LIFE-HISTORY AND DISEASE ECOLOGY OF THE BROWN-NOSED COATI (*NASUA NASUA*) AND THE CRAB-EATING FOX (*CERDOCYON THOUS*) IN THE BRAZILIAN PANTANAL

A Dissertation
presented to
the Faculty of the Graduate School
University of Missouri

In Partial Fulfillment
Of the Requirements for the Degree
Doctor of Philosophy

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by

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July 2010

The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

LIFE-HISTORY AND DISEASE ECOLOGY OF THE BROWN-NOSED COATI (*NASUA NASUA*) AND THE CRAB-EATING FOX (*CERDOCYON THOUS*) IN THE BRAZILIAN PANTANAL

presented by Natalie Olifiers
a candidate for the degree of Doctor of Philosophy
and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Matthew E. Gompper

Professor David Galat

Professor Brenda Beerntsen

Professor Joshua Millspaugh

“It would be possible to describe everything scientifically, but it would be without meaning, as if you described a Beethoven symphony as a variation of wave pressure”

A. Einstein

In memory of my grandmothers,

Sydinea Sulser
Hulda Doberstein Olifiers
Maria José Oliveira Moura

ACKNOWLEDGEMENTS

First, I thank my advisor, Dr. Matthew Gompper, for his unconditional support, his friendship, patience and excitement with the project. I am also grateful to Dr. Paulo S. D'Andrea, who advised me in the intricate field of "inter-scientist relationships" and funded a great part of this work. Dr. Gompper and Dr. D'Andrea gave me the freedom and recognition I needed to build my self-confidence as a scientist.

Thanks to Rita Bianchi, who helped me in every single step of this journey. I have no words to acknowledge her for sharing so many responsibilities, for her friendship and hard-work in the field.

My students, Elisa Pucu, Ana Gomes, Wagner Lopes and Viviane Rodrigues were hard-working and also very patient in teaching me how to be an advisor. Thanks also to Fernada Alves, Nilson Xavier, Juliane Saab and Renata Calixto, for their friendship and all the help in the field and laboratory. These students make every day in the Nhumirim Ranch a day to be remembered. During three months, Laís Grego kindly taught me how to identify the blood cells, which was such an important part of the work. There were also many temporary trainees, friends, visitors and volunteers who helped in the field for short periods and I thank all of them.

Vitor Rademaker and Fabiana Rocha helped in the first steps of the project. Fabiana taught me the basics for handling carnivores while Vitor shared responsibilities during the "Earthwatch phase" and, in some way, he was the person who first stimulated me to investigate the field of disease ecology.

Thanks to every cowboy, driver or cook who fed us, rescued us from unexpected conditions, or spent time with us capturing animals at the Nhumirim Ranch. Thanks to Marcos Tadeu, Armindo, Henrique, Roberto, Ricardo, Batista, Odilza and Antônio, for their patience and flexibility to solve the countless little difficulties my work brought to their daily routine at Embrapa and Nhumirim Ranch.

My family gave me the psychological support I needed, even though they are still wondering what exactly I was doing so far from home and for so long. My brother Carlos Henrique funded me in my last semester, while my brother Eric made my life much easier giving me access to his university electronic sources.

I also thank to Dr. Guilherme Mourão for his collaboration in the project, the access to his facilities at Embrapa and the material he provided for the fieldwork. At FIOCRUZ, Bernardo Teixeira, Michele Maria and Rosana Gentile helped on many occasions. I am also grateful to Arnaldo Maldonado and Joyce for helping with the helminth identification.

I express my gratitude to all students and researchers from the Laboratory of Trypanosomatids, Fiocruz/RJ. Thanks to Daniela Rozas, Andre Roque, Samanta, Kerla, Miguel, Marcos and Carlos for their outstanding technical support. Dr. Ana Jansen provided all the help, expertise and access to the facilities for the health and trypanosomatid analyses. Dr. Heitor Herrera was the first contact with Embrapa/Pantanal and contributed with his expertise during the project. I also thank Paulo Cançado from UFRRJ who helped in the field, identified the tick species and trained/co-advised one of my students.

I have many friends in the US who helped me in several ways. Karthik Masagounder shared the office with me for a long time and helped me with little things that actually made a lot of difference. Aniruddha Belsare kindly spent a couple of hours with me discussing some of the health data. My committee members, Dr. David Galat, Dr. Joshua Millspaugh and Dr. Brenda Beerntsen were always ready to help me and I am grateful to them too. Carlos Silva, Lori Thweatt and Elina Silva were my US family and provided me with a good environment for writing. My room was always available, no matter how long I was absent. I also thank Matt’s family, who welcomed me in their house for a couple of weeks when I arrived.

Finally, I thank my dear boyfriend Alberto Maurer, who stood by me since the very beginning, always helping me to understand others and to find solutions for practical and complex problems. With his expertise in management, in some way he taught me to manage myself a little better. He also probably deserves a prize for being so patient, for having waited for me all this time, and for having spent so much time and money flying to see me and flying me to see him.

This project was funded by IOC-FIOCRUZ/RJ, EMPRAPA, FUNDECT, EARTHWATCH INSTITUTE, CNPq and the University of Missouri.

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GENERAL INTRODUCTION

Infectious diseases can be significant threats to public health (Binder *et al.*, 1999; Smith *et al.*, 2009), and in the last decades, there have been a number of outbreaks of new pathogens in humans, with more than 70% coming from wildlife (Jones *et al.*, 2008). Parasites can have important effects on wildlife as well, affecting individuals, populations, communities and even entire ecosystems (Hudson *et al.*, 2002; Smith *et al.*, 2009). For instance, parasites may decrease health of their hosts, regulate population numbers and contribute to local extinctions (Anderson & May, 1978; May & Anderson, 1978; Scott & Dobson, 1989; Dobson & Hudson, 1992; Hudson *et al.*, 1998). Yet, despite their role in several levels of life organization, there is still a relatively poor understanding of the impact parasites have on the health and demography of wild hosts. This is not only a reflection of a superficial knowledge about basic ecology of wild hosts and their parasites, but also a lack of attention to critical questions linking ecology and parasitology.

The paucity of studies on the brown-nosed coati (*Nasua nasua*: Linnaeus, 1766) and the crab-eating fox (*Cerdocyon thous*; Linnaeus, 1766) exemplifies how we still know little about the interplay between wild hosts and their parasites. These carnivores are very abundant and widespread in South America and they harbor several parasites that infect wildlife, domestic animals, and humans (Ferriolli & Barreto, 1968; Miles *et al.*, 1983; Lainson *et al.*, 1989; Nunes & Oshiro, 1990; Oshiro *et al.*, 1990; Nunes *et al.*, 1993; Gompper & Decker, 1998; Courtenay & Maffei, 2004). Despite this, little is known about the life-history of these carnivores, the dynamic of their parasites, and how parasites affect host health. Coatis, for example, have been suggested as important reservoirs of *Trypanosoma cruzi* and *T. evansi* (Kinetoplastida: Trypanosomatidae) in the Brazilian Pantanal wetlands (Herrera *et al.*, 2004; Herrera *et al.*, 2008). These protozoans are of health and economic importance since they cause Chagas disease in humans and "Mal de Cadeiras" disease in horses, respectively. The effect of trypanosomatids on the coati health and the potential effect of these parasites on the population dynamics of coatis are, however, only superficially known (Silva *et al.*, 1999; Herrera *et al.*, 2001; Herrera *et al.*, 2002; Alves *et al.*, 2010).

Understanding the effects of parasites on hosts requires not only knowledge about the parasites themselves, but also about the ecology of their hosts. This dissertation was developed with this aim in mind. It is part of a larger collaborative effort with a diverse group of researchers examining the population biology and disease ecology of several species of midsized carnivores, and the epidemiology of parasites of public health concern in the Pantanal region, Brazil. The dissertation is divided in four chapters: the first and second chapters regard, respectively, the development of a tool for aging coatis and crab-eating foxes and the collection of basic information on the life-history of these species in the study area. Information gathered in these two chapters was then used in the following sections. In the third chapter, I focus on ectoparasites of coatis and foxes, particularly on ticks. I investigate how abiotic factors and the host attributes described in chapters 1 and 2 influence tick abundance and prevalence on hosts. This chapter can be considered a model for investigating the relative importance of biotic and abiotic factors in parasite dynamics. Finally, in chapter 4 I focus on how hemoparasites and gastro-intestinal parasites affect coati health. There are few studies which have investigated the effects of multiple parasites on direct measures of the health parameters of free-ranging hosts. In this sense, this last chapter can also be viewed as a model for future studies focusing in the interplay between parasites and health of wild, free-ranging mammals.

For a better picture of the broader project, additional publications from this collaborative effort should be referred. In these, the reader will find additional information regarding the behavior of coatis and foxes (Bianchi, 2009; Olifiers *et al.*, 2009), their ectoparasites (Cançado, 2008), and the dynamics of *T. cruzi* and *T. evansi* in coatis from the Pantanal (Herrera *et al.*, 2008; Alves *et al.* 2010).

Chapter 1

ESTIMATING AGE OF BROWN-NOSED COATIS AND CRAB-EATING FOXES FROM THE PANTANAL REGION, BRAZIL

ABSTRACT

Conservation and management of animal populations often requires knowledge of their age structure, but this information is usually difficult to discern. Here I propose a method to estimate age of brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) based on teeth condition and body size measurements. Thirty-one coatis and 45 foxes of known-age captured in the Pantanal region were sexed, weighed, measured and their dental eruption and wear characterized and quantified 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 those individuals were then used in a discriminant analysis to generate functions 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. In coatis it was mainly explained by the body size measurements, whereas in crab-eating foxes it was represented mainly by teeth condition index. During model validation, individuals were on average classified with 88% and 80% average confidence for coatis and foxes, respectively. This method is as accurate as other methods that are commonly

applied to assess age, but it is less subjective. It is also less invasive than methods requiring tissue removal such as counting teeth cementum annuli. The method outlined here should be useful for age estimation of other populations, as long as the models are validated with a subset of animals from the region studied.

INTRODUCTION

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, and other crucial life-history parameters.

Several methods or combination of them have been proposed for aging species of the order Carnivora. 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 annuli layers in the premolar or canine tooth of carnivores is an alternative technique that can be used after the 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, all of them generally suffer from some degree of lack of validation. Method validation requires a dataset 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 scattered. In addition, if the method is based on cementum annuli 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). Similarly, tooth-wear criteria are usually considered to have low precision, sometimes overestimating the age of young animals or underestimating the ages of older animals (Erickson *et al.*, 1964; Spinage, 1973); this makes model validation by age class necessary.

Recently, there have been attempts to compare different techniques or test their reliability for gray wolves (Gipson *et al.*, 2000), black bears (McLaughlin *et al.*, 1990; Beck, 1991; Keay, 1995; Harshyne *et al.*, 1998; Costello *et al.*, 2004), and raccoons (Fiero & Verts, 1986). Nonetheless, for most species, a formal validated method remains lacking. This is especially true for most of the South American carnivores, including some of the most abundant ones, such as the brown-nosed coati (Procyonidae: *Nasua nasua*) and the crab-eating fox (Canidae: *Cerdocyon thous*). Here I propose and validate a non-invasive method to estimate age for these two species of carnivores based on teeth condition and body size measurements.

MATERIAL AND METHODS

Study area

Field work was conducted at the Nhumirim Ranch (18º59’S, 56º39’W), a 4400ha research station of The Brazilian Agricultural Research Corporation (Embrapa) located in the Nhecolândia sub-region of Pantanal, Brazil. The Pantanal is the largest seasonal floodplain in the world. It has two marked seasons – a wet season (October to March) and a dry season (April to September). The region is characterized by sandy soil with a mosaic vegetation of semi-deciduous forest, dispersed shrub vegetation, and seasonally flooded fields (Rodela, 2006). Permanent and temporary ponds and “salinas” (salt water ponds) occur throughout the region. Human population density is low (< 2 people per km^2) 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; Alho & Lacher 1991; Bianchi 2009; Desbiez & Borges, 2010). At Nhumirim Ranch, these two species have an omnivorous generalist diet, with foxes feeding predominantly on arthropods, vertebrates, and fruits, and coatis feeding mainly on arthropods and fruits (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 500m apart in the study area. At each node of the grid a wire box live-trap (1m x 0.40m x 0.50m) 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 in 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 (Nasco Rototags®), measured, sexed, and weighed them. 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 were recorded. In addition, we photographed the frontal and lateral view of the dentition of each captured animal (Fig. 1 and 2). After handling procedures, we monitored the animals until they had recovered from the anesthesia 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 # 183/2005 – CGFAU/LIC; last license # 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 animals (Table 1) and classified incisor, canine, and pre-molar and molar condition in up to three qualitative categories (Table 2; Fig. 1 and 2). Values of teeth condition (Table 2) for each animal were then summed to quantify a teeth condition index with minimum value equal to zero (a very young individual) 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 adult males. We therefore included this ratio in the analyses of coatis (Table 1).

Table 1. Body size and canine measurements taken from coatis and foxes captured at the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

Variable	**Description**
Weight	Mass in grams (within 50g for animals less than 5Kg and to the nearest 100g for more than 5Kg)
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	Crown height of the right upper and lower canines, measured from apex to base of enamel (taken with calipers to within 0.01mm)

[a] Pregnant females had neck circumferences and axillary girth raw measurements substituted by their values before becoming pregnant.

Table 2. Characterization of teeth condition through tooth eruption and teeth wear in coatis and foxes from the Nhumirim Ranch – Pantanal/Brazil from 2005 to 2009. Values between 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 were often flat before superior ones (Fig. 1B);

[b] This category was not used for *Nasua nasua* because incisors lack sharp ridges in this species;

[c] For *Cerdocyon thous*, sharp = 0 and flat = 1 because canines, premolars, and molars were never as flat as in *N. nasua* (see Fig. 2).

[d] Premolars and molars were characterized together because we did not observe clear differences in wear between them.

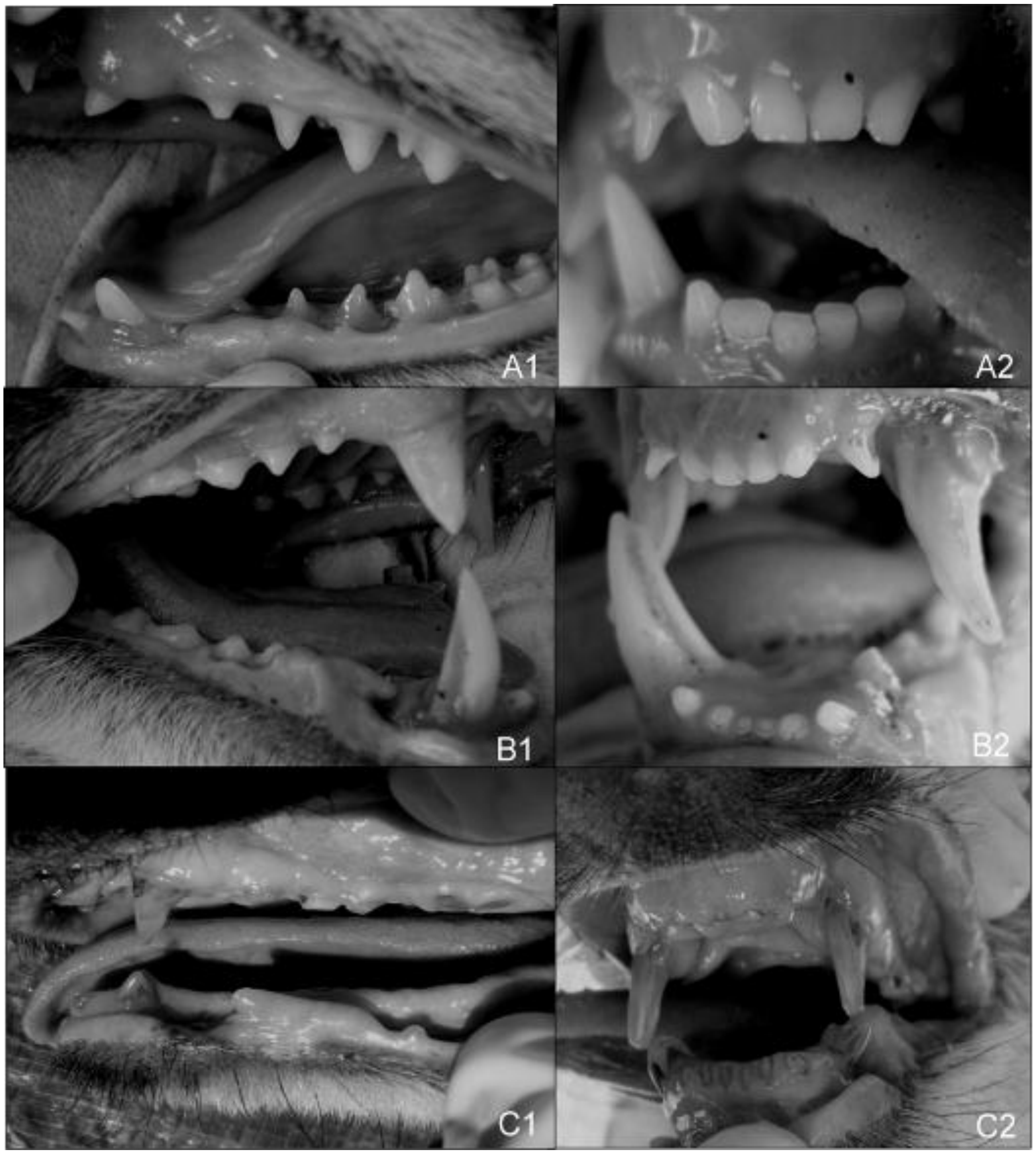

Fig. 1. Lateral and frontal views of the dentition of three *Nasua nasua* from the Nhumirim Ranch, Pantanal/Brazil. A1 and A2. A 1.2-yr-old coati with teeth index = 1.0. All teeth are sharp and dentition is complete, but canines are still growing. B1 and B2. A 2 to 3-yr-old coati with teeth index = 5.0. 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. A >5-yr-old coati with teeth index = 11.0. All teeth are very flat.

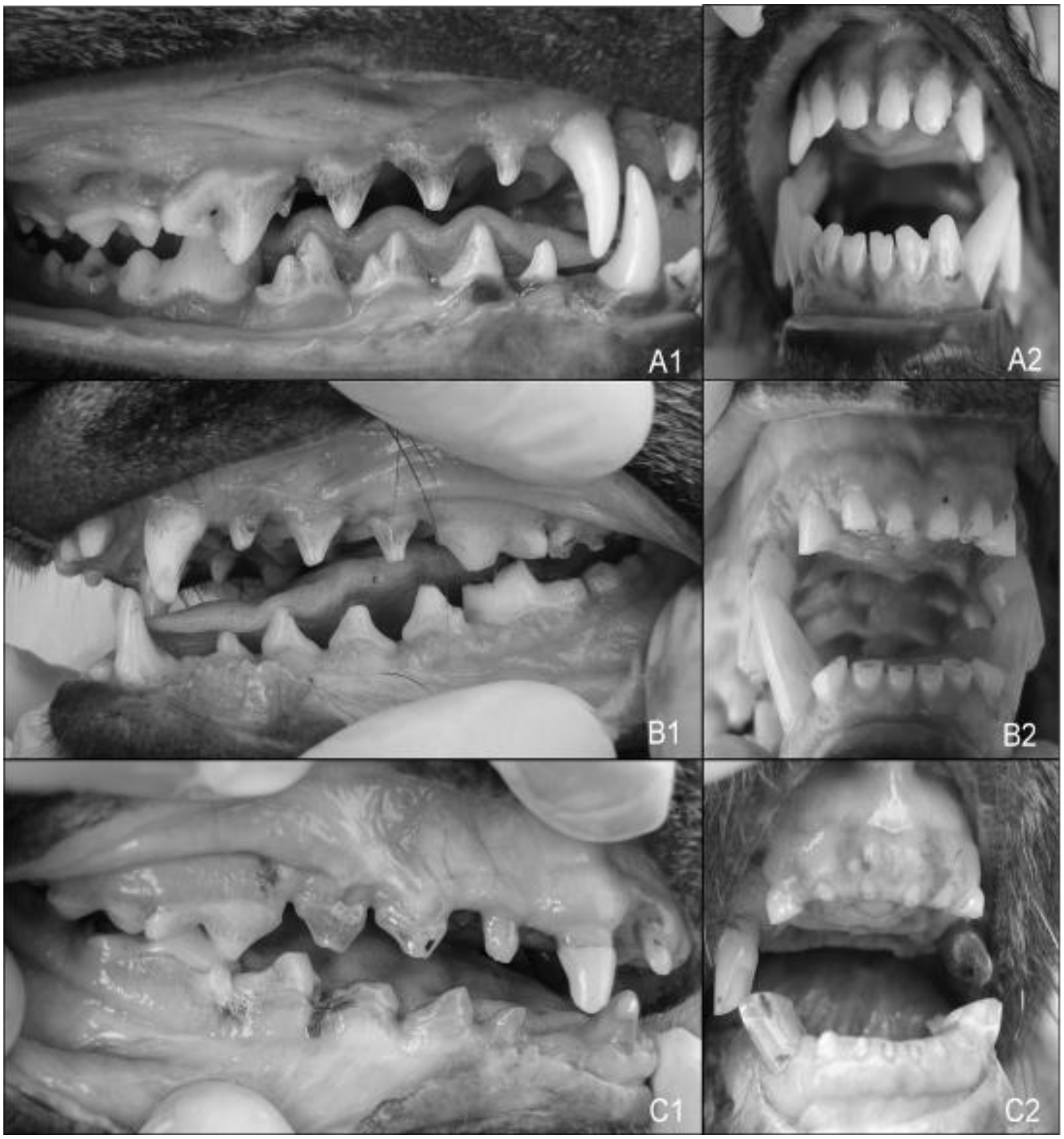

Fig. 2. Lateral and frontal views of the dentition of three *Cerdocyon thous* from the Nhumirim Ranch, Pantanal/Brazil. A1 and A2. Dentition of a 1-yr-old fox with teeth index = 1.5. All teeth are sharp, incisors show sharp ridges that project slightly beyond the teeth, and dentition is complete. Tartar is present in moderate quantity. B1 and B2. A >2-yr-old fox with teeth index = 9.0. Molars and pre-molars are flat, canines are very flat and tartar is present in moderate quantity. C1 and C2. Dental arcade of one of the oldest foxes captured (teeth index = 13); molars and pre-molars are flat, canines are broken, incisors are very flat.

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 the correlation matrix. 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 analyses (DA) (*F* to enter = 1; *F* to remove = 0; *a priori* probabilities were the same for all groups). The models generated were then validated using a 3-fold cross validation process in which we randomly divided the data set into three equal size subsets and used one to validate models generated with the other two subsets. Validation was accessed by computing the number of misclassifications obtained for each of the three models generated. We also generated a general model comprising all of the dataset. Classifications are not a priori predictions for the general model, but rather *post hoc* classifications. Therefore, misclassifications for the general model were investigated only as a diagnostic tool for assessing differences between models due to sample size.

Coatis and foxes are pulse breeders in the Pantanal (Chapter 2) so aging ≤ 1-year-old individuals is straightforward. In addition, because animals were recaptured up to 4 times/yr over 4 years, ages of many animals were known with a precision of approx. 6 months. Foxes of known-age were classified in four age categories: ≤ 0.5 yr old, 0.6 to 1.0 yr, 1.1 to 2.0 yr, and > 2.0 yr old. Due to small sample size, coatis were classified in three categories: ≤ 0.5 yr old, 0.6 to 2.0 yr, and > 2.0 yr old. For both species, sexual dimorphism was tested using the first PCA factor (which mainly represents body size measurements for both species) on 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 analysis to estimate age for this species. Coati males, however, 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 $\chi^2_{\ 1}$=0.18, P=0.669; females vs. both sexes: Yates corrected χ^2_1=0.60; P=0.437).

We captured only two animals with known age between 2 and 4 yr old, thus animals in the last age category were mostly > 4 yr 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 twice 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 indexes before including it on the subsequent DA, thus avoiding pseudo-replication.

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. Following averaging of measurements from known-age individuals that had more than one capture within an age class, 34 coati captures and 64 fox captures were utilized 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 ≤ 0.5 yr 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 ≥ 1-yr-old males. Tooth wear and yellowing started after 1 year in both species. The first three factors of the PCA for *C. thous* and the first two factors for *N. nasua* explained over 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. The second factor for foxes was mainly represented by tail length whereas for coatis it was represented mainly by the ratio of lower to upper canine height. Factor 3 in foxes had less than 10% contribution for the total data variance and was not strongly correlated with any body size measurement.

