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Study of defensive behavior of a venomous snake as a new approach to understand snakebite

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Snakebites affect millions of people worldwide. The majority of research and management about snakebites focus on venom and antivenom, with less attention given to snake ecology. The fundamental factor in snakebites is the snakes' defensive biting behavior. Herein we examine the effects of environmental variables (temperature, time of day, and human stimulus) and biological variables (sex and body size) on the biting behavior of a medically significant pit viper species in Brazil, *Bothrops jararaca* (Viperidae), and associate it with the epidemiology of snakebites. Through experimental simulations of encounters between humans and snakes, we obtained behavioral models applicable to epidemiological situations in the State of São Paulo, Brazil. We found a significant overlap between behavioral, morphological, environmental, and epidemiological data. Variables that increase snakebites in epidemiological data also enhance the tendency of snakes to bite defensively, resulting in snakebites. We propose that snakebite incidents are influenced by environmental and morphological factors, affecting the behavior of snakes and the proportion of incidents. Thus, investigating behavior of snakes related to snakebite incidents is a valuable tool for a better understanding of the epidemiology of these events, helping the prediction and, thus, prevention of snakebites.

Keywords Snakebite, Behavior, Epidemiology, Ecology

Snakebite is a potentially fatal condition caused by toxins in the venom of venomous snakes and remains a significant public health concern worldwide, affecting millions of people each year[1].The World Health Organization (WHO) classifies snakebite as a high-priority neglected tropical disease due to its impact on human populations. While much research has been conducted on venom properties, antidotes, and incidence analysis, there has been limited emphasis on the ecological and behavioral aspects of snakebite[2,3].

Antipredator behavior is a vital ecological and behavioral aspect to be studied in snakebite, since encounters between humans and snakes often trigger defensive responses from the snakes, such as biting, which can result in envenomation[1]. Although there is a possibility of "dry bites" (i.e., snakebites without venom injection)[4], defensive snakebites require a larger quantity of venom than predatory bites, potentially leading to more severe envenomation[5]. However its importance, there is a lack of comprehensive studies that associate antipredator behavior with snakebite.

Defensive biting behavior is influenced by various intrinsic and environmental factors, such as body size[6,7], sex[8,9], type of aversive stimulus[10,11], body temperature[12,13] and various extrinsic factors[7,8]. Depending on the context and species of each snake, these variables can impact the defensive repertoire in different ways.

Understanding the ecological and behavioral factors influencing snakebite incidence is crucial for effective control and prevention measures[3]. A dynamic understanding of venomous snake ecology can enhance the management of snakebite risks. However, research in this area has been limited, hampering efforts to accurately map, predict, and mitigate snakebite[2,3].

This study aims to address this research gap by investigating the intrinsic and environmental factors that influence defensive behavior and its relationship to snakebite epidemiology. The model snake species studied,

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the pit viper *Bothrops jararaca*, is the most prevalent venomous snake species in southeastern Brazil[14,15]. This snake is the cause of most snakebite cases in the region and represents a significant proportion of incidents in the country, totaling around 20,000 envenomations annually[16,17].

To achieve this goal, we analyzed whether intrinsic and environmental factors influence the defensive behavior of *B. jararaca* and its association with snakebite epidemiology (Fig. 1). More specifically, it investigated the morphological properties of this snake, such as sex, size, life stage, type of aversive stimulus, environmental temperature, and time of day, to assess their impact on the probability of snakebite and tolerance of *B. jararaca* to aversive stimuli and how this relates to snakebite epidemiology. Additionally, we explored epidemiological data scenarios to apply the associations between the behavior of *B. jararaca*, size, temperature, time of day, and the incidence of snakebite. Our hypothesis is that all the environmental and intrinsic factors that have higher representativeness in snakebite cases will also influence the biting behavior of *B. jararaca*, increasing this snake's tendency to bite defensively, as this behavior may be the causal mechanism between the two variables (ecological and epidemiological).

Results

Behavioral influence of intrinsic and environmental factors

Sex, size, stimulus type, and time of day

We found that both intrinsic and environmental factors can influence the tendency to bite defensively and tolerance of *B. jararaca* (Tables S1, S2, S3, S4). Tendency to bite was defined as a high probability of biting. The interaction between snake size, sex, type of human stimulus, and time of day had a clear impact on bite probability ($z = -2.978$; $p = 0.002$) (Fig. 2a) and tolerance to the stimulus ($z = 2.714$; $p = 0.007$) (Fig. 2b). Importantly, we did not observe a significant difference between captive and wild *B. jararaca* in their propensity to bite ($z = -0.648$, $p = 0.517$) and tolerance ($z = -0.125$, $p = 0.90$). Smaller females were more prone to bite with non-physical stimulation during the morning period. For males, the situation was the opposite, and males with smaller body sizes than smaller females had lower probability of biting. However, these smaller males when touched during the morning period had a higher probability of biting, similar to females (Fig. 2a). Additionally, tolerance, defined as the number of stimuli required until the first bite, was affected by various variables. We found that when there is physical contact, both male and female snakes tended to be intolerant to the stimulus, regardless of size and time of day. However, when there was no physical contact, smaller males were more tolerant than larger ones during the morning period ($z = 3.220$; $p = 0.00128$) (Fig. 2b).

We observed that the body region where physical contact occurred also influenced the likelihood of a snake biting. When the contact occurred on the head, the probability of a defensive bite was significantly higher compared to contact made on the midbody or tail ($z = -88.87$, $p < 0.001$). The probability of a *B. jararaca* biting when touched on the head was approximately 44%, whereas contact on the midbody and tail decreased the probability to 20% ($z = -1010.77$, $p < 0.001$) and 11% ($z = -1804.28$, $p < 0.001$), respectively (Fig. 3).

Figure 1. Natural situations under which *Bothrops jararaca* snakebite chances tend to increase. (**A**) The snake bites defensively when stepped on or cornered. (**B**) Juvenile snakes are more prone to bite than adults and frequently rest on vegetation where they are difficult to spot. (**C**) The camouflaging effect of the color pattern of a snake basking among vegetation close to worked land is a risk factor for snakebite. (**D**) The snake often basks on recently worked land at the edges of plantations, another risk factor for snakebite.

