The effects of prenatal corticosterone and thermal environment on numerical discrimination abilities in a lizard

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## Abstract

Animals use quantitative judgements in several ecological contexts. From social behaviour to navigating their environment, quantitative abilities can be crucial in animals’ behaviour. Since prenatal conditions are predicted to impact brain development, quantitative competence may also be affected by the early environment. Factors like temperature or stress-related hormones transmitted by the parents can play a prominent role in shaping cognitive traits. In addition, abrupt temperature changes can lead to the transmission of stress-related hormones to the offspring, making it essential to study the combined effects of stress hormones and thermal conditions during development. Here, we investigated the effects of prenatal corticosterone (CORT) and temperature on numerical discrimination in the Common garden skink (*Lampropholis guichenoti*). We manipulated CORT levels and incubation temperature of eggs employing a 2x2 factorial design, and then assessed numerousness through a spontaneous choice test with food as the relevant stimulus. In a repeated measures design, animals were subjected to five different tests with different items and ratios (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 2 VS 4). We hypothesized that CORT exposure and cold temperatures would constraint performance in the numerical task. Contrary to our expectations, we found no evidence of numerical discrimination for any treatments. These findings suggest that *L. guichenoti* may not use numerical cues to make foraging decisions and that early conditions do not affect their behaviour towards different quantities of food.

## Significant statement

Discriminating between quantities is crucial for animal behaviour, yet the impact of early environmental factors on the development of quantitative competence remains largely unexplored. In this study, we investigated for the first time the effects of prenatal corticosterone exposure and incubation temperature on numerical discrimination in a reptile. Contrary to our predictions, we did not observe any evidence of numerical discrimination regardless of incubation conditions. Our results align with previous studies showing that reptiles consuming live prey struggle to differentiate prey numbers in spontaneous choice tests. These results open new avenues for exploring the evolution of numerical abilities in reptiles and identifying factors influencing the development of quantitative skills in this group. Further research should explore alternative methods, such as trained tasks, to better understand the cognitive mechanisms underlying numerical competence in reptiles.

## Introduction

The ability to discern between more and fewer quantities is a fundamental cognitive skill widely observed across the animal kingdom ([Vonk and Beran 2012](#ref-vonk2012bears); [Stancher et al. 2015](#ref-stancher2015numerical); [Beran and Parrish 2016](#ref-beran2016capuchin)). Numerical cognition is considered adaptive in various ecological contexts, contributing to social decisions, mate selection, or foraging ([Lucon-Xiccato and Dadda 2017](#ref-lucon2017individual); [Nieder 2018](#ref-nieder2018evolution)). For instance, female lions (*Panthera leo*) use numerical information to assess the risk of confronting rival groups ([McComb et al. 1994](#ref-mccomb1994roaring)), while other animals use these abilities to navigate their environment ([Chittka and Geiger 1995](#ref-chittka1995can)), locate potential mates ([Carazo et al. 2009](#ref-carazo2009quantity)), or to make optimal foraging decisions ([Uller et al. 2003](#ref-uller2003salamanders)). Given the adaptive advantages of numerical and quantity discrimination, research has extensively explored the ability of different species to differentiate between quantities under different contexts and paradigms.

Numerical competences are often evaluated by considering the types of cues animals use ([Plotnik et al. 2019](#ref-plotnik2019elephants); [Cooper et al. 2024](#ref-cooper2024tell)), the ecological contexts in which these abilities are deployed ([McComb et al. 1994](#ref-mccomb1994roaring); [Chittka and Geiger 1995](#ref-chittka1995can); [Uller et al. 2003](#ref-uller2003salamanders); [Carazo et al. 2009](#ref-carazo2009quantity)), and the neural mechanisms that underlie them ([Nieder 2005](#ref-nieder2005counting)). The later have demonstrated that non-verbal numerical representations in mammals activate an extensive cortical network, with the prefrontal cortex and the intraparietal sulcus being particularly relevant ([Nieder 2005](#ref-nieder2005counting)). This connection between numerosity and brain physiology underscores the importance of understanding how brain development influences numerical abilities.

An animal’s brain is particularly sensitive to environmental inputs during early stages of development ([Coomber et al. 1997](#ref-coomber_independent_1997); [Zhu et al. 2004](#ref-zhu_prenatal_2004)), with long-lasting effects on cognitive abilities ([Zhu et al. 2004](#ref-zhu_prenatal_2004); [Amiel et al. 2014](#ref-amiel_egg_2014)). For instance, increases in glucocorticoids (GCs) - the main groups of hormones mediating the response to stressors ([Sapolsky et al. 2000](#ref-sapolsky_how_2000)) - during early life can alter animals’ performance in spatial, associative, or reversal learning tasks ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Crino et al. 2014](#ref-crino_corticosterone_2014-learn); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn); [Bebus et al. 2016](#ref-bebus_associative_2016); [Lui et al. 2017](#ref-lui2017chronic)). These effects have been shown to vary depending on factors such as dose, subjects’ sex, or type of cognitive test employed by Farrell et al. ([2015](#ref-farrell_developmental_2015-learn)), but little is known about how exposure to GCs during development can affect quantitative abilities. At the same time, the thermal early environment plays a prominent role in brain development and cognitive abilities in ectotherms ([Coomber et al. 1997](#ref-coomber_independent_1997); [Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Amiel et al. 2017](#ref-amiel_effects_2017); [Vila Pouca et al. 2018](#ref-vila_pouca_incubation_2018), [2019](#ref-vila_pouca_quantity_2019)). For example, Port Jackson shark incubated at elevated-temperatures learned faster in a quantity discrimination task ([Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)). However, the effects of early thermal environment on numerical abilities in other taxa remains largely unexplored.

Given that abrupt temperature changes can increase GC levels in vertebrates, leading to elevated GCs in offspring ([Racic et al. 2020](#ref-racic_effects_2020); [Crino et al. 2023](#ref-Crino_2023)), it is essential to study the combined effects of early-life GCs and thermal conditions on cognitive development. Understanding how these factors influence quantitative abilities could reveal important insights into the ecological relevance of numerosity. To fill this gap, we used the common garden skink (*Lampropholis guichenoti*) as a model to test the effects of prenatal corticosterone (CORT) - the main GC in reptiles ([Crino et al. 2023](#ref-Crino_2023)) - exposure and thermal environment on numerical discrimination abilities.