Teeth condition index and scores of PCA factors 1 and 3 were kept in all models generated for *C. thous* after running the DA. Scores of factors 1 and 2 as well as teeth condition index were kept in all generated models for *N. nasua*. Overall, teeth condition 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 teeth condition 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 mainly by body measurements (factor 1 of the PCA) and, with less degree, by 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 Lambdas for variables in models of age determination for coatis and foxes captured at the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009. "Models 1 to 3" is the average of the Partial Wilk's Lambdas values for the three model subsets generated with two-thirds data subsets. The general model comprises the whole dataset. Lower partial Wilk's Lambdas implies higher variable contribution to the model.

Species	**Variables**	**Models 1 to 3**	**General model**
Nasua nasua	Factor 1	0.36	0.36
	Teeth condition	0.57	0.56
	Factor 2	0.81	0.83
Cerdocyon thous	Teeth condition	0.14	0.15
	Factor 1	0.85	0.85
	Factor 3	0.90	0.91

Table 4. Step-down test of discriminant functions (roots) of the general models that describe age categories for coatis and foxes captured at the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009. The first row (0 roots removed) represents significance test for all roots; the second line reports significance of the remaining roots, after removing the first root, and on the third after removing the first and second roots. Wilk's Lambda of discriminant functions is also shown.

Species	**Roots removed**	**Wilks' Lambda**	**Chi-square**	**df**	***P* - level**
Nasua nasua	0	0.10	69.40	6	< 0.001
	1	0.67	12.09	2	0.002
Cerdocyon thous	0	0.10	139.82	9	< 0.001
	1	0.82	12.17	4	0.016
	2	0.99	0.06	1	0.804

The first discriminant function for foxes discriminated mostly between animals older than 2 yr old and the other age categories; for coatis, it discriminated between 0.6 to 2.0 yr old and the other age categories (Fig. 3 and 4). Although having a rather small discriminative power, the second function in coatis discriminates mainly between animals of 0.6 to 2.0 yr old and other age categories. For foxes, the second function discriminates mainly between individuals > 2.0 yr old and those with up to 2.0 yr old.

From 70% to 100% of the data classifications were correct in the 3-fold cross validation process for both species (Table 5). The 1.1 to 2.0-yr-old age category in foxes was the only one showing a rather high number of misclassifications (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 as an upper limit of the true percentage of correct classifications only. Given that the number of correct classifications obtained with the general models differs a maximum of 6.8% from those obtained with the 3-fold cross validation process (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.

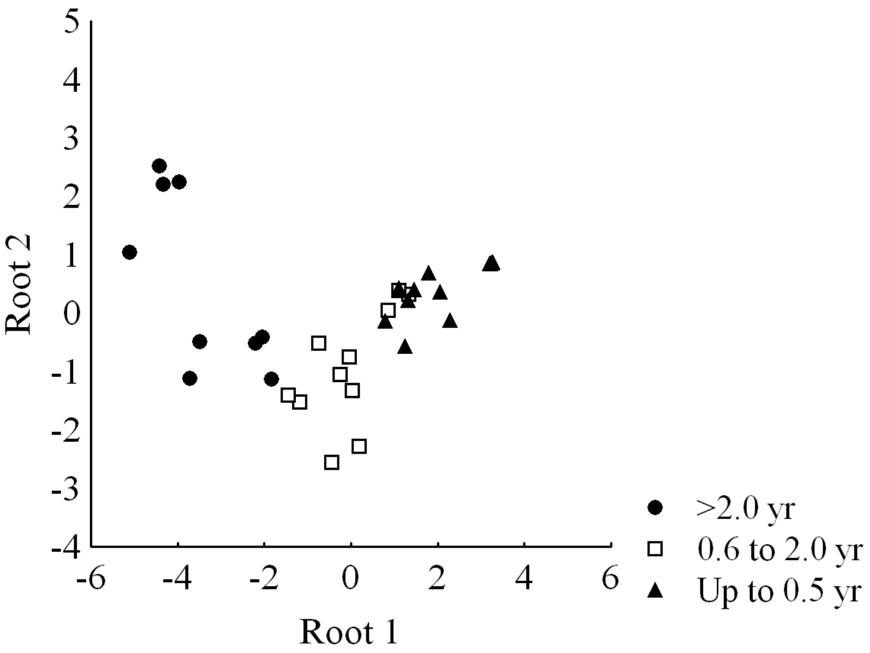

Fig. 3. The two first functions (roots) discriminating between age categories of coatis (*N. nasua*) captured in Nhumirim Ranch, Pantanal/Brazil between 2005 and 2009.

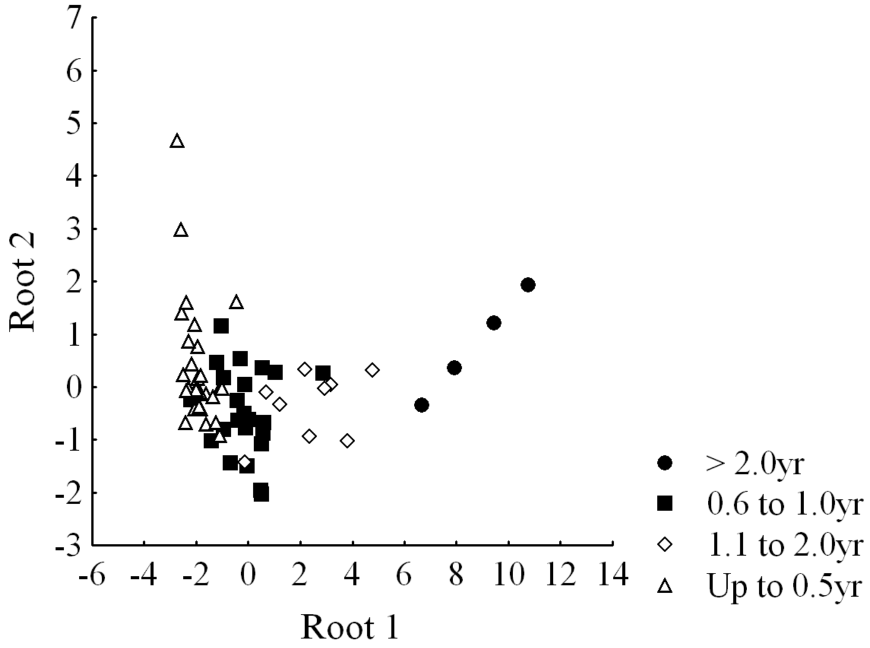

Fig. 4. The two first functions (roots) discriminating between age categories of *C. thous* captured in Nhumirim Ranch, Pantanal/Brazil between 2005 and 2009.

Table 5. Mean number of correct classifications obtained in the 3-fold cross validation process for the discriminant functions describing age categories for coatis and foxes captured at the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009. The mean number of cases classified is shown between brackets.

Species	**Age category**	**Mean number of correct classifications**
Nasua nasua	≤ 0.5 yr	4.3 (4.7)
	0.6 to 2.0 yr	3.0 (3.7)
	> 2.0 yr	2.7 (3.0)
Cerdocyon thous	≤ 0.5 yr	8.0 (9.3)
	0.6 to 1.0 yr	6.0 (8.0)
	1.1 to 2.0 yr	2.0 (3.0)
	> 2.0 yr	1.3 (1.3)

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, coyotes, sea otters, spotted hyenas, leopards, and other carnivores (Garshelis, 1984; Stander, 1997; Landon *et al.,* 1998; Van Horn *et al.,* 2003), and it has been considered accurate for aging wolves with up to 4 yr 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 annuli 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 annuli layers. In addition, export permits to have analyses conducted elsewhere may be cumbersome and expensive 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 of carnivores have elements of subjectivity (Gipson *et al.*, 2000). Subjectivity, however, can be minimized via a validation process. Models generated in this study to estimate age of coatis and foxes correctly classified individuals with 67% to 100% confidence (Table 5). If we combine the second and third age categories for foxes, the probability of age misclassifications would be 18% or less for this species.

The misclassifications in >2-yr-old coatis were caused by two 2.2 yr old females that had teeth condition and body measurements very similar to animals in the 0.6 to 2.0-yr age category. If nipple condition is used as an additional criteria to classify animals in age categories, those two females would have been classified correctly as > 2.0 yr old, given that coati females do not 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 extremely rare or non-existent, 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 Fig. 1B and C are both > 2.0 yr old, coati C is clearly older than coati B. Thus the >2.0-yr-old category is a particularly broad age group in which one can find animals with teeth condition indexes varying from 2.5 to 11.5. Depending on study goals, one could split this age category into multiple age classes.

For the few studies providing any validation of a method proposed to age carnivores in age categories ≤ 4 yr old, accuracy of age determination for animals varied from 60% to 100% (Grau *et al.,* 1970; Costello *et al.,* 2004). In a study comparing 5 techniques for estimating age in raccoons, accuracy based on pair-wise comparisons of aging methods for animals up to 4 yr old varied from 31 to 100% (Fiero & Verts, 1986). Therefore, age estimates for coatis and foxes provided by this method are at least as accurate as those offered by other methods for other carnivore species. Unfortunately, the 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 allow its use for live animals without extracting teeth. We believe that the models generated here can be used to estimate the age of brown-nosed coatis and crab-eating foxes captured in Central and South America, as long as the models are validated with a subset of animals from the studied area. The method can also be used as a framework to generate age estimation methods for other species.

In the Appendix 1, we outline the steps necessary to estimate age of coatis and foxes using the methodology presented here.

Chapter 2

LIFE-HISTORY OF BROWN-NOSED COATIS AND CRAB-EATING FOXES IN THE PANTANAL OF BRAZIL

ABSTRACT

Information on life history traits is central for testing ecological theories and providing baseline data for population dynamics studies. Here I provide information on reproduction, morphology, growth, and survival for crab-eating foxes (Canidae: *Cerdocyon thous*) and brown-nosed coatis (Procyonidae: *Nasua nasua*) from the Brazilian Pantanal. Between 2005 and 2009, we captured and monitored 79 foxes and 139 coatis. We fitted Gompertz sigmoid curves to body size data by age category on both species and calculated survival using the Kaplan-Meier staggered entry procedures. An extended breeding season occurred for both species. Seventeen percent of the foxes first breed at approx. 1 year old, but most female foxes and coatis did not reproduce until their second year of life. Sex ratio did not differ from 1:1 for foxes, but was male biased in coatis. Each species and sex reached its asymptote of head-body length at the age of possible first reproduction, although foxes continued to gain body mass for six months thereafter. Mortality in foxes was distributed evenly among age classes whereas in coatis it was concentrated in > 2-year-old individuals. Growth rates and growth asymptotes

were associated with the timing of reproduction in both species, whereas morphological differences were linked to their respective social systems.

INTRODUCTION

Life history parameters are a series of species-specific features related to reproduction, survival, and growth that evolved in response to ecological factors (Stearns, 1976; Congdon & Gibbons, 1990; Congdon *et al*., 1994). They reflect life strategies and trade-offs that each species, population or sex applies in response to selective features in the environment. Information on life history traits is fundamental for testing ecological theories and for providing the basis for conservation and management activities. Despite this recognition, there is a considerable lack of knowledge of life history features for many wild species. Obtaining reproductive information such as age-specific survival is essential to forecast variation in demographic parameters necessary for informed management of a species (Conroy & Carrol, 2009). In addition, because the size of an animal is closely related to most aspects of its biology (Peters, 1983), growth patterns also turn out to be an important life-history trait (Stearns, 1992). By analyzing growth curves, it is possible to calculate growth rates, maximum sizes and ages at maximum growth, as well as to estimate species energetics (Ackerman *et al.*, 1986), productivity (Robinette *et al*., 1961) and age (Laundré & Hernandez, 2002).

The brown-nosed coati (Procyonidae: *Nasua nasua*) and the crab-eating fox (Canidae: *Cerdocyon thous*) are amongst the most abundant and widespread carnivores in South America (Eisenberg & Thorington, 1973; Schaller, 1983; Robinson & Redford, 1986; Alho *et al.*, 1987; Bianchi, 2009), but little is known about their life history traits. In this study, we provide information on reproduction, morphometrics, survival, and extrapolate growth curves for crab-eating foxes and brown-nosed coatis from the Pantanal region, Brazil.

The crab-eating fox occurs from southern Panama to northern Argentina and Uruguay (Courtenay & Maffei, 2004). It is putatively monogamous with social groups comprising a breeding pair with pups and sometimes adult-sized offspring from previous years (Macdonald & Courtenay, 1996; Courtenay & Maffei, 2004; Bianchi, 2009). Information on reproductive and growth traits for this species is primarily derived from one work with captive animals (Brady, 1978), a report on its sociobiology on Marajó Island, Brazil (Macdonald & Courtenay, 1996), and scattered observations from other sites (Brady, 1979; Parera, 2002; Faria-Correa *et al.*, 2009). The brown-nosed coati occurs from Colombia to northern Argentina (Gompper & Decker, 1998). It is a polygynous species with a complex social structure in which adult females and immature individuals form bands. Some adult males may stay with bands out of the breeding season, while others are primarily solitary for most of the year, joining the bands only during the reproductive season (Resende *et al.*, 2004; Hirsh, 2007; Bianchi, 2009). After the breeding season, pregnant females leave their bands and give birth in a nest, generally constructed in a tree (Olifiers *et al.*, 2009). Most of the knowledge on its reproduction and ontogeny is from captive animals (Gompper & Decker, 1998) and from a few studies in the wild (Beisiegel, 2001; Bonatti, 2006; Hirsch, 2007).

Coatis and foxes differ not only in social structure (small vs. large groups) but also in the extent of their size dimorphism, with foxes being relatively monomorphic and coatis being highly dimorphic in size (Macdonald & Courtenay, 1996; Chapter 1). Here we examine coatis and foxes morphology in greater depth and ask how these between-sex-similarities for foxes and between-sex-differences for coatis are related to other life history traits, such as growth patterns, sex ratio and survival.

MATERIAL AND METHODS

Study area

Field work was conducted at Nhumirim Ranch (18º59'S, 56º39'W), a 4400ha research station of The Brazilian Agricultural Research Corporation (Embrapa) located in the Nhecolândia sub-region of Pantanal, Brazil. The Pantanal is the largest seasonal floodplain in the world and has a distinct wet season from October to March and dry season from April to September. The region is characterized by sandy soil with a mosaic vegetation of semi-deciduous forest, dispersed shrub vegetation, and seasonally flooded fields (Rodela, 2006). Permanent and temporary ponds and "salinas" (salt water ponds) occur throughout the region. Human population density is low (< 2 people per km^2) 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, and densities of brown-nosed coati and crab-eating fox are among the highest reported (Alho *et al*., 1987; Mittermeier *et al.*, 1990; Alho & Lacher, 1991; Bianchi, 2009; Desbiez & Borges, 2010).

Capturing and handling procedures

From November 2005 to February 2009 we captured coatis and foxes up to four times per year (2738 trap-nights distributed among 11 trapping sessions) using wire box live-traps (1m x 0.40m x 0.50m) in a 7.2Km^2 trapping grid. In addition, we occasionally placed traps out of the grid to capture specific individuals. Traps were baited with bacon, set late in the afternoon, and checked in the following morning.

We anesthetized individuals with an intramuscular injection of Zoletil$^{@}$50 (Virbac®; tiletamine hydrochloride and zolazepan hydrochloride, 10 mg/Kg) and ear-tagged them with numbered colored tags (Nasco Rototags®). Body size measurements, body mass, the reproductive condition of females (apparent or non-enlarged nipples) as well as tooth eruption and wear were recorded as described in Chapter 1. A subsample of eight foxes and 13 coatis were radio-collared. We monitored animals until recovery from the anesthesia and released them at the capture site.

Beginning in 2007, we established camera-traps (Tigrinus®) between and during trapping sessions in the study area (2238 trap-nights). Cameras were baited with bacon and bobcat urine (*Lynx rufus*) and rebaited every two days. Whenever triggered, cameras would take a picture every 10 seconds, which facilitated identification of marked individuals by ear-tag color and number. Information collected with camera traps and radio-collared animals was opportunistically used to improve estimates of birthing season, proportion of females that were reproductively active each year, and survivorship. In February 2009, all radio-collars were removed from foxes. Coatis remained radio-collared for a follow-up study. Animal procedures were approved by the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA, first license #183/2005 – CGFAU/LIC; last license #11772-2) and University of Missouri Animal Care and Use Committee (protocol #4459).

Demographic parameters

We calculated fecundity as the proportion of ≥ 9-months-old females that were pregnant, lactating or had swollen nipples (indicating recently weaning). Mating and birthing seasons were extrapolated by back-calculating data on pregnant and lactation females with information on gestation period and growth obtained in captivity (Brady, 1978; Gompper & Decker, 1998). We calculated the sex ratio as the total proportion of trapped unique males/females and compared it between seasons (wet and dry) and age (*N. nasua*: ≤ 2yr old and > 2 yr old; *C. thous:* ≤ 1yr old, 1-2 yr old, and > 2yr old) using χ^2 tests ($\alpha = 0.05$). Average (±SD) sex ratio per excursion was also calculated.

Growth, morphometrics and survival

We used the Levenberg-Marquardt algorithm in Statistica 6.0 to fit three growth curves to the body mass (g) and head-body length (mm) of coatis and foxes: the Gompertz sigmoid asymptotic model curve, the logistic curve and the von Bertalanffy equation (von Bertalanffy, 1938; Kingsley, 1979; Ricker, 1979; Zullinger *et al.*, 1984). We did not fit the commonly-applied generalized logistic or Richards' curve (Richards, 1959) due to high colinearity between descriptors (Zullinger *et al.*, 1984). We compared the fit of these models using the Akaike Information Criteria (AIC) computed from the least squares regression statistics (Burnham & Anderson, 2002). After choosing the best model, data on coatis were re-analyzed by sex to investigate how sexual dimorphism in body size occurs in this species. Data on crab-eating foxes was not separated by sex because this species is not dimorphic (Chapter 1; see results). Within and between-species differences on model parameters were compared using an unpaired t test ($\alpha = 0.05$).

For growth curve analyses, we divided coatis and foxes into eight age categories (Table 6). Age categories one to five included known-age individuals, whereas animals with estimated age were used in the last three relative age categories only (> 2 yr old). Age of unknown-age individuals was calculated based on body size measurements and a teeth condition index (TCI) that measures dental development and the degree of teeth worn (Chapter 1). Individuals with a TCI of 0−4 were placed in age category 6 (2−3 yr old); those with TCI of 4.5−8 in age category 7 (3−4 yr old), and individuals with a TCI > 8 were classified as > 4 yr old (age category 8). Information on recaptured animals was occasionally used in consecutive age classes, but whenever an individual had two or more body mass and head-body measurements taken within the same age category, we averaged these values to avoid pseudo-replication. Pregnant females were excluded from both growth curve and body size calculations.

Ultimately, we selected Gompertz models for further analysis (see results). The Gompertz model is described as $M(t) = A(e^{-e\,(-k\,(t-I))})$, where M = body size at age category t, A = asymptotic body size, k = a growth-rate constant, and I = the age at the inflection point. By assigning the A, K and I values in the Gompertz formula and testing different values of t (age category), we were able to estimate the age at which $M \approx A$, *i.e.*, the age at which the body mass or head-body length asymptote is reached. It should be noted that we used (asymmetrical) age categories (see Table 6), so that k and I are useful for comparisons between species and sexes in this study only and do not provide a precise growth pattern for these species.

We calculated survivorship for coatis and foxes using the Kaplan-Meier staggered entry procedure with right-censoring, due to radio-collar failure and marked individuals lost during the study (Kaplan & Meier, 1958; Pollock *et al.*, 1989). The number of foxes at risk of death in age categories 1 to 5 was the total number of marked animals, and deaths were represented by marked (but not radio-collared) individuals occasionally found dead. Survival estimates from age categories 1 to 5 should therefore be interpreted as maximum survival rates since the probability of capturing/recapturing animals is higher than finding dead animals by chance. Survival of immature coatis was not estimated because we did not find dead young or juvenile coatis. For adults (animals in age categories 6 to 8), animals at risk of death were the total number of radio-collared individuals in each age category, and only deaths of radio-collared individuals were considered. For the survival analyses, we supplemented the dataset by including individuals (7 coatis and 20 foxes) with estimated age between 0.6 and 2.0 yr old (age categories 3 to 5).

RESULTS

We captured 79 foxes (44 males and 35 females) and 139 coatis (96 males and 43 females). Recapture rates in traps were 65% for foxes (20/35 females and 31/44 males) and 36% for coatis (16/43 females and 34/96 males). Fifty-two percent of foxes and 31% of coatis were recaptured at least once in distinct excursions (around 3 months apart). We also recaptured 21 foxes and 20 coatis in camera-traps.

Table 6. Age-specific survival (S) and cumulative survival (Cum. S) of *C. thous* and *N. nasua* in Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

		C. thous				*N. nasua*			
Age category	**Age (yr)**	**N**	**S**	**Cum. S**	**95%CI**	**N**	**S**	**Cum. S**	**95%CI**
1	0−0.2	1	1.00	1.0	−	6	1	1.00	−
2	0.21−0.5	6	1.00	1.0	−	8	1	1.00	−
3	0.51−1.0	15	0.93	0.93	0.81−1.0	7	1	1.00	−
4*	1.1−1.5	12	0.83	0.78	0.55−1.0	10	1	1.00	−
5*	1.51−2.0					13	1	1.00	−
6	2−3	2	1.00	0.78	0.55−1.0	3	0.67	0.67	0.13−1.0
7	3−4	4	0.75	0.58	0.21−0.58	2	0.50	0.33	0.0−0.87
8	>4.0	5	0.60	0.35	0.02−0.68	6	0.67	0.22	0.0−0.60

* Data on age categories 4 and 5 were pooled for foxes.

Reproduction and sex ratio

Foxes first reproduced in the first breeding season after their birth (≤ 1yr old), but this occurred only for two females (17%), whereas all others gave birth in the second breeding season or later (Yates corrected χ^2 =7.83; d.f.=1; P=0.052). We observed pairs of foxes comprised of <1-year-old individuals on three occasions, but it was not possible to determine whether they were breeding pairs or siblings that had not yet dispersed. On one occasion, a young male was captured with a 2-year-old female, but soon after the female died and we could not determine whether they had reproduced. All female coatis started reproducing at the second year of age or later (Yates corrected χ^2 =32.87; d.f.=1; p<0.001). On average, 47% (36 to 64%) of the adult female foxes (≥ 9 months old) and 58% (45 to 79%) of the adult female coatis (≥ 2 yr old) reproduced from 2006−07 to 2008−09. For the set of females that were ≥ 9 months old observed in consecutive years, 4 foxes (50%) reproduced during 2 or 3 consecutive years, and 3 coati females (43%) reproduced during 2 consecutive years.

The first pregnant foxes were observed in late August. Given a gestation length of 56 days (Brady, 1978) and assuming those females were in the middle of their gestation period, the mating season for crab-eating foxes in Pantanal began by mid-July. Lactating females were observed in November and January. However, we photographed one pup of 40 to 70 days old on May 21st, 2008 and captured one pregnant female in February 5th, 2009. Pregnant coatis were observed in mid-September, lactating ones by mid-November, and the first juveniles born in the season were seen on November. Assuming that observed pregnant females were in the middle of their gestation and that the gestation period for the species is 74−77 days (Gompper & Decker, 1998), the reproductive season would start by early August, but continue through November, given that we captured one female with three young (260−430 grams) in early February.

Sex ratio per excursion (2.4±1.8) and the total sex ratio of coatis was biased to males (total: 2.2 males per female; $\chi^2 = 10.87$; d.f =1; $P<0.001$) and this bias was not related to season or age ($\chi^2_{\text{season x age}} = 2.85$; d.f.=3; $P=0.415$). Sex ratio per excursion in crab-eating foxes was 1.4±0.7 males/females. Sex ratio in foxes was not different from 1:1 (total: 1.3 males per female; $\chi^2 = 0.63$; d.f.=1; $P<0.427$) and did not differ between seasons or age ($\chi^2_{\text{season x age}} = 4.28$; d.f.=5; $P=0.510$).

Growth curves, body size measurements and survival

Coatis are dimorphic in size, as all body size measures differed between adult males and females (Table 7). Differences between coati males and females varied from 8% (tail and head length) to 62% (body mass) and 103% (lower canine height). In contrast, foxes were mostly monomorphic in size; even though axillary girth and head width were 3−4% larger on male foxes than females, no differences were found for the remaining body measurements (Table 7). All body measurements showed relatively low coefficient of variation (≤ 12%), except for body mass (≤ 15%) and lower canine height (≤ 17%).

Table 7. Mean (SD) body size measurements and lower canine height for adult coatis (*N. nasua*) and crab-eating foxes (*C. thous*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009. Values are from animals that had reached their respective growth asymptotes. F = females; M = males.

