

# Animal Behaviour

## Early environmental conditions do not impact behavioural flexibility in an invasive and non-invasive lizard species

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<b>Abstract:</b>	<p>Behavioural flexibility is expected to be crucial for animals adapting to environmental changes. However, the conditions experienced during early development can profoundly impact behavioural flexibility making it unclear how populations will respond to novel circumstances. Stressful situations faced by the parents can have a direct impact on animals' cognition through the transmission of glucocorticoids - stress-related hormones that affect offspring cognition. At the same time, stressful conditions can influence parental behaviour during nesting and consequently the thermal developmental conditions they experience. Here, we investigated the interactive effects of prenatal corticosterone (CORT) and temperature on behavioural flexibility in two lizard species, <i>Lampropholis delicata</i> and <i>L. guichenoti</i>. We manipulated prenatal CORT levels and incubation temperature in a 2x2 factorial design, and then assessed behavioural flexibility through a reversal learning task. We hypothesized prenatal CORT and cold temperatures to impair with lizards' performance in the reversal task. Given <i>L. delicata</i>'s success as an invasive species, we expected this species to behave more flexibly and be less affected by early environmental conditions. Contrary to our expectations, behavioural flexibility appears to be robust to prenatal temperature and CORT in both species. The lack of difference in reversal learning between <i>L. delicata</i> and <i>L. guichenoti</i> suggests that novel environments are unlikely to influence flexible behavioural learning and that behavioural flexibility itself is unlikely to explain differences in invasion success between these species.</p>
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Dear Editor,

Please consider our manuscript entitled “*Early environmental conditions do not impact behavioural flexibility in an invasive and non-invasive lizard species*” for publication in **Animal Behaviour**.

Behavioural flexibility – the ability of individuals to adjust behaviour adaptively in response to internal or external changes in the environment – has been shown to be important for animals adapting to novel circumstances. However, environmental conditions experienced during early development can also profoundly impact brain development and learning making the role of behavioural flexibility during adaptation to environmental changes process unclear. Robustness of flexible learning strategies to early environments, particularly temperature and stress, may provide a resolution to this perplexing problem but has not been tested before. Using a multi-species experimental approach, where we manipulate both early temperature and stress during development, we show that behavioural flexibility is indeed robust to early developmental environments in an invasive and non-invasive lizard species. The lack of difference in reversal learning between these two species also points to behavioural flexibility being less important to the invasive process in these lizards compared to work in mammals and birds where it seems quite critical.

Our novel findings have broad implications for the fields of cognitive ecology and invasion biology, and we think they will stimulate new research paths into the role that developmental environments play on learning and cognition that are important in understanding animal’s response to environmental changes. As such, we think this is well suited for a general journal such as *Animal Behaviour*. We hope that you agree this is also the case.

We confirm that this manuscript has not been published elsewhere and is not currently under consideration for publication in any other journal. All authors have approved the manuscript for submission and declare no conflicts of interest.

Thank you for considering our manuscript for publication. We look forward to hearing from you.

Sincerely,

Pablo Recio Santiago  
PhD Student  
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# Early environmental conditions do not impact behavioural flexibility in an invasive and non-invasive lizard species

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## Abstract

Behavioural flexibility is expected to be crucial for animals adapting to environmental changes. However, the conditions experienced during early development can profoundly impact behavioural flexibility making it unclear how populations will respond to novel circumstances. Stressful situations faced by the parents can have a direct impact on animals' cognition through the transmission of glucocorticoids - stress-related hormones that affect offspring cognition. At the same time, stressful conditions can influence parental behaviour during nesting and consequently the thermal developmental conditions they experience. Here, we investigated the interactive effects of prenatal corticosterone (CORT) and temperature on behavioural flexibility in two lizard species, *Lampropholis delicata* and *L. guichenoti*. We manipulated prenatal CORT levels and incubation temperature in a 2x2 factorial design, and then assessed behavioural flexibility through a reversal learning task. We hypothesized prenatal CORT and cold temperatures to impair with lizards' performance in the reversal task. Given *L. delicata*'s success as an invasive species, we expected this species to behave more flexibly and be less affected by early environmental conditions. Contrary to our expectations, behavioural flexibility appears to be robust to prenatal temperature and CORT in both species. The lack of difference in reversal learning between *L. delicata* and *L. guichenoti* suggests that novel environments are unlikely to influence flexible behavioural learning and that behavioural flexibility itself is unlikely to explain differences in invasion success between these species.

