

# Thermal reception in the Mexican Lance-head rattlesnake, *Crotalus polystictus*

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The sensory systems of Boidae and Crotalinae snakes detect subtle differences of thermal infrared energy. The complexity of this ability involves neurophysiological mechanisms with interspecific differences in the anatomy of thermoreceptor organs and functionally in thermal detection ranges and thermal thresholds, with ecological correlations that influence the thermo-reception. However, little is known about the information these snakes obtain and use from infrared radiation. We analyzed the behavioral response of adult Mexican Lance-head Rattlesnakes (*Crotalus polystictus*) to static thermal stimuli, evaluating the influence of distance from the snake of the thermal stimuli, and its lizard-like or mouse-like shape. The results reveal that *C. polystictus* is able to detect static thermal stimuli located from 20 to 200 cm away. Head movements and tongue-flicks were the most frequently performed behaviors, which suggests they are behaviors that can facilitate the detection of subtle differences in temperature of static stimuli. In addition, we suggest that stimulus shape and temperature are important in the timing of head orientation and frequency of tongue-flicks. We discuss the possible methodological and sensory implications of this behavioral response in *C. polystictus*.

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7   **Abstract**

8   The sensory systems of Boidae and Crotalinae snakes detect subtle differences of thermal  
9   infrared energy. The complexity of this ability involves neurophysiological mechanisms with  
10   interspecific differences in the anatomy of thermoreceptor organs and functionally in thermal  
11   detection ranges and thermal thresholds, with ecological correlations that influence the thermo-  
12   reception. However, little is known about the information these snakes obtain and use from  
13   infrared radiation. We analyzed the behavioral response of adult Mexican Lance-head  
14   Rattlesnakes (*Crotalus polystictus*) to static thermal stimuli, evaluating the influence of distance  
15   from the snake of the thermal stimuli, and its lizard-like or mouse-like shape. The results reveal  
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19   stimuli. In addition, we suggest that stimulus shape and temperature are important in the timing  
20   of head orientation and frequency of tongue-flicks. We discuss the possible methodological and  
21   sensory implications of this behavioral response in *C. polystictus*.

## 22 Introduction

23 The Viperidae and Boidae snakes are families, widely recognized for their ability to detect subtle  
24 fluctuations in infrared thermal radiation ( $<0.003^{\circ}\text{C}$ ; Bullock & Diecke, 1956; Ebert, 2007;  
25 Bakken & Krochmal, 2007). The thermal difference between objects, allows these snakes to  
26 assimilate thermal information (Shine & Li-Xin., 2002; Krochmal & Bakken, 2003; Van Dyke &  
27 Grace, 2010), which converges with visual information in the optical tectum, to create an  
28 environmental thermal map (Newman & Hartline, 1981, 1982; Campbell et al., 2002).

29 The neurophysiological mechanisms these snakes use to detect thermal contrasts have  
30 been widely studied (Newman & Hartline, 1982; Berson & Hartline, 1988; Goris, 2011). It is  
31 known that these mechanisms help snakes find optimal sites for thermoregulation (Bakken &  
32 Krochmal, 2007), perceive images during the ecdysis process (King & Turmo, 1997), and detect  
33 both predators and prey (Noble & Schmidt, 1937; Molenaar, 1992). However, the functionality  
34 of this mechanism is limited by a snake's morphology and its interaction with the environment.  
35 For example, variables such as pit diameter and depth may influence the sharpness, strength and  
36 contrast of an image and may be correlated with snake habits, habitats, and prey activity periods  
37 (Bakken & Krochmal, 2007).

38 Other investigations have focused on the pit membrane irradiance, which is a function of  
39 source dimensions, source-background temperature contrast, and distance (Bakken & Krochmal,  
40 2007)). Although the general knowledge about snake thermoreception is widespread, scientists  
41 know little about the amount of information that snakes obtain from the infrared spectrum and  
42 studies are pending to clarify the effect of prey shape and distance as variables that may be  
43 involved in a snake's reaction and its ability to detect temperature differences.

44           Only a few behavioral studies have explored the detection of snake thermal stimuli. The  
45 present work investigates the thermal detection, measured as the thermal irradiance contrast of a  
46 target against the background at different distances, and the resulting ability of the Mexican  
47 Lance-head Rattlesnake (*Crotalus polystictus*) to detect two forms of prey stimulus using a  
48 behavioural approach. The advantage of behavioral studies is that the reception and assimilation  
49 of thermal signals is displayed as a whole across the behavioural reaction of the snakes, while  
50 studies of thermal infrared imaging in the facial pit, center only on certain features of the thermal  
51 perception or processing (Ebert & Westhoff, 2006). *Crotalus polystictus* is a medium-sized snake  
52 with a total length of up to 800 mm (Fig. 1). It is endemic to Mexico (specifically the  
53 neovolcanic axis) and subject to special protection (SEMARNAT, 2010). Habitat includes  
54 grasslands and pine-oak forests between 1450 and 2600 m elevation (Armstrong & Murphy,  
55 1979; Campbell & Lamar, 1989). It feeds mainly on small endothermic vertebrates such as mice,  
56 and ectothermic vertebrates such as lizards. Both have been reported as the main prey for the  
57 only well-studied population in Mexico (Meik et al., 2012). It is active in both day and nighttime.

## 58 **Material & Methods**

59 This study received the approval of the ethics committee of the Universidad Autónoma del  
60 Estado de México (Number 4047/2016SF). All subjects were treated humanely on the basis of  
61 guidelines outlined by the American Society of Ichthyologists and Herpetologists (ASIH, 2004).

