UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

João Miguel Alves Nunes

Comportamento defensivo de Jararacas:
uma abordagem ecológica e epidemiológica

São José do Rio Preto
2022

Comportamento defensivo de Jararacas:
uma abordagem ecológica e epidemiológica

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biodiversidade, junto ao Programa de Pós-Graduação de Biodiversidade, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

Financiadora: CAPES

Orientador: Profª. Drª. Otávio Augusto Vuolo Marques
Coorientador: Profª. Drª.Selma Maria Almeida Santos

São José do Rio Preto
2022

Alves-Nunes, João Miguel.
Comportamento defensivo de Jararacas: Uma abordagem ecológica e epidemiológica / João Miguel Alves Nunes. -- São José do Rio Preto, 2022

Orientador: Otávio A.V. Marques
Coorientador: Selma M. Almeida Santos
Dissertação (mestrado) - Universidade Estadual Paulista (Unesp),
Instituto de Biociências, Letras e Ciências Exatas, São José do Rio Preto

1. Otávio A.V. Marques. 2. Paulo S. Bernade. 3. RicardoJ. Sawaya. Comportamento defensivo de Jararacas: Uma abordagem ecológica e epidemiológica

João Miguel Alves Nunes

Comportamento defensivo de Jararacas:
uma abordagem ecológica e epidemiológica

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre Mestrado acadêmico, junto ao Programa de Pós-Graduação em Biodiversidade, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista “Júlio de Mesquita Filho”, Câmpus de São José do Rio Preto.

Financiadora: CAPES

Comissão Examinadora

Prof. Dr. Otávio A. Vuolo Marques
UNESP – Câmpus de São José do Rio Preto
Orientador

Prof. Dr. Ricardo Jannini Sawaya
UFABC

Prof. Dr. Paulo Sérgio Bernarde
UFAC

São José do Rio Preto
05 de setembro de 2022

AGRADECIMENTOS

Sempre gostei de ler os agradecimentos dos trabalhos que lia. Mas acho que colocar em palavras aqui não chega nem perto do tanto que gostaria de agradecer certas pessoas.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 0001

Gostaria de agradecer ao meu Deus por ter me acompanhado e me dado saúde para realizar e continuar este trabalho (algo muito relevante para mim rsrsrsr). Agradeço a minha família, Alessandra Cristina (mãe), Reinaldo Murilo (pai), Beatriz (irmã), meus avós e tios, por serem pacientes comigo e me apoiarem nas aventuras e decisões que tomei.

Algumas pessoas foram fundamentais para que este trabalho ocorresse. O principal deles foi o Adriano Fellone. Um ser humano ímpar, justo e trabalhador. Como um técnico é o mais genial cientista que o laboratório de ecologia e evolução do Butantan teve e irá ter. Agradeço por ter percebido isso e tê-lo não só como colega de trabalho, mas amigo.

Agradeço a professora e co-orientadora Selma M. Almeida Santos. Como uma mãe, me abraçou e me acolheu num dos momentos mais difíceis e conturbados da minha relação no laboratório. Sempre demostrou apoio e me deu muito suporte com todas as minhas ideias malucas.

Agradeço ao meu Orientador, Otávio A.V. Marques. Muitas pessoas o criticam, porém, eu tive o prazer de poder conhecer mais dele e de sua trajetória e perceber como somos parecidos. Sou grato por ele ter me apoiado em tudo que propus, me deu suporte e respaldo para continuar trabalhando, mesmo depois de meu histórico de acidentes, obrigado pela confiança. Obrigado por ser um ótimo pesquisador e orientador.

Gostaria de agradecer outros professores que foram fundamentais para não só este trabalho, mas para mudar a minha carreira. Agradeço, ao professor Genoilson B. Alves, pois desde o ensino médio me incentivou e me mostrou esta carreira. Além disso, gostaria de agradecer aos professores Alexandre Adalardo de Oliveira; Adriana Maria Zanfortin Martini e Camila de Toledo Catanho por oferecerem uma das melhores disciplinas que existe e me moldar como um cientista, sempre falo que não tem palavras para agradecer o tanto que me ajudaram. Agradecer a professora Eliane Gonçalves de Freitas pois me ajudou no ínicio das minhas ideias confusas e fazer meus olhos brilharem com a carreira.

Além disso, gostaria de agradecer aos técnicos do laboratório como: Kelly, Cris, Cintia, Fabiano e Bruno, por terem tido uma grande paciência comigo e sempre estarem disponíveis para me ajudar.

Agradeço aos médicos e enfermeiros do hospital vital Brazil que me salvaram e me deram suporte durantes os acidentes que obtive realizando este trabalho.

Agradeço aos professores que fizeram parte da minha banca de qualificação e defesa, como Fausto Barbo (meu professor preferido na universidade), Leticia Sueiro, Carlos Medeiros (sempre fui seu fã, desde o hospital), Marcio Martins, Ricardo Sawaya (um dos professores mais incríveis e do bem que conheço) e ao Professor Paulo Sérgio Bernade que ainda não o conheci pessoalmente, mas admiro o seu trabalho.

É entendendo a complexidade da natureza que podemos a simplicidade humana.

RESUMO

Uma das relações mais importantes entre os seres vivo na natureza é a predação. A partir dela é que surgem os comportamentos defensivos. Essa classe comportamental é amplamente abrangente e diversificada entre os répteis, principalmente entre as serpentes. As adaptações comportamentais para se defender sofrem influência de diversos fatores ambientais e intrínsecos aos animais, tornando-se um meio pelo qual a evolução pode agir. Além disso, é através de um destes comportamentos que desencadeiam os acidentes causados por serpentes peçonhentas. O objetivo deste trabalho foi entender o comportamento defensivo sob uma perspectiva ecológica e utilizá-lo como ferramenta no entendimento da epidemiologia de acidentes ofídicos. Com base nisso, o grupo *Bothrops* se tornou um excelente objeto de pesquisa. Dentro deste grupo, *Bothrops jararaca* é uma das serpentes de maior representatividade em acidentes ofídicos do país além de possuir uma história evolutiva de espécies relacionados que podem exercer influência no comportamento defensivo. *Bothrops insularis* e *Bothrops alcatraz*, são duas espécies endêmicas de ilhas, com origens e parentesco com *B. jararaca* semelhantes, porém possuem ecologias e pressões de predação diferentes. O trabalho foi divido em dois capítulos. O primeiro trata da associação entre fatores bióticos e abióticos no comportamento defensivo e padrões epidemiológicos, utilizando a *B. jararaca*. O segundo capítulo, visa entender se o comportamento defensivo da jararaca continental e as jararacas insulares sofre influência dos tipos de estímulos predatórios e como são os padrões comportamentais exibidos. Com base nisso, foi obtido um estudo do comportamento defensivo abrangente e relevante**.**

PALVRAS-CHAVE: Comportamento defensivo. Jararaca. Jararacas insulares. Epidemiologia. Ecologia

ABSTRACT

One of the most important relationships between living beings in nature is predation. It is from these defensive behaviors arise from it. This behavioral class is wide-ranging and diverse among reptiles, especially snakes. The behavioral adaptations to defend themselves are influenced by several environmental factors and intrinsic to the animals, becoming a means by which evolution can act. Furthermore, it is through one of these behaviors that accidents caused by venomous snakes are triggered. The objective of this work was to understand defensive behavior from an ecological perspective and use it as a tool to understand the epidemiology of ophidian accidents. Based on this, the *Bothrops* group became an excellent object of research. Within this group, *Bothrops jararaca* is one of the most representative snakes in ophidian accidents in the country, besides having an evolutionary history of related species that may influence defensive behavior. *Bothrops insularis* and *Bothrops alcatraz* are two endemic species of islands, with similar origins and kinship with *B. jararaca*, but with different ecologies and predation pressures. The paper is divided into two chapters. The first deals with the association between biotic and abiotic factors in defensive behavior and epidemiological patterns, using *B. jararaca*. The second chapter, aims to understand if the defensive behavior of the continental jararaca and the insular jararacas is influenced by the types of predatory stimuli and what the behavioral patterns exhibited are like. Based on this, a comprehensive and relevant study of defensive behavior was obtained.

KEY WORDS: Defensive behavior. Jararaca. Insular jararaca. Epidemiology. Ecology

Sumário

1. CHAPTER 1: Association between defensive bite behavior and snakebite epidemiology: A new tool for understanding epidemiological data

1.2 INTRODUCTION

Snakebite is a potentially fatal disease caused by toxins in the bite of a venomous snake. About 4.5-5.4 million people are bitten annually by snakes worldwide (WHO, 2021). Snakebite has been classified by the World Health Organization (WHO) as a high-priority neglected tropical disease. Most of the research on snakebites is focused on venom - its properties, effects, and antidotes (LANCET, 2019). The rest focuses mainly on the analysis of incidence and individual risk factors (GUTIERREZ *et al*., 2017), however, studies related to snake ecology remain a less discussed topic.

Snakebite shares many epidemiological similarities with zoonoses. Understanding the ecology of hosts and vectors, as well as pathogens, has been critical to the control of zoonoses (MORRI *et al*., 2019). There is very little research that relates snake ecology to snakebites. The epidemiological understanding of ophidian accidents remains limited, which hinders efforts to accurately map, predict, and mitigate the risk of ophidian accidents (MORRI *et al*., 2019)). Therefore, a more dynamic understanding of the ecological determinants of venomous snakes will greatly enhance control. Developing the ability to interpret the impact of ecological, behavioral, and environmental parameters may provide new tools to reduce human-snake contact and prevent the impact of ophidian envenomation on communities (WHO, 2021).

One of the important ecological behavioral variables for the study related to snake bites is antipredator behavior. However, to date, there has been no study that relates the two topics with the property. From the accidental encounter between a human and a snake, the snake will defend itself and may use one of its antipredator behavior mechanisms (biting) and poison the humans (GUTIERREZ *et al*., 2017). Therefore, when studying biting behavior, we are studying the event that generates the poisoning. This behavior is highly influenced by various intrinsic or environmental factors (STANKOWICH & BLUMSTEIN, 2005). Defensive tactics can change in frequency depending on body size (WHITAKER *et al*., 2000; SHINE et al., 2002), sex (SHINE *et al*., 2000; WEBB *et al*., 2001), aversive stimulus (SCUDDER & CHIZAR, 1977; LLEWELYN *et al*., 2010), body temperature (MORI & BURGHARDT, 2004; WHITFORD *et al*., 2020), and a variety of extrinsic factors (SHINE *et al*., 2000, 2002). These variables can

influence the defensive repertoire in different ways, depending on the context and species of each snake.

Bothrops jararaca is the most common species occurring in the southeast region of Brazil, being responsible for the majority of accidents, about 90% of snakebite cases in the country (FRANÇA & MALAQUE, 2003; RIBEIRO & JORGE, 1997). Due to the diversification of environments of occurrence, cryptic coloration, and high relative abundance, *B. jararaca* has high representativeness of ophidian accidents in Brazil, being of great interest to public health (PUZZI *et al*., 2008). Sazima (1988, 1992) studying the defensive behavior of *B. jararaca* in the field suggested that the observed behavioral patterns of this species might coincide with the epidemiological data of snakebites, suggesting the study of the ecology of this species to understand and predict the epidemiological data.

Therefore, our goal is to analyze whether intrinsic and environmental aspects interfere with the defensive behavior of *B. jararaca*, and how this is associated with the epidemiology of both snakebites. More specifically, to analyze whether morphological properties, sex, life stage, and environmental aspects such as aversive stimulus, environmental temperature, and time of day interfere with the probability of attack and tolerance of *B. jararaca*. Furthermore, to investigate the association between data on the probability of strike of *B. jararaca* and the average number of snake bites caused by this species.

1.3. METHODS

1.3.1. **Study Animal**

We used 116 specimens of the *Bothrops jararaca*. Seventy-five adult snakes (37 females and 38 males: > 10 years old), 13 juveniles (4 males and 9 females: 1.5 years old), and 29 newborns (13 males and 16 females: 14 days years old). Specimens were born in captivity, except for twenty-five adult snakes (10 females and 15 males) that came from the wild in cities in São Paulo state, which were at least 2 years in captivity. All animals were kept in individual boxes (45x30x15), containing a cardboard substrate and water pot, at a temperature of around 25 °C, under a light and dark photoperiod of 12:12. Biometric data were collected from the snakes as SVL of Snout-Vent length (cm).

1.3.3 **Behavioral Tests**

To perform the behavioral tests of aggressiveness, confrontations between a human and the snake were performed, simulating an accident. The confrontation tests were carried out between October and November 2021, a period of the year that the number of accidents caused by this species increased (SILVA *et al.*, 2003), and it is also the period of higher daily activity of the animals (Siqueira *et al.*, 2021). For the confrontations, a 2.025 m² (1.5m (length) X 1.35m (width) X 1m (height)) arena was set up, with an aluminum plate as the base, and Styrofoam walls. For each behavioral test, the snakes were left in the arena for up to 15 minutes in order to cease exploratory behaviors and habituate to the test arena. The confrontation experiments were performed with and without physical contact between the animal's body and humans. For the contact stimulus, the tester wore an appropriate safety boot and performed 30 steps by lightly touching three regions of the animal's body (head, midbody, and tail) in random order at 3-second intervals. In contrast, for the non-contact stimulus, the tester stepped 5 cm away from the snake body regions, randomizing the order, without touching the animal. The experiments were conducted during two periods of the day. In the morning period, the tests were performed between 8 and 11 am. At night, the tests were performed between 6 and 10 pm. A break of five days was given between the stimulus in order to avoid stress and learning influences (GLAUDAs *et al.*, 2006). In each test, the snake's temperature was measured with an infrared thermometer **(**ST-620, Incoterm**)** at a distance of 2 meters to avoid stress before the confrontations. The temperature of the animals ranged from 15°C to 23°C. All experiments were recorded in order to analyze later, using a film camera (HDR-PJ200, Sony).

1.3.4 **The temperature of occurrence of *Bothrops jararaca***

To find out the temperature at which *B. jararaca* occurs in the wild, we collected the distribution data of *Bothrops jararaca* according to Nogueira et al.(2019). An analysis was performed using a point sample tool of *QGis 3.18 software*. Each point was applied under a raster layer with climate data. For the high and cold temperature data, the bioclimatic variables BIO5 (maximum temperature of the warmest month) and BIO6 (minimum temperature of the coldest month) were used, respectively. The climate data were acquired from *WorldClim* version 2.1 between the years 1970 and 2000. This version was released in January 2020. After collecting the temperatures of *B. jararaca* occurrence, the average was taken to be able to make the predictions of the probabilities of bites.

1.3.5 **Statistical Analysis**

1.3.5.1 **Behavioral tests**

The statistical analyses were based on the experimental design. The categorical predictor variables were the sex of the animal (two levels: male and female), human stimulus (two levels: with and without physical contact), period of the day (two levels: morning and night) and the continuous independent variables were size (SVL) and temperature (°C) of the animal. The response variables were **bite probability and tolerance.** The bite probability was defined by the number of successes in a certain number (n) of trials (number of Bernoulli events). The number of attacks (bites) was defined as success whereas the number of confrontations (n=30) minus the number of attacks was defined as failure (all anti-predator behaviors that did not involve attacking and biting the man were considered as failure). Tolerance was defined as the number of stomps with or without physical contact that the animal endured until the human received the first bite. The Variance Inflation Factor (VIF) index was used to find some collinearity between the predictor variables, considering the VIF value 4 as the acceptable limit between the variables (Shrestha, 2020). However, there was no collinear relationship between the variables.

Due to the nature of these variables and based on the experimental design, a Generalized linear mixed model (GLMM) with binomial distribution and logit link function was used for the bite probability data and for the tolerance data, GLMM with Poisson distribution and log link function was used. All data were subsequently exponentiated for the interpretation of the results. Since the model does not support the analysis of the additive and integrative properties of the five variables, models were built without temperature, and subsequently included temperature in the contexts where the contact and without human contact. For the general model, where the additive relationship and integration of sex, day period, human stimulus, and size were analyzed, the variables temperature, "ID" of each animal, and origin (born or not in captivity) were used as random variables, since the experiments were performed with the same animals and at different temperatures, thus eliminating certain pseudoreplication and experimental design problems. For the models involving temperature, we evaluated the additive and integrative relationships between the fixed variables temperature, size, sex, and period of the day in each human stimulus. In this case, only the animals' ID and origin were used as random variables.

For the life stage analyses, 116 animals were used. The model was also a GLMM with binomial and Poisson distributions, for bite probability and tolerance, respectively. The fixed variables were life stage, day period, and sex, and the random variables were animal size, individual, and environment of origin. Using ANOVA tables and AIC values, the minimum adequate model was selected. In addition, we performed Tukey's Test for Post-hoc Analysis, in order to find significant relationships between the variables. All models were subjected to data dispersion analysis, homoscedasticity, and outline testing using model diagnostic values and plots, with the help of the "DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models" package in R.

To predict the bite probabilities was used the estimated coefficients of the minimum adequate models, multiplying by the minimum temperature of 15°C and maximum temperature of 30°C, and multiplying size by the average size of 85 cm of the *B. jararaca* population.This size was chosen because it is the average size between males and females, therefore the size of *B. jararaca* is most likely to be found in the wild.