	***C. thous* (F)**[a]	***C. thous* (M)**[a]	**Range**	***N. nasua* (F)**[b]	**Range**	***N. nasua* (M)**[b]	***Range***
Body mass (g)	6794 (763.5)	6746 (613.7)	5563-8250	3501 (388.4)**	2870-4140	5662 (833.3)	3680-7470
Axillary girth (mm)	356 (15.0)*	371 (16.9)*	338-397	278 (21.4)**	235-330	334 (17.9)	310-373
Neck circumference (mm)	231 (10.6)	236 (13.1)	220-248	185 (16.1)**	165-234	228 (27.0)	198-345
Head-body length (mm)	713 (50.4)	732 (39.3)	555-800	568 (30.3)**	510-614	647 (38.6)	550-710
Tail length (mm)	320 (21.4)	313 (20.6)	270-355	468 (32.6)**	395-537	505 (25.0)	450-565
Head length[c] (mm)	168 (6.8)	170 (6.8)	156-185	159 (6.3)**	150-170	172 (9.5)	155-190
Head width[d] (mm)	80 (3.4)*	83 (3.1)*	75.6-85.8	64 (4.5)**	48.4-72.2	81 (4.4)	71.7-90.0
Shoulder height (mm)	394 (10.8)	400 (11.7)	371-421	266 (14.5)**	230-287	300 (18.7)	271-356
Lower canine height (mm)				7.5 (1.3)**	4.6-9.7	15 (2.0)	11.3-19.4

Between-sex comparisons using unpaired t-test: *P<0.05; **P<0.001.

[a] Body mass, thorax and neck circumferences were taken from > 1.5-yr-old foxes; other measurements were taken from foxes with > 1yr old. Range is for both sexes together. Sample size varied from 10 to 19 individuals.

[b] All measurements were taken for coati females in age category 6 or higher (> 2 yr old) and coati males in age category 7 or higher (>3 yr old). Sample size varied from 21 to 28 individuals.

[c] Maximum distance from tip of rhinarium to posterior edge of the external occipital protuberance, measured along sagittal midline.

[d] Maximum distance from the widest point of the zygomatic arch in each side of the head.

We used 82 body mass measurements from 60 crab-eating foxes (25F and 35M) and 122 body mass measurements from 108 coatis (35F and 73M) to build growth curves. Due to missing data, head-body measurements were collected from fewer individuals (59 foxes and 101 coatis). All three growth models fit the body mass and head-body length of coatis and foxes equally well (ΔAIC < 2; Table 8). The Gompertz model was always the second ranked model for all traits and both species, whereas the logistic and von Bertalanffy models fluctuated between being the first or third ranked models (Table 8). Therefore, we chose the Gompertz model to describe growth curves, facilitating comparisons between and within species. Crab-eating foxes reached the head-body length asymptote between 0.5 and one year old (age category 3), but kept gaining weight until between one and 1.5 yr old (age category 4; Fig.5a and b). Growth rate and body mass gain approached zero at age category 7 in coati males, whereas females stopped growing and gaining weight at age category 6 (Fig. 5). Both coati sexes showed similar head-body and mass growth rates (k) and both reached their maximum growth rates (I) at the same age category (Table 9).

Six crab-eating foxes (3F and 3M) and four coatis (3M and 1F) died during the study due to unknown causes. Fox mortalities occurred from age category 2 (0.2−0.5 yr old) to 8 (> 4 yr old; Table 6). In contrast, all coatis deaths occurred in animals > 2 yr old (age categories 6 to 8) and all mortalities occurring during and just prior to the breeding season (May-June).

Table 8. Akaike Information criteria (AIC) values and percentage of variance explained (% variance) for growth models fitted to body 36mass (g) and head-body length (mm) of foxes and coatis in Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

Species	**Models**	**Body mass (g)**				**Head-body length (mm)**			
		% variance	**R**	**AIC**	**ΔAIC**	**% variation**	**R**	**AIC**	**ΔAIC**
C. thous	Logistic	0.57	0.76	481.3	0.7	0.39	0.63	266.6	0.0
	Gompertz	0.58	0.76	481.0	0.3	0.39	0.63	266.7	0.1
	Von Bertalanffy	0.58	0.76	480.7	0.0	0.39	0.63	266.8	0.2
N.nasua	Logistic	0.63	0.79	736.5	0.0	0.64	0.80	370.3	0.0
	Gompertz	0.62	0.79	736.8	0.3	0.64	0.80	370.5	0.2
	Von Bertalanffy	0.60	0.77	738.1	1.6	0.64	0.80	370.8	0.5

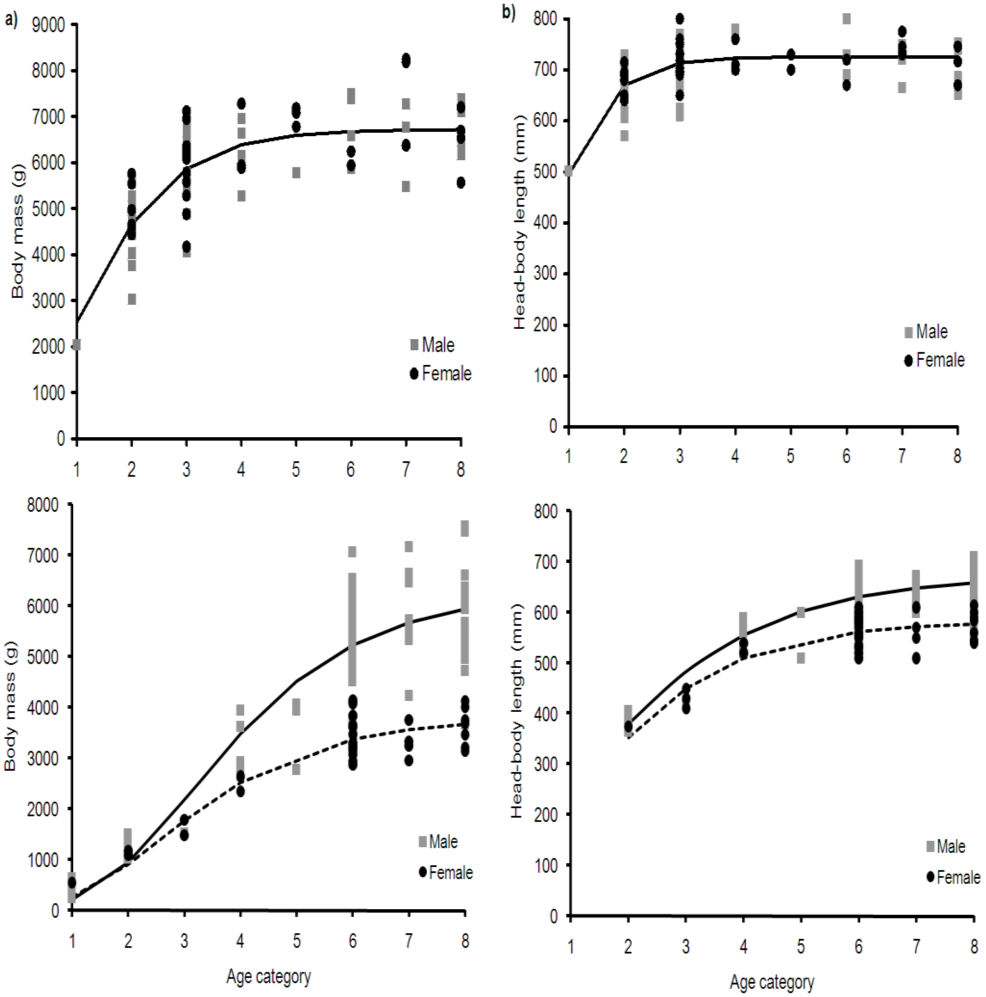

Fig. 5. Gompertz growth curves for body mass and head-body length of (a, b) *C. thous* and (c, d) *N. nasua* captured in Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

Table 9. Parameters of the Gompertz growth curve and their standard errors (SE) for data on body mass (g) and head-body length (mm) of coatis (*N. nasua*) and foxes (*C. thous*) captured in Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009. A = asymptotic body mass or head body length, k = growth-rate constant, and I = the age at the inflection point.

Dependent variable	**Parameters**	***C. thous***		***N. nasua* - males**		***N. nasua* - females**	
		Estimate	SE	Estimate	SE	Estimate	SE
	A	6720	154.3	6296[a]	325.3	3802[a]	199.0
Body mass (g)	K	1.0[b]	0.2	0.6	0.1	0.6	0.1
	I	1.0[b]	0.2	3.1	0.2	2.6	0.2
	A	726	6.7	672[a]	14.2	583[a]	12.7
Head-body length (mm)	K	1.6[b]	0.3	0.5	0.1	0.7	0.2
	I	0.4[b]	0.2	1.0	0.2	1.0	0.4

[a] Significant difference between sexes (P<0.001).

[b] Significant difference between species (P<0.01). For between species comparisons, we pooled data for both coati sexes to obtain the Gompertz parameters for the species. The parameter A was not compared between species because each has distinct ranges of body measurements.

DISCUSSION

Reproduction and sex ratio

Although foxes can reproduce at less than 1 year of age (Brady, 1978; Macdonald & Courtenay, 1996), more than 80% reproduced for the first time at the second year of life or later in the Nhumirim Ranch. Most females may not be mature to enter estrus at < 1 year old. Alternatively, because yearlings may remain with their parents for up to 15 months (Bianchi, 2009), the presence of an older female may inhibit estrus among younger females, as has been observed in other canids (Brady, 1978; Hradecký, 1985; Asa & Valdespino, 1998). Reproduction among coatis is less flexible than observed for foxes: coati females start reproducing at the second year of life and earlier reproduction may be rare or non-existent.

The breeding and birthing seasons were similar for both species and their onset agrees with that observed in other studies (Macdonald & Courtenay, 1996; Hirsch, 2007; Costa, Mauro & Silva, 2009; Faria-Correa *et al.*, 2009). Two foxes (15% of reproducing females), however, showed late pregnancy in the birthing season. Although there are no substantial studies on the reproductive and sociobiology of this species, several reproductive strategies for canids have been reported. For instance, female island foxes (*Urocyon litoralis*) that are paired with a male at the end of the reproductive season may show late pregnancy perhaps due to late induced estrus (S. Timm, pers. comm. referenced in Asa *et al.*, 2007). Alternatively, female bush dogs may reenter estrus after losing their litter (Porton *et al.*, 1987). It is unknown, however, if *C. thous* is polyestral and whether it shows induced estrus or induced ovulation. Notwithstanding, the length of the breeding season in crab-eating fox populations is variable (Brady, 1979; Faria-Correa *et al.*, 2009), and it may breed twice a year or show aseasonal reproduction in captivity (Brady, 1978; Porton *et al.*, 1987). Evidence that crab-eating foxes reproduce more than once a year in the wild remains inconclusive (Brady, 1979; Macdonald & Courtenay, 1996; Faria-Correa *et al.*, 2009).

In the Pantanal and other areas in Brazil (Bonatti, 2006), the reproductive season of *N. nasua* is longer than in Argentina (Hirsh, 2007) and less synchronous from that reported for *N. narica* (Kaufmann, 1962; Russell, 1982; Binczik, 2006). The extended reproductive season for both brown-nosed coatis and crab-eating foxes support the hypothesis that breeding season may vary substantially according to latitude and study area. Below 20°N or S, for instance, many canids show reproductive asynchrony (Valdespino, 2007); the slight annual variation in photoperiod in the tropics (a variable that may influence reproductive activity in many species; Sadleir, 1969) and fluctuations in climatic factors (*e.g.* precipitation) or food availability from year to year may influence the timing and span of the reproductive season of species.

The sex ratio in crab-eating foxes is not skewed, as also described in other studies (Montgomery & Lubin, 1978; Faria-Correa *et al.*, 2009), but sex ratio in coatis was highly biased to males, which contrasts with earlier findings (Redford & MacLean, 1993; Costa *et al.*, 2009). Sex ratios of adult *N. narica* from Barro Colorado Island (Panama) is not different from 1:1 (Kaufmann, 1962; Gompper, 1997), whereas in Arizona (USA) and Jalisco (Mexico) it is biased toward females, probably as a consequence of the high mortality caused by predation of adult solitary males (Hass & Valenzuela, 2002). Adult male coatis are larger than females, are usually solitary, and show different patterns of home range (Bianchi, 2009). Thus, sex ratio skew among adults might be expected due to capture biases. However, bias toward males also occurred among < 2-yr-old coatis (40M and 13F), a phase during which coatis body size dimorphism is still not apparent and individuals of both sexes travel together in bands. In addition, in the two entire litters of young coatis (less than 6 weeks) we were able to capture, the proportion of females:males was 1:5 and 1:3, respectively. Therefore, we do not believe that sexual bias among coatis was a sampling artifact. To the contrary, the sex bias may exist at birth or during the first weeks of life. There are many mechanisms leading to bias in sex ratio, but our knowledge of how sex ratio adjustments occur in mammals is still rudimentary (Clutton-Brock & Iason, 1986; Godfray & Werren, 1996). In many polygynous vertebrates, males compete more strongly for access to mates than do the females, and male fitness may be more strongly influenced by parental investment than is female fitness; moreover, parents may bias the sex ratio of their offspring toward the sex whose production will have the greatest effect on their own fitness (Trivers & Willard, 1973; Clutton-Brock & Iason, 1986). In this coati population, sex ratio bias to males or parental investment on males may be high because this sex has the greatest effect on the parental fitness.

Growth and morphometrics

This is the first study to provide substantial information on age-specific body measurements for crab-eating foxes and brown-nosed coatis. Although sample size of adult females is not large, values presented in Table 7 are the averages of multiple measurements taken from the same individuals (up to 10 measures taken from an individual) over the course of the study. Variation in body mass was larger than in other body size measurements for both species. This is expected because body mass is more subjected to seasonal and annual variation in food quality and availability, behavior, and parasite burden (see Chapter 4). The high variation coefficient for lower canine length is because it is progressively worn and therefore smaller in old animals.

A 3−4% intersexual dimorphism on linear measurements of body size is commonly found for fox species (Hildebrand, 1952; Prestrud & Nilssen, 1995; Vidal *et al.*, 2009), as it was found for crab-eating foxes (Table 7). Such differences are relatively small and were observed in only 25% of the body size measurements. Thus, this species may be considered monomorphic in size. Sexual dimorphism in size may result from a combination of factors, including sexual selection, selection for decreased female size, differential predation rates and abiotic factors (Karubian & Swaddle, 2001; Isaac, 2005). Notwithstanding, differences between coati sexes occur not only on body size, but also on canine size and the extent of sociality, suggesting that life history differences between coati sexes may be at least partially attributed to the effects of sexual selection (Selander, 1965; Orians, 1969; Charlesworth, 1980). In polygynous species, there is a strong selection pressure on male traits that are important in obtaining mates (Arnold & Wade, 1984), leading to sex differences in morphology (for example, large lower canines) and behavior (the coati male's solitary habits) that are usually accompanied by distinct age-specific mortalities between males and females (Clinton & Leboeuf, 1993). The ratio of male:female lower canine in coatis from the Nhumirim Ranch is 2.00 (Table 7), the highest amongst 45 species of carnivores and the highest recorded for *Nasua* spp. (Gittleman & VanValkenburgh, 1997; Binczik, 2006). If only individuals in age category 6 are considered (adults with teeth in the best condition), such ratio reaches 2.29. Carnivore canine tooth dimorphism is related with mating systems, with more polygynous species exhibiting greater dimorphism (Gittleman & VanValkenburgh, 1997). Such species likely experience high competition among males for mates (Emlen & Oring, 1977; Hirsh, 2007), which seems to be particularly marked in *N. narica* (Binczik, 2006). The absence of size dimorphism between sexes in crab-eating foxes, on the other side, is likely a reflection of monogamy, paternal care and lower within-sex competition for mates (Kleiman, 1977; Bekoff *et al.*, 1981; Prestrud & Nilssen, 1995; Vidal *et al.*, 2009).

Both coatis and foxes reach their head-body length asymptote at the age category when they start reproducing, but foxes gain weight until the following age category after reaching the head-body size asymptote. Among brown-nosed coatis, males and females grow at similar rates and show maximum growth at similar ages (Table 9). However, males reach the body mass and head-body length asymptote one age category later than females (age category 7), when most males probably start attempting to reproduce (Binczik, 2006). Trade-offs between reproduction and somatic investment are predicted by life history theory (Stearns, 1976; Tuomi *et al*., 1983), and a reduction in body size growth in mammals may occur with sexual maturity (Green & Rothstein, 1991). Investment in reproduction is particularly high for females: pregnant females need to invest in uterine, placental and mammary tissues, and lactation is usually considered the most expensive aspect of reproduction (Gittleman & Thompson, 1988; Pontier *et al.*, 1989). Apart from this, coati males and females differ on the asymptotic size only, with males reaching a final larger body size than females and also reproducing later than females. The deferred reproduction and the longer growth period in males may be a strategy to minimize the costs of reproduction associated with a strong within-sex competition for mates (Selander, 1965; Clinton & LeBoeuf, 1993).

Finally, foxes show higher growth rates than coatis (Table 9) which may be a consequence of their small litter size associated with biparental care, as well as their more carnivorous diet associated with higher metabolic rates than in coatis (Case, 1978; Chevillardhugot, *et al.* 1980; Hennemann *et al.*, 1983; Gittleman & Oftedal, 1987; Bianchi, 2009).

Survivorship

Around 20% of the adult males showed scars, and during the mating season, four adult coati males were found injured probably due to agonistic encounters with other males; the same has been observed for *N. narica* (Hass & Valenzuela, 2002; Binczik, 2006). Hirsch (2007) also observed fights between *N. nasua* males during the breeding season in Argentina. Although information on survival of coatis is limited, all male deaths occurred among > 2-yr-old individuals during the two months leading up to the breeding season. Similarly, Rocha (2006) observed that 3 out of 4 radio-collared adult males died during or just before the 2005-2006 reproductive season at Nhumirim Ranch, although the cause of these deaths was unclear. Despite mortality among adult males, we did not observe bias toward females in the adult sex ratio, as observed for *N. narica* in Arizona and Jalisco by Hass & Valenzuela (2002), who also verified a higher mortality rates in solitary adults than on adults in bands, suggesting that living in band may provide anti-predator benefits.

We did not access mortality of immature coatis, but mortality of less than 3-months-old *N. nasua* in Argentina was reportedly low (Hirsh, 2007). Among *N. narica* nestlings in Costa Rica (Saénz, 1994), mortality is high due to predation by capuchin monkeys (Newcomer & Defarcy, 1985; Fedigan, 1990; Perry & Rose, 1994), and in the USA, Mexico and Panama, mortality is also high, but due to unknown causes (Gompper, 1994; Hass & Valenzuela, 2002). There are no capuchin monkeys in the Pantanal, and although diverse species may prey on young coatis, the ocelots – which are very abundant in the study areas – rarely prey upon coatis (Bianchi, 2009).

The survivorship curve for crab-eating foxes showed no sharp age-specific differences although its interpretation is limited due to large confidence limits (Table 6). Mortality in this species may be better distributed between age-classes and less or not related to predation or agonistic encounters. Because crab-eating foxes are overall monomorphic, mortality linked to sexual selection pressures is not expected to be strong in this species. Rather, mortality among adult crab-eating foxes is usually related to hunting (Berta & Marshall, 1978; Macdonald & Courtenay, 1996), and mortality among pups may be associated to resource availability. In the Nhumirim Ranch, hunting of foxes is not likely to be occurring and food availability does not seem to be a limiting factor. Parasites, on the other side, may be an important source of mortality. Necropsy of two foxes found dead showed more than 100 Acanthocephala sp. individuals in their intestine, and eggs of at least 6 other genera of gastro-intestinal helminths were found in feces of captured foxes; in addition, we detected fox exposure to protozoans (*Trypanosoma cruzi* and *T. evansi*) and canine distemper (unpublished data).

Coatis and foxes have very distinct social structures that are associated to their life history traits investigated. Between-sex differences in coatis are linked to distinct growth curves, skewed sex ratios, and possibly biased survival rates for males and females, and such differences may be partially related to sexual selection. Crab-eating foxes, in turn, are monomorphic in body size and have an equal sex ratio. Males and females mature at the same age and show mortality patterns that do not seem to be associated with competition for mates; these are typical traits usually found among monogamous canid species with biparental care.

Chapter 3

RELATIVE IMPORTANCE OF BIOTIC AND ABIOTIC FACTORS FOR TICK ABUNDANCE AND PREVALENCE ON BROWN-NOSED COATIS AND CRAB-EATING FOXES FROM THE PANTANAL, BRAZIL

ABSTRACT

Tick abundance and prevalence is correlated with a diversity of environmental and host characteristics. However, the relative importance of these factors is not well understood. Here we examined the importance of biotic and abiotic factors in predicting abundance and prevalence of three species of *Amblyomma* spp. ticks (*A. parvum*, *A. cajannense* and *A. ovale*) on brown-nosed coatis (Procyonidae: *Nasua nasua*) and crab-eating foxes (Canidae: *Cerdocyon thous*) from the Pantanal region, Brazil. We created linear models accounting for seasonal dynamics (temperature and precipitation) and host characteristics (species, sex, age and body size) to investigate the relative importance of these variables on the abundance of larvae, nymphs, replete adult females, and adult tick of each species on hosts. All tick species and developmental stages showed aggregated distributions and the ratio of males/females tick on hosts was male-biased. Overall, temperature and precipitation were the most important predictors of tick abundance. Abundance of *Amblyomma* larvae and nymphs was higher during the cold and dry months whereas adults of *A. parvum* were abundant during the summer. Biotic predictors were also important; tick abundance and prevalence varied primarily with host species and host behavior. *Amblyomma ovale* infested coatis more than foxes, whereas *A. parvum* did not show host species differences. The abundance and prevalence of *A. cajannense* was very low in both host species. Crab-eating foxes harbored more immature stages and replete ticks than coatis, possibly because coatis show allogrooming. Differences between coati sexes were likely related to behavioral differences between adult males - which are usually solitary - and coati females, which live in social groups. Further investigation should focus on long-term, community-level studies of host-tick systems, considering additional extrinsic biotic predictors on tick abundance and prevalence.

INTRODUCTION

Parasites inhabiting a host population are influenced by an interplay of intrinsic (*e.g.* parasite life history) and extrinsic (*e.g.* host ecology or environmental factors) features (Poulin, 2006; Krasnov *et al.*, 2007). Tick abundance, for instance, is highly influenced by local temperature, photoperiod, and site characteristics (Daniel, 1978; Adler *et al.*, 1992; Lindsay *et al.*, 1999; Randolph & Storey, 1999; Lindstrom & Jaenson, 2003; Monello & Gompper, 2007). Much less is known about the role of host features in modulating tick abundance. While features of the host are known to correlate with the extent of parasitism by ticks, the relative importance of such factors has rarely been investigated, especially on wild hosts in their natural environments (Monello & Gompper, 2007; Brunner & Ostfeld, 2008; Monello & Gompper, 2010).

Recent work suggests that host age, sex, host size, individual behavior, and the extent of host social contact may predict the likelihood of tick feeding success and the abundance of some tick developmental stages on their hosts (Adler *et al.*, 1992; Monello & Gompper, 2007; Boyer *et al.*, 2010; Monello & Gompper, 2010). In fact, abundance or intensity (number of parasites per infected host) of many parasites tends to increase with host age and may stabilize or even decline in older individuals. Such stabilization or decline may occur if acquired immunity or mortality of old, heavily parasitized host is important (Hudson & Dobson, 1995; Wilson *et al.*, 2002). Likewise, parasite abundance or intensity may change according to host sex: in mammals and some other *taxa*, males tend to be more heavily infected than females, perhaps due to differences in immune function (Schuurs & Verheul, 1990; Poulin, 1996; Zuk & McKean, 1996; Schalk & Forbes, 1997; Hughes & Randolph, 2001a) and behavior (Boyer *et al.*, 2010). Adult tick intensity may also increase on social or gregarious hosts because ticks can detect and seek out CO_2 emission sources (Ostfeld *et al.*, 1996; Anderson *et al.*, 1998; McMahon & Guerin, 2002; Cançado *et al.*, 2008), host odor (Crooks & Randolph, 2006), and host sound (Webb, 1979). Hence, high local host densities may attract more questing ticks (Monello & Gompper, 2010).

Regardless of the factors acting on parasite abundance, most macroparasites, including ticks, show aggregated distribution in their host populations, with the majority of parasite population concentrated into 20% or less individual hosts (Shaw *et al.*, 1998; Shaw *et al.*, 2003; Monello & Gompper, 2007; Poulin, 2007; Brunner & Ostfeld, 2008). The degree of aggregation and the extent of tick infestation have important consequences for parasite persistence on hosts and for vector-borne pathogen transmission (Perkins *et* *al.*, 2003). Such aggregation across a host population may also determine the impact of parasites as a regulating force on host population (Wilson *et al*., 2002).