62 In order to analyze the behavioral response of *C. polystictus* (Cope, 1865) during presentation of  
63 a static thermal stimulus that was different in both shape and distance, we used eight adults  
64 males (mean  $\pm$  1 Standard Deviation, *SD*: Snout Vent Length SVL=  $518.4 \pm 137.5$  mm; body  
65 weight =  $236.2 \pm 79.9$  g) provided by Herpetarium “Reptilium” of the Zacango Zoo in Toluca,

66 México. The snakes were kept inside the herpetarium and individually housed in glass terrariums  
67 (50 x 30 x 30 cm) containing water dishes, *ad libitum*, and logs. The terrariums were housed at  
68 an ambient room temperature of 20-28°C, and were exposed to a 12 h light/12 h dark  
69 photoperiod. The snakes were fed every 12 days with mice (*Mus musculus*) of approximately  
70 10% of their body weight to stimulate their appetite during the trials (Ebert & Westhoff, 2006).  
71 Five days after mouse ingestion the snakes defecated and during the next seven days the snakes  
72 were tested. When the tests were finished, the snakes were fed again.

### 73 **Thermal stimuli**

74 To evaluate the effect of prey shape in the thermal reception of the snakes, we used the forms of  
75 the two main prey of *C. polystictus*, a mouse-like shape (6 cm<sup>2</sup>) and a lizard-like shape (7 cm<sup>2</sup>).  
76 Specifically both thermal stimuli were built with resistors (22 Ω) (Fig. 2; Martínez-Vaca, 2015)  
77 and attached to an electronic switch (Solid State Swich, SSR 10 amp). The temperature of both  
78 thermal stimuli was adjusted and constantly monitored with a thermal sensor (thermocouple K -  
79 270 -1372 °C), which was attached onto the surface of prey-like element and connected to an  
80 intelligent temperature programming device (XMT-7100 , Nanning Giant Mechanical and  
81 Electrical Technologies, China) so that we could adjust and control the stimuli temperature.

82         Although the geometry of both thermal stimuli is complex, with many resistors  
83 presenting various angles between the long axis of the resistor and the vector from the target to  
84 the snake, Pit vipers respond behaviourally to different forms of thermal contrast (Van Dyke &  
85 Grace, 2010), particularly, this structure of thermal prey has been tested in Viperidae snake  
86 *Ophryacus undulates*, demonstrating that they can thermally simulate both prey (Martínez-Vaca,  
87 2015) and provoke behavioral response of snake during presentation of static thermal stimulus.

88 To generate a temperature difference of  $5^{\circ}\text{C}$  between the artificial stimuli and the room  
89 temperature, the thermal stimuli were maintained at  $29 \pm 1^{\circ}\text{C}$  and the room temperature at  $24 \pm$   
90  $1^{\circ}\text{C}$ , with the thermal sensor and the temperature programming device, previously described.  
91 The absolute temperatures in  $^{\circ}\text{C}$  was used in thermal radiation calculations. The total radiant flux  
92 emitted from both stimuli and the irradiance contrast was calculated using the adjusted model  
93 Stefan-Boltzmann as in de Cock Bunning (1983). We calculated the irradiance contrast for both  
94 stimuli located 20 cm from the snake (minimum distance, as described below) and 200 cm  
95 (maximum distance). The lizard-like stimulus had a thermal contrast of 0.012 to  $0.00012 \text{ W cm}^2$ ,  
96 and the mouse-like shape was 0.010 to  $0.00010 \text{ W/cm}^2$ , where  $\text{W cm}^2$  represents watts per square  
97 centimeter.

## 98 **Experimental tank**

99 The experimental glass tank (220 x 20 x 20 cm) was divided into two compartments:  
100 compartment A in which the experimental snake was maintained during the test (20 x 20 x 20  
101 cm; Fig. 3), and compartment B (200 x 20 x 20 cm) into which an experimental thermal stimulus  
102 was placed. Compartment B was marked every 20 cm, from 0 to 200 cm, (Fig. 3). The two  
103 compartments were separated by a metal mesh barrier (5 mm pore) located at the 0 cm mark that  
104 prevented the snake from touching the thermal stimulus and decreased the visual perception of it  
105 as well. To eliminate external vibrations (Van Dyke & Grace, 2010) the experimental tank was  
106 placed on a 20 mm thick Polystyrene sheet. The tank was covered with dark paper to prevent the  
107 snakes from seeing the observer (Stone, Ford & Holtzman, 2000). Experimental tanks were  
108 housed in a room with an air temperature of  $24 \pm 1^{\circ}\text{C}$ .

## 109 **Experimental procedure**

110 We placed a stimulus (lizard-like or mouse-like) in compartment B of the experimental tank 15  
111 minutes before testing, and at any one of ten distances (20, 40, 60, 80, 100, 120, 140, 160, 180  
112 and 200 cm) from the mesh barrier that separated the two compartments. Ten minutes before the  
113 test, one *C. polystictus* was extracted from its terrarium and placed into compartment A of the  
114 experimental tank (Ebert & Westhoff, 2006). The test started after the snake adjusted to the new  
115 surroundings and ceased moving about. The snake was videotaped (SONY DCR-DV203, 12  
116 megapixels, Dolby Laboratories, Japan) for four consecutive minutes. The first two minutes of  
117 video recording was a no-thermal contrast treatment in which the thermal stimulus was kept at  
118  $24 \pm 1^\circ\text{C}$ , similar to room temperature. At two minutes, the heating mechanism to the thermal  
119 stimulus was turned on and it gradually increased the temperature of the stimulus to  $29 \pm 1^\circ\text{C}$  in  
120  $86 \pm 3.4$  s ( $34.8 \pm 3.7$  s to up the first  $^\circ\text{C}$  and  $11.8 \pm 1.9$  s between each posterior  $^\circ\text{C}$ ). During this  
121 time, the snake was videotaped (for a total of two minutes) to record its behavioral response to  
122 the stimulus (Ebert & Westhoff, 2006; Van Dyke & Grace, 2010). After the video recording  
123 finished, we removed snake from the experimental tank and placed back into its terrarium. The  
124 experimental tank was cleaned with Neutrox® odorless soap (Specialized Materials and Supplies  
125 S.A of C.V, Jalisco, México) and another individual was placed in it for the next test.