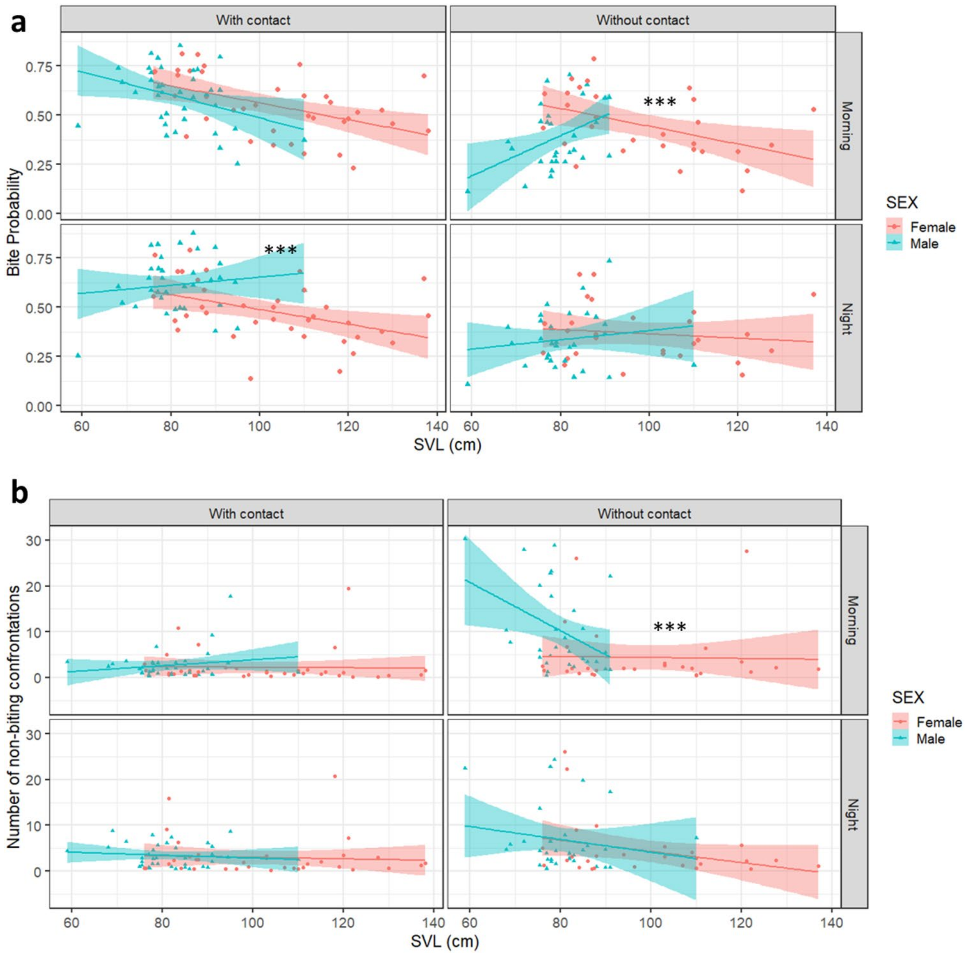

Figure 2. Biting probability and tolerance are influenced by *Bothrops jararaca* size (SVL), sex, type of human stimulus, and period of the day. (**a**) Biting probability. (**b**) Tolerance (number of confrontations with or without non-bite contact). ***clear difference in bite probability and tolerance between sexes ($p < 0.001$).

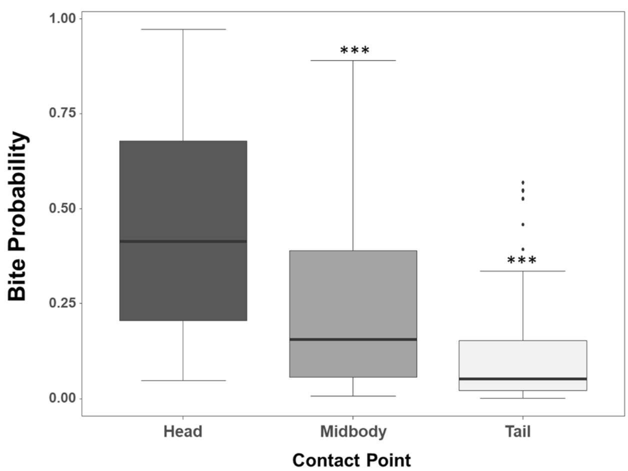

Figure 3. Probability of bite with physical contact on the head, midbody, and tail of *Bothrops jararaca*. ***clear difference in bite probability between tail and midbody with the stimulus made on the head ($p < 0.001$). The bottom and upper lines of the box represent the second and third quartiles, respectively, and the central line is the median. Whiskers indicate the dispersion of the data (95% of probability values), excluding outliers.

Temperature
In the context of physical contact between humans and snakes, the temperature of the snake influenced the probability of biting. The temperature of females during both nighttime and daytime periods showed a positive correlation with biting probability, i.e., warmer animals had a greater tendency to bite ($z = 3.868$, $p = 0.001$) (Fig. 4). However, for males, the opposite was observed during the nighttime since warmer snakes were less likely to bite ($z = 14.840$; $p < 0.001$) (Fig. 4).

In addition to the effects mentioned, we observed that the probability of *B. jararaca* biting changed with life stage when contacted by the boot. Newborns showed a higher biting probability than adults, with a probability 2.17 times higher than adults ($z = 3.533$; $p < 0.001$). On the other hand, juveniles showed no clear differences from adults in biting probability ($z = 1.362$; $p = 0.17$) (Fig. 5a). The interaction between the life stage and sex proved to be important. The time of day did not influenced the probability of biting in a given life stage ($z = -0.710$; $p = 0.478$). However, when we analyzed the interaction between life stage and sex, we found a significant difference in the biting probability between the sexes of newborns and adults, both during the day and at night (Newborns: $z = -2.211$; $p = 0.027$; Adults: $z = 2.783$; $p = 0.005$), but not among juveniles ($z = -0.619$; $p = 0.536$). In general, newborn females were the most prone to bite stage, regardless of the time of day (Fig. 5b).

Epidemiological data of snakebite by Bothrops jararaca
The interaction between environmental and morphological variables modified the probability of snakebite by *B. jararaca*. We applied our model to an epidemiological scenario occurring in the State of São Paulo, Brazil. It is known that the body size of *B. jararaca* differs according the region within the state. The average body size of *B. jararaca* in the coastal region is smaller than that of the upland population[18,19]. As we observed that the biting tendency is influenced by size and temperature, we used this information to predict the probability of a snakebite based on body size the regional temperature. We calculated the model for temperatures of 22 °C and 18.3 °C for the coastal and upland regions, respectively, using the environmental temperature map of *B. jararaca* occurrence (Fig. S1). Our model indicates that snakes from the coast have a higher likelihood of biting compared to those from the uplands (Fig. 6A).

As the snakes from the coast could be more prone to bite than those from the uplands, we investigated the incidence of bothropic envenomation in these two regions. As expected, more snakebites occur in the coastal areas than in the uplands (Table S5; Fig. 6B, 6C). We utilized the population data from the rural area, a region where people become more vulnerable to snakebites[20]. An average of 500 snakebites per 100,000 inhabitants annually was recorded, while in the uplands, the average was 278 ($z = --2.986$, $p = 0.0028$) (Fig. 6B). The size of the population inhabiting rural areas, as well as their interaction with the geographical region (coast and upland), has not demonstrated any correlation with the incidence of snakebites ($z = -1.629$, $p = 0.1032$).

Another important factor is the association between the number of annual snakebites in the municipalities of São Paulo and their average temperatures. We found a positive correlation: municipalities with higher annual temperatures also had higher rates of snakebites (Table S6). For each degree increase in temperature, the odds of a snakebite increase by approximately 28.27% ($z = 2.416$, $p = 0.01$) (Fig. S2). It is worth noting that the variance explained by the random variable (number of inhabitants in each municipality) was lower than the variance explained by predictor variables such as temperature and region (upland and coastal) (population variance: 0.0250; predictor variance: 0.0364). This indicates that the predictor variables have a greater impact on the variation in the outcome than the variation between different populations.