## Introduction

Behavioural flexibility describes the ability of individuals to adjust behaviour adaptively in response to changes in the internal or external environment (Brown & Tait, 2010). It comprises a suite of behaviours, including problem-solving or innovation (Brown & Tait, 2010), that can be beneficial for adapting to novel circumstances (Szabo et al., 2020; Szulkin et al., 2020). Species with greater behavioural flexibility are expected to be more resilient to environmental changes because behaving flexibly is predicted to be crucial for exploiting new resources and facing new challenges (Chapple et al., 2012; Szabo et al., 2020; Szulkin et al., 2020; Wright et al., 2010). For example, survival of cane toads in semi-arid regions of Australia depend on adjusting their hydration activities to include daytime hours, rather than relying solely on nighttime (Webb et al., 2014); while successful colonisation of new environments by birds is related to higher frequency of foraging innovations (Sol & Lefebvre, 2000). Although there is limited understanding of the physiological mechanisms involved in animals' ability to behave flexibly, relative brain size has been shown to be a good predictor of behavioural flexibility in mammals, birds, reptiles and amphibians (Amiel et al., 2011; Sol et al., 2008; Sol & Lefebvre, 2000). This suggests that behavioural flexibility is likely to be underpinned by complex neural mechanisms, and that alterations in these mechanisms may impact animals' ability to respond to novel situations. The brain is considered especially sensitive to environmental changes during the early stages of development (Zhu et al., 2004), creating uncertainty about how the conditions faced during early life might impact behavioural flexibility.

The conditions in which organisms develop can be altered by the circumstances faced by their parents. For instance, exposing threespine sticklebacks females to a model predator during ovulation, affected their offspring's tendency to use social cues (Feng et al., 2015). Under stressful situations, animal's sensory systems detect and transmit environmental signals to instigate

adaptive physiological and behavioural adjustments – responses that are mediated by glucocorticoids (GCs) (Sapolsky et al., 2000). Glucocorticoids can be transmitted to offspring and influence their phenotype and development through transgenerational effects (Crino et al., 2023). In addition to the environments experienced by parents, offspring also experience potentially stressful environmental conditions that can interact with or amplify parental effects. For example, human disturbance alters snapping turtles' nest-site choice, ultimately impacting the temperature at which eggs are incubated (Kolbe & Janzen, 2002). Because animals' responses to abrupt changes in temperature are mediated by GCs (Crino et al., 2023), and this response can affect animals' decision-making while nesting, GCs transmission and early thermal environment are predicted to interact to shape offspring phenotype. Both GCs and temperature are known to exert potent effects on brain development (Amiel et al., 2017; Coomber et al., 1997; Jonson et al., 1976; Zhu et al., 2004) and cognition (Clark et al., 2014; Lui et al., 2017), but little is known about how they may interact to affect behavioural flexibility. Since behaving flexibly is essential for facing novel environments, studying the interactive effect of early environment on behavioural flexibility is crucial if we aim to understand animals' capability to adapt to environmental changes.