Traditionally, research on numerical abilities has been mainly focused on mammals, birds, and fishes ([Nieder 2005](#ref-nieder2005counting); [Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)), but recent studies demonstrate that reptiles are also competent in numbers. For example, red-footed turtles (*Chelonoidis carbonaria*) rapidly learned to associate colour with higher volumes of food ([Soldati et al. 2017](#ref-soldati2017long)), Hermann’s tortoises (*Testudo hermanni*) proved to discriminate between big and small food quantities in a spontaneous choice test ([Gazzola et al. 2018](#ref-gazzola2018continuous)), and freshwater turtles (*Trachemys scripta* and *Mauremys sinensis*) showed high skills when tested in a trained quantity discrimination task ([Lin et al. 2021](#ref-lin2021superior), [2024](#ref-lin2024trained)). For example, the lizard *Podarcis siculus* failed to discriminate between different numbers of food items in a spontaneous choice test ([Miletto Petrazzini et al. 2017](#ref-miletto2017quantitative)), but succeeded in a trained quantity discrimination task ([Miletto Petrazzini et al. 2018](#ref-miletto2018quantity)). In contrast, rock lizards (*Iberolacerta cyreni*) preferred larger quantities of crickets when presented five versus ten items ([Recio et al. 2021](#ref-recio_prey_2021)). Indeed, the literature evidences basic quantitative abilities in reptiles that may vary with the type of cue and test employed.

At the same time, reptiles’ cognition is significantly influenced by the environment in which they develop. Incubating lizards at elevated temperatures has been shown to improve cognitive abilities in consistency with elevated neuronal density in some areas of the brain ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Amiel et al. 2017](#ref-amiel_effects_2017)); and thermal environment impaired cognition only under incubation temperatures beyond lizards’ natural range Abayarathna and Webb ([2020](#ref-abayarathna_effects_2020)). The effects of prenatal CORT on cognitive abilities in reptiles is less understood, but in other taxa, high levels of GCs are tipically related to cognitive impairments ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004)). However, the combined influence of prenatal CORT and thermal environment remains unknown, particularly on numerical abilities.

Our study aimed to address this gap by manipulating CORT concentration in *L. guichenoti* eggs and incubating them under one of two temperature regimes (Cold – 23ºC ± 3ºC or Hot – 28ºC ± 3ºC) in a 2x2 factorial design. After hatching, we assessed the lizards’ numerical discrimination abilities in a spontaneous choice test, where they were presented with different numbers of crickets. Based on other studies that explored the cognitive effect of prenatal GCs or thermal early environment in reptiles and other taxa, we hypothesized that lizards incubated at higher temperatures would outperform those incubated at lower temperatures in the numerical discrimination task ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Amiel et al. 2017](#ref-amiel_effects_2017); [Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)). Additionally, we predicted that increased CORT levels would impair numerical competence ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004)), with more pronounced effects at lower temperatures. Consistent with Weber’s law ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)), we expected that lizards incubated at higher temperatures would successfully discriminate between quantities even at high ratios (e.g. 3 VS 4), whereas event the lowest ratios (e.g. 1 VS 4) could be challenging for those exposed to CORT or incubated at lower temperatures.

## Methods

#### Husbandry

*Breeding colony* – The lizards tested in out experiment came from a breeding colony established in the lab in 2019. The colony consists of approx. 180 adults of and *L. guichenoti* housed in commonal containers (41.5 L x 30.5 W x 21 H cm) with six lizards (2 males and 4 females) per enclosure. Enclosures are provided with non-stick matting, shelter, and several small water dishes filled daily, and lizards are fed mid-size crickets (*Acheta domestica*) three days a week. Crickets are dusted with calcium weekly and multivitamins and calcium biweekly. Room temperatures are set to 22-24ºC, but to ensure a temperature gradient, we employ a heat chord and a heat lamp following a 12 h light:12 h dark cycle, keeping the warm side of enclosures at 32ºC.

*Eggs collection and incubation* – Between mid-October 2023 to the end of February 2024, we placed small boxes (12.5 L x 8.3 W x 5 H cm) with moist vermiculite inside in one extreme of the communal enclosures (see above) to provide females with a place to lay the eggs. We checked for eggs in the boxes three days a week. Eggs’ length and width were measured with a digital calliper to the nearest 0.1 mm, while mass was recorded with a (OHAUS, Model spx123) digital scale ± 0.001g error. Eggs were assigned a clutch number and an individual identity. Then we treated eggs with CORT or vehicle (see CORT and Temperature manipulation below) and placed them in individual cups (80 mL) with moist vermiculite (12 parts water to 4 parts vermiculite). We covered the cups with cling wrap to retain moisture and left the eggs in LATWIT 2X5D-R1160 incubators at two different temperatures (see CORT and Temperature manipulation below) until hatching.

*Hatchlings* – Incubators were checked three times a week for the presence of hatchlings. Once hatchlings were collected, we measured SVL and Tail Length (TL) with a ruler to the nearest mm, and weighed them with a (OHAUS, Model spx123) digital scale ± 0.001g error. Hatchlings were then placed in individual enclosures (18.7L x 13.2W x 6.3H cm) provided with non-stick matting and a small water dish. During this period, they were sprayed with water daily and received 3-6 small *A. domestica* crickets three times a week. All care otherwise follows similar protocols to adults (see above).

#### Manipulating Early Thermal and CORT Environments

We manipulated CORT concentration in eggs and incubated them under one of two temperature regimes (Cold – 23ºC ± 3ºC or Hot – 30ºC ± 3ºC) in a 2x2 factorial design ([Fig. 1](#fig-Methods) A). We first allocated eggs to one of two different treatments: in the CORT treatment, eggs were topically supplied with 5µL of CORT dissolved in 100% Ethanol (vehicle) at a final (10 pg CORT/mL) concentration; in the Control treatment eggs received an equal volume of the vehicle. CORT concentration employed in the CORT treatment represents two standard deviations above the mean natural concentration obtained in eggs from both species (non-published data). Then, eggs were incubated in one of the two previously mentioned temperature regimes (Cold or Hot) until hatching. These temperatures represent the upper and lower limit of the natural incubation temperatures ([Qualls and Shine 2000](#ref-qualls2000post)). The number of eggs per clutch assigned to each hormone and temperature treatment was counterbalanced in both species.