126 The tests were conducted at night, from 1900 to 2400 hours in order to not alter the  
127 snakes' circadian rhythm and to coincide with the period of greatest activity recorded for *C.*  
128 *polystictus* (Armstrong & Murphy, 1979). The thermal stimuli (lizard-like and mouse-like), the  
129 ten distances to which the stimuli were placed (from 20 to 200 cm) and the eight experimental  
130 snakes were randomly alternated. Three replications per distance were performed, each with a  
131 different snake. To minimize habituation, each snake was tested only one time in a day, with  
132 three days between each test.

### 133 **Evaluated behaviors and statistical analysis**

134 To evaluate the ability of *C. polystictus* to detect static artificial thermal stimuli, in two different  
135 shapes, and ten different distances, we observed and recorded the following behaviors displayed  
136 by *C. polystictus*: (1) head orientation (change of head position in direction of stimulus, Ebert &  
137 Westhoff, 2006); (2) tongue-flick (tongue protrudes from the mouth swinging up and down,  
138 Gove & Burghardt, 1983); and (3) pupil orientation (change of pupil position in direction of  
139 stimulus, Martínez-Vaca, 2015). Additionally, we measured the frequency and latency of each  
140 behavior. Latency is defined as the time between when the thermal stimulus was offered and the  
141 behavior was displayed.

142 The behavior frequencies were transformed by square root (Zar, 1984) and were analyzed  
143 with a three-way ANOVA (temperature: 24 and 29°C; distance: 20 - 200 cm; and stimulus  
144 shape: lizard or mouse) for each behavior. Because statistical test resolution can be affected by  
145 sample size and inter-individual variability, it was decided to corroborate the maximum distance  
146 of thermal detection (200 cm) with a two-way ANOVA (stimulus shape, and stimulus  
147 temperature.).

148 For latency analysis, we used only behaviors registered when the stimulus temperature  
149 reached 29°C due to the interests in the responses caused by the thermal stimulation and because  
150 only some snakes showed a minimal and individual behavioral activity at room temperature  
151 (24°C). Thus, we proceeded to perform a two-way ANOVA (stimulus shape and distance).  
152 Finally, because in natural conditions the behavioral response to a thermal stimulus involves a  
153 combination of different sensory systems, we applied a three-way ANOVA (stimulus shape,  
154 distance, and behaviors) for latency and frequency at 29°C. When the significance level was  $\leq$



155 0.05, we used the post-hoc Student-Newman-Keuls test. We used the statistical program Sigma  
156 Stat version 3.5, and for all results we reported behavior frequencies transformed by square root  
157 (Zar, 1984). Data are represented as the mean  $\pm$  1 *SE*.

## 158 Results

159 Each behavior in *C. polystictus* showed different frequencies, but neither shape nor distance of  
160 stimulus had any effect on the frequencies of each behavior (three-way ANOVA, behavior,  $F_2 =$   
161 4.92,  $P = 0.009$ ; distance,  $F_9 = 0.82$ ,  $P = 0.59$ ; shape,  $F_1 = 0.35$ ,  $P = 0.55$ ). The interactions  
162 between behavior frequencies, distance, and shape were not significant ( $P > 0.05$ , Student-  
163 Newman-Keuls *post hoc*).

164 The three-way ANOVA indicated that the head orientation and the tongue-flick had the  
165 highest frequencies (2.32 and 2.27, respectively; Fig. 4B). The latency was similar for all  
166 distances ( $F_9 = 1.91$ ,  $P = 0.06$ ), and for all registered behaviors ( $F = 2.58$ ,  $P = 0.08$ ), however,  
167 there is a slight tendency ( $P = 0.08$ ) in which the pupil orientation was the first behavior  
168 performed in response to the thermal increase of the stimulus (Fig. 4A).

169 Although few snakes had a minimum behavioral activity in the absence of thermal  
170 contrast (when both stimulus temperature and room temperature were at 24°C). The snakes  
171 responded more frequently to both stimulus shapes (lizard and mouse) when they gradually  
172 increased in temperature to 29°C (tongue-flicks,  $F = 37.81$ ,  $P = 0.001$ ), head orientation ( $F =$   
173 56.89,  $P = 0.001$ ), and pupil orientation ( $F = 48.81$ ,  $P = 0.001$ ; Fig. 5).

174 Only at 20 cm from the stimulus did the snakes direct more tongue-flicks to the mouse-  
175 like shape than to the lizard-like shape (20 cm; Fig. 6). The latency of the snakes was lower for  
176 the lizard-like stimulus (49 s), than for the mouse-like (67 s;  $F = 3.89$ ,  $P = 0.055$ ; Fig. 7). Neither

177 the stimulus shape nor its distance influenced the latency of the snakes (distance  $F_9 = 1.14$ ,  $P =$   
178  $0.35$ ; distance-stimulus shape  $F_9 = 1.68$ ,  $P = 0.12$ ).

179 In general, the stimulus distance did not have a significant effect on the frequency of  
180 behaviors registered, but when the stimulus was at 200 cm, the behavioral response was still  
181 greater at 29°C than at 24°C stimulus temperature: tongue flicks ( $F = 8.20$ ,  $P = 0.02$ ), head  
182 orientation ( $F = 18.84$ ,  $P = 0.002$ ), and pupil orientation ( $F = 9.75$ ,  $P = 0.01$ ) (Fig. 8). At 29°C  
183 the tongue flick and head orientation showed similar frequencies for both lizard-like and mouse-  
184 like shapes. Only the frequency of pupil orientation was greater for mouse-like than for lizard-  
185 like stimuli ( $F = 5.02$ ,  $P = 0.055$ ; Fig. 8).

## 186 Discussion

187 The objective of this study was to analyze the ability of the snake *Crotalus polystictus* to detect  
188 and recognize static thermal stimuli in two different shapes, ten different distances, and two  
189 different temperatures. The results show that this species can detect static thermal stimuli by  
190 thermal contrast of relative warm objects (29°C) against a cooler background (24°C). This  
191 species also shows a shorter time to respond to the lizard-like stimulus, and a more frequent  
192 behavioral response to the mouse-like stimulus. In general, the behavioral response was not  
193 different between the experimental stimulus distances.

