley Reserve (South Africa), where the charity Save China's Tigers breeds and prepares South China tigers (*P. t. amoyensis*) for reintroduction into protected areas in China. Tigers were confined to predator-proof fenced outdoor camps ranging from 0.4 to 100 ha within the 33,000 ha reserve. Camps complied with the National Norms and Standards for predators in South Africa (Botha 2005). Tigers either hunted free-ranging prey within fenced camps (e.g., aardwolf, *Proteles cristata*; blesbok, *Damaliscus pygargus*; Cape porcupine, *Hystrix africaeaustralis*; Chacma baboon, *Papio ursinus*), or were fed fresh carcasses of antelope (e.g., springbok, *Antidorcas marsupialis*; blue wildebeest, *Connochaetes taurinus*; common eland, *Taurotragus oryx*) and warthog (*Phacochoerus africanus*).

Data collection took place between September 2012 and May 2014, where tigers were immobilized with a combination of medetomidine (50-100 µg/kg) and ketamine (1-2 mg/kg), weighed, measured, and fitted with GPS collars for other study objectives (Fabregas et al. in prep). We measured gum-line recession with a digital caliper (error, ±0.02 mm) for 12 captive-bred tigers (Table 1), where birth dates were known for all individuals. Measurements were taken only once per individual, obtaining one left and one right measurement per tiger. Gum-line recession (Currier 1979; Laundré et al. 2000) was defined as the distance (mm) from the cementumenamel junction to the gingiva (i.e., gum-line) on the left and right upper canines (Spinage 1973; Currier 1979). The mean of both measurements was calculated and used in the analyses. Each tiger was measured only once. All the protocols were approved by the University of Pretoria Animal Ethics Committee (V053-12).

A *t*-test for mean comparison was performed between males and females to assess the effect of sex in gum-line recession. A linear regression (stepwise method) (Zar 1999) was used to calculate the equation to determine tiger age from gum-line recession. All statistical tests were performed with SPSS software (IBM Corp 2011), and statistical significance was set at 0.05.

Results

Mean ages (±SE) for males and females were $56.71\pm$ 12.50 months and 60.57 ± 17.34 months respectively, confirming no differences in the ages between sexes (p=0.860). A *t*-test for mean comparison discarded a possible effect of sex on gum-line recession as no significant differences were found in gum-line recession between males and females (3.33±1.63 mm for males, 2.87±1.16 mm for females, p=0.820).

Linear regression of age and gum-line recession showed a significant slope (p=0.001) and indicated a good fit ($R^2=0.91$) (Fig. 1). Four tigers were 23 months old at the time of data collection and showed 0 values for gum-line recession. The predictive equation to estimate the age in tigers older than 23 months was

$$Age(months) = 20.47 + 12.60$$

 \times mean gum-line recession (mm)

where the 95 % confidence interval was ± 10.49 for the constant and ± 2.43 for the mean gum-line recession coefficient.

When analyzing the residuals for observed versus predicted age using the linear model (Table 1), we found no significant relationship between the observed age and the error of predicted age (Pearson's coefficient, r=0.39), indicating that the error of the estimation was independent of the age of the tiger.

Discussion

Gum-line recession is a physiological aspect of aging (Spinage 1973), and a correlation between both variables was expected. We found a strong relationship between gum-line recession and age in tigers older than 2 years, where our correlation coefficient (R^2 =0.91) was higher than that obtained by Laundré and colleagues (2000) when tested with cougars (R^2 =0.81). This technique must be applied with caution when used with dead specimens as gums may recede as carcasses dry out (Laundré et al. 2000). A correction factor however could be calculated to account for this (Laundré et al. 2000).

The error of the model was independent of tiger age, indicating that the estimation is equally accurate for all age classes greater than 23 months. Ages of younger individuals can be estimated from body measurements (e.g., Smuts et al. 1978). However, as an animal reaches sexual maturity growth rate progressively declines (Winsor 1932; Zullinger et al. 1984) becoming more difficult to accurately estimate age based on body size measurements. Gum-line recession can be used at any time in two year old tigers or older without compromising accuracy.