#### Numerical discrimination task

Two weeks before starting the experimen phase (see below), lizards were moved to the experimental arena for acclimatation. The arenas were individual medium-size (41 L x 29.7 W x 22 H cm) plastic containers provided with a shelter (9 L x 6 W x 1.5 H cm) on one of the extremes and a water dish in the center ([Fig. 1](#fig-Methods) B). The new enclosures were placed in 7 different racks in two rooms. Each rack was associated with its own CCTV system (device model DVR-HP210475), allowing us to record lizards’ behaviour during the experiment. The number of lizards per species and treatment in each rack was counterbalanced to control for any effect of the room or the position of the lizard in the rack. During acclimatation and throughout the experiment, lizards were fed with only one cricket daily, dusted with calcium and multivitamins (see protocol below), and water was supplied *ad libitum*. The temperature of both rooms was set to between 22-24ºC, but we also provided a temperature gradient using a heat cord along the center of the arena and heat lamps in a 12 h light: 12 h dark cycle.

The numerical discrimination test consisted in a series of two-choice trials where lizards where presented with different number of food items. Each choice was placed on one of two sides of a white 3D printed platform (see [Fig. 1](#fig-Methods) B, C) and then covered with a transparent Petri dish to prevent the lizard from obtaining the food. We performed five tests that differed in the ratio between both choices: 1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4. We never used more than four items because higher numbers are considered to be represented by a different system ([Nieder 2005](#ref-nieder2005counting); [Cantlon et al. 2009](#ref-cantlon2009beyond); [Hyde 2011](#ref-hyde2011two)). The food consisted of frozen small crickets (A. domestica) thawed and dusted with calcium and multivitamins. We decided to use frozen crickets to avoid movement that would affect lizards’ behaviour. Lizards were fed frozen crickets during one month before the beginning of the experiments for habituation to frozen prey. Two weeks before the experiments, to habituate lizards to the platform and the Petri dish, the frozen cricket was placed on top of the Petri dish on one of the sides of the platform, simulating the conditions of the experiment (see [Fig. 1](#fig-Methods) B). The side where the food was placed was changed every day to avoid any side bias during the trials. However, to further control for that, the side where we placed the higher number of crickets was randomised between trials and individuals. We also counterbalanced the number of individuals per treatment tested in the same type of trial each day. The crickets used during the experiment were all the same size, but to control for other quantitative traits ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)), we changed the orientation of the crickets as shown in [Fig. 1](#fig-Methods) D. In addition, after the experiments, we conducted another set of trials to test whether the orientation of the crickets could affect lizards’ choice, but we found no effect on orientation (see Supplementary Material - Prey orientation test).

All tests were done between 10 and noon when the lizards were most active. Each trial was performed daily for five days between the 17th and 22nd of May 2024. We recorded three main variables: i) Latency, defined as the time between the start of the test - when the platform was left in the enclosure - until the first interaction with one of the options; ii) First choice of the lizard, recorded as 1 if the lizard interacted first with the larger amount or 0 if not; and iii) Interest shown for the higher amount of food (detailed below). We considered that the lizard interacted with the food when it touched the Petri dish with its snout or when the front half of the body was on top of the platform. For five minutes after the first interaction with one of the options, we recorded the time spent interacting with that option for a maximum of five minutes; we did this for both amounts of food independently of what was the first choice. We measured the interest shown for the higher amount of food by substracting the time interacting with the option with fewer crickets to the time spent interacting with the larger amount of food. If there was no interaction with one of the options during the test, we considered the time spent as 0 for that option. When there was no interaction with any of the choices, both Latency, First choice, and Interest were noted as NA. We also considered Interest as NA in a few cases where the animals managed to lift the dish and eat the prey; Latency and First choice were recorded and employed in the analyses. We excluded from the analyses all the individuals that did not make any choice in three or more tests.

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| Fig 1— Experimental design of the study. Panel (**A**) shows the early environment manipulation procedures. (**B**) illustrates the arena where the tests were performed, while panel (**C**) indicates the measurements of the platform used for the experiments. Finally, panel (**D**) displays the types of numerical tests used and the orientation of the crickets in each test. |

#### Statistical analyses

In our analyses, we fitted a multivariate Bayesian multilevel model using the brm function from the *brms* package ([Bürkner 2017](#ref-burkner2017brms)) in R extension (version 2.8.2) ([R Core Team 2021](#ref-R)). We ran four parallel MCMC chains of 4000 iterations with a warmup period of 1000 iterations. We modelled the three main recorded behaviours: Latency (log-transformed), Choice, and Interest (see above) as the response variables, and test (1 VS 4, 1 VS 3, 2 VS 4, 2 VS 3, 3 VS 4), hormone (CORT versus Control), incubation temperature (Cold versus Hot) and their interaction as fixed factors. We also included in our predictor variables of sex, and age (centered to 0). We included lizard identity and clutch as random factors. We used the posterior distributions of parameters from these models to test for differences in the ability to discriminate between different number of crickets between treatments and species, for each of the numerical tests. We calculated the contrast between treatments for each test using pmcmc values and estimated whether predicted Choice and Interest differed from 0.5 or zero, respectively, using pmcmc. We considered statistical significance if pmcmc < 0.05.

#### Ethical note

Subjects were collected from a breeding colony that had been kept in the lab since 2019. All animals in this experiment were provided humane laboratory housing, thermoregulation opportunities, and light and humidity conditions (see above for details). Lizards in the colony received five-seven crickets per individual three times a week, while the subjects in the experiment were fed once per day. All lizards were handled minimally to avoid stress. Experimental procedures and laboratory housing complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee (A2022\_33). Upon completion of experimental trials, lizards were kept in their enclosure and will be euthanized following standard humane procedures once they reach the human-ending point.

## Results

Our results showed an effect of sex and age on Latency (mean estimated effect of sex = -0.46, 95% CI = [-0.9, -0.02]; mean estimated effect of age = 0.06, 95% CI = [0.01, 0.11]; see Table S1 in Supplementary Material), where males and older individuals made, overall, faster decisions. However, we did not see such effects on Choice or Interest (see Table S1 in Supplementary Material). Nevertheless, when we analyse the effect of treatment on performance, for every variable recorded, we control for sex by averaging the predicted values for males and females, and we control for age by including the centered age in the model. These results are summarized in [Fig. 2](#fig-results) and Table 1.

