

Estimating age of carnivores from the Pantanal region of Brazil

Authors: Olifiers, Natalie, de Cassia Bianchi, Rita, D'Andrea, Paulo Sérgio, Mourão, Guilherme, and Gompper, Matthew E.

Source: Wildlife Biology, 16(4): 389-399

Published By: Nordic Board for Wildlife Research

URL: https://doi.org/10.2981/09-104

The BioOne Digital Library (<u>https://bioone.org/</u>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<u>https://bioone.org/subscribe</u>), the BioOne Complete Archive (<u>https://bioone.org/archive</u>), and the BioOne eBooks program offerings ESA eBook Collection (<u>https://bioone.org/esa-ebooks</u>) and CSIRO Publishing BioSelect Collection (<u>https://bioone.org/csiro-ebooks</u>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Estimating age of carnivores from the Pantanal region of Brazil

Natalie Olifiers, Rita de Cassia Bianchi, Paulo Sérgio D'Andrea, Guilherme Mourão & Matthew E. Gompper

Conservation and management of animal populations often require knowledge about the age structure, but this information is usually difficult to discern. We propose a method to estimate the age of carnivores based on dental condition and body size measurements, and we apply the method to populations of brown-nosed coatis Nasua nasua and crab-eating foxes Cerdocyon thous in the Brazilian Pantanal. We sexed, weighed and measured 31 coatis and 45 foxes of known-age, and characterized and quantified their pattern of dental eruption and wear for the construction of a teeth condition index. Scores of the first factors of a principal component analysis including data on six body size measurements and the teeth condition index of the individuals were then used in a discriminant analysis to generate functions that can be used for estimating age of animals of unknown age. Models were validated using subsets of individuals of known age through a 3-fold cross validation process. The first functions accounted for over 90% of the discriminatory power for both species. Whereas in coatis, the first function was mainly explained by the body size measurements, in crab-eating foxes it was represented mainly by the teeth condition index. During model validation, individuals were on average classified with 88 and 80% average confidence for coatis and foxes, respectively. Our method is as accurate as other methods that are commonly applied to assess age, but less subjective. While it requires animal capturing, it is less invasive than methods requiring tissue removal such as analyses of teeth cementum annuli. The method we outlined can be used for age estimation of other populations, as long as the models are validated with a subset of animals from the region studied. It could also be useful as a model for estimating age of other carnivore species.

Key words: age, body size, brown-nosed coati, Cerdocyon thous, crab-eating fox, dental condition, Nasua nasua, Pantanal

Natalie Olifiers & Matthew E. Gompper, Department of Fisheries and Wildlife Sciences, University of Missouri, Columbia, Missouri 65211-7240, USA - e-mail addresses: natolifiers@yahoo.com.br (Natalie Olifiers); gompperm@ missouri.edu (Matthew E. Gompper)

Rita de Cassia Bianchi, Programa de Pós-Graduação em Ecologia e Conservação, Centro de Ciências Biológicas e da Saúde, UFMS, 79070-900, Campo Grande, MS, Brazil - e-mail: rc_bianchi@yahoo.com.br

Paulo Sérgio D'Andrea, Laboratório de Biologia e Parasitologia de Mamíferos Silvestres Reservatórios, Instituto Oswaldo Cruz, FIOCRUZ, Av. Brazil, 4365 Manguinhos, Rio de Janeiro, RJ, 21040-360, Brazil - e-mail: dandrea@ioc. fiocruz.br

Guilherme Mourão, Laboratório de Vida Silvagem, Centro de Pesquisa Agropecuária do Pantanal, Embrapa Pantanal, Rua 21 de Setembro, 1880, Corumbá, MS, 79320-900, Brazil - e-mail: gui@cpap.embrapa.br

Corresponding author: Natalie Olifiers

Received 26 November 2009, accepted 27 October 2010

Associate Editor: John W. Laundré

Age determination is essential for investigations of many aspects of life history and demography. Conservation and management of wildlife require knowledge of the age structure of populations to estimate growth rates, life span, age of maturity, fitness and other crucial life-history parameters. Several methods or combinations of methods have been proposed for aging species of the order Car-

© WILDLIFE BIOLOGY 16:4 (2010)

nivora. Body weight and body measurements, reproductive status, tooth wear and tooth replacement may allow an approximation of the age of live animals (Grau et al. 1970, Gipson et al. 2000). Other age indicators such as morphological development of the skull, ossification of radius, ulna and cranial sutures (Sanderson 1961, Junge & Hoffmeister 1980), baculum morphology (Sanderson 1961, Johnson 1970), as well as testicle and ovary mass may be more precise (Erickson et al. 1964), but are invasive methods that cannot be applied to live animals. Counting the cementum layers in a premolar or canine tooth of carnivores is an alternative technique that can be used after a tooth is removed and sectioned. This technique requires specific laboratory expertise, but is a commonly used method for estimating age of a variety of carnivore species, especially in North America where this expertise is accessible (Stoneberg & Jonkel 1966, Willey 1974, Root & Payne 1984, King 1991, Ballard et al. 1995).