1.3.6 **Epidemiological data**

1.3.6.1 **Epidemiological data from the coast and plateau of São Paulo**

To analyze the data on accidents caused by *B. jararaca* on the coast and plateau of São Paulo was used data on bothropic accidents from the Brazilian government's Information System for Notifiable Diseases (SINAN). The number of cases of bothropic accidents (accidents caused by snakes belonging to the *Bothrops* genus) per year between 2009 and 2017 were collected from the thirty municipalities in São Paulo that had the most cases. To differentiate the municipalities between geographic regions, it was defined that cities occurring below 80 meters of altitude belonged to the coast (lowland) and municipalities above 80 meters of altitude as plateau (highland).

The number of accidents was estimated and corrected using the population number of each municipality (IBGE, 2021). The number of accidents per 100,000 habitats was estimated. We fitted a GLMM using the 'glmmTMB' R package (BROOKS *et al*., 2017) using the negative binomial family (nbinom1) which was selected from alternatives based on tests of overdispersion, Akaike's information criterion, AIC, values, and inspection of model diagnostic plots. The fixed variable was geographic region (two levels: plateau and coast) and the random variables were accident year and municipality identification.

1.3.6.2 **Association between size and jararacas involved in snakebites**

The *B. jararaca* involved in accidents were obtained from the Alphonse Richard Hoge herpetological collection of Instituto Butantan. These are snakes that people who have been bitten bring with them when they go to the Vital Brazil hospital at the Butantan Institute. We collected the sex and size of 422 individuals of *B. jararaca* involved in accidents from 1969 to 2005 in the plateau of São Paulo state. We performed a histogram between which body size was more frequent in accidents.

1.3.6.3 **Epidemiological data from São Paulo associated with temperatures**

In this test, we wanted to investigate whether there was an association between the temperature of the municipalities and the average number of bothropic accidents. For this, we collected the temperature of the municipalities evaluated in the previous analyses using the Point sample tool, similar to the method used to acquire the occurrence temperatures of *B. jararaca*. In this case, a raster layer was used with the climate variable BIO1 (Annual Average Temperature) provided by *WorldClim* version 2.1. With the data of **mean annual temperatures** and the **number of bothropic accidents** provided by SINAN, a GLMM with Poisson distribution and log-link function was performed.

1.4 **RESULTS**

1.4.1. Behavioral influence of intrinsic and environmental factors

We found that both the snake's intrinsic and environmental factors are able to influence the aggressiveness and tolerance of *B. jararaca* (Table S1,S2,S3,S4). There was an interaction between sexual, morphological and environmental variables influencing both bite probability and tolerance. Sex, time of day, and type of human stimulus (with or without contact), when evaluated alone, had no clear influence on both the likelihood of biting and tolerance of *B. jararaca*, but their interaction clearly interfered with the animals' behavior. The period of the day has an influence on the aggressiveness of females regardless of the stimulus, while males were influenced by the period of the day only when there is no physical contact ($z = 3.204$; $p = 0.0295$) Females subjected to physical contact with humans are more likely to bite during the morning than at night ($z = 3.692$; $p = 0.0055$) as well as when there is no physical contact ($z = 4.263$; $p = 0.0005$) (Fig 1).

Physical contact between man and snake increased the likelihood of bite for both females (z = 3.900; p= 0.0024) and males (z = 6.777; p <.0001), regardless of the period of the day (Fig 1). Moreover, males, when subjected to human stimulation without physical contact, are more tolerant than females (z= 3.220; p= 0.00128).

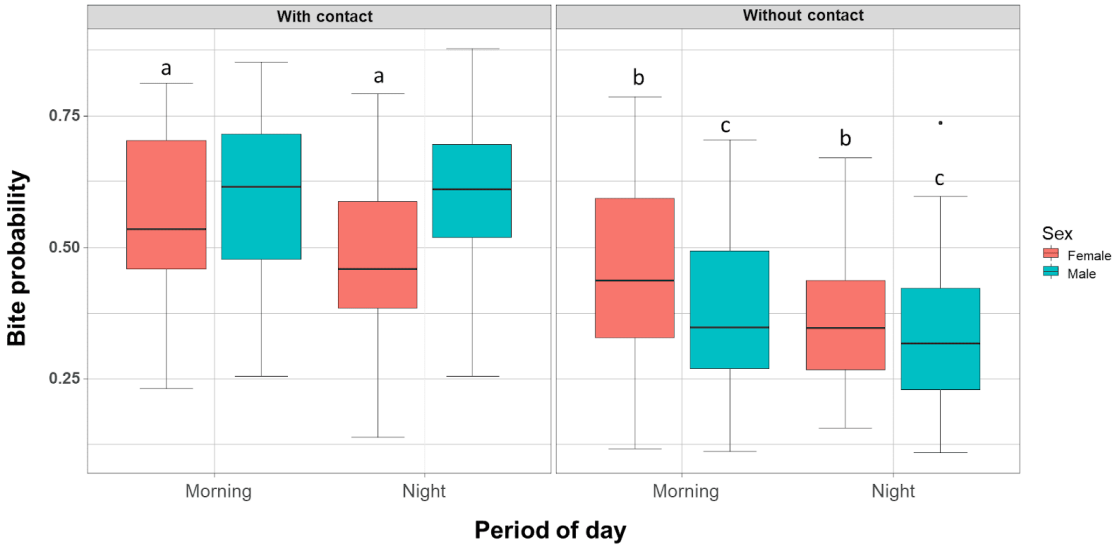


Figure 1. Biting probability of adult males and females of *B. jararaca* subjected to human stimulation with and without contact during the morning and night periods. a: clear statistical difference (p<0.05) found between females subjected to physical contact at different times of the day. b: clear statistical difference (p<0.05) found between females subjected to stimulation without physical contact at different times of the day. c: clear statistical difference (p<0.05) found between males subjected to stimulation without physical contact at different times of the day.

We observed that animal size influenced bite probability when snakes are subjected to physical contact, showing a negative correlation, i.e. smaller snakes are more likely to attack than larger ones (z= -2.922; p= 0.003481), but when there is no contact, size does not interfere with attack probability (z= -0.216, p= 0.828811) (Fig 2). In addition, the number of times the animal tolerated the stimulus, regardless of whether or not it had contact, body size had no

significant influence (z= -0.991, p= 0.32186).

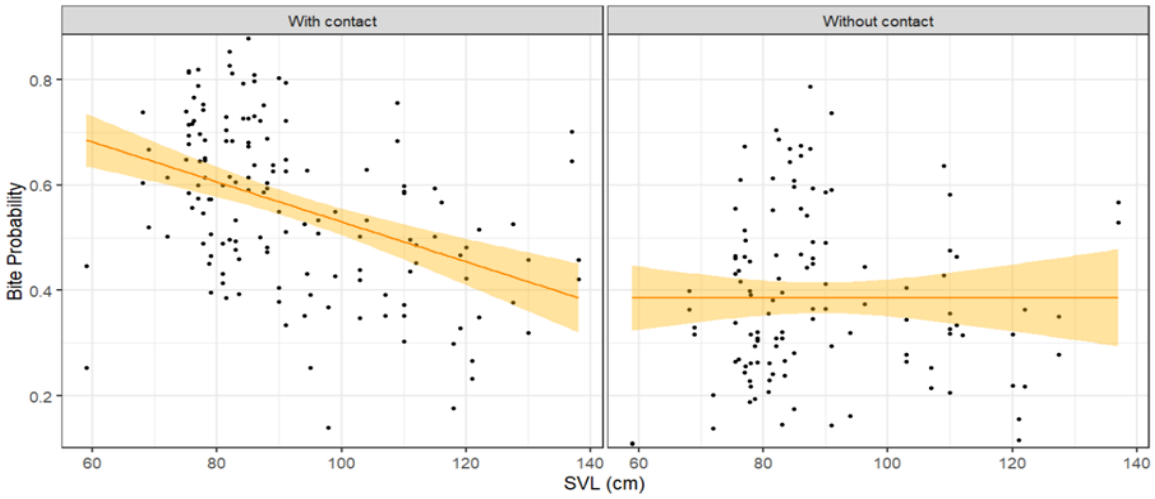


Figure 2. Effect of size (SVL) and human stimulus on the probability of bite *B. jararaca* adults.

In addition, the interaction between animal size, sex, type of human stimulus, and time of day showed a clear interference in bite probability (z= -2.978; p= 0.002901) (Fig3) and tolerance (z= 2.714; p= 0.00664) (Fig4). In general, smaller females are more likely to bite mainly when they are touched during the morning period, however, for males, the opposite occurs. Smaller males are less likely to bite. Nonetheless, there is still an exception, males that are touched during the morning period, are more likely to bite, just like females (Fig3).

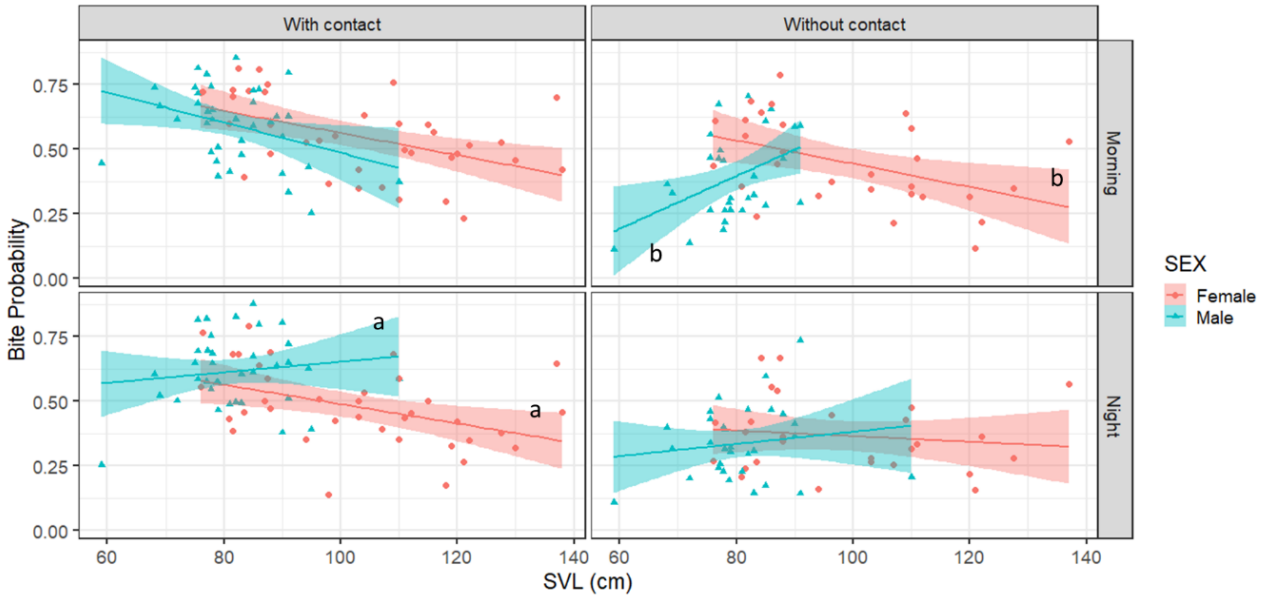


Figure 3. Biting probability being influenced by animal size (SVL), sex, type of human stimulus and period of the day. a; b: Clear statistical difference between female and male lines ($p < 0.05$).

Furthermore, when there is physical contact between humans and *B. jararaca*, both males and females, regardless of size and time of day, have a tendency to be intolerant to stimulation. However, when there is no physical contact, smaller males are more tolerant than larger ones during the morning period ($z = 3.220$; $p = 0.00128$) (Fig4).

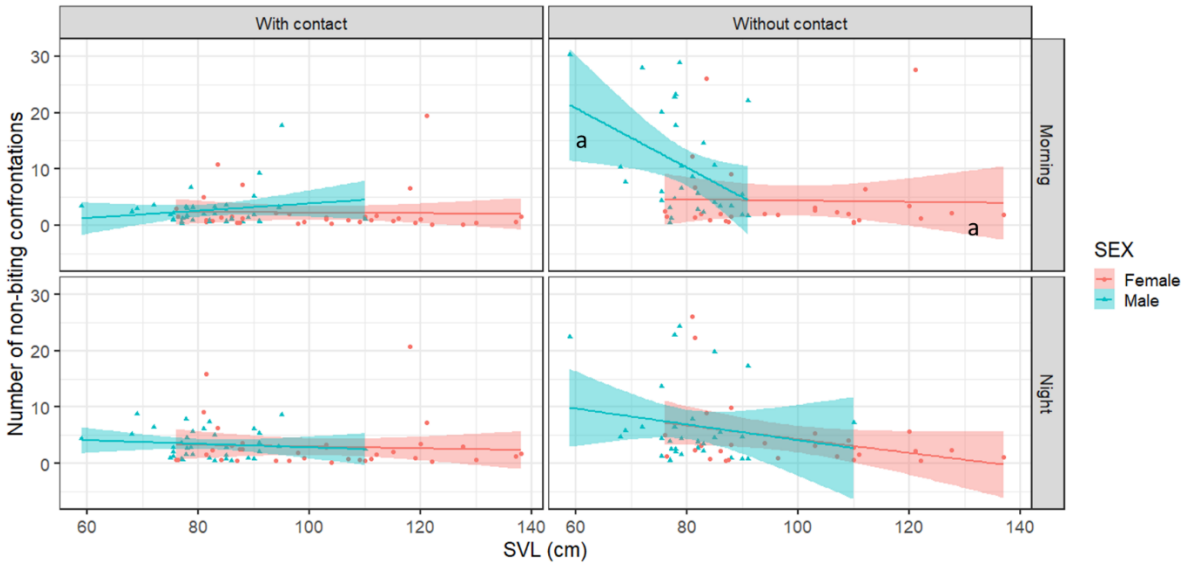


Figure 4. Tolerance (number of confrontations with or without non-bite contact) of *B. jararaca* is influenced by animal size (SVL), sex, type of human stimulus, and time of day. a: Clear statistical difference between female and male lines ($p < 0.05$).

Additionally, we found that the probability of a bite is also influenced by the part of the animal's body where the physical contact is made. When there is contact with the head the probability of attack is higher than in the middle and on the tail ($z = -88.87$, $p < 0.00001$). Contact in the middle of the body and tail decreases the odds of a bite by 78.039% ($z = -1010.77$, $p<0.00001$) and 93.320% ($z = -1804.28$, $p<0.00001$) respectively, than when there is contact with the head.

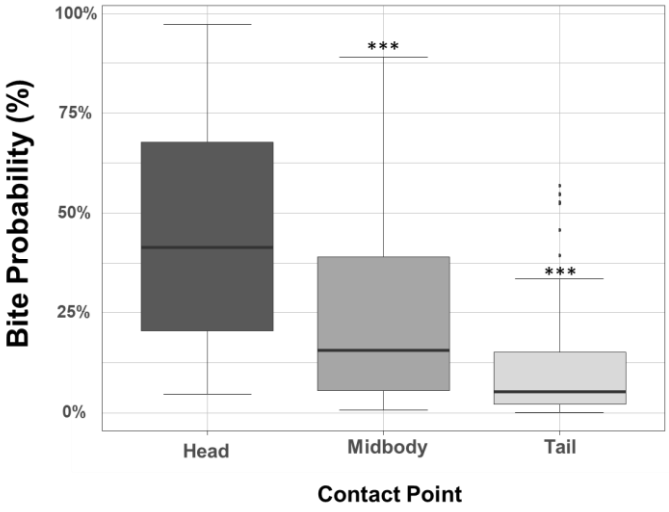


Figure 5. Probability of bite when there is physical contact with the head, midbody and tail of *B. jararaca.* *** clear difference in bite probability between tail and midbody with the stimulus made on the head (p<0.001).

In the context of physical contact between humans and snakes, the temperature of the animal influenced the probability of attack. Females in both nighttime and daytime periods, animal temperature showed a positive correlation, i.e., warmer animals have a higher tendency to bite (z = 3.868, p = 0.000110) (Fig 6). But for males, during the night period, there is a negative correlation, in which warmer animals are less likely to attack (z= 14.840; p<0.00001) (Fig6).