194 Under our experimental protocol, the maximum distance for thermal stimulus was 200  
195 cm and *C. polystictus* was able to respond behaviorally to this maximum distance. This suggests  
196 a high thermal sensitivity, exceeding more than two or three times the maximum detection  
197 distance reported for other vipers, such as 66.6 cm in *Agkistrodon rhodostoma* (de Cock  
198 Bunning, 1983) and 100 cm in *Crotalus atrox* (Ebert & Westhoff, 2006). However, *Trimeresurus*

199 *flavoviridis* has the ability to respond to thermal stimuli located 500 cm away (Goris and  
200 Terashima, 1973). This shows the need to implement experimental designs in *C. polystictus* to  
201 investigate whether or not the snake is able to detect thermal stimulation at greater distances than  
202 our 200 cm experimental distance.

203         With respect to the characteristics of prey items, it is known that the shape and size of the  
204 stimuli are relevant to execute predatory attacks in semiaquatic colubrid snakes (Macias García  
205 & Drummond, 1995). However, this information is scarce in rattlesnakes which, to a large  
206 extent, perceive prey thermally. In general our results show a certain ability of *C. polystictus* to  
207 detect and differentiate heat energy between two different shapes, showing a shorter time to  
208 respond to the lizard-like stimulus but a more frequent behavioral response to the mouse-like  
209 stimulus. This ability to differentiate the two thermal energy sources is possibly associated with  
210 the amount of heat radiated and the thermal contrast. The lizard-like stimulus presents a surface  
211 temperature of a solid three-dimensional prey with 13 resistors. It exceeds 0.00002 W cm<sup>2</sup> of  
212 thermal contrast as compared to the surface temperature of the mouse-like stimulus (12 resistors).  
213 And although in general, the behavioral response of *C. polystictus* did not show differences  
214 between stimulus distances, the mouse-like stimulus provoked more tongue-flicks at 20 cm  
215 distance and more pupil orientation at 200 cm. This suggests that thermal information may be  
216 used by the snake to detect endothermic prey in both natural and laboratory settings.

217         Viperine snakes integrate sensory systems, as the pit organs, that respond to thermal  
218 infrared radiation of surrounding surfaces (Bullock & Cowles, 1952), complemented with visual  
219 stimuli. In this study, the visual perception of *C. polystictus* was limited by using a mesh screen  
220 placed between it and the stimuli suggests that the increase in the pupil orientation frequency to  
221 200 cm is a behavior needed to obtain visual information that complements the thermal stimulus.

222 This raises the possibility that the surface temperature of three-dimensional prey of the mouse-  
223 like stimulus generates a greater thermal contrast and facilitates the perception of distant stimuli.  
224 Under these assumptions, we should mention that thermal sharpness is favored by short distance  
225 whereas thermal contrast is favored by long distances such as 200 cm.

226         The subtle way of initiating the detection of a thermal stimulus by means of the pupil  
227 orientation by *C. polystictus* manifests a stealth behavior of great biological relevance for cryptic  
228 animals (Cundall & Greene, 2000) whose survival depends on their camouflage and immobility.  
229 The study of head orientation is important because the behavior can result in greater frequency of  
230 detection of contrast of immobile thermal objects (Goris & Terashima, 1973). Head orientation  
231 and movement is integrated with tongue-flicks for chemo-recognition and chemical confirmation  
232 of stimuli previously detected via other sensory systems.

233         The register of certain behavioral activity of *C. polystictus* at 24°C of room temperature is  
234 due to the exploratory and basal nature exhibited by some snakes after certain periods of  
235 inactivity. Nevertheless, the artificial stimulus temperature, and the increased behavioral  
236 response with 5°C of thermal contrast between stimulus and room temperature, suggest that  
237 rattlesnakes are capable of detecting the radiance contrast (temperature contrast) between warm  
238 stimulus and cooler background, defined as positive contrast (Van Dyke & Grace, 2010). The  
239 functional implication of this ability to detect thermal contrast suggests that rattlesnakes may  
240 maximize potential thermal contrast of prey with background temperature through the selection  
241 of foraging sites. Such site selection could be particularly relevant to rattlesnakes preying  
242 diurnally on ectothermic prey (lizards) and nocturnally on endothermic prey (mice). Our results  
243 support the hypothesis that rattlesnakes have a high thermal sensitivity based on thermal contrast  
244 favored by relevant distances such as 200 cm.

245 Methodologically, the use of static stimuli made with electrical resistance, provides an  
246 effective and economic way of performing thermal contrast experiments with snakes to the  
247 exclusion of other sensory systems. It is also a practical method to provide different forms and  
248 evaluate thermal recognition (Martínez-Vaca, 2015).

## 249 **Conclusions**

250 The Mexican Lancehead Rattlesnake (*C. polystictus*) has the ability to recognize static thermal  
251 stimuli of different shapes up to 200 cm away. The stimulus shape and its interaction with the  
252 distance, shape the execution of the behavioral responses to thermoreception with an increase in  
253 behavioral response at 29°C from 24°C. The lizard-like stimulus resulted in a lower latency to its  
254 detection while the mouse-like stimulus resulted in a greater frequency of tongue-flicks at 20 cm  
255 and pupil orientation at 200 cm. The behaviors snakes exhibited more frequently for the  
256 perception of the static thermal stimuli were the head orientation and tongue-flicks.

257 This research provides relevant information on the complexity of the snake thermal  
258 sensory system, proposing effective, practical, and economic ways to evaluate the behavioral  
259 response to thermal stimuli. It is clear that scientists need to improve the experimental designs to  
260 continue generating knowledge about the sensorial and behavioral ecology of these snakes. We  
261 propose that new studies need to be carried out that consider inter and intraspecific  
262 ecomorphological comparisons and that manipulate variables involved in thermoreception such  
263 as size, shape, irradiation, kinematics, mobility, and distance of thermal stimuli.

