

HECTOR RIBEIRO BENATTI

**Comparison of morphometric patterns and blood biochemistry  
in capybaras (*Hydrochoerus hydrochaeris*) of human-modified  
landscapes and natural landscapes**

São Paulo

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HECTOR RIBEIRO BENATTI

**Comparison of morphometric patterns and blood  
biochemistry in capybaras (*Hydrochoerus hydrochaeris*) of  
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**Advisor:**

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Accordinging:

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Marcelo B. Labruna, Ph.D.

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**Parecer Consubstanciado da Comissão de Ética no Uso de Animais FMVZ (ID 005948)**

A Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, no cumprimento das suas atribuições, analisou e **APROVOU** a Alteração do cadastro (versão de 19/fevereiro/2020) da proposta acima referenciada.

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### Committee Members

Prof. \_\_\_\_\_

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## **DEDICATION**

I dedicate this work to my parents, Cristina and Renato and to my brothers Diogo and Renan, for the 11504 days of support and unconditional love. Thank you for being my references.

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*“Life is short and potential studies infinite. We have a much better chance of accomplishing something significant when we follow our passionate interests and work in areas of deepest personal meaning”*

– Stephen Jay Gould (1941 – 2002)

## RESUMO

BENATTI, H. R. **Comparação de padrões morfométricos e bioquímica do sangue em capivaras (*Hydrochoerus hydrochaeris*) de paisagens modificadas pelo homem e paisagens naturais.** 2020. 90 f. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

A capivara (*Hydrochoerus hydrochaeris*) é o maior roedor do mundo, podendo atingir, até 100 kg, com o peso médio de indivíduos adultos de 50 kg. No sudeste brasileiro a capivara tem papel fundamental na manutenção da Febre Maculosa Brasileira (FMB), uma vez que funciona como hospedeiro amplificador para a bactéria *Rickettsia rickettsii*, agente causador da doença. Dentre as características necessárias para amplificação do agente, alta prolificidade e geração de novos indivíduos suscetíveis à rickettsemia são essenciais no cenário epidemiológico da FMB. Muitas paisagens modificadas pelo homem no sudeste do Brasil vivenciam expansão horizontal e vertical das populações de capivaras nas últimas décadas devido ao grande suprimento de alimentos, graças à expansão das monoculturas, como por exemplo, as canavieiras. Supôs-se, então, que as capivaras nas paisagens modificadas pelo homem (HMLs) estão aumentando suas reservas corporais, levando-as a um quadro de obesidade, quando comparadas às capivaras nas paisagens naturais (NLs) do bioma Pantanal do Brasil. Também se observou que a densidade populacional de capivaras em uma determinada área de ocupação é muito mais alta nas HMLs do que nas NLs, possivelmente por conta de um maior sucesso reprodutivo devido à oferta abundante de alimentos. Conjecturou-se, pois, que a obesidade dos animais em HMLs possa estar gerando distúrbios bioquímicos de natureza nutricional e metabólica. Esses distúrbios, somados a maiores capacidades reprodutivas, podem, portanto, aumentar as taxas de reposição de indivíduos, fazendo a manutenção da FMB no sudeste brasileiro. Objetivando responder a essas hipóteses, durante os anos 2015–2019, foram capturadas capivaras em sete HMLs no sudeste do Brasil, e em duas NLs do bioma Pantanal, todas com populações estabelecidas de capivaras. Foram realizadas coletas de sangue, mensuração de comprimento total, altura e circunferências de pescoço, tórax e abdômen, para comparar os resultados entre as populações, sendo as capivaras de NLs o grupo controle e as de HMLs o grupo experimental. Os resultados mostraram que os animais em HMLs eram mais pesados do que os animais nas NLs, e, apesar de mais pesados, eles não apresentavam medidas lineares maiores, excluindo qualquer tipo de interferência do tamanho nos valores de massa corporal dos indivíduos. Curiosamente, as capivaras de HMLs mostraram circunferências do pescoço e tórax maiores que aquelas registradas para capivaras em NLs. Além disso, o presente trabalho registrou um novo limite superior de peso para a espécie (105,2 kg). Ainda, os resultados encontrados nos exames de sangue demonstraram que, dos

onze fatores bioquímicos analisados, cinco (albumina, creatina quinase, colesterol, frutossamina e proteína total) foram significativamente diferentes entre as populações e dois (cálcio e aspartato aminotransferase) foram limítrofes. A análise de fatores bioquímicos direta ou indiretamente relacionados à obesidade comprova que as populações de capivaras de HMLs estão desenvolvendo, devido ao excesso de gordura, uma série de distúrbios bioquímicos de ordem metabólica e nutricional, que podem levar ao aumento das taxas de mortalidade nessas populações. Soma-se a isso o fato de que roedores expostos a dietas altamente energéticas tendem a exibir maior sucesso reprodutivo.

Palavras-chave: capivara; paisagens modificadas pelo homem; paisagens naturais; padrão morfométrico; perfil bioquímico.

## ABSTRACT

BENATTI, H. R. **Comparison of morphometric patterns and blood biochemistry in capybaras (*Hydrochoerus hydrochaeris*) of human-modified landscapes and natural landscapes.** 2020. 90 f. Thesis (Doctorate in Sciences) – School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, 2020.

The capybara (*Hydrochoerus hydrochaeris*) is the largest rodent in the world, reaching up to 100 kg, with an average adult weight of 50 kg. In southeastern Brazil, capybaras occupy a fundamental role in maintaining Brazilian spotted fever (BSF), since it functions as an amplifying host for the bacterium *Rickettsia rickettsii*, the agent of the disease. Among the characteristics necessary for amplification of the agent, high prolificacy and generation of new individuals susceptible to rickettsemia are essential in the epidemiological scenario of BSF. Many human-modified landscapes (HMLs) in southeastern Brazil have experienced horizontal and vertical expansion of capybaras populations in recent decades due to the large supply of food, thanks to the expansion of crops, as for example, sugarcane. It was conjectured, then, that capybaras in HMLs are increasing their body reserves, leading them to a picture of obesity, when compared to capybaras in Natural Landscapes (NLs) of the Brazilian Pantanal biome. It was also observed that the population density of capybaras in a given occupation area is much higher in HMLs than in NLs, possibly due to greater reproductive success due to the abundant supply of food. It was conjectured, therefore, that the obesity of animals in HMLs may be generating biochemical disorders of nutritional and metabolic nature. These disorders, added to greater reproductive capacities, can therefore increase the replacement rates of individuals, maintaining the BSF in southeastern Brazil. To answer these hypotheses, during the years 2015–2019, capybaras were captured in seven HMLs in southeastern Brazil, and in two NLs of the Pantanal biome, all with established populations of capybaras. Blood collection, measurement of total length, height and circumferences of neck, thorax and abdomen were performed to compare the results between populations, with NLs capybaras being the control group and HMLs being the experimental group. The results showed that animals in HMLs were heavier than animals in NLs, and, despite being heavier, they did not present larger linear measurements, excluding any type of size interference in the individuals' body mass values. Interestingly, HMLs capybaras showed larger neck and chest circumferences than those recorded for NLs capybaras. In addition, the present work registered a new superior weight limit for the species (105.2 kg). The results

found in blood tests showed that, of the eleven biochemical factors analyzed, five (albumin, creatine kinase, cholesterol, fructosamine and total protein) were significantly different between populations and two (calcium and aspartate aminotransferase) were borderline. The analysis of biochemical factors directly or indirectly related to obesity proves that the capybara populations of HMLs are developing, due to excess fat, a series of biochemical disorders of metabolic and nutritional order, which can lead to increased mortality rates in these populations. In addition, rodents exposed to high-energy diets exhibit greater reproductive success.

**Keywords:** capybara; human-modified landscapes; natural landscapes; morphometric pattern; biochemical profile.

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# 1. Background, hypotheses and objectives

## 1.1. INTRODUCTION

### i. Capybara (*Hydrochoerus hydrochaeris*)

Capybara is a mammal native to Latin America and belongs to the order Rodentia, family Caviidae and genus *Hydrochoerus*. In this region, it has a wide distribution ranging from eastern Panama to Uruguay, including Brazil (ALHO et al, 1987). Only two species are known, *Hydrochoerus isthmius* (Colombia, Venezuela, Ecuador and Panama), and *Hydrochoerus hydrochaeris*, the later in all South American countries except for Chile. In Brazil, capybaras are widely distributed all over the country, except for its very scarce presence in the Caatinga biome (JIMÉNEZ, 1995; MOREIRA et al., 2013) (Fig. 1).



Figure 1 – Distribution map of the genus *Hydrochoerus* spp. (Based on JIMÉNEZ, 1995; MOREIRA et al., 2013)

Capybaras are robust, semi-aquatic animals, being considered the largest extant rodent in the world, with adults reaching between 50 and 60 kg and at most 1.35 m in length (MOREIRA et al., 2013).

They are strict herbivores with cecum-colic digestion, dependent on symbiosis with fermenting microorganisms (fungi, bacteria and protozoa); therefore, they have a well developed cecum (MOREIRA et al., 2013) (Fig. 2). Because of this, the capybara digestion process is very efficient, highlighting a high capacity to digest fibrous foods. Its digestive system, as a whole, is quite bulky, reaching an average capacity of 8.8L - 10L (OJASTI, 1973; BARROS MORAES et al., 2002; RODRIGUES et al., 2006). The diet basically consists of grazing grasses, also aquatic plants such as water hyacinths (BARRETO and QUINTANA, 2012). Interestingly, an adult capybara (40 kg) tends to consume about 3 to 5 kg of grass / day, and a young one (20 kg) consumes approximately 2 kg (SILVA, 1986).

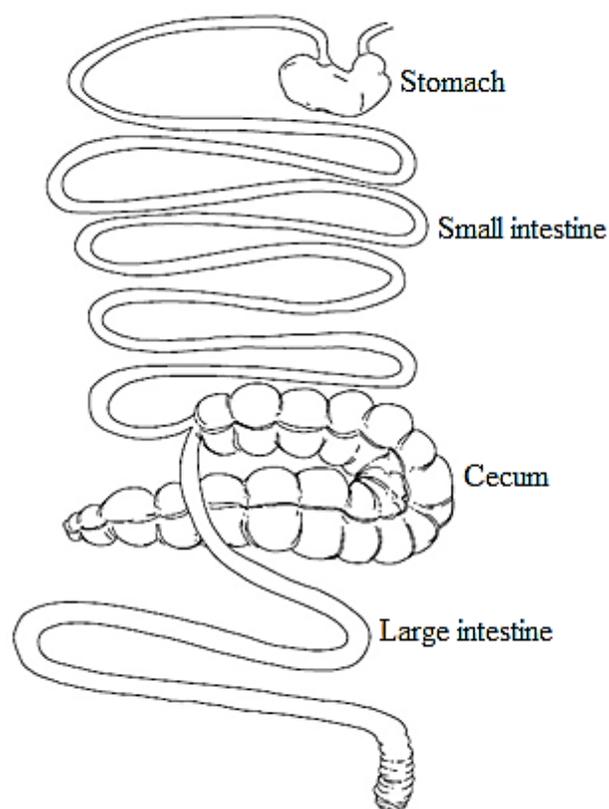


Figure 2 - Schematic drawing of the digestive tract of capybaras. Adapted from Moreira et al (2013).

In natural situations, as occurs in the Brazilian Pantanal, during the water season and high food supply, the capybara selects the food and ingests smaller quantities, while in the dry season the selection is lower, but the intake is higher (HUME, 1989 ). In the State of São Paulo, where capybaras are close to sugarcane monocultures, this behavior of food selection can be reduced, since the amount of energy from the pasture is the main factor when capybaras choose what to eat (CORRIALE et al., 2011), and the supply of sugarcane is constant throughout the year (LORA et al., 2006).

The capybara has a gestation period of 147-156 days (150 on average), yielding litters of 4 to 8 individuals. Sexual maturity is influenced by the climate, availability of resources

and lifestyle (OJASTI, 1973). According to Zara (1973), captive animals reach maturity at 15 months old, while López-Barbella (1984) states that wildlife animals reach the same status between 6 and 12 months old.

In the wild, females start their reproductive life before males, when they reach body weight between 30 and 40 kg (OJASTI, 1973), which happens between 1.5 and 2 years. This condition can represent a problem in areas with strong anthropic action, where the food supply (e.g., crops) is vast and this weight can be reached before the age of 18 months; therefore, there is a possibility that the reproductive life of capybaras in these areas may begin earlier, increasing reproductive success (SHANER et al., 2018), and thus, increasing the size of the groups. In addition, males and females, weighing over 50 kg, have a higher reproductive performance (SILVA, 1986).

## **ii. Aspects of group formation and occupation of areas**

Capybaras are social animals that form their groups close to water collections - rivers, lakes, flooded regions - because their sweat glands are not well developed (HERRERA, 2013); therefore, they need water bodies to regulate body temperature (homeothermy). In addition to thermal regulation, water bodies are a vital resource for capybara, not only for hydration, but also to escape from predators, mating and consumption of aquatic plant species (MACDONALD, 1981).

Capybara live in groups composed by individuals of both sexes and all ages, with a varying number of individuals, from 5 to 100 per group, the latter being an atypical situation, outside the natural standards. Most socially stable groups do not reach more than 30 individuals (HERRERA et al., 2011). The size of the group is directly related to the reproductive success of the population (HERRERA and MACDONALD, 1989); however, in higher densities of larger societies this relationship is lost, since, possibly, the ideal size of the group has been exceeded (SALAS, 1999).

## **iii. Habitat loss, emergence and reemergence of diseases**

The human needs to use land for food production causes the environment to be constantly modified, rapidly reducing natural areas and increasing areas of monoculture or

pasture. This sudden change in the environment causes a massive decrease in native fauna. In the most optimistic speculations, from 1970 until now, the populations of mammals, birds, reptiles, amphibians and fish fell by almost 30% (LOH, 2008).

In Brazil, the destruction of native forests for the implantation of monocultures and pastures is a historical process, which took place in the country over the centuries (FAUSTO, 1994). In southeastern Brazil, one of the focus regions of the present study, the expansion of agriculture fields has caused a decrease in native fauna habitat areas (GHELER-COSTA et al. 2012) providing, however, an alternative food source of high energy for capybaras (BOVO et al. 2016). The advance of sugarcane crops caused a decrease in the habitat areas of native fauna. Along with these conditions, in the last decades, the State of São Paulo has been experiencing both horizontal and vertical expansion of capybaras populations. In the first case, capybaras started to occupy many areas where they did not exist before; in the second case, population densities have increased significantly in places of already established populations (VERDADE and FERRAZ, 2006; FERRAZ et al., 2007).

It is known that changes in biodiversity, regardless of the biome, have the potential to affect the risk of exposure to diseases in plants and animals, including humans. Every infectious disease includes at least one pathogen and a host, and in the case of vector-borne diseases, there is one more species involved (KEESING, et al., 2006). According to Keesing et al. (2006) the loss of biodiversity can affect the transmission of infectious diseases by changing: (i) the abundance of the host or vector; (ii) the behavior of the host, the vector or the parasite; (iii) the environmental conditions for the host or vector. In some cases, a combination of more than one factor.