Whereas we identified an association among epidemiological, morphological, and behavioral data in this scenario, these data are subject to confounding factors. For instance, the snakebite data used, provided by the

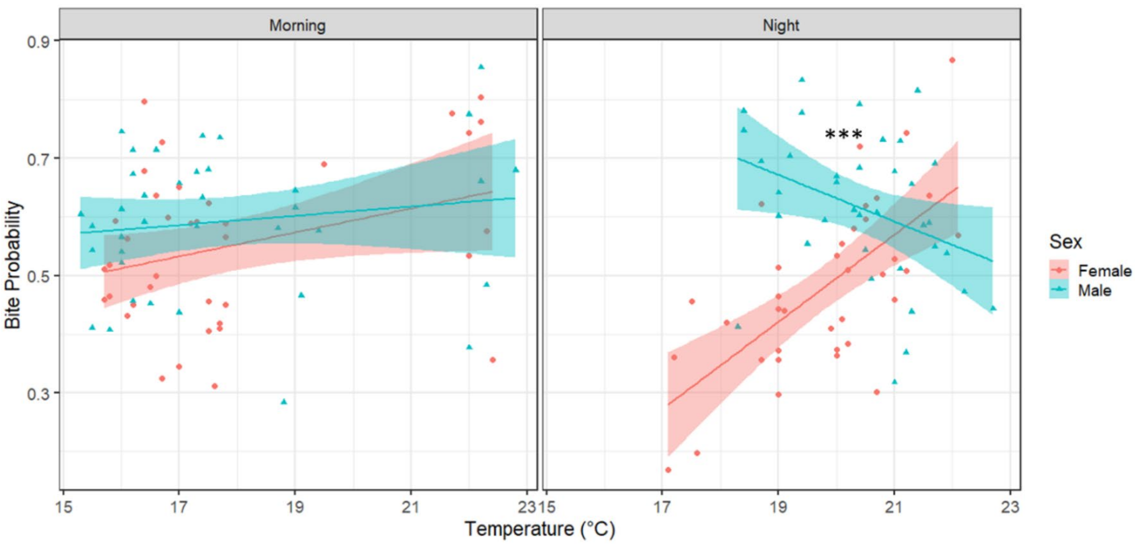

Figure 4. Influence of body temperature on biting probability of *Bothrops jararaca* females and males in morning and evening periods. ***Clear difference in bite probability between sexes ($p < 0.001$).

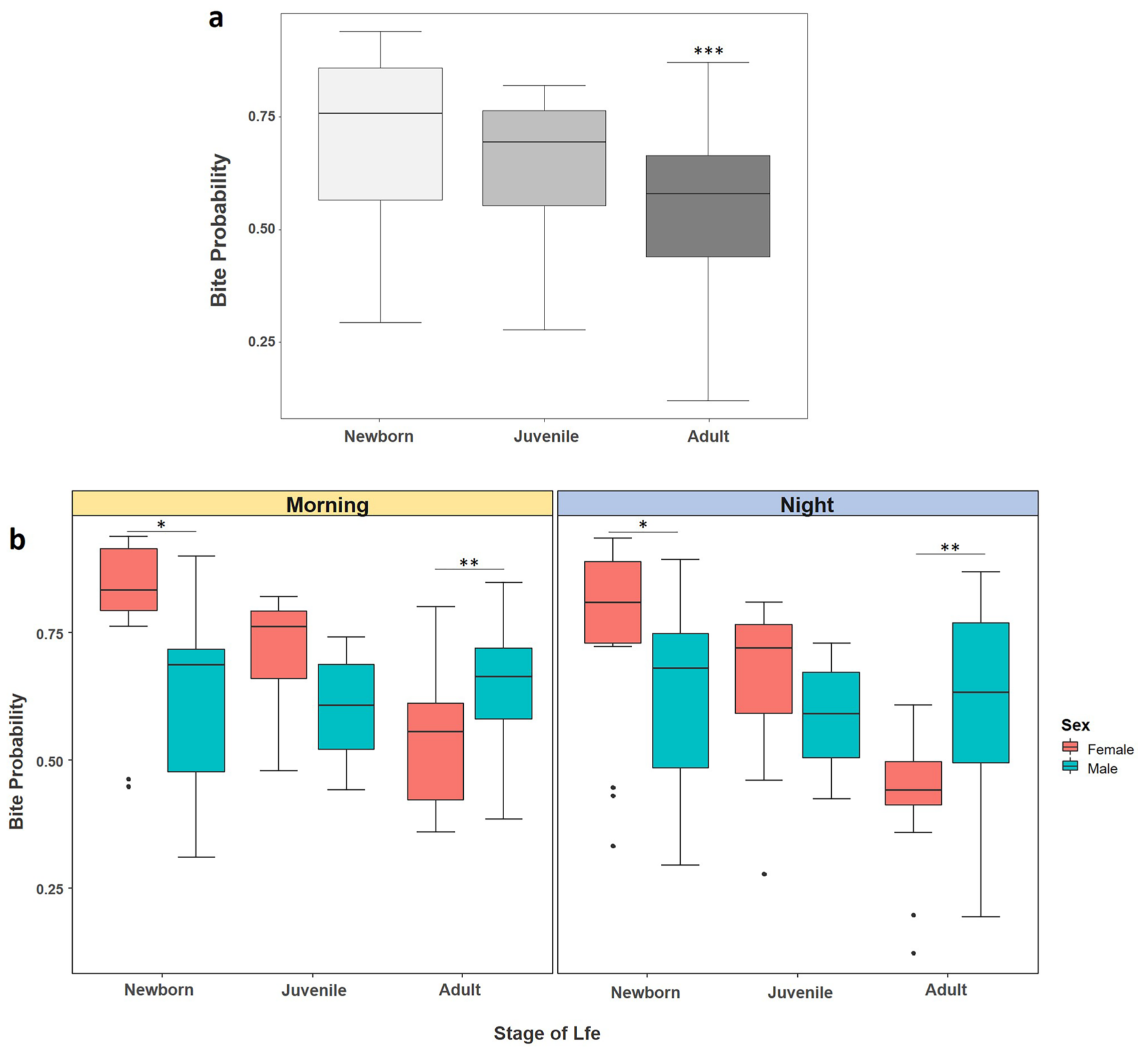

Figure 5. (**a**) The probability of biting with life stage of *Bothrops jararaca*. *** Clear statistical difference in probabilities between adults and newborns ($p < 0.001$). (**b**) Biting probability of *B. jararaca* of different sexes, life stages, and times of the day. **Clear statistical difference between adult males and females both day and night ($p < 0.01$). *Clear statistical difference between newborn males and females both day and night ($p < 0.05$). The bottom and upper lines of the box correspond to the second and third quartiles, respectively, and the central line represents the median. Whiskers indicate the dispersion of the data (95% of probability values), excluding outliers.

public health agency, only encompass information on snakebites from any species within the genus *Bothrops*. Consequently, we conducted analyses of our models in a more specific context, focusing solely on snakebites caused by *B. jararaca*. Using snakes involved in snakebite incidents exclusively from upland areas of São Paulo, we found that both males and females were smaller than the average size of the *B. jararaca* population in the uplands. The average size of males involved in snakebite incidents was 760 mm, while the average size of the male population sampled by our collection was 821 mm. Similarly, the average size of females involved in snakebites was 880 mm, while the average size of the female population was 1,130 mm (Fig. 7).

Another relevant result obtained from these data was the identified association between gender and time of day of the envenomation by *B. jararaca* (X-square = 12.05; df = 4; $p = 0.017$). In the morning, females account for 64% of snakebites. In the afternoon, the difference between genders decreased with females causing 58% of snakebites. On the other hand, during the night, the proportion reverses, with males accounting for 57% of envenomations (Table S7).