Regardless of the method used, many approaches suffer from some degree of lack of validation. Method validation requires a data set of animals with known age to which age estimates can be contrasted so that the reliability of age estimates can be verified. For many species this information is missing. In addition, if the method is based on cementum annulus counts or tooth wear, it is usually necessary to validate the method with a known-age animal set from the same study area, given that deposition of cementum layers and tooth wear may vary with animal diet and region (Costello et al. 2004).

There have been attempts to compare different techniques or test their reliability for gray wolves Canis lupus (Gipson et al. 2000), black bears Ursus americanus (McLaughlin et al. 1990, Keay 1995, Harshyne et al. 1998, Costello et al. 2004) and raccoons Procyon lotor (Fiero & Verts 1986). Nonetheless, for most species, a formal validated method remains absent. This is especially true for most of the South American carnivores, including some of the most abundant ones such as the brown-nosed coati Nasua nasua and the crab-eating fox Cerdocyon thous. Here we propose and validate a method to estimate age for these two species of carnivores based on dental condition and body size measurements. Our method can be used as an approach for estimating age of other species of carnivores.

Material and methods

Study area

We conducted our field work at the Nhumirim Ranch (18°59' S, 56°39' W), a 4,400 ha research station of The Brazilian Agricultural Research Corporation, Embrapa, located in the NhecoIndia subregion of Pantanal, Brazil. The Pantanal is the largest seasonal flood plain in the world. It has two

390

marked seasons: a wet season (October-March) and a dry season (April-September). Human population density is low (< 2 people/km²) and the main economic activity is cattle ranching (Adamoli 1987). The Pantanal region has a high diversity and density of medium to large-sized mammals including such Carnivora as brown-nosed coati and crab-eating fox (Alho et al. 1987, Mittermeier et al. 1990, Trolle, 2003, Bianchi 2009, Desbiez & Borges 2010). At Nhumirim Ranch these two species have an omnivorous generalist diet, with crab-eating foxes feeding predominantly on arthropods, vertebrates and fruit, and coatis feeding mainly on arthropods and fruit (Bianchi 2009).

Capturing and handling procedures

From December 2005 to February 2009 we captured coatis and foxes up to four times per year as part of a broader study of the ecology and parasitology of these species. We established a grid of 36 trap stations spaced 500 m apart in the study area. At each node of the grid a wire box live trap of $1 \times 0.40 \times 0.50$ m was baited with a piece of bacon. In addition, we occasionally placed traps out of the grid to capture specific individuals. Traps were set late in the afternoon and checked the following morning. Capture sessions were usually performed every 3-4 months.

We anesthetized individuals with an intramuscular injection of Zoletil@50 (Virbac[®]; tiletamine hydrochloride and zolazepan hydrochloride, 10 mg/Kg), ear-tagged, equipped with subcutaneous transponders (ISO FDX-B, 134.2 Khz, Animal-TAG[®]), measured, sexed and weighed them. We recorded the reproductive condition of females (apparent or non-enlarged nipples) and males (descended or non-descended scrotum) as well as tooth eruption and teeth wear. In addition, we photographed the frontal and lateral views of the dentition of each captured animal (Figs. 1 and 2). After handling procedures, we monitored the animals until they recovered from anaesthesia and then released them at the site of capture. All animal procedures were approved by the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA, first license number 183/2005 - CGFAU/LIC; last license number 11772-2) and University of Missouri Animal Care and Use Committee (protocol #4459).

Body size measurements and teeth condition index We took six body size measurements from captured

© WILDLIFE BIOLOGY 16:4 (2010)

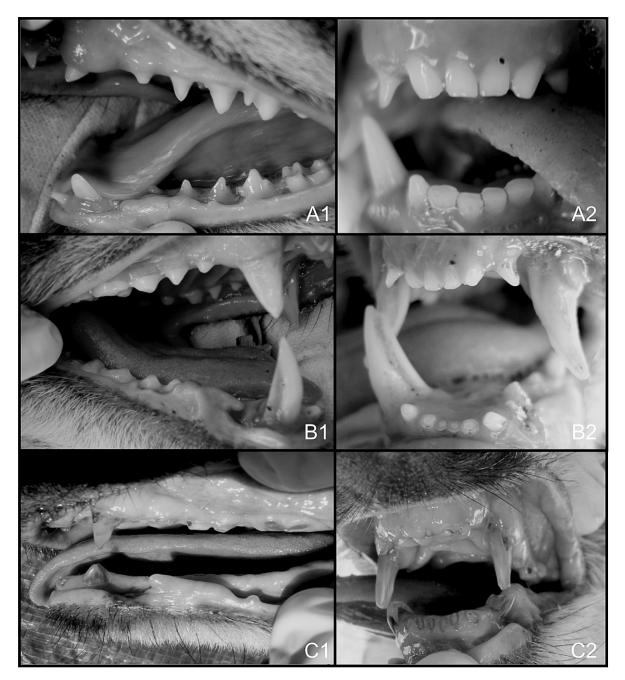


Figure 1. Lateral (A1-C1) and frontal (A2-C2) views of the dentition of three brown-nosed coatis from the Nhumirim Ranch, Pantanal, Brazil. A1 and A2 show a 1.2-year old coati with a teeth index of 1.0. All teeth are sharp and the dentition is complete, but canines are still growing. B1 and B2 show a 3-year old coati with a teeth index of 5.0. Molars and pre-molars are flat, canines are sharp, upper incisors are sharp and lower incisors are flat; one canine is broken. C1 and C2 show a > 5-year old coati with a teeth index of 11.0. All teeth are very flat.