Here we examined the relative importance of extrinsic biotic and abiotic factors on the abundance and prevalence of three species of ticks on two carnivores that exhibit distinct degrees of sociality. We sampled *Amblyomma cajannense*, *A. parvum* and *A. ovale* ticks infesting the brown-nosed coatis (Procyonidae: *Nasua nasua*) and the crab-eating fox (Canidae: *Cerdocyon thous*) in the Pantanal wetlands, and assessed the relative importance of temperature, precipitation and host species, age, sex and size in predicting the abundance of different tick developmental stages of *Amblyomma* species. In addition, we investigated how the degree of parasite aggregation varied according to host and tick species as well as tick developmental stages.

A small number of *Amblyomma* spp. ticks have been studied in South America, mostly on domestic animals (Guglielmone *et al.*, 1990a; Labruna *et al.*, 2002; Castagnolli *et al.*, 2003; Oliveira *et al.*, 2003; Cabrera & Labruna, 2009). Like other ixodids, many *Amblyomma* species have a three-host life cycle, with larvae and nymphs probably engorging on small to medium-sized hosts and adults feeding on larger species (Oliver, 1989; Guglielmone *et al.*, 2003; Nava *et al.*, 2006; Cançado, 2008). *Amblyomma parvum* is found from Mexico to Argentina (Nava *et al.*, 2008b), whereas *A. cajannense* and *A. ovale* are found from southern US to Argentina (Guglielmone *et al.*, 2003). *Amblyomma parvum* and *A. cajannense* have one generation a year with distinct seasonal parasitic stages (larvae, nymphs and adults). Immature stages infest hosts during months of low temperature and rainfall, while adults are abundant on hosts during periods of high rainfall and temperature (Guglielmone *et al.*, 1990a; Labruna *et al.*, 2002a; Oliveira *et al.*, 2003; Nava *et al.*, 2008a; Labruna *et al.*, 2009). *Amblyomma cajannense* is the most prevalent *Amblyomma* species in the Neotropics (Estrada-Pena *et al.*, 2004) and it is a vector of *Rickettsia rickettsii*, which causes the Brazilian spotted fever (Guedes *et al.*, 2005; Sangioni *et al.*, 2005). The seasonal phenology of *Amblyomma ovale* is less understood, but the species is recognized as a potential vector for *Hepatozoon canis* (Forlano *et al.*, 2005).

The two host species investigated are among the most common South American carnivores (Alho *et al.*, 1987; Mittermeier *et al.*, 1990; Alho & Lacher, 1991; Bianchi, 2009). They have distinct social systems and life history patterns, which may ultimately influence the extent of parasitism by ticks: crab-eating foxes are monomorphic in size and live in small family groups comprising a breeding pair with pups and sometimes adult-sized offspring from previous years (Macdonald & Courtenay, 1996; Courtenay & Maffei, 2004; Bianchi, 2009). In contrast, brown-nosed coatis are dimorphic in size (Chapter 2) and have a complex social structure in which adult females and immature individuals form bands of up to 30 individuals in the Pantanal (Lacher *et al.*, 1987), while most adult males are solitary, joining the bands only during the reproductive season (Resende *et al.*, 2004; Hirsh, 2007; Bianchi, 2009).

Aside from the potential effects of host age, sex and size on tick component-population, species of ticks may show host preference (Slowik & Lane, 2009), which can lead to heterogeneities in tick abundance and prevalence on distinct host species. As a consequence, we hypothesize that prevalence and abundance of tick species may differ for coatis and foxes. Furthermore, intrinsic differences between tick developmental stages and the behavioral differences between coatis and foxes may result in additional variability in tick response to biotic predictors. Questing larvae, for instance, have low mobility, are usually highly clumped in the environment (Ostfeld *et al.*, 1996), and show an “ambush strategy”, in which they wait and attach to a passing host (Sonenshine, 1993; Oorebeek *et al.*, 2009). Host infestation by tick larvae, therefore, will not likely to be related to the degree of host sociality *per se*. Conversely, we hypothesize that tick abundance may be reduced on coati band members due to a “dilution effect”, in which the host per-capita infestation is decreased (Gompper, 2004; Côté & Poulin, 1995; Fauchald *et al.*, 2007). In addition, because coatis exhibit allogrooming (Kaufmann, 1962; Vicente *et al.*, 2007) we expect tick abundance to be reduced among individuals of a band (Gompper, 2004), when comparing to solitary males.

Regardless of the role biotic factors on tick component-population parameters, we anticipate a relationship between temperature and tick abundance, with high abundances of adult ticks during the summer and high abundances of immature stages during the winter (Guglielmone *et al.*, 1990a; Mangold *et al.*, 1994; Oliveira *et al.*, 2003; Szabo *et al.*, 2007; Nava *et al.*, 2008a). Abiotic factors are closely related to the life cycle and abundance of questing ticks through the effects of temperature and humidity on tick behavior and survival (Needham & Teel, 1991). As a consequence, when biotic and abiotic factors are analyzed together, we hypothesize that temperature and precipitation will be more important than biotic factors because the number of ticks feeding on hosts is at least partially a function of the total number of questing ticks available to infest hosts (Brunner & Ostfeld, 2008).

MATERIAL AND METHODS

Study area

Field work was conducted at Nhumirim Ranch (18º59’S, 56º39’W), a 4400ha research station of The Brazilian Agricultural Research Corporation (Embrapa) located in the Nhecolândia sub-region of the Pantanal, Brazil. The Pantanal is the largest seasonal floodplain in the world and it has two marked seasons – a wet season (October to March) and a dry season (April to September). The region is characterized by sandy soil with a mosaic vegetation of semi-deciduous forest, dispersed shrub vegetation, and seasonally flooded fields (Rodela, 2006). Permanent and temporary ponds and “salinas” (salt water ponds) occur throughout the region. Human population density is low (< 2 people per km^2) and the main economic activity is cattle ranching (Adamoli, 1987). Densities of coatis and foxes are high in the study area (Alho *et al.*, 1987; Lacher *et al.*, 1987; Desbiez & Borges, 2010), with coatis and foxes showing relatively small home ranges (foxes: 1.4 to 1.8 Km^2 ; coatis: 0.9 to 2.5 Km^2), and adult, solitary coati males showing smaller home ranges than females (males: 0.9 to 1.2 Km^2; females: 2.1 to 2.5 Km^2; Bianchi, 2009).

Capture procedures and tick quantification

From May 2006 to February 2009 we captured coatis and foxes up to four times per year (see Fig. 8), using wire live-traps (1m x 0.40m x 0.50m) placed in a trapping grid of 7.2 Km^2 and with occasional traps placed out of the grid. Traps were baited with bacon, set late in the afternoon and checked in the following morning. We anesthetized individuals with an intramuscular injection of Zoletil$^@$50 (Virbac®; tiletamine hydrochloride and zolazepan hydrochloride, 10 mg/Kg) and ear-tagged them with numbered colored tags (Nasco Rototags®). Body size and mass measures as well as tooth eruption and wear were recorded (Chapter 1). We monitored the animals until recovery from the anesthesia and released them at the capture site. Animal procedures were approved by the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA, first license #183/2005 – CGFAU/LIC; last license #11772-2) and University of Missouri Animal Care and Use Committee (protocol #4459).

We estimated the total number of ticks on hosts by direct 3 minute counts and collected tick samples by searching the ears, between the digits, and on the ventral and dorsal parts of the animals. In addition, tick collection was supplemented via 10 strokes with a flea comb from the base of the neck to the tip of the tail on the dorsal region of animals (modified from Monello & Gompper, 2009). This was done to increase sample collection from the back and tail of the animal where direct counts and removal are less efficient. Collected ticks were stored in sealed plastic bags and later conserved in ethanol 70%. At the laboratory, adult ticks were examined using a dissecting scope and identified to species (Barros-Battesti *et al.*, 2006). Because immature stages of most *Amblyomma* species from Brazil remain undescribed, nymphs and larvae were classified as *Amblyomma* spp. Adults ticks were separated by sex, and classified as non-replete (not engorged with blood) or replete (engorged). We estimated the absolute number of nymphs and larvae, as well as adults of each tick species (*A. cajannense, A. parvum* and *A. ovale*) on individual hosts by multiplying the collected number of each tick species or stage by the total number of ticks observed during the 3 minutes count (all species and stages combined) and then dividing this by the total number of ticks of all species and stages collected from an individual host.

Host characteristics and abiotic factors

Hosts were divided into five age categories (≤ 0.5yr, 0.51-2yr, 2-3 yr, 3-4 yr and > 4yr) based on body size measurements and a teeth condition index that measures dental development and the degree of teeth wear (Chapter 1 and 2). Host body size was quantified by the scores of the first factor of a principal component analysis including data on six body size measurements (Chapter 1).

We obtained data on daily precipitation and daily average temperature for the study site from INMET (National Institute of Meteorology) and averaged it for the 30 days prior to tick collection on individual hosts. This was performed because tick response to abiotic factors is likely a function of temperature and precipitation variation within a wider period of time than the single day of tick collection on the host, and because tick infrapopulations are probably the result of multiple host-tick encounters occurring over the past several days or weeks.

Measures of parasitism

Host prevalence was estimated as the number of hosts infested with nymphs, larvae or adult ticks divided by the total number of capture events (see below). Abundance was the total (extrapolated) number of tick species or developmental stage (larvae, nymphs, replete adult females) found on each host individual. We calculated confidence intervals for prevalence (Sterne's exact method), mean abundance (bootstrap confidence intervals with 2000 replications), and the aggregation index of discrepancy D (Poulin, 1993) using the program Quantitative Parasitology 3.0 (Reiczigel & Rózsa, 2005). Prevalences were compared between host species, host sexes, host age categories and coati bands *vs.* solitary males using Fisher's Exact tests, and mean abundances were compared between host species, host sex and coati bands *vs.* solitary males using Bootstrap t-tests. For quantitative comparisons between host age categories, we used the Median test. Finally, we calculated the sex ratio of the tick species (excluding *A. cajannense*, for which sample sizes were small) on the hosts as the proportion of males/females and used the Sign test to compare the number of males and females infesting hosts.

Trapping effort occurred up to 4 times a year, and so some individuals were recaptured on multiple occasions. For host individuals examined more than one time, we assessed whether such samples were pseudo-replicated by investigating the residuals of tick abundance *vs.* time correlation plots. Since residuals were large and tick abundance markedly changed over time, host capture events were considered independent samples. Moreover, tick collection on a same individual host was performed in an average three-month interval, which is sufficient time for turn-over of the feeding tick infrapopulation. This allowed an increased sample size for model testing (see below). However, we also ran all general models for tick species and developmental stage using solely data from the first tick collection on hosts, and the overall results (not shown) were similar to those obtained from models carried out with multiple samples from individual hosts.

Models

We created generalized linear models with negative binomial distributions accounting for seasonal dynamics (temperature and precipitation) and host characteristics (host sex, age and size) to investigate the relative importance of these factors on tick abundance. The abundance of nymphs, larvae, replete adult females and each adult tick species were the dependent variables in the models, which were generated according to a two-level and a three-stage process. Because each *Amblyomma* species may parasitize both host species, we created a) general-level models, in which host species was treated as an additional independent variable and b) host species-level models, in which analyses were conducted for coatis and foxes separately. Host species-level modeling was performed only when host species was found to be an important variable in the respective general model (*i.e.*, patterns of parasitism differed for the two host species). Candidate models represented independent hypothesis and were created following a three-stage procedure: for both analysis levels (general and host species-levels), a) biotic and abiotic global sub-model were created (Table 10, models 2 and 4) and compared to the global model containing all biotic and abiotic variables (Table 10, model 1); b) the contribution of variables in the best supported sub-model was then further analyzed through the generation of additional nested models (*e.g.* Table 10, models 3 and 5); c) the important variables (those that solely or in combination improved the model fit when compared to the better performing global sub-model) were pre-selected and subsequently included in all additional models, which contained all combinations of variables from the less important (second ranked) sub-model (Table 10, *e.g.* model 6).

Table 10. *A priori* models representing tick abundance for larvae, nymphs, replete adult females and each tick species on coatis and foxes in the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009.

Model #	Hypothesis	Model*
Variation on tick abundance is related to biotic and abiotic factors:		
1	General and host-species global models	[Species]** + Age + Sex + Size + Temp + Prec
Biotic models - variation on tick abundance is related to:		
2	A combination of all host characteristics (biotic sub-model)	[Species]**+ Age + Sex + Size
	Host age-related differences	Age
	Host sex-related differences	Sex
3	Host size-related differences	Size
	Host species	Species
	Combinations of two or more host characteristics	Age + Sex + Size; [Species]** + Age + Sex; [Species]** + Sex + Size; [Species]** + Sex, etc
Abiotic models - variation on tick abundance is related to:		
4	A combination of abiotic variables (abiotic sub-model)	Temp + Prec; Temp x Prec
5	Temperature (^{o}C)	Temp
	Precipitation (mm)	Prec
6	Additional candidate models	Temp + Age + Sex + Size
	Ex: when the abiotic global model was the best sub-model and	Temp + Age + Sex
	temperature was the most important (pre-selected) variable in it	Temp + Age + Size
		Temp + Sex
		Temp + Age
		Temp + Size

* Structure was similar for all models; *e.g.*, Age + Sex + Size was β_0 (intercept) + β_1 (Age) + β_2 (Sex) + β_3 (Size);

** “Species” is included in the general models but not on the host species-level models.

Model fit was compared using Akaike Information Criterion corrected for small sample size (AICc) and when needed, for overdispersion (QAICc). Models were ranked based on the difference between the best approximating model (model with the lowest AICc or QAICc) and all others in the set of candidate models (ΔAICc, ΔQAICc). Models with differences within two units of the best-fitting model have strong empirical support and were considered competitive models (Burnham & Anderson, 2002). When there was no clear best-fitting model, the relative importance of each predictor was quantified by adding the Akaike weights across all the models in the set of competitive models that included that predictor or combination of predictors (variable weights). When the set of competitive, best-fitting models included the intercept-only model, we did not proceed with model interpretation.

Due to small sample size, abundance of replete adult females was analyzed at the *Amblyomma* genus level and the abundance of *A. cajannense* was investigated for the general model only (not dividing by host species). For larvae abundance, the dependent variable was $\log_2 (n + 1)$-transformed due to excessive overdispersion (c-hat > 6), and age categories 1 and 2 for coatis (≤ 2 yr old individuals) were pooled in the species-level models due to limited host sample size.

RESULTS

We collected 129 tick abundance samples from 67 foxes and 147 tick abundance samples from 100 coatis. We identified 3724 larvae, 3811 nymphs, 128 replete adult females, 37 adult *A. cajannense*, 840 adult *A. parvum* and 280 adult *A. ovale*. The sex ratio for adult *A. parvum* and *A. ovale* were biased to males (*A. parvum* sex ratio: 2.8; Z=5.27; N=189; P<0.001; *A. ovale* sex ratio: 1.6; Z=2.48; N=110; P=0.01), and all tick species and developmental stages showed aggregated distributions (Table 11; $D \geq 0.6$), with D increasing with tick abundance ($R_{Pearson}=0.65$; N=12; P=0.021), but decreasing with tick prevalence ($R_{Pearson} = -0.87$; N=12; P<0.001).

Prevalences

Amblyomma parvum was the most prevalent species on coatis and foxes, followed by *A. ovale* and *A. cajannense* (Table 11). Prevalence of *A. ovale* was higher on coatis and prevalence of *A. parvum* was the same for both host species; larvae, nymphs and replete adult females were more prevalent on foxes than coatis (Table 11). When comparing host sexes, prevalence of *A. parvum* on both host species and prevalence of *A. ovale* on foxes were higher on female hosts than on males (Table 12). Conversely, prevalence of replete ticks tended to be higher on male (Table 12) than female coatis and higher on solitary adults coatis than band members (P=0.04; Fig. 6a). Prevalence of larvae on ≤ 0.5-yr-old foxes was lower than on older individuals (P=0.01; Fig. 7a). Conversely, prevalence of *A. parvum* was high in ≤ 0.5-yr-old foxes, especially when compared with age category 5 (P=0.04; Fig.7b). Finally, prevalence of larvae on coatis tended to be lower in age category 5 when compared to previous age categories (P=0.07; Fig. 7c).

Table 11. Prevalence and mean abundance of *Amblyomma* species and developmental stages on coatis (*N. nasua*; N = 137 to 147 sampling events) and foxes (*C. thous*; N = 122 to 129) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. D = discrepancy index. Replete = engorged adults females of *Amblyomma* spp.

Tick stage/species - Host species	Prevalence (%)	95% CI	Mean abundance	95% CI	D
Larvae - *Cthous*	62.0*	53.1−70.2	29.9***	22.1−40.6	0.76
Larvae – *N. nasua*	49.0*	40.8−57.2	9.5***	6.0−17.0	0.86
Nymphs - *C. thous*	88.4*	81.5−93.2	20.1	16.4−24.8	0.58
Nymphs – *N. nasua*	78.2*	70.8−84.4	17.7	13.6−23.2	0.73
A. cajannense - *C. thous*	3.9	1.5−8.8	0.1	0.0−0.3	0.96
A. cajannense – *N. nasua*	8.2	4.7−13.9	0.3	0.1−0.6	0.95
A. parvum - *C. thous*	69.8	61.3−77.2	4.1	3.3−5.3	0.62
A. parvum - *N. nasua*	68.0	59.9−75.2	3.9	3.2−4.8	0.62
A. ovale - *C. thous*	27.9***	20.7−36.4	0.6**	0.4−0.9	0.82
A. ovale - *N. nasua*	50.3***	42.2−58.5	2.1**	1.5−2.9	0.75
Replete - *C. thous*	39.3**	31.0−48.4	0.7**	0.5−0.8	0.71
Replete - *N. nasua*	22.6**	16.4−30.6	0.4**	0.2−0.5	0.83

*P≤0.05; **P<0.01; ***P≤0.001.

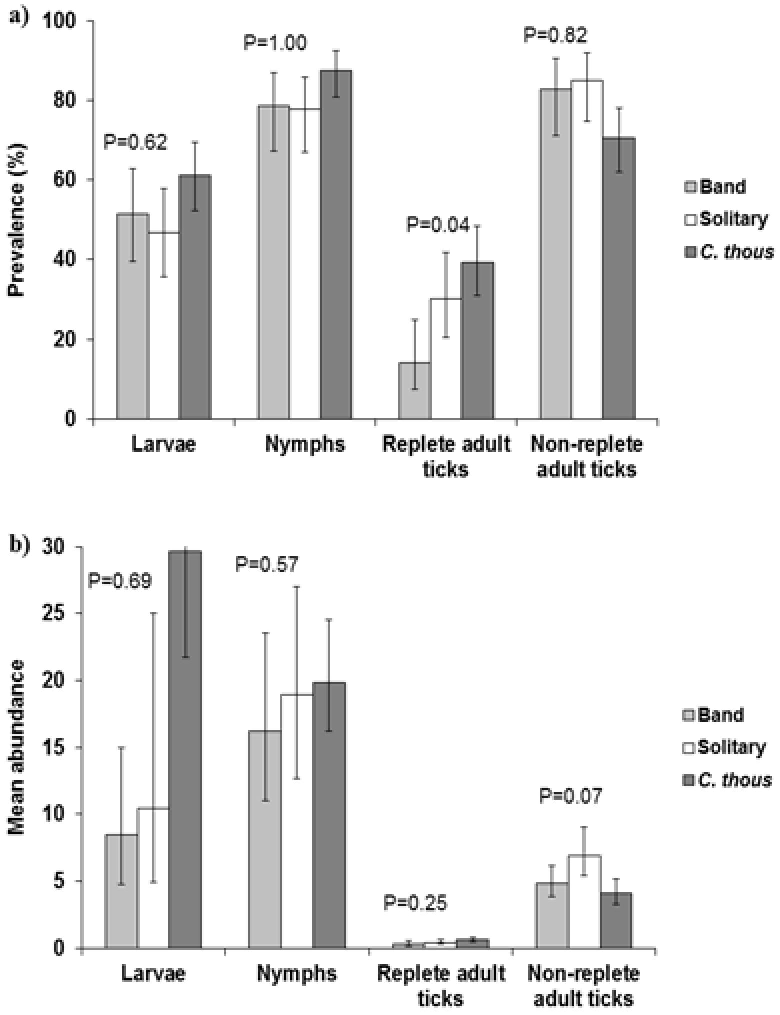

Fig. 6. Prevalence and mean abundance (± 95% confidence intervals) of immature stages and adult replete and non-replete ticks on coati band members (immature coatis and females), solitary individuals (adult males) and crab-eating foxes (*C. thous*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. P-values are for comparisons between coati bands and solitary individuals.

Table 12. Prevalence and mean abundance of *Amblyomma* species and developmental stage in male and female coatis (*N. nasua*: M=101 to 107; F=36 to 40 samples) and foxes (*C. thous*: M=67 to 71; F=55 to 58) at the Nhumitim Ranch, Pantanal from 2006 to 2009. Replete = engorged adult females of *Amblyomma* spp.

	Males				**Females**			
Host - *C. thous*	Prevalence (%)	95%CI	Mean abundance	95%CI	Prevalence (%)	95% CI	Mean abundance	95%CI
Larvae	63.4	51.4−74.1	27.6	18.6−39.9	60.3	47.4−72.6	32.9	19.5−53.9
Nymphs	90.0	81.1−95.3	22.4	17.5−29.8	86.2	75.1−93.5	17.2	12.7−24.2
A. cajannense	2.8	0.5−9.7	0.1	−	5.2	1.4−14.4	0.1	0.0−0.3
A. parvum	59.2***	47.2−70.4	3.9	3.0−5.8	82.8***	70.8−90.8	4	2.9−6.0
A. ovale	21.1*	13.0−32.3	0.4	0.2−0.8	36.2*	24.9−49.1	0.8	0.5−1.4
Replete	35.8	25.3−48.2	0.5	0.4−0.8	43.6	30.8−57.3	0.8	0.5−1.2
Host - *N. nasua*								
Larvae	51.4	41.9−60.8	9.1	5.0−19.0	42.5	27.7−58.8	10.6	5.1−21.6
Nymphs	78.5	69.7−85.7	19.3	14.2−25.8	77.5	62.0−88.0	13.4	7.9−22.3
A. cajannense	9.3	5.0−16.7	0.4	0.1−0.8	5.0	0.9−17.2	0.1	0.0−0.1
A. parvum	62.6**	52.8−71.6	3.7	2.9−5.0	82.5**	67.7−91.6	4.3	3.2−5.5
A. ovale	51.4	41.9−60.8	2.5**	1.8−3.5	47.5	32.3−62.8	1.1**	0.7−1.6
Replete	26.7*	18.7−36.1	0.4	0.3−0.6	11.1*	3.9−26.1	0.3	0.1−0.8

*P<0.08; **P<0.05;***P<0.01.