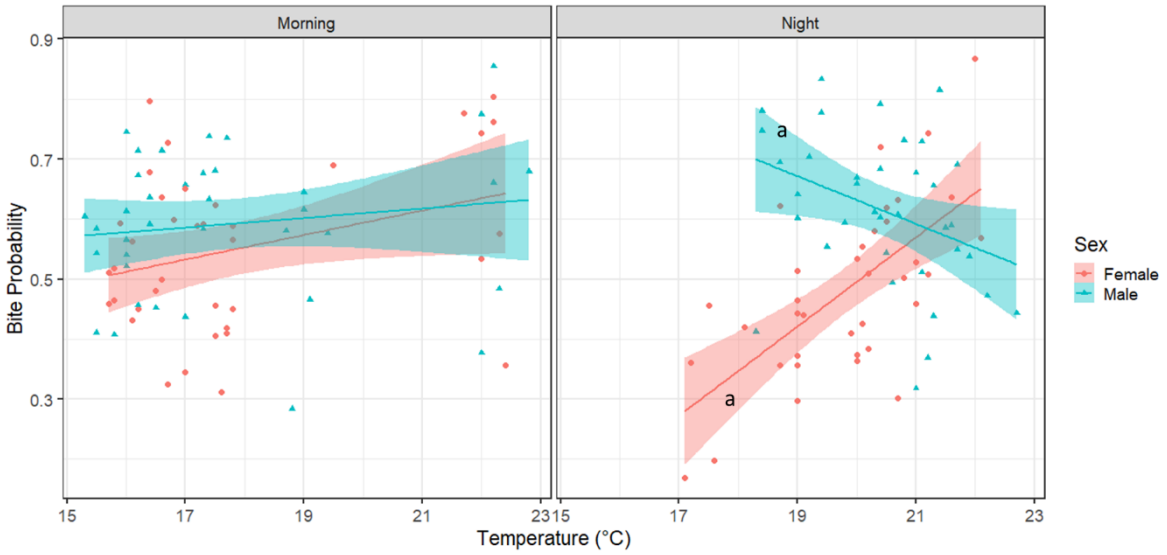


Figure 6. Influence of body temperature on the bite probability of females and males of *B. jararaca*, in morning and evening periods. a: Clear statistical difference between female and male lines ($p < 0.05$).

In addition to these effects mentioned, when the animals are stepped on, we observed that the probability of biting changes according to the life stage of *B. jararaca*. Newborns have a higher bite probability than adults, with a bite probability 2.17 times higher than the adult chance ($z = 3.533$; $p = 0.00041$). Youngsters, on the other hand, have no clear differences from adults ($z = 1.362$; $p=0.17310$) (fig.7). Stimulus tolerance, on the other hand, was not found to have a clear difference between life stages.

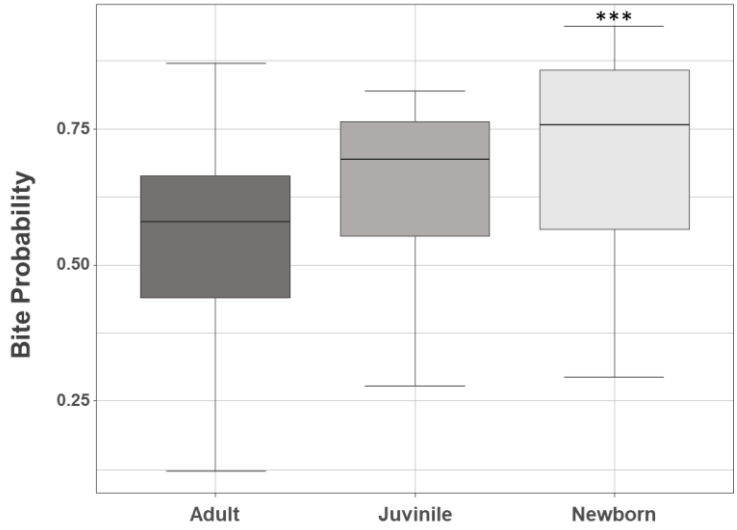


Figure 7. Probability of bite depends on the life stage of *B. jararaca.* *** Clear statistical difference in probabilities between adults and newborns ($p<0,001$).

The interaction between life stage and sex proved to be important. The time of day did not influence the probability of biting in the life stage ($z = -0.710$; $p=0.477779$). However, when we analyzed the interaction between life stage and sex, both during the day and at night, there was a significant difference in the probability of biting between the sexes of newborns and adults (*Newborns:* $z = -2.211$; $p=0.027050$; *Adults:* $z = 2.783$; $p=0.005392$) but not among juveniles ($z = -0.619$; $p=0.53570$). In general, newborn females were more aggressive than other animals, regardless of the time of day (Fig. 8).

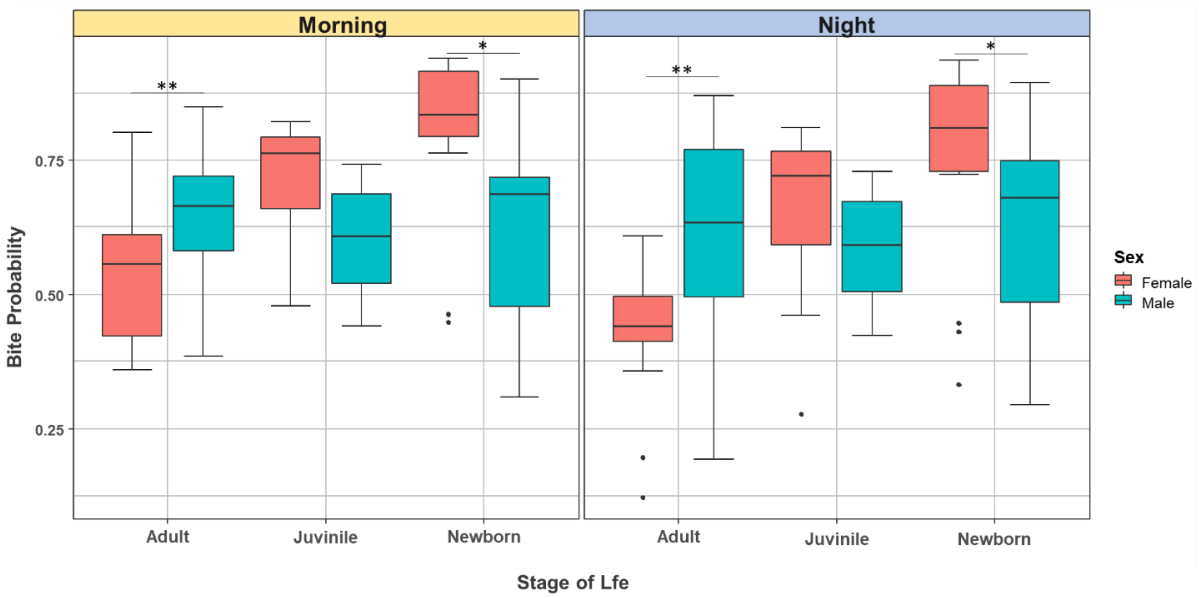


Figure 8. Biting probability of *B. jararaca* of different sexes, life stages and times of day. ** Clear statistical difference between adult males and females both day and night ($p<0.01$). * Clear statistical difference between newborn males and females both day and night ($p<0.05$).

1.4.2. Bite Probability Prediction

We obtained the local temperature of each point of occurrence of *B. jararaca*. The average minimum temperature was about 15°C while the average maximum temperatures chosen were 30°C (Figure S1). With these temperatures we were able to predict the probabilities of biting for males and females, average size 85 cm (average size of a *B. jararaca* population), when there is or is no contact with humans, at night and in the morning (table1). We observed that the greatest probability of attack is during the morning periods at higher temperatures, but it is possible to observe that the probability of bite suffers much influence from the interaction between the multiple environmental and intrinsic variables of the animal (table1).

A

Data	Male ♂							
	SVL (85 cm)							
	Morning Period				Night Period			
	Minimal temperature (15°C)		Maximal temperature (30°C)		Minimal temperature (15°C)		Maximal temperature (30°C)	
Stimulus	With contact	Without contact	With contact	Without contact	With contact	Without contact	With contact	Without contact
Probability of Bite	51%	90%	83%	100%	86%	25%	19%	57%

B

Data	Female ♀							
	SVL (85 cm)							
	Morning Period				Night Period			
	Minimal temperature (15°C)		Maximal temperature (30°C)		Minimal temperature (15°C)		Maximal temperature (30°C)	
Stimulus	With contact	Without contact	With contact	Without contact	With contact	Without contact	With contact	Without contact
Probability of Bite	53%	41%	91%	88%	14%	23%	96%	63%

Table 1. Predicted bite probability for each in each scenario where there is or is not physical contact between male and female snakes of size 85 cm and humans, at different periods of the day and different temperatures. (A) Male; (B) Female.

Another interesting result was that Moraes (2008) showed that jararacas from the coast are smaller than those from the highlands. Due to finding an influence of size on the aggressiveness of *B. jararaca*, we performed bite probability predictions for body size of the populations. As already predicted by the model, animals from the coast obtained higher probabilities of attack than those from the plateau (Table S5).

1.4.3. Epidemiological data of snakebites by *Bothrops jararaca*

1.4.3.1. Epidemiological data from the coast and plateau of São Paulo

Since *B. jararaca* from the coast are probably more aggressive than those from the highlands, we investigated the number of bothropic accidents from these regions. As expected, more bothropic accidents occur on the coast than on the plateau (Table S6; Figure S2). On the coast, an average of 36.75 accidents per 100 thousand inhabitants occurs annually, while on the plateau, 10.64, and the odds of an accident occurring on the plateau are lower than on the coast ($z = -4.571$, $p = 0.00000485$) (fig 9).

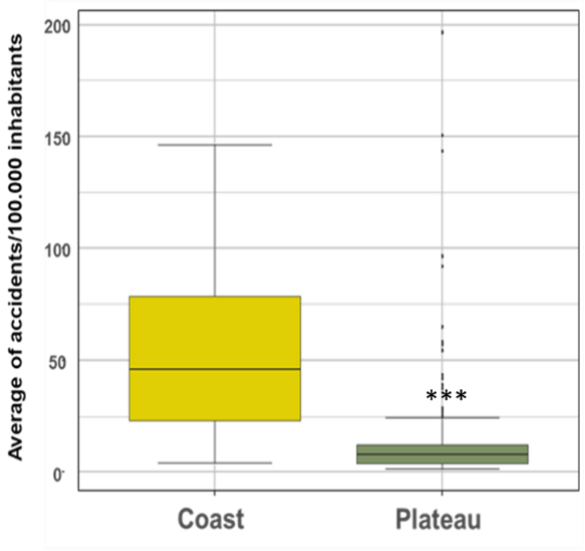


Figure 9. Average number of bothropic accidents from 2009 to 2017, on the coast and plateau of the state of São Paulo. *** clear statistical difference in the average number of accidents between the plateau and coastal regions (p<0.001)

1.4.3.2.Epidemiological data from São Paulo associated with temperatures

We found an association between the number of annual accidents originating in both sexes per 100,000 inhabitants of the municipalities of São Paulo and their temperature. A positive relationship was found, in which municipalities with higher average annual temperatures also had higher accident rates (Table S7). For each degree of temperature, the odds of snakebite occurrence increase about 28.27% (z=2.416, p = 0.0157) (Fig10).

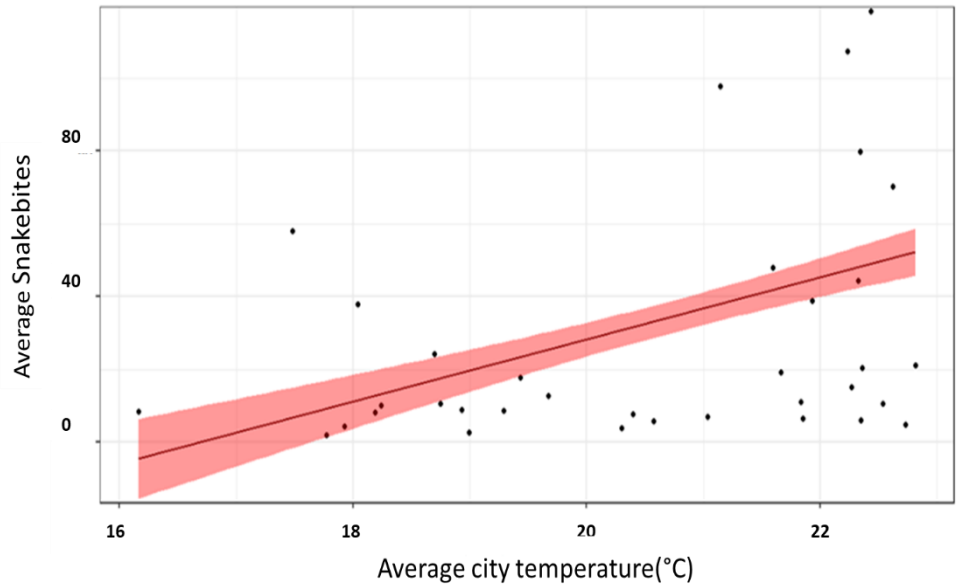


Figure 10. Association between the average number of bothropic accidents in the municipalities of São Paulo and their mean annual temperature.

1.4.3.3.Association between size and jararacas involved in snakebites

In addition, we found that both female and male jararaca involved in accidents in the São Paulo Plateau, tend to be smaller than the average population size of *B. jararaca* sampled by the collection The average size of the males most involved in accidents is 760 mm while the average male population size sampled by collection can range between 785 mm and 875 mm (Moraes, 2008; Siqueira *et al*., 2018). The average size of the female population of *B. jararaca* from the plateau of São Paulo ranges between 1097 mm and 1236.7 mm, and the average size of females involved in accidents is 880 mm (Moraes, 2008; Siqueira *et al*., 2018) (fig.11).

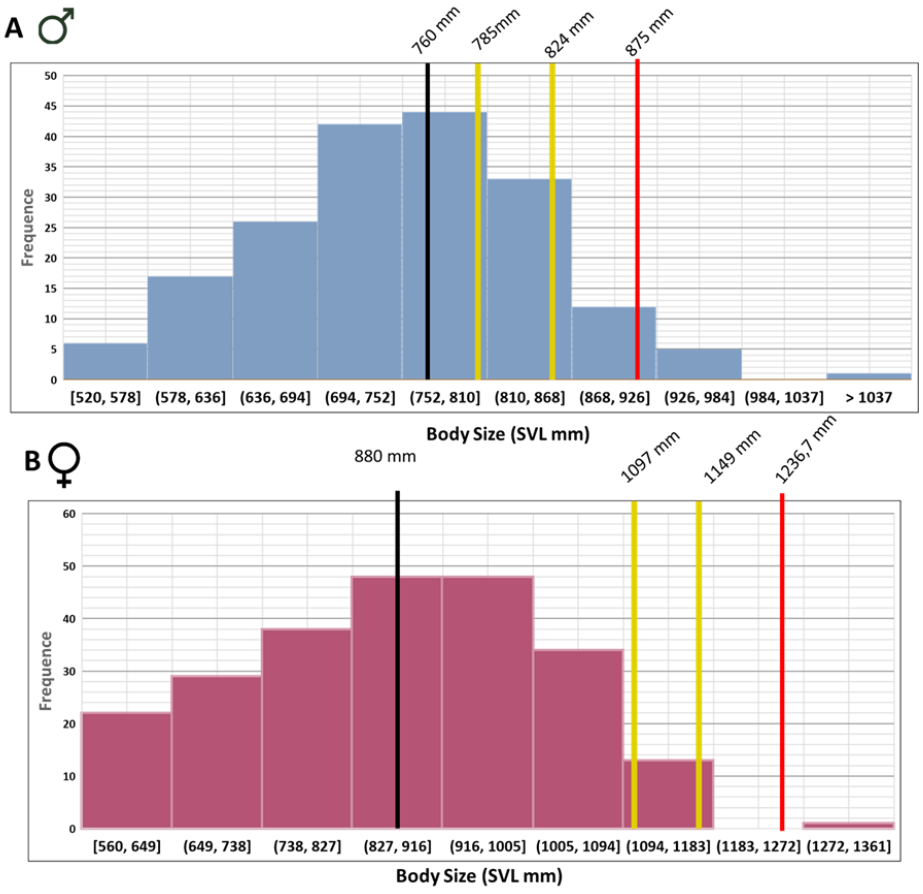


Figure 11. Histogram of the size of *Bothrops jararaca* involved in accidents in the São Paulo lowlands. A- Males; B- Females. Black bar: mean size of snakes involved in accidents. Red bar: mean size of *B. jararaca* from the São Paulo plateau (Moraes, 2008). Yellow bars: mean size of two populations from the municipality of São Paulo (Siqueira *et al*., 2018)**.**

1.5. DISCUSSION

This is the first study to demonstrate the link between the influence of intrinsic and environmental factors on snake aggressiveness and epidemiological data on snakebites. The aggressiveness of snakes, and its relationship to the chance of causing an accident, was defined as the probability of effecting a bite, with anti-predator behavior being responsible for the poisoning in humans. We found that this probability is influenced by the context in which the snake is. The probability changes depending on the integration of snake size, sex, life stage, type of aversive stimulus, what region of the body is touched, the temperature of the animal, and time of day. Our results are in agreement with evidence that animals have the ability to assess the risk of being eaten and respond in the best way (LIMA & DILL, 1990; IBANEZ et al., 2014). Furthermore, according to the snake's natural history, context influences defensive mechanisms in different ways. For example, habitat use, snakes that use the sit-wait strategy (such as *B. jararaca*) use more crevices and holes and therefore, instead of fleeing, can perform more varieties of behaviors than snakes that are active foragers (LLEWELYN,WEBB & SHINE, 2010).