Discussion

This is the first study to explore the relationship between intrinsic and environmental factors, snake tendency to bite defensively, and epidemiological data on snakebite. We define tendency to bite as the probability of biting, this anti-predator behavior leading to human envenomation.

Some studies on viperids report a difference in the antipredator behavior of captive snakes compared to those in the wild[22]. However, in our study, we found that the propensity to bite and tolerance do not differ significantly between snakes in captivity and their wild counterparts[23–25]. Our results show that the probability a

Bite probability of *Bothrops jararaca* (Mean ± SD) [%]							
Female ♀				Male ♂			
Morning		Night		Morning		Night	
Upland	Coast	Upland	Coast	Upland	Coast	Upland	Coast
55.5 ± 5.5	64.5 ± 10.5	39 ± 41	63 ± 6	62.5 ± 6.5	70 ± 6	78 ± 6	53 ± 12

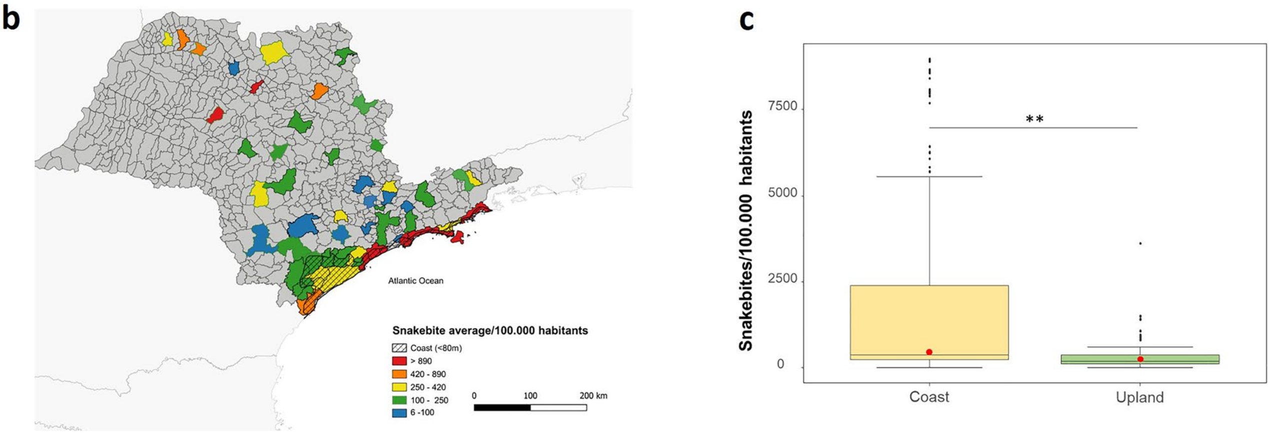

Figure 6. (**a**) Biting probabilities (%) obtained from size-based models of coastal and upland populations of *Bothrops jararaca*. (**b–c**) Bothropic envenoming of São Paulo from 2009 to 2022. (**b**) Map of the distribution of *Bothrops* snakebite incidence in the State of São Paulo, Brazil. The average number of snakebites on the coast and uplands of the state of São Paulo. ***Clear statistical difference in the average number of snakebite cases between the uplands and coast ($p < 0.001$). The red circles indicate the average of snakebites per 100,000 inhabitants on the coast and in the uplands. The average sizes of *B. jararaca* populations were obtained from literature[18,19,21].

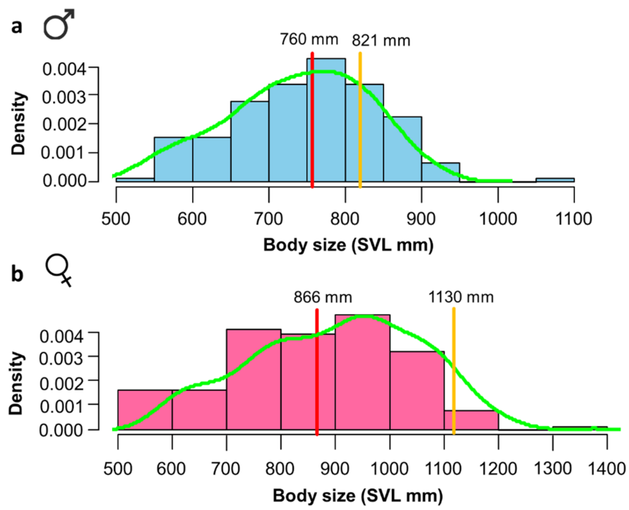

Figure 7. Probability density of body size distribution of *Bothrops jararaca* involved in snakebite in São Paulo uplands. (**a**) Males; (**b**) Females. Red line: average size of snakes involved in snakebite. Yellow line: the average size of snakes from São Paulo uplands[18,19,21]. Green line: probability density line.

of biting depends on factors such as snake size, sex, life stage, type of aversive stimulus, body region touched, environment temperature, and time of day. Additionally, we found a strong association between defensive bite behavior and snakebite incidence. The environmental and intraspecific factors influencing the increase in the probability of behavioral bites are the same as those associated with a higher incidence of snakebite events in epidemiological studies.

The main variable that influenced the tendency of *B. jararaca* to bite was body size, and epidemiological data support this as more snakebite cases are due to small snakes than those due to large ones[26]. Body size is known to influence bite chance in several snake species[7,25,27–30] although for certain species this relationship is unclear[31]. In general, smaller snakes tend to be more prone to bite than larger snakes[7,25,30], which may be due to predation pressure these snakes experience in the wild[9,32–35]. For *B. jararaca* this idea is also supported by the detail that newborn venom has a higher lethal activity in chicks[36]. As birds are important predators[37], a more effective venom of the newborns is advantageous against these predators. In addition to body size, when touched on the head the tested individuals were more prone to bite. Predation on venomous snakes is risky but more effective when the predator aims at the head or neck[37,38]. Therefore, people stepping on the head or the frontal part of a small *B. jararaca* greatly increases the snakebite risk.

For ectothermic vertebrates such as snakes, body temperature stands out as one of the most crucial factors influencing predator avoidance behavior, as it regulates the physiological capability of the snake to detect, repel, or escape from predators[8]. Additionally, a correlation has been identified between climatic cycles associated with El Niño and snakebites by *Bothrops asper* in Costa Rica: snakebites corresponded to meteorological changes, possibly attributed to the impact of meteorological fluctuations on snake biology[39].

Ambient temperatures influences behavior in various ways. Snakes in colder environments tend to display more defensive behaviors (as tendency to bite) than those in warmer conditions[12]. At higher temperatures, the snake's metabolism is more accelerated, improving locomotor escape[12,40]. It is more advantageous to flee, as biting would expose the snake's most vital body part[11]. Nonetheless, we found that *B. jararaca* has a higher propensity to defensive bite in warmer temperatures. One study[41] report something similar: an increase in the bite frequency in three species of colubrid snakes in lab conditions. Similar result is reported for *Thamnophis sirtalis*[42] , although the snakes were tested in boxes that restricted locomotor behaviors such as fleeing. The positive relationship between temperature and tendency to bite can be explained by the fact that speed, accuracy, and distance of the strike are reduced at low temperatures (suboptimal temperatures)[40,43]. At higher temperatures, the bite is more effective[13]. *Bothrops jararaca* showed a positive association between increased temperature and tendency to bite, although males changed their strategy at night: at higher temperatures, their propensity to defensive bite decreased. As nighttime is the most active period of this viperid and a possible period of higher predation pressure[26,37,44], males prefer escape behaviors under high temperatures as their locomotor capacities are greater than under low temperatures.