animals (Table 1) and classified incisor, canine and premolar and molar condition in up to three qualitative categories (Table 2; see Figs. 1 and 2). We then summed values of the dental condition (see Table 2) for each animal to quantify a teeth condition index with minimum value equal to zero

© WILDLIFE BIOLOGY 16:4 (2010)

(very young individuals) and maximum value equal to 12 for coatis and 13 for foxes (very old individuals). In coati males, the ratio of the height of lower and upper canines may help discriminate between age categories, given that the lower canines are 2.5 times the size of upper canines in adults. We

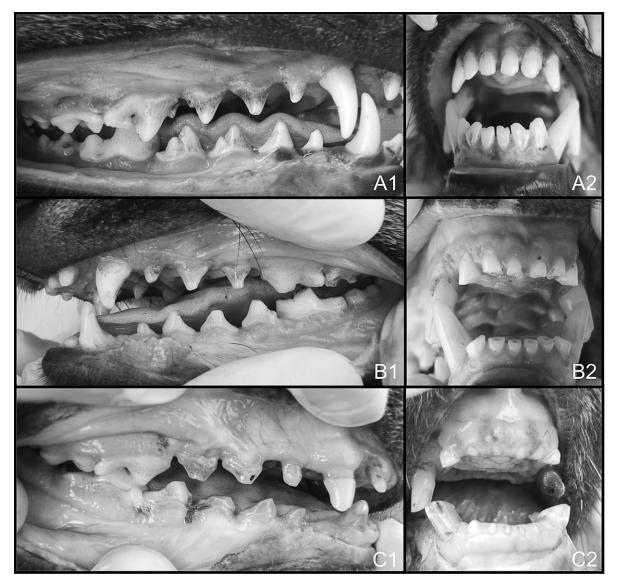


Figure 2. Lateral (A1-C1) and frontal (A2-B2) views of the dentition of three crab-eating foxes from the Nhumirim Ranch, Pantanal, Brazil. A1 and A2 show the dentition of a 1-year old fox with a teeth index of 1.5. All teeth are sharp, incisors show sharp ridges that project slightly beyond the teeth and the dentition is complete. Tartar is present in moderate quantity. B1 and B2 show a >2-year old fox with a teeth index of 9.0. Molars and premolars are flat, canines are very flat and tartar is present in moderate quantity. C1 and C2 show the dental arcade of the oldest fox captured (with a teeth index of 13); molars and premolars are flat, canines are broken and incisors are very flat.

therefore also included this ratio in the analyses of coatis (see Table 1).

Data analyses

To reduce the number of variables and to avoid redundancy in subsequent analyses, we ran a principal component analysis (PCA) with body size variables (and the ratio between upper and lower canines in coatis) based on a correlation matrix (Statistica 6.0). We then used the factor scores of the first PCA factor axes explaining > 80% of the cumulative data variation and the teeth condition index of individuals in a forward stepwise discriminant analysis (DA; F to enter=1, F to remove=0; *a priori* probabilities were the same for all groups; Statistica 6.0). We validated the generated models using a 3-fold cross validation process in which we randomly divided the data set into three equal-sized subsets and used one to validate models generated with the other two subsets. Validation was accessed

© WILDLIFE BIOLOGY 16:4 (2010)

Table 1. Body size and canine measurements taken from carnivores captured in the Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009.

Variable	Description		
Weight	Mass in g (within 50 g for animals < 5 kg and to the nearest 100 g for > 5 kg)		
Head-body length	Distance between the tip of the nose to inflection point of tail		
Shoulder height	Distance between point of shoulder blade to tip of toe		
Tail length	Distance between inflection point with body to tip of flesh		
Neck circumference	Circumference of neck, measured midway between shoulders and head		
Axillary girth	Thorax circumference at the axilla ^a		
Height of upper and lower canine	Minimum crown height of the right upper and lower canines, measured from the apex to the base of enamel (taken with calipers to within 0.01mm)		

^a Pregnant females had weight, neck circumferences and axillary girth raw measurements substituted by their values before becoming pregnant.

by computing the number of misclassifications obtained for each of the three models created. We also generated a general model comprising all of the data set. Classifications are not *a priori* predictions for the general model, but rather *post hoc* classifications. Therefore, we investigated misclassifications for the general model only as a diagnostic tool

Table 2. Characterization of teeth condition through tooth eruption and teeth wear in carnivores captured in the Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009. Values in brackets are scores that are summed to yield the teeth condition index.