1.5.1. *Influence of body size*

The main variable that influenced the aggressiveness of the *Bothrops jararaca* was body size. When stepped on, the smaller animals were more likely to bite. Epidemiological data show that more accidents occur with small jararaca (SAZIMA, 1988). Therefore, the rate of accidents caused by smaller snakes can be explained by their high aggressiveness. Body size is known to influence aggression in many snakes (BOGERT, 1941; CARPENTER & GILLINGHAM, 1975; GUTZKE et al., 1993; HAILEY & DAVIES, 1986; SHINE et al., 2002; SWEET, 1985, ROTH & JOHNSON, 2004), although for certain species this relationship is unclear (DELANEY, 2019). In general, smaller snakes tend to be more aggressive than larger snakes (SHINE et al., 2002; SWEET, 1985, ROTH & JOHNSON, 2004). Possibly, this is due to the predatory pressure these animals experience in the wild. Small snakes are generally more preyed upon (BLOMBERG & SHINE, 2000; JANZEN et al., 2000; SHINE et al., 2001; TUCKER et al., 1999; VITT, 2000). Furthermore, as we found for *B. jararaca*, other snake species touched on the head also become more aggressive, since most predatory attacks that confer greater risk and are more successful by predators are performed on the more frontal part of the snake's body (LANGKILDE et al., 2004). Therefore, the risk of a human being suffering a bite by stepping on the front part of a small jararaca is very high.

We found other data that may explain the relationship between body size, behavior, and accident epidemiology. Moraes (2008) found that the population of *B. jararaca* was smaller in body size in the coastal plain than the population in the plateau in São Paulo state, Brazil. Predictions of bite probabilities using body size from these populations showed that the smaller snakes in the coast were more aggressive. The data found of botropic accidents in São Paulo state showed that the coast was the region with the highest number of accidents. Thus, the influence of body size on the aggressiveness of *B. jararaca* would explain this epidemiological profile. However, this association should be carefully analyzed, since it does not explain causality, since the data provided by SINAN are for accidents caused by any snake belonging to the *Bothrops* genus, and not exclusively for the *B. jararaca* species. Another factor is that we do not know the abundance of pitvipers in different regions of the state of São Paulo, and possibly more accidents occur on the coast because the region has a larger number of snakes than the highlands. More studies are needed to investigate this relationship between snakebites on the coast and the plateau of São Paulo for a full understanding. Nevertheless, looking exclusively at accident data caused by *B. jararaca* in the São Paulo plateau, we found that most accidents are caused by smaller animals than the average found for the population. Thus, another evidence that the influence of body size on *B. jararaca* behavior can lead to an increase in accidents.

1.5.2. *Influence of sex and life stage*

The factor that most impacts the size of the snake is the sex. *Bothrops jararaca* presents sexual dimorphism, with females being larger than males (CAMPBELL & LAMAR, 2004). In the adult stage we observed that, depending on the context, males and females of *B. jararaca* showed quite different probabilities of attack and tolerance. The interaction between body size, stimulus type, and time of day for females has the same standard effect, with smaller females being more aggressive and less tolerant. On the other hand, males show a more flexible pattern of behavior, exhibiting strong context dependence. In general, males have a positive correlation between size and aggression, with larger snakes being more intolerant. However, only in the morning, with physical contact, does the relationship between size and aggression change.

Our hypothesis for this change is that the aversive stimulus was performed in the context of increased vulnerability. The stimulus with physical contact is one of the most dangerous stimuli for the snake, as possibly its primary defensive strategies would have been unsuccessful. Furthermore, the morning period is the time when the snake would normally be at rest or in

reclusion (SAZIMA, 1988; CAMPBELL & LAMAR, 2004). Under all this context, both females and smaller males tend to be more aggressive. We know that small snakes have lower locomotor performance than larger ones (SCRIBNER AND WEATHERHEAD, 1995; CARRIER,1996; FINKLER & CLAUSSEN, 1999; KELLEY et al., 1997), so instead of performing escape, which is one of the main ways to avoid predation (LIMA & DILL,1990), choosing aggressive behaviors such as biting may be more effective.

Our data showed that generally adult males tend to be more aggressive than females. Due to sexual dimorphism in this species, with males being smaller than females, this factor probably increases the aggressiveness of adult males. However, epidemiological data show that there are more snakebites caused by females than by males (RIBEIRO et al., 1995; SAZIMA,1988; RIBEIRO & JORGE,1997). Therefore, there would be another factor to explain these data. Most of the accidents caused by *B. jararaca* are carried out by juvenile animals, with a representation of up to 56% of the cases (RIBEIRO et al., 1995; SAZIMA, 1988). Furthermore, for every 137 cases of accidents caused by juveniles and adults of *Bothrops jararaca*, 72 (52.55%) were caused by juvenile females while only 5 (3.64%) were caused by juvenile males (RIBEIRO et al., 1995). We found that the life stage influences the aggressiveness of *B. jararaca*. Young snakes were more aggressive than adults. Additionally, the newborn females proved to be more aggressive than the male young and any other group. Therefore, the high probability of biting by female young of *B. jararaca* leads to the high representativeness of this group in the epidemiological profile. This relationship shows again that the study of snake defensive behavior is strongly linked to snake accident data and can be used to understand epidemiological profiles.

1.5.3. *Influence of time of day and temperature*

According to Sazima (1988, 1992), envenomations caused by *B. jararaca* are more frequent during the morning hours. During the day, both females and males are more likely to attack than during the night. Again, the data between environmental and behavioral factors are interacting and explaining certain snake accident patterns. In addition, the morning period is the period of highest human activity, when there are more encounters with snakes (SAZIMA, 1988, 1992; SILVA et al.,2003; LIMA et al.,2009). This congruence between the period of higher human activity and the high probability of *B. jararaca* bite may result in a higher incidence of accidents.

Another factor of great relevance is the effect of temperature. We found that *B. jararaca* has a higher propensity to attack in warmer temperatures. Keogh and DeSerto (1994) observed something similar in the laboratory; there was an increase in the frequency of bites in three colubrid snake species studied. Schieffelin and De Queiroz (1991) also found the same relationship for *Thamnophis sirtalis*, although they tested the snakes in wooden boxes that restricted locomotor behaviors such as escape. This positive relationship between temperature and aggressivity can be explained by the fact that the speed, accuracy and distance of the strike (bite) are reduced at low temperatures (suboptimal temperatures) (GREENWALD, 1974; CITADINI & NAVAS, 2013), meaning that at higher temperatures, the bite becomes more effective (WHITFORD et al., 2020).

This is the first study investigating some thermal effects on viper behavior on the South American continent, a previous study investigated a dipsadid snake from southeast Brazil (CITADINI & NAVAS, 2013). For ectothermic species such as snakes, body temperature is one of the most important influences on predator avoidance behavior, as the temperature will determine the animal's physiological ability to detect, repel or escape a predator (SHINE et al., 2000). According to a review by Mori and Burghardt (2004), snakes in colder environments tend to exhibit more aggressive behaviors (where the distance between the snake and the predator decreases) than in warmer temperatures. This pattern occurs because in higher temperatures the snake's metabolism is more accelerated, improving locomotor performance for escape (MORI & BURGHARDT, 2004; CITADINI & NAVAS, 2013) and it is more preferable to flee than to expose itself during a bite, because by biting, the snake exposes its most vital body part (LLEWELYN et al.,2010). Although, in general, *B. jararaca* has a positive association between increased temperature and aggression, males during the night change strategy, and at higher temperatures, decreasing the propensity to attack. This may be due to the fact that, as *B. jararaca* males are smaller and slender snakes, being at night the most active period of the species and possible period of higher predation pressure (SAZIMA,1988,1992; HARTMANN et al.,2003) and under high temperatures, they prefer escape behaviors since their locomotor capacities are higher.

This thermal influence on the behavior of *B. jararaca* has a strong association with the epidemiological data. The municipalities with the highest rates of bothropic accidents in São Paulo are also the cities with the highest average annual temperatures. Furthermore, according to Sazima (1988,1992), more accidents caused by *B. jararaca* occur in warmer seasons and periods of the year. Therefore, we found another example of an association between the

influence of abiotic factors, biting behavior, and epidemiology, but this association cannot be confused with causality. During the warmer months, there is also an increase in *B. jararaca* (PONTES *et al*., 2009; ROCHA *et al*., 2014; SIQUEIRA *et al*., 2021) activity and possibly due to this an increase in accidents, i.e., snake bites during this period may be a sum effect of increased aggressiveness and activity. The influence of temperature on the incidence of snakebites has gained strength in the literature. In Costa Rica a correlation was found between climate cycles involved with El Niño and snakebites by *Bothrops asper*, snakebites follow meteorological changes, and these patterns likely reflect the impact of meteorological fluctuations on snake biology (CHAVES *et al*.,2015).

Therefore, it can be observed that both biotic and abiotic variables can influence the aggressiveness and the likelihood of the snake performing a bite. There is a strong association between the epidemiological data of snakebites and the behavioral data studied. This study demonstrates that the probability of *B.jararaca* attacking and biting a human being is highly context-dependent, but certain patterns were found. During the daytime and in warmer temperatures, which are the periods when most accidents occur, animals become more aggressive. Snakes have a greater tendency to bite when stepped on, primarily on the anterior part of the body. Young snakes are more likely to bite than adults, especially young females, which would explain why this group is the most involved in accidents. Furthermore, the predictions of bite probabilities using body size show a strong association with the accident data investigated. So by studying the behavior that generates the envenomation, we can understand and predict epidemiological trends. Thus, it is possible to use the defensive behavior of snakes as a new tool to study snakebites. This tool can be used in studies such as Goldstein et al (2021) who integrated human and snake ecological factors to predict snakebites. Just as in malaria, where we cannot fail to study the biological aspects of the mosquito vector, we also cannot fail to study the behavioral aspects of snakes that cause snake bites (Murray et al, 2020). More research work with this integrative perspective is needed if we are to understand, predict, and prevent snake bites.

1.6. REFERENCES

BLOMBERG, S.P, SHINE, R. Size-based predation by kookaburras (*Dacelo novaeguineae*) on lizards (*Eulamprus tympanum*: Scincidae): what determines prey vulnerability? Behav Ecol Sociobio l48:484-489, 2000.

BOGERT CM,. Sensory cues used by rattlesnakes in their recognition of ophidian enemies. Ann NY Acad Sci41:329-343, 1941.

BROOKS, M. E., KRISTENSEN, K., VAN BENTHEM, K. J., MAGNUSSON, A., BERG, C. W., NIELSEN, A., ... & BOLKER, B. M. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R journal, 9(2), 378-400, 2017.

CAMPBELL, J.A. & W.W. LAMAR. The venomous reptiles of the Western Hemisphere. Volume I. Ithaca, New York, p. 476, 2004.

CARPENTER CC, GILLINGHAM JC. Postural responses to kingsnakes by crotaline snakes. Herpetologica31:293-302, 1975.

CARRIER, D.R. Ontogenetic limits on locomotor performance. Physiol Zool69:467-488, 1996.

CHAVES, L. F., CHUANG, T. W., SASA, M., & GUTIÉRREZ, J. M. Snakebites are associated with poverty, weather fluctuations, and El Niño. Science advances, 1(8), e1500249,2015.

CITADINI, J.M.; NAVAS, C.A. Inter-individual variation and temperature-dependent antipredator behavior in the snake *Tomodon dorsatus* (Dipsadidae). Behavioural Processes 97, p. 11–17, 2013.

CLUTTON-BROCK, T.H. The evolution of parental care. Princeton, New Jersey: Princeton University Press, 1991.

DELANEY,D.M. Antipredation behavior covaries with body size in Neotropical snakes. Amphibia-Reptilia 40: 437-445, 2019.

FINKLER MS, CLAUSSEN DL. Influence of temperature, body size, and inter-individual variation on forced and voluntary swimming and crawling speeds in *Nerodia sipedon* and *Regina septemvittata*. J Herpetol33:62-71, 1999.

FRANÇA, FOS, MALAQUE, CMS. Acidente botrópico. In: Cardoso, JLC, França, FOS, Wen, FH, Malaque, CMS, Haddad Jr. V. (Eds), Animais peçonhentos no Brasil: biologia, clínica e terapêutica dos acidentes, Sarvier, São Paulo, pp. 72–86 ,2003.

GLAUDAS, X., WINNE, C. T., & FEDEWA, L. A. Ontogeny of anti-predator behavioral habituation in cottonmouths (Agkistrodon piscivorus). Ethology, 112(6), 608-615, 2006.

GOLDSTEIN, E., ERINJERY, J. J., MARTIN, G., KASTURIRATNE, A., EDIRIWEERA, D. S., DE SILVA, H. J., ... & IWAMURA, T. Integrating human behavior and snake ecology with agent-based models to predict snakebite in high risk landscapes. PLoS neglected tropical diseases, 15(1), 2021.

GREENWALD, O.E. Thermal Dependence of Striking and Prey capture by Gopher Snakes. Copeia,v.1974 n.1, p. 141 – 148, 1974.

GUTZKE WHN, TUCKER C, MASON RT. Chemical recognition of kingsnakes by crotalines: effects of size on the ophiophage defensive response. Brain Behav Evol41:234-238, 1993.

HAILEY, A. DAVIES P.M.C.Effects of size, sex, temperature and condition on activity metabolism and defence behaviour of the viperine snake, *Natrix maura.* J Zool 208:541-558, 1986.

HARTMANN, PA, HARTMANN, MT, & GIASSON, LOM. Uso de habitat e alimentação em juvenis de Bothrops jararaca (Serpentes, Viperidae) na Mata Atlântica do sudeste do Brasil. Phyllomedusa: Journal of Herpetology , 2 (1), 35-41, 2003.

IBANEZ, A.; LÓPES,P.; MARTÍN,J. Inter-Individual variation in antipredator hiding behavior of Spanish terrapins depends on sex, size, and coloration. Ethology, v.120, n.8, p. 742 – 752, 2014.

JANZEN FJ, TUCKER JK, PAUKSTIS GL. Experimental analysis of an early life-history stage: Avian predation selects for larger body size of hatchling turtles. J Evol Biol13:947-954, 2000.

KELLEY, K.C.; ARNOLD SJ, GLADSTONE J. The effects of substrate and vertebral number on locomotion in the garter snake *Thamnophis elegans*. Funct Ecol11:189-198, 1997.

KEOGH, J.S.; DE SERTO, F.P. Temperature dependent defensive behavior in three species of North American colubrid snakes. J Herpetol 28: 258-261, 1994.

LANCET, T. Snakebite-emerging from the shadows of neglect. Lancet (London, England), 393(10187), 2019.

LANGKILDE, T., R. SHINE, R. MASON, T. Predatory attacks to the head vs. body modify behavioral responses of garter snakes. Ethology 110:937–947, 2004.

LAYNE, J.R, JR, FORD NB. Flight distance of the queen snake, *Regina septemvittata*. J Herpetol18:496-498, 1984.

LIMA, J. S., MARTELLI JÚNIOR, H., MARTELLI, D. R. B., SILVA, M. S. D., CARVALHO, S. F. G. D., CANELA, J. D. R., & BONAN, P. R. F. Perfil dos acidentes ofídicos no norte do Estado de Minas Gerais, Brasil. Revista da Sociedade Brasileira de Medicina Tropical, 42, 561-564, 2009.

LIMA,S.L;DILL, L.M.B.Behavioural decisions made under risk of predation: a review and prospectus. Canadian Journal of Zoology, v.68, n.4, p.619-640,1990.

LLEWELYN, J.; WEBB, J.K.; SHINE, R. Flexible defense: context-dependent antipredator responses of two species of Australian elapid snakes. Herpetologica, 66(1), p.1–11, 2010.

MAGURRAN, A.E.; NOWAK MA. Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata*. Proc R Soc Lond B246:31-38, 1991.

MATIAS, N. R., ALVES, M. L. M., ARAUJO, M. L. D., & JUNG, D. M. H. Variação morfométrica em *Bothropoides jararaca* (Serpentes, Viperidae) no Rio Grande do Sul. Iheringia. Série Zoologia, 101, 275-282, 2011.

MORAES, R.A. Variations in morphological and ecological characters in *Bothrops jararaca* (Serpentes: Viperidae) populations in São Paulo State. Master's Thesis, University of São Paulo, São Paulo, 2008.

MORI, A., & BURGHARDT, G. M. Thermal effects on the antipredator behaviour of snakes a review and proposed terminology. Herpetological Journal, 14(2), 79-87, 2004.

MURRAY, K. A., MARTIN, G., & Iwamura, T. Focus on snake ecology to fight snakebite. The Lancet, 395(10220), e14, 2020.

NOGUEIRA, C. C., ARGÔLO, A. J., ARZAMENDIA, V., AZEVEDO, J. A., BARBO, F. E., BÉRNILS, R. S., ... & MARTINS, M. Atlas of Brazilian snakes: verified point-locality maps to mitigate the Wallacean shortfall in a megadiverse snake fauna. South American Journal of Herpetology, 14(sp1), 1-274, 2019.

PONTES JAL, PONTES RC AND ROCHA CFD. The snake community of Serra do Mendanha, in Rio de Janeiro State, southeastern Brazil: composition, abundance, richness and diversity in areas with diferent conservation degrees. Braz J Biol 69(3): 795-804, 2009.