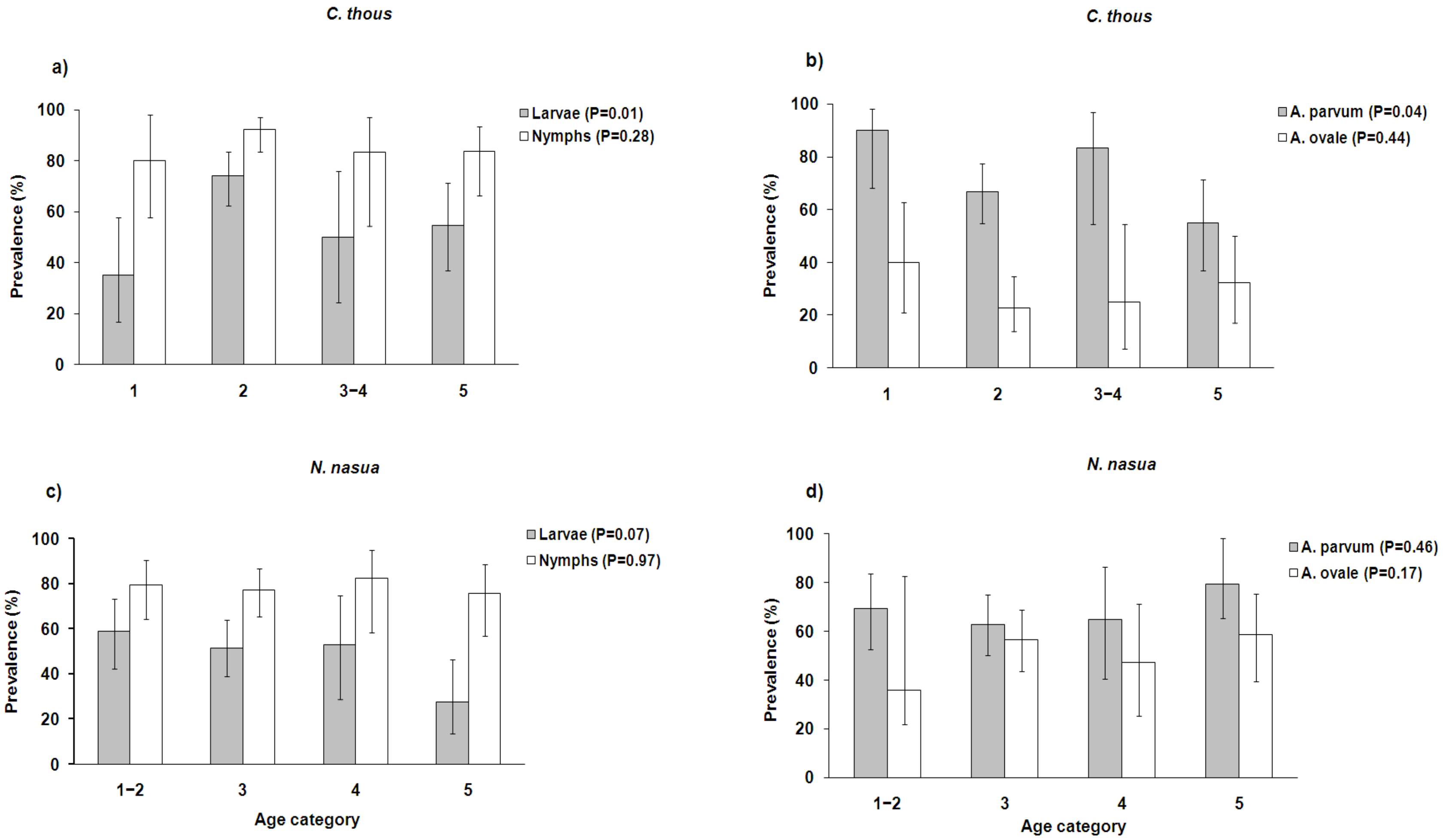

Fig. 7. Prevalence (± 95% confidence intervals) of immature stages and adults of *Amblyomma parvum* and *A. ovale* on coatis (a, b) and foxes (c, d) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Age categories are: 1 (≤ 0.5 yr old), 2 (0.51-2 yr old), 3 (2-3 yr old), 4 (3-4 yr old) and 5 (> 4 yr old). P-values for Fisher's Exact test are shown.

Tick abundance and model rankings

The mean abundance of *Amblyomma* spp. larvae per host was higher during early winter (May − July) and was followed by a peak of nymphs in August − September (Fig. 8a). Adults of *A. parvum* peaked in the summer (January – February), but adult *A. ovale* fluctuation was less cyclic (Fig. 8b). Fluctuations in abundance of *A. cajannense* were not evaluated due to small sample sizes.

Host age was the only biotic predictor related to the abundance of nymphs (Table 13), as ≤ 0.5-yr-old individuals (mainly foxes, given that there were only 2 coatis in this age category) showed relatively low number of nymphs (Median test for foxes $\chi^2 = 15.33$; d.f.=3; P=0.002; Fig. 9a). Host age was also present in the best-fitting model for the abundance of *Amblyomma* larvae on coatis (Table 15), which was somewhat larger in animals at age category 3 ($\chi^2 = 7.15$; d.f.=3; P=0.07; Fig 9c). This biotic variable was not present in the single best model predicting abundance of larvae in foxes, but prevalence (see previous section) and abundance ($\chi^2 = 15.79$; d.f.=3; P=0.001) of larvae in ≤ 0.5-yr-old foxes were also low compared to the following age category (Fig. 7a and 9a). Moreover, fox size was present in the second best models for larvae on foxes (not shown; ΔAIC = 2.07) and age and size were correlated ($R_{\text{Spearman}} = 0.38$; N = 129; $P < 0.05$).

Host size was related to the abundance of *A. parvum* and *A. ovale* on foxes (Table 15 and 16), with young, small individuals showing higher abundances of *A. parvum* (Fig. 9b). In fact, abundance of adult *A. parvum* was higher on ≤ 0.5-yr-old foxes and relatively constant on older individuals, and age and size were correlated. Abundance of *A. ovale* on foxes was too low to more fully assess the effect of host size on this tick abundance and host age was not related to *A. ovale* abundance on foxes (Fig. 9b). Host size was also present in general models for abundance of replete ticks, but when analyzing the effect of this variable on each host species separately, we found that host size was secondarily important for foxes and the interpretation for coatis was compromised because the intercept-only model was the top model (Table 15). There was a slight correlation between host size and abundance of replete ticks (R=0.13; N=276; p=0.03) because coatis are smaller than foxes, and foxes showed higher prevalences and abundance of replete ticks than coatis (Table 11). Although host size was present in other supported models, these differed from simpler models by just one parameter and had similar values of maximized log-likelihood (Table 15).

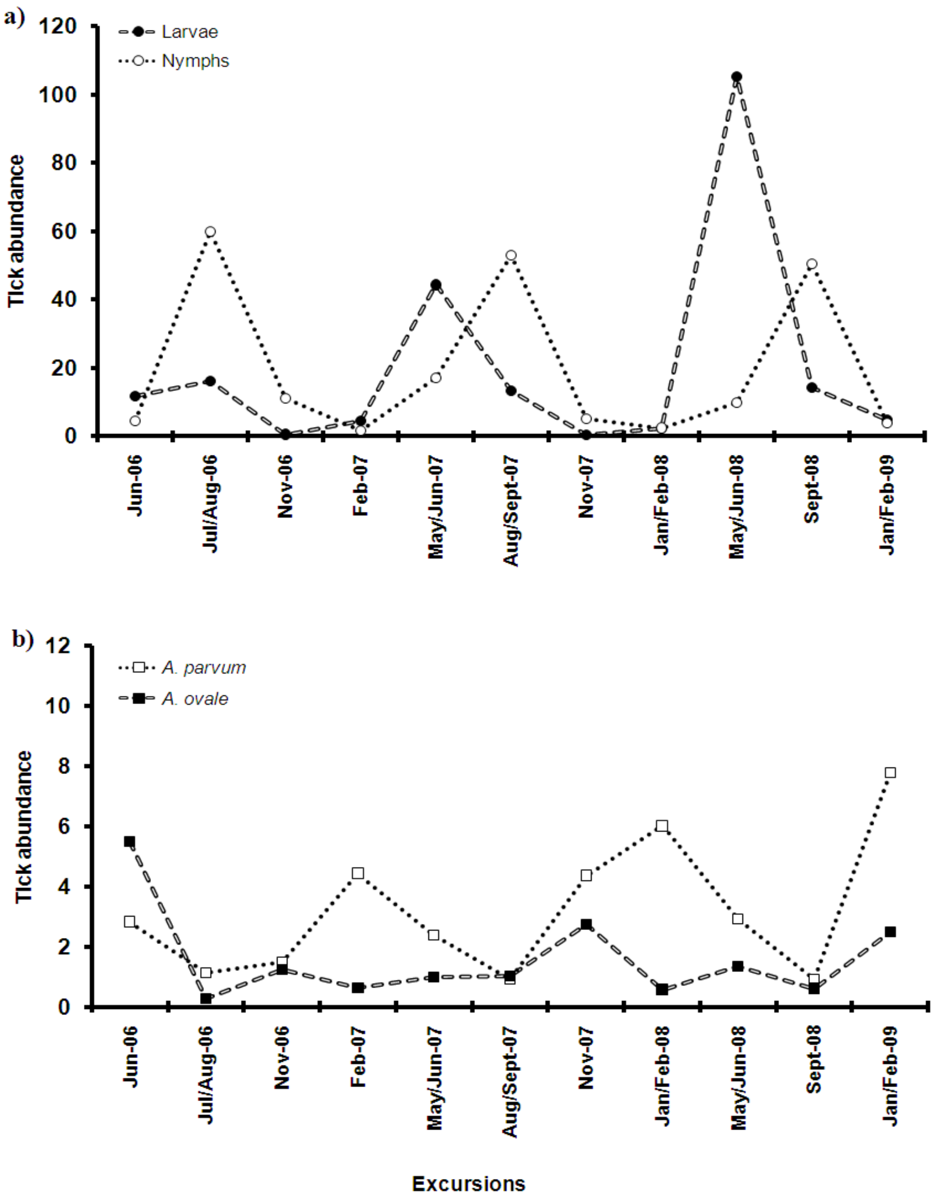

Fig. 8. Average extrapolated abundance per host of a) larvae and nymphs of *Amblyomma* spp. and b) adults of *A. parvum* and *A. ovale* collected on coatis and foxes captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Lines connecting points are for visual improvement only.

Table 13. Ranking of general best-fitting models describing the abundance of tick species and tick developmental stages on foxes (*C. thous*) and coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Pre-selected variables (see text) are shown in bold. Log(*l*) = log-likelihood of the models; k = number of parameters in the models.

Model	**log(l)**	**AICc/ QAICc**	**k**	**ΔAICc/ ΔQAICc**	**AICc/QAICc Weight**
A. parvum					
Temp + Prec + Size	-518	1047	5	0.0	0.23
Temp + Prec + Sex	-519	1048	5	0.9	0.15
Temp + Prec	-520	1048	4	1.3	0.12
Temp + Prec + Species	-519	1048	5	1.3	0.12
Temp + Prec+ Species + Sex	-518	1048	6	1.6	0.10
Temp + Prec + Species + Size	-518	1049	6	1.9	0.09
A. ovale					
Species + Temp + Prec	-185	380	5	0.0	0.31
Species + Temp x Prec	-186	381	3	0.6	0.18
Species + Prec	-186	381	4	0.7	0.22
Species	-188	381	3	1.2	0.17
A. cajannense					
Intercept only	-38	77	1	0.0	0.40
Prec + Species	-35	79	4	1.9	0.16
Nymphs					
Temp + Prec + Age	-797	1606	6	0.0	0.65
Larvae					
Temp + Size	-529	1064	3	0.0	0.16
Temp + Species	-529	1064	3	0.4	0.13
Temp	-530	1065	2	1.0	0.10
Temp + Prec	-530	1065	3	1.5	0.08
Temp + Species + Size	-529	1066	4	1.7	0.07
Intercept only	-532	1066	1	1.8	0.07
Temp + Species + Sex	-529	1066	4	1.9	0.06
Temp + Sex + Size	-529	1066	4	1.9	0.06
Replete females					
Temp + Species	-202	412	4	0.0	0.31
Temp + Size	-203	413	4	1.3	0.16
Temp + Species + Size	-202	413	5	1.3	0.16
Temp + Species + Sex	-202	414	5	1.7	0.13
Temp + Sex + Size	-202	414	5	2.0	0.11

Table 14. Variable weight (Var. weight) and relative variable weight (Rel. var. weight) for the best-fitting general models describing the abundance of tick species and tick developmental stages on foxes (*C. thous*) and coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Data for the two host species are analyzed together. Pre-selected variables (see text) are shown in bold.

General models	Var. weight	Rel. var. weight
A. parvum		
Temp + Prec	0.82	0.56
Size	0.32	0.22
Species	0.31	0.22
Sex	0.25	0.17
A. ovale		
Species	0.75	0.42
Prec	0.61	0.34
Temp	0.43	0.24
Replete females		
Temp	0.88	0.41
Species	0.60	0.28
Size	0.43	0.20
Sex	0.24	0.11

Table 15. Ranking of separate host-species level best-fitting models describing the abundance of tick species and tick developmental stages on foxes (*C. thous*) and coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Pre-selected variables (see text) are shown in bold. Log(*l*) = log-likelihood of the models; k = number of parameters in the models.

Model	**log(l)**	**AICc/ QAICc**	**k**	**ΔAICc/ ΔQAICc**	**AICc/QAICc Weight**
A. parvum/C. thous					
Temp + Prec + Size	-297	602	4	0.0	0.25
Temp + Prec + Size + Sex	-296	603	5	0.3	0.22
Temp + Prec	-298	603	3	0.4	0.21
Temp + Prec + Sex	-298	603	4	1.1	0.14
A. parvum/N.nasua					
Temp	-241	489	3	0.0	0.44
Temp + Sex	-241	490	4	1.4	0.22
Temp + Size	-241	490	4	1.9	0.17
A. ovale/C. thous					
Age + Sex + Size + Temp	-87	193	9	0.0	0.52
A. ovale/N. nasua					
Prec + Sex	-150	308	4	0.0	0.31
Larvae/*C. thous*					
Temp + Prec	-259	523	3	0.0	0.45
Larvae/*N. nasua*					
Temp + Age	-212	436	6	0.0	0.30
Temp	-216	437	3	1.3	0.15
Temp + Size + Age	-212	438	7	1.7	0.13
Temp + Sex + Age	-212	438	7	1.8	0.12
Replete adult females/*C. thous*					
Temp + Sex	-131	269	3	0.0	0.22
Temp	-133	269	2	0.2	0.20
Temp + Sex + Age	-131	270	4	1.1	0.13
Temp + Sex + Size	-131	270	4	1.1	0.13
Temp + Size	-132	271	3	1.7	0.09
Temp + Prec	-132	271	3	2.0	0.08
Replete adult females/*N. nasua*					
Intercept only	-72	147	1	0.0	0.21
Temp + Size	-70	147	3	0.0	0.20
Temp	-72	147	2	0.9	0.13
Prec	-72	148	2	1.2	0.12
Temp x Prec	-72	148	2	1.2	0.11
Temp + Sex + Size	-70	149	4	2.0	0.07

Table 16. Variable weight (Var. weight) and relative variable weight (Rel. var. weight) for the best-fitting species-level models describing the abundance of tick species and tick developmental stages on foxes (*C. thous*) and coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Pre-selected variables (see text) are shown in bold.

Species-level models	Var. weight	Rel. var. weight
A. parvum/C. thous		
Temp + Prec	0.82	0.50
Size	0.47	0.28
Sex	0.36	0.22
A. parvum/N. nasua		
Temp	0.82	0.68
Sex	0.22	0.18
Size	0.17	0.14
Larvae/*N. nasua*		
Temp	0.70	0.56
Age	0.54	0.44
Size	0.13	0.10
Sex	0.12	0.10
Replete adult females/*C. thous*		
Temp	0.83	0.48
Sex	0.47	0.27
Size	0.22	0.13
Age	0.13	0.07
Prec	0.08	0.05

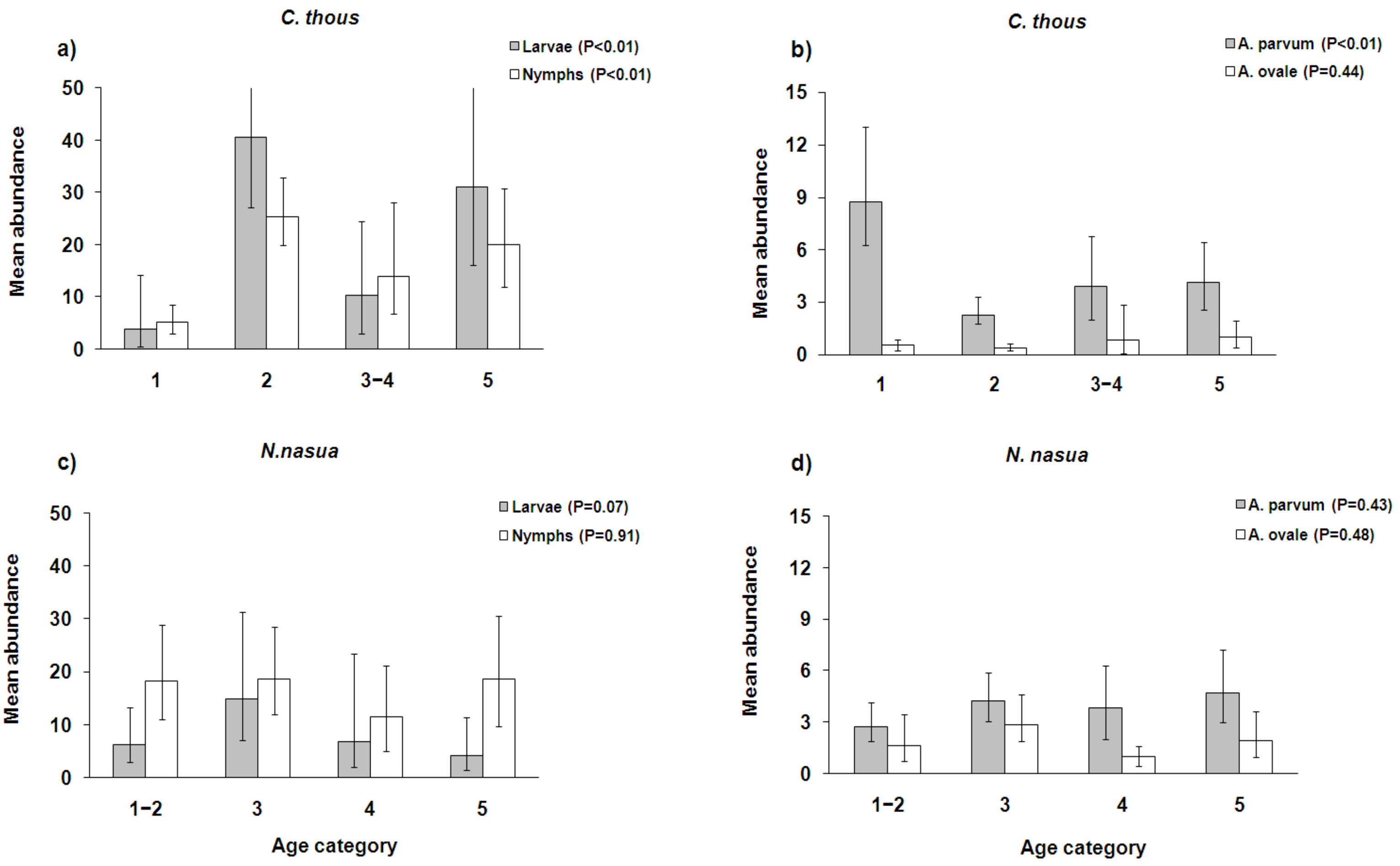

Fig. 9. Mean abundance (± 95% confidence intervals) of immature stages and adults of *Amblyomma parvum* and *A. ovale* on coatis (a, b) and foxes (c, d) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. Age categories are: 1 (≤ 0.5 yr old), 2 (0.51-2 yr old), 3 (2-3 yr old), 4 (3-4 yr old) and 5 (> 4 yr old). P-values for comparisons between age classes (Median test) are shown.

DISCUSSION

Abiotic factors

Overall, abiotic factors were the most important variables underlying tick abundance. Biotic factors also played an important role for some tick species and developmental stages, but these were always in association with abiotic predictors in the best-fitting models. Temperature was the most important variable affecting abundance of tick developmental stages and *Amblyomma* species, as observed in other studies (Guglielmone *et al.*, 1990a; Mangold *et al.*, 1994; Oliveira *et al.*, 2003; Szabo *et al.*, 2007; Nava *et al.*, 2008a). Precipitation was also an important predictor in many of the selected explanatory models (Tables 13 to 16). Although precipitation was correlated with temperature (R=0.67; N=276; P<0.001), these variables nonetheless had an additive effect on tick abundance (Table 13 and 15). Furthermore, precipitation was the single abiotic variable best explaining abundance of *A. ovale* adults on coatis and was more important than temperature in the general model for this tick species (Table 13 and 15). This suggests that the cycle of *A. ovale* in the Pantanal may be more closely related to precipitation than is seen for other tick species. Precipitation did not appear in the single selected model explaining *A. ovale* abundance on foxes, but abundance of *A. ovale* on this host species was not high (Table 11), which may compromise this result to some extent.

The importance of abiotic variables on tick abundance is reflected in the cyclical abundance of larvae, nymphs, as well as *A. parvum* adults (Fig. 8). Seasonal variation in abundance of immature stages and adults of *A. parvum* throughout the year was similar to previous studies conducted in other areas of Brazil and Argentina (Guglielmone *et al.*, 1990b; Mangold *et al.*, 1994; Oliveira *et al.*, 2000; Labruna *et al.*, 2002b; Oliveira *et al.*, 2003; Nava *et al.*, 2008a). *Amblyomma parvum* and *A. cajannense* abundance in South America show peaks of larvae and nymphs during the cold and dry months and high abundance of adults in the warm and rainy season. This allows synchronization of egg incubation to periods of high moisture and vegetation cover, which increases hatch rates of eggs and provides suitable conditions for larva diapause (Labruna *et al.*, 2009). The cycle of *A. ovale*, however, seemingly differs from that observed for *A. parvum*, showing decreased seasonal variation. However, the small sample size did not allow us to more fully investigate this pattern. In addition, the fact that precipitation was the most important variable for abundance of *A. ovale* on coatis suggests that this species may have a distinct seasonal life cycle that our data collection and analysis was unable to more fully discern.

Host species

Despite the high relative importance of abiotic factors on tick abundance, we observed a wide variation in tick abundance in response to biotic predictors. Host species was an important variable affecting tick abundance (except for nymph abundance), indicating that coatis and foxes were not equivalent hosts. Host behaviour, home range size and use, microhabitat preferences, and time spent active all likely affect host-tick encounter rates and, consequently, prevalence and abundance of ticks on their hosts. For instance, grooming may decrease the number of tick on hosts and may have reduced prevalence of replete ticks on coatis (Mooring *et al.*, 1996; Shaw *et al.*, 2003; Gompper,

2004) . We do not know, however, which species of immature *Amblyomma* spp. occur on coatis and foxes, as different tick species and developmental stages may show preference for specific hosts. For instance, coatis and foxes had similar values of adult *A. parvum* prevalence and abundance, whereas *A. ovale* was more abundant and prevalent on coatis than foxes (Table 11). *Amblyomma parvum* and *A. cajannense* are generalists, infesting many host species, whereas *A. ovale* seems to be particularly common among carnivores (Guglielmone *et al.*, 2003; Labruna *et al.*, 2005; Cançado, 2008), and has been found on both *Nasua* species (Kaufmann, 1962; Cançado, 2008; Gompper, 2004). The low abundance of *A. cajannense* on foxes and coatis compared to other host species suggests that this is not a common tick on carnivores from the Pantanal, although immature stages of this species may commonly occur on them (Labruna *et al.*, 2005), and this has been the most abundant species captured by CO_2 traps in the study area (Cançado *et al.*, 2008). Although activity patterns, home range size and host use of specific habitats preferred by ticks can also affect tick abundance on hosts (Boyer *et al.*, 2010), foxes and coatis have similar home ranges and habitat preferences in the Pantanal (Bianchi, 2009) and the most striking difference between these species is their behaviour. Although we do not know whether coatis and foxes differ in their immunological response to tick infestation, differences on abundance and prevalence of ticks species and tick developmental stages on coatis and foxes are likely to be result of the interplay between tick preference and host behaviour.

Host sex and behavior

The relation between host sex and the prevalence and abundance of *Amblyomma* ticks was not straightforward and overall did not conform to the hypothesis that males show higher parasitemias than females due to immune suppression. Prevalence measures of immature stages were the same for both sexes, whereas females showed high prevalence of *A. parvum* for both species (Table 12). Abundance of *A. ovale* and prevalence of replete ticks, however, were higher on coati males than females (Table 12). Although physiological and immune response differences may underlie higher abundances of parasites in males than females (Schuurs & Verheul, 1990; Schalk & Forbes, 1997; Hughes & Randolph, 2001b; Gompper, 2004; Monello & Gompper, 2007; see Chapter 4) and might have contributed to higher abundance of *A. ovale* on coati males than females. However, we would then expect higher values on males than females foxes as well, but these measures were usually equal to or higher on females (Table 12). Differences in tick abundance between coati sexes are therefore most probably related to behavioral differences between sexes.

Prevalence of replete ticks and overall abundance of non-replete ticks were higher on adult solitary males than on coati bands (Fig. 6; Table 12). Allogrooming among individual band members may help to decrease prevalence of replete ticks (Gompper, 2004), since grooming is a common activity among coatis (Kaufmann, 1962; Hirsch, 2007) that can significantly reduce tick burdens (Shaw *et al.*, 2003; Slowik & Lane, 2009). Furthermore, a “dilution effect” may occur when questing ticks encounter coati bands, leading to lower per-capita tick abundance among members of a band. Abundance of replete ticks, however, was not clearly lower on coati band members since it was very low on both species (Table 11; Fig. 6b).

Host age and size

Host age appeared in several top models, but these results are at least partially an artifact. Foxes with ≤ 0.5 yr old had low immature tick abundance because by the time questing nymphs and larvae are abundant (July-September), most of the foxes born in the previous birthing season were > 0.5 yr old (Chapter 2), which also underlies the high abundances of larvae and nymphs on foxes in age category 2 (Fig. 9a). Likewise, the peak of *A. parvum* abundance on young foxes (Fig. 9b) may occur because young foxes start leaving the dens and travelling with their family when the abundance of questing adult *A. parvum* is high.