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| Fig 2— Mean estimates of log-latency (Latency), the probability of choosing first the larger number of crickets (Choice), and the estimated interest for the larger number of crickets (Interest) per each of the treatments for each of the numerical tests performed. The x-axis represents the estimate, and the y-axis is the density of the estimates. The different colours indicate the different treatments. Points and bars represent the mean and standard deviation of the mean of the estimates, respectively. Vertical dashed lines in Choice and Interest graphs values 0.5 and 0, respectively. |

Temperature, CORT, or their interaction contrasts were not significant in any of the numerical tests (Table 1). We did not observe any clear pattern indicating relevant effects of early environment on any of the tests ([Fig. 2](#fig-results)). Except for Hot-CORT lizards in 1 VS 3 tests or Cold-Control lizards in 2 VS 4 tests, the predicted probability of choosing the greater number of crickets first was not different from 0.5 ([Fig. 2](#fig-results); but also see Table S2 in Supplementary Material). Similarly, the predicted interest for the larger number of crickets was not different from 0 in any of the tests ([Fig. 2](#fig-results); but also see Table S2 in Supplementary Material).

*Table 1. Effects of temperature, CORT, and their interaction on latency, choice, and interest shown for the higher amount of food in each of the numerical discrimination tests. The table shows the comparisons for each predictor (Temperature = [meanHot - meanCold]; Hormone = [meanControl - meanCORT]; Interaction = [(meanHot-Control - meanHot-CORT) - (meanCold-Control - meanCold-CORT)]) and the pmcmc value of the comparisons. In bold, the effects where pmcmc < 0.05.*

|  | | Tests | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| Variable | Predictor | 1VS4 | 1VS3 | 2VS4 | 2VS3 | 3VS4 |
| Latency | Temperature | -0.3 (p= 0.58) | -0.2 (p= 0.63) | -0.4 (p= 0.44) | -0.4 (p= 0.40) | -0.1 (p= 0.86) |
|  | Hormone | -0.3 (p= 0.64) | -0.1 (p= 0.80) | 0 (p= 1.00) | -0.2 (p= 0.75) | -0.3 (p= 0.47) |
|  | Interaction | -0.8 (p= 0.20) | -0.5 (p= 0.39) | -0.5 (p= 0.41) | -0.4 (p= 0.49) | 0 (p= 0.96) |
| Choice | Temperature | 0.1 (p= 0.68) | 0.2 (p= 0.52) | 0 (p= 0.94) | -0.3 (p= 0.24) | 0.2 (p= 0.39) |
|  | Hormone | 0 (p= 0.97) | 0 (p= 0.93) | 0 (p= 0.86) | 0 (p= 0.83) | 0.1 (p= 0.56) |
|  | Interaction | -0.3 (p= 0.24) | -0.4 (p= 0.09) | 0.1 (p= 0.67) | -0.2 (p= 0.36) | -0.1 (p= 0.62) |
| Interest | Temperature | -11.8 (p= 0.69) | 22.1 (p= 0.62) | -5.2 (p= 0.88) | -22.2 (p= 0.69) | 4.1 (p= 0.91) |
|  | Hormone | -1.2 (p= 0.96) | -11.6 (p= 0.80) | 1.2 (p= 0.97) | 2.7 (p= 0.96) | 40 (p= 0.19) |
|  | Interaction | 19.4 (p= 0.63) | -56 (p= 0.16) | 34.9 (p= 0.36) | -69.7 (p= 0.08) | -23.8 (p= 0.56) |

## Discussion

Our results indicate that lizards did not discriminate between different numbers of crickets in any of the tests and that their performance did not vary across treatments. We did not see changes in Latency in any of the tests, and treatment did not affect lizards’ time to make a decision either. Furthermore, in most cases, the estimated probability of choosing the option with more crickets first did not differ from random (0.5), with significant differences emerging only in two tests for two of the four treatments; yet no clear pattern was observed. These findings were further reinforced by similar results regarding the interest for the larger number of food items that did not differ from zero in any of the tests or groups. Altogether, these findings suggest that *L. guichenoti* did not use numerical or other potential quantitative cues we were not controlling for to make foraging decisions.

Our experiment employed a spontaneous choice test to investigate *L. guichenoti*’s numerical abilities. This test is considered a reliable method to assess animal numerosity, as it allows for observing natural behaviour and acquiring ecological issues related to numerousness and quantity discrimination ([Nieder 2005](#ref-nieder2005counting); [Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). However, one of the main caveats of this approach is that motivation plays a key-role in animals’ performance ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous); [Bisazza et al. 2014](#ref-bisazza2014experim)). Here, we discarded the trials where the lizards did not interact with any of the options, so the observed results are based exclusively on those where the lizards chose. Therefore, we do not consider motivation a factor affecting our results. Moreover, spontaneous choice tests are based on the assumption that animals should select the most appropriate quantity, which may not necessarily be the largest one ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). Although spontaneous choice tests employing food assume animals prefer larger amounts of food, this may not hold if increased handling costs are involved ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). Nevertheless, in that scenario, we would still expect animals to exhibit a preference, even if for the smaller amount of food, or to observe some influence of age on their choice or interest, as older animals are typically larger and may be more adept at handling a greater number of prey items.

Another consideration is the limitation of spontaneous choice tests to control for other quantitative cues that may correlate with numerosity ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous)). Although we controlled for some quantitative information by rotating crickets in the option with fewer items to increase total area, we can not rule out the possibility that lizards could use other quantitative cues. If that were the case, however, we would still see some type of discrimination between choices unless the cues were conflicting ([Cooper et al. 2024](#ref-cooper2024tell)). Indeed, it is possible that *L. guichenoti* uses multiple quantitative cues simultaneously during foraging decisions, and when those cues conflict, the preference for one cue over another might vary between individuals, potentially producing the results observed here. Alternatively, *L. guichenoti* may not rely on numerical cues for foraging decisions, instead utilizing other types of information.