 Table 1
 Gum-line recession values (i.e., mean value between left and right gum-line recession of the upper canine teeth) for tigers in the sample and their corresponding observed and predicted age when using a linear regression model

Tiger ID	Sex	Gum-line recession (mm)	Observed age (months)	Predicted age (months)	Difference (months)
1	М	5.21	61	86.13	-25.13
2	М	2.87	56	56.58	-0.58
3	F	2.09	37	46.81	-9.81
4	М	0.00	23	20.47	2.53
5	М	0.00	23	20.47	2.53
6	F	1.53	29	39.75	-10.75
7	F	0.00	23	20.47	2.53
8	F	0.00	23	20.47	2.53
9	F	5.42	114	88.78	25.22
10	F	2.65	64	53.87	10.13
11	F	8.44	134	126.78	7.22
12	М	8.57	122	128.41	-6.41

Several techniques have been used to estimate age in wild tiger studies with mixed accuracy. Some authors apply only one method (e.g., width of the front paw pad: Smirnov and Miquelle 1999), but a combination of other techniques is often used, such as tooth wear and/or tooth eruption (Kerley et al. 2003; Goodrich et al. 2010, 2011), body mass and measurements (Goodrich et al. 2010; Kerley et al. 2003), behavioral cues (Kerley et al. 2003), and birth dates of young of radiocollared mothers (Goodrich et al. 2011). With the exception of the latter, all the other techniques can only classify animals in three (Goodrich et al. 2010) or four broad age categories (Goodrich et al. 2011). Our model provided reliable estimates



Fig. 1 Linear regression for the tigers participating in the study (n=12). Four animals were 23 months old when data was collected and showed no gum-line recession

of age for tigers within 1-year age classes in 10 out of 12 tigers, increasing accuracy over current methods.

The use of dental characteristics as indicators of age obtained from captive animals (e.g., tooth wear) may not be applicable for wild animals due to different food preferences (Laws 1968; Goddard 1970). Our study, however, was conducted with captive tigers that fed on wild prey, which included hide, bones, intestines, and any other body part that tigers would consume (see "Materials and methods" section). Similar physical and chemical processes to influence teeth as that for wild animals are therefore assumed.

For an age estimation technique to be utilized it should be accurate, the data should be easy to collect and handled in the field, and should be fairly inexpensive (Currier 1979). Our results show that gum-line recession is closely related to age in tigers, is fast and easy to measure, is not expensive or timeconsuming, and does not require heavy or bulky equipment for its measurement. We believe that gum-line recession can be used to provide reliable estimates of age for wild tigers. Its validity for other carnivore species should be confirmed.

Acknowledgments We thank the University of Pretoria for funding the leading author in conducting this and other related studies of tigers, as well as Save China's Tigers for providing the animals and logistic support to gather data. The manuscript greatly benefited from comments by Dr. Gary Koehler and Dr. Henk Bertschinger.

Ethical standards The authors declare that the experiments followed the National Norms and Standards for predators in South Africa, and all the protocols were approved by the University of Pretoria Animal Ethics Committee (V053-12).

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

References

- Ashman DL, Christensen ML, Hess ML, Tsukamoto GK, Wichersham MS (1983) The mountain lion in Nevada. report W-48-15. Nevada Department of Wildlife, Reno
- Botha P (2005) Draft national norms and standards for the sustainable use of large predators issued in terms of section 9(1) of the National Environmental Management: Biodiversity Act (Act No 10 of 2004). https://www.environment.gov.za/sites/default/files/gazetted_ notices/nema_largepredators_sustainableuse_g27214gon72.pdf Accessed 4 May 2014
- Chundawat RS, Habib B, Karanth U, Kawanishi K, Ahmad Khan J, Lynam T, Miquelle D, Nyhus P, Sunarto S, Tilson R, Wang S (2011) *Panthera tigris*. IUCN red list of threatened species. Version 2014.2 http://www.iucnredlist.org/details/15955/0 Accessed 13 May 2014
- Currier MJP (1979) An age estimation technique and some normal blood values for mountain lions (*Felis concolor*). Dissertation, Colorado State University
- Dinerstein E, Loucks C, Wikramanayake E, Ginsberg J, Sanderson E, Seidensticker J et al (2007) The fate of wild tigers. Biosci 57:508– 514