For small species, such as rodents, host size may be an important predictor of tick abundance because of the limited surface for tick attachment. However, host size by itself did not explain abundance of ticks on coatis and foxes. The importance of fox size to the abundance on *A. parvum*, for instance, is likely a result of young foxes (age category 1) having high abundances compared to older age classes (age categories 2 to 5). Foxes stop growing between 6 months and 1 year old, even though they can keep gaining weight until 1.5 yr old (Chapter 2). As a consequence, the largest differences in fox size occur between individuals in age category 1 (≤ 0.5 yr old) and the remaining age categories, when the abundance of *A. parvum* on foxes is constantly lower (Fig 10b).

Aggregation and sex ratios

Aggregation is expected for component populations of most parasites (Shaw & Dobson, 1995; Poulin, 2007). All tick species and tick developmental stages showed aggregated distributions (Table 11). Adults of *A. parvum* showed the lowest degrees of aggregation, which agrees with the low host specificity of this species. Although *A. cajannense* showed high D indices, its prevalence and abundance on coatis and foxes was very low (Table 11), and the index D is negatively correlated to prevalence and abundance (Poulin, 1993).

Intensity of *A. ovale* and *A. parvum* were male-biased, a result documented for other *Amblyomma* species (Oliveira *et al.*, 2003; Cançado, 2008). In some *Amblyomma* species, adult males attach to hosts prior to females, and the rate of female fixation may be influenced by the presence of feeding males (Gladney, 1971; Rechav *et al.*, 1997). Moreover, males can remain attached for longer periods than females (Labruna *et al.*, 2002a; Pinter *et al.*, 2002), which would increase the probability of finding males in samples collected from hosts. However, sex ratios are male-biased even in questing adult ticks attracted by CO_2 traps (Cançado *et al.*, 2008).

CONCLUSIONS

The life cycle of *Amblyomma* ticks infesting coatis and foxes in the Pantanal is closely related to temperature and precipitation. While feeding on hosts, however, additional host features are important predictors of tick abundance. The role of extrinsic biotic factors on tick abundance and prevalence, however, is not straightforward and varies by host species, tick species and tick developmental stage (Monello & Gompper, 2007; Brunner & Ostfeld, 2008). The exact causes of some of these most conservative of patterns in parasite ecology, including parasite aggregation, remain unclear for many host-tick systems. Host species and host behavior, however, had important effects on tick prevalences and abundances and were comparatively more important than other extrinsic biotic predictors such as host age, sex and size. Even though the effects of these parameters on tick component population parameters have not been exhaustively investigated, there is good evidence that additional biotic factors, such as host home range size and use, activity pattern and habitat use also deserve attention (Boyer *et al.*, 2010). Further investigation should therefore focus on more fully describing the life cycle of poorly known species of ticks, such as *A. ovale,* and on the development of long-term, community-level studies of host-tick systems that consider additional biotic and abiotic predictors for tick abundance and prevalence.

Chapter 4

EFFECTS OF *TRYPANOSOMA* SPP. AND OTHER PARASITES ON THE HEALTH OF FREE-RANGING COATIS

ABSTRACT

Predicting the relative effects of parasites on host health is critical for understanding whether parasites influence individual hosts and potentially limit host populations. Hematological parameters and measures of host body condition have long been used in the medical and veterinary sciences for assessments of the health of individuals, but have rarely been used in an ecological context to understand the effects of parasitic species on the health of free-ranging wild species. We examined the relative importance of hemoparasites and gastro-intestinal parasites on the health of free-ranging coatis from the Brazilian Pantanal. We used coati body condition and hematological parameters as response variables in linear models that were compared using an information theoretic approach. Predictors were high parasitemias by *Trypanosoma cruzi*, *T. evansi* (Kinetoplastida: Trypanosomatidae) and abundance of microfilariae (hemoparasites), as well as presence and abundance of cestodes, nematodes, acanthocephalans and coccidians (gastro-intestinal parasites). In addition, we analyzed how host health changed with host sex, seasonality (reproductive vs. non-reproductive season). Based on both model rankings and focused examination of the effects of particular parasitic species, we observed clear associations between high *Trypanosoma*

spp. high parasitemias and changes in hematological parameters and body condition of coatis. These associations were best predicted by models that also incorporated seasonality and host sex. Body condition decreased in coatis with high parasitemias of *T. evansi*, especially during the reproductive season. Total red blood cell counts and packed cell volume decreased in males with high *T. evansi* parasitemias, but females showed a more variable response. For *T. cruzi*, monocytes decreased in infected individuals, while eosinophils decreased mainly in males with high parasitemias. Total white blood cell counts and neutrophils decreased in males with high parasitemias for both *Trypanosoma* species during the reproductive season, whereas females again showed variable responses to infection. Females therefore apparently handle acute infection better than males. In addition, an overall decrease in health occurred during the breeding season, when coatis are under reproductive stress and may be more prone to infection. High abundances of microfilariae in the bloodstream, and cestode eggs and coccidian oocysts in feces were associated with decreased coati health, but its effect size was comparatively small. Hematological parameters along with body condition were good predictors of direct measures of the health of coatis. While few studies have used hematological parameters to investigate the effects of parasites on wild host health, this study shows the potential of these measures as an approach to better understand the ecological relevance of parasite-host interactions.

INTRODUCTION

Parasites may influence several aspects of the life history of their hosts, including fecundity and survival (Ebert *et al.*, 2000; Meagher & Dudek, 2002; Vandegrift *et al.*, 2008). In most cases, these parameters are negatively associated with the presence or intensity of parasites (Meagher & Dudek, 2002; Kreuder *et al.*, 2003). As secondary effects, host abundance may decline, placing the host population at risk of extinction (Tompkins & Begon, 1999; Muths *et al.*, 2003; Bradshaw & Brook, 2005). These consequences are primarily mediated by the effects of the parasites on host health. As the host mounts an immune response to infection while maintaining homeostasis, energetic resources that might otherwise be directed towards reproduction or relied on to enhance survival must instead be directed to fight the parasites and their consequences. Thus, evaluating host health is critical for understanding how parasites influence hosts at the individual and population levels. Despite this, studies on the impact of parasites on the health of wild species are usually limited to correlations between body condition and parasite presence or intensity (Rodriguez & Carbonell, 1998; Newey *et al.*, 2004; Costa & Macedo, 2005; Lello *et al.*, 2005; Vervaeke *et al.*, 2005; Hakkarainen *et al.*, 2007; Vicente *et al.*, 2007).

A parasitic infection, however, may not necessarily have a negative impact on host health if it does not elicit a strong immune response or if it precludes or diminishes other, more severe infections. In fact, concomitant infections by multiple parasites are the rule in wild populations (Poulin & Morand, 2004), and several parasites have an antagonistic effect on one another (Cox, 2001). Despite this, the role of simultaneous infection by multiple parasites on host health is usually overlooked (Bordes & Morand, 2009). Furthermore, traditional diagnostic measures of immune response (*i.e.* IgG antibodies detection) may mean little for assessments of host health if exposure and associated responses occurred in the past.

On the other hand, fluctuations in hematological parameters are good predictors of fitness (Beldomenico *et al.*, 2008a) and have been used as indicators of health in medicine and veterinary for decades (Jain, 1993; Tizard, 2004). Hematological parameters fluctuate in response to parasitological and immunological stressors. Peripheral neutrophils are indicators of acute inflammatory response, as their numbers increase rapidly due to cytokines released during tissue injury (Jain, 1993; Tizard, 2004). An increase in the number of circulating young neutrophils or band cells (shift to left) also occurs when demand from the bone marrow increases and a large percentage of band cells are released in the bloodstream (Jain, 1993). Lymphocytes are the effectors of acquired immunity and proliferate in response to antigenic stimuli. Their circulating levels therefore may be useful indicators of current immunological investment (Jain, 1993; Beldomenico *et al.,* 2008b). Peripheral monocyte numbers increase due to subacute and chronic inflammatory responses, usually caused by bacterial and protozoan infections (Jain, 1993). The role of eosinophils is not clearcut, but they are involved in the regulation of allergic and inflammatory responses, taking active part in almost all types of inflammatory processes; eosinophilia (increased number of circulating eosinophils) can be found as a response to several parasitic infections (Jain, 1993; Ovington & Behn, 1997). Basophils are usually rare in the bloodstream and the mechanisms leading to changes in their numbers are not completely understood. Their function is related to the sensitivity of membrane receptors to a variety of substances, and a slight increase in their numbers may be seen in association with conditions causing an increase in the numbers of eosinophils (Jain, 1993).

In this study, we examined the relative importance of hemoparasites and gastro-intestinal parasites on the health of free-ranging coatis (Procyonidae: *Nasua nasua*) from the Brazilian Pantanal region as a model to investigate the effect of concomitant infection on the host health. We characterized coati body condition and quantified hematological parameters to investigate the effect of high parasitemias by *Trypanosoma cruzi*, *T. evansi* (Kinetoplastida: Trypanosomatidae) and microfilariae (hemoparasites), as well as presence and abundance of cestodes, nematodes, acanthocephalans and coccidians (gastro-intestinal parasites) on coati health. In addition, we analyze how health condition changes according to host sex and seasonality (reproductive vs. non-reproductive season).

Coatis are among the most common South American carnivores and they harbor several species that infect wildlife, domestic animals, and humans (Ferriolli & Barreto, 1968; Miles *et al.*, 1983; Lainson *et al.*, 1989; Oshiro *et al.*, 1990; Nunes *et al.*, 1993; Gompper & Decker, 1998). Parasites of coatis and their impact on host health have been initially investigated, with some emphasis given to *T. cruzi* and *T. evansi* infection (Silva *et al.*, 1999; Herrera *et al.*, 2001; Herrera *et al.*, 2004; Alves *et al.* 2010). These protozoans are of public health and economic importance since they cause Chagas disease in humans and “Mal de Caderas” disease in horses, respectively. Both species infect a broad range of wild and domestic mammal species, but coatis have been suggested as important reservoirs for both *Trypanosoma* species (Nunes *et al.*, 1993 ; Silva *et al.*, 1995; Herrera *et al.*, 2008; Alves *et al.*, 2010).

While *T. evansi* and *T. cruzi* cause anemia and a decrease in total leukocyte counts in coatis (Silva *et al.*, 1999; Herrera *et al.*, 2002; Alves *et al.*, 2010), gastro-intestinal parasites from coatis are poorly known and their effect on host health has not been investigated. Cestodes and acanthocephalans sequester nutrients before absorption by the host intestinal tract and may decrease host condition when in high intensities. Cestodes may cause diarrhea, whereas high intensities by acanthocephalans may result in peritonitis and death (Kreuder *et al.*, 2003). Coccidian protozoans develop in epithelial cells of the alimentary tract and can cause chronic diarrhea and enteritis in immuno-compromised animals (Bowman, 2003). Hookworms, in turn, ingest blood and can cause anemia and loss of body condition (Kassai, 1999). Some species of filarid nematodes, such as *Dirofilaria immitis*, can cause loss of body condition and anemia, among other serious clinical signs (Sevimli *et al.*, 2007). Moreover, concomitant infection by multiple species of parasites is common and may worsen host health condition (Cox, 2001; Bordes & Morand, 2009).

High parasitemias of hemoparasites and strongylids (*e.g.* hookworms) are expected to alter the hematological profile of coatis since they can cause anemia, whereas microfilariae and the abundance of gastro-intestinal parasites are most probably related to body condition. The microfilaria is a stage in the life cycle of certain parasitic nematodes, such as filarid worms. The adult nematodes may invade tissues such as the lungs, heart and the lymphatic system, affecting host body condition (Sevimli *et al.*, 2007). In addition to the decrease in the number of red blood cells and packed cell volume caused by *T. evansi* (Silva *et al.*, 1999; Herrera *et al.*, 2002), alterations in red blood cell morphology and in the number of circulating metarubricytes (young, nucleated erythrocytes) are also expected (Rue *et al.*, 2000). *Trypanosoma cruzi* can also cause anemia and variable changes in leukocyte numbers (Repka *et al.*, 1985; Pung *et al.*, 1988; Melo & Machado, 2001; Santos *et al.*, 2007).

Host sex and season can also mediate how parasite burdens ultimately influence host health. Seasonal changes in host behavior and contact rates, for instance, influence host-parasite interactions and host immune defenses (Altizer *et al.*, 2006). Additionally, in mammals and some other *taxa*, males are more heavily parasitized than females, due to differences in androgen levels, immune function and physiological stress (Schuurs & Verheul, 1990; Poulin, 1996; Zuk & McKean, 1996; Schalk & Forbes, 1997; Hughes & Randolph, 2001). Because males may harbor more parasites than females, we anticipate a stronger effect of parasites on the heath of male coatis. In addition, males may be under particular strong physiological stress during the mating season and we therefore anticipate a weak immune response against parasites during that season.

The effect of *T. evansi* and *T. cruzi* high parasitemias on coatis packed cell volume and total white blood cell counts was investigated in a preliminary study (Alves *et al.,* 2010). Here, we perform a follow-up examination of how these parasites affect body condition, total number of red blood cells and differential leukocyte counts, while concomitantly accounting for seasonal and sex differences in host response to parasitism.

MATERIALS AND METHODS

Study area

Field work was conducted at Nhumirim Ranch (18º59’S, 56º39’W), a 4400ha research station of The Brazilian Agricultural Research Corporation (Embrapa) located in the Nhecolândia sub-region of the Pantanal, Brazil. The Pantanal is the largest seasonal floodplain in the world and is characterized by sandy soil with a mosaic vegetation of semi-deciduous forest, dispersed shrub vegetation, and seasonally flooded fields (Rodela, 2006). Permanent and temporary ponds and “salinas” (salt water ponds) occur throughout the region. Human population density is low (< 2 people per km^2) and the main economic activity is cattle ranching (Adamoli, 1987). In the Nhumirim Ranch, the breeding season of coatis starts by early August and extends to late November (Chapter 2).

Capture procedures

From May 2006 to February 2009 we captured coatis up to four times per year, using wire box traps (1m x 0.40m x 0.50m) placed in a trapping grid of 7.2 Km^2 and with occasional traps placed out of the grid. Traps were baited with bacon, set late in the afternoon and checked in the following morning. We anesthetized individuals with an intramuscular injection of Zoletil$^@$50 (Virbac®; tiletamine hydrochloride and zolazepan hydrochloride, 10 mg/Kg) and ear-tagged them with numbered colored tags (Nasco Rototags®). Body size measures, body mass, as well as tooth eruption and wear were recorded and used to age individuals (Chapter 1 and 2). Animals were monitored until recovery from the anesthesia and released at the capture site.

We collected blood samples (5mL) from the cephalic vein and stored it in Vacutainer® tubes with EDTA. In addition, we prepared eight blood smears from captured animals and collected fecal samples from beneath traps or via fecal loop. Handling and sample collection procedures were standardized to minimize biases in the hematological profiles caused by an alarm response due to capture stress (Beldomenico *et al.*, 2008a).

Capture procedures were approved by the Brazilian Government Institute for Wildlife and Natural Resources Care (IBAMA, first license #183/2005 – CGFAU/LIC; last license #11772-2) and University of Missouri Animal Care and Use Committee (protocol #4459). Appropriate biosafety techniques and individual protection equipment were used during animal handling and sample manipulation.

Hematology

Packed cell volume (PCV), total red blood cell (RBC) counts and total white blood cell (WBC) counts were quantified within 8 hours of blood collection. A subsample of whole blood was diluted 1:200 in distilled water, placed in a Neubauer chamber where RBCs were immediately counted. Total WBC counts were done in the Neubauer chamber after diluting whole blood at 1:20 in a 4% acid acetic solution for 10 minutes. RBC and WBC counts were performed according to Voigt (2000).

Blood smears were air-dryed and fixed with methanol (Merk®). At the Laboratory of Trypanosomatid Biology/FIOCRUZ, slides were stained with Giemsa’s azure eosin methilene blue solution (Merk®) for differential leukocyte counts and observation of blood cell morphology. We diluted 10mL Giemsa’s azure eosin methylene blue solution with 190mL buffer solution (pH 6.8-7.0) and stained the smears for 15−20 minutes. We counted and classified 100 leukocytes across the entire cell monolayer (crossing technique; Voigt, 2000). For animals showing leukocytosis (>25,000 WBC/cu mm), we counted 200 leukocytes to improve accuracy of estimates. In addition, we characterized RBC morphology (cell shape and color) and recorded the number of metarubricytes found during leukocyte counts.

Parasitology

We used the microhematocrit centrifuge technique (MHCT; Woo, 1970) and hemoculture with blood subsamples from the captured animals to assess high parasitemia infections by trypanosomatids (Alves *et al.*, 2010) and microfilariae. We estimated the average number of microfilariae in 10 randomly chosen 100x microscope fields during MHCT, which was also used to search for trypomastigote forms. Hemoculture was used to assay for epimastigote forms of *T. cruzi* and for isolating them for subsequent molecular characterization, as described elsewhere (Herrera *et al.*, 2008; Alves *et al.*, 2010). *Trypanosoma evansi* was directly identified in the stained blood smears. Positive hemoculture and/or MHCT indicate high parasitemias.

We searched for the presence of eggs and oocysts in coati feces by mixing a small quantity of fresh feces with physiologic solution and investigating it using a microscope (400x). In addition, a subsample of 1−3 grams of feces was weighed to the nearest 0.01g, suspended in 20mL of 10% formalin and filtered through a gauze mesh. The diluted sediment was then divided in two 15mL tubes: one was kept as a backup sample while the contents of the second tube were analyzed by sugar solution flotation (sugar density

1.27) and sedimentation techniques (Foreyt, 2001). After sedimentation, the pellet was suspended with 1mL of 10% formalin and a subsample of 80μL from this solution was placed on a slide for analysis (Monteiro *et al.*, 2007). Slides from the sugar flotation and sedimentation techniques were analyzed at 100x and all egg and oocyst types were photographed under 400x. Eggs and oocyst were measured (maximum length and width) and classified into morphotypes; whenever possible, they were identified at the family or genus level. Identification at the species level was not possible due to lack of information on gastro-intestinal parasites of coatis.

Beginning in August 2007, we quantified eggs and oocysts found in fecal samples. The total number of each egg and oocyst found with the sedimentation technique was extrapolated to the total weight of feces present in the 15mL tube (usually 1.5 grams). To this total, we added the eggs found during the sugar flotation done with the same material.

Statistical analyses

Variation in coati body condition was measured as the standardized residuals from a linear regression between body mass (g) and head-body length (mm) of individuals. The residuals were calculated for males and females separately because coatis are dimorphic in size (Chapter 2). Only coatis > 2 yr old and non-pregnant, non-lactating individuals were considered in analyses. To reduce the number of variables and to avoid redundancy in subsequent analyses, we ran a principal component analysis (PCA) with Log_{10}RBC and PCV values. We then used the scores of the first PCA factor axis on subsequent analyses. Total WBC and leukocyte differential numbers were log-transformed for normalization prior to analyses, except for band cells, which showed a negative binomial distribution and were analyzed accordingly.

Red blood cells in the individual blood smears were characterized as altered or normal. They were qualified as altered when polycromasia and poikilocytosis were predominant or when more than 3 metarubricytes/100 leukocytes were found in blood smears. The RBC morphology was then compared using one-tailed Fisher's Exact tests between categories of qualitative variables (host sex, season and *T. cruzi* and *T. evansi* high parasitemias).

We considered four groups of gastro-intestinal parasites in the *taxa*-specific analyses: Eimeriidae spp (coccidians), Oligacanthorhynchidae sp. (acanthocephalans), Strongylida spp. (hookworms) and Cestoda spp. (see Fig. 10). Other groups had very low prevalences and were not analyzed.

We used an information theoretic approach to contrast the relative performance of models that predict measures of host health. We created general and generalized linear models accounting for seasonal dynamics (reproductive vs. non-reproductive season), host sex, and parasite abundance to investigate the relative importance of these factors for body condition and hematological parameters of individual coatis. The standardized residuals of body size (body condition), the PCA factor representing PCV and RBC, total WBCs and each leukocyte type count were the response variables in the models. Basophils were not analyzed because they were rarely encountered. The number of band cells was modeled using generalized linear models with a negative binomial distribution and log link. Data from recaptured individuals were eliminated from data modeling to avoid pseudo-replication, but were included in average and 95% confidence interval estimation. For recaptured animals showing low and high *T. cruzi* parasitemias in different field excursions (N=7), we compared hematological variables between periods of low and high parasitemias using paired t-test. The same procedure could not be performed for *T. evansi* because only three individuals showed conversion of *T. evansi* parasitemias.

Because combining all independent variables in a single set of models would result in an overly complex analysis with many models and a reduced sample size (not all information was available for every individual) we ran two separate sets of analyses that are described below.

Hemoparasite models

Sample size for hemoparasites were larger than for gastro-intestinal parasites, so we first contrasted a set of models with hemoparasites (*T. cruzi*, *T evansi* and microfilariae) and extrinsic variables (host sex and seasonality). In this set of models, high parasitemias by *T. cruzi* and *T. evansi*, host sex, and seasonality were qualitative descriptors and average microfilariae/field was a quantitative variable. We first created hemoparasite and extrinsic global sub-models (Table 17, models 2 and 9) that were compared to the global model containing all variables (Table 17, model 1). The contribution of variables in the most important sub-model (the one showing lower Akaike criteria) was then further analyzed through the generation of additional nested models (Table 17; models 3−8 and 10−11). Finally, the important variables (those that solely or in combination improved the model fit when compared to the better performing global sub-model) were pre-selected and subsequently included in all additional models, which contained all possible combination of variables from the less important sub-model (Table 17; model 12). We included host sex in all models for which WBCs, lymphocyte counts and the PCA factor were the response variables because these are known to vary with sex (unpublished data), as occur for many other species (Jain, 1993). Interaction terms between high parasitemias by *T. cruzi*, *T. evansi* and sex were included *a posteriori* in additional models whenever investigation of predictor vs. response variable plots revealed possible interactions between these variables (Table 17; model 13).

Candidate models represented independent hypotheses, and model fit was compared using Akaike Information Criterion corrected for small sample size (AICc) and for overdispersion when needed (QAICc). Models were ranked based on the difference between the best approximating model (model with the lowest AICc or QAICc) and all others in the set of candidate models (ΔAICc, ΔQAICc). Models with differences within two units of the top model have strong empirical support and were considered competitive models (Burnham & Anderson, 2002). When there was no clear best-fitting model, the relative importance of each predictor was quantified by adding the Akaike weights across all the models in the set of competitive models that included that predictor (variable weight). When the set of competitive, best-fitting models included the intercept-only model, we did not proceed with model analysis and interpretation.

Table 17. *A priori* models representing hematological profile (HP) and body condition (BC) of coatis in the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009.

	Hypothesis	**Model***
Variation on HP and BC is related to hemoparasites and extrinsic factors:		
1	Global model	*T. cruzi* + *T. evansi* + Mf + Season + Sex
Hemoparasite model - variation on HP and BC is related to:		
2	A combination of all hemoparasites (hemoparasite sub-model)	*T. cruzi* + *T. evansi* + Mf
3	High parasitemias by *T. cruzi*	*T. cruzi*
4	High parasitemias by *T. evansi*	*T. evansi*
5	High abundance of microfilaria	Mf
6-8	Combination of two or more hemoparasites	*T. cruzi* + *T. evansi*; *T. cruzi* + Mf; *T. evansi* + Mf
Extrinsic variables - variation on HP and BC is related to:		
9	A combination of extrinsic variables (extrinsic sub-model)	Season + Sex
10	Seasonality	Season
11	Host sex	Sex
Additional candidate models:		
12	Ex: when the extrinsic model was the best sub-model and Season was the most important (pre-selected) variable in it	Season + *T. cruzi* + *T. evansi* + Mf Season + *T. cruzi* + *T. evansi* Season + *T. evansi* + Mf Season + *T. evansi* Season + Mf Season + Mf
Additional models with interaction terms:		
13	Ex: when TE, TC, Sex and Season were present in the final best-fitting models	*T. evansi* x Sex + *T. cruzi* + Season *T. cruzi* x Sex + *T. evansi* + Season *T. evansi* x Season + *T. cruzi* x Season *T. evansi* x *T. cruzi* x Season

* Structure was similar for all models; *e.g.*, Season + *T. cruzi* + *T. evansi* was β_0 (intercept) + β_1 (Season) + β_2 (*T. cruzi*) + β_3 (*T. evansi*);

Gastro-intestinal parasite models

Predictors for these set of models were the total number of eggs or oocysts for each parasite group found divided by fecal sample weight. Since sample size was small, we did not run models with all possible combination of gastro-intestinal parasites and hemoparasites. Instead, we added each gastro-intestinal parasite predictor to the best-fitting hemoparasite model (see above) and observed whether the model fit was improved. In this way, we accessed the relative importance of hemoparasites, gastro-intestinal parasites and extrinsic variables on coati health.