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## 271 **References**

272 American Society of Ichthyologists and Herpetologists (ASIH). 2004. Guidelines for the use of  
273 live amphibians and reptiles in field research. ASIH, Herpetologists League, Society for the  
274 Study of Amphibians and Reptiles. Available at <https://>

275 [www.aaalac.org/accreditation/Guidelines\\_for\\_Use\\_of\\_Live\\_Amphibians\\_and\\_Reptiles.pdf](https://www.aaalac.org/accreditation/Guidelines_for_Use_of_Live_Amphibians_and_Reptiles.pdf)

276 Armstrong BL, Murphy JB. 1979. The natural history of Mexican rattlesnakes. Special  
277 publication No. 5. University of Kansas, Museum of Natural History.

278 Bakken GS, Krochmal AR. 2007. The imaging properties and sensitivity of the facial pits of  
279 pitvipers as determined by optical and heat-transfer analysis. *The Journal of the Experimental*  
280 *Biology* 210:2801–2810. DOI:10.1242/jeb.006965.

281 Berson DM, Hartline PH. 1988. A Tecto-Rotundo-Telencephalic pathway in rattlesnakes:  
282 evidence for a forebrain representation of the infrared sense. *Journal of Neuroscience* 8:1074–  
283 1088.

284 Bullock TH, Diecke FPJ. 1956. Properties of an infrared receptor. *Journal of Physiology* 134:47–  
285 87.

286 Bullock TH & Cowles RB. 1952. Physiology of an infrared receptor: the facial pit of pit vipers.  
287 *Science* 115:541–543.

- 288 Campbell J, Lamar WW. 1989. *The Venomous Reptiles of Latin America*. Ithaca and London:  
289 Cornell University Press.
- 290 Campbell AL, Naik RR, Sowards L, Stone MO. 2002. Biological infrared imaging and sensing.  
291 *Micron* 33:211–225. DOI: 10.1016/S0968-4328(01)00010-5.
- 292 Cundall D, Greene HW. 2000. Feeding in snakes. In: Schwenk K, ed. *Feeding: Form, Function,*  
293 *and Evolution in Tetrapod Vertebrates*. San Diego: Academic Press, 293–333.
- 294 de Cock Bunning T. 1983. Thermal sensitivity as a specialization for prey capture and feeding in  
295 snakes. *American Zoologist* 23:363–375. DOI: 10.1093/icb/23.2.363.
- 296 Ebert J. 2007. Infrared sense in snakes. Behavioral and anatomical examinations (*Crotalus atrox*,  
297 *Phyton regius*, *Corallus hortulanus*). D. Phil. Thesis, Rheinische Friedrich-Wilhelms University.
- 298 Ebert J, Westhoff G. 2006. Behavioral examination of the infrared sensitivity of rattlesnakes  
299 (*Crotalus atrox*). *Journal of Comparative Physiology A* 192:941–947. DOI: 10.1007/s00359-  
300 006-0131-8.
- 301 Macias García C, Drummond H. 1995. Components of Visual Prey Recognition by the Mexican  
302 Aquatic Garter Snake *Thamnophis melanogaster*. *Ethology* 101:101–111. DOI: 10.1111/j.1439-  
303 0310.1995.tb00349.x.
- 304 Goris RC. 2011. Infrared organs of snakes: An integral part of vision. *Journal of Herpetology*  
305 45:2–14. DOI: 10.1670/10-238.1.
- 306 Goris RC, Terashima S. 1973. Central response to infra-red stimulation of the pit receptors in a  
307 crotaline snake, *Trimeresurus flavoviridis*. *Journal of Experimental Biology* 58:59–76.

- 308 Gove D, Burghardt GM. 1983. Context-correlated parameters of snake and lizard tongue-  
309 flicking. *Animal Behaviour* 31:718–723. DOI: 10.1016/S0003-3472(83)80227-9.
- 310 King RB, Turmo JR. 1997. The effects of ecdysis on feeding frequency and behavior of the  
311 common garter snake (*Thamnophis sirtalis*). *Journal of Herpetology* 31:310–312. DOI:  
312 10.2307/1565405.
- 313 Krochmal AR, Bakken GS. 2003. Thermoregulation is the pits: use of thermal radiation for  
314 retreat site selection by rattlesnakes. *Journal of Experimental Biology* 206:2539–2545. DOI:  
315 10.1242/jeb.00471.
- 316 Martínez-Vaca LO. 2015. Respuesta conductual ante estímulos químicos, térmicos y auditivos  
317 en la serpiente *Ophryacus undulatus*. D. Phil. Thesis, Universidad Veracruzana, México.
- 318 Meik JM, Setser K, Mociño-Deloya E, Lawing AM. 2012. Sexual differences in head form and  
319 diet in a population of Mexican Lance-headed Rattlesnakes, *Crotalus polystictus*. *Biological*  
320 *Journal of the Linnean Society* 106:633–640. DOI: 10.1111/j.1095-8312.2012.01881.x.
- 321 Molenaar GJ. 1992. Anatomy and Physiology of infrared sensitivity of snakes. In: Gans C,  
322 Ulinsky PS, ed. *Sensorimotor integration. Biology of the Reptilia*. Vol. 17. Chicago: University  
323 of Chicago Press, 367–453.
- 324 Newman EA, Hartline PH. 1981. Integration of visual and infrared information in bimodal  
325 neurons in the rattlesnake optic tectum. *Science* 213:789–791.
- 326 Newman EA, Hartline PH. 1982. The infrared "vision" of snakes. *Scientific American* 246:116–  
327 127.