PUZZI,M.B;VICARIVENTO, N.B;XAVIER,A. Acidentes Ofídicos.Revista Científica de Medicina Veterinária.Ano VI-Número 10, 2008.

RIBEIRO, L.A.; JORGE, M.T. Acidente por serpentes do gênero *Bothrops*: série de 3.139 casos. Revista da Sociedade Brasileira de Medicina Tropical 30(6):475-480, 1997.

RIBEIRO, L.A.; JORGE, M.T.; Iversson,L.B. Epidemiology of poisonons snake-bites: a study of cases assisted in 1988.Rev. Saúde Pública, Vol 29(5), 1995.

ROCHA, M. C., HARTMANN, P. A., WINCK, G. R., & CECHIN, S. Z. Seasonal, daily activity, and habitat use by three sympatric pit vipers (Serpentes, Viperidae) from southern Brazil. Anais da Academia Brasileira de Ciências, 86, 695-706, 2014.

ROTH,E.D., JOHNSON, J.A., Size-based variation in antipredator behavior within a snake (*Agkistrodon piscivorus*) population. Behavioral Ecology, Volume 15, Issue 2, p. 365–370, 2004.

SAZIMA,I.Natural history of the jararaca pitviper,*Bothrops jararaca*, in southeastern Brazil,Pp. 199-216. In:J.A.Campbell & E.D. Brodie-Jr (Eds).Biology of Pitvipers.Selva, Tyler, Texas, 452p. 1992.

SAZIMA,I.Um estudo de biologia comportamental da jararaca, *Bothrops jararaca*, com uso de marcas naturais.Mem.Isnt.Butantan 50(3):83-99, 1988.

SCHIEFFELIN, C.D.; DE QUIEROZ ,A.M. Temperature and defense in the common garter snake: warm snakes are more aggressive than cold snakes. Herpetologica 47: 230-237, 1991.

SCRIBNER, SJ. WEATHERHEAD, PJ. Locomotion and antipredator behaviour in three species of semi-aquatic snakes. Can J Zool73:321-329, 1995.

SCUDDER, K. M., & CHISZAR, D. Effects of six visual stimulus conditions on defensive and exploratory behavior in two species of rattlesnakes. The Psychological Record, 27(3), 519-526, 1977.

SHINE, R. Reproduction in Australian elapid snakes I. Testicular cycles and mating seasons. Australian Journal of Zoology 25:647-653. 1977a

SHINE, R.. Reproduction in Australian elapid snakes II. Female reproductive cycles. Australian Journal of Zoology 25:655-666. 1977b.

SHINE R, SUN L, FITZGERALD M, KEARNEY M. Antipredator responses of free-ranging pit vipers (*Gloydius shedaoensis*, Viperidae). Copeia 2002:843–850, 2002.

SHINE, R.; LEMASTER, M.P, MOORE, I.T, OLSSON, M.M, MASON, R.T. Bumpus in the snake den: effects of sex, size, and body condition on mortality of red-sided garter snakes. Evolution55:598-604, 2001.

SHINE, R.; OLSSON MM, LEMASTER MP, MOORE IT, MASON RT. EFFECTS of sex, body size, temperature, and location on the antipredator tactics of free-ranging gartersnakes. Behav Ecol11:239-245, 2000.

SHRESTHA, N. Detecting multicollinearity in regression analysis. American Journal of Applied Mathematics and Statistics, 8(2), 39-42, 2020.

SILVA,C.; JORGE, M.T.; RIBEIRO,L.A. Epidemiology of snakebite in a central region of Brazil. Toxicon 41:125-255, 2003.

SINAN. Sistema de informações de agravos de notificação - Sinan. In: Fundação Nacional de Saúde. Anais do Seminário de Vigilância Epidemiológica. Brasília: Ministério da Saúde; 2021.

SIQUEIRA, L. H., BANCI, K. R., & MARQUES, O. A. Seasonal activity of Bothrops jararaca (Serpentes, Viperidae): optimizing foraging while avoiding predators. South American Journal of Herpetology, 20(1), 67-74, 2021.

SIQUEIRA, L.H.C.; MARQUES, O.A.V. Effects of Urbanization on *Bothrops jararaca* Populations in São Paulo Municipality, Southeastern Brazil. Journal of Herpetology, Vol. 52, No. 3, 299–306, 2018.

SWEET S.S. Geographic variation, convergent crypsis and mimicry in gopher snakes (*Pituophis melanoleucus*) and western rattlesnakes (*Crotalus viridis*). J Herpetol19:55-67,1985.

TUCKER, J.K, FILORAMO, N.I, JANZEN, F.J. Size-biased mortality due to predation in a nesting freshwater turtle, *Trachemys scripta*. Am Midl Nat141:198-203, 1999.

VITT, L.J. Ecological consequences of body size in neonatal and small-bodied lizards in the neotropics. Herpetol Monogr14:388-400, 2000.

WHITAKER, P.B.; SHINE, Responses of free-ranging brownsnakes (*Pseudonaja textiles*:Elapidae) to encounters with humans. Wildl Res26:689-704, 1999.

WHITFORD, M. D., FREYMILLER, G. A., HIGHAM, T. E., & CLARK, R. W.The effects of temperature on the defensive strikes of rattlesnakes. Journal of Experimental Biology, 223(14), 2020.

LLEWELYN, J.; WEBB, J. K.; SHINE. Flexible defense: Context-dependent antipredator responses of two species of Australian elapid snakes. Herpetologica, v. 66, n. 1, p. 1-11, 2010.

1.7. SUPPLEMENTARY

Table S1. **Results from models evaluating bite probability of *Bothrops jararaca***. Bold p-values indicate p-value <0.005. The ":" sign indicates interaction between the variables. Intercept - Sex (Female); period (morning); stimulus (with contact).

Variables	Estimate	Std. Error	z value	p
Intercept	2.461.206	0.755381	3.258	**0.001121**
Size	-0.021211	0.007258	-2.922	**0.003473**
Period (Night)	-0.721446	0.652966	-1.105	0.269214
Sex (Male)	0.328993	1.284531	0.256	0.797859
Stimulo (Without contact)	-0.398589	0.664294	-0.600	0.548494
Size:Period (Night)	0.002298	0.006321	0.364	0.716218
Size:Sex (Male)	-0.007681	0.014347	-0.535	0.592377
Period (Night):Sex (Male)	-2.575.040	1.147.446	-2.244	**0.024823**
Size:Stimulo (Without contact)	-0.001438	0.006654	-0.216	0.828915
Sex (Male):Stimulo (Without contact)	-0.256090	0.982291	-0.261	0.794319
Sex (Male):Stimulo (Without contact)	-5.451.948	1.408.503	-3.871	**0.000109**
Size:Period (Night):Sex (Male)	0.037541	0.013122	2.861	**0.004223**
Size:Period (Night):Stimulo (Without contact)	0.001642	0.009715	0.169	0.865827
Size:sexo[T.macho]:Stimulo (Without contact)	0.063930	0.016706	3.827	**0.000130**
periodo[T.noite]:Sex (Male):Stimulo (Without contact)	5.077.277	1.883.375	2.696	**0.007021**
Size:Period (Night):Sex (Male):Stimulo (Without contact)	-0.065873	0.022119	-2.978	**0.002901**

Table S2.**Results from models evaluating Tolerance of *Bothrops jararaca***.Bold p-values indicate p-value <0.005. The ":" sign indicates interaction between the variables. Intercept - Sex (Female); period (morning); stimulus (with contact).

Variables	Estimate	Std. Error	z value	p
Intercept	1.311.860	1.252.070	1.048	0.29475
Size	-0.012226	0.012344	-0.990	0.32193
Period (Night)	-0.510108	1.253.034	-0.407	0.68394
Sex (Male)	-0.581745	2.160.288	-0.269	0.78771
Stimulo (Without contact)	-0.909060	1.181.489	-0.769	0.44164
Size:Period (Night)	0.009725	0.012474	0.780	0.43561
Size:Sex (Male)	0.012233	0.024544	0.498	0.61820
Period (Night):Sex (Male)	2.462.122	1.961.299	1.255	0.20935
Size:Stimulo (Without contact)	0.012244	0.012283	0.997	0.31884
Sex (Male):Stimulo (Without contact)	0.026997	1.603.552	0.017	0.98657
Sex (Male):Stimulo (Without contact)	6.334.954	1.967.602	3.220	**0.00128**
Size:Period (Night):Sex (Male)	-0.030511	0.022309	-1.368	0.17141
Size:Period (Night):Stimulo (Without contact)	-0.001412	0.016410	-0.086	0.93144
Size:sexo[T.macho]:Stimulo (Without contact)	-0.070609	0.022982	-3.072	**0.00212**
periodo[T.noite]:Sex (Male):Stimulo (Without contact)	-7.048.247	2.619.868	-2.690	**0.00714**
Size:Period (Night):Sex (Male):Stimulo (Without contact)	0.082478	0.030387	2.714	**0.00664**

Table S3.**Model results evaluating the probability of *Bothrops jararaca* bite in the context of human-snake physical contact.**Bold p-values indicate p-value <0.005. The ":" sign indicates the interaction between the variables. Interception - Sex (female); period (morning).

Variables	Estimate	Std. Error	z value	p
Intercept	-42.173.629	43.577.336	-0.968	0.33315
Temperature	0.3497483	0.2425218	1.442	0.14927
Sex (Male)	32.383.640	43.966.747	0.737	0.46140
Period (Night)	93.135.196	29.069.854	3.204	**0.00136**
Size	0.0172241	0.0419770	0.410	0.68157
Temperature:Sex (Male)	-0.5437417	0.2354026	-2.310	**0.02090**
Temperature:Period (Night)	-0.6175680	0.1475027	-4.187	**2.83e-05**
Sex (Male):Period (Night)	22.075.287	38.059.657	0.580	0.56190
Temperature:Size	-0.0022482	0.0023217	-0.968	0.33288
Sex (Male):Size	-0.1007757	0.0478157	-2.108	**0.03507**
periodo[T.noite]:Size	-0.1124419	0.0144287	-7.793	**6.55e-15**
Temperature:Sex (Male):Period (Night)	0.0993972	0.1928686	0.515	0.60630
Temperature:Sex (Male):Size	0.0096653	0.0024649	3.921	**8.81e-05**
Temperature:Period (Night):Size	0.0067916	0.0006121	11.095	**< 2e-16**
Sex (Male):Period (Night):Size	0.0425056	0.0262369	1.620	0.10522
Temperature:Sex (Male):Period (Night):Size	-0.0047416	0.0010560	-4.490	**7.12e-06**

Table S4..Model results evaluating the probability of *Bothrops jararaca* bite in the context of human-snake without physical contact.Bold p-values indicate p-value <0.005. The ":" sign indicates the interaction between the variables. Interception - Sex (female); period (morning).

Variables	Estimate	Std. Error	z value	p
Intercept	-42.173.629	43.577.336	-0.968	0.33315
Temperature	0.3497483	0.2425218	1.442	0.14927
Sex (Male)	32.383.640	43.966.747	0.737	0.46140
Period (Night)	93.135.196	29.069.854	3.204	**0.00136**
Size	0.0172241	0.0419770	0.410	0.68157
Temperature:Sex (Male)	-0.5437417	0.2354026	-2.310	**0.02090**
Temperature:Period (Night)	-0.6175680	0.1475027	-4.187	**2.83e-05**
Sex (Male):Period (Night)	22.075.287	38.059.657	0.580	0.56190
Temperature:Size	-0.0022482	0.0023217	-0.968	0.33288
Sex (Male):Size	-0.1007757	0.0478157	-2.108	**0.03507**
periodo[T.noite]:Size	-0.1124419	0.0144287	-7.793	**6.55e-15**
Temperature:Sex (Male):Period (Night)	0.0993972	0.1928686	0.515	0.60630
Temperature:Sex (Male):Size	0.0096653	0.0024649	3.921	**8.81e-05**
Temperature:Period (Night):Size	0.0067916	0.0006121	11.095	**< 2e-16**
Sex (Male):Period (Night):Size	0.0425056	0.0262369	1.620	0.10522
Temperature:Sex (Male):Period (Night):Size	-0.0047416	0.0010560	-4.490	**7.12e-06**

Table S5. *Bothrops jararaca* bite probability predictions for each average population size in São Paulo according to Moraes (2008). A- Predictions made for males; B- for females

A

Human Stimulus	**Regions**	
	Coast (SVL=1120,7 ±114,4 mm)	**Plateau (SVL=1236,7 ± 62,8 mm)**
	Bite Probability (%)	**Bite Probability (%)**
Without contact	32% - 45%	29% - 36%
With contact	46% - 58%	43%- 49%

B

Human Stimulus	Regions	
	Coast (SVL=850,7 ± 55,7 mm)	Plateau (SVL=875,5 ± 45,6 mm)
	Bite Probability (%)	**Bite Probability (%)**
Without contact	40% -50%	43% -51%
With contact	54% - 62%	53% - 60%

Table S6.Result of model estimating the amount of snakebites caused by *Bothrops* in the regions of São Paulo State, Brazil between 2009 and 2017 .Bold p values indicate p<0.005.

Variables	Estimate	Std. Error	z value	p
Coast	36.874	0.2168	17.005	**< 2e-16**
Plateau	-13.223	0.2732	-4.839	**0.0000013**

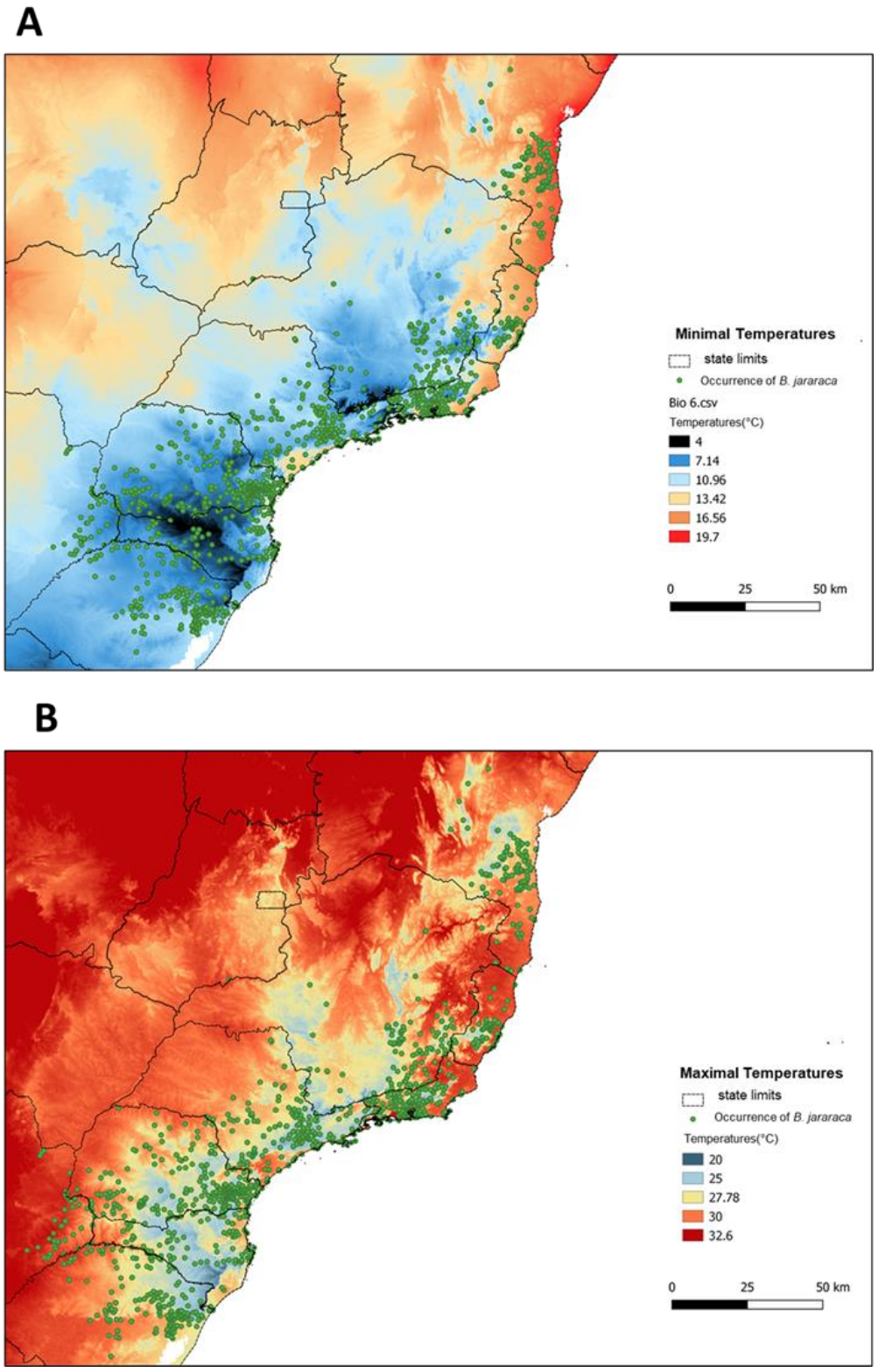


Figure S1.*Bothrops jararaca* distribution map plotted on a map with the temperatures of occurrence. A- map with minimum temperatures. B- Map with maximum temperatures.