Here, we studied the effects of prenatal temperature and corticosterone (CORT) (the main GC in reptiles) (Crino et al., 2023), on behavioural flexibility in two species of reptiles, the delicate skink (*Lampropholis delicata*) and the common garden skink (*L. guichenoti*). Both species have similar life-histories and overlapping distributions (Chapple et al., 2011, 2014), but only *Lampropholis delicata* has been successful in colonising different areas around the globe (Baker, 1979; Chapple, Miller, et al., 2013; Chapple et al., 2015). The differences in invasion success between both skinks may be driven by disparities in behavioural flexibility - a prediction supported in other invasive species (Amiel et al., 2011; Chapple et al., 2012; Sol et al., 2008; Sol & Lefebvre, 2000; Wright et al., 2010). However, the relative impact of early environments on behavioural flexibility remains unexplored. Early environments are known to affect suites of morphological and behavioural traits differently in each species (Carrasco et al., 2023; Kar et al., 2023), but we are uncertain about the interactive role of maternal CORT and early temperatures on behavioural flexibility. We manipulated CORT and incubation temperature in both species and then subjected hatchlings to a reversal learning task. We predicted that both cold incubation temperatures and CORT treatment will impair behavioural flexibility (Clark et al., 2014; Lui et al., 2017), and the combination of both treatments to have the most detrimental effect on behavioural flexibility. Since invasive species are expected to behave more flexibly (Amiel et al., 2011; Chapple et al., 2012; Sol et al., 2008; Sol & Lefebvre, 2000; Wright et al., 2010), we predicted that *L. delicata*, on average, will perform more proficiently in the task compared to *L. guichenoti* and that behavioural flexibility in *L. delicata* will be more robust to early environmental conditions.

## Methods

### *Husbandry*

**Breeding colony** – We tested juveniles originating from a breeding colony established in the lab since 2019. A total of 270 and 180 adults of *L. delicata* and *L. guichenoti*, respectively, were established in containers (41.5 L x 30.5 W x 21 H cm) with six lizards (2 males and 4 females) per enclosure. Enclosures had non-stick matting, a shelter, and several small water dishes. Water was given daily, and they were fed approx. 40 mid-size crickets (*Acheta domestica*) per

enclosure three days a week. Crickets were dusted with calcium weekly and multivitamin and calcium biweekly. Using a heat chord and a heat lamp we created a temperature gradient and kept lights on a 12 h light:12 h dark cycle. UVA/UVB lighting was also provided. Room temperatures were set to 22-24°C, and warm side of enclosures reached 32°C.

*Eggs collection and incubation* – Between mid-October 2022 to the end of February 2023, we provided females with a place to lay eggs by means of small boxes (12.5 L x 8.3 W x 5 H cm) with moist vermiculite inside that were placed in one extreme of the communal enclosures (see above). We checked for the presence of eggs in the boxes three days a week. After collection, we measured the length and width of eggs with a digital caliper to the nearest 0.1 mm and weighed them with a digital scale  $\pm 0.001$ g error (OHAUS, Model spx123). We also recorded the clutch and egg identities. Eggs were then treated with CORT or vehicle (see CORT and Temperature manipulation below) and were placed in individual cups (80 mL) with moist vermiculite (12 parts water to 4 parts vermiculite). The cups were covered with cling wrap to retain moisture and left in LATWIT 2X5D-R1160 incubators at two different temperatures (see CORT and Temperature manipulation below) until hatching.

*Hatchlings* – Eggs in the incubator were checked three times a week for hatchlings. After hatchling, we measured juveniles' SVL and Tail Length (TL) with a ruler to the nearest mm and weighed them with a digital scale  $\pm 0.001$ g error. We then placed hatchlings in individual enclosures (18.7L x 13.2W x 6.3H cm) and provided them with non-stick matting and a small water dish. During this period, they were sprayed with water every day and received 3-6 small *A. domestica* crickets three times a week. All care otherwise followed similar protocols to adults (see above). Two weeks before starting the training phase (see below), lizards were moved to an experimental arena for acclimation. The arenas were individual medium size (41 L x 29.7 W x 22 H cm) plastic containers with a shelter (9 L x 6 W x 1.5 H cm) on one of the side and a water dish on the other. These new enclosures were placed in two rooms across 7 different racks associated to 7 different CCTV systems (device model DVR-HP210475) that allowed us to record their behaviour during the experiment (see details below). 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 acclimation and throughout the experiment, lizards were fed with only one cricket per day dusted with calcium and multivitamin (see protocol below), and water was supplied ad libitum. We provided a temperature gradient by means of a heat cord and heat lamps in a 12 h light: 12 h dark cycle. The temperature of both rooms was set to between 22-24°C.