Several lines of study demonstrated that the loss or reduction of biodiversity tends to increase the transmission of pathogens and the emergence or reemergence of diseases. This pattern has occurred in several ecosystems, with different pathogens, hosts and vectors. Several studies confirmed the theory of the correlation between environmental changes and the reduction of biodiversity and disease transmission: West Nile fever (EZENWA et al., 2006; SWADDLE and STAVROS, 2008; ALLAN et al., 2009); hantavirus (DIZNEY et al., 2009; CLAY et al., 2009); Lyme disease (OSTFELD et al., 2006; LOGIUDICE et al., 2008; KEESING et al., 2009), among others. For Brazilian spotted fever (BSF), Polo et al. (2015)

demonstrated that the increase and expansion of the sugarcane monoculture was directly related to the territorial expansion of human cases of the disease.

#### **iv. Capybaras and the Brazilian spotted fever**

Brazilian spotted fever, caused by the bacterium *Rickettsia rickettsii*, is the most severe and most prevalent tick-borne disease affecting humans in Brazil (LABRUNA, 2009). From 2001 to 2018, 978 cases of BSF were confirmed in the state of São Paulo, with a 50% lethality rate (LUZ et al. 2019). The more severe clinical picture is often due to failures in the initial diagnosis (DE LEMOS et al., 2001).

In the southeastern region of Brazil, *R. rickettsii* is transmitted mainly by the tick *Amblyomma sculptum*, and by *Amblyomma aureolatum* in a few areas with different epidemiological conditions. In the state of São Paulo, *A. aureolatum* is the main vector in the metropolitan region of São Paulo, whereas *A. sculptum* is the main vector in degraded areas of the countryside of the state (GUEDES et al., 2005; PINTER and LABRUNA, 2006; LABRUNA, 2009; LUZ et al. 2019).

While *R. rickettsii* is maintained in tick populations through transovarial and transstadial passages, it is known that the infection by *R. rickettsii* has some deleterious effect on ticks. Therefore, the *R. rickettsii*-infection rates tend to drop over consecutive tick generations, since the mortality of infected ticks is greater than in uninfected ticks (BURGDORFER, 1988; LABRUNA et al., 2011). Accordingly, the successful maintenance of *R. rickettsii* in a tick population depends on the participation of a vertebrate amplifying host, which once primarily infected, will develop a transient bacteremia (rickettsemia) for a few days, when new cohorts of *R. rickettsii*-infected ticks are created (BURGDORFER, 1988).

To be considered a competent amplifying host, the vertebrate must: (i) be abundant in areas endemic for the agent; (ii) be the main host for the vectors; (iii) be susceptible to infection by *R. rickettsii*; (iv) once infected, must develop ricketsemia for a period of time sufficient to transmit the agent to other ticks that may parasitize it; (v) be prolific, therefore,

produce descendants susceptible to the infection (LABRUNA, 2009). The capybara fulfills all the attributes listed above.

In southeastern Brazil, where there are the highest numbers of BSF cases in Brazil, the anthropogenic actions have provided to capybaras a favorable environment for the formation of large groups (often over 30 animals), as previously mentioned (VERDADE and FERRAZ, 2006). In addition, with the loss of their natural habitat, capybaras are increasingly approaching areas of human occupation (parks, university campuses, closed condominiums), contributing to the constant maintenance of BSF in these areas (LABRUNA, 2013). In addition, in a recent study, Polo et al. (2017) reported that the dynamics of population densities of capybaras and *A. sculptum* in anthropogenic areas in the countryside of southeastern Brazil have a direct relationship in the spread of BSF in these regions.

## 1.2. HYPOTHESES

It is hypothesized that the abundant food supply for capybaras in HMLs may be generating obese animals with biochemical disorders of nutritional and metabolic nature due to excess weight. These disorders could therefore be increasing the replacement rates of individuals susceptible to *R. rickettsii* in the population. In addition, the disorderly increase in groups of capybaras is essential for the maintenance of *A. sculptum* in the environment, especially in areas endemic to BSF (LUZ et al. 2019).

In order to analyze such conjectures, the following hypotheses were elaborated:

(I) For BODY MASS / SIZE ratio

H0: The BODY MASS / SIZE ratio of capybaras in HMLs does not show statistical difference with the same relation of capybaras in NLs.

H1: The mass / size ratio of capybaras in HMLs exhibits statistical difference with the same ratio of capybaras in NLs. Animals in HMLs are expected to be no larger; however, to be heavier than capybaras in NLs.

(II) For serum biochemical profile

1. Liver and kidney profile

H0: The serum biochemical profile for liver enzymes (AST and AKP) and urea of capybaras in HMLs does not show significant difference in relation to capybaras in NLs. In other words, the biochemical profile of capybaras from different areas does not show significant differences.

H1: The serum biochemical profile for liver enzymes (AST and AKP) and urea of capybaras in HMLs shows a significant difference in relation to capybaras in NLs. In other words, the biochemical profile of capybaras in different areas shows a significant difference.

2. Protein (total protein and serum albumin) and energy (fructosamine) metabolism and serum electrolytes (phosphorous and calcium)

H0: Capybaras present in HMLs do not show any difference in protein and energy metabolism when compared to capybaras in NLs; capybaras from both landscapes do not exhibit differences regarding serum electrolyte concentrations.

H1: Capybaras present in HMLs exhibit differences in protein and energy metabolism when compared to capybaras in NLs; capybaras from both landscapes exhibit some difference regarding to serum electrolyte concentrations.

### **1.3. OBJECTIVES**

Therefore, the general objective of the present study was to analyze a possible difference in the data obtained from body mass, body measurements and biochemical profile of capybaras captured in the Brazilian Pantanal (NLs), which will be our control group, and from capybaras sampled in HMLs in the state of São Paulo.

Specific objectives:

(I) To measure the total length, height and circumferences of the neck, thorax and abdomen of capybaras among HMLs and NLs; to determine body mass indexes of capybaras from the two landscapes and to evaluate possible differences between them.

(II) To evaluate the biochemical profile of capybaras from HMLs and NLs based on eleven biochemical variables directly or indirectly related to increases in body energy reserves; to compare variables between capybaras from the two landscapes and to evaluate possible differences between them.

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## **1.5. FIGURES LIST**

Figure 1 – Distribution map of the genus *Hydrochoerus* spp. (Based on JIMÉNEZ, 1995; MOREIRA et al., 2013). (p. 18)

Figure 2 - Schematic drawing of the digestive tract of capybaras. Adapted from Moreira et al (2013). (p. 19)

## **2. Human-modified landscapes sustain heavier population of free-living capybaras (*Hydrochoerus hydrochaeris*) in Brazil**

### **2.1. ABSTRACT**

The capybara (*Hydrochoerus hydrochaeris*) is the largest extant rodent in the world and, according to the literature, adults can reach between 50 and 60kg. Many human-modified landscapes in southeastern Brazil have experienced horizontal and vertical expansion of capybaras populations in recent decades due to the large supply of food. It is conjectured that there is an increase in the energy reserves of capybaras in the human-modified landscapes when compared to capybaras in natural landscapes of the Pantanal biome of Brazil. During 2015–2019, we captured capybaras in seven human-modified landscapes in the state of São Paulo, southeastern Brazil, and in two natural landscapes of the Pantanal biome, all with established populations of capybaras. Our results showed that animals in human-modified landscapes ( $61.2 \text{ kg} \pm 16.1$ ) were heavier than animals in natural landscapes ( $54.2 \text{ kg} \pm 11.2$ ), moreover the body measures as total length and height of both groups were similar, excluding any kind of interference in the body mass values of individuals. Interestingly, capybaras from human-modified landscapes showed circumferences of the neck and thoracic measurements ( $60.9 \text{ cm} \pm 7.1$ ;  $90.4 \text{ cm} \pm 9.3$ , respectively) above those recorded for capybaras in natural landscapes ( $55.9 \text{ cm} \pm 5.5$ ;  $86.5 \text{ cm} \pm 7.4$ , respectively), indicating a possible relationship with body mass gain obtained by these animals. We concluded that the capybaras of human-modified landscapes were heavier when compared to capybaras of natural landscapes, which live in areas with low anthropogenic action. These higher body weights in human-modified landscapes are due to the constant availability of highly energetic foods, for example: sugarcane, very common in the state of São Paulo. Moreover, unlike natural landscapes, in anthropized areas these animals have limited movement to these food sources without any concern for predators. All of these elements lead to an increase in energy reserves in capybaras.

### **2.2. INTRODUCTION**

The capybara (*Hydrochoerus hydrochaeris*) is the largest living rodent in the world, reaching 50-60 kg in average (MOREIRA et al. 2013), being able to live socially in groups

formed from 5 to 100 individuals per group, the latter being an atypical situation outside the natural standards. Around 30 individuals typically compose the most socially stable groups known (HERRERA et al. 2011). Capybaras are native to all South American countries (except for Chile), most intensely from northern Venezuela to the mouth of the la Plata River in Argentina (FRAILEY 1986, VUCETICH et al. 2010, MOREIRA et al. 2013). The presence of capybaras in west of Andes in Panama, Colombia and Venezuela is thought represented by another species, smaller than the *H. hydrochaeris*, *Hydrochoerus isthmius* (MOREIRA et al. 2013).

In southeastern Brazil, the expansion of agriculture fields has caused a decrease in native fauna habitat areas (GHELER-COSTA et al. 2012) providing, however, an alternative food source of high energy for capybaras (BOVO et al. 2016). Along with these conditions, the state of São Paulo has experienced a horizontal and vertical expansion of capybara populations in recent decades (VERDADE and FERRAZ 2006, FERRAZ et al. 2007, BOVO et al. 2016), and this highly nutritional agriculture crops are possibly enabling an increase in the energy reserves of these animals, thereby increasing their body conditions.

Body condition is an important determinant of an animal's individual fitness and its implications are of great interest to ecologists, since animals with good body conditions and higher energetic reserves are more apt to survive and perpetuate the species (GREEN 2001). Besides, several authors have positively correlated higher energy body reserves with greater reproductive success (ATKINSON and RAMSAY 1995, DOBSON and MICHENER 1995, WAUTERS and DHONDT 1995).

The horizontal and vertical expansion of capybara populations across human-modified landscapes, made possible a better survival condition and the greater reproductive success is contributing to the increase of the encounter rates between humans and capybara populations, increasing conflicts and hazards regarding public safety and human health (LABRUNA 2013).

The present study aimed to compare the body conditions of capybaras across human-modified landscapes (HMLs) in the state of São Paulo and capybaras from natural landscapes (NLs) in the Pantanal biome of the states of Mato Grosso and Mato Grosso do Sul. For this

purpose, we analyzed linear measurements (body weight, height, length and perimeters) that are commonly used for rodents (SCHULTE-HOSTEDDE et al. 2001). While more accurate measurements regarding fat amount determination require the material to be destroyed and dissolved in solvents (DOBUSH et al. 1985), such lethal procedure was not possible in the present study.

## **2.3.MATERIALS AND METHODS**

### **Ethics approval and consent to participate**

This study was conducted under the approval of the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine of the University of São Paulo (project number 5948070314), in accordance with the regulations/guidelines of the Brazilian National Council of Animal Experimentation (CONCEA). Field capture of capybaras were authorized by the Brazilian Ministry of the Environment (permit SISBIO Nos. 43259–6) and by the São Paulo Forestry Institute (Cotec permit 260108–000.409/2015).

### **Study areas**

Captures of free-ranging capybaras were carried out during 2015-2019 in seven municipalities of the state of São Paulo (HMLs), and in two municipalities (NLs), one in the state of Mato Grosso and another one in the state of Mato Grosso do Sul (Table 1, Fig. 1). Capybara sampling was primarily performed for another study related to the epidemiology of Brazilian spotted fever - BSF (caused by the bacterium *Rickettsia rickettsii*), for which capybaras were consider to play a role as amplifying hosts of the bacterium to the capybara tick, *Amblyomma sculptum*, as described elsewhere (LUZ et al. 2019). We also included capybaras captured in two additional HMLs, Avaré and Tatuí, which were not included in the study of Luz et al. (2019). Among HMLs, sampled capybaras belonged to groups that were established adjacent to or within human settlements, such as farms, university campus, public or private parks, where capybaras had constant access to artificial pastures and crops. In NLs, sampled capybaras belonged to groups established in areas with no artificial pastures or crops, and with minimal anthropic changes during the last few decades. Detailed description of all sampled areas shown in Table S1.

**Table 1 – Location of capybara captures and sampling.**

Location	Municipality/State	Approximate Coordinate
<i>Human Modified Landscapes (HMLs)</i>		
University of São Paulo campus of Piracicaba	Piracicaba/SP	22°43'05.1"S 47°36'39.6"W
Carioba sewage treatment station	Americana/SP	22°42'35.7"S 47°20'24.5"W
Federal University of São Carlos campus of Araras	Araras/SP	22°18'34.4"S 47°23'00.2"W
Private Company	Tatuí/SP	23°22'42.4"S 47°55'01.0"W
University of São Paulo campus of Pirassununga	Pirassununga/SP	21°56'52.4"S 47°27'13.6"W
University of São Paulo campus of Ribeirão Preto	Ribeirão Preto/SP	21°10'03.9"S 47°51'30.7"W
Avaré State Park	Avaré/SP	23°05'59.2"S 48°54'32.9"W
<i>Natural Landscapes (NLs)</i>		
Pantanal of Poconé	Poconé/MT	16°29'35.6"S 56°26'20.8"W
Pantanal of Nhecolândia	Corumbá/MS	19°14'53.5"S 57°01'34.0"W