Table S7. Result of the model relating the amount of annual snakebites and the average temperature of the municipalities of São Paulo.

Variable	Estimate	Std. Error	z value	p
(Intercept)	-24.667	21.198	-1.164	0.2446
Temperature	0.2490	0.1031	2.415	**0.0157**

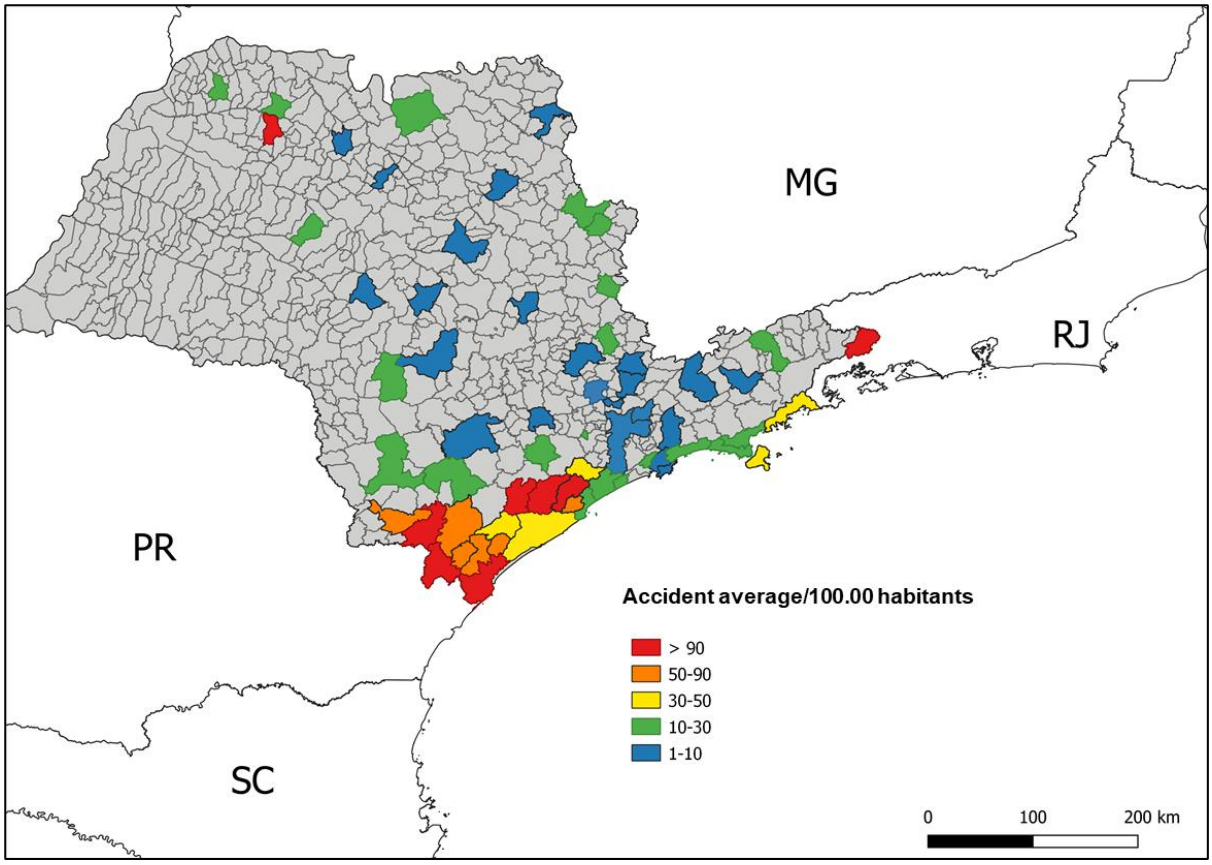


Figure S2. Map of the average incidence of Snakebites caused by *Bothrops* in the State of São Paulo, Brazil, between 2009 and 2017.

2.CHAPTER 2: The influence of predatory stimulus on the antipredator behavior of island pitvipers

2.1.INTRODUCTION

One of the most relevant interactions between organisms in a community is the prey-predator relationship. Predation is considered responsible for the development of several adaptations in organisms (VERMEIJ,1982). Among several adaptations, the behavioral ones deserve attention, especially the actions that avoid the predation of the organism, i.e. the anti-predator behaviors. (EDMUNDS, 1974). Snakes have a wide variety of predators, both invertebrates and vertebrates, with different sizes, degrees of specialization, capture tactics, metabolic rates, and life habits (GREENE,1988; MARTINS, 1996; POUGH et al.,2003). Therefore, no single antipredator behavior fits all cases (GREENE,2000), resulting in an extensive defensive repertoire and one of the most elaborate described in reptiles (GREENE,1988).

Antipredator behavior is influenced by intrinsic (e.g., age, reproductive condition, and experience or learning) and extrinsic (e.g., habitat characteristics, temperature, and social context) factors (reviewed by Endler, 1986) (STANKOWICH & BLUMSTEIN, 2005). Among these factors, the type of predator and the predation pressure has great influence. Snakes respond in different ways depending on the type of predatory stimulus, its color, presence of eyes, specie, size, and temperature (BURGHARDT & GREENE, 1988; LANGKILDE et al., 2004; SHINE & MASON, 2000; WITAKER & SHINE, 1999; SCUDDER & CHIZAR, 1977).

When isolated from predators, expensive and no longer functional antipredator behavior by selection can be eliminated (KAVALIERS, 1990; MAGURRAN et al ., 1995; MAGURRAN, 1999). Generally, islands lose many predators because these environments have many limitations and are therefore unable to support predators to the same extent as the mainland environment (BLUMSTEIN, 2002). Therefore, for prey to maintain anti-predator behavior in the absence of predators is considered costly (MAGURRAN, 1999), consequently it is expected to be eliminated by selection if there is no benefit.

The pitviper *Bothrops alcatraz* and the golden lancehead *Bothrops insularis* are snake species endemic to Alcatrazes Island and Queimada Grande Island, respectively located about 30 to 35 km off the coast of the state of São Paulo, Brazil (MARQUES et al., 2002; MARQUES,

2021). Both species have similar origins (MARQUES et al., 2002; MARQUES, 2021). During the Pleistocene (11000 years ago), the sea level oscillated many times, which caused the separation of the islands from the continent separating the pitviper populations (MARQUES et al., 2002; MARQUES, 2021). Genetic studies show that both *B. alcatraz* and *B. insularis*, have great similarity with common mainland pitviper *Bothrops jararaca*, and it is suggested that the two island species shared the same ancestor with *B.jararaca* (MARQUES et al., 2002; MARQUES, 2021; ZAHER et al.,2019).

All three species suffer different predation pressures. *B. jararaca*, being the most widespread species and occurring on the continent faces a wider range of predators, both aerial and terrestrial (CORDERO & NICOLAS, 1986; WAGLER, 1824). As for the island species, there is little known about the predation they suffer. On both islands, we observed bird species that prey on snakes, but there is only evidence of predation by birds on *B. insularis*, since on Queimada Grande Island there are no terrestrial predators (MARQUES, 2021). On Alcatrazes Island there is the presence of both terrestrial and aerial predators, but so far no observation of predation (Fig 1) (MARQUES et al., 2002; MARQUES, 2021).

These species with their respective evolutionary and ecological histories provide an appropriate setting to study how different predatory stimuli can interfere with antipredator behavior. Therefore, our goal is to understand the influence that predatory stimulus type has on the antipredator behavior of island pitvipers. Specifically to assess the influence of terrestrial and avian predatory stimuli on the frequency of each behavior in the antipredator repertoire.

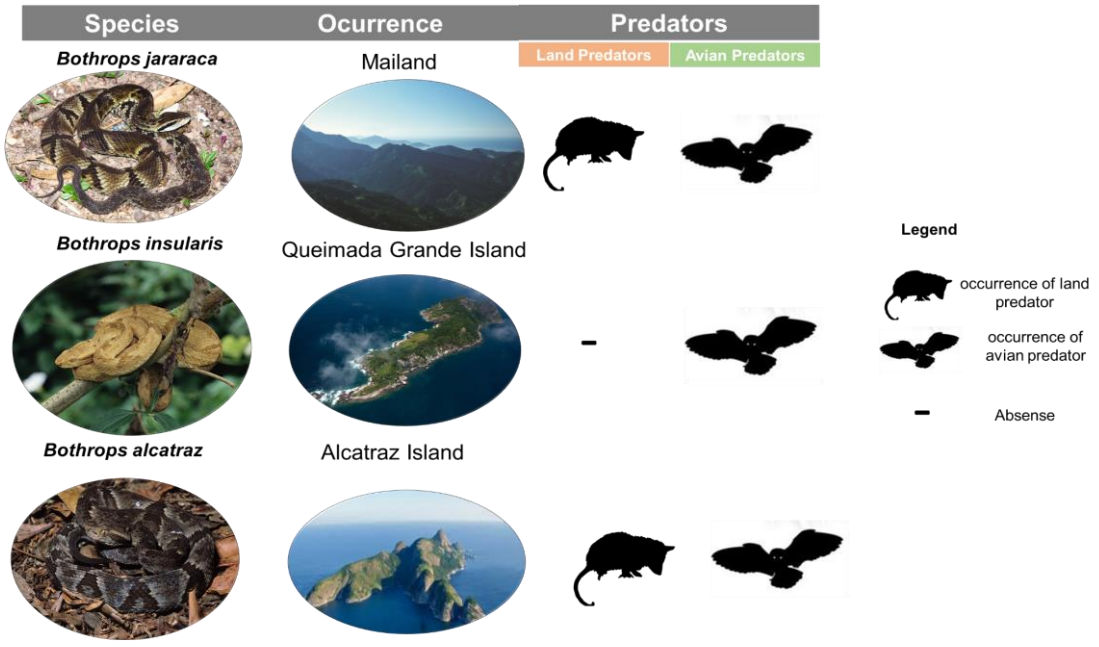


Figure 1. Type of predators existing in the natural habitat of each species

2.2.METHODS

2.2.1. Study Animal

We used a total of 47 snakes, including 20 individuals of *B. jararaca* (10 males and 10 females). 19 individuals of *B. insularis* (10 males and 9 females) and 8 individuals of *B. alcatraz* (3 males and 5 females). All animals were born in captivity and housed in the Laboratory of Ecology and Evolution of the Instituto Butantan. Every animal is considered an adult by their sexual maturity (Shine et al., 1977 a,b; Matias et al., 2011). The animals were maintained at a temperature around 25 °C, under a light and dark photoperiod of 12: 12. This fact removes the effect of previous experience that each animal may have had in the different environments that species occupy.

2.2.2. Behavioral Tests

To perform the behavioral tests, we conducted confrontations with the snakes using avian and land predator models as stimuli. For the confrontations, a 2.025 m² (1.5m (length) X 1.35m (width) X 1m (height)) arena was set up, with an aluminum plate as a base, and Styrofoam walls. For the confrontations, a 2.025 m² (1.5m (length) X 1.35m (width) X 1m (height)) arena was set up, with an aluminum plate as a base, and Styrofoam walls. All experiments were conducted between 5 pm and 8 pm at a temperature around 23 C. For each behavioral test, the snakes were left in the arena for up to 15 minutes in order to cease exploratory behaviors and habituate to the test arena.

To simulate a terrestrial predator, we used a cloth and cotton model with 42 cm and 24 cm and morphological characteristics of the opossum, *Didelphis albiventris*, since it is known to exert predatory pressure on *B. jararaca* (Oliveira & Santori., 1999). In addition, a bottle with water at 37°C was placed to simulate the typical internal temperature of mammals. Using a 1.5 m wooden stick attached to the predator model, 30 advances of the model toward the animal's head were executed with a 2 s interval, touching the snake's body.

To simulate an avian predator, we used a taxidermized burrowing owl (*Athene cunicularia*) with its wings spread. There are reports that this species of owl can also prey on these species in addition to occurring on the islands (Macarrão, 2010; Marques, 2021). At each behavioral test, the model was warmed with the help of heaters so that the temperature was around 37°C to 39 °C. With the help of a fishing rod, the owl model was attached and confronted the snake 30 times with a 3 s interval, touching the snake's body. The confrontations were performed from top to bottom simulating an aerial attack. The tester was about 3.5 meters away

from the snake. An interval of seven days was given between stimulation in order to avoid stress and learning influences (Glaudas *et al.*, 2006). All experiments were recorded for later analysis using a film camera (HDR-PJ200, Sony).

The observed behaviors were classified according to Greene (1988), Sazima (1988), Duvall et al (1985), and Araujo and Martins (2006) (Supplementary1). In addition, following Mori and Burghardt (2004) apparent function behavioral classification, the behaviors were divided into three behavioral classes for simplification in demonstrating the results. Threatening behaviors (gaping; body flatten and strike) are responses that involve any behavioral element to prevent predation by showing danger to the predator. Cryptic behaviors (immobility and head hiding) are an attempt to remain imperceptible to the predator. Escape behaviors (lee; blunt escape and cocking) are based on increasing the distance between the snake and the predator.

2.2.3. Statistical Analysis

Statistical analyses were based on the experimental design. The fixed categorical predictor variables were species (three levels: *B. jararaca*, *B. insularis*, and *B. alcatraz*) and predatory stimulus (two levels: terrestrial and avianpredator). The response variables were the frequency of each antipredator behavior during the 30 confrontations with each predator.

Due to the nature of these variables and based on the experimental design, a generalized linear mixed model (GLMM) with poisson distribution and log link function was used for each behavior. Only for body flattening a GlmmTMB model with a negative binomial distribution (nbinom1) was used. Since the experiments were performed with the same animals and the sex that was not of our interest to investigate, we placed these two variables as random variables in the models, thus eliminating problems of pseudoreplication and experimental design. Using ANOVA tables and AIC values, the minimum adequate model was selected. In addition, we performed Tukey's Test for Post-Hoc Analysis to find significant relationships between variables. All models were subjected to data dispersion analysis, homoscedasticity, and delineate tests using model diagnostic values and plots, with the help of the package "DHARMa: residual diagnostics for hierarchical (multilevel/mixed) regression models" in R.

2.3.RESULTS

2.3.1. Escape behaviors

The behaviors of species belonging to the escape category were strongly influenced by the predatory stimulus. All species flee more frequently when confronted with a terrestrial predator (opossum) than with an avian predator (owl) (z=5.233; p <0.0001) (Fig. 2, top graphic). *Bothrops insularis*, independent of the predatory stimulus, showed a higher flee rate than the other species (z=4.218; p<0.0001), with a chance of fleeing about 85% higher than *B. jararaca*. Similar pattern was observed for the blunt escape data. All studied species fledged more when confronted with the terrestrial predatory stimulus (z= 6.626; p <.0001) (Fig.2 , middle graphic). *Bothrops. jararaca*, regardless of the predatory stimulus, had no clear difference in frequency when compared to *B. alcatraz* (z= -0.999; p= 0.3177). However, *B. insularis*, again, showed higher escape frequencies than the other species, with odds about 97% higher than *B. jararaca* (z= 2.298; p= 0.0215).

The cocking behavior showed results with different patterns. This behavior was influenced by the species type in different ways depending on the predatory stimulus. The dependence between variables was significant. *Bothrops insularis*, as well as *B. alcatraz*, showed no difference depending on the type of predatory stimulus (*B. insularis*- z= 0.391, p= 0.9988; *B. alcatraz*- z=-1.169, p=0.8516). However, the different predatory stimuli influenced the frequency of cocking of *B. jararaca* (z= -3,936 ; p<0.0001).