RESULTS

One hundred and two blood samples from 70 adult coatis (22 females and 48 males) were analyzed for the presence of *Trypanosoma* spp., microfilariae and hematological parameters. Forty-four percent of the coatis showed high parasitemias of *T. cruzi* and 29% of *T. evansi* (considering only one capture per individual). Three individuals (2F and 1M) showed concomitant high parasitemias by both *T. cruzi* and *T. evansi*. Microfilariae were found in 93% of the blood samples analyzed, with abundance varying from 0 to 17.9 microfilariae/microscope field. We analyzed 31 samples from 4 females and 27 coati males for abundance of gastro-intestinal parasite eggs or oocysts. Coccidians were the most prevalent and abundant gastro-intestinal parasites in coatis, followed by cestodes, acanthocephalans and strongylids (Fig. 10); other parasites showed prevalences of less than 10% (not shown).

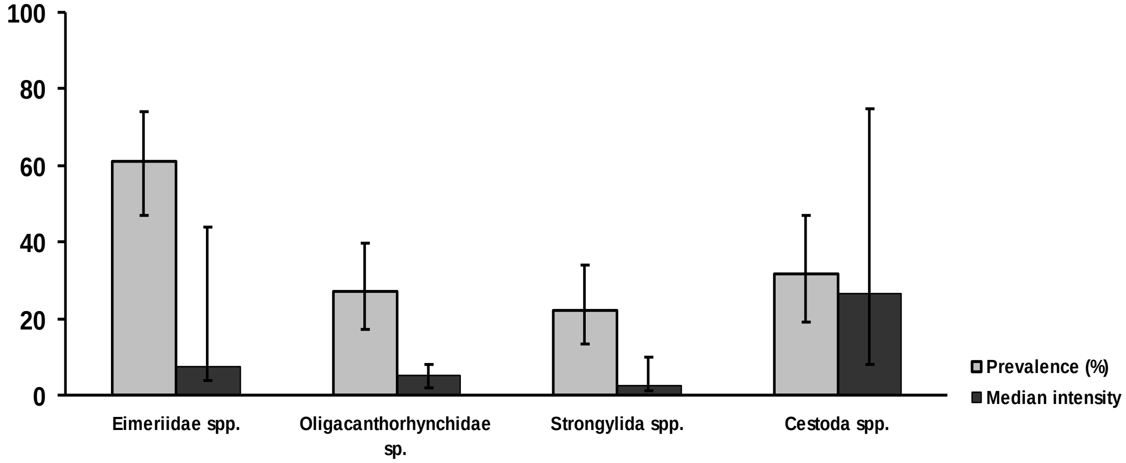

Fig. 10. Prevalence and median intensity of oocyst or ova of gastro-intestinal parasites in coatis from the Nhumirim Ranch, Pantanal/Brazil. Bars represent 95% Sterne's confidence intervals.

Body condition was best predicted by season, high parasitemias by *T. evansi*, and abundance of microfilaria (Table 18 and 19). Body mass decreased during the reproductive season (β = -0.57) and with high parasitemias by *T. evansi* (β = -0.63) and microfilaria (β = -0.06; Fig. 11). An average decrease of 350 grams was observed in coatis with high parasitemias by *T. evansi*. This represents a 6 to 10% decrease in the total body mass of males and females, respectively. The average weight loss approached 800 grams for animals with high parasitemias during the reproductive season (15 to 23% loss of body mass on males and females, respectively).

Total number of RBCs and PCV values was best predicted by *T. evansi* high parasitemias (β = -1.19) and microfilariae abundance (β = -0.06), whereas host sex was *a priori* included in all models (β_{females} = -0.11; Table 18 and 19). RBC and PCV values decreased in males with high parasitemias by *T. evansi*, while females showed variable response (large confidence limits; Fig. 12). Even though *T. cruzi* high parasitemias seems to contribute to RBC and PCV variation (Table 18 and 19), it did not influence RBC and

PCV values by itself (Fig. 12); instead, its contribution is represented by the double-infected male (TC+TE+), which showed an even stronger decrease in PCV and RBC. The two double infected females, in turn, did not seem to respond differently from those with high parasitemias by *T. evansi* only (TC-TE+; Fig. 12).

Table 18. Ranking of general best-fitting models describing body condition and hematological parameters on coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. k = number of parameters in the models. Pre-selected variables (see text) are shown in bold. See Table 17 for abbreviations.

Model	Log(l)	AICc/ QAICc	k	ΔAICc/ ΔQAICc	AICc/QAICc Weight
Body condition					
Season + Mf + TE	-93.3	197.5	5	0.00	0.25
Season + Mf + TE + TC	-92.8	199.0	6	1.42	0.12
Season + TE	-95.2	199.1	4	1.56	0.12
RBC & PCV					
Sex + Mf + TE	-95.2	201.3	5	0.00	0.24
Sex + TC + TE + Mf	-94.3	202.0	6	0.73	0.17
Sex x TE + Mf	-94.6	202.4	6	1.15	0.14
Sex + TE	-97.2	203.0	4	1.68	0.10
Sex + TE + Mf + Season	-94.8	203.0	6	1.72	0.10
Season + Sex + TC + TE + Mf	-93.6	203.1	7	1.80	0.10
WBC					
Sex x TE + TC	-31.4	76.1	6	0.00	0.68
Sex x TE	-33.5	77.8	5	1.70	0.29
Neutrophils					
TC x Season + Sex x **TE** x Season	-38.0	102.4	11	0.00	0.70
Band cells					
Season + Sex x TE + Mf	-468.9	415.3	7	0.00	0.34
Season + Sex x TE + Mf + TC	-467.1	416.3	8	1.05	0.20
Eosinophils					
TE + Season	-85.2	178.9	4	0.00	0.39
Sex x TE + Season	-83.3	179.9	6	0.95	0.25
TE + Season + Sex	-84.6	180.1	5	1.20	0.22
Monocytes					
Intercept	-73.1	150.3	2	0.00	0.32
Season	-72.8	152.0	3	1.63	0.14
Lynphocytes					
Intecept	-76.4	157.1	2	0.00	0.46
Sex	-76.4	159.2	3	2.15	0.16

Table 19. Variable weight (Var. weight) and relative variable weight (Rel. var. weight) for the best-fitting models describing body condition and hematological parameters on coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. k = number of parameters in the models. Pre-selected variables (see text) are shown in bold. RBC & PCV = PCA factor scores for red blood cell count (cu mm) and packed cell volume (%). See Table 17 for abbreviations.

Variables	**Var. weight**	**Rel. var. weight**
Body condition		
Season	0.49	0.33
TE	0.49	0.33
Microf	0.38	0.25
TC	0.12	0.08
RBC & PCV		
Sex + TE + Mf	0.85	0.65
TC	0.26	0.20
Season	0.20	0.15
WBC		
Sex	0.97	0.37
TE	0.97	0.37
TC	0.68	0.26
Band cells		
Season	0.53	0.23
Microf	0.53	0.23
Sex	0.53	0.23
TE	0.53	0.23
TC	0.20	0.09
Eosinophils		
TE	0.86	0.39
Season	0.86	0.39
Sex	0.46	0.21

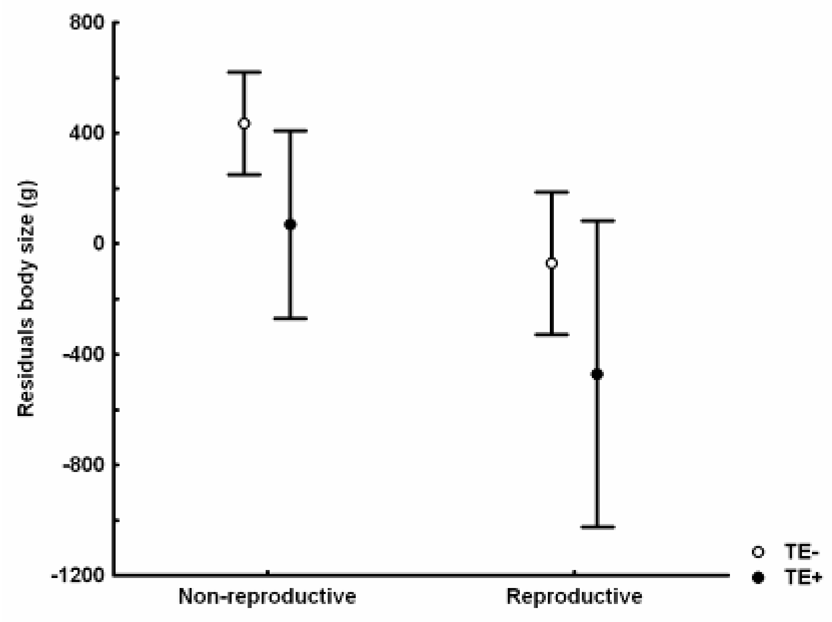

Fig. 11. Body condition of coatis, measured as the residuals of a linear regression between body mass (g) and head-body length (mm), as a function of the presence (TE+) or absence (TE-) of high parasitemias by *T. evansi*, during the reproductive and non-reproductive season in the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

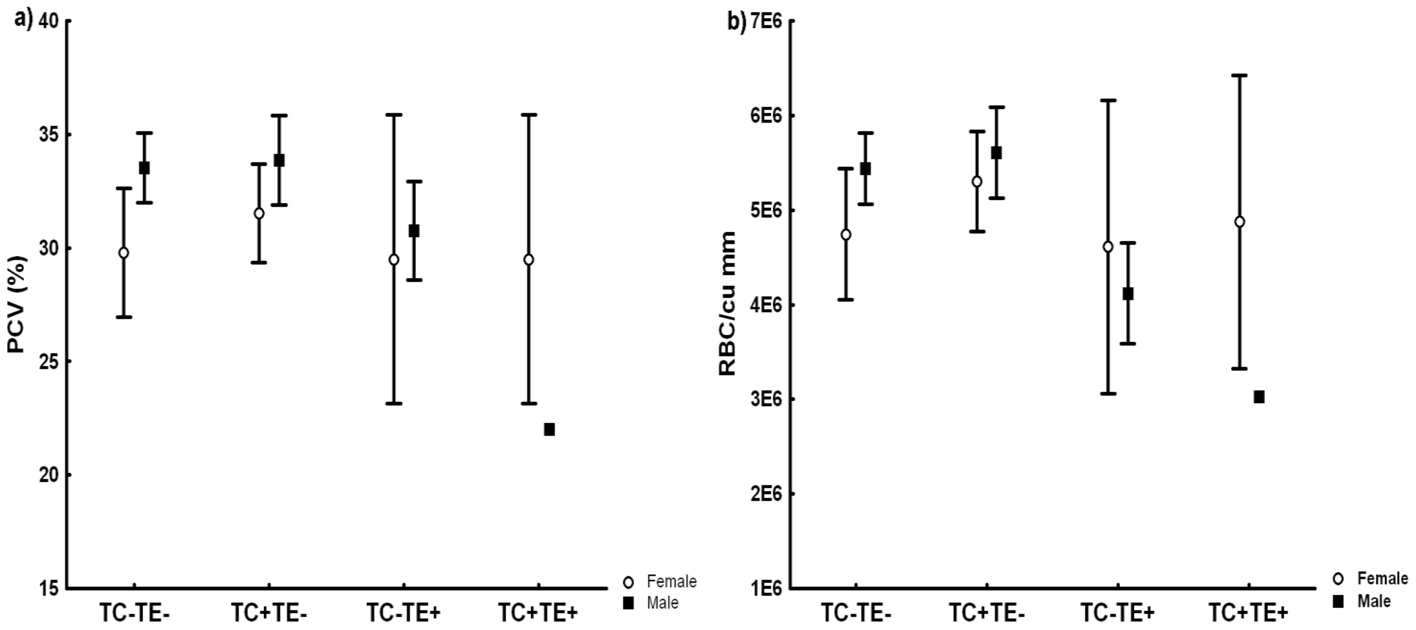

Fig. 12. a) Packed cell volume (PCV) and b) total red blood cells count (RBC) as a function of the presence (+) or absence (-) of high parasitemias by *T. evansi* (TE) and *T. cruzi* (TC) in coati males (filled squares) and females (open circles) from the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

In addition, season had a contribution to this pattern (Table 18 and 19): males with high parasitemias by *T. evansi* showed a decrease in PCV and RBC values during the reproductive season, while in the non-reproductive season such decrease was less evident (Fig. 13). Females with high parasitemias by *T. evansi*, in turn, had variable responses (large confidence intervals) during the non-reproductive season, but a possible decrease in RBC and PCV values during the reproductive season (Fig. 13).

The decrease in the number of RBC and PCV were accompanied by alterations in the shape and color of RBC, with 25% of coatis with high *T. evansi* parasitemias showing altered RBCs (P=0.005). Alternatively, individuals with high parasitemias by *T. cruzi* did not show alterations on RBC (P=0.447). There was no differences between sexes (P=0.539) or seasons (P=0.339) in the morphology of RBCs.

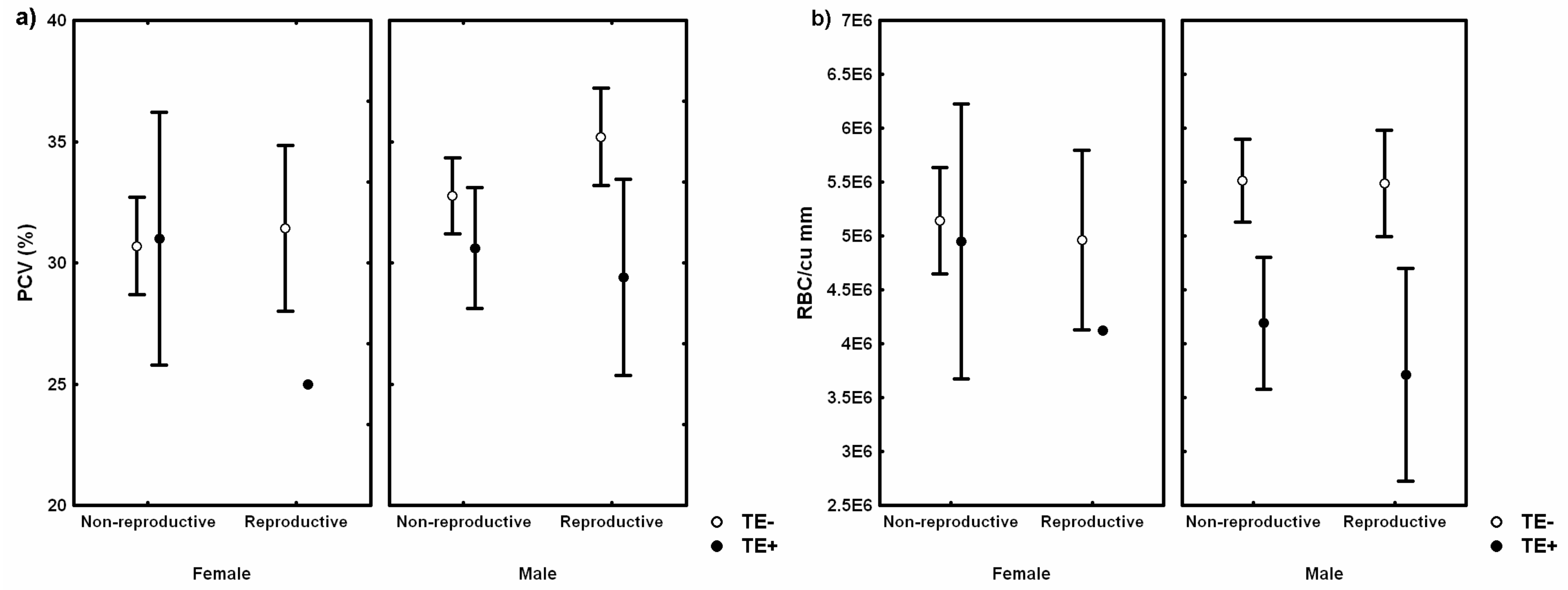

Fig. 13. a) Packed cell volume (PCV) and b) total red blood cell counts (RBC) as a function of the presence (filled circles) or absence (open circles) of high parasitemias by *T. evansi* (TE) and season (reproductive vs. non-reproductive) in coati males and females from the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

Total WBC count were best predicted by both *Trypanosoma* species (Table 18 and 19), with a decrease in WBC was particularly evident among males with high parasitemias by *T. evansi* (Fig. 14a). Females with high parasitemias by *T. evansi*, in turn, showed variable WBC responses (larger confidence limits; Fig. 14a). The influence of *T. cruzi* and *T. evansi* on WBC is reflected on the number of neutrophils, which was also influenced by these two parasites (Table 18). WBC and neutrophils decreased in coatis with high parasitemias by both *Trypanosoma* species (Table 18; Fig. 14). The double-infected male (TC+TE+) showed an even more pronounced reduction on WBC and neutrophil counts, whereas females with high parasitemias by both *Trypanosoma* species did not respond differently from single-high parasitemia ones (TC+TE- or TC-TE+; Fig. 14). The variable “Season” showed an interaction with presence of both *Trypanosoma* species high parasitemias on predicting the number of neutrophils (Table 18). During the reproductive season, the number of neutrophils decreased with high parasitemias by *Trypanosoma* species, while in the non-reproductive season it remained constant (Fig. 15).

The number of band cells was best predicted by season, sex, abundance of microfilariae and high parasitemias by *T. evansi*, while *T. cruzi* high parasitemias had a lower contribution (Table 18 and 19). During the non-reproductive season, the number of band cells remained constant in both coati sexes, despite high parasitemias by *T. evansi* or *T. cruzi* (Fig. 16 and 17). During the reproductive season, males had more band cells than females, but their numbers decreased with high parasitemias by *T. evansi*, whereas females with *T. evansi* and/or *T. cruzi* high parasitemias showed an increase in the number of band cells (Fig. 16 and 17). In addition, band cell numbers decreased with high abundances of microfilariae (β = -0.21).

Circulating eosinophil numbers decreased in males with high parasitemias by *T. evansi* in both seasons, whereas females showed variable response during the non-reproductive season and a possible decrease in the reproductive season (Fig. 18). None of the variables analyzed predicted variation on the total number of monocytes and lymphocytes, for which the intercept-only model was among the best-fitting models (Table 18). However, when comparing the hematological profile of the same individuals showing high and low parasitemias by *T. cruzi*, we detected a decrease in the number of monocytes with *T. cruzi* high parasitemias (paired t-test = -2.41; d.f=6; p=0.053; Fig. 19), while other leukocyte numbers did not change with high parasitemias by *T. cruzi*.

In general, incorporation of data on gastro-intestinal parasites did not result in a more supported model, except for abundance of cestode eggs, which was related to an increase in the number of eosinophils (β=0.004), and abundance of coccidian oocysts (Eimeriidae spp.), which was associated with a decrease in the number of neutrophils (β=-0.002; Table 20).

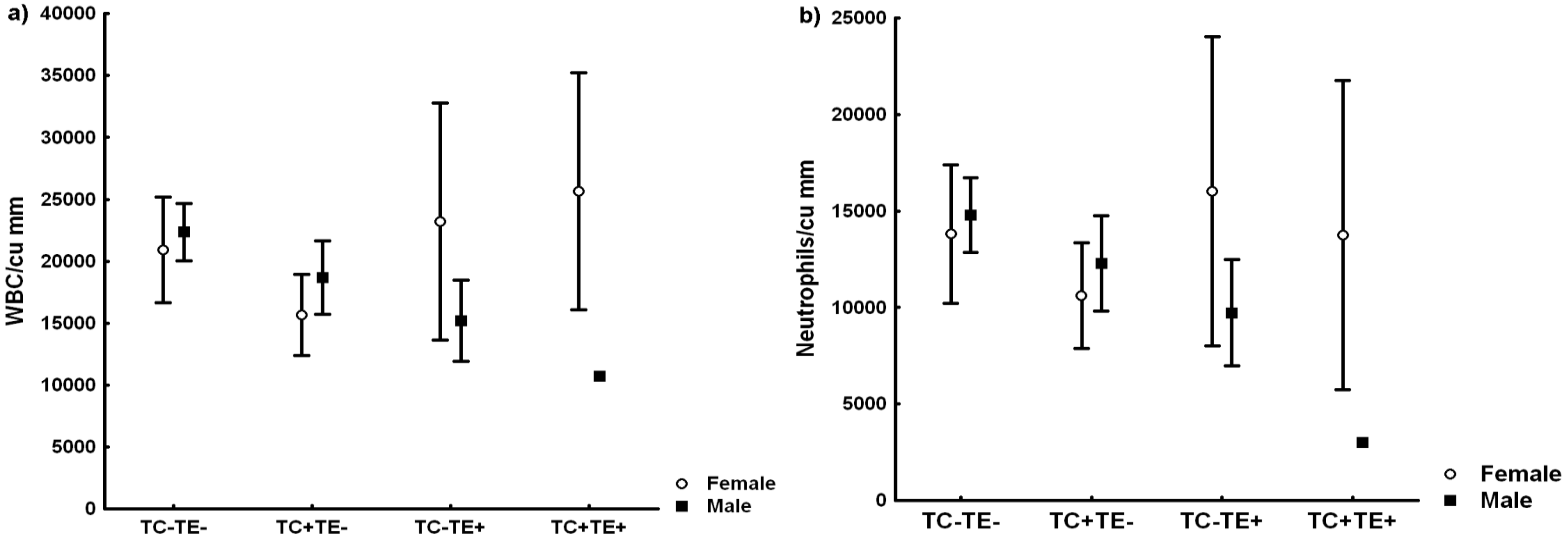

Fig. 14. a) Total white blood cell counts (WBC) and b) number of neutrophils as a function of the presence (+) or absence (-) of high parasitemias by *T. evansi* (TE) and *T. cruzi* (TC) in coati males (filled squares) and females (open circles) from the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

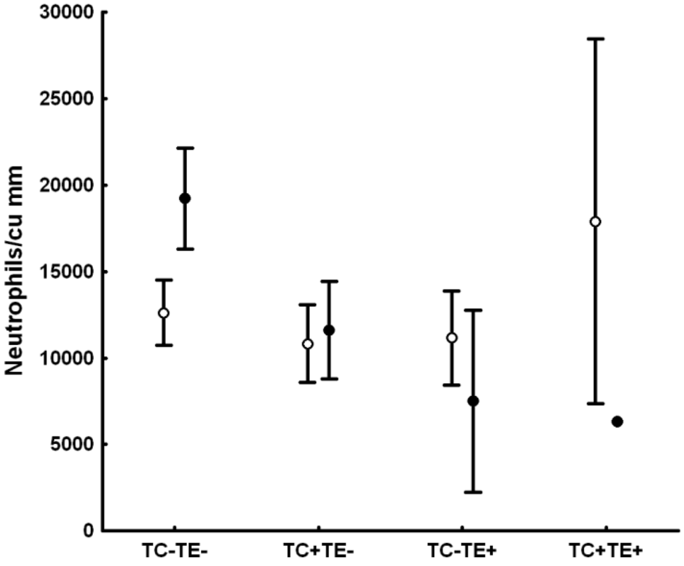

Fig. 15. Number of neutrophils as a function of the presence (+) or absence (-) of high parasitemias by *T. evansi* (TE) and *T. cruzi* (TC) in coatis during the reproductive (filled circles) and non-reproductive (open cicles) seasons in the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

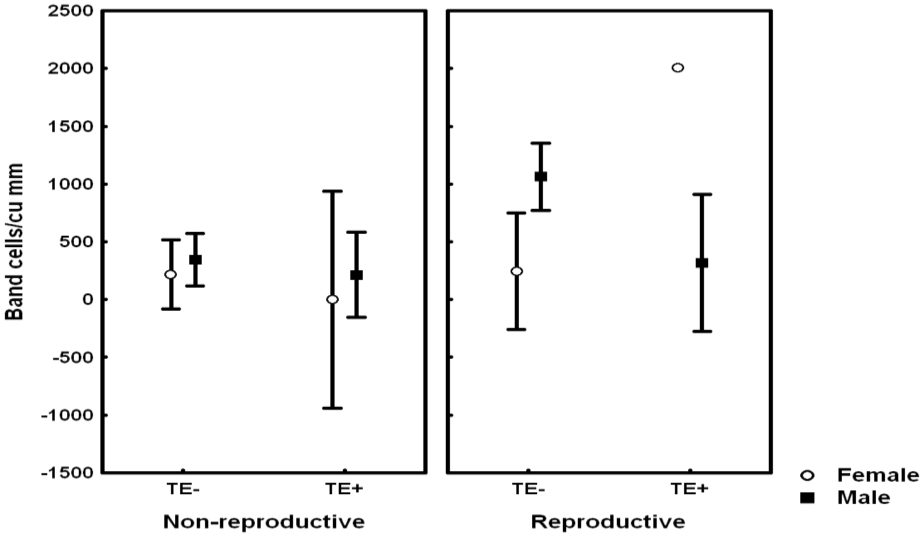

Fig. 16. Number of band cells as a function of the presence (+) or absence (-) of high parasitemias by *T. evansi* (TE) and season (reproductive vs. non-reproductive) in coati males (filled squares) and females (open circles) from the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

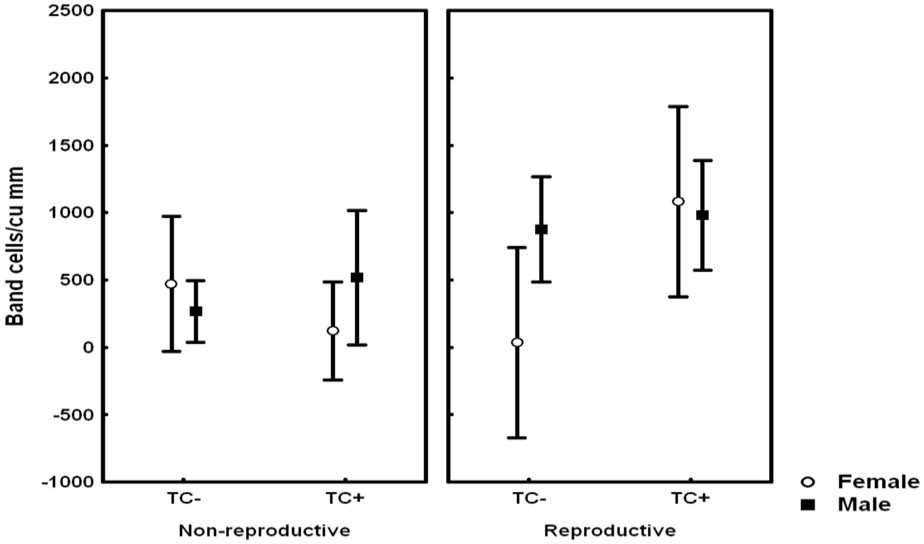

Fig. 17. Number of band cells as a function of the presence (+) or absence (-) of high parasitemias by *T. cruzi* (TC) and season (reproductive vs. non-reproductive) in coati males (filled squares) and females (open circles) from the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

Table 20. Ranking of general best-fitting models including gastro-intestinal parasites and describing body condition and hematological parameters on coatis (*N. nasua*) captured at the Nhumirim Ranch, Pantanal/Brazil from 2006 to 2009. k = number of parameters in the models. See Table 17 for abbreviations.