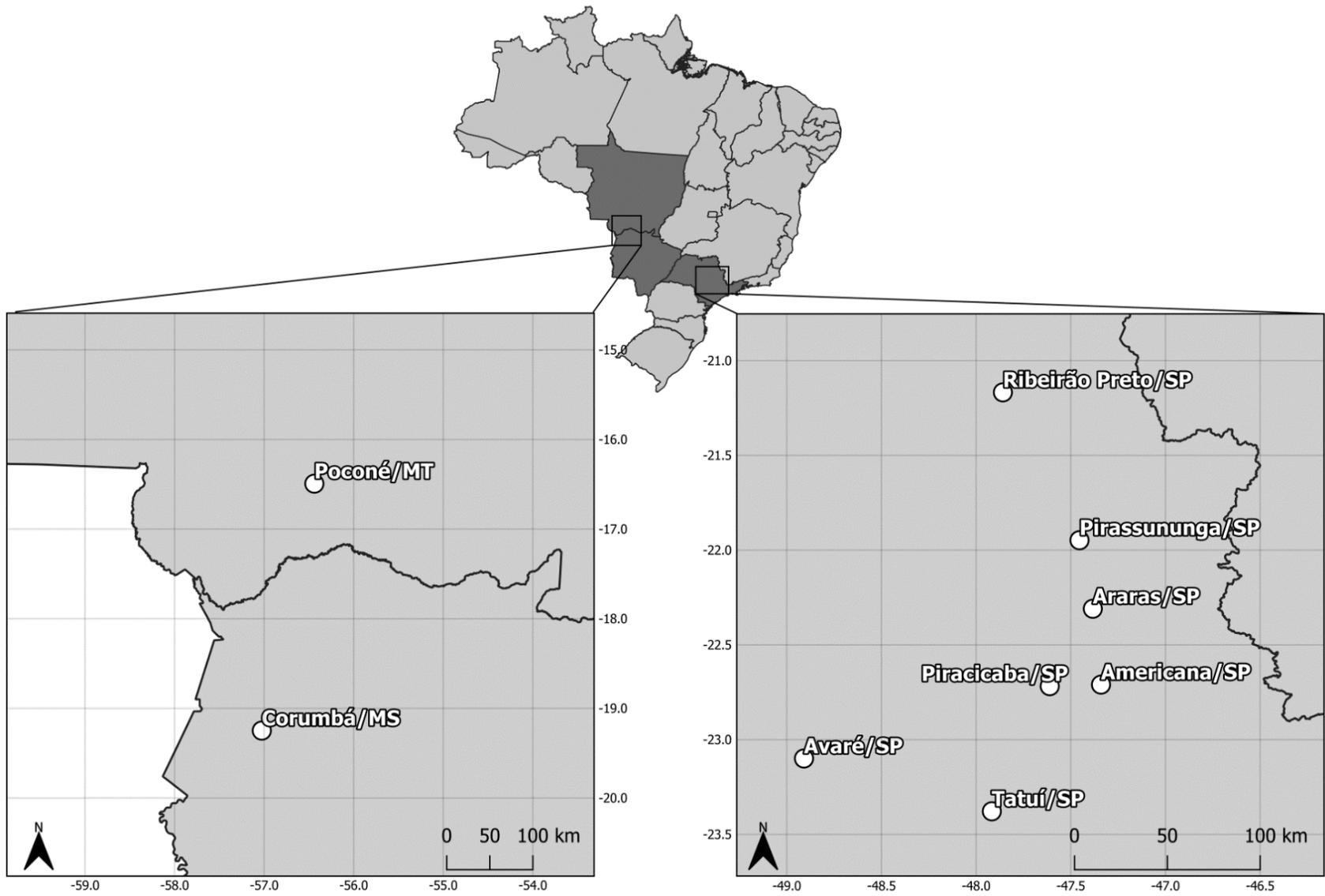


Figure 1 - map representing each collection area

## **Animal capture and containment**

Capybaras were captured by using 16 to 20 m<sup>2</sup> - corrals baited with sugar cane and green corn. Once closed in the corral, animals were physically restrained by a net catcher and anaesthetized with an intramuscular injection of a combination of ketamine (10 mg/kg) and xylazine (0.2 mg/kg). In Mato Grosso do Sul, corrals were not effective, thus capybaras were captured by anesthetic darting via a CO<sub>2</sub>-injection rifle (Dan-Inject model JM Standard, Denmark) using the same chemicals described above (LUZ et al. 2019). In an attempt to interfere the minimum possible in animal health, all animals were always captured and handled in the early evening.

Under anesthesia, animals were subjected to biometrical measures (described below) and identified with a subcutaneous microchip (Alflex model P/N 860005–001, Capalaba, Australia). After recovering from anesthesia, capybaras were released at the same capture site, as described elsewhere (LUZ et al. 2019).

## **Biometrical measures**

For such analyses, only adult animals weighing >35.0 kg (MOREIRA et al. 2013) were measured, since body condition index should not be used to compare individuals of different ages, as it is supposed to reflect or to be validated by the amount of “absolute” or “percentage” energy tissue. This is because, at each stage of growth and development, the proportional or absolute amount of energy reserves can be expected to change with normal growth processes, even in an ideal environment (KOTIAHO 1999).

For each sampled capybara, we measured the length, height and perimeter (neck, thorax and abdomen) of the body (in centimeters), as well as the body weight (kg), as commonly applied to biometric studies of rodents (PERGAMS and LAWLER 2009).

The total length of the animals were measured from the end of the muzzle to the end of the last vertebra, and the height was measured from the highest point of the withers (with the capybara's paw stretched is measured from the tip of the largest finger to the upper margin of the scapula). The neck perimeter was measured at the angle of the mandible. The chest perimeter was measured at the height of the armpit and the circumference of the abdomen at

the umbilical line (Fig. 2). For all measures a polyester tape 150 cm long and 1 cm precision was used (Corrente Coats, Brazil).

Weighing was done with the animal wrapped in a nylon net (weight 2.4 kg). The scale used was a digital dynamometer with a precision of 0.1 kg (Pesola model PCS0300, Hatton Rock, UK); it was attached to the net through a metal hook and then the net / animal set was suspended using a 1.5 m-aluminum bar.

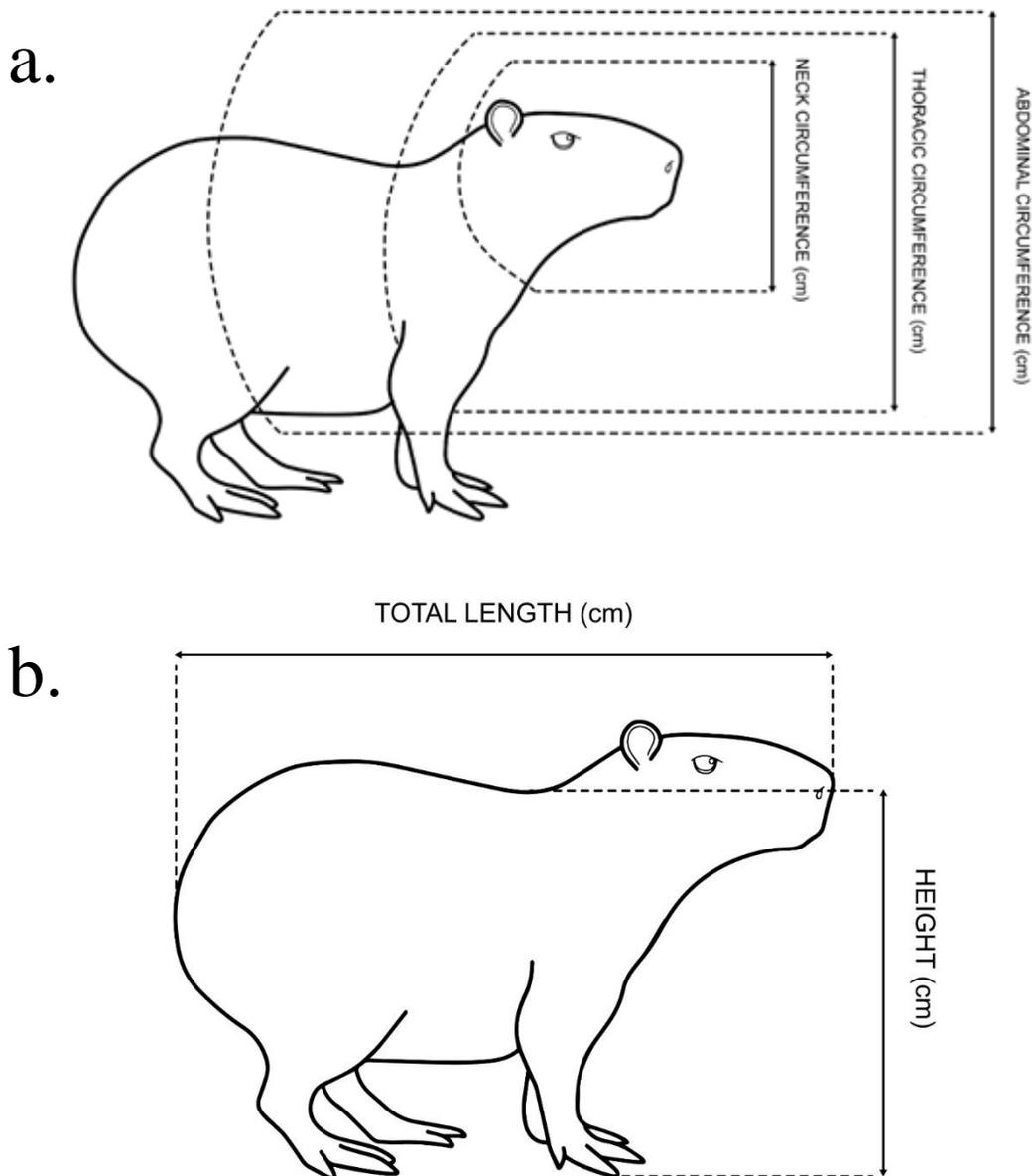


Figure 2 - Representation of the measures taken from the capybaras. A. the linear measurements. B. the circumference measurements.

## **Definition of Body Condition Index (BCI)**

Jakob et al. (1996) pointed out that the calculation of the body condition of animals is a common goal for several studies, in order to compare absolute size (or other measurement) with body mass; however, authors used several terminologies to indicate the same thing. In the present work, the adopted terminology was the "body condition index" (BCI), which was firstly proposed by Hepp et al. (1986).

To our knowledge, information on body condition index (BCI) for the evaluation of large rodents are absent in the literature. Therefore, ratios previously applied to small rodents were extrapolated for capybaras, as follows:

- I.  $BM / TL$ : Body mass (BM) divided by total length (TL) (LUNN and BOYD 1993, HUOT et al. 1995).
- II.  $BM / TL^2$ : Body mass (BM) divided by the square of the total length ( $TL^2$ ) (TIERNEY et al. 2001, BERCOVITCH et al. 2003).
- III.  $BM / TL^3$ : Body mass (BM) divided by the cube of the total length ( $TL^3$ ) (HUOT et al. 1995).
- IV.  $resBM / TL^2$ : Residual of linear regression of body mass (resBM) divided by the square of total length ( $TL^2$ ) (CAVALLINI 1996).
- V.  $resBM / TL^3$ : Residual of the linear regression of the body mass (resBM) divided by the cube of the total length ( $TL^3$ ) (TELLA et al. 1997, MCELROY et al. 2007).

## **Statistical analysis**

Data were evaluated using descriptive statistics, using violin-type density graphs and statistical summaries describing the groups through means and standard deviations for group comparison (HMLs versus NLs).

Differences between capybaras from HMLs and NLs were assessed using the Student's *t*-test for parametric distributions or non-parametric Wilcoxon test. Normality was verified by the Shapiro-Wilk test and for all analyzes 5% was used as significance level.

For statistical analysis, by standardization, only the most recent recaptures were included in the calculations.

All analyzes were performed using R software (R Core Team, 2017, Vienna, Austria).

### **Fat amount estimative**

An assumption of fat amount was estimated by calculating the residual index, which is the regressed body mass over body size. The residual index was calculated after data were appropriately transformed to meet regression assumptions. The residual distance from the point of the individual to the regression curve works as an estimate of the body condition, i.e. the distance from the point to the curve can be a good estimate of the extra mass accumulation, in this case, the fat (GOULD 1975).

## **2.4. RESULTS**

A total of 218 adult capybaras were sampled, 174 in HMLs and 44 in NLs (Table 2). Among these, 52 were recaptures. A total of 134 capybaras were analyzed in HMLs, and 32 in NLs. These capybaras were represented by 127 females and 39 males (Table 2).

**Table 2 – Number of adult capybaras sampled by locality of human-modified landscapes (HMLs) and natural landscapes (NLs) in Brazil**

Groups	Municipality	Number of adult capybaras				
		Total sampled	Recaptures	Included in the study		
				Males	Females	Total
HMLs	Piracicaba	38	13	3	22	25
	Americana	12	2	5	5	10
	Araras	17	2	1	14	15
	Tatuí	14	0	3	11	14
	Pirassununga	66	13	14	39	53
	Ribeirão Preto	22	10	4	8	12
	Avaré	5	0	1	4	5
	TOTAL	174	40	31	103	134
NLs	Poconé	20	10	4	6	10
	Corumbá	24	2	4	18	22
	TOTAL	44	12	8	24	32

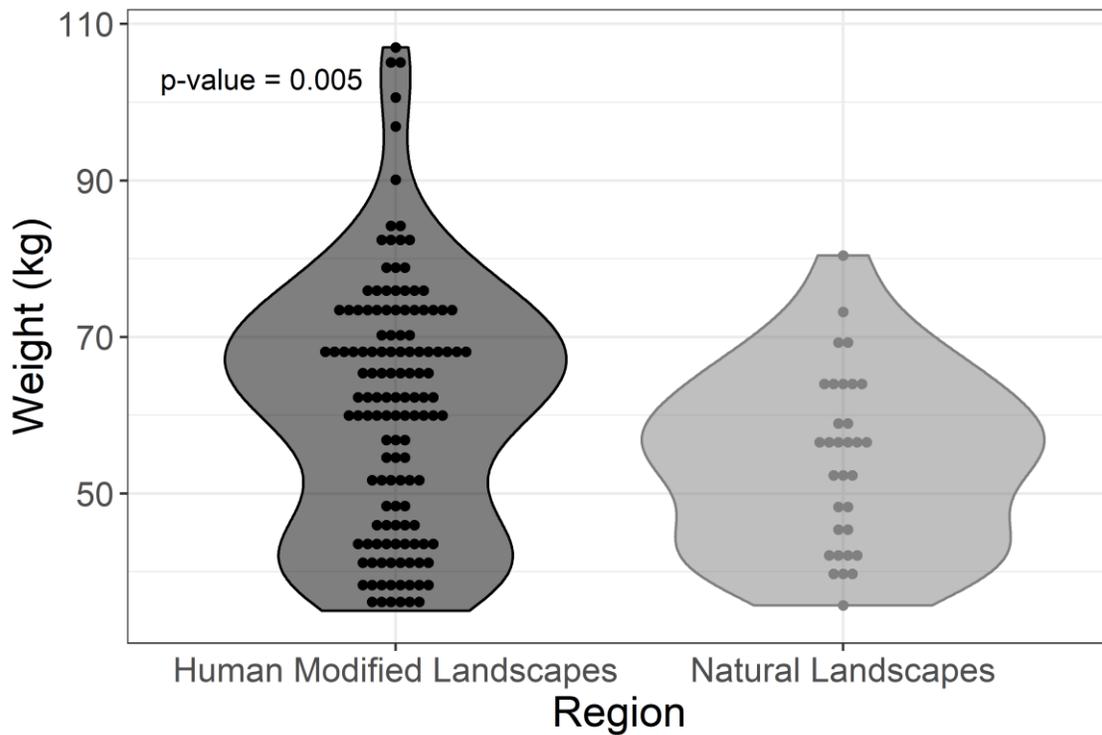
HMLs – Human-modified landscapes; NLs – Natural landscapes

### **Biometrics and weighing**

We recorded a weight range in adult capybaras of 35 - 105.2 kg, with three female capybaras, weighing 105.2, 104.8, and 100.6 kg were the overall heaviest animals, all captured in the HML, in Pirassununga municipality.

Within the same experimental groups (HML or NL), there was no difference between weights and measures of male and female capybaras.

The weight of adult capybaras of HMLs was, on average, 7 kg higher than the weight of animals from NLs ( $p = 0.005$ ), with no significant differences in height and total length to explain this difference (Fig. 3 and 4; Table 3).