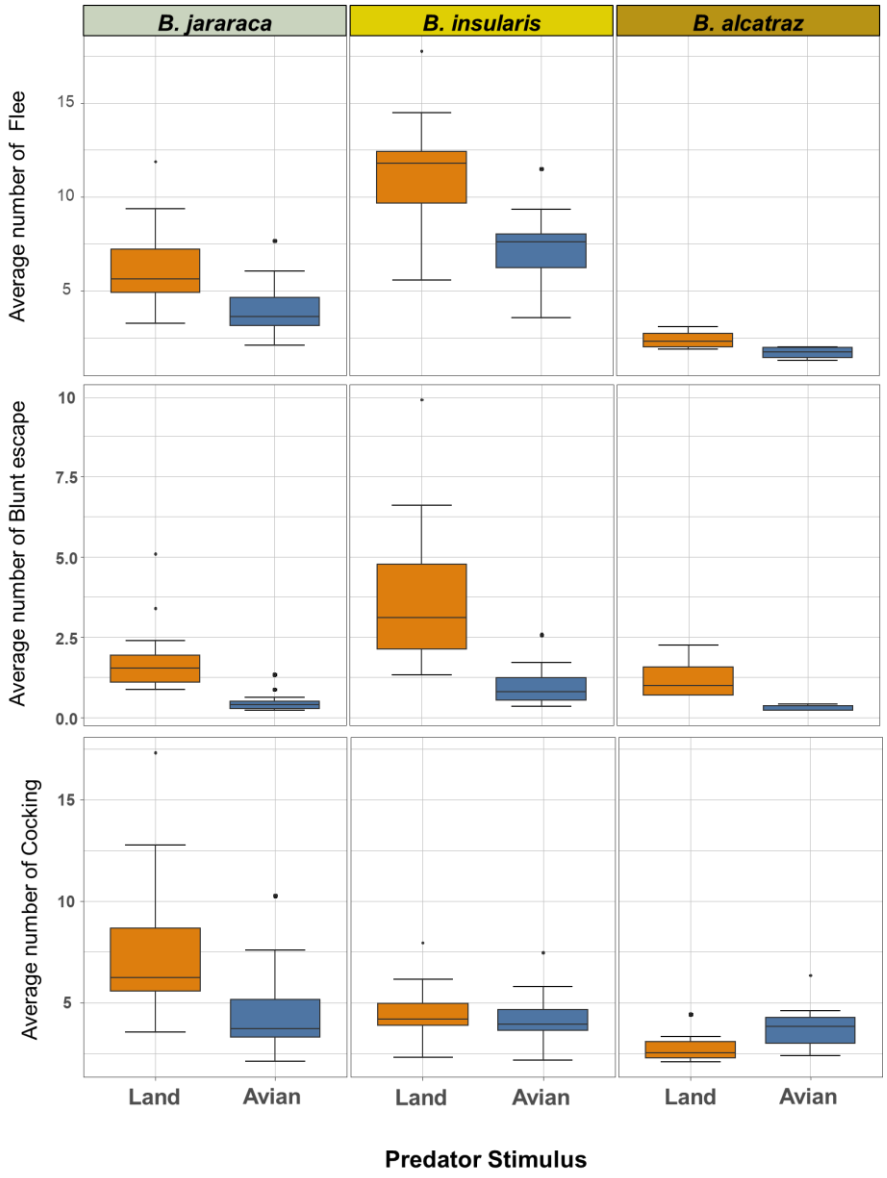


Figure 2. Influence of predatory stimulus types (Land and Aerial) on escape category behaviors of *B. jararaca, B. insularis* and *B. alcatraz.* Upper graph - Flee behavior. Middle graph- Blunt escape behavior. Lower graph - Cocking behavior. *** significant difference in the frequency of behaviors in relation to the type of predatory stimulus ($p<0.001$).

2.3.2. Cryptic behaviors

There was a difference in the frequency of cryptic behaviors such as immobility and hiding the head. Among the species, *B. alcatraz*, was one of the species that showed the least tendency to show any cryptic behavior (Fig. 3). The type of predatory stimulus influenced immobility behavior only for *B. jararaca*, which showed a higher tendency to immobility for

avian predator (*B. jararaca*: z= 3.234, p= 0.00122; *B. insularis*: z= -0.263, p= 0.9998; *B. alcatraz*: z= 1.656, p= 0.5612). In addition, *B. jararaca* showed the highest rate of immobility among the species studied. All species showed no clear difference in the frequency of head-hiding behavior when subjected to different stimuli (*B. jararaca*: z= -0.144, p= 0.88577; *B. insularis:* z= 1.667, p= 0.5535; *B. alcatraz*: z= -0.135 , p= 1.000). However, *B. insularis* subjected to the terrestrial predatory stimulus has the highest rate of head-hiding behavior among species (z= 2.792, p= 0.00524).

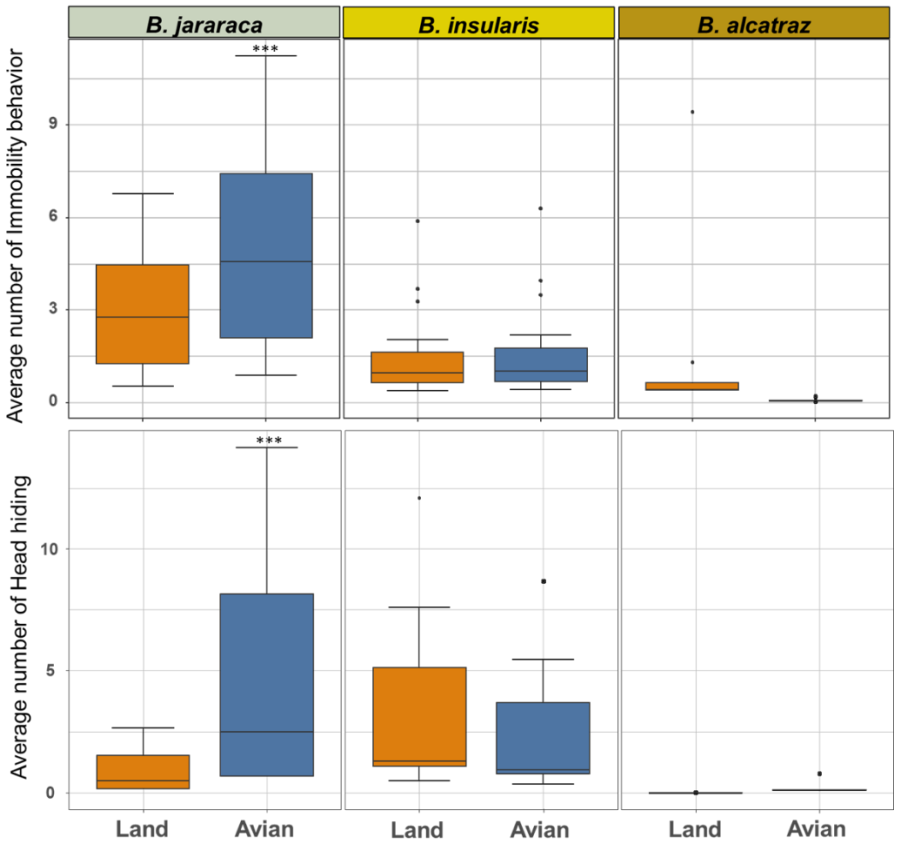


Figure 3. **Influence of predatory stimulus types on cryptic category behaviors of *B. jararaca, B. insularis* and *B. alcatraz.*** Upper graph - Immobility behavior. Lower graph - Head hiding behavior. *** clear difference in the frequency of behaviors in relation to the type of predatory stimulus (p<0.001).

2.3.3. Threatening behaviors

All behaviors involving a threat signal, such as strike, gaping and body flatten, were influenced by the type of predatory stimulus in different ways among the species. Gapping behavior was strongly influenced by the type of predator. For *B. jararaca and B. insularis* it

was more frequent to perform gaping for terrestrial predator (z= -3.803; p= 0.000143), although the difference was not significant for the latter (z= -1.905; p= 0.056742). On the other hand, *B. alcatraz*, had a change in pattern, as it more frequently gaped to an aerial predatory stimulus (z= 2.265; p= 0.02352) (Fig 4. upper graph).

For strike behavior, the interaction between predator type and species was significant. All species showed a change in attack frequency to the different predators. *Bothrops jararaca* and *B. insularis* showed the same pattern, with more strikes to terrestrial than avian predators stimuli (*B. jararaca*: z= 2.684, p= 0.007273; *B. insularis*: z= 4.860, p<0.0001). In contrast, *B. alcatraz* showed the opposite pattern. When faced with the avian predator, it performed more strikes than with the land stimulus (z=-2.217, p= 0.026609). Among the species, *B. alcatraz* proves to be the most aggressive, with the highest strike behavior rates. The odds of *B. alacatraz* to strike a terrestrial predator are about 75% and 49% higher than *B. insularis* and *B. jararaca*, respectively. While for an aerial predator, *B. alcatraz* has about 44% more odds than *B. insularis* to perform strike behavior. When compared to *B. jararac*a the difference of 27% chance is not significant (z= -1.604; p= 0.108618) (Fig 4 , middle graph).

Another behavior strongly influenced by predator type was body flattening behavior. One more time, *B. alcatraz* had a different behavioral pattern than the other species and did not show anybody flattening. Both *B. jararaca* and *B. insularis*, had a large difference in body flattening rate during encounters with predator types. Both species performed more body flattening when faced with the avian predatory stimulus (z= 6.638, p<0.0001). Furthermore, among the species, the golden lancehead was the one that flattens the body most independently of the predator (avianpredator: z= -3.31, p= 0.000929; land predator: z= 3.169, p= 0.00153) (Fig4 bottom graph)

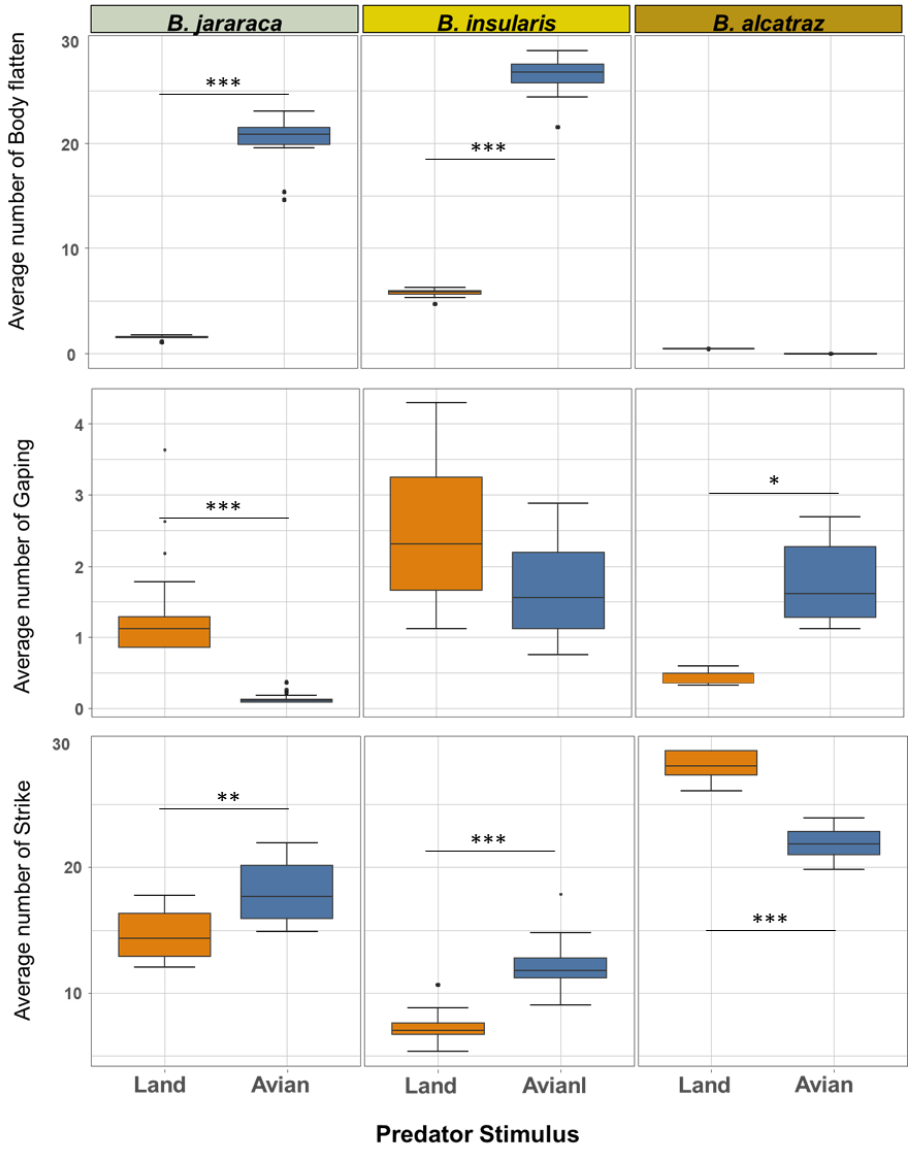


Figure 4. Influence of predatory stimulus types on escape threatening behaviors of *Bothrops jararaca, B. insularis* and *B. alcatraz.* Upper graph - Gaping behavior. Middle graph- Strike behavior. Lower graph - Body flatten behavior. *** clear difference in the frequency of behaviors in relation to the type of predatory stimulus ($p<0.001$).

2.4.DISCUSSION

A previous study evaluating the defensive behavior of five species of the genus *Bothrops* found quantitative differences in behavioral categories which appear to be an adaptive response to more or less predation pressure of avian predators since each species studied occupies distinct habitats (ARAUJO et al., 2006). Our study simulating avian and terrestrial predators showed in fact that these different predatory stimuli influenced the frequency of each category of antipredatory behavior. Furthermore, the species studied *Bothrops jararaca* and their closest species that inhabit islands also responded differently to the same predatory stimulus. Snakes can change their antipredator behavior during a snake's life depending on the rate of predation pressure experienced (AUBRET et al,. 2011). These authors verified that adult snakes exposed to a higher risk of predation showed higher bite rates. On the other hand, there is no association between behavior and predator richness among neonates (AUBRET et al., 2011). Thus, behavioral change can be attributed to learning processes with predators. All snakes used in the present study were born in captivity, so they had no contact with natural predators and did not need to change behaviors by ontogeny. Therefore, we can state that the antipredator behaviors for these species were not influenced by previous experiences with predators.

In the context of escape behaviors, cocking behavior is the most differentiated. According to Morri and Burghardt (2004), escape behaviors are strategies that increase the distance between a snake and a predator. Among the categories of behaviors, it is common for the snake to turn its head to the opposite side of the predatory stimulus. However, cocking is the only behavior that while increasing the distance between the snake and the stimulus object, the snake's head continues to direct attention with its head toward the predator. *Bothrops jararaca* was the only species that suffered influence in the frequency of cocking depending on the predatory stimulus. Possibly, this is explained because *B. jararaca*, hypothetically, is the species that presents the greatest diversity of predators, and cocking behavior is highly relevant to avoid predation by some type of predator that the other species do not confront (SCHALK & COVE, 2018).

For the other escape behaviors, such as flee and blunt escape, all species exhibited a similar pattern of behavior. Snakes exhibited these behaviors more often in the face of terrestrial predators. Possibly because this type of predator is on the same physical plane as the snake, and can make more attacks than an avian predator in a short period of time. Therefore, snakes would tend to seek shelter as quickly as possible through escape, since behaviors that

involve moving away from the predatory stimulus are the main ones used by snakes (STANKOWICH & BLUMSTEIN, 2005; LLEWELYN et al.,2010).

Bothrops insularis used fleeing and sudden blunt behaviors most frequently among the species studied. This behavioral characteristic may be due to an attribute that most distinguishes it from other species. Its yellowish coloration, which is out of tune with the environment. Jackson (1976) observed that striped or differently colored snakes tend to escape more than those with spotted coloration. Snakes with spotted patterns tend to be more cryptic and stationery because their coloration pattern favors camouflage.

Based on this, *B. alcatraz* and *B. jararaca* which have spotted coloration should use cryptic behaviors more frequently. Although, *B. alcatraz* did not show any cryptic behaviors. *B. jararaca* corroborated this hypothesis, as it was one of the species that invested in this behavioral class the most. Therefore, the coloration of the animal, should not be the only explanation, other variables of the ecology and natural history of the species not yet known influence the decision-making.

It was found that the species tended to use more cryptic behaviors in contexts involving avian predator attacks. Wilgers and Horne (2007) demonstrated using artificial snakes that detection by avian predators is impaired when there is no movement by the snake, as these predators visually identify their prey by the movement it makes. Furthermore, *B. jararaca* demonstrated to hide its head more often from avian predators than terrestrial predators. Birds of prey such as the Laughing falcon (*Herpetotheres cachinnans*) attack their prey by going toward the animal's head (MEDRANO-VIZCAÍNO, 2019). This difference may be due to the fact that the snake tends to hide its most vital part (i.e., the head) precisely in avian attacks with higher head attack rates (LLEWELYN *et al*.,2010).

Threat behavior tends to be aimed at persuading the predator that the animal is dangerous (GRENNE, 1988; MORI & BURGHARDT, 2004). Within this class of behaviors, the strike is one of those that are most influenced by abiotic and biotic factors (WHITAKER *et al*., 2000; SHINE et al., 2002; MORI & BURGHARDT, 2004). Both *B. jararaca* and *B. insularis* showed higher strike frequency to avian stimuli. On the other hand, *B. alcatraz,* was more aggressive to terrestrial predators, possibly due to this species suffering greater predation pressure by terrestrial predators such as tegu lizards (*Salvator merianae*) that are abundant on the island (MUSCAT et al., 2016). Among the three species, *B. alcatraz* was the most aggressive, presenting the highest strike rate. This species is very small, and has similarities with the young of *B. jararaca*, having pedomorphic characteristics (MARQUES *et al*., 2002). Snake size is one of the main variables that interfere with strike behavior. Generally, by

suffering more predation pressure, small snakes tend to be more aggressive and have higher strike rates (BLOMBERG & SHINE, 2000; JANZEN *et al.*, 2000; SHINE *et al*., 2001; Tucker *et al*., 1999; Vitt, 2000). This may explain this typical behavior of *B. alcatraz*. Another important factor is that we do not know the predation rate and there are no reports of predation of this species on the island, only the occurrence of potential terrestrial and avian predators on the island of Alcatrazes (MARQUES, 2021). Therefore, the striking and frequent strike behavior of this species may be influenced by several unknown factors that still need to be studied.