Interestingly, half of the studies demonstrating numerosity in reptiles employed trained quantity discrimination tasks ([Soldati et al. 2017](#ref-soldati2017long); [Miletto Petrazzini et al. 2018](#ref-miletto2018quantity); [Lin et al. 2021](#ref-lin2021superior), [2024](#ref-lin2024trained); [Tomonaga et al. 2023](#ref-tomonaga2023slowly)). In the remaining successful studies, the relevant stimuli involved either vegetables ([Gazzola et al. 2018](#ref-gazzola2018continuous); [Szabo et al. 2021](#ref-szabo_spontaneous_2021)) or large quantities (e.g., 5 vs. 10)([Recio et al. 2021](#ref-recio_prey_2021)). However, when *Podarcis siculus* were presented different numbers of *Musca domestica* larvae in a spontaneous choice test, the lizards failed to discriminate between quantities ([Miletto Petrazzini et al. 2017](#ref-miletto2017quantitative)) despite showing high quantitative skills when size of prey was the main cue ([Miletto Petrazzini et al. 2017](#ref-miletto2017quantitative)), or when tested using training procedures ([Miletto Petrazzini et al. 2018](#ref-miletto2018quantity)). Hence, it is possible that further studies exploring numerosity in *L. guichenoti* using different methodologies or contexts, such as larger quantities ([Nieder 2005](#ref-nieder2005counting); [Recio et al. 2021](#ref-recio_prey_2021)), inclusion of chemical stimuli ([Carazo et al. 2009](#ref-carazo2009quantity); [Plotnik et al. 2019](#ref-plotnik2019elephants); [Recio et al. 2021](#ref-recio_prey_2021)), using relevant stimuli like conspecifics ([Carazo et al. 2009](#ref-carazo2009quantity); [Lucon-Xiccato and Dadda 2017](#ref-lucon2017individual)), or use training procedures ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous); [Bisazza et al. 2014](#ref-bisazza2014experim); [Soldati et al. 2017](#ref-soldati2017long); [Miletto Petrazzini et al. 2018](#ref-miletto2018quantity); [Lin et al. 2021](#ref-lin2021superior), [2024](#ref-lin2024trained); [Tomonaga et al. 2023](#ref-tomonaga2023slowly)), may yield more conclusive results.

We did not find any effect of prenatal conditions on *L. guichenoti*’s ability to use numerical information to make foraging decisions. We anticipated that lower incubation temperatures and exposure to CORT will impair lizards’ performance, especially in those tests with higher ratios following Weber’s Law ([Agrillo and Bisazza 2014](#ref-agrillo2014spontaneous); [Nieder 2018](#ref-nieder2018evolution)). However, our treatments did not impact lizards' behaviour, suggesting that early environmental conditions do not affect numerical competence in this species. Our predictions were based on previous studies showing the effects of prenatal temperature and GCs on cognitive abilities in reptiles and other groups ([Szuran et al. 1994](#ref-szuran_water_1994); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014); [Lui et al. 2017](#ref-lui2017chronic); [Amiel et al. 2017](#ref-amiel_effects_2017)). However, these effects can be species-specific, varying across cognitive domains and test types ([Szuran et al. 1994](#ref-szuran_water_1994); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn); [Bebus et al. 2016](#ref-bebus_associative_2016); [Dayananda and Webb 2017](#ref-dayananda_incubation_2017); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020); [Haq et al. 2021](#ref-haq_prenatal_2021)).

In this context, it is important to consider that both GCs and thermal environment can exert differential effects on distinct regions of the brain. For example, incubation temperature impacts mitochondrial activity and cell density in specific brain nuclei ([Coomber et al. 1997](#ref-coomber_independent_1997); [Amiel et al. 2017](#ref-amiel_effects_2017)). Similarly, impairments in spatial learning - but not in other learning tasks - in rats ([Haq et al. 2021](#ref-haq_prenatal_2021)) are likely associated with the greater density of GCs receptors mammals’ hippocampi compared to other regions of the brain ([Siegel 2006](#ref-siegel_2006)). While it remains unknown which regions of the reptiles’ brain encode numerical information, our results suggest that relevant regions may be unaffected by the prenatal conditions tested here. However, researchers have proposed that non-verbal number representation is processed by two types of systems: one for small sets (≤ 4) that tracks and encodes objects as individual entities, and another and another for larger numerosities that operate through estimation processes ([Nieder 2005](#ref-nieder2005counting); [Cantlon et al. 2009](#ref-cantlon2009beyond); [Hyde 2011](#ref-hyde2011two)). Our data indicates that at least those brain regions involved in discrete numerical representation might be robust to the effect of prenatal CORT and temperature in *L. guichenoti*. Studies using higher quantities of food items may help elucidate the effects of prenatal conditions on *L. guichenoti*’s numerical abilities. Alternatively, it is also plausible that treatment effects are masked by the overall poor performance across most of the tests, which could be a consequence of the species’ reliance on another type of information for foraging rather than numerical information. Thus, future research should investigate the effects of prenatal CORT and thermal environment on numerical competence by employing trained quantity discrimination tasks ([Soldati et al. 2017](#ref-soldati2017long); [Miletto Petrazzini et al. 2018](#ref-miletto2018quantity); [Lin et al. 2021](#ref-lin2021superior), [2024](#ref-lin2024trained); [Tomonaga et al. 2023](#ref-tomonaga2023slowly)).

In conclusion, to the best of our knowledge, our study is the first to explore the impact of prenatal conditions on numerical abilities in reptiles. Our findings suggest that *L. guichenoti* does not rely on numerical information for foraging decisions and that their performance does not vary across different prenatal treatments. This implies that early environmental conditions, such as incubation temperature and CORT exposure, may not affect numerosity in this species. However, it is critical to note that the methodologies employed in this study may not have fully captured the nuances of numerical cognition in *L. guichenoti*. Therefore, we recommend that future studies employ a broader array of tests and paradigms, including training-based tasks, larger sets of stimuli, and other sensory modalities, to more comprehensively assess numerical competence in this and related species. Such work will be pivotal in unravelling early environmental factors' effects on reptiles' cognitive abilities and advancing our understanding of numerosity across taxa.

## Data accessibility

All data, data description, and R code are available in public repository <https://github.com/Pablo-Recio/CORT-Temp_Numerical>.

## Declaration of AI use

We declare Chat GPT was used for questions related to coding and data analyses. All other parts of the manuscript were written by the authors and those parts where AI was used were checked and modified accordingly by the authors.

## Authors’ contributions

PR: conceptualization, methodology, data collection, data curation, formal analysis, writing—original draft, writing—review and editing; DCL: data collection, writing—review and editing; OC: conceptualization, writing—review and editing; CF: conceptualization, funding acquisition, writing—review and editing; AYP: data collection, writing—review and editing; DN: conceptualization, methodology, funding acquisition, project administration, resources, supervision, writing—review and editing.  
All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

## Conflict of interest declaration

We declare we have no competing interests.