**Figure 3 - graphic representation of the weight distribution in individuals according to the capture area**

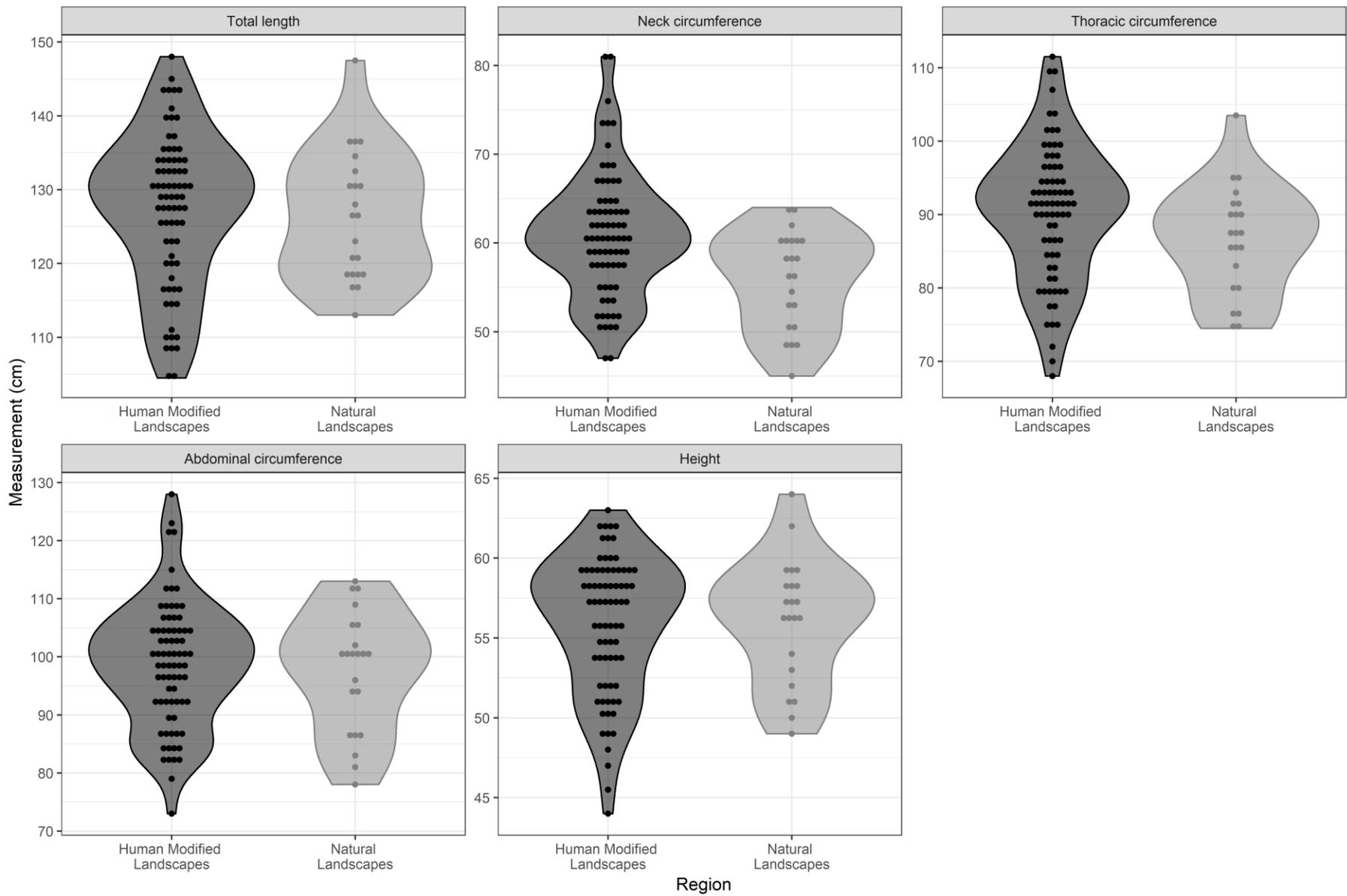


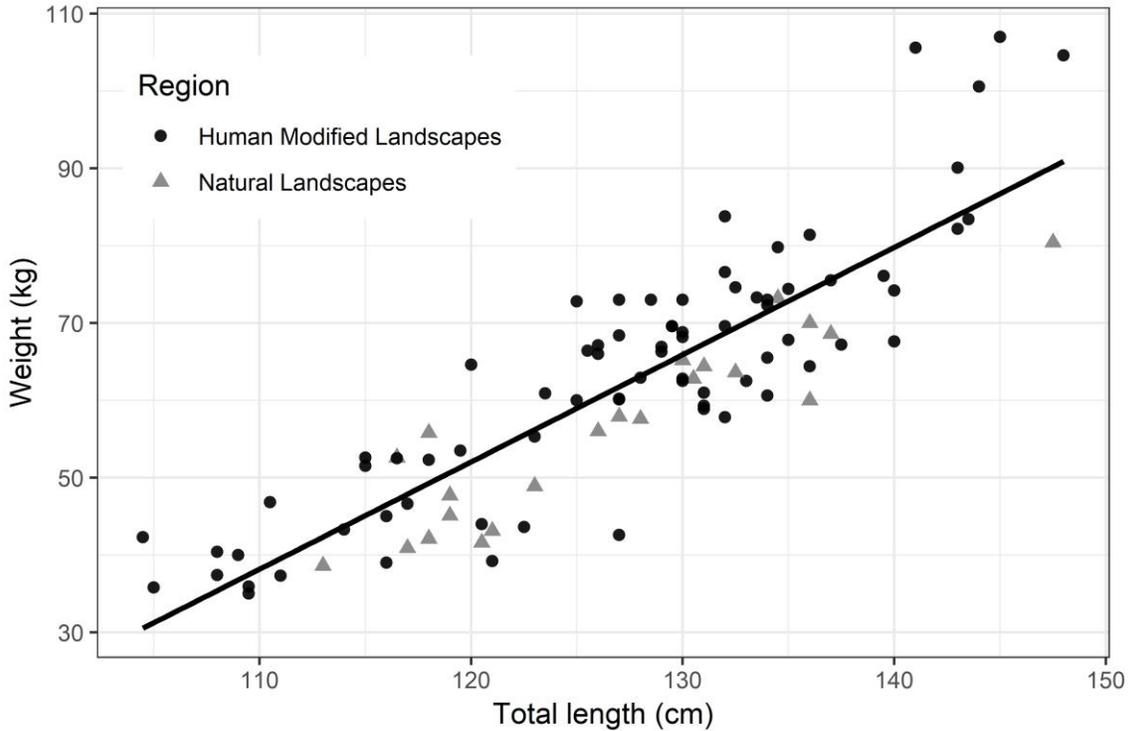
Figure 4 - graphic representation of the measurement's distribution of individuals according to the capture area

**Table 3 – comparison of biometric parameters between capybaras from human-modified landscapes (HMLs) state and natural landscapes (NLs)**

Measurement	Region	Mean ( <i>SD</i> )	Median ( <i>IQR</i> )	<i>P</i> -Value
Height	HMLs	55.9 (4.3)	57 (5.9)	0.8
	NLs	56.1 (3.9)	56.8 (5.1)	
Total Length	HMLs	127.3 (10.3)	129.2 (13.6)	0.69
	NLs	126.4 (8.7)	126.5 (13.1)	
Abdominal circumference	HMLs	98.7 (10.7)	99.5 (11.8)	0.6578
	NLs	97.6 (10.3)	100.2 (15.5)	
Neck circumference	HMLs	60.9 (7.1)	60.8 (6.8)	0.000966
	NLs	55.9 (5.5)	57.2 (8.5)	
Thoracic circumference	HMLs	90.4 (9.3)	91.5 (11.6)	0.049
	NLs	86.5 (7.4)	87.5 (10.1)	
Weight	HMLs	61.2 (16.1)	62.7 (25.8)	0.005
	NLs	54.2 (11.2)	55.7 (18.4)	

*SD*: Standard deviation.; *IQR*: Interquartile range; HMLs – Human-modified landscapes; NLs – Natural landscapes

By performing weight regression as a function of total length, height and gender, it was found that only total length explains a significant portion of weight variance. Thus, the residue of weight regression as a function of total length functions as an approximation of the amount of fat deposited an important variable in animal weight composition (Fig. 5). Note: the  $r^2$  of both models is about the same 75%.



**Figure 5 - graphic representation of the distribution of weight in relation with the total length of capybaras according to the region**

Regarding the circumference measurements, we observed statistical differences in two of the three measurements: neck and chest. Abdominal circumference did not differ between populations (Table 3).

**Definition of Body Condition Index (BCI)**

By comparing all the BCI, there was a statistical difference between the two groups, and the BCI of animals across HMLs was significantly higher than the group of capybaras resident in NLs (Table 4). Regardless of the formula used to calculate the body condition index, there was always differences between the two groups, and animals from HMLs had higher body index (Fig. 6).

**Table 4 - Body Condition Index average values of capybaras from human-modified landscapes (HMLs) and natural landscapes (NLs)**

Formula	Region	Mean (SD)	P-value
MC/CT	HMLs	0.4928 (0.0965)	0.005
	NLs	0.4410 (0,0656)	
MC/CT <sup>2</sup>	HMLs	0,0038 (0.0005)	<0.001
	NLs	0.0035 (0,0004)	
MC/CT <sup>3</sup>	HMLs	3.03E+5 (3.64E+3)	<0.001
	NLs	2.75E+5 (2.62E+3)	
Residue	HMLs	1.37 (8.01)	<0.001
	NLs	-4.74 (4.89)	
resMC/CT <sup>2</sup>	HMLs	8.75E+5 (0.00048)	<0.001
	NLs	-2.9E+11 (0.00031)	
resMC/CT <sup>3</sup>	HMLs	7.18E+1 (3.79E+3)	<0.001
	NLs	-2.28E+3 (2.57E+3)	

BM / TL: Body mass (BM) divided by total length (TL); BM / TL<sup>2</sup>: Body mass (BM) divided by the square of the total length (TL<sup>2</sup>); BM / TL<sup>3</sup>: Body mass (BM) divided by the cube of the total length (TL<sup>3</sup>); resBM / TL<sup>2</sup>: Residual of linear regression of body mass (resBM) divided by the square of total length (TL<sup>2</sup>); resBM / TL<sup>3</sup>: Residual of the linear regression of the body mass (resBM) divided by the cube of the total length (TL<sup>3</sup>)

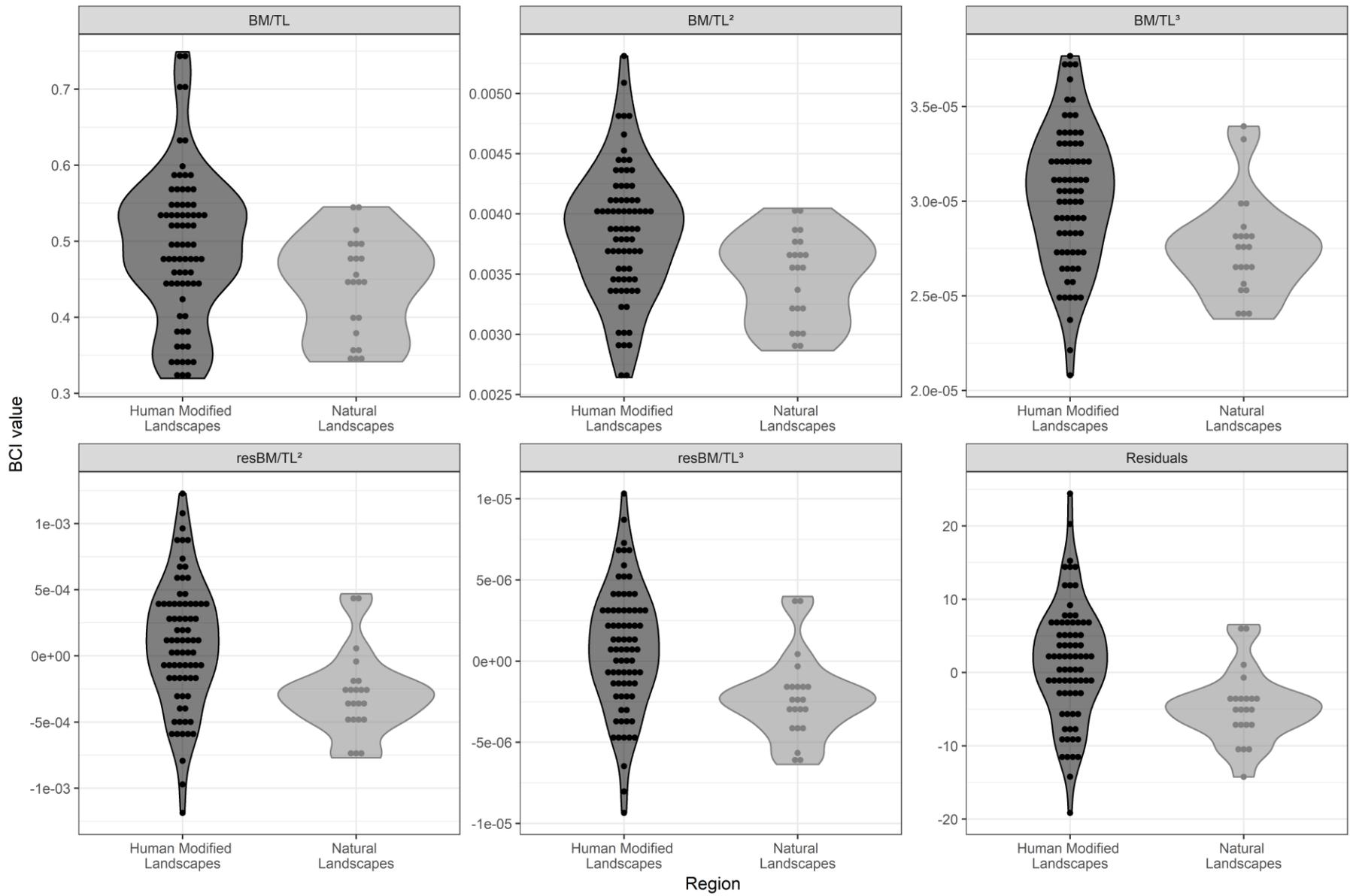


Figure 6 - graphic representation of the distribution of Body Condition Indexes according to the capture area

## 2.5. DISCUSSION

It was observed that HML capybaras are heavier than NL capybaras, although the total length and height do not differ statistically. Previous work has recorded weights of these animals, but to our knowledge, none of them compares populations from different areas.

Mones and Ojasti (1986) recorded in Llanos, Venezuela, a weight range in adults between 35 - 65 kg, while Alho (1986) recorded in the Brazilian Pantanal, capybaras of up to 70 kg. Interestingly, the sampling sites of Alho (1986) are in the same region as the present study, where we recorded in the Brazilian Pantanal (NLs), a 79.8 kg animal, almost 10 kg heavier than the old limit registered for the species in the local.

Other authors record species boundaries without defining location, as is the case with MacDonald et al. (2007), in which he defines the weight range for adult capybaras between 35 - 66 kg with an average of 50 kg. Moreira et al. (2013) defines the weight range between 35 - 100kg for the species, also with an average of 50kg.