- Gay SW, Best TL (1996) Age-related variation in skulls of the puma (*Puma concolor*). J Mamm 77:191–198
- Goddard J (1970) Age criteria and vital statistics of a black rhinoceros population. E Afr Wildl J 8:105–122
- Goodrich JM, Miquelle DG, Smirnov EN, Kerley LL, Quigley H, Hornocker MG (2010) Spatial structure of Amur (Siberian) tigers (*Panthera tigris altaica*) on Sikhote-Alin Biosphere Zapovednik. Russia J Mamm 91:737–748
- Goodrich JM, Seryodkin IV, Miquelle DG, Kerley LL, Quigley HB, Hornocker MG (2011) Effects of canine breakage on tiger survival, reproduction and human–tiger conflict. J Zool 285:93–98
- IBM Corp (2011) IBM-SPSS statistics for windows, version 20.0. IBM Corp, Armonk
- Jhala YV, Gopal R, Quereshi Q (2008) Status of tigers, co-predators and prey in India: National Tiger Conservation. Authority and Wildlife Institute of India, New Delhi and Dehradun
- Karanth KU (2003) Tiger ecology and conservation in the Indian subcontinent. J Bombay Nat Hist Soc 100:169–189
- Karanth KU, Stith BM (1999) Prey depletion as a critical determinant of tiger population viability. In: Seidensticker J, Christie S, Jackson P (eds) Riding the tiger: tiger conservation in human-dominated landscapes. Cambridge University Press, Cambridge, pp 100–113
- Kerley LL, Goodrich JM, Miquelle DG, Smirnov EN, Quigley HB, Hornocker MG (2003) Reproductive parameters of wild female Amur (Siberian) tigers (*Panthera tigris altaica*). J Mamm 84:288– 298
- Landon DB, Waite CA, Peterson RO, Mech LD (1998) Evaluation of age determination techniques for gray wolves. J Wildl Mgmt 62:674–682
- Laundré JW, Hernandez L, Streubel D, Altendorf K, Lopez-Gonzalez C (2000) Ageing mountain lions using gum-line recession. Wildl Soc Bull 28:963–966
- Laws RM (1952) A new method of age determination for mammals. Nature 169:972–973
- Laws RM (1968) Dentition and ageing of the hippopotamus. E Afr Wildl J 6:19–52
- Linhart SB, Knowlton FF (1967) Determining age of coyotes by tooth cementum layers. J Wildl Mgmt 31:362–365

- Linkie M, Chapron G, Martyr D, Holden J, Leader-Williams N (2006) Assessing the viability of tiger subpopulations in a fragmented landscape. J Appl Ecol 43:576–586
- Miquelle DG, Smirnov EN, Merrill TW, Myslenkov AE, Quigley HB, Hornocker MG et al (1999) Hierarchical spatial analysis of Amur tiger relationships to habitat and prey. In: Seidensticker J, Christie S, Jackson P (eds) Riding the tiger: tiger conservation in humandominated landscapes. Cambridge University Press, Cambridge, pp 71–99
- Morell V (2007) Can the wild tiger survive? Science 317:1312-1314
- Roulichová J, Anděra M (2007) Simple method of age determination in red fox, Vulpes vulpes. Folia Zool 56:440–444
- Schweikher FP (1930) Ectocranial suture closure in the hyaenas. Amer J Anat 45:443–460
- Shaw HG (1986) Mountain lion field guide. Special report No 9. Arizona Game and Fish Department, Phoenix
- Smirnov EN, Miquelle DG (1999) Population dynamics of the Amur tiger in Sikhote-Alin Zapovednik, Russia. In: Seidensticker J, Christie S, Jackson P (eds) Riding the tiger: tiger conservation in human-dominated landscapes. Cambridge University Press, Cambridge, pp 61–70
- Smuts GL, Anderson JL, Austin JC (1978) Age determination of the African lion (*Panthera leo*). J Zool 185:115–146
- Spinage CA (1973) A review of the age determination of mammals by means of teeth, with especial reference to Africa. E Afr Wildl J 11: 165–187
- Stander PE (1997) Field age determination of leopards by tooth wear. Afr J Ecol 35:156–161
- Van Horn RC, McElhinny TL, Holekamp KE (2003) Age estimation and dispersal in the spotted hyena (*Crocuta crocuta*). J Mamm 84:1019– 1030
- Winsor CP (1932) The Gompertz curve as a growth curve. PNAS 18:1-8
- Zar JH (1999) Biostatistical analysis, 4th edn. Prentice Hall, Upper Saddle River
- Zullinger EM, Ricklefs RE, Redford KH, Mace GM (1984) Fitting sigmoidal equations to mammalian growth curves. J Mamm 65: 607–636