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# Suplementary Material

#### Model results

*Table S1.- Summary of the model fitted*

For loglatency

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| b\_loglatency\_Intercept | 6.47 | 6.47 | 0.31 | 5.96 | 7.00 | 1.00 | 4547.93 | 6871.87 |
| b\_loglatency\_test\_type1VS3 | -0.03 | -0.03 | 0.21 | -0.37 | 0.31 | 1.00 | 5851.20 | 8069.68 |
| b\_loglatency\_test\_type2VS4 | -0.13 | -0.13 | 0.21 | -0.46 | 0.21 | 1.00 | 5607.53 | 8779.16 |
| b\_loglatency\_test\_type2VS3 | -0.02 | -0.02 | 0.21 | -0.36 | 0.33 | 1.00 | 5792.88 | 8099.56 |
| b\_loglatency\_test\_type3VS4 | -0.07 | -0.07 | 0.21 | -0.42 | 0.27 | 1.00 | 5394.76 | 8113.94 |
| b\_loglatency\_tempHot | 0.05 | 0.05 | 0.45 | -0.69 | 0.80 | 1.00 | 4240.55 | 6468.22 |
| b\_loglatency\_cortControl | 0.09 | 0.09 | 0.35 | -0.50 | 0.66 | 1.00 | 3501.90 | 6160.76 |
| b\_loglatency\_sexm | -0.46 | -0.45 | 0.22 | -0.82 | -0.10 | 1.00 | 5456.02 | 7259.40 |
| b\_loglatency\_age | 0.06 | 0.06 | 0.02 | 0.02 | 0.10 | 1.00 | 5242.87 | 6844.75 |
| b\_loglatency\_test\_type1VS3:tempHot | -0.03 | -0.03 | 0.30 | -0.52 | 0.46 | 1.00 | 5246.33 | 7514.61 |
| b\_loglatency\_test\_type2VS4:tempHot | -0.18 | -0.18 | 0.30 | -0.67 | 0.31 | 1.00 | 5432.60 | 8115.85 |
| b\_loglatency\_test\_type2VS3:tempHot | -0.25 | -0.25 | 0.30 | -0.74 | 0.25 | 1.00 | 5517.51 | 7473.31 |
| b\_loglatency\_test\_type3VS4:tempHot | -0.10 | -0.11 | 0.30 | -0.60 | 0.39 | 1.00 | 5247.17 | 7570.16 |
| b\_loglatency\_test\_type1VS3:cortControl | 0.04 | 0.04 | 0.29 | -0.43 | 0.53 | 1.00 | 5376.57 | 7715.18 |
| b\_loglatency\_test\_type2VS4:cortControl | 0.15 | 0.16 | 0.29 | -0.33 | 0.64 | 1.00 | 5492.31 | 8062.19 |
| b\_loglatency\_test\_type2VS3:cortControl | -0.04 | -0.04 | 0.29 | -0.52 | 0.44 | 1.00 | 5459.05 | 7755.73 |
| b\_loglatency\_test\_type3VS4:cortControl | -0.38 | -0.38 | 0.29 | -0.87 | 0.09 | 1.00 | 5264.74 | 8063.37 |
| b\_loglatency\_tempHot:cortControl | -0.78 | -0.78 | 0.49 | -1.58 | 0.03 | 1.00 | 3491.24 | 5908.08 |
| b\_loglatency\_test\_type1VS3:tempHot:cortControl | 0.25 | 0.25 | 0.42 | -0.43 | 0.94 | 1.00 | 5103.28 | 7366.37 |
| b\_loglatency\_test\_type2VS4:tempHot:cortControl | 0.28 | 0.28 | 0.42 | -0.40 | 0.97 | 1.00 | 4860.78 | 7733.09 |
| b\_loglatency\_test\_type2VS3:tempHot:cortControl | 0.37 | 0.37 | 0.42 | -0.32 | 1.05 | 1.00 | 5243.01 | 8532.21 |
| b\_loglatency\_test\_type3VS4:tempHot:cortControl | 0.74 | 0.75 | 0.42 | 0.05 | 1.44 | 1.00 | 5119.54 | 7726.59 |

For choice

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| b\_choice\_Intercept | -0.68 | -0.68 | 0.58 | -1.64 | 0.26 | 1.00 | 4717.07 | 6912.29 |
| b\_choice\_test\_type1VS3 | 0.24 | 0.24 | 0.69 | -0.89 | 1.38 | 1.00 | 5106.43 | 7093.98 |
| b\_choice\_test\_type2VS4 | 0.71 | 0.70 | 0.69 | -0.42 | 1.86 | 1.00 | 4984.93 | 7514.81 |
| b\_choice\_test\_type2VS3 | 1.12 | 1.10 | 0.73 | -0.06 | 2.35 | 1.00 | 4902.86 | 6926.45 |
| b\_choice\_test\_type3VS4 | 0.33 | 0.32 | 0.70 | -0.82 | 1.48 | 1.00 | 5219.20 | 7158.11 |
| b\_choice\_tempHot | 1.13 | 1.12 | 0.83 | -0.23 | 2.50 | 1.00 | 4283.18 | 6599.41 |
| b\_choice\_cortControl | 0.61 | 0.60 | 0.74 | -0.59 | 1.84 | 1.00 | 3922.51 | 6114.01 |
| b\_choice\_sexm | -0.06 | -0.06 | 0.30 | -0.54 | 0.43 | 1.00 | 14505.46 | 8697.98 |
| b\_choice\_age | -0.04 | -0.04 | 0.03 | -0.09 | 0.01 | 1.00 | 12392.71 | 8820.47 |
| b\_choice\_test\_type1VS3:tempHot | 0.80 | 0.79 | 1.04 | -0.88 | 2.55 | 1.00 | 4972.18 | 6745.87 |
| b\_choice\_test\_type2VS4:tempHot | -1.33 | -1.33 | 1.00 | -2.98 | 0.29 | 1.00 | 4886.89 | 7191.48 |
| b\_choice\_test\_type2VS3:tempHot | -1.77 | -1.77 | 1.04 | -3.49 | -0.09 | 1.00 | 4289.06 | 6024.53 |
| b\_choice\_test\_type3VS4:tempHot | -0.13 | -0.13 | 1.02 | -1.80 | 1.57 | 1.00 | 4538.82 | 7185.57 |
| b\_choice\_test\_type1VS3:cortControl | 0.48 | 0.47 | 0.99 | -1.15 | 2.12 | 1.00 | 4938.94 | 7818.41 |
| b\_choice\_test\_type2VS4:cortControl | -0.70 | -0.69 | 0.98 | -2.32 | 0.90 | 1.00 | 4749.40 | 7672.85 |
| b\_choice\_test\_type2VS3:cortControl | 0.15 | 0.16 | 1.04 | -1.53 | 1.85 | 1.00 | 4980.80 | 8127.50 |
| b\_choice\_test\_type3VS4:cortControl | 0.14 | 0.13 | 1.00 | -1.50 | 1.77 | 1.00 | 4881.82 | 7388.95 |
| b\_choice\_tempHot:cortControl | -1.33 | -1.32 | 1.06 | -3.06 | 0.40 | 1.00 | 3598.45 | 6418.88 |
| b\_choice\_test\_type1VS3:tempHot:cortControl | -0.65 | -0.64 | 1.44 | -3.01 | 1.71 | 1.00 | 4947.05 | 7258.07 |
| b\_choice\_test\_type2VS4:tempHot:cortControl | 1.82 | 1.79 | 1.41 | -0.52 | 4.12 | 1.00 | 4832.87 | 7213.76 |
| b\_choice\_test\_type2VS3:tempHot:cortControl | 0.25 | 0.25 | 1.45 | -2.12 | 2.59 | 1.00 | 4689.39 | 7011.19 |
| b\_choice\_test\_type3VS4:tempHot:cortControl | 0.81 | 0.80 | 1.44 | -1.57 | 3.20 | 1.00 | 4767.29 | 7038.46 |