Variable	Description		
Upper and lower incisors	White (0), yellow (1) Intact (0), broken or rooted (1) Sharp (0), flat (0.5), very flat (1) ^a Sharp ridges present (0), sharp ridges absent (1) ^b		
Canines	White (0), yellow (1) Intact (0), broken or rooted (1) Sharp (0), flat (0.5), very flat (1) ^c		
Premolars and molars ^d	White (0), yellow (1) Intact (0), broken or rooted (1) Sharp (0), flat (0.5), very flat (1) ^c		
Overall condition of teeth	Tartar: Absence (0), moderate quantity (0.5), high quantity (1) Dentition: Incomplete (0), Complete (1)		

^a Upper and lower incisors were characterized separately because the lower incisors were often flat before the superior incisors (see Fig. 1B);

^b This category was not used for brown-nosed coati because incisors lack sharp ridges in this species;

- ^c For crab-eating fox, sharp = 0 and flat = 1 because canines, premolars and molars were never as flat as in brown-nosed coati (see Fig. 2).
- ^d Premolars and molars were characterized together because we did not observe clear differences in wear between them.

© WILDLIFE BIOLOGY 16:4 (2010)

for assessing differences between models due to sample size.

Coatis and foxes are pulse breeders (Macdonald & Courtenay 1996, Gompper & Decker 1998, Hirsch 2007) so aging \leq 1-year old individuals is straightforward. In addition, because animals were recaptured up to four times/year over 3.2 years, we knew ages of many animals with a precision of approximately six months. We classified crabeating fox of known-age into four age categories: \leq 0.5-year old, 0.6-1.0-year, 1.1-2.0-years and > 2.0-years old. Due to the small sample size, we classified coatis into three categories: ≤ 0.5 -year old, 0.6-2.0-years and > 2.0-years old. For both species, we tested sexual dimorphism using the first PCA factor (which mainly represents body size measurements for both species, as shown below) in a 2-way ANOVA ($\alpha = 0.05$). Crab-eating foxes do not show sexual dimorphism ($F_{3,64} = 0.877, P = 0.458$) and data on both sexes can be used in one DA to estimate age for this species. Coati males are larger than females ($F_{3,30}=4.379$, P=0.024), which would justify a separate DA for males and females. However, we present the results of one analysis including both coati sexes, as there were no significant differences in the number of misclassifications obtained for analyses performed with males or females only vs both sexes together (males vs both sexes: Yates corrected: $\chi_1^2 = 0.18$, P = 0.669; females vs both sexes: Yates corrected: $\chi_1^2 = 0.60$, P = 0.437) and sample sizes for coatis was somewhat small for some age and sex classes.

We captured only two coatis with known age between two and four years old. Thus, animals in the last age category for both species were mostly > 4-years old. These individuals corresponded to the first animals captured at the beginning of the survey that were recaptured at the end of the study. Information on recaptured animals was occasionally used in consecutive age classes. However, when an individual had two or more body size and canine measurements taken within an age class, we averaged its PCA factor score values as well as its teeth condition indices before including it in the subsequent DA, thus avoiding pseudoreplication.

Results

We captured 74 foxes on 308 occasions and 106 coatis on 238 occasions. From these samples, 31 coatis and 45 foxes were known-age individuals that were captured 51 and 85 times, respectively. Known-age individuals were those captured during the first months of life or captured at the beginning of the study as adults and recaptured in subsequent years. Following averaging of measurements from known-age individuals that had been captured more than once within an age class, we utilized 34 coati captures and 64 fox captures to generate the discriminant functions.

Most known-age foxes (N=23) that did not have fully erupted teeth (i.e. they lacked a complete dentition) were < 6 months old. However, seven individuals had completely developed dentition before that age. In coatis, dentition may be complete in < 1-year-old individuals, although lower canines may still be growing in \geq 1-year-old males. Tooth wear and yellowing started after one year of life in both species. The first three factors of the PCA for crab-eating fox and the first two factors for brownnosed coati explained > 80% of the data variation and were included in the subsequent DAs for each species. The first factor represented mostly body size measurements for both species, since all correlations between raw body size variables and the first factors were > 0.78, except for tail length in foxes. The second factor for foxes was mainly represented by tail length (correlation between this variable and the factor 2 axis = 0.91), whereas for coatis it was represented mainly by the ratio of lower to upper canine height (correlation = 0.84). Factor 3 in foxes had < 9.5% contribution for the total data variance and was not strongly correlated with any body size measurement. The teeth condition index and scores of PCA factors 1 and 3 were kept in all models generated for crab-eating fox after running the DA. Scores of factors 1 and 2 as well as the teeth condition index were kept in all generated models for brown-nosed coati. Overall, the condition of the teeth was the most important variable discriminating between age categories in foxes. Whereas in coatis, the factor representing body size measurements (factor 1) was the most important variable for discrimination between age classes (Table 3).

Two discriminant functions were statistically significant for both species (Table 4), and both the condition of the teeth and body measurements were important for discriminating between age categories in coatis and foxes. In coatis, 92% of all discriminatory power was explained by the first function, which was represented by body measurements (factor 1 of the PCA) and, with less degree, by the teeth condition index. The teeth condition index in foxes was the variable that mostly explained the first function, which accounted for over 97% of the discriminatory power.