Another threatening behavior is gaping. This behavior was most often used by *B. insularis.* Gapping behavior is used by arboreal and diurnal snake species (MARTINS *et al*., 2008). Although the Golden Lancehead uses low vegetation, it is the most arboreal species among the three (AMARAL, 1921; MARQUES. 2021). In addition, it is also the species that has more diurnal habits than the other species that are more nocturnal (SAZIMA,1992; MARQUES et al., 2002b; SIQUEIRA et al. 2021). These data corroborate these previous studies. Both *B. insularis* and *B. jararaca* exhibited more gaping to land stimuli. This data can be explained because gaping is a behavior that increases the size of the animal on an x-axis, and is possibly more effective for predatory confrontations that occur on the same spatial plane.

It was observed, that both *B. jararaca* and *B. insularis*, flattened more when confronted with avian predatory stimulus. Body flattening behavior expands the snake's body laterally (GREENE, 1988). In a view from above, the snake appears larger when flattened. Bird attacks occur overhead and often in daylight (MEDRANO-VIZCAÍNO, 2019). Terrestrial and diurnal snakes tend to flatten out more than other species (MARTINS *et al*., 2008).

Antipredator behavior can be lost after the loss of a key predator, i.e., loss of a trait after relaxed selection (LATHI *et al*., 2009), or it can persist for many generations (BYERS, 1997). If unnecessary antipredator behavior has substantial costs when expressed, then the loss of predators should lead to rapid trait loss (BLUMSTEIN et al., 2004). Thus, island species that have usually lower predation rates than mainland pitvipers would tend to lose or decrease the frequency of some antipredator behaviors. We found that defensive behavioral repertoire persists in island pitvipers, but the frequencies of these behaviors differ amongst species and predatory stimuli and they still exhibit some similarities in response patterns to predators.

This persistence of antipredator behaviors is consistent with the multipredator hypothesis. This hypothesis attempts to explain the persistence of antipredator behavior under relaxed selection (BLUMSTEIN, 2006). This hypothesis assumes that antipredator behavior has pleiotropic effects and that the behaviors may be genetically linked. Once such a set of traits

is created, the loss of a specific predator but the persistence of others would prevent the antipredator behavior from disappearing.

The multipredator hypothesis has been found in some kangaroos and wallabies, in which the loss of all predators apparently led to a rapid loss of antipredator behavior, while the loss of only one or two predators had a limited effect on the expression of antipredator. behavior for the missing species (BLUMSTEIN & DANIEL, 2002, BLUMSTEIN *et al*., 2004). In addition, the evolutionary persistence of rattlesnake (*Crotalus* spp.) recognition in California ground squirrels (*Spermophilus beecheyi*) (reviewed in COSS & GOLDTHWAITE 1995; COSS 1999) suggested that rattlesnake recognition abilities can be maintained for over 70000 years after rattlesnake isolation. *B. insularis* and *B. alcatraz* are separated from the mainland about 11000 years (MARQUES *et al*. 2002; WÜSTER *et al*. 2005). Therefore, the integrity of the antipredator behaviors of the isolated species, based on the multi-predator hypothesis, must be because some type of predator still exerts pressure on these populations, or if not, they are still relatively young populations and would not yet have lost the defensive repertoire.

Another explanation that may elucidate the permanence of antipredator behavior is the "phantom predator past hypothesis" (PECKARSKY & PENTON 1988; BYERS 1997). This hypothesis states that a species subjected to past selection for antipredator behavior by a predator that exerted strong predatory pressure will maintain antipredator behavior if it is not too costly to do so (e.g., NEILL 1990). The ancestral marsupials of opossums have been in South America for at least 10 million years and the ancestral lineage of the genus *Didelphis* for 3 million years (DIAS & PERINI, 2018). These animals are major predators of snakes, including jararacas, because they are resistant to the toxins in their venoms (PERALES et al., 1986; MELO & SUAREZ-KURTZ, 1988). Therefore, these american marsupials probably exerted a strong predation pressure and selection of defensive behaviors on the ancestral lineages of the south american pitvipers since the early Pleistocene, which still persists in the current lineages.

This was the first study to investigate the antipredator behavior of insular species in South America. However, more comparative behavioral studies of these species are needed, as well as extending these studies to other *Bothrops spp*. island species that are being described (BARBO et al., 2012; BARBO et al., 2016). Experimental studies conducted with animals in the wild need to be conducted for a complete understanding of antipredator behavior providing a general understanding used for the conservation of these species.

2.5. REFERENCES

AMARAL A. Contribuição para o conhecimento dos ophideos do Brasil – Parte II: Biologia da nova espécie, Lachesis insularis. Memórias do Instituto Butantan 1: 40 – 44, 1921.

ARAUJO, M. S.; MARTINS, M. Defensive behaviour in pit vipers of the genus Bothrops (Serpentes, Viperidae). The Herpetological Journal, v. 16, n. 3, p. 297-303, 2006.

AUBRET, Fabien; MICHNIEWICZ, Radika J.; SHINE, Richard. Correlated geographic variation in predation risk and antipredator behavior within a wide-ranging snake species (*Notechis scutatus*, Elapidae). Austral Ecology, v. 36, n. 4, p. 446-452, 2011.

BARBO, Fausto E. et al. A new and threatened insular species of lancehead from southeastern Brazil. Herpetologica, v. 68, n. 3, p. 418-429, 2012.

BARBO, Fausto E. et al. Another new and threatened species of lancehead genus *Bothrops* (Serpentes, Viperidae) from Ilha dos Franceses, Southeastern Brazil. Zootaxa, v. 4097, n. 4, p. 511-529, 2016.

BLOMBERG, S.P, SHINE, R. Size-based predation by kookaburras (*Dacelo novaeguineae*) on lizards (*Eulamprus tympanum*: Scincidae): what determines prey vulnerability? Behav Ecol Sociobio l48:484-489, 2000.

BLUMSTEIN, Daniel T. Moving to suburbia: ontogenetic and evolutionary consequences of life on predator-free islands. Journal of Biogeography, v. 29, n. 5-6, p. 685-692, 2002.

BLUMSTEIN, Daniel T. The multipredator hypothesis and the evolutionary persistence of antipredator behavior. Ethology, v. 112, n. 3, p. 209-217, 2006.

BLUMSTEIN, Daniel T.; DANIEL, Janice C.; SPRINGETT, Brian P. A test of the multi-predator hypothesis: rapid loss of antipredator behavior after 130 years of isolation. Ethology, v. 110, n. 11, p. 919-934, 2004.

BURGHARDT, Gordon M.; GREENE, Harry W. Predator simulation and duration of death feigning in neonate hognose snakes. Animal Behaviour, v. 36, n. 6, p. 1842-1844, 1988.

BYERS, J. A. American Pronghorn: Social Adaptations and the Ghosts of Predators Past. Univ. Chicago Press, Chicago, IL.1997.

BYERS, John A. American pronghorn: social adaptations and the ghosts of predators past. University of Chicago Press, 1997.

CORDERO, G.A.R.; NICOLAS, R.A.B .Feeding habits of the opossum (Didelphis marsupialis) in northern Venezuela. Field Zool New Ser 39: 125–131, 1986.

COSS, Richard G. Effects of relaxed natural selection on the evolution. Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms, eds SA Foster and JA Endler (New York, NY, p. 180-208, 1999.

COSS, Richard G.; GOLDTHWAITE, Ronald O. The persistence of old designs for perception. Perspectives in ethology, v. 11, p. 83-148, 1995.

DIAS, C.A.R & PERINI, F.A.Biogeography and early emergence of the genus *Didelphis* (Didelphimorphia, Mammalia). Zoologica Scripta 47(4). 2018.

GLAUDAS, X., WINNE, C. T., & FEDEWA, L. A. Ontogeny of anti-predator behavioral habituation in cottonmouths (*Agkistrodon piscivorus*). Ethology, 112(6), 608-615, 2006.

GREENE HW. Antipredator mechanisms in reptiles. In: Defense and life history (Gans C, Huey RB, eds). New York: Alan R. Liss; 1–134, 1988.

JACKSON, James F.; INGRAM III, William; CAMPBELL, Howard W. The dorsal pigmentation pattern of snakes as an antipredator strategy: a multivariate approach. The American Naturalist, v. 110, n. 976, p. 1029-1053, 1976.

JANZEN FJ, TUCKER JK, PAUKSTIS GL. Experimental analysis of an early life-history stage: Avian predation selects for larger body size of hatchling turtles. J Evol Biol13:947-954, 2000.

KAVALIERS, M. Responsiveness of deer mice to a predator, the short-tailed weasel: population differences and neuromodulatory mechanisms. Physiological Zoology, 63(2), 388-407, 1990.

LAHTI, David C. et al. Relaxed selection in the wild. Trends in ecology & evolution, v. 24, n. 9, p. 487-496, 2009.

LANGKILDE, Tracy; SHINE, Richard; MASON, Robert T. Predatory attacks to the head vs. body modify behavioral responses of garter snakes. Ethology, v. 110, n. 12, p. 937-947, 2004.

LLEWELYN, John; WEBB, Jonathan K.; SHINE, Richard. Flexible defense: Context-dependent antipredator responses of two species of Australian elapid snakes. Herpetologica, v. 66, n. 1, p. 1-11, 2010.

MACARRÃO, A. 2010. Avifauna da Ilha da Queimada Grande, SP: diversidade, estrutura trófica e sazonalidade. Unpublished MSc thesis, Universidade Estadual Paulista, São José do Rio Preto, Brazil.

MAGURRAN, A. E. Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms. 1999.

MARQUES O. A. V., MARTINS M., and SAZIMA I.A jararaca da Queimada Grande. Ciência Hoje 31: 56-59, 2002.

MARQUES, Otavio Augusto Vuolo et al. A ilha das cobras: biologia, evolução e conservação da jararaca-ilhoa na Queimada Grande. 2021.

MARQUES, Otavio AV; MARTINS, Marcio; SAZIMA, Ivan. A new insular species of pitviper from Brazil, with comments on evolutionary biology and conservation of the Bothrops jararaca group (Serpentes, Viperidae). Herpetologica, v. 58, n. 3, p. 303-312, 2002b.

MARTINS, Marcio; MARQUES, Otavio AV; SAZIMA, Ivan. How to be arboreal and diurnal and still stay alive: microhabitat use, time of activity, and defense in Neotropical forest snakes. South American Journal of Herpetology, v. 3, n. 1, p. 58-67, 2008.

MEDRANO-VIZCAÍNO, Pablo. Predating behavior of the Laughing falcon (Herpetotheres cachinnans) on the venomous Amazonian pit viper Bothrops atrox (the use of roads as a prey source). BioRisk, v. 14, p. 25, 2019.

MELO PA, SUAREZ-KURTZ G. Release of sarcoplasmic enzymes from skeletal muscle by *Bothrops jararaca* venom: antagonism by heparin and by the serum of South American marsupials. Toxicon 26(1): 87–95. 1988.

MORI, Akira; BURGHARDT, Gordon M. Thermal effects on the antipredator behaviour of snakes a review and proposed terminology. Herpetological Journal, v. 14, n. 2, p. 79-87, 2004.

MUSCAT, EDELCIO; OLMOS, Fabio; ROTENBERG, ELSIE. *Salvator merianae* (black and white tegu) scavaging around the nests of *Fregata magnificens* (frigatebird). Herpetological Bulletin, v. 135, p. 36-37, 2016.

NEILL, W. E. Induced vertical migration in copepods as a defense against invertebrate predation. Nature 345, 524—526.1990.

OLIVEIRA, Maria Ermelinda; SANTORI, Ricardo Tadeu. Predatory behavior of the opossum *Didelphis albiventris* on the pitviper *Bothrops jararaca.* Studies on Neotropical Fauna and Environment, v. 34, n. 2, p. 72-75, 1999.

PECKARSKY, B. L. & PENTON, M. A. Why do *Ephemerella nymphs* scorpion posture: a ‘‘ghost of predation past’’? Oikos 53, 185—193.1988.

PERALES J, MUÑOS R, MOUSSATCHE H . Isolation and partial characterization of a protein fraction from the opossum (*Didelphis marsupialis*) serum, with protecting property against the *Bothrops jararaca* venom. An Acad Brasil Cienc 58:155–162. 1986.

SAZIMA,I.Natural history of the jararaca pitviper,*Bothrops jararaca*, in southeastern Brazil,Pp. 199-216. In:J.A.Campbell & E.D. Brodie-Jr (Eds).Biology of Pitvipers.Selva, Tyler, Texas, 452p. 1992.

SCHALK, Christopher M.; COVE, Michael V. Squamates as prey: Predator diversity patterns and predator-prey size relationships. Food Webs, v. 17, p. e00103, 2018.

SCUDDER, Kent M.; CHISZAR, David. Effects of six visual stimulus conditions on defensive and exploratory behavior in two species of rattlesnakes. The Psychological Record, v. 27, n. 3, p. 519-526, 1977.

SEGHERS, B. H., SHAW, P. W., & CARVALHO, G. R. The behavioral diversity and evolution of guppy, *Poecilia reticulata*, populations in Trinidad. Advances in the Study of Behavior, 24, 155-202, 1995.

SHINE R, SUN L, FITZGERALD M, KEARNEY M. Antipredator responses of free-ranging pit vipers (*Gloydius shedaoensis*, Viperidae). Copeia 2002:843–850, 2002.

SHINE, R.; LEMASTER, M.P, MOORE, I.T, OLSSON, M.M, MASON, R.T. Bumpus in the snake den: effects of sex, size, and body condition on mortality of red-sided garter snakes. Evolution55:598-604, 2001.

SHINE, Richard et al. Effects of sex, body size, temperature, and location on the antipredator tactics of free-ranging gartersnakes (*Thamnophis sirtalis*, Colubridae). Behavioral Ecology, v. 11, n. 3, p. 239-245, 2000.

SIQUEIRA, L. H., BANCI, K. R., & MARQUES, O. A. Seasonal activity of *Bothrops jararaca* (Serpentes, Viperidae): optimizing foraging while avoiding predators. South American Journal of Herpetology, 20(1), 67-74, 2021.

STANKOWICH, Theodore; BLUMSTEIN, Daniel T. Fear in animals: a meta-analysis and review of risk assessment. Proceedings of the Royal Society B: Biological Sciences, v. 272, n. 1581, p. 2627-2634, 2005.

TUCKER, J.K, FILORAMO, N.I, JANZEN, F.J. Size-biased mortality due to predation in a nesting freshwater turtle, *Trachemys scripta*. Am Midl Nat141:198-203, 1999.

venom: antagonism by heparin and by the serum of South American marsupials. Toxicon 26(1): 87–95. 1988.

VITT, L.J. Ecological consequences of body size in neonatal and small-bodied lizards in the neotropics. Herpetol Monogr14:388-400, 2000.

WAGLER,J. Serpentum brasiliensium spcies novae. Franc.Seraph. Hubschmann, Monachii, 1824.

WHITAKER, P.B.; SHINE, Responses of free-ranging brownsnakes (*Pseudonaja textiles*:Elapidae) to encounters with humans. Wildl Res26:689-704, 1999.

WILGERS, Dustin J.; HORNE, Eva A. Spatial variation in predation attempts on artificial snakes in a fire-disturbed tallgrass prairie. The Southwestern Naturalist, v. 52, n. 2, p. 263-270, 2007.

WÜSTER W., DUARTE M. R., and SALOMÃO, M. G. Morphological correlates of incipient arboreality and ornithophagy in island pitvipers, and the phylogenetic position of . Journal of Zoology 266: 1 – 10, 2005

ZAHER, Hussam et al. Large-scale molecular phylogeny, morphology, divergence-time estimation, and the fossil record of advanced caenophidian snakes (Squamata: Serpentes). PloS one, v. 14, n. 5, p. e0216148, 2019.

2.6.SUPPLEMENTARY MATERIAL

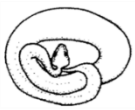
Table of behaviors		
Escape behavior		
Behavior	**Description**	**Figure**
Flee	The snake, with its anterior body oriented toward the ground, moves in the opposite direction of the stimulus, increasing the distance between them. The movement can be serpentine (lateral undulation) or anterolateral delocalization (sidewinding)	
Blunt escape	The snake through a quick thrust withdraws the entire front part of its body from the ground, in the opposite direction of the stimulus,	
Cocking	The snake remains with the anterior body region retracted in a sigmoid curve, facing the stimulus source and recoiling with undulations of the posterior body portions. The head is elevated, and the tail flaps constantly. This behavior can be included in what Greene (1988) defined as "frontal display"	
Cryptic behaviors		
Immobility behavior	The snake stays still and may be in a loose or coiled position	
Head hiding	The snake positions its head or part of it in the space between the spirals of its coiling, covering it with one of the turns of its body	
Threatening behaviors		
Gaping	The snake partially opens its mouth, then closes it, without throwing any strikes. The body may be coiled or not	
Strike	The snake projects its head rapidly toward the stimulus, with wide abduction of the mouth, as the curves of the anterior body region stretch.	
Body flatten	The snake performs dorso-ventral compression along its body, mainly on its posterior part	

Table 1. table of antipredator behaviors and their explanation