Our work defines that *H. hydrochaeris* must have separate weight averages depending on the type of environment it occupies (HML or NL). In both cases, we obtained different values from those reported in the literature. For the HMLs we obtained a range of 35 - 105.2 kg, registering a new weight limit for the species, with an average of 61.2 kg. For NLs capybaras the weight range is 35 - 79.8 kg, with an average of 54.2 kg.

The five body index showed differences between the sampled groups, revealing that capybaras in the HMLs had higher BCI than capybaras in the NLs; therefore, higher fat reserves. We can infer that the extra weight of animals from HMLs comes from excess fat, since most of the fat in a capybara is subcutaneous (BRESSAN et al. 2002). The measurements of the perimeters indicated higher fat deposits in animals in the HMLs.

Even though Jakob et al. (1996) stated that results vary dramatically according to the BCI chosen to calculate and to analyze body condition, in the case of our work, this was not a problem, since the main idea was to show that there was a difference in weight and BCI between

the two sampled groups (HMLs and NLs), and all of our BCI results showed that difference between the populations.

Since body condition is treated as a measure of energy status, animals in better conditions for survival are expected to have higher energy reserves (usually fat) (SCHULTE-HOSTEDDE et al. 2001). Therefore, based in individual energy reserves, we can infer that capybaras from HMLs have better survival conditions than animals from NLs. Firstly, due to the vast supply of food offered by crops (e.g., sugarcane, corn) throughout the state of São Paulo (FERRAZ et al. 2007). Secondly, besides the presence of pumas (*Puma concolor*), a natural predator of capybaras in HMLs (ANGELIERI, et al. 2016), heavier animals are able to some extent avoid predation, despite having impaired locomotion (TROMBULAK 1989).

In addition to the possible better survival conditions of capybaras in HMLs, it is known that both natural and sexual selection tend to favor larger and heavier individuals (SELK et al. 1988, BLANCKENHORN 2000). The obvious, although fundamental, generalization is that the greater the availability of limiting resources, the larger the population size (MOREIRA et al. 2013). A trait that responds to food availability, such as body mass, is likely to covary with fitness at the phenotypic level; i.e., individuals that have access to more food are heavier and reproduce more (SCHLUTER et al. 1991).

There are studies with domestic cattle considering body condition and reproductive efficiency, the higher the BCI, the better the reproductive efficiency (PRYCE et al. 2001). This association stems from the fact that the corpus luteum might be directly activated by cholesterol, a progesterone precursor (ROCHE et al. 2007). There are also reports of relationship between blood fatty acids and prostaglandin production. The direct effect of positive energy balance (energy consumed minus energy required for maintenance) on luteinizing hormone secretion has been demonstrated in heifers for over 30 years (IMAKAWA et al. 1987). Therefore, there is a great possibility that capybaras in HMLs, due to access to abundant food supply, are reproducing on larger scales than the animals of NLs, thus forming larger groups.

Population growth either horizontally or vertically has led to greater approximations between capybara and man. This approach in the HMLs of the state of São Paulo has been

routinely covered by the media (Gazeta de Piracicaba 2019, Jornal da USP 2019, Estadão 2019). It is not uncommon for humans and capybaras to live in the same environment, for example, university campus, luxury residential parks, municipal parks and, in some cases, even within the city, next to water collections (VERDADE and FERRAZ 2006, BOVO et al. 2016, ROCHA et al. 2017, PASSOS NUNES et al., 2019). Such proximity has brought certain losses with regard to public health and safety (LABRUNA 2013, ABRA et al. 2019, PASSOS NUNES et al. 2019).

Among HMLs, capybaras are major hosts for the tick *A. sculptum*, the vector of *R. rickettsii*, the agent of Brazilian Spotted Fever (BSF) in the countryside of the state of São Paulo. BSF is the main and most lethal tick-borne disease of humans in Brazil (LABRUNA 2009). The clinical picture is severe and often due to failures in the initial diagnosis, of fatal course (DE LEMOS et al. 2001). From 2001 to 2018, over 978 cases of BSF were confirmed of which 489 had a fatal outcome, a 50% fatality rate (LUZ et al. 2019).

## **CONCLUSION**

We concluded that capybaras of HMLs in the state of São Paulo are heavier, but not larger, when compared to capybaras of NLs in the Brazilian Pantanal, which reinforces the mass gain of animals in HMLs. Also, capybaras in HMLs had larger circumferences of the neck and chest, region where these animals commonly have subcutaneous fat accumulation. This increase in energetic reserves has enabled the spread of capybara populations in the HMLs, which might have brought several consequences regarding health and public safety, as well as deleterious consequences for capybaras themselves.

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**S1 Table. Areas where capybaras were sampled in the present study. The description of all areas (except for Tatuí and Avaré) have been recently reported by Luz et al. (2019)**

Municipality (State) <sup>a</sup>	Classification <sup>b</sup>	Description
Piracicaba (São Paulo)	Human-modified landscape	Highly anthropic area in the University of São Paulo campus of Piracicaba; human-modified landscape composed by sugar cane and corn crops interposed by livestock artificial pastures and degraded riparian forests along the Piracicaba River. Elevation around 586 m.
Americana (São Paulo)	Human-modified landscape	Highly anthropic area in the Carioba sewage treatment station; human-modified landscape composed by abandoned grass fields (no livestock) and degraded riparian forests along the Piracicaba River. Elevation around 511 m.
Araras (São Paulo)	Human-modified landscape	Highly anthropic area in the Federal University of São Carlos campus of Araras; human-modified landscape composed by sugar cane crops interposed by livestock artificial pastures and regenerating riparian forests along an artificial lake. Elevation around 659 m.
Pirassununga (São Paulo)	Human-modified landscape	Highly anthropic area in the University of São Paulo campus of Pirassununga; human-modified landscape composed by sugar cane and corn crops interposed by livestock artificial pastures and a regenerating riparian forests along an artificial lake. Elevation around 596 m.
Tatuí (São Paulo)	Human-modified landscape	Highly anthropic area in a private company area, a car testing field, consisted by eucalyptus reforestation, artificial pasture and small water bodies. No livestock. Elevation around 625 m.
Ribeirão Preto (São Paulo)	Human-modified landscape	Highly anthropic area in the University of São Paulo campus of Ribeirão Preto; human-modified landscape composed by grass fields (no livestock) and degraded forests along two streams. Elevation around 550 m.
Avaré (São Paulo)	Human-modified landscape	Highly anthropic area in the Avaré State Park; human-modified landscape composed by grass fields (no livestock) and reforestation vegetation along an artificial lake. Elevation around 768 m.
Poconé (Mato Grosso)	Natural landscape	Natural landscape composed by seasonally flooded scrublands and forests, few above high-water level forests interposed by natural lakes and rivers; no livestock or open grass lands. Elevation around 125 m.
Corumbá (Mato Grosso do Sul)	Natural landscape	Natural landscape composed by seasonally flooded grass lands (natural pastures) interposed by natural lakes and high-water level forests; livestock present at low densities. Elevation around 105 m.

<sup>a</sup> areas are indicated in the map of Fig. 1

<sup>b</sup> classification according to the degree of anthropization

**Reference:**

Luz R. R. et al. Epidemiology of capybara-associated Brazilian spotted fever. **PLoS Neglected Tropical Diseases** 2019; 13(9):e0007734.

## 2.7. LIST OF ABBREVIATIONS

HML – human-modified landscapes

NL – natural landscapes

BCI – body condition index

BSF – Brazilian spotted fever

IACUC – Institutional Animal Care and Use Committee

CONCEA – Brazilian National Council of Animal Experimentation

CO<sub>2</sub>– carbon dioxide

BM – body mass

TL – total length

resBM – residual of the linear regression of the body mass

## 2.8. FIGURES LIST

FIGURE 1 - map representing each collection area. (p. 38)

FIGURE 2 - representation of the measures taken from the capybaras. A. the linear measurements. B. the circumference measurements. (p. 40)

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S1 TABLE. Areas where capybaras were sampled in the present study. The description of all areas (except for Tatuí and Avaré) have been recently reported by Luz et al. (2019). (p. 58)

### **3. Comparison of biochemical aspects related to obesity in free-ranging capybaras of Human-Modified Landscapes and Natural Landscapes**

#### **3.1. ABSTRACT**

Capybaras (*Hydrochoerus hydrochaeris*) are the largest rodents in the world, reaching weights over 100 kg, with averages varying according to the region the population occupies. Recent studies have shown that capybaras that inhabit Human-Modified Landscapes (HMLs) are significantly heavier than capybaras in Natural Landscapes (NLs) and this weight gain is due to the accumulation of fat. It is conjectured that overweight individuals tend to develop metabolic disorders; therefore, the aim of the present study was to analyze sanguineous biochemical factors directly or indirectly related to the overweight of HML animals in relation to NL capybaras. The results found by our group demonstrated that, of the eleven biochemical factors analyzed, five were significantly different between populations and two were borderline. Therefore, our results suggest that HML populations are developing, due to excess fat, a range of biochemical disorders of metabolic and nutritional order, which can lead to increased mortality rates in these populations and higher population replacement rates, a factor extremely important for the epidemiology of Brazilian spotted fever.

#### **3.2. INTRODUCTION**

Capybara (*Hydrochoerus hydrochaeris*) is the largest rodent in the world. Adults can reach weights above 100 kg, with averages of 50 - 60 kg among different habitat types (MOREIRA et al., 2013; BENATTI et al., 2020). Capybaras live in social groups composed by animals of both sexes and all ages, with a variable number of individuals, from 5 to 100 per group, the latter being an atypical situation, outside the natural standards, knowing that the most socially stable groups are only 30 individuals (HERRERA et al., 2011). These rodents are found in all South American countries, except for Chile, most intensely from northern Venezuela to southwestern Uruguay, including Brazil (FRAILEY, 1986; VUCETICH et al., 2010; MOREIRA et al., 2013).

Although not common practice in Brazil, other South American countries exploit the capybara's zootechnical potential for meat production, as they have a high reproduction rate, fast growth, high density tolerance and easy handling, which allows working with alternatives and sustainable production (QUINTAM et al., 1994). Commercial interest in the species has spurred a few studies to determine the hematological profile of captive capybaras (AROUCA et al. 2000; VAN DEN HEIJDEN, 2000; MUÑOZ and MONTOYA, 2001, MADELLA et al., 2006).

More recently, given the visibility that the species has gained due to the constant approximation towards humans settlements (VERDADE and FERRAZ, 2006; BOVO et al., 2016, ROCHA et al., 2017), new studies have been conducted aiming at hematological and biochemical profiles (MATUS and PULIDO, 2010; CHIACCHIO et al., 2014; MÉNDEZ and BARRAGÁN, 2014).

Besides the standardization of reference values in physiological parameters, necessary to form bases for bioclimatic models of animal, environment and human interrelation (MATUS and PULIDO, 2010), the monitoring of capybaras in areas cohabitated by humans is important for population management, aiming to reduce negative ecological interactions for both species (CHIACCHIO et al., 2014). Those negative effects has been studied mainly in the role of capybaras in the ecology of a highly lethal tick-borne disease, Brazilian spotted fever (BSF) (LABRUNA, 2013; LUZ et al., 2019), and the impact of these animals on public safety, especially when groups are close to highways (ABRA et al., 2019).

A recent study showed that capybara populations from human-modified landscapes (HMLs) have higher body mass indices, attributed to the higher fat deposition, when compared to capybaras from pristine habitats like the Brazilian Pantanal, which are considered natural landscapes (NLs) (BENATTI et al., 2020). While several authors positively correlated obesity with metabolic disorders and higher mortality rates when compared to a healthy population (ANDRES, 1980; SOLOMON and MANSON, 1997; BENDER; 1998; SCHULTE et al., 1999; KATZMARZYK and ARDERN, 2004), it has also been conjectured that animals with higher

energy reserves have more reproductive efficiency than those with low reserves (PRYCE et al. 2001; ROCHE et al. 2007).

Given the importance of capybara in the epidemiology of BSF (LABRUNA, 2013; LUZ et al., 2019), the present study analyzed factors that could be associated with the expansion of the disease in southeastern Brazil. Thus, the aim of this study was to compare the biochemical profile of capybaras between HMLs and NLs. The biochemical parameters chosen for analyses are directly or indirectly related to the obesity of HML-capybaras.

### **3.3.MATERIALS AND METHODS**

#### **Study sites**

Free-ranging capybaras were captured in nine areas: seven municipalities in the state of São Paulo, southeastern Brazil, (HMLs); one area in the state of Mato Grosso do Sul and one in the state of Mato Grosso (NLs), the last two within the Pantanal biome in mid-western Brazil. Ecological characteristics and details on the capture methods in these areas have been described elsewhere (LUZ et al., 2019; BENATTI et al., 2020; LOPES et al., 2020). Briefly, animals were captured in corrals, where they were anesthetized (LUZ et al., 2019; BENATTI et al., 2020). The capybaras were captured in Mato Grosso do Sul by active search, without corrals, as described by Luz et al (2019). Under the effect of dissociative anesthetic they were submitted to sample collections.

Capybara populations in the HMLs inhabited areas with strong anthropic action, close to buildings, highways, crops (corn, sugar cane), cultivated grasses, within condominiums and university campuses. In contrast, capybaras residing in the NLs lived in areas with very low anthropogenic action (BENATTI et al, 2020; LOPES et al, 2020), and for this reason, they were considered the control group of the present study. Only adult animals were analysed.

#### **Blood collection and serum biochemistry**

Blood samples (5 to 10 ml of venous blood) were collected through the cranial cava, saphenous or cephalic vein. These samples were immediately stored in a vacuum tube

containing separator gel for subsequent serum centrifugation. In the laboratory, sera were transferred to two 1.5 ml polypropylene tubes, in order to keep all the samples in duplicates, and then frozen at -20° C for further analyses.

Biochemical variables were determined on a Labtest ® biochemical analyzer, Labmax 240 model. To verify the presence of possible obesity-related metabolic disorders, the biochemical profile of adult capybaras was performed. The following serum elements were analyzed: triglycerides, cholesterol, fructosamine, albumin, total protein, aspartate aminotransferase, alkaline phosphatase, creatine kinase, urea, calcium, and phosphorous. All protocols were performed according to the manufacturer's instructions of each kit.

For liver profile, measurements of aspartate aminotransferase (BioSystems® – 11531, Barcelona, Spain) and alkaline phosphatase (BioSystems® – 11593, Barcelona, Spain) were performed. Obese patients tend to develop liver steatosis, which would lead to increases in liver enzymes, aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Even if there is no clinical condition of steatosis, the simple deposition of excess fat can lead to increases in these enzymes (THRALL, 2012).

For renal profile, measurements of urea (BioSystems® – 11541, Barcelona, Spain) were performed. Obese patients tend not to have large changes in serum urea concentrations, but since the intent is an overview of the animal's general picture, it is important to determine the animal's renal function. Urea is directly related to muscle mass and obese patients have less muscle mass, which may lead to decreased or fluctuating serum urea levels (THRALL, 2012).