Table 3. Values of Partial Wilk's λ for variables in models of age determination for the two species of carnivores studied in the Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009. 'Models 1-3' are the average of the Partial Wilk's λ values for the three model subsets generated with $\frac{1}{2}$ data subsets. The general model comprises the whole data set. Lower partial Wilk's λ implies higher variable contribution to the model.

Table 4. Step-down test of discriminant functions (roots) of the general models that describe age categories for the two species of carnivores in the Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009. The first row (Roots removed) represents significance test for all roots; the second line reports significance of the remaining roots, after removing the first root, and the third line after removing the first and second roots. Wilk's λ of discriminant functions are also shown.

Roots

394

Species	Variables	Models 1-3	General model
Brown-nosed coati	Factor 1	0.36	0.36
	Teeth condition	0.57	0.56
	Factor 2	0.81	0.83
Crab-eating fox	Teeth condition	0.14	0.15
	Factor 1	0.85	0.85
	Factor 3	0.90	0.91

Species	removed	Wilk's $\boldsymbol{\lambda}$	χ^2	df	P - level
Brown-nosed coati	0	0.10	69.40	6	< 0.001
	1	0.67	12.09	2	0.002
Crab-eating fox	0	0.10	139.82	9	< 0.001
	1	0.82	12.17	4	0.016
	2	0.99	0.06	1	0.804

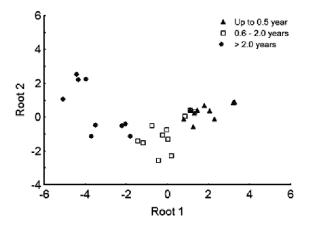


Figure 3. The two first functions (roots 1 and 2) discriminating between age categories of brown-nosed coatis captured in Nhumirim Ranch, Pantanal, Brazil during December 2005-February 2009.

The first discriminant function for foxes mostly discriminated between animals > 2.0-years old and the other age categories; for coatis, it discriminated between the three age categories (Figs. 3 and 4). Although having a rather small discriminative power, the second function in coatis discriminated mainly between animals of 0.6-2.0-years old and other age categories. For foxes, the second function discriminated between individuals with > 2.0 and up to two years old and the remaining age categories.

From 67 to 100% of the data classifications were correct in the 3-fold cross validation process for both species (Table 5). The 1.1-2.0-years old age category in foxes showed the higher number of

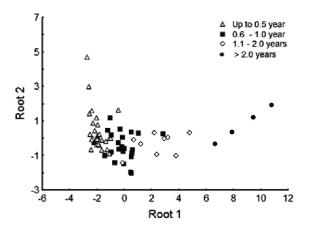


Figure 4. The two first functions (roots 1 and 2) discriminating between age categories of crab-eating foxes captured in Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009.

© WILDLIFE BIOLOGY 16:4 (2010)

Table 5. Mean number of correct classifications obtained in the 3fold cross validation process for the discriminant functions describing age categories for two species of carnivores in the Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009. The mean number of cases classified is shown in brackets.

Species	Age category (in years)	Mean number of correct classifications
Brown-nosed coati	≤ 0.5	4.3 (4.7)
	0.6-2.0	3.0 (3.7)
	> 2.0	2.7 (3.0)
Crab-eating fox	≤ 0.5	8.0 (9.3)
	0.6-1.0	6.0 (8.0)
	1.1-2.0	2.0 (3.0)
	> 2.0	1.3 (1.3)

misclassifications (see Table 5). For the general models, 84.4 and 85.3% of the classifications were correct for foxes and coatis, respectively. However, for the general models, these classifications are not *a priori* predictions and the obtained percentage of correct classifications should be interpreted with caution. Given that the number of correct classifications obtained with the general models differs by a maximum of 6.8% from those obtained with the 3-fold cross validation process ($\frac{2}{3}$ of the data), validation of the general models with an extra data subset from the study area would likely provide a similar number of correct classifications.

Discussion

This is the first attempt to provide a non-invasive methodology to estimate age of two of the most abundant members of the South American carnivore community. Age estimates from tooth wear and tooth eruption pattern have been proposed for wolves Canis lupus, coyotes Canis latrans, sea otters Enhydra lutris, spotted hyenas Crocuta crocuta, leopards Panthera pardus and other carnivores (Garshelis 1984, Stander 1997, Landon et al. 1998, Van Horn et al. 2003). It has been considered accurate for aging wolves up to four years old, and across most of the lifespan of spotted hyenas (Landon et al. 1998, Van Horn et al. 2003). Its accuracy has even been considered comparable to cementum annulus counts, although its precision is usually believed to be lower (Linhart & Knowlton 1967, Bowen 1982, Gipson et al. 2000).

Regardless of its lower precision, estimating age

of free-living carnivores through tooth wear and eruption pattern (whether associated or not with body measurements) may be the only option available in much of the world, where there are currently no laboratories specialized in analysing cementum layers and export permits may be cumbersome to obtain. Furthermore, despite its usual good precision, counting cementum annuli requires training to remove animal teeth and special care to avoid animal injury, which makes it impractical in some cases (Goodwin & Ballard 1985, Dimmick & Pelton 1994).