The environmental and morphological differences among populations of *B. jararaca* in the state of São Paulo, provide a practical example of applying the behavioral model to these variables. Two studies[18,19] report that *Bothrops* spp. from the coastal region of São Paulo have smaller body sizes than those from uplands By employing the behavioral model, we predicted the probabilities of snakebite incidents based on average body size and occurrence temperature. We found that smaller snakes along the coast were more prone to bite, which results in significantly higher number of snakebites compared to the uplands. Additionally, coastal municipalities in São Paulo with higher rates of bothropic envenomation also have higher annual average temperatures. This indicates an association between the influence of temperature and body size on the biting tendency of *B. jararaca* and the observed epidemiological profile. However, it is important to emphasize that this association does not establish causality, as other factors may also contribute to our findings.

Firstly, the data provided by SINAN[45] include any snakebites involving snakes of the genus *Bothrops*, not exclusively *B. jararaca*. Another point to consider is the unknown abundance of these viperids in different regions of São Paulo. Furthermore, epidemiological data indicates that envenomations by *B. jararaca* are more common in the warmer months[26], coinciding with an increase in its activity[26,37,46–48] and the vacation period that leads to higher influx of people to the coastal region for leisure events. Another relevant factor is that some municipalities, especially in the upland areas such as the São Paulo city, are highly urbanized, making encounters with snakes less common among the population. However, a survey of snake fauna in the municipality of São Paulo showed that *B. jararaca* is the third most abundant species and is widespread throughout the whole area, including parks in the central area that are used by the population for leisure activitiest[49]. Furthermore, our data indicate that the number of individuals residing in rural or urban areas did not affect the differential incidence of snakebites between coastal and upland regions. Nonetheless, it is possible that there are more cases of snakebite incidents on the coast due to the presence of favorable environmental and anthropogenic factors, leading to an increase in snakebite occurrences in that region. Moreover, the average temperature in the regions (coastal and upland) may not strongly influence the body temperatures and behavioral levels of snakes found by humans. Yet, occurrence temperatures of *B. jararaca* are used as a reliable proxy for studying behavioral patterns[50]. Thus, biting behavior of *B. jararaca* in these areas may be another factor contributing to this epidemiological profile. Further research is needed to investigate the relationship between the incidence of snakebite events on the coast and in the uplands.

It seems that body size influences the behavior of *B. jararaca*, which, in turn, affects the epidemiology of snakebite incidents. Specifically, when examining only the envenomations caused by *B. jararaca* in the upland region of São Paulo from the Vital Brazil hospital,Instituto Butantan, we observed that most snakebites were caused by individuals smaller than the population average. Other studies also report that smaller snakes were the most common cause of snakebite[15,26]. Our hypothesis is that biting behavior explains the link between morphology (size) and snakebite epidemiology. In other words, the small size of *B. jararaca* that causes most snakebites may be due to their high propensity to bite. However, these data must also be interpreted with caution since snakes brought by bite victims can lead to biased sampling. Body size of *B. jararaca* is significantly influenced by gender, females being larger than males[37,51]. We found that adult males and females display different bite probabilities and tolerance, depending on the context. Specifically, smaller females tend to be more prone to bite and less tolerant, whereas males display a more flexible behavior with a positive correlation between body size and tendency to bite. However, during physical contact in the morning, the relationship between body size and tendency to bite changes, and males behave similarly to females. We hypothesize that this behavioral change is due to the aversive stimulus in a context of increased vulnerability. Physical contact is one of the most dangerous stimuli for snakes, as their primary defensive strategies (camouflage, fleeing) may not be successful. Additionally, during the morning, when *B. jararaca* would habitually rest or hide[37], both females and smaller males tend to be more prone to bite. This may be because smaller snakes have lower locomotor performance than larger ones[52–55] and may choose defensive behaviors such as biting instead of fleeing, the latter behavior being one of the main ways to avoid predation.

Adult *B. jararaca* males were more prone to bite than females, although epidemiological data showed that females actually cause more snakebite cases than males, which indicates another contributing factor. Most envenomations are caused by juveniles[26], accounting for up to 56% of cases[56]. Of every 137 bites caused by juveniles and adults, 72 (52.55%) were caused by juvenile females, while only 5 (3.64%) were caused by juvenile males[56]. We found that life stage affects the tendency of *B. jararaca* to bite, younger individuals displaying more tendency to bite than adults. Notably, among all life stages, newborn females were the most prone to bite. However, males became more defensive as they grow. Our hypothesis is that when they are young and the size of both sexes is similar, females are innately more prone to bite. However, when adults, due to sexual dimorphism, i.e., smaller males, body size becomes the determining factor. This indicates that the higher probability of snakebite caused by juvenile females contributes to their high representation in epidemiological profiles. This relationship underscores the importance of studying defensive snakebite behavior to better understand epidemiological profiles of snakebite.

Poisonings due to *B. jararaca* are more frequent in the morning than in the afternoon or evening, despite its nocturnal habits[26]. During the day, both females and males are more prone to defensive bite than during the night. However, during the night, males become more aggressive than females. Our data from 422 envenomations showed a significant association between gender and time of day. Females caused envenomations during the morning, and males at night. Once again, the data indicates that environmental and behavioral factors interact to explain patterns of snakebite incidents. Moreover, the morning is the period of highest human activity in places where *B. jararaca* is found, which tends to increase the chances of encounters with these snakes[26,37,57,58]. The correspondence between the peak of human activity and the higher likelihood of encounters with *B. jararaca* results in a greater incidence of snakebites.

In conclusion, we observed that both biotic and abiotic variables could influence the likelihood of *B. jararaca* tendency to defensive bites. There was a strong correlation between the snakebite epidemiological data and the behavioral data reported herein. This study highlights that the likelihood of jararaca biting people is highly dependent on contextual factors. These snakes tend to become more prone to bite during the day and in warmer temperatures when most snakebites occur. They are more likely to bite when stepped on, especially on the anterior part of the body. Young snakes, particularly young females, are more prone to biting, which explains why this size class is the major cause of snakebite. Additionally, we found a significant association between body size and the probability of biting, as evidenced by the accident data. Therefore, the study of the behavior that leads to envenomation is crucial to understanding and predicting epidemiological trends, and can be used as a new tool to study snakebites. For example, a study[59] integrated human and snake ecological factors to predict snakebites. As with malaria, where the biology of the mosquito vector must be studied, we cannot overlook the importance of studying the behavior of snakes involved in snakebite[3]. We propose that governments and organizations focused on combating snakebite invest more in studying ecological and behavioral features of venomous snakes. With these data, models can be developed to predict which regions are more disposed to snakebite. This information would aid the management of public health agencies by providing knowledge to distribute antivenom serum to priority areas. Nonetheless, more research with an integrative perspective in other regions and venomous species is needed to understand, predict, and prevent snakebites.

Materials and methods

Ethics

All procedures were approved by the Ethics Committee on Animal Use of the Butantan Institute (CEUAIB) and are in accordance with the guidelines of the National Council for the Control of Animal Experimentation (CONCEA) for the care and use of animals in research (CEUA 8,444,011,020).