Model	**Log(l)**	**AICc/ QAICc**	**k**	**ΔAICc/ ΔQAICc**	**AICc/QAICc Weight**
Body condition					
Season + TE + Mf	-39.2	91.0	5	0.00	0.29
Season + TE + Mf + Strongilyda	-38.0	91.7	6	0.73	0.20
Season + TE + Mf + Eimeriidae	-38.1	91.8	6	0.79	0.20
Intercept	-44.1	92.7	2	1.67	0.13
Season + TE + Mf + Cestoda	-38.6	92.9	6	1.90	0.11
RBC & PCV					
TE + Mf + Sex	-39.4	91.5	5	0.00	0.39
Intercept	-44.0	92.6	2	1.05	0.23
WBC					
Intercept	-19.2	42.7	2	0.00	0.61
TE x Sex + TC	-14.9	45.2	6	2.46	0.18
Neutrophils					
Season x TC + Season x TE x Sex + Eimeriidae	-11.8	54.7	10	0.00	0.75
Intercept	-26.5	57.5	2	2.79	0.19
Band cells					
Sex x TE + Season + Mf	-89.2	197.4	7	0.00	0.76
Sex x TE + Season + Mf + Cestoda	-89.1	201.1	8	3.64	0.12
Eosinophils					
Season + TE + Cestoda	-30.7	73.9	5	0.00	0.73
Season + TE	-33.6	77.0	4	3.02	0.16
Monocytes					
Intercept	-25.1	54.7	2	0.00	0.36
Eimeriidae	-24.4	55.8	3	1.08	0.21
Oligacanthorhynchidae	-24.6	56.3	3	1.61	0.16
Strongylida	-24.7	56.5	3	1.78	0.15
Lymphocytes					
Intercept	-30.7	65.8	2	0.00	0.42
Sex + Eimeriidae	-28.4	66.3	4	0.46	0.33

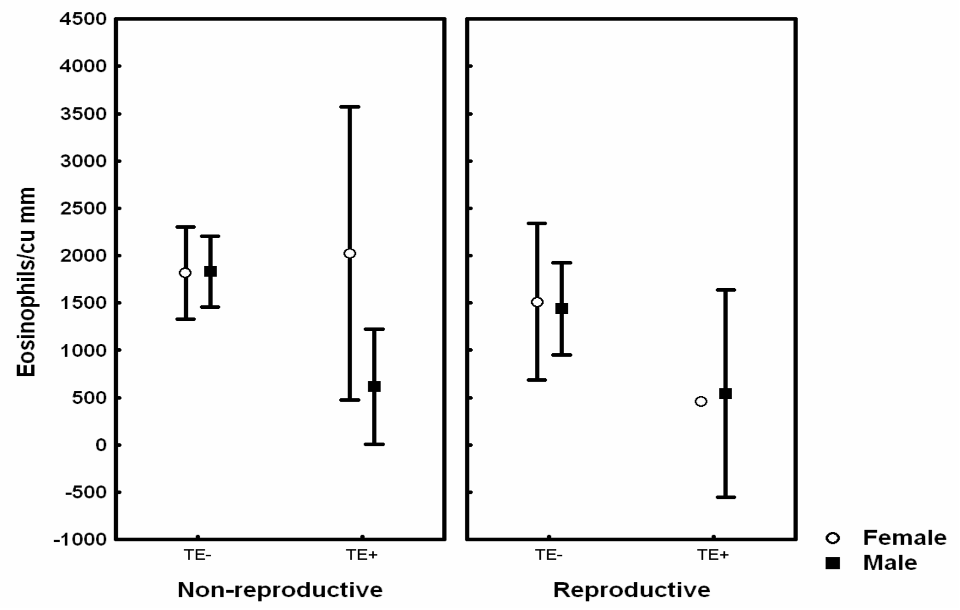

Fig. 18. Number of eosinophils as a function of the presence (TE+) or absence (TE-) of high parasitemias by *T. evansi* in coatis during the reproductive and non-reproductive seasons in the Pantanal. Bars are 95% confidence limits.

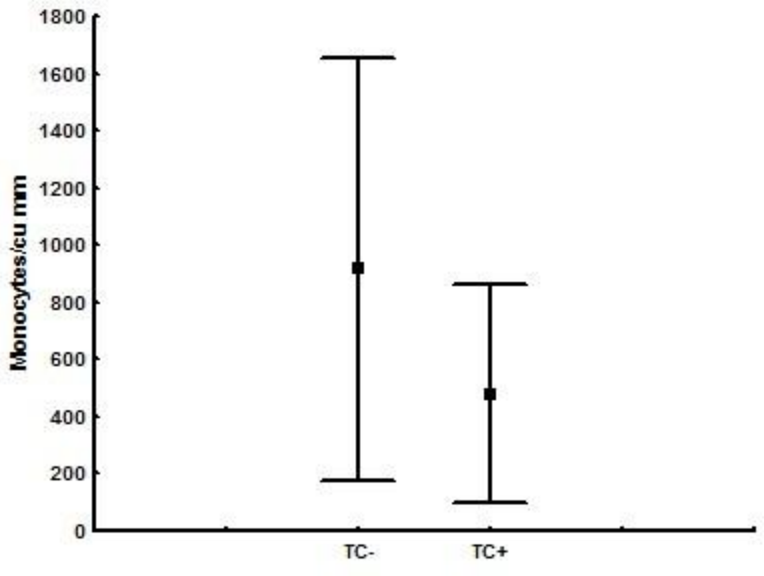

Fig. 19. Number of monocytes as a function of the presence (TC+) or absence (TC-) of high parasitemias by *T. cruzi* in coatis from the Nhumirim Ranch, Pantanal/Brazil. Bars are 95% confidence limits.

DISCUSSION

Changes in hematological parameters may arise for a variety of reasons and must be interpreted with caution (Jain, 1993; Beldomenico *et al.*, 2008a). Nonetheless, we observed clear associations between high *Trypanosoma* parasitemias and changes in these parameters and in the body condition of free-ranging coatis. In addition, microfilaria abundance and perhaps additional parasites seem to have a synergistic negative effect on coati health, especially among males and during the reproductive season.

Trypanosoma evansi

Trypanosoma evansi was associated with a decrease in the number of RBCs and in PCV of males (Table 18; Fig. 12), and these changes were accompanied by alterations in the overall morphology of RBCs and number of metarubricytes. RBC and PCV values of coatis with high *T. evansi* parasitemias were similar to those found in other studies (Silva *et al.*, 1999; Herrera *et al.*, 2002). This parasite causes a macrocytic normochromic anemia in coatis that with time may become normocytic normochromic, *i.e.*, anemia without morphological changes in RBCs (Silva *et al.*, 1999; Herrera *et al.*, 2002). Many species of mammals suffer from chronic anemia caused by *T. evansi* infection (Onah *et al.*, 1996; Arias *et al.*, 1997; Rue *et al.*, 2000; Cadioli *et al.*, 2006) and RBC morphological changes were also reported in infected dogs (Rue *et al.*, 2000). Yet, despite the hematological alterations, RBC and PCV values were not much below those reported for captive uninfected animals (Silva *et al.*, 1999; Fowler & Cubas, 2001), suggesting that even though some free-ranging coatis may develop severe anemia, most of them do not show large decreases in RBC and PCV values.

Changes in total WBCs due to infection by *T. evansi* in coatis have not been examined as closely as have changes in RBC and PCV (Silva *et al.*, 1999; Alves *et al.*, 2010). We observed that the decrease in total WBCs was mainly caused by a reduction in the number of neutrophils (Table 18; Fig. 14) in males with high parasitemias of *T. evansi*. These individuals also showed a reduced number of eosinophils (Table 18; Fig. 18), but this finding is of unclear significance, since the role of eosinophils is not fully understood (Hogan *et al.* 2008). However, a decrease in the number of eosinophils (eosinopenia) may be a consequence of acute infection (Jain, 1993).

In addition, we observed an average decrease of 350 grams in body mass of coatis with high parasitemias of *T. evansi* and such decrease approached 800 grams during the reproductive season. This weight loss reflects a broader energetic challenge that may be biologically meaningful for reproduction and survival of coatis (Newey *et al.*, 2004; Lello *et al.*, 2005; Pioz *et al.*, 2008).

Trypanosoma cruzi

High parasitemias of *T. cruzi* itself was not associated with changes in RBC or PCV values of coatis, except perhaps in the single male also infected with *T. evansi* (TC+TE+; Fig. 12). Total WBCs and neutrophils slightly decreased in coatis with high parasitemias of *T. cruzi* (Table 18; Fig. 14), whereas the decrease in the average number of monocytes was more pronounced (Fig. 19). Infection by *T. cruzi* has variable effects on host hematological parameters of different species (Repka *et al.*, 1985; Pung *et al.*, 1988; Melo & Machado, 2001), but monocytes and lymphocytes are believed to play an important role in halting *T. cruzi* during the early, acute stage of infection (Melo & Machado, 2001; Padilla *et al.*, 2009). Therefore, if the hosts were able to mount an adequate immune response against this parasite, an increase in the number of these circulating cells would be expected. Thus, the decrease in the number of neutrophils and monocytes of animals with high parasitemias of *T. cruzi* may indicate that coatis do not have an adequate immune response to acute infection of *T. cruzi*. However, coatis kept high *T. cruzi* parasitemias for 7.5 months on average (N = 8), and some individuals were infected and re-infected with multiple *T. cruzi* strains (Alves *et al.*, 2010). Therefore, despite the decrease in total WBCs, neutrophils and monocytes, coatis seem to handle the acute phase of *T. cruzi* infection, at least for the temporal span investigated in this season.

Other parasites

Coatis are wild hosts for *Dirofilaria immitis* in Argentina (Vezzani *et al.*, 2006) and *Brugia guyanensis* in French Guiana (Orihel, 1964); in Brazil, they have been reported infected with *D. repens* and *D. incrassata* (Vieira *et al.*, 2008). The impact of most of these filarids in wild species is usually unknown. Infection by *D. immitis* cause moderate normocytic normochromic anemia in dogs by reducing RBC, hemoglobin concentration and PCV values (Harvey *et al.*, 1982; Atwell & Buoro, 1983). Neutrophilia, eosinophilia and monocytosis have also been reported in *D. immitis* infected individuals (Sevimli *et al.*, 2007). Clinical signs for infected canids vary from moderate to severe, including weakness, anorexia, dehydration, weight loss and death (Ishihara *et al.*, 1988; Sacks & Blejwas, 2000). In addition, macaques experimentally infected with *D. repens* showed peaks of eosinophilia (Wong, 1976). We found a slight contribution of abundance of the microfilariae to reduced body condition, RBC and PCV values, as well band cells numbers in coatis. However, the effect size of microfilaria abundance was relatively low compared to the effects of high parasitemias by *T. evansi*, *T. cruzi* and the effect of seasonality and a decrease in the band cell numbers was not expected as a result of infection. Given the high prevalences of microfilaria in adult coatis and the absence of animals with clinical signs of infection, we believe this parasite by itself has a minor effect on coati health, but may have a synergistic effect when concomitant infection by *T. cruzi* and *T. evansi* occurs.

Overall, gastro-intestinal parasites were not associated with hematological parameters or decreased body condition, even though other studies have found an association between the presence or abundance of such parasites and a decrease in host body condition (Rodriguez & Carbonell, 1998; Newey *et al.*, 2004; Vervaeke *et al.*, 2005; Hakkarainen *et al.*, 2007; Vicente *et al.*, 2007; Varela-Stokes *et al.*, 2008). Our sample size, however, was small. Abundance of cestode eggs was weakly associated with increased numbers of eosinophils (Table 20). In fact, eosinophilia may occur as a response to cestode infection; the large size of helminths preclude them to be phagocytized; as a consequence, eosinophils apparently release toxin molecules that combat helminths extracellularly (Ovington & Behn, 1997), even though the exact role of eosinophils in controlling parasitic helminth infection is still unclear (Hogan *et al.*, 2008). Abundance of coccidian oocysts was also slightly associated with decreased number of circulating neutrophils in coatis (Table 20). Coccidian infection can cause diverse alterations in the blood parameters of experimentally infected hosts (Rose & Ogilvie, 1979; Worliczek *et al.* 2010) and their effect on coatis was never been investigated. The association between this parasite and a decrease in the number of neutrophils may actually be a consequence of animals in poor condition (perhaps due to *Trypanosoma* spp. infection) being predisposed to infection (Beldomenico *et al.,* 2008b).

Seasonality and sex differences

Overall, coati body condition decreased during the reproductive season (Fig. 11), and the relation between high parasitemias by hemoparasites and changes in the hematological parameters was stronger during that season, especially on males (Fig 13, 15, 16 and 17). A reduction in body fat during the reproductive season has also been observed for *N. narica* from Tikal National Park, Guatemala and is probably a reflection of reproductive stress, perhaps associated to a reduction on food consumption (Binczik, 2006). During the breeding season, poor nutrition and investment in reproduction weaken the immune system in ways that could affect the ability of hosts to defend against infectious diseases (Altizer *et al.,* 2006).

We observed that females apparently cope better with infection by *T. evansi* and *T. cruzi* than do males. The decline in hematological parameters related to *T. cruzi* and *T. evansi* high parasitemias was usually stronger in males, especially during the reproductive season. In humans, women are more resistant than men to infection by *T. cruzi* (Brabin & Brabin, 1992) and are more efficient at clearing peripheral *T. cruzi* parasitemias (Hoff *et al.,* 1979). Estrogen seems to play an important role in resistance to *T. cruzi* (Prado Jr. *et al.,* 1998; Santos *et al.,* 2007) and many other parasites, whereas testosterone depresses the immune system of males (Schuurs & Verheul, 1990; Zuk &

McKean, 1996). In addition, coatis are polygynous, show sexual dimorphism, and males strongly compete for females during the reproductive season (Chapter 2), which sometimes result in wounds. Given these behavioral and morphological characteristics, hematological differences between coati males and females are expected to be pronounced (Klein, 2000). This would also contribute for an even stronger decrease in immune response during the mating season, when testosterone levels in coati males peak (Binczik, 2006). In the Pantanal, however, female coatis show higher parasitemias by *T. cruzi* than males, even though both sexes are equally exposed to infection (Alves *et al.*, 2010). It is unclear why coati females show higher parasitemias than males but still seem to manage the infection better than males.

The distinct response of males and females to high parasitemias by *T. cruzi* and *T. evansi* has important implications for future studies: host sexes should be analyzed separately for the effect of *Trypanosoma* species. If this is not possible, host sex should be at least reported. In addition, given that coatis have been suggested as important reservoirs of *T. cruzi* and *T. evansi* in the Pantanal, further research should focus on how these parasites affect fecundity and survival of coatis, and indirectly, the population dynamics of coatis. There is also a lack of information on how transmission occurs among coatis and why females show high parasitemias than males. This information is essential to clarify the role of coatis as a reservoir of *T. cruzi* and *T. evansi* the Pantanal region.

CONCLUSIONS

There is an increasing need for investigation of the impact of multiple parasites on host health (Lello *et al.*, 2005; Bordes & Morand, 2009). Hematological parameters, along with body condition, were closely related to the health of coatis and offer an opportunity to better understand how parasites interact with hosts and cause disease. Few studies have used a combination of such hematological parameters to investigate the effect of parasites on wild host health. Interpretation of such parameters, however, is complex. The effect of host sex, season and the synergistic effect of parasites on coati health highlights the need to consider such a broad approach when analyzing the health of wild animals.

GENERAL CONCLUSION

In chapter 1, I presented a non-invasive method to estimate age of coatis and crab-eating foxes. The method can potentially be applied to estimate age of coatis and foxes from other areas and can be used as a framework to generate age estimation methods for other species. In the ensuing chapters, this method was utilized to generate age estimates that were used to describe how coatis and foxes grow (chapter 2), and how parasite burdens change with host age (chapter 3). In chapter 2, I provided information on reproduction, morphology, growth, and survival for coatis and foxes. I showed, for instance, that growth rates and asymptotes were associated with the timing of reproduction in both species, and that morphological differences between the species were associated with their respective social systems. Part of this information was again used in the subsequent chapter, where I described the complexities of the life cycle of three species of ticks on the two hosts, and how each tick species and developmental stage responded to host characteristics and abiotic factors. Even though abiotic factors were more important for predicting tick abundance, biotic variables - mainly host species and behavior - were also important. Chapter 3 illustrates how the response of parasites to extrinsic factors as well as factors intrinsic to the host can be intricate and variable, and stresses the importance of long-term, community-level studies of host-tick systems. It also emphasizes the need for further investigation of poorly known species, such as *Amblyomma ovale*. Given the relatively high abundances of this tick species on coatis and the high densities of coatis in the Pantanal, this carnivore may function as a good model for future studies of *A. ovale*, especially considering the basic ecological information that has accumulated from this and associated studies.

Finally, in chapter 4 I investigated the effect of parasites on the health of coatis by focusing on an array of hematological parameters and body condition. We found that these indices of host health were closely related to parasite abundance and parasitemia in coatis. Overall, the health of coatis decreased with high parasitemias of hemoparasites, particularly *T. cruzi* and *T. evansi*. In addition, males seem to be proportionally more affected by high parasitemias than females, especially during the reproductive season. Gastro-intestinal parasites, in contrast, had relatively little impact on host health, although sample size was small for this specific analysis.

Surprisingly, there are few studies relating hematological parameters with ecological or parasitological information of wild populations, even though these parameters have been routinely used to characterize the health of humans and domestic animals for decades (Newson & Chitty, 1962; Mihok & Schwartz, 1989; Silva *et al.*, 1999; Altizer *et al.*, 2006; Huitu *et al.*, 2007; Beldomenico *et al.*, 2008a; Beldomenico *et al.*, 2008b). This lack of health studies reflects a “natural” gap between different scientific fields, such as ecology, parasitology and epidemiology. For this reason, there is an increasing need for developing interdisciplinary studies, so that these gaps can be filled (Daszak *et al.*, 2004; Daszak *et al.*, 2007). This is particularly true given the emerging and re-emerging diseases which have received increasing attention due to their consequences to public health and wildlife (Binder *et al.*, 1999; Daszak, 2000; Smith *et al.*, 2009). In Brazil, the gap between research in these respective areas is especially great. There is little national incentive for multidisciplinary studies and the few interdisciplinary groups studying the effect of parasites on wild hosts and their populations are isolated, independent initiatives. This dissertation and the broader project from which it is part of is one of the few exceptions. The fact that it was developed with midsized carnivores and in an area still considered relatively unknown to the international scientific community made it even more challenging. Thus, this dissertation, along with the other projects that together shaped the larger project represents our contribution to what we hope will be the rise of conservation medicine in Brazil.

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APPENDICES

APPENDIX 1

Here we describe how to transform data on 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 index = 1; Weight = 2340g; Head−body length = 520mm; tail length = 470mm; shoulder height = 240mm; neck circumference = 165mm; axillary girth = 225; and the ratio between upper and lower canine height = 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 correlation matrix and therefore the raw variables must be standardized first, i.e. each variable must be diminished from its mean and divided by its standard deviation of that variable (Table 21).

Example: standardized weight for Coati 1 = (2340 − 4319)/1585 = −1.249.

Next, the standardized values must be transformed in factor scores for use in the classification functions. To do so, multiply each standardized value by each of its respective factor score coefficient (Table 22) and then sum the values.

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

$[-1.249 \times (-0.180)] + [-0.761 \times (-0.179)] + [-0.111 \times (-0.164)] + \ldots = 0.924$

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

Table 21. Mean and standard deviation of body measurements of brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) and the ratio between upper and lower canine height in coatis (U/L) captured in the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

Variables	*Nasua nasua*		*Cerdocyon thous*	
	Mean	SD	Mean	SD
Weight (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
Thorax perimeter	294	47	354	30
U/L	1.30	0.35		

Table 22. Factor score coefficients of the Principal Component Analyses run for body measurements and for the ratio between upper and lower canine height (U/L) of brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) captured in Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

Variables	*Nasua nasua*		*Cerdocyon thous*		
	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
Thorax perimeter	-0.179	0.074	-0.235	0.242	-0.439
U/L	-0.101	-1.098			

Now the factor scores and the teeth condition index can be used in the classification functions of the discriminant analysis (Table 23). 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):

$$S_i = c_i + w_{i1} \times x_1 + w_{i2} \times x_2 + ... + w_{im} \times x_m$$

where

subscript i = respective group;

subscripts 1, 2, ..., m = m 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;

S_i= the resultant classification score.

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

$S_{\leq 0.5\text{yr}} = -10.143 + (6.584 \times 0.924) + [(-1.847) \times (-0.765)] + 0.066 \times 1 = -2.712$

Remember that the teeth condition index (equal to “1” in this case) was included as a separate variable in the models and therefore is not a factor score and does not need to be standardized. Once the classification scores for a case are computed, classify 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 yr = − 2.712; 0.6 to 2.0 yr = − 0.611; > 2.0 yr = −5.983. According to the classification scores, this animal is

0.6 to 2.0 yr old because this age category has the highest classification score for this animal.

Mahalanobis distances and posterior classification probabilities can also be computed for the cases to be classified. For a complete description of these procedures, see Legendre and Legendre (1998).

Table 23. Classification functions for age determination of brown-nosed coatis (*Nasua nasua*) and crab-eating foxes (*Cerdocyon thous*) captured in the Nhumirim Ranch, Pantanal/Brazil from 2005 to 2009.

Species	**Variables**	**≤ 0.5 yr**	**0.6 to 2.0 yr**		**> 2.0 yr**
N. nasua	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
		≤ 0.5 yr	0.6 to 1.0 yr	1.1 to 2.0 yr	> 2.0 yr
C. thous	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

VITA

Natalie Olifiers was born and raised in Rio de Janeiro, Brazil. Natalie graduated in biology from the Federal University of Rio de Janeiro in 2000 and immediately began her Masters in Ecology, Conservation and Wildlife Management at the Federal University of Minas Gerais (UFMG). During her Masters, she studied the effects of forest fragmentation on small mammal communities. After that, Natalie worked for one year developing a new tracking trap for mammals. Soon after, she joined the Oswaldo Cruz Foundation (FIOCRUZ/RJ) to work as a technical coordinator in an international cooperation (German - Brazil) for studying the effect of forest fragmentation on small mammal communities. In the meantime, she started working as a principal investigator on a project of disease ecology in the Pantanal wetlands. In 2005, she joined the University of Missouri to obtain her PhD in the Department of Fisheries and Wildlife Sciences.