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Defensive plasticity in South American rattlesnakes *Crotalus durissus*

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Reptiles exhibit defensive responses in diverse ways, influenced by situational factors. Snakes, such as the South American rattlesnake *Crotalus durissus*, display complex defensive responses, adapting tactics based on circumstances when encountering potential predators. Environmental and intrinsic parameters shape snake behaviour during predator encounters. This study employs distinct stimuli to investigate behavioural changes and whether snakes adapt defensive strategies. We compare the behaviours of rattlesnakes before and after an aversive stimulus involving physical contact. Following encounters with higher-risk stimuli, snakes become more defensive. Findings reveal that snakes alter strategies based on threat intensity. This phenomenon might be influenced by the potential cost of defense and prior interactions with predators. The study underscores the intricate nature of snake defensive behaviour.

Keywords: behavioural plasticity, antipredator behaviour, aversive stimulus, behavioural cost, active behaviours

Many animals possess the ability to assess and behaviourally influence the risk of predation (Ibáñez et al., 2014; Lima & Dill, 1990), consequently generating defensive responses in different ways, depending on circumstantial factors (Sazima, 1988). Therefore, it is possible to hypothesise the existence of a component of choice in the mechanisms used by the individual when confronted with a dangerous situation (Sih et al., 2012).

Snakes can exhibit a complex repertoire of defensive responses, switching from one tactic to another depending on the conditions at the moment of encountering a potential predator (Llewelyn et al., 2010; Stankowich & Blumstein, 2005). Currently, it is known that many environmental parameters determine how snakes modulate their defensive behaviour in various situations of predator encounter, such as temperature (Citadini & Navas, 2013; Webb et al., 2001), distance from refuge (Stankowich & Blumstein, 2005) and intrinsic parameters such as sex (King, 2002), ontogeny (Vitt, 2000) and body condition (Andrén, 1982). Venomous snakes are ideal for investigating changes in defensive strategy, as they incur high costs associated with striking (e.g. venom expenditure, potential prey damage, increased vulnerability to predators due to extended head and neck, muscle fatigue (Hayes et al., 2002), and they possess a wide variety of warning behaviours (Greene, 1988).

The South American rattlesnake *Crotalus durissus* is a snake with a highly extensive distribution, spanning from the northern to the southern regions of the South American continent. Due to its high adaptability, it easily occupies urban areas and is susceptible to attacks from predators belonging to various animal groups, including birds, mammals and even other squamates (Flores-Villela et al., 1990; Daltry et al., 1996). Similar to other members of the Crotalinae subfamily, rattlesnakes possess an infrared sensory system in their facial organs (Gracheva et al., 2010; Noble & Schmidt, 1937) enabling them to detect electromagnetic radiation invisible to most other animals (Goris, 2011).

The ability to distinguish the degree of danger in a predatory attack and how this ability changes with experience remains relatively unexplored by science. Given this, we enquire whether snakes belonging to the species *C. durissus* have the ability to alter their defensive repertoire according to the level of danger posed by a predatory attack, and whether a change in behaviour patterns is possible after undergoing experiences of greater peril. Our hypothesis is based on the notion that *C. durissus* will modify its defensive behaviours when confronted with different predatory stimuli. Furthermore, following exposure to a more dangerous stimulus, we posit that the snakes would become more aggressive, exhibiting increased active behaviours in order to evade their predator.

For this purpose, four snakes of the *C. durissus* species (2 males and 2 females) were used, approved by the ethics committee for the use of animals (CEUA-8444011020) originating from natural environments in the state of São Paulo, Brazil. All animals were adults with snout-vent lengths corresponding to the adult phase (>

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83 cm SVL)(Barros et al., 2012). The experiments were conducted between 20 and 24 July 2020, with two days post-collection, from 14:00 h to 16:00 h, in a room at 25 °C. The experimental test involved placing the animal in the centre of an arena measuring 2.025 m^2 (1.5 m x 1.35 m x 1 m), constructed with an aluminum base plate and foam walls according to Alves-Nunes et al. (2023). Each individual snake was allowed to acclimate in the centre of the arena for 15 minutes individually. The experiments were carried out once for each animal, involving a total of three stimuli.