- 328 Noble GK, Schmidt A. 1937. The structure and function of the facial and labial pits of snakes.  
329 *Proceedings of the American Philosophical Society* 77:263–288.
- 330 SEMARNAT. 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010. Protección  
331 ambiental. Especies nativas de México de flora y fauna silvestres. Categorías de riesgo y  
332 especificaciones para su inclusión, exclusión o cambio. Lista de especies en riesgo. Available at  
333 [http://dof.gob.mx/nota\\_detalle\\_popup.php?codigo=5173091](http://dof.gob.mx/nota_detalle_popup.php?codigo=5173091) (accessed 01 february 2018).
- 334 Shine R, Li-Xin S. 2002. Arboreal ambush site selection by pit-vipers *Gloydius shedaoensis*.  
335 *Animal Behaviour* 63:565–576. DOI: 10.1006/anbe.2001.1928.
- 336 Stone A, Ford N, Holtzman D. 2000. Spatial learning and shelter selection by juvenile spotted  
337 pythons, *Antaresia maculosa*. *Journal of Herpetology* 34:575–587.
- 338 Van Dyke JU, Grace MS. 2010. The role of thermal contrast in infrared-based defensive  
339 targeting by the copperhead, *Agkistrodon contortrix*. *Animal Behaviour* 79:993–999. DOI:  
340 10.1016/j.anbehav.2010.01.012.
- 341 Zar JH. 1984. Biostatistical analysis. 2nd edition. New Jersey: Prentice Hall International.

# Figure 1

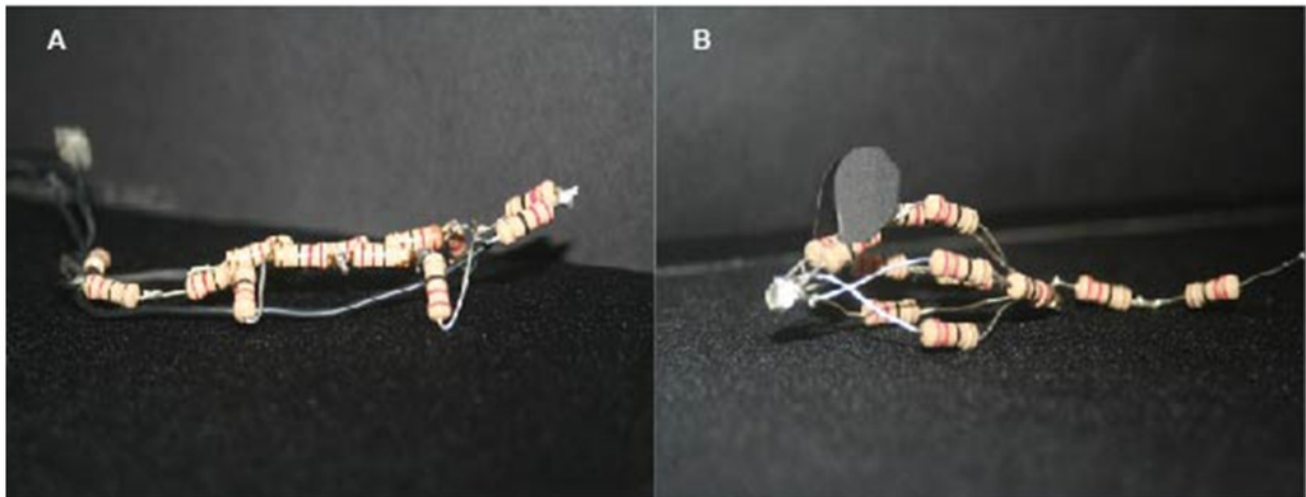
Adult Mexican Lancehead Rattlesnake, *Crotalus polystictus*. (Photo by O Martínez Vaca-León).



## Figure 2

Static thermal stimuli built with electrical resistance in two different shapes, (A) lizard-like, and (B) mouse-like.

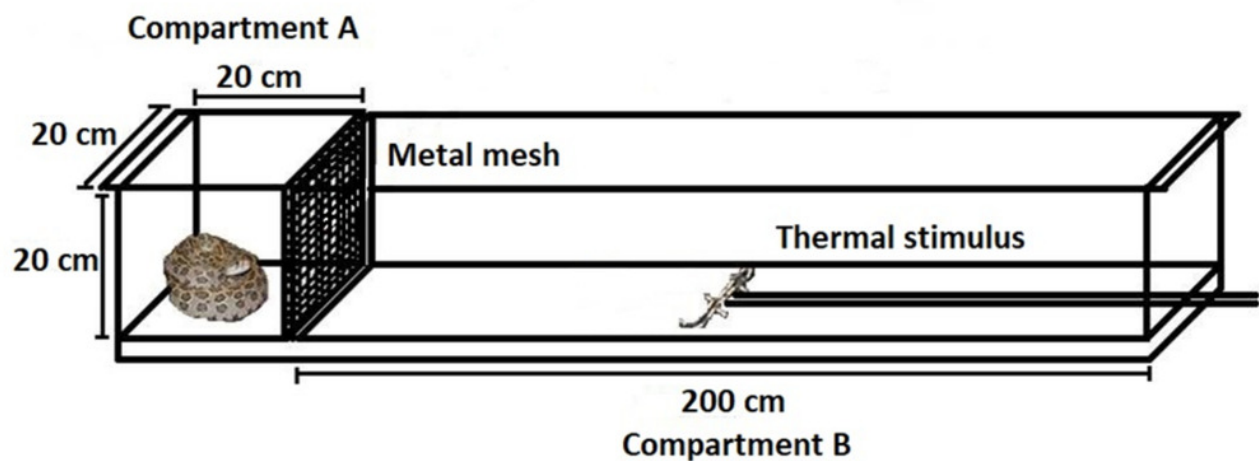
Both stimuli are coupled to an electronic switch, a thermal sensor, and an intelligent temperature programming device to adjust and control the stimulus temperature.



## Figure 3

The experimental glass in which the experimental *C. polystictus* was housed during the test.