For compared interest

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| b\_comparedinterest\_Intercept | 12.58 | 12.75 | 20.03 | -20.62 | 45.52 | 1.00 | 4226.87 | 6914.84 |
| b\_comparedinterest\_test\_type1VS3 | -27.45 | -27.46 | 24.85 | -68.46 | 13.12 | 1.00 | 5057.66 | 7251.20 |
| b\_comparedinterest\_test\_type2VS4 | 4.45 | 4.37 | 25.04 | -36.11 | 45.40 | 1.00 | 4955.22 | 7144.03 |
| b\_comparedinterest\_test\_type2VS3 | -18.02 | -18.08 | 25.03 | -59.03 | 23.55 | 1.00 | 4608.81 | 7655.14 |
| b\_comparedinterest\_test\_type3VS4 | -38.45 | -38.44 | 25.60 | -80.23 | 3.89 | 1.00 | 4974.12 | 7540.36 |
| b\_comparedinterest\_tempHot | -21.42 | -21.25 | 28.74 | -68.59 | 25.65 | 1.00 | 3724.65 | 6038.13 |
| b\_comparedinterest\_cortControl | -10.87 | -10.78 | 25.67 | -53.25 | 31.15 | 1.00 | 3414.61 | 5708.73 |
| b\_comparedinterest\_sexm | -8.60 | -8.64 | 9.32 | -23.92 | 7.03 | 1.00 | 17607.01 | 8664.01 |
| b\_comparedinterest\_age | -0.68 | -0.67 | 0.97 | -2.25 | 0.91 | 1.00 | 15664.51 | 9250.40 |
| b\_comparedinterest\_test\_type1VS3:tempHot | 71.62 | 71.63 | 35.19 | 13.31 | 129.60 | 1.00 | 4600.90 | 6586.40 |
| b\_comparedinterest\_test\_type2VS4:tempHot | -1.40 | -1.38 | 35.97 | -59.74 | 58.37 | 1.00 | 4594.12 | 6529.68 |
| b\_comparedinterest\_test\_type2VS3:tempHot | 34.51 | 34.43 | 35.89 | -23.35 | 93.63 | 1.00 | 4206.05 | 6855.33 |
| b\_comparedinterest\_test\_type3VS4:tempHot | 37.54 | 37.66 | 36.94 | -22.35 | 97.98 | 1.00 | 4702.14 | 7554.35 |
| b\_comparedinterest\_test\_type1VS3:cortControl | 27.46 | 27.64 | 35.35 | -30.54 | 86.37 | 1.00 | 4866.97 | 7599.14 |
| b\_comparedinterest\_test\_type2VS4:cortControl | -5.20 | -5.27 | 35.16 | -62.72 | 52.41 | 1.00 | 4362.77 | 6797.39 |
| b\_comparedinterest\_test\_type2VS3:cortControl | 48.43 | 48.30 | 35.49 | -9.54 | 106.81 | 1.00 | 4047.11 | 6637.73 |
| b\_comparedinterest\_test\_type3VS4:cortControl | 63.11 | 62.88 | 35.72 | 4.22 | 122.72 | 1.00 | 4618.01 | 7533.53 |
| b\_comparedinterest\_tempHot:cortControl | 19.41 | 19.72 | 36.63 | -40.29 | 79.85 | 1.00 | 3346.75 | 5960.45 |
| b\_comparedinterest\_test\_type1VS3:tempHot:cortControl | -75.56 | -75.33 | 50.52 | -157.46 | 7.56 | 1.00 | 4608.18 | 7070.71 |
| b\_comparedinterest\_test\_type2VS4:tempHot:cortControl | 15.19 | 15.16 | 50.30 | -69.06 | 97.00 | 1.00 | 4217.24 | 6394.78 |
| b\_comparedinterest\_test\_type2VS3:tempHot:cortControl | -89.54 | -88.78 | 50.35 | -173.98 | -6.66 | 1.00 | 3896.04 | 6636.15 |
| b\_comparedinterest\_test\_type3VS4:tempHot:cortControl | -43.38 | -43.71 | 51.44 | -127.24 | 41.39 | 1.00 | 4491.70 | 6562.71 |