To analyze fat metabolism, measurements of cholesterol (Labtest® – 76-2/100, Minas Gerais, Brazil) and triglycerides (Labtest – 87-2/250, Minas Gerais, Brazil) were performed. These parameters were chosen because they tend to be increased in obese animals (THRALL, 2012). When increased, they generate insulin resistance, or a diminished cellular response to a given plasma insulin concentration (CLARK and HOENIG, 2016). Insulin, one of the main anabolic hormones, having its action diminished due to the increase of triglycerides and cholesterol, muscle catabolism rates may increase, consequently leading to serum creatine

kinase (CK) increases (Guyton and Hall, 2006), which we analyzed (ByoSystems® – 21790, Barcelona, Spain).

Because of this possible insulin resistance we measured the amount of serum fructosamine (ByoSystems® – 11046, Barcelona, Spain). Fructosamine is glucose conjugated with albumin present in the blood (MURPHY et al., 1997). Fructosamine gives a seven to ten day overview of blood glucose behavior. Since obese patients have insulin resistance, there is a possibility of increased fructosamine (VAN LIEW et al., 1993).

Protein metabolism analyzes were performed by measuring total protein (Labtest® – 99-250, Minas Gerais, Brazil) and albumin (Labtest® – 19-1/250, Minas Gerais, Brazil). Albumin is related to fructosamine. In addition, today the World Health Organization already considers obesity to be a pro-inflammatory or inflammatory state (WORLD HEALTH ORGANIZATION, 2000), and albumin is an acute, but a negative, phase inflammatory protein, that is, in an inflammation picture given by obesity, albumin falls, recording a serum decrease in values, as well as a possible fluctuation in total protein measurement (THRALL, 2012).

Overweight patients have metabolic dysfunction, which may lead to alterations in the hypothalamus-pituitary-adrenal axis, leading to increases in cortisol. Cortisol, by definition, is a catabolic hormone, causing increased muscle catabolism rates, which, again, may increase serum CK concentrations. Increased blood CK may cause proximal tubular damage to the kidneys, causing changes in serum phosphorus levels (Labtest® – 12-200, Minas Gerais, Brazil) (GUYTON and HALL, 2006).

And finally, obese animals have lower bone and muscle densities when compared to non-obese individuals, so we can have changes in the total blood calcium concentrations of obese animals, registering lower levels of it (LATIMER, 2011). Because of this, serum calcium measurement was performed (BioSystems® – 11570, Barcelona, Spain).

### **Statistical analysis**

Data were evaluated by descriptive statistics, using violin-type density graphs and statistical summaries to describe the groups through means and standard deviations for group comparison.

Differences between capybaras from HMLs and NLs were assessed using the Student's *t*-test for parametric distributions or non-parametric Wilcoxon test. Normality was verified by the Shapiro-Wilk test and for all analyzes 5% was used as significance level.

All analyzes were performed using R software (R Core Team, 2017, Vienna, Austria).

### **Ethical statements**

This study has been approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine of the University of São Paulo (approval number 5948070314), in accordance with the regulations/guidelines of the Brazilian National Council of Animal Experimentation (CONCEA). Field capture of capybaras and collections of ticks were authorized by the Brazilian Ministry of the Environment (permit SISBIO Nos. 43259–6) and by the São Paulo Forestry Institute (Cotec permit 260108–000.409/2015).

## **3.4. RESULTS AND DISCUSSION**

The biochemistry variables were measured in a total of 161 free-ranging adult capybaras, being 129 from HMLs and 32 from NLs (Table 1). The results of the statistical analyzes of the biochemical parameters compared between the two groups (HMLs and NLs) are shown in Table 2.

**Table 1 – Number of capybaras sampled according to gender and localities**

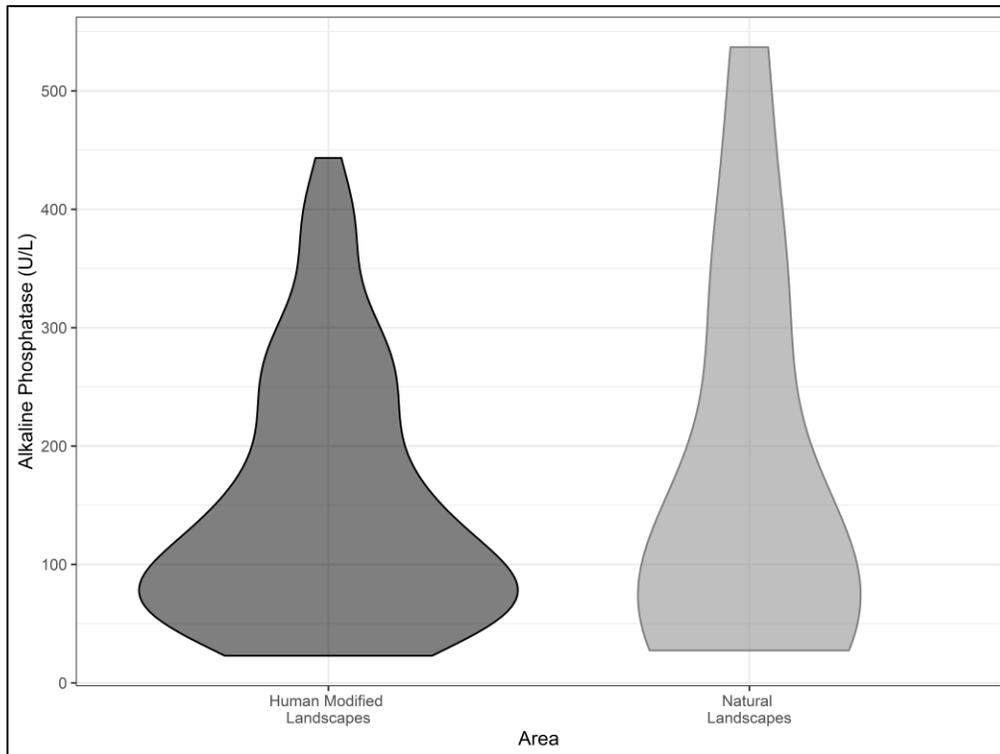
Groups	Municipality	Males	Females	Total sampled
Human-modified landscapes (HMLs)	Piracicaba	3	30	33
	Americana	6	6	12
	Araras	0	8	8
	Tatuí	3	9	12
	Pirassununga	7	34	41
	Ribeirão Preto	4	14	18
	Avaré	1	4	5
	TOTAL		24	105
Natural landscapes (NLs)	Poconé	2	7	9
	Corumbá	5	18	23
	TOTAL		7	25

**Table 2 – Comparisons of biochemical parameters between capybaras from human-modified landscapes (HMLs) and natural landscapes (NLs)**

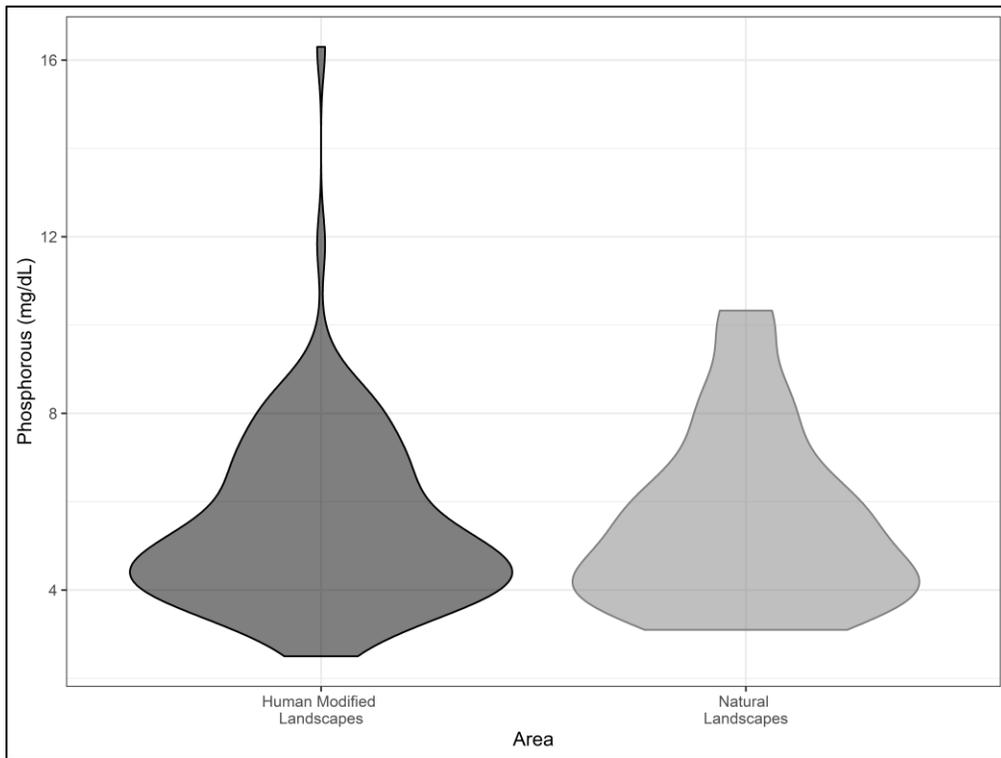
Serum Biochemical Parameter	Region	Mean (SD)	Median (IQR)	<i>P</i> -value
Albumin (g/dL)	HMLs	2.8 (0.4)	2.9 (0.6)	6.00E-04
	NLs	2.5 (0.4)	2.5 (0.7)	
Alkaline Phosphatase (U/L)	HMLs	151.1 (104.7)	115.3 (148.2)	0.7756
	NLs	171.5 (163.9)	92.8 (244.9)	
Aspartate Aminotransferase (U/L)	HMLs	21.7 (25)	14 (8.4)	0.0597
	NLs	13.6 (6.9)	11.8 (10.4)	
Calcium (mg/dL)	HMLs	10.3 (1.2)	10.5 (1.3)	0.0523
	NLs	10.9 (0.9)	10.8 (1)	
Creatine Kinase (U/L)	HMLs	261 (733.2)	87.3 (100.3)	<0.0001
	NLs	61.4 (77)	35 (48.2)	
Cholesterol (mg/dL)	HMLs	50.7 (16.7)	47.1 (14.5)	<0.0001
	NLs	37.6 (15.4)	34.1 (16.2)	
Fructosamine (mmol/L)	HMLs	200.9 (53.5)	190 (68)	0.0332
	NLs	175.6 (33.9)	175 (42)	
Phosphorous (mg/dL)	HMLs	5.5 (2)	5 (2.5)	0.9901
	NLs	5.5 (2)	5.4 (2.3)	
Total Protein (g/dL)	HMLs	6 (0.6)	6 (0.7)	0.0137
	NLs	6.3 (0.6)	6.5 (1)	
Triglycerides (mg/dL)	HMLs	83.6 (100.4)	50.9 (57.4)	0.9345
	NLs	76 (72.7)	55.9 (61)	
Urea (mg/dL)	HMLs	26.1 (16.7)	24.2 (19.4)	0.2498
	NLs	28.6 (10.4)	25.4 (10.5)	

SD: Standard Deviation.; IQR: Interquartile Range

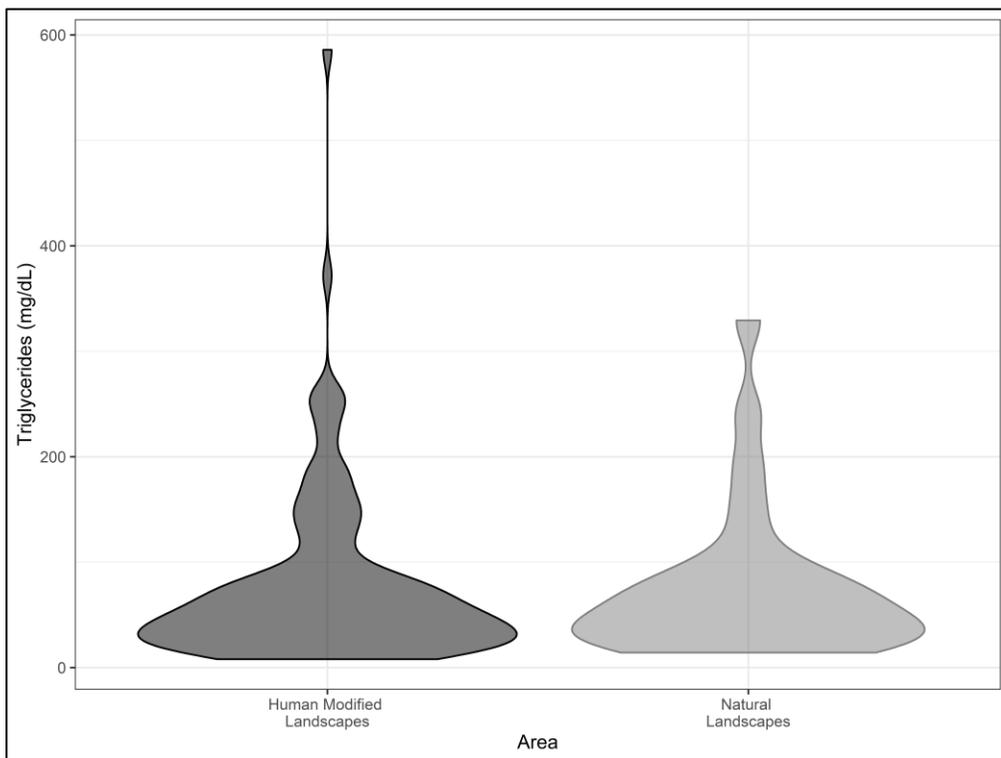
Biochemical factors such as ALP, phosphorus, triglycerides and urea showed no statistical differences between the two capybara groups (Figs. 1 - 4). However, the present ALP values (HMLs =  $151.1 \pm 104.7$  U / L; NLs =  $171.5 \pm 163.9$  U / L) were lower than the values reported for free ranging capybaras in Paz de Ariporo ( $235.75 \pm 144.83$  U / L), Colombia (Álvarez-Méndez and Barragán, 2014). The values of phosphorus (HMLs = NLs =  $5.5 \pm 2$  mg / dL) and triglycerides (HMLs =  $83.6 \pm 100.4$  U / L; NLs =  $76 \pm 72.7$  U / L) are similar to the values reported by Matus and Pulido (2010) for captive capybaras ( $5.081 \pm 3.99$  mg / dL for phosphorus and  $89.15 \pm 89.24$  U / L for triglycerides). The urea was compared with results available in the literature by converting the results found by our group to blood urea nitrogen (BUN), which consists of multiplying the value found for serum urea by 0.467 (stoichiometric calculation; molecular weights: urea 60.06 g / mol; nitrogen 14 g / mol), since that found in the literature for the species is the BUN. Therefore, converted from urea to BUN we have the following results: HMLs =  $12.18 \pm 7.79$  mg / dL; NLs =  $13.3 \pm 4.85$ , which in both cases, are above the results recorded by Matus and Pulido (2010).