Study animal

We studied a sample of 116 *Bothrops jararaca* individuals, including 75 adults (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 old). All snakes, except for 25 adults (10 females and 15 males), were born in captivity. These 25 adults came from the wild in São Paulo state and had been in captivity for two weeks. The snakes were individually housed in boxes (45 × 30 × 15 cm), supplied with a cardboard substrate and a water pot, and maintained at a temperature of about 25 °C under a 12:12 light–dark photoperiod. We measured the snout-vent length (SVL) of each snake to the nearest mm with a malleable ruler.

Behavioral tests

During behavioral tests of bite tendency, we carried out confrontations between a human and a snake, simulating a snakebite scenario. The confrontations were carried out between October and November 2021, which is a period when snakebites by *B. jararaca* increase[57]. It is also a period of higher daily activity for snakes[48]. We set up a 2.025 m^2 arena (1.5 m length × 1.35 m width × 1 m height), 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 min to habituate to the test arena. We carried out the confrontation experiments with and without physical contact of the snake's body. For the contact stimulus, we used safety boots and softly stepped, on the head, mid-body, and tail (ten times in each region) randomly at 3-s intervals. For the non-contact stimulus, the researcher stepped with the boot near the snake, within 5 cm of the snake's body regions, randomizing the order without touching the animal. We carried out the experiments during two-day periods. In the morning, the tests were between 8:00 and 11:00 h and at night, they were between 18:00 and 22:00 h. An interval of five days was given between the test to avoid stress and learning influences[60]. In each test, we measured the snake's temperature using an infrared thermometer (ST-620, Incoterm) at a distance dependent on the size of the animal—approximately 2 m for larger specimens and 50 cm for juveniles. Furthermore, we measured the temperature of both the animal and substrate of arena, and the difference was insignificant (≅ 0.3 °C) The temperature of the test snakes ranged from 15 to 23 °C. We recorded all tests with a film camera (HDR-PJ200, Sony) for later analyses.

Statistical analysis

Behavioral tests

The categorical predictor variables included the sex of the snake (two levels: male and female), the human stimulus (two levels: with and without physical contact), and the period of the day (two levels: morning and night). The continuous independent variables were the size (SVL) and temperature (°C) of the animal. The probability of biting was defined by the number of Bernoulli events, which consisted of the number of successes (bites) in a given number (n) of trials (n = 30 confrontations). The difference between the number of confrontations and the number of bites was defined as "no bite", including all anti-predator behaviors that did not involve biting the researcher. Tolerance was defined as the number of boot placements in the arena with or without physical contact that the snake allowed until biting the boot. To test for possible collinearity among predictor variables, we used the Variance Inflation Factor (VIF) index, with a VIF value of 4 considered the acceptable limit among variables[61]. No collinear relationships were detected.

We used a generalized linear mixed model (GLMM) with binomial distribution and logit function for the bite probability data, while a Poisson distribution with log link function was used for the tolerance data. The data were exponentiated for interpretation purposes. As the model does not support the analysis of the additive and interactive properties of all five predictor variables, we built separate models with and without the temperature variable. The general model incorporated gender, time of day, human stimulus, and size as fixed effects, while temperature, animal identification, and origin (whether born in captivity or not) were utilized as random effects. In models that included temperature as one of the predictor variables, the fixed effects analyzed were temperature, gender, time of day, and size, under the human foot step stimulus. In this scenario, we employed snake ID and origin as random effects. The distinction between these models was necessary, as a model with quintuple interaction is intricate and presents challenges in the biological interpretation of results. Consequently, we used a separate model to scrutinize the effect of temperature in a context of heightened danger, specifically when a human steps on the snake.

We used 116 individuals for the life stage analysis. The model employed was a GLMM with binomial and Poisson distributions for bite probability and tolerance, respectively, with the assistance of the 'lme4' package. The fixed variables included life stage, day period, and sex, while the random variables comprised animal size, individual, and environment of origin. We selected the minimum adequate model using ANOVA tables and AIC values. Furthermore, we performed Tukey's Test for Post-hoc Analysis to identify significant relationships between the variables. To ensure data accuracy, we conducted data dispersion analysis, homoscedasticity, and outline testing using model diagnostic values and plots. We used the "DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models" package in R for this purpose.

Epidemiological data

Epidemiological data from the coast and upland of São Paulo

We applied the behavioral models we obtained to gain insights into epidemiological data. The size of *B. jararaca* can vary depending on the geographic region (coast and upland) in the state of São Paulo, southeastern Brazil[18,19,21]. To analyze data on snakebite due to *B. jararaca* in the coast and upland regions of São Paulo, we collected data on bothropic envenoming from the Brazilian government's Information System for Notifiable Diseases (SINAN). We collected the number of cases of bothropic envenoming (bites caused by snakes belonging to the genus *Bothrops*) per year between 2009 and 2022 from the thirty municipalities in São Paulo with the highest case numbers. To differentiate between the municipalities in terms of their geographic regions, we defined that cities below 80 m a.s.l. belonged to the coast (lowland) and those above 80 m a.s.l. were classified as uplands[18].

The number of snakebites was estimated and adjusted based on the human population inhabiting only the rural area of each municipality[62,63]. The number of snakebites per 100,000 residents was then calculated. We used the 'glmmTMB' R package[64] to fit a generalized linear mixed-effects model with a negative binomial distribution (nbinom2) selected from alternative distributions based on tests of overdispersion and AIC values. Model diagnostic plots were also inspected to assess model fit. The fixed effect was the geographic region, with two levels (upland and coast) and rural population number of each municipality. The random effects included snakebite year and municipality ID.

To complete the behavioral model, we needed temperature data on the occurrence of *B. jararaca* in São Paulo. We obtained distribution data for this snake[65] and used a point sample tool in QGis 3.18 software to analyze the data. Each point was applied under a raster layer with temperature data. We used BIO5 (maximum temperature of the warmest month) and BIO6 (minimum temperature of the coldest month) bioclimatic variables to represent the maximum and minimum temperature data, respectively. The temperature data were acquired from *WorldClim version 2.1* between the years 1970 and 2000, which was released in January 2020. After collecting the temperatures of *B. jararaca* occurrence, we calculated the average temperature to predict the probability of snakebites occurring.

Linear models allow for predictions based on the estimated coefficients. Thus, to predict the probabilities of snakebites according to variables of body size and occurrence temperature of *B. jararaca*, we formulated a predictive equation. The formula involves multiplying the estimated coefficients from the generated model (i.e., the model with interactive effects between temperature, gender, time of day, and size) by the average temperature obtained from the highlands and the average temperature from the coast. Additionally, we multiply the estimated coefficients of the model by the body size described in two reports[18,19] for *B. jararaca* in different regions (highlands and coastal). The employed model was based on the context in which contact with the snake occurs.

Epidemiological data from São Paulo associated with temperatures
To investigate the association between the temperature of municipalities and the average number of bothropic envenomings, we collected temperature data for the municipalities evaluated in previous analyses using the Point sample tool, similar to the method used to obtain the occurrence temperatures of *B. jararaca*. We used a raster layer with the climate variable BIO1 (Annual Average Temperature) provided by *WorldClim* version 2.1. A Generalized Linear Mixed Model (GLMM) with Poisson distribution and a log-link function was fitted using the 'glmmTMB' package in R[64]. The model aimed to predict the number of bothropic snakebite incidents per 100,000 inhabitants, sourced from SINAN, with the average temperature obtained from each municipality as the predictor variable. Municipality ID was included in this model as a random variable.