Subsequently, the first stimulus (Stimulus 1) was administered, involving a plastic bottle containing 37 °C water, mimicking the temperature of its natural predators. The stimulus object was approached toward the snake's head without making physical contact, at a constant speed with 1-second intervals. The duration of the experiment ranged from one to four minutes. Following stimulus 1, a one-minute interval was given. After this interval, the animals were exposed to a higher-stress stimulus involving physical contact (Physical stimulus). This was achieved using a snake catcher to apply pressure to three regions of the snake (tail, mid-body and behind the head), each stimulus lasting five seconds with a 3-second interval between each stimulus. The duration of the stimulus ranged between one and four minutes. Following the conclusion of this phase, another 1-minute interval was provided, allowing the snake to rest.

Immediately after this, the animals were subjected to a third stimulus (Stimulus 2). This stimulus was similar to the first, where a warm bottle approached the snake without contact. These stimuli represent relevant cues for predation events carried out by opossums, which are homeothermic predators that engage in compression of all three regions of the snake's body (Almeida-Santos et al., 2000). This investigation aimed to determine whether the snake's behaviour changed in response to the same stimulus before and after a more perilous experience.

The experiments were recorded and subjected to behavioural analysis, involving documentation of the duration of all behaviours and the duration of predatory stimuli in seconds. The behaviours were categorised into two behavioural groups: passive and active (Citadini & Navas, 2013; Silva, 2016). Passive behaviours encompassed all immobility-related behaviours or actions that increased the distance between the snake and the predatory stimulus (immobility, body retraction, coiling, hiding the head, retracting the head, moving away and fleeing). On the other hand, active behaviours comprised actions involving alert signals and a reduction in the distance between the snake and the aggressor (thrashing, body rotation, striking, biting, strike pose and sigmoid position) (Figures S1 & S2, see supplementary material).

The data were subjected to analysis using a Generalised Linear Mixed Model (GLMM), employing a binomial distribution and a logit link function. This analysis was conducted using the "lme4" package in R version 4.2.2. This approach was chosen because the snake has only two possible behavioural decision options (active or passive). The fixed variable was the type of experiment (with three levels of stimulus: Stimulus 1, physical stimulus and Stimulus 2. The random effect was the individual snake itself. The variance explained by the random variable was lower than the predictor variable (type of stimulus), with a proportion of explained variance of 0.176 and 0.824, respectively. Therefore, the type of stimulus explains more variance in the data than the individual behavioural differences of *C. durissus*.

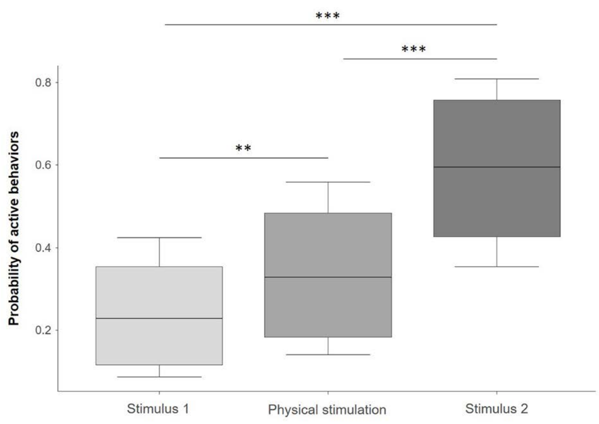

Figure 1. Probabilities of active behaviours of *Crotalus durissus*. Active behaviour probabilities depending on the stimulus. Stimulus 1 (no physical contact), Physical stimulus and Stimulus 2 (no physical contact). Asterisk indicates that the results show a clear difference in the frequency of behaviours in relation to the type of predatory stimulus among each species (*** $p < 0.001$; ** $p < 0.01$).