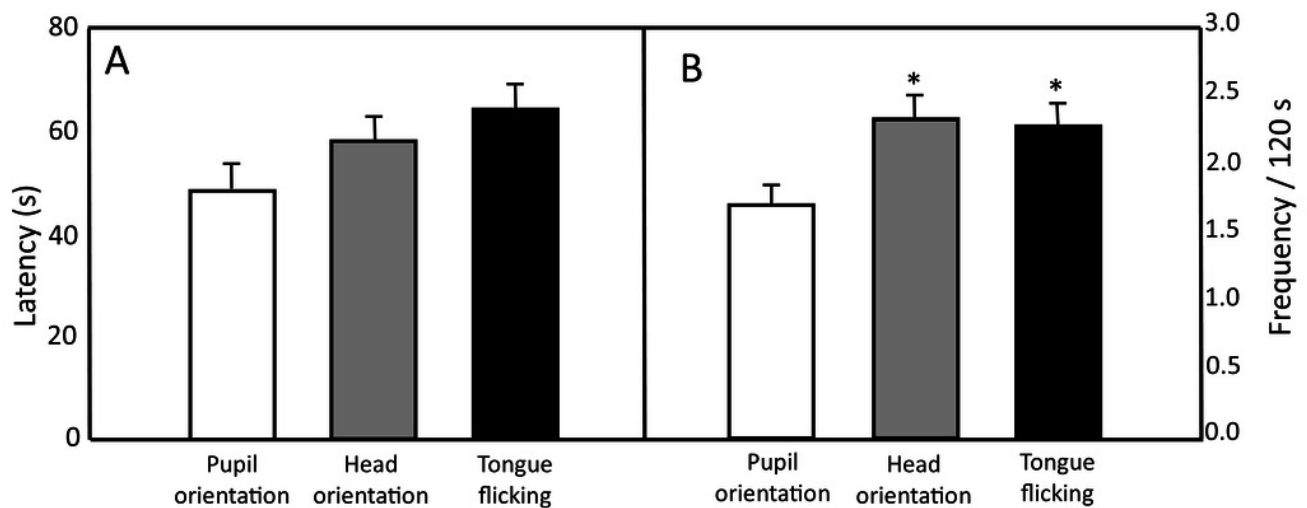
The experimental glass tank was divided into: compartment A, where the experimental snake was maintained during the test, and compartment B into which an experimental thermal stimulus was placed. The two compartments were separated by a metal mesh barrier.



## Figure 4

Behaviors displayed by *C. polystictus* in response to artificial thermal stimuli.

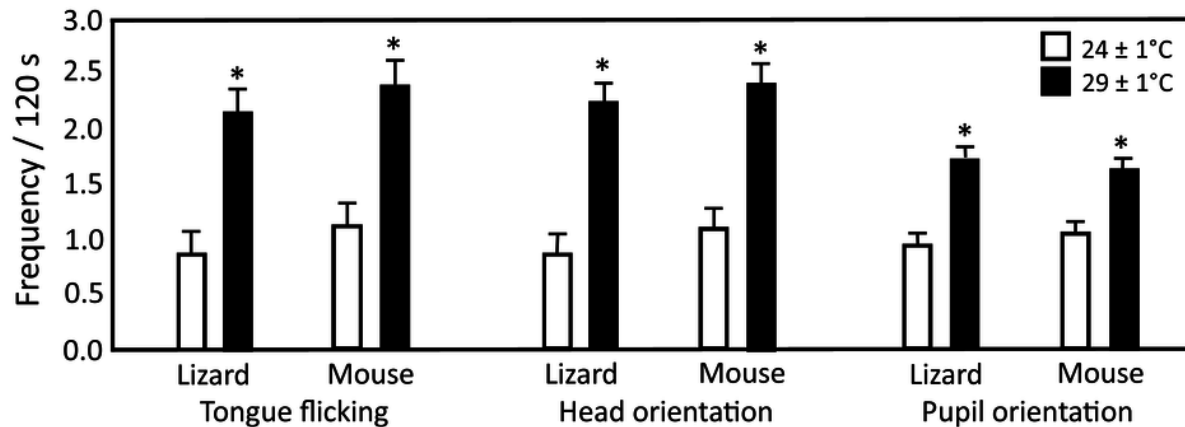
(A) Mean latency to displayed behavior  $\pm 1 SE$ . (B) Mean frequencies (square root transformed)  $\pm 1 SE$ . (\* $P < 0.05$  vs Pupil orientation).



## Figure 5

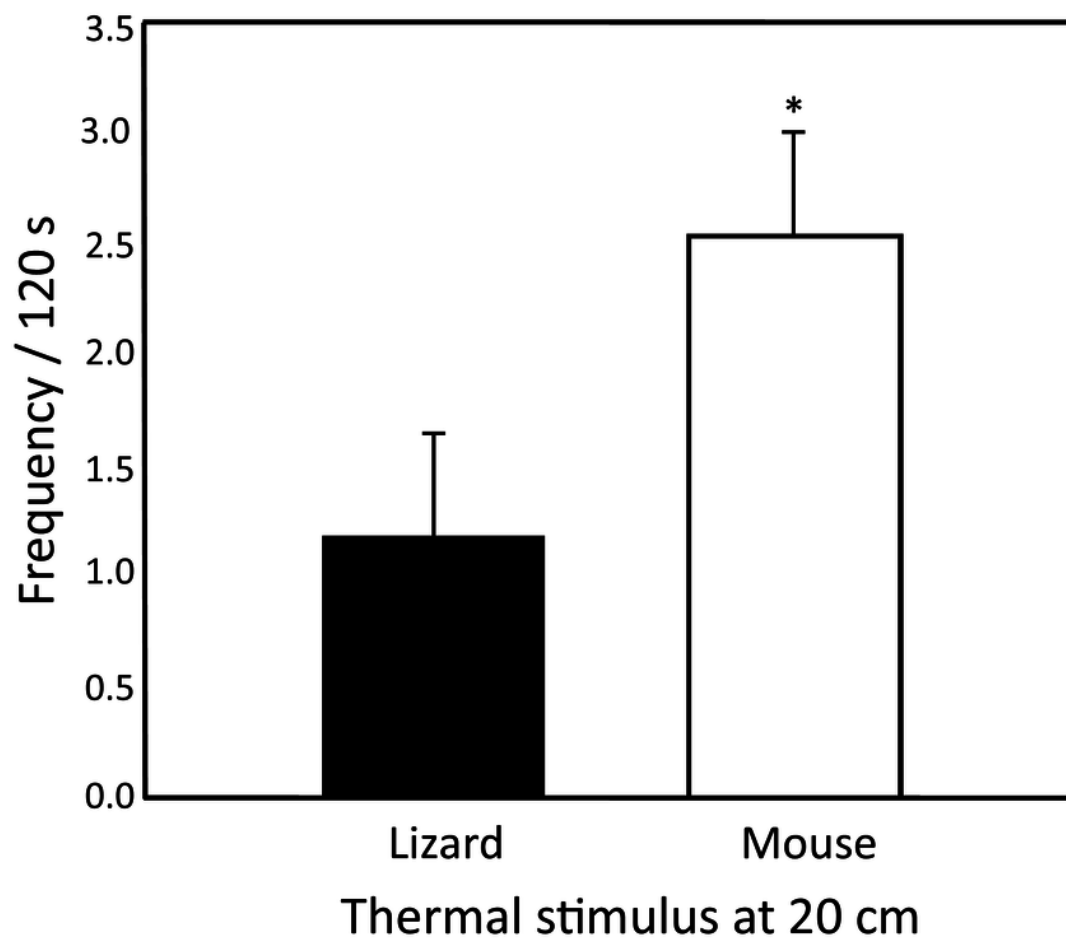
Mean frequencies (square root transformed)  $\pm 1$  SE of tongue-flicks, head orientation, and pupil orientation displayed by *C. polystictus* in response to lizard-like and mouse-like thermal stimuli at 24° and 29 °C.