Association between size, period of day and snakes involved in snakebite
Bothrops jararaca individuals involved in snakebite were obtained from the Alphonse Richard Hoge herpetological collection of the Instituto Butantan. These snakes were brought by bitten people, who sought treatment at the Vital Brazil hospital at the Instituto Butantan. We obtained data on the sex, size, and the time of day of snakebite from 422 snakes that caused snakebites between 1969 and 2005 in the upland region of the state of São Paulo. We created a histogram and a probabilistic density analysis of body size to investigate the sizes of snakes involved in snakebite incidents in this region. Additionally, to investigate the association between snake sex and the time of day of the envenomation, we conducted a contingency table and chi-square analysis.

Data availability
The authors declare that the supporting data are made available to the members of the Editorial Board and reviewers in accordance with the Journal's guidelines.

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References
1. Gutiérrez, J. M. *et al.* Snakebite envenoming. *Nat. Rev. Dis. Primers* **3**, 1–21 (2017).
2. Lancet, T. Snakebite—Emerging from the shadows of neglect. *Lancet* **393**, 2175. https://doi.org/10.1016/S0140-6736(19)31232-2 (2019).
3. Murray, K. A., Martin, G. & Iwamura, T. Focus on snake ecology to fight snakebite. *Lancet* **395**, 100043. https://doi.org/10.1016/S0140-6736(19)32510-3 (2020).
4. Naik, B. S. "Dry bite" in venomous snakes: A review. *Toxicon* **133**, 63–67. https://doi.org/10.1016/j.toxicon.2017.04.015 (2017).
5. Hayes, W. K., Herbert, S. S., Rehling, G. C. & Gennaro, J. F. Factors that influence venom expenditure in viperids and other snake species during predatory and defensive contexts. *Biology of the Vipers* (2002) 207–233.
6. Whitaker, P. B. & Shine, R. Responses of free-ranging brownsnakes (*Pseudonaja textilis*: Elapidae) to encounters with humans. *Wildl. Res.* **26**, 689–704 (1999).
7. Shine, R., Sun, L. X., Fitzgerald, M. & Kearney, M. Antipredator responses of free-ranging pit vipers (*Gloydius shedaoensis*, Viperidae). *Copeia* **3**, 843–850 (2002).
8. Shine, R., Olsson, M. M., Lemaster, M. P., Moore, I. T. & Mason, R. T. Effects of sex, body size, temperature, and location on the antipredator tactics of free-ranging gartersnakes (*Thamnophis sirtalis*, Colubridae). *Behav. Ecol.* **11**, 239–245 (2000).
9. Webb, J. K., Brown, G. P. & Shine, R. Body size, locomotor speed and antipredator behaviour in a tropical snake (*Tropidonophis mairii*, colubridae): The influence of incubation environments and genetic factors. *Funct. Ecol.* **15**, 561–568 (2001).
10. Scudder, K. M. & Chiszar, D. Effects of six visual stimulus conditions on defensive and exploratory behavior in two species of rattlesnakes. *Psychol. Rec.* **27**, 519–526 (1977).
11. Llewelyn, J., Webb, J. K. & Shine, R. Flexible defense: Context-dependent antipredator responses of two species of Australian elapid snakes. *Herpetologica* **66**, 1–11 (2010).
12. Mori, A. & Burghardt, G. M. Thermal effects on the antipredator behaviour of snakes: A review and proposed terminology. *Herpetol. J.* **14**, 79–87 (2004).
13. Whitford, M. D., Freymiller, G. A., Higham, T. E. & Clark, R. W. The effects of temperature on the defensive strikes of rattlesnakes. *J. Exp. Biol.* **223**, jeb223859 (2020).
14. Cardoso, J. L. C., França, F. O. S., Wen, F. H., Málaque, C. M. S. & Haddad, V. Jr. Animais peçonhentos no Brasil: Biologia, clínica e terapêutica dos acidentes. *Rev. Inst. Med. Trop. Sao Paulo* **45**, 475–480 (2003).
15. Ribeiro, L. A. & Jorge, M. T. Acidente por serpentes do gênero Bothrops: Série de 3.139 casos. *Rev. Soc. Bras. Med. Trop.* **30**, 475–480 (1997).
16. de França, F. O. S. Associação da venenemia e da gravidade em acidentes botrópicos, no momento da admissão no Hospital Vital Brazil, do Instituto Butantan, SP, com variáveis epidemiológicas, clínicas e laboratoriais. *Rev. Soc. Bras. Med. Trop.* **31**, 495–496 (1998).
17. Puzzi, M. B. *et al.* Acidentes ofídicos. *Revista Científica Eletônica De Medicina Veterinária* **6**, 1–7 (2008).
18. de Moraes, R. A. *Variações em caracteres morfológicos e ecológicos em populações de Bothrops jararaca (Serpentes: Viperidae) no estado de São Paulo* (Universidade de São paulo, 2008).
19. Siqueira, L. H. C., Piantoni, C. & Marques, O. A. V. Morphological variation in the common lancehead populations: Sexual dimorphism and ontogenetic patterns. *J. Zool.* **318**, 283–296 (2022).
20. Schneider, M. C. *et al.* Snakebites in rural areas of Brazil by race: Indigenous the most exposed group. *Int. J. Environ. Res. Publ. Health* **18**, 9365 (2021).