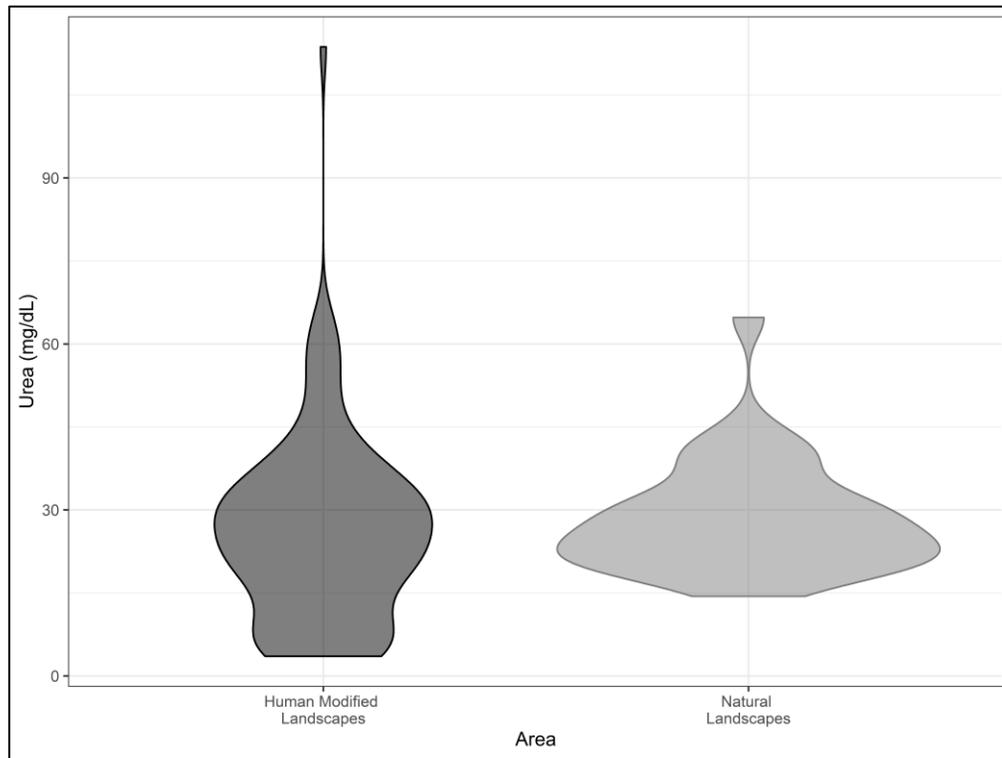
**Figure 1 - Graphical representation of the serum concentration of alkaline phosphatase for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**



**Figure 2 - Graphical representation of the serum concentration of phosphorous for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**

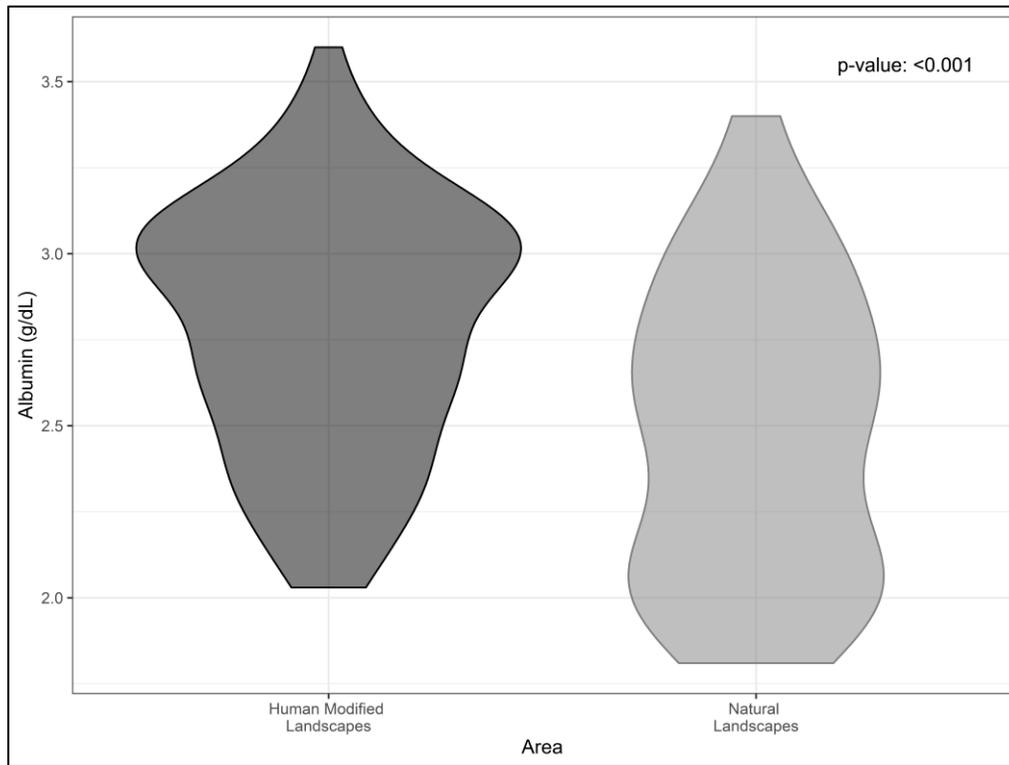


**Figure 3 - Graphical representation of the serum concentration of triglycerides for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**



**Figure 4 - Graphical representation of the serum concentration of urea for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**

Significant differences of biochemical parameters between animals from HMLs and NLs were observed for albumin, CK, cholesterol, fructosamine and total protein. Some parameters were borderline: calcium ( $P$ -value = 0.0597) and AST ( $P$ -value = 0.0523). The values found for serum albumin (Fig. 5) in this study (HMLs =  $2.8 \pm 0.4$  g / dL; NLs =  $2.5 \pm 0.4$  g / dL) are close to those found in free-living capybaras ( $2.54 \pm 0.25$  g / dL) by Álvarez-Méndez and Barragán (2014); however, differ from the values recorded by Matus and Pulido (2010) for captive capybaras ( $3.175 \pm 0.28$  g / dL). Normally, the increase in serum albumin can be directly attributed to the contribution in the diet (THRALL, 2012). Since HMLs capybaras are exposed to an unrestricted food supply (LOPES et al. 2020), this could justify the increase in albumin recorded in these animals.

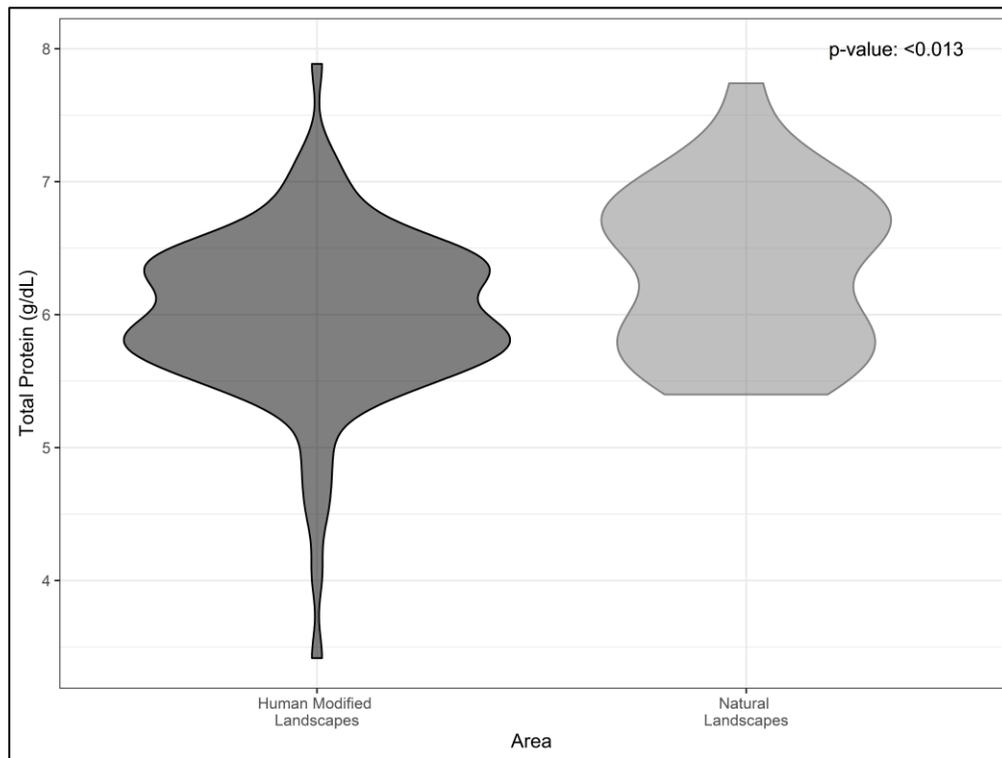


**Figure 5 - Graphical representation of the serum concentration of albumin for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**

Notwithstanding the difference in diet, the population of capybaras in NLs is subjected to the presence of natural predators - jaguar (*Panthera onca*), puma (*Puma concolor*), caiman alligators (*Caiman* spp.) and anacondas (*Eunectes* spp.) - (LORD, 1994; PORFÍRIO, 2009; CAVALCANTI et al., 2010; MOREIRA et al., 2013), while animals in HMLs have only the puma as natural predators, although on scales lower than NL capybaras (ANGELIERI, et al. 2016). The presence of predators exerts an 'intimidation' effect on potential prey which, in the case of the present study, is the capybara. This intimidation effect may lead to changes in the hypothalamic–pituitary–adrenal axis, generating a picture of chronic stress (MARKETON and GLASER, 2008). This intimidation impairs the foraging of herds, preventing animals from taking full advantage of available food (CLINCHY et al., 2013). In addition to the primary stress caused by fear of predation, another determining factor for the animal's physical condition is directly related to the fact described above about feeding efficiency. Physiological changes may

be observed in association with variation in food intake (BECKERMAN, WIESKI and BAIRD, 2007), food demand (HAWLENA and SCHMITZ, 2010) and conversion efficiency (MCPEEK, 2004), all of which may be affected by the presence of predators.

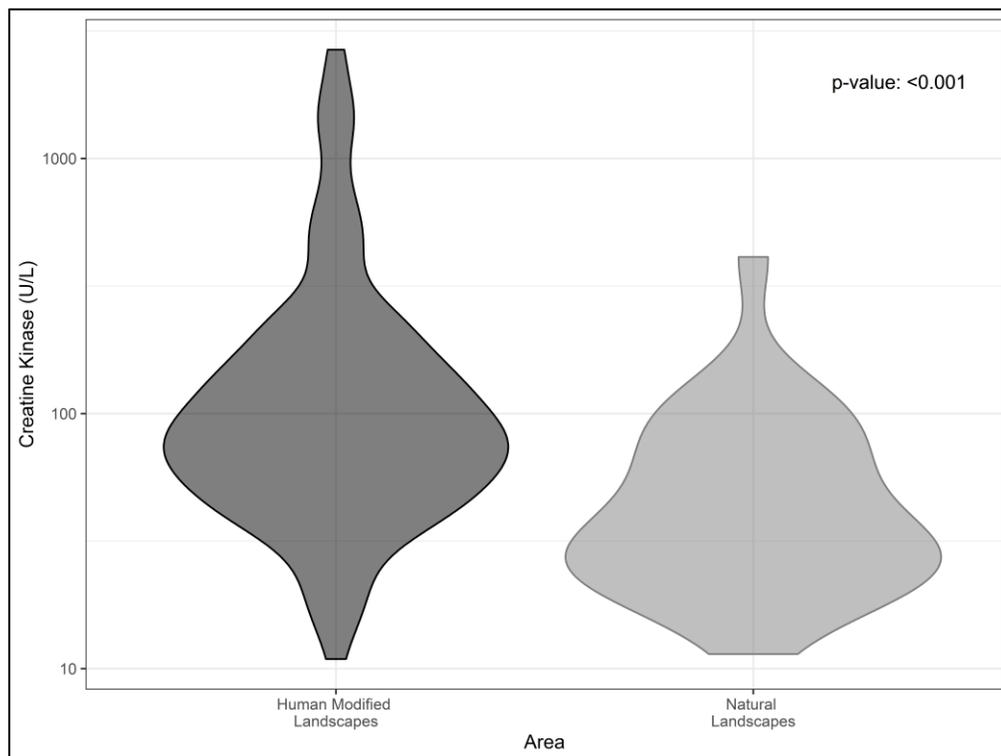
Well-established studies dealing with stress-associated changes in plasma immunoglobulin levels demonstrate that chronic stress increases the amount of immunoglobulins in the blood, leading to an increase in total protein (KIECOLT-GLASER et al., 1984; GLASER et al., 1986). That picture would justify higher values of total protein (Fig. 6) for the capybaras of NLs ( $6.3 \pm 0.6$  g / dL) when compared to the capybaras of HMLs ( $6 \pm 0.6$  g / dL).



**Figure 6 - Graphical representation of the serum concentration of total protein for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**

While the CK values recorded in our study varied widely, both within NLs and HMLs capybaras (Fig. 7 – for better visualization the graph was built in log<sub>10</sub> scale), the results were much higher than those previously reported by Álvarez-Méndez and Barragán (2014) ( $2.59 \pm$

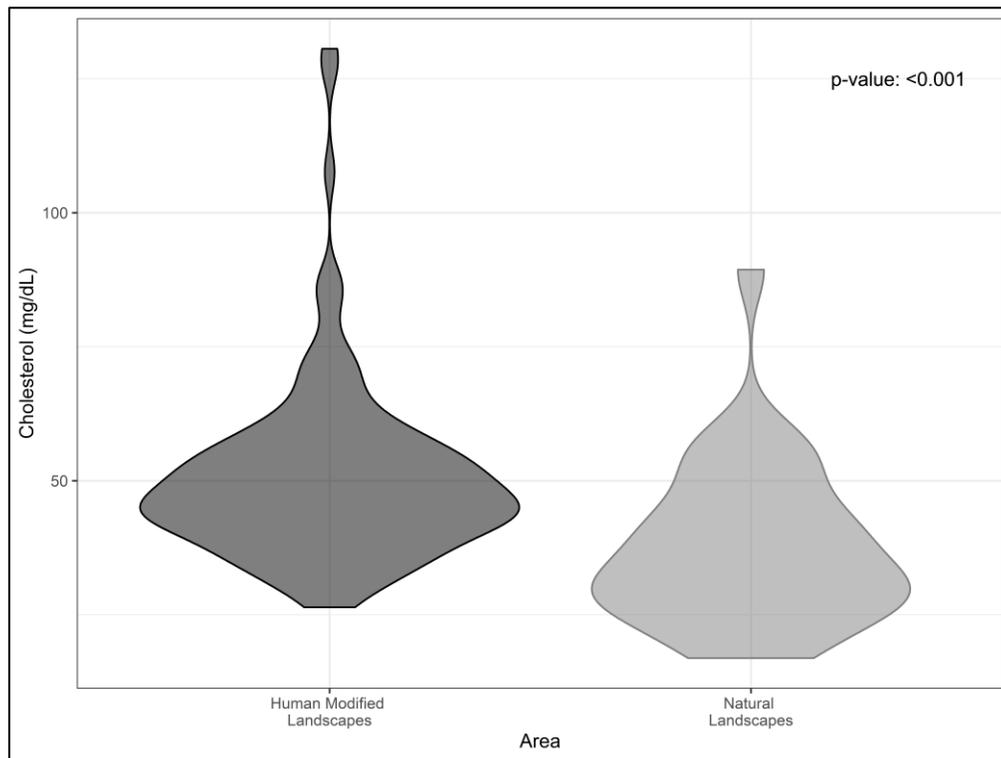
0.36 U / L). A possible explanation for this is the capture myopathy that can generate significant increases in CK in the blood (PATERSON, 2007). In the study conducted by Álvarez-Méndez and Barragán (2014), capybaras were contained for a few minutes, without anesthetic restraint, only for physical examination and blood collection. In the case of our study, capybaras captured in corrals, sometimes were confined hours until we sampled all animals. This may justify the stress and capture myopathy and the serum increases in CK.