All techniques available for estimating age in carnivores have elements of subjectivity (Gipson et al. 2000). Subjectivity, however, can be minimized via a validation process. Models generated in our study to estimate age of coatis and foxes correctly classified individuals with 67-100% confidence (see Table 5). If we combine the second and third age categories for foxes, the probability of age misclassifications would be $\leq 18\%$ for this species.

The misclassifications in > 2.0-year-old coatis were caused by two 2.2-year-old females that had teeth condition and body measurements very similar to animals in the 0.6-2.0-years age category. If nipple condition is used as an additional criteria to classify animals in age categories, the two females would have been classified correctly as > 2.0-years old, given that coati females rarely reproduce before the second year of life, and therefore do not have apparent nipples before that age (Kaufmann 1962). Because exceptions to this pattern are rare or nonexistent, we believe the use of nipple condition as an *a posteriori* criterion to classify individuals in age categories is appropriate in these cases.

We did not create additional age categories or analyze males and females separately due to sample size limitations. However, additional relative age classes within the absolute age categories already established by the method proposed could be easily defined. For instance, although coatis shown in Figs. 1B and 1 C are both > 2.0-years old, coati C is clearly older than coati B. Thus, the > 2-years old category is a particularly broad age group in which one can find animals with teeth condition indices varying from 2.5 to 11.5. Depending on the study goals, one could split this age category into multiple age classes.

For the few studies providing any validation of a method proposed to classify carnivores up to four years old, accuracy of age determination for animals varied from 60 to 100% (Grau et al. 1970, Harshyne

et al. 1998, Costello et al. 2004). In a study comparing five techniques for estimating age in raccoons, accuracy based on pair-wise comparisons of aging methods for animals up to 4-years old varied from 31 to 100% (Fiero & Verts 1986). Therefore, age estimates for coatis and foxes provided by our method are at least as accurate as those offered by other methods for other carnivore species. Unfortunately, our study length and sample size did not allow us to provide more precise age categories for these two species. Nevertheless, this is a first step to provide a non-invasive methodology to estimate age for these species with a reduced degree of subjectivity and good accuracy.

Management implications

Our method combines dental and body size measures in a discriminatory analytical framework to allow the aging of two common South American carnivore species. The methodology is as accurate, or more so, than most methods currently used for many species, has relatively low subjectivity and is relatively non-invasive, which is useful for working with live animals without extracting teeth. We believe that the models we generated can be used to estimate the age of brown-nosed coatis and crabeating foxes captured in other areas as long as the models are validated with a subset of animals from the studied area. Our method can also be used as a framework to generate age estimation for other species.

In Appendix I we outline the steps necessary to estimate age of coatis and foxes using the methodology that we presented here.

Acknowledgements - we are grateful to the trainees and Embrapa workers for their assistance with the field work. We are also grateful to B.T. Beerntsen, J.J. Millspaughand and D. Galat for comments on previous versions of the manuscript. Funds were provided by CNPq (Process # 484501), FIOCRUZ and Embrapa Pantanal. Doctoral grants were provided by the University of Missouri to N. Olifiers and by CAPES to R.C. Bianchi.

References

- Adamoli, J. 1987: Vegetação do Pantanal. In: Allem, A.C. & Valls, J.F.M. (Eds.); Vegetação do Pantanal, EM-BRAPA-CENARGEN, Brasília, pp. 23-25. (In Portuguese).
- Alho, C.J.R., Lacher, J.T.E., Campos, Z.M.S. & Gonçalves, H.C. 1987: Mamíferos da Fazenda Nhumirim, sub-região de Nhecolândia, Pantanal do Mato Grosso

do Sul: I - levantamento preliminar de espécies. - Revista Brasileira de Zoologia 4(2): 151-164. (In Portuguese).