\*  $P < 0.05$  vs thermal stimuli 24°  $\pm$  1°C.



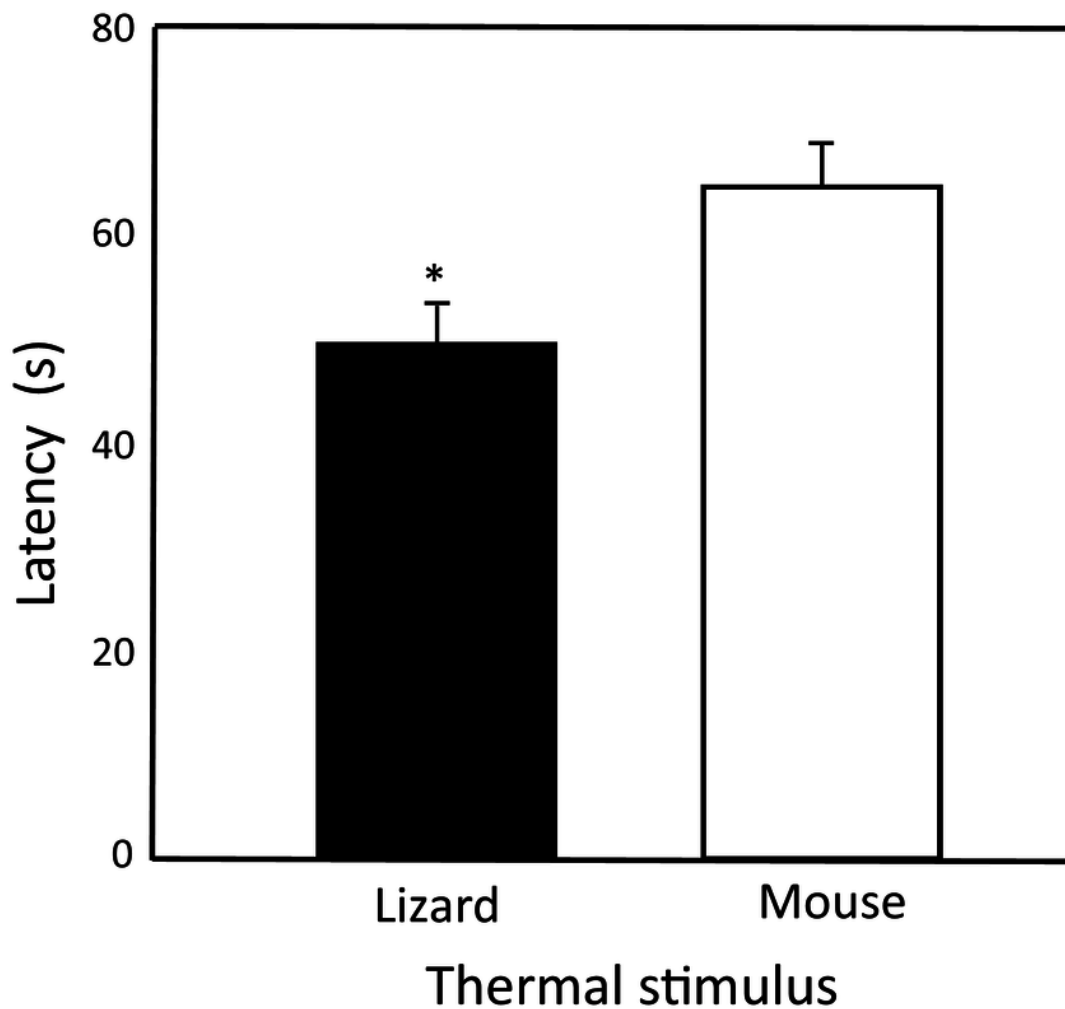
## Figure 6

Mean frequencies (square root transformed)  $\pm 1$  SE of tongue-flicks displayed by *C. polystictus* in response to lizard-like and mouse-like thermal stimuli 20 cm away.



## Figure 7

Mean latency  $\pm 1$  SE of head orientation displayed by *C. polystictus* in response to lizard-like and mouse-like thermal stimuli at 29 °C.





## Figure 8

Mean frequencies (square root transformed)  $\pm 1$  SE of behaviors displayed by *C. polystictus* in response to lizard-like and mouse-like thermal stimuli at 24° and 29°C and 200 cm away.

\*  $P < 0.05$  vs thermal stimuli at 24°C; Pupil orientation: mouse vs lizard to 29°C, Student-Newman-Keuls post hoc.