### *Manipulating Early Thermal and CORT Environments*

To test empirically the effect of early environment we manipulated CORT concentration in eggs and incubated them under one of two temperature regimes (Cold – 23°C  $\pm$  3°C or Hot – 30°C  $\pm$  3°C) in a 2x2 factorial design (Fig. 1 A). We first allocated eggs into one of two different treatments: in the CORT treatment, eggs were topically supplied with 5 $\mu$ L of CORT dissolved in 100% Ethanol (vehicle) at a final concentration of 10 pg CORT/mL; in the Control treatment eggs received an equal volume of the vehicle. CORT concentration employed in the CORT treatment represents 2 standard deviations above the mean natural concentration obtained in eggs from both species (non-published data). Eggs were then 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 (Cheetham et al., 2011; Qualls &



Shine, 2000). The number of eggs per clutch assigned to each hormone and temperature treatment were counterbalanced in both species.

### *Quantifying Behavioural Flexibility*

The learning test was divided in three main phases: a habituation phase where lizards had to learn to eat a frozen-thawed cricket from white 3D-printed polylactic acid (PLA) ramps (9 L x 4 W x 5 H cm); a colour association task where lizards had to associate the food reward with one of two colours (red or blue) (Fig. 1 Associative task); and finally, to quantify behavioural flexibility, we reversed the previously trained colour (red or blue) so that the lizards needed to learn to associate the food with the opposite colour (Fig. 1 Reversal task).

The habituation phase was divided into three stages: in the first stage, the reward, a small, frozen cricket (*A. domestica*) was placed in an opaque petri dish (3D x 1.6H cm) (Fig. 1 Habituation phase, Stage 1); in the second stage, the petri dish with the cricket was placed on top of the white 3D printed ramps (Fig. 1 Habituation phase, Stage 2); and in the third stage, the cricket was left inside a well (3D x 1.75H cm) on top of the ramp (Fig. 1 Habituation phase, Stage 3). Trials in every stage lasted one hour, beginning when the feeding block (petri dish, ramp, or both) was placed inside the enclosure and finishing when we took the feeding block away. We recorded whether the cricket had been consumed or not and we considered a stage as completed if the lizards ate the crickets in at least 5 out of 6 trials. This phase lasted 38 days, and only in one case a lizard did not progress to the next phases because it did not pass the acquisition criteria.

In the colour association (Associative task in Fig. Fig. 1) and reversal task (Reversal task in Fig. 1 B), we used three ramps that were identical in shape and size but different in the colour. The colours of these ramps were green, red, and blue, as previous studies demonstrate that squamates can discriminate between these colours (Baden & Osorio, 2019). However, to control for potential colour biases, we assigned one group of lizards within each treatment to associate food in the blue ramp followed by the red ramp in the reversal, while in the other group the order was reversed. As in the last stage of the habituation phase, we placed the cricket inside the wells of the ramps, but then two of them were covered with 3D-printed lids (3D x 0.5H cm) so prey was only accessible in one ramp. The food reward was placed in all three ramps to avoid lizards using prey chemical cues that were released in the closed wells through a series of small holes on the top of the lids. In all trials, the position of the feeders was changed randomly to ensure subjects were using colour rather than spatial cues for the association. Lizards were tested in the association task once a day for 35 days and once a day for 40 days in the reversal.

The full experiment was performed between the 6<sup>th</sup> of March until the 26<sup>th</sup> of June 2023. Tests were made between 11 to 12 am, when the lizards were active. Trials in the learning phases (colour associative task and reversal tasks) were recorded with different CCTV systems always using the same camera per individual. We recorded whether the animal chose the correct ramp first or not. We considered that a choice was made if the head of the lizard was inside the well of one of the ramps. We considered a trial failed if there was no choice in one hour of recording. Those trials were scored as NA. Here, we analysed the data of the reversal task as a measure of behavioural flexibility (Brown & Tait, 2010).