21. Siqueira, L. H. C. & Marques, O. A. V. Effects of urbanization on *Bothrops jararaca* populations in São Paulo municipality. *South-eastern Braz. J. Herpetol.* **52**, 299–306 (2018).
22. Greene, H. W. Antipredator mechanisms in reptiles. *Bio. Reptil.* **16**, 1–152 (1988).
23. Araújo, M. S. & Martins, M. The defensive strike of five species of lanceheads of the genus *Bothrops* (Viperidae). *Braz. J. Biol.* **67**, 327–332 (2007).
24. Place, A. J. & Abramson, C. I. Habituation of the rattle response in western diamondback rattlesnakes. *Crotalus atrox. Copeia* https://doi.org/10.1643/CE-06-246 (2008).
25. Roth, E. D. & Johnson, J. A. Size-based variation in antipredator behavior within a snake (*Agkistrodon piscivorus*) population. *Behav. Ecol.* **15**, 365–370 (2004).
26. Sazima, I. Um estudo de biologia comportamental da jararaca *Bothrops jararaca*, com uso de marcas naturais. *Mem. Inst. Butantan* **50**, 83–99 (1988).
27. Carpenter, C. C. & Gillingham, J. C. Postural responses to kingsnakes by crotaline snakes. *Herpetologica* **31**, 293–302 (1975).
28. Gutzke, W. H., Tucker, C. & Mason, R. T. Chemical recognition of kingsnakes by crotalines: Effects of size on the ophiophage defensive response. *Brain Behav. Evol.* **41**, 234–238 (1993).
29. Hailety, 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).
30. Sweet, S. S. Geographic variation, convergent crypsis and mimicry in gopher snakes (*Pituophis melanoleucus*) and western rattlesnakes (*Crotalus viridis*). *J. Herpetol.* **19**, 55–67 (1985).
31. Delaney, D. M. Antipredation behavior covaries with body size in Neotropical snakes. *Amphib. Reptil.* https://doi.org/10.1163/15685381-20191125 (2019).
32. Janzen, F. J., Tucker, J. K. & Paukstis, G. L. Experimental analysis of an early life-history stage: Avian predation selects for larger body size of hatchling turtles. *J. Evol. Biol.* **13**, 947–954 (2000).
33. Blomberg, S. P. & Shine, R. Size-based predation by kookaburras (*Dacelo novaeguineae*) on lizards (*Eulamprus tympanum*: Scincidae): What determines prey vulnerability?. *Behav. Ecol. Sociobiol.* **48**, 484–489 (2000).
34. Tucker, J. K., Filoramo, N. I. & Janzen, F. J. Notes and discussion: Size-biased mortality due to predation in a nesting freshwater turtle Trachemys scripta. *Am. Midl. Nat.* **141**, 198–203 (1999).
35. Vitt, L. J. Ecological consequences of body size in neonatal and small-bodied lizards in the neotropics. *Herpetol. Monogr.* https://doi.org/10.2307/1467053 (2000).
36. Zelanis, A. *et al.* Analysis of the ontogenetic variation in the venom proteome/peptidome of *Bothrops jararaca* reveals different strategies to deal with prey. *J. Proteome Res.* **9**, 2278–2291 (2010).
37. Sazima, I. Natural history of the jararaca pitviper, *Bothrops jararaca*, in southeastern Brazil. in *Biology of pitvipers* (1992).
38. Oliveira, M. E. & Santori, R. T. Predatory behavior of the opossum *Didelphis albiventris* on the pitviper *Bothrops jararaca*. *Stud. Neotrop. Fauna Environ.* **34**, 72–75 (1999).
39. Chaves, L. F., Chuang, T. W., Sasa, M. & Gutiérrez, J. M. Snakebites are associated with poverty, weather fluctuations, and El Niño. *Sci. Adv.* **1**, e1500249 (2015).
40. Citadini, J. M. & Navas, C. A. Inter-individual variation and temperature-dependent antipredator behavior in the snake *Tomodon dorsatus* (Dipsadidae). *Behav. Process.* **97**, 11–17 (2013).
41. Keogh, J. S. & DeSerto, F. P. Temperature dependent defensive behavior in three species of North American colubrid snakes. *J. Herpetol.* **28**, 258–261 (1994).
42. Schieffelin, C. D. & De Queiroz, A. Temperature and defense in the common garter snake: Warm snakes are more aggressive than cold snakes. *Herpetologica* **47**, 230–237 (1991).
43. Greenwald, O. E. Thermal dependence of striking and prey capture by gopher snakes. *Copeia* **1974**, 141–148 (1974).
44. Hartmann, P. A., Hartmann, M. T. & Giasson, L. O. M. Uso do hábitat e alimentação em juvenis de *Bothrops jararaca* (Serpentes, Viperidae) na Mata Atlântica do sudeste do Brasil. *Phyllomedusa J. Herpetol.* **2**, 35–41 (2003).
45. SINAN. Sistema de informações de agravos de notificaçã. *Fundação Nacional de Saúde. Anais do Seminário de Vigilância Epidemiológica* (2023).
46. Pontes, J. A. L., Pontes, R. C. & Rocha, C. F. D. The snake community of Serra do Mendanha, in Rio de Janeiro State, southeastern Brazil: Composition, abundance, richness and diversity in areas with different conservation degrees. *Braz. J. Biol.* **69**, 795–804 (2009).
47. 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. *Acad. Bras. Cienc.* **86**, 695–706 (2014).
48. Siqueira, L. H. C., Banci, K. R. S. & Marques, O. A. V. Seasonal activity of *Bothrops jararaca* (Serpentes, Viperidae): Optimizing foraging while avoiding predators. *South Am. J. Herpetol.* **20**, 67–74 (2021).
49. Barbo, F. E., Marques, O. A. V. & Sawaya, R. J. Diversity, natural history, and distribution of snakes in the municipality of São Paulo. *South Am. J. Herpetol.* **6**, 135–160 (2011).
50. Díaz-Ricaurte, J. C., Serrano, F. C., Guevara-Molina, E. C., Araujo, C. & Martins, M. Does behavioral thermal tolerance predict distribution pattern and habitat use in two sympatric Neotropical frogs?. *PLoS One* **15**, e0239485 (2020).
51. Flores-Villela, O., Campbell, J. A. & Lamar, W. W. Venomous reptiles of Latin America. *Copeia* **1990**, 900 (1990).
52. Scribner, S. J. & Weatherhead, P. J. Locomotion and antipredator behaviour in three species of semi-aquatic snakes. *Can. J. Zool.* **73**, 321–329 (1995).
53. Carrier, D. R. Ontogenetic limits on locomotor performance. *Physiol. Zool.* **69**, 467–488 (1996).
54. Finkler, M. S. & Claussen, D. L. Influence of temperature, body size, and inter-individual variation on forced and voluntary swimming and crawling speeds in *Nerodia sipedon* and *Regina septemvittata*. *J. Herpetol.* **33**, 62–71 (1999).
55. Kelley, K. C., Arnold, S. J. & Gladstone, J. The effects of substrate and vertebral number on locomotion in the garter snake *Thamnophis elegans*. *Funct. Ecol.* **11**, 189–198 (1997).
56. Ribeiro, L. A., Jorge, M. T. & Iversson, L. B. Epidemiologia do acidente por serpentes peçonhentas : Estudo de casos atendidos em 1988 * Epidemiology of poisonons snake-bites : A study of cases assisted in 1988. *Rev. Saude Publ.* **29**, 380–388 (1995).
57. Da Silva, C. J., Jorge, M. T. & Ribeiro, L. A. Epidemiology of snakebite in a central region of Brazil. *Toxicon* **41**, 251–255 (2003).
58. Lima, J. S. *et al.* Perfil dos acidentes ofídicos no norte do Estado de Minas Gerais Brasil. *Rev. Soc. Bras. Med. Trop.* **42**, 561–564 (2009).
59. Goldstein, E. *et al.* Integrating human behavior and snake ecology with agent-based models to predict snakebite in high risk landscapes. *PLoS Negl. Trop. Dis.* **15**, e0009047 (2021).
60. Glaudas, X., Winne, C. T. & Fedewa, L. A. Ontogeny of anti-predator behavioral habituation in cottonmouths (*Agkistrodon piscivorus*). *Ethology* **112**, 608–615 (2006).
61. Shrestha, N. Detecting multicollinearity in regression analysis. *Am. J. Appl. Math. Stat.* **8**, 39–42 (2020).
62. SEADE, F. S. E. de A. de D. População. *Retratos de São Paulo* (2010).
63. IBGE. População. *Instituto Brasileiro de Geografia e Estatística* (2021).
64. Brooks, M. E. *et al.* glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400 (2017).
65. Nogueira, C. C. *et al.* Atlas of brazilian snakes: Verified point-locality maps to mitigate the wallacean shortfall in a megadiverse snake fauna. *South Am. J. Herpetol.* **14**, 1–274 (2019).

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Author contributions
J.M.AN., A.F., and O.A.V.M. designed research; J.M.AN., A.F., performed research; J.M.AN. analyzed data; and J.M.AN., A.F., S.M.AS., C.R.M., I.S., O.A.V.M wrote the paper.

Competing interests
The authors declare no competing interests.

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