**Figure 7 - Graphical representation of the serum concentration of creatine kinase for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**

In our study, NL capybaras had a serum cholesterol value ( $37.6 \pm 15.4$  mg / dL) close to those recorded by Matus and Pulido (2010) for captive capybaras ( $39.78 \pm 26.67$  mg / dL). However, capybaras sampled in HMLs showed serum cholesterol values quite high in relation to the above ( $50.7 \pm 16.7$  mg / dL) (Fig. 8). This is possibly related to the calorie- restricted diet that the capybaras from NLs and those from the study by Matus and Pulido (2010) were subjected to. In the first study, NLs capybaras did not have access to a high-calorie diet such as

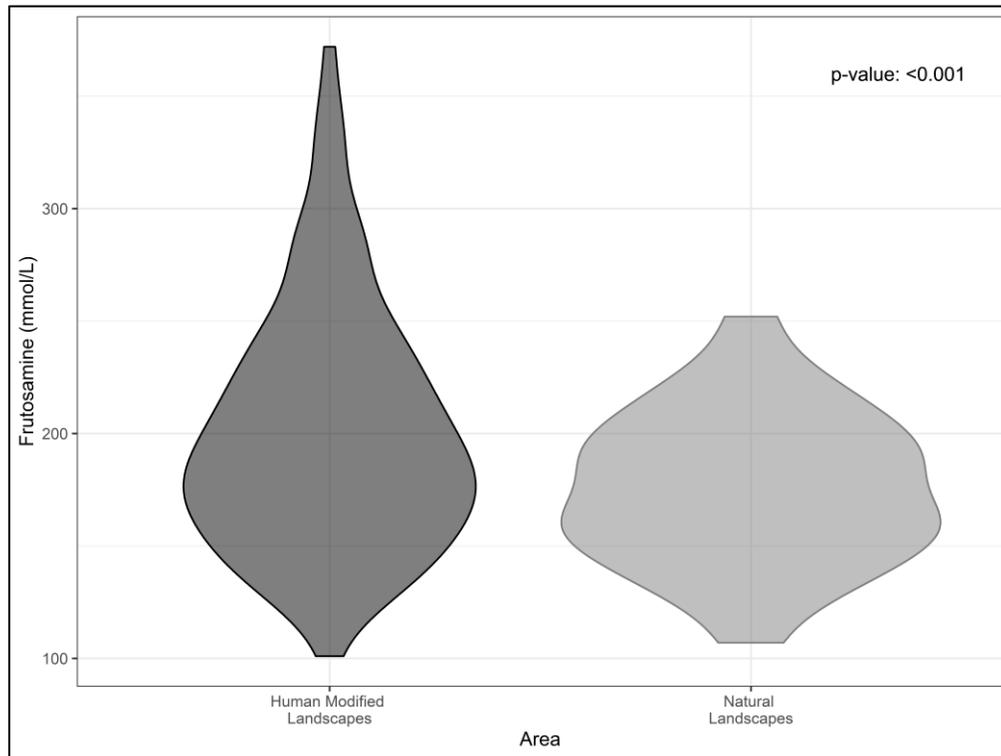
crops (LOPES et al., 2020; DIAS et al., 2020), while HML capybaras had free access to several crop fields, such as sugar cane and corn, highly energetic sources of food (LOPES et al., 2020; DIAS et al., 2020). A study carried out with Fischer 344 Rats showed that populations exposed to non-restricted diets had higher serum cholesterol levels when compared to populations with dietary restrictions (VAN LIEW et al., 1993).



**Figure 8 - Graphical representation of the serum concentration of cholesterol for capybaras from Human-Modified Landscapes and Natural Landscapes in Brazil**

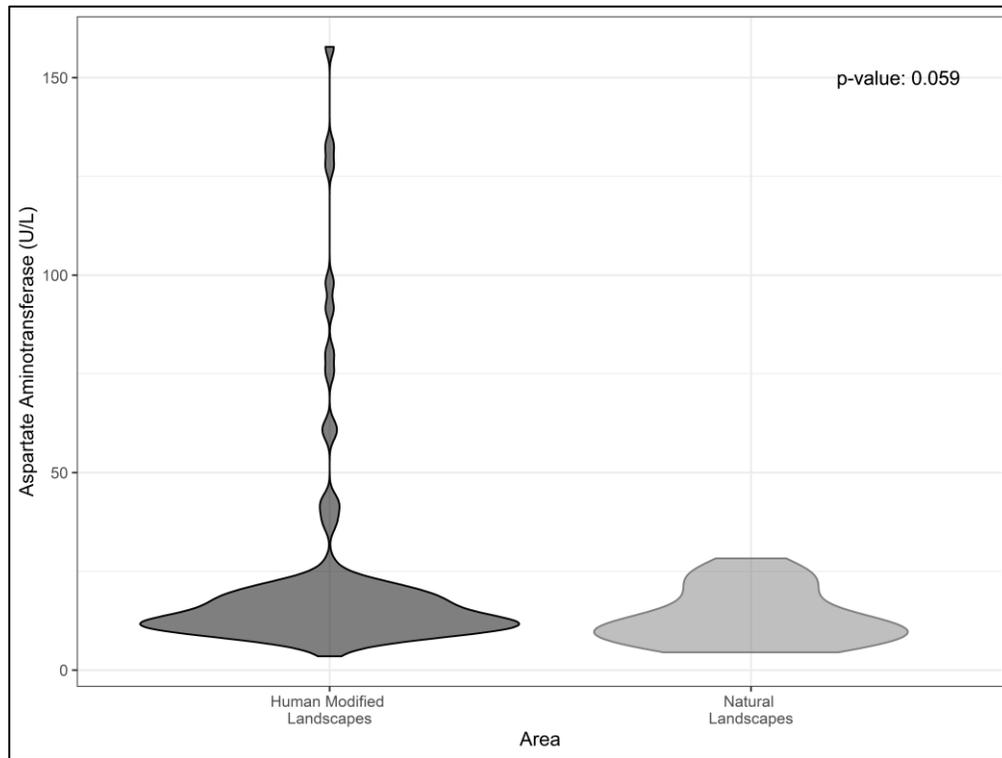
To our knowledge, this is the first work to measure fructosamine in capybaras. As previously mentioned, fructosamine gives a seven to ten-day overview of blood glucose behavior. Since obese patients have insulin resistance, our hypothesis that capybaras from HMLs, exposed to high-calorie diets, would have higher fructosamine values than capybaras from NLs (VAN LIEW et al., 1993) was confirmed. Capybaras from HMLs showed levels of

serum fructosamine above 200 mmol/L, while capybaras from NLs showed levels of 175 mmol/L (Fig. 9).



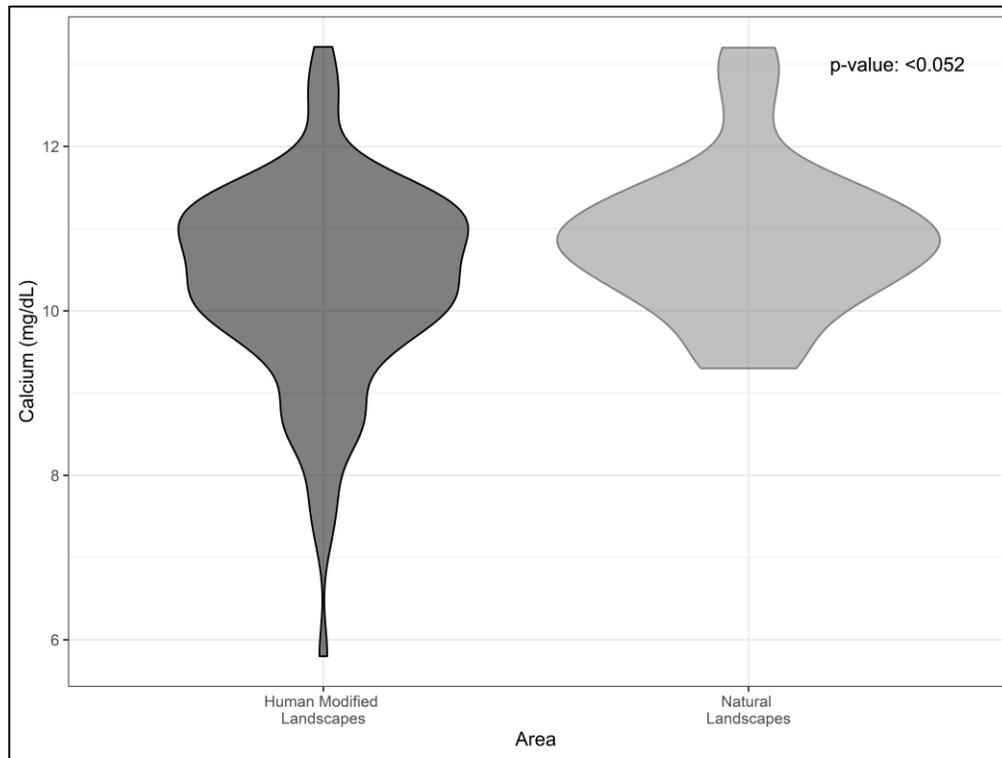
**Figure 9 - Graphical representation of the serum concentration of fructosamine for the Human-Modified Landscapes and Natural Landscapes capybara populations**

As previously defined, aspartate aminotransferase increases due to cell death for several reasons, including hepatic steatosis, but it can also increase due to muscle damage (THRALL, 2012). A recent study (LOPES et al., 2020) demonstrated that the activity and displacement of capybaras are lower in HMLs and in NLs. This decrease in the patterns of displacement and activity can generate long periods in which the animals remain in decubitus in the HMLs, justifying the borderline increase in AST due to muscle injury. In addition, the recently reported obesity of capybaras in HMLs (BENATTI et al. 2020) would justify the increase in liver enzyme due to steatosis in these animals (Fig. 10).



**Figure 10 - Graphical representation of the serum concentration of aspartate aminotransferase for the Human-Modified Landscapes and Natural Landscapes capybara populations**

Several recently published studies have highlighted a negative association between obesity and bone density or quality, confirming that excess fat is a determining factor in decreasing bone density, thus decreasing the amounts of total calcium circulating in the blood (ROSEN and BOUXSEIN, 2006; AKRIDGE et al., 2007; CHEN et al., 2010). These statements support our findings that obese capybaras from HMLs had, albeit statistically borderline, lower amounts of total circulating calcium ( $10.3 \pm 1.2$  mg/dL) than capybaras from NLs ( $10.9 \pm 0.9$  mg/dL) (Fig. 11). Changes in calcium, however, were not sufficient to cause changes in the parathormone-bone matrix axis to the point of causing variations in circulating levels of phosphorus, taking into account that the populations did not show changes in renal function since there was no difference between the nitrogen compounds; i.e., urea (THRALL, 2012).



**Figure 11 - Graphical representation of the serum concentration of calcium for the Human-Modified Landscapes and Natural Landscapes capybara populations**

Our biochemical analyzes showed that there were marked differences between the populations of obese capybaras from HMLs and non-obese capybaras from NLs. These biochemical differences, as discussed above, may be causing metabolic disorders of a nutritional nature in HMLs' populations, causing, as conjectured, higher mortality rates among these animals and, consequently, opening space for a greater population replacement of the species given a greater reproductive efficiency in animals with higher energy reserves (PRYCE et al., 2001; ROCHE et al., 2007). As recently demonstrated by mathematical models (POLO et al., 2017), this higher population replacement rate is crucial for the maintenance and expansion of BSF, since the capybara functions as an amplifying host of the disease-causing bacterium, *Rickettsia rickettsii*, to the tick vector population (*Amblyomma sculptum*).

We have chosen 11 biochemical analyzes (each one of them justified above) to determine the serum profile of capybaras from HMLs and compare them with those of the NLs in order to

verify possible differences in animal health. As a limitation in our analyses, it is noteworthy that the statistical differences between capybara groups do not necessarily infer practical difference in clinical parameters, and it is also necessary to explore the effect-size as well as the different interpretations over the confidence interval of both groups (AMRHEIN, GREENLAND and MCSHANE, 2019).

### **3.5. CONCLUSION**

Analyses of biochemical parameters on capybaras showed significant alterations in animals from HMLs, when compared to animals from NLs. Of the eleven parameters analyzed, five (albumin, creatine kinase, cholesterol, fructosamine and total protein) showed a significant difference and two (calcium and aspartate aminotransferase) were borderline, suggesting that the population residing in HMLs exhibit metabolic disfunctions related to obesity. These conditions may be related to a higher reproductive capacity of capybaras among HMLs, which in turn, could have direct implications in the maintenance of Brazilian spotted fever in southeastern Brazil.

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### **3.7.LIST OF ABBREVIATIONS**

HML – human-modified landscapes

NL – natural landscapes

BSF – Brazilian spotted fever

AST – aspartate transaminase

AKP – alkaline phosphatase

CK – creatine kinase

IACUC – Institutional Animal Care and Use Committee

CONCEA – Brazilian National Council of Animal Experimentation

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## 4. Conclusions

Prior to this work, the literature on morphological and biochemical aspects of capybaras was related only to unique populations, whether of captive or free-ranging animals. Herein, free-ranging capybaras from HMLs and NLs were compared for the first time.

By analyzing over one-hundred adult capybaras, it was showed that morphological aspects cannot be compared between populations of regions with different degrees of anthropogenic action, since it was demonstrated that capybaras living in regions with high anthropogenic interference are significantly heavier than capybaras in regions closer than where the literature considers natural for the species. This overweight is due to the accumulation of subcutaneous fat. In HMLs, the average weight for the species is more than 10 kg higher than previously recorded in the literature for the species as a whole. The average weight of the populations and their Body Condition Index varied according to the degree of anthropization of the region they occupy. In addition, the present study recorded new weight superior limits for capybaras: 105.2 kg, more than five kilograms above previous records.

The increase in weight and energy reserves can be linked to a state of obesity in the capybara populations among HMLs, when compared to the NLs, the control group representing most of the available literature records. Analyzes of biochemical factors directly or indirectly related to obesity corroborate this statement, since the populations of capybaras from HMLs, exposed to high-energy diets, presented alterations related to metabolic disorders due to overweight, as already established in the literature.

These factors, obesity and disorders related to it, may be related to the maintenance of BSF in the state of São Paulo, which was the model state for HMLs, since these possible changes may be influencing directly on the population replacement rates of capybaras, promoting the constant maintenance of the disease.

Finally, studies about the reproductive capacity of capybaras within HMLs and NLs are warranted. If the reproductive capacity is, in fact, higher among HMLs, it would prove the

theory of higher replacement rates of susceptible individuals and, therefore, the maintenance of BSF endemism.