Other effects

| variable | mean | median | sd | q5 | q95 | rhat | ess\_bulk | ess\_tail |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| sd\_clutch\_\_loglatency\_Intercept | 0.34 | 0.34 | 0.19 | 0.04 | 0.67 | 1.01 | 716.16 | 2313.13 |
| sd\_lizard\_id\_\_loglatency\_Intercept | 0.81 | 0.81 | 0.10 | 0.64 | 0.97 | 1.00 | 1725.51 | 2777.99 |
| sd\_clutch\_\_choice\_Intercept | 0.26 | 0.23 | 0.18 | 0.02 | 0.60 | 1.00 | 3913.70 | 5803.67 |
| sd\_lizard\_id\_\_choice\_Intercept | 0.59 | 0.60 | 0.25 | 0.13 | 0.99 | 1.00 | 2047.51 | 3110.77 |
| sd\_clutch\_\_comparedinterest\_Intercept | 9.92 | 9.22 | 6.32 | 1.03 | 21.27 | 1.00 | 4065.36 | 6333.93 |
| sd\_lizard\_id\_\_comparedinterest\_Intercept | 9.31 | 8.34 | 6.38 | 0.81 | 20.85 | 1.00 | 3847.09 | 5748.30 |
| sigma\_loglatency | 0.65 | 0.65 | 0.03 | 0.61 | 0.70 | 1.00 | 12611.43 | 8861.89 |
| sigma\_comparedinterest | 78.78 | 78.70 | 3.03 | 73.99 | 83.90 | 1.00 | 15359.76 | 8383.11 |
| lprior | -29.26 | -29.25 | 0.08 | -29.40 | -29.13 | 1.00 | 8343.29 | 9738.65 |
| lp\_\_ | -3440.63 | -3440.02 | 18.68 | -3472.06 | -3410.58 | 1.00 | 2890.39 | 5974.94 |

#### Treatments effects

*Table S2.- Estimated mean probability of choosing first the higher amount (Choice) and the mean interest for the higher amount of food (Interest) per treatment group for each of the numerical tests performed. p indicates the pmcmcm value (p) of testing the hypothesis Choice ≠ 0.5, and Interest ≠ 0, which would indicate a preference towards one of choices (> towards higher amounts, < towards lower amounts) of the comparissons. In bold, the groups where pmcmcm < 0.05*

| Test | Treatment | Est. mean | p | Est. mean | p |
| --- | --- | --- | --- | --- | --- |
| 1VS4 | Cold-CORT | 0.34 | 0.22 | 8.24 | 0.68 |
|  | Cold-Control | 0.48 | 0.85 | -2.58 | 0.89 |
|  | Hot-CORT | 0.60 | 0.46 | -13.22 | 0.52 |
|  | Hot-Control | 0.43 | 0.61 | -4.69 | 0.81 |
| 1VS3 | Cold-CORT | 0.39 | 0.41 | -19.15 | 0.34 |
|  | Cold-Control | 0.64 | 0.26 | -2.71 | 0.89 |
|  | **Hot-CORT** | **0.79** | **< 0.05** | 30.91 | 0.12 |
|  | Hot-Control | 0.63 | 0.32 | -8.66 | 0.67 |
| 2VS4 | Cold-CORT | 0.50 | 0.99 | 12.68 | 0.54 |
|  | Cold-Control | 0.48 | 0.85 | -3.60 | 0.85 |
|  | Hot-CORT | 0.45 | 0.73 | -10.03 | 0.61 |
|  | Hot-Control | 0.55 | 0.72 | 8.62 | 0.66 |
| 2VS3 | Cold-CORT | 0.59 | 0.51 | -9.68 | 0.63 |
|  | **Cold-Control** | **0.75** | **< 0.05** | 27.84 | 0.15 |
|  | Hot-CORT | 0.45 | 0.70 | 2.97 | 0.88 |
|  | Hot-Control | 0.38 | 0.35 | -29.22 | 0.14 |
| 3VS4 | Cold-CORT | 0.41 | 0.51 | -30.03 | 0.15 |
|  | Cold-Control | 0.59 | 0.49 | 21.87 | 0.25 |
|  | Hot-CORT | 0.64 | 0.30 | -14.05 | 0.52 |
|  | Hot-Control | 0.69 | 0.14 | 14.03 | 0.49 |

#### Testing potential side biases

*Table S3.- Number of individuals per treatment that chose the right (R) or left (L) side in each of the numerical tests. p-value indicates the result of the binomial test comparing the number of choices between sides. In bold, the groups where p-value < 0.05*

|  | 1 VS 4 | 1 VS 3 | 2 VS 4 | 2 VS 3 | 3 VS 4 |
| --- | --- | --- | --- | --- | --- |
| Control-Cold (n = 20) | R = 12 | L = 8 , p = 0.5 | R = 12 | L = 8 , p = 0.5 | R = 9 | L = 11 , p = 0.82 | R = 12 | L = 8 , p = 0.5 | R = 12 | L = 8 , p = 0.5 |
| Control-Hot (n = 20) | R = 12 | L = 8 , p = 0.5 | R = 5 | L = 15 , p = 0.04 | R = 9 | L = 11 , p = 0.82 | R = 10 | L = 10 , p = 1 | R = 11 | L = 9 , p = 0.82 |
| CORT-Cold (n = 20) | R = 11 | L = 9 , p = 0.82 | R = 11 | L = 9 , p = 0.82 | R = 11 | L = 9 , p = 0.82 | R = 10 | L = 10 , p = 1 | R = 11 | L = 9 , p = 0.82 |
| CORT-Hot (n = 20) | R = 11 | L = 9 , p = 0.82 | R = 9 | L = 11 , p = 0.82 | R = 9 | L = 11 , p = 0.82 | R = 10 | L = 10 , p = 1 | R = 11 | L = 9 , p = 0.82 |

#### Prey orientation test

Because we were concerned that the orientation of the crickets could affect the lizards’ choice, we performed one simple choice test to investigate this. We used the same platform and Petri dish as in the numerical discrimination task, but we placed one cricket on each side of the platform, one oriented vertically in respect to the shelter position (see [Fig. 1](#fig-Methods) B) and the other one horizontal to the shelter. We perform a unique trial randomising the position of the orientation of the cricket. We recorded the amount of individuals that chose the cricket oriented vertically and horizontally. Results are shown in the table below.

*Table S4. Number of individuals per treatment that chose the cricket oriente horizontally (Horizontal) or vertically (Vertical) in our control tests. p-value indicates the result of the binomial test comparing the number of choices between both choices.*

|  | Horizontal | Vertical | p-value |
| --- | --- | --- | --- |
| Control-Cold (n = 20) | 8 | 12 | 0.503 |
| Control-Hot (n = 20) | 7 | 13 | 0.263 |
| CORT-Cold (n = 20) | 12 | 8 | 0.503 |
| CORT-Hot (n = 20) | 9 | 11 | 0.824 |

#### Checking the model plots

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