- Ballard, W.B., Matson, G.M. & Krausman, P.R. 1995: Comparison of two methods to age gray wolf teeth. - In: Carbyn, L.N., Fritts, S.H. & Seip, D.R. (Eds.); Ecology and conservation of wolves in a changing world. Canadian Circumpolar Institute Occasional Publication No. 35: 455-459.
- Bianchi, R.C. 2009: Ecologia de mesocarnívoros em uma área no Pantanal Central, Mato Grosso do Sul. - PhD thesis, Universidade Federal do Mato Grosso so Sul, Campo Grande, Brazil, 206 pp. (In Portuguese).
- Bowen, W.D. 1982: Determining age of coyotes, *Canis latrans*, by tooth sections and tooth wear patterns. Canadian Field-Naturalist 96(3): 339-341.
- Costello, C.M., Inman, K.H., Jones, D.E., Inman, R.M., Thompson, B.C. & Quigley, H.B. 2004: Reliability of the cementum annuli technique for estimating age of black bears in New Mexico. - Wildlife Society Bulletin 32(1): 169-176.
- Desbiez, A.L.J. & Borges, P.A.L. 2010: Density, habitat selection and observations of South American coati *Nasua nasua* in the central region of the Brazilian Pantanal wetland. Small Carnivore Conservation 42: 14-18.
- Dimmick, R.W. & Pelton, M.R. 1994: Criteria of sex and age. - In: Bookout, T.A. (Ed.); Criteria of sex and age. The Wildlife Society, Bethesta, Maryland, USA, pp. 169-214.
- Erickson, A.W., Neilor, J. & Petrides, G.A. 1964: The black bear in Michigan. - Michigan State University, Agricultural Experiment Station, East Lansin, USA, 102 pp.
- Fiero, B.C. & Verts, B.J. 1986: Comparison of techniques for estimating age in raccoons. - Journal of Mammalogy 67(2): 392-395.
- Garshelis, D.L. 1984: Age Estimation of Living Sea Otters. - Journal of Wildlife Management 48(2): 456-463.
- Gipson, P.S., Ballard, W.B., Nowak, R.M. & Mech, L.D. 2000: Accuracy and precision of estimating age of gray wolves by tooth wear. Journal of Wildlife Management 64(3): 752-758.
- Gompper, M.E. & Decker, D.M. 1998: *Nasua nasua*. Mammalian Species 580: 1-9.
- Goodwin, E.A. & Ballard, W.B. 1985: Use of tooth cementum for age-determination of gray wolves. Journal of Wildlife Management 49(2): 313-316.
- Grau, G.A., Sanderson, G.C. & Rogers, J.P. 1970: Age determination of raccoons. - Journal of Wildlife Management 34(2): 364-371.
- Harshyne, W.A., Diefenbach, D.R., Alt, G.L. & Matson, G.M. 1998: Analysis of error from cementum annuli age estimates of known-age Pennsylvania black bears. -Journal of Wildlife Management 62(4): 1281-1291.
- Hirsch, B.J. 2007: Within-group spatial position in ringtailed coatis (*Nasua nasua*): balancing predation, feeding success, and social competition. - PhD thesis. Stony Brook University, New York, USA, 349 pp.
- Johnson, A.S. 1970: Biology of the raccoon (Procyon lotor

© WILDLIFE BIOLOGY 16:4 (2010)

varius Nelson and Goldman) in Alabama. - Auburn University Agricultural Experiment Station Bulletin 402: 1-148.

- Junge, R. & Hoffmeister, D.F. 1980: Age determination in raccoons from cranial suture obliteration. - Journal of Wildlife Management 44(3): 725-729.
- Kaufmann, J.H. 1962: Ecology and social behavior of the coati, *Nasua narica*, on Barro Colorado Island, Panama.
 University of California Publications in Zoology 60: 95-222.
- Keay, J.A. 1995: Accuracy of cementum age assignments for black bears. - California Fish and Game 81(3): 113-121.
- King, C.M. 1991: A Review of Age-Determination Methods for the Stoat *Mustela erminea*. - Mammal Review 21(1): 31-49.
- Landon, D.B., Waite, C.A., Peterson, R.O. & Mech, L.D. 1998: Evaluation of age determination techniques for gray wolves. - Journal of Wildlife Management 62(2): 674-682.
- Linhart, S.B. & Knowlton, F.F. 1967: Determining age of coyotes by tooth cementum layers. - Journal of Wildlife Management 31(2): 362-365.
- Macdonald, D.W. & Courtenay, O. 1996: Enduring social relationships in a population of crab-eating zorros, *Cerdocyon thous*, in Amazonian Brazil (Carnivora, Canidae). Journal of Zoology 239: 329-355.
- McLaughlin, C.R., Matula, G.J., Cross, R.A., Halteman, W.H., Caron, M.A. & Morris, K.I. 1990. Precision and accuracy of estimating age of Maine black bears by cementum annuli. - International Conference on Bear Research and Management 8: 415-419.
- Mittermeier, R.A., Camara, I.G., Padua, M.T.J. & Blanck, J. 1990: Conservation in the Pantanal of Brazil. - Oryx 24: 101-112.
- Root, D.A. & Payne, N.F. 1984: Evaluation of Techniques for Aging Gray Fox. - Journal of Wildlife Management 48(3): 926-933.
- Sanderson, G.C. 1961: Techniques for determining age of raccoons. Illinois Natural History Survey 45: 1-16.
- Stander, P.E. 1997: Field age determination of leopards by tooth wear. African Journal of Ecology 35: 156-161.
- StatSoft, I. 2001: STATISTICA. StatSoft, Inc., Tulsa, Oklahoma, USA.
- Stoneberg, R.P. & Jonkel, C.J. 1966: Age determination of black bears by cementum layers. - Journal of Wildlife Management 30(2): 411-414.
- Trolle, M. 2003: Mammal survey in the southeastern Pantanal, Brazil. - Biodiversity and Conservation 12: 823-836.
- Van Horn, R.C., McElhinny, T.L. & Holekamp, K.E. 2003: Age estimation and dispersal in the spotted hyena (*Crocuta crocuta*). - Journal of Mammalogy 84(3): 1019-1030.
- Willey, C.H. 1974: Aging black bears from first premolar tooth sections. - Journal of Wildlife Management 38(1): 97-100.

Appendix I

In this appendix we describe how to transform data on brown-nosed coati and crab-eating fox body size and canine height ratio in principal component factor scores, and how to subsequently use these scores in the discriminant classification functions for estimating age of a given animal.