It was observed that there is a clear difference in the probability of snakes displaying active behaviours as the intensity of the stimulus varies (Fig. 1). Moreover, a change in the behavioural pattern in response to the same stimulus was observed after undergoing the physical contact stimulus ($z = 7.647$; $p < 0.001$). The chance of rattlesnakes exhibiting active behaviours after physical contact is approximately five times higher than before the more stressful stimulus ($z = 9.185$; $p < 0.001$). In the experiments with Stimulus 1, the four most common behaviours were head-turning (21.34%), striking (16.85%), coiling (15.16%) and fleeing (14.04%). In the Physical stimulus, the most performed behaviours were biting (19.62%), head turning (16.82%), inspection (13.40%) and striking (11.21%). In Stimulus 2, the most common behaviours were striking (38.34%), head turning (14.38%), fleeing (12.78%) and coiling (10.32%).

As predicted, the snakes exhibited a change in defensive behaviour in response to different stimuli. It is known that other snakes also respond differently to various types of stimuli (Herzog et al., 1989; Scudder & Chiszar, 1977; Arnold & Bennett, 1984) described something similar for *Thamnophis radix*, where the snakes became more aggressive when the simulated predator attacks were more severe. These observations suggest that individuals make behavioural decisions based on different contexts, assessing costs and benefits when confronted by a predator

(Endler, 1986). Furthermore, another aspect that can influence defensive behaviour is the physiological state in which the snake finds itself. The baseline plasma level of corticosterone, a stress-related hormone in snakes, is associated with a higher propensity to attack. However, this behaviour has not been correlated with the magnitude of the snake's corticosterone increase (Herr et al., 2017). It would be of great interest to conduct hormonal analyses on the animals before and after physical confrontations to observe the relationship between the animal's hormonal state and behavioural changes.

Furthermore, we observed a change in behaviour patterns in response to the same stimulus after undergoing a more perilous predatory encounter. Stimulus 1 and Stimulus 2, being the same stimulus and not involving high risk, could have led to habituation in animals and reduction in their active behaviours, as observed in Glaudas (2004). However, this was not the case. The animals increased the display of active defensive behaviours. We deduce that this effect could have been due to the fact that the animals had experienced a more dangerous encounter.

Snakes possess the ability to adjust their defensive behaviour according to discrimination between high and low-risk threats (Glaudas et al., 2006). If a previous experience with a potential predatory stimulus suggests that the risk of predation is low or non-existent, the best strategy might involve not engaging in active defense (e.g. striking) and instead minimising associated costs by investing in more passive defenses to avoid confrontation. Conversely, if the risk of predation is very high, the cost of defense might be trivial compared to the individual's need to maximise its survival. Therefore, factors such as risks and previous experiences with a predator could influence an individual's ultimate decision, as may have been the case with *C. durissus*.

An unexamined factor that could have effects is the degree of muscle exhaustion the animals experienced. Arnold & Bennett (1984) assessed *T. radix* and found that the snakes exhibited fleeing behaviour when encountering the investigator until high levels of lactate were reached. Then, they changed their behaviour, adopting one of their defensive displays. This suggests that *C. durissus* might indeed possess defensive plasticity according to the type of predator and the level of danger they present. However, more studies are necessary that involve more individuals of different sexes and ages. In addition, investigating whether muscle fatigue could also be a factor that influences defensive behaviour would be beneficial.

Therefore, we present evidence that the South American rattlesnake *C. durissus* alters its defensive behaviour in response to the degree of perceived threat, suggesting an adaptation in assessing each predatory situation and responding with varying degrees of defensiveness. This research provides insights into the behavioural plasticity and the variation in defensive responses of *C. durissus* in the face of perceived threats. The study demonstrates that the snakes adapt their reactions contingent upon the intensity of the threat, suggesting a sophisticated capacity for risk assessment and behavioural modification. This contributes to an enhanced comprehension of the underlying mechanisms of animal behaviour in prey-predator interaction contexts.

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