Suppose the following data for an individual coati (Coati 1): Teeth condition index = 1; weight = 2,340 g; head-body length = 520 mm; tail length = 470 mm; shoulder height = 240 mm; neck circumference = 165 mm; axillary girth = 225 mm; and the ratio between the upper and lower canine height is 1.4. First, calculate the factor scores for each of the two factors representing the raw data. Remember that the principal component analysis is based on the correlation matrix and therefore the raw variables must be standardized first; i.e. each value must be diminished from the mean and divided by the standard deviation of that variable (Table 6).

Example : standardized weight for Coati 1 =

$$(2, 340 - 4, 319)/1, 585 = -1.249.$$

Next, the standardized values must be transformed into factor scores for use in the classification functions. To do so, multiply each standardized value by each of its respective factor score coefficient (Table 7) and then sum the values.

Example: the factor score of Coati 1 corresponding to Factor 1 would be:

Table 6. Mean and standard deviation (SD) of body measurements (in mm) of brown-nosed coatis and crab-eating foxes, and the ratio between the upper and the lower canine height (U/L) in coatis captured in the Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009.

	Brown-no	sed coati	Crab-eating fox	
Variables	Mean	SD	Mean	SD
Weight (in g)	4319	1585	6257	1097
Head-body length	587	88	713	51
Tail length	476	54	315	25
Shoulder height	273	38	388	24
Neck perimeter	201	37	228	17
Axillary girth	294	47	354	30
U/L	1.30	0.35		

Table 7. Factor score coefficients of the Principal Component Analyses run for body measurements (in mm) and for the ratio between the upper and the lower canine height (U/L) of brownnosed coatis and crab-eating foxes captured in Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009.

	Brown-nosed coati		Crab-eating fox		
Variables	Factor 1	Factor 2	Factor 1	Factor 2	Factor 3
Weight	-0.180	0.030	-0.241	0.084	-0.149
Head-body length	-0.179	0.122	-0.209	-0.013	0.697
Tail length	-0.164	0.203	-0.108	-1.024	-0.157
Shoulder height	-0.175	0.179	-0.206	-0.050	0.781
Neck perimeter	-0.163	0.035	-0.230	-0.111	-0.654
Axillary girth	-0.179	0.074	-0.235	-0.242	-0.439
U/L	-0.101	-1.098			

$$[-1.249 \times (-0.180)] + [-0.761 \times (-0.179)]$$

+ $[-0.111 \times (-0.164)] + \ldots = 0.924$

The new factor scores for Coati 1 would then be: Factor 1 = 0.924; Factor 2 = -0.765.

Now the factor scores and the teeth condition index can be used in the classification functions of the discriminant analysis (Table 8). The classification functions are utilized to determine to which group a case most likely belongs. Each function allows us to compute classification scores for each case for each group, by utilizing the formula (Statistica 6.0; StatSoft, I. 2001):

 $S_i = c_i + w_{i1} \times x_1 + w_{i2} \times x_2 + \ldots + w_{ij} \times x_j$

where subscript i = respective group, subscripts 1, 2, ..., j = j variables, c_i = constant for the ith group, w_{ij} = weight for the jth variable in the computation of the classification score for the ith group, x_j = observed value for the respective case for the jth variable and S_i = the resultant classification score.

Example: for Coati 1, the classification scores for age category ≤ 0.5 year would be:

$$\begin{split} S_{\leq 0.5 year} &= -10.143 + (6.584 \times 0.924) \\ &\quad + \left[(-1.847) \times (-0.765) \right] + (-0.066) \\ &\quad \times 1 = -2.712 \end{split}$$

Remember that the teeth condition index (equal to '1' in this case) was included as a separate variable in the models; therefore, it is not a factor score and does not need to be standardized. Once the classification scores for a case are computed, classify

© WILDLIFE BIOLOGY 16:4 (2010)

Species	Variables	≤ 0.5 year	0.6 - 2.0 years		> 2.0 years
Brown-nosed coati	Factor 1	6.584	2.459		-0.482
	Factor 2	-1.847	-1.436		0.381
	Teeth condition index	-0.066	0.207		1.663
	Constant	-10.143	-2.967		-6.909
Crab-eating fox		≤ 0.5 year	0.6 - 1.0 year	1.1 - 2.0 years	> 2.0 years
	Factor 1	1.422	0.548	0.579	1.575
	Factor 3	0.337	1.239	1.888	3.651
	Teeth condition index	0.606	2.324	4.985	12.074
	Constant	-2.328	-3.807	-11.905	-62.203

Table 8. Classification functions for age determination of brown-nosed coatis and crab-eating foxes captured in Nhumirim Ranch, Pantanal, Brazil, during December 2005-February 2009.

the case as belonging to the group for which it has the highest classification score.

Example: for Coati 1, the classification scores are: for ≤ 0.5 year = -2.712, 0.6 - 2.0 years = -0.611 and

> 2.0 years = -5.983. According to the classification scores, this animal is 0.6-2.0 years old because this age category has the highest classification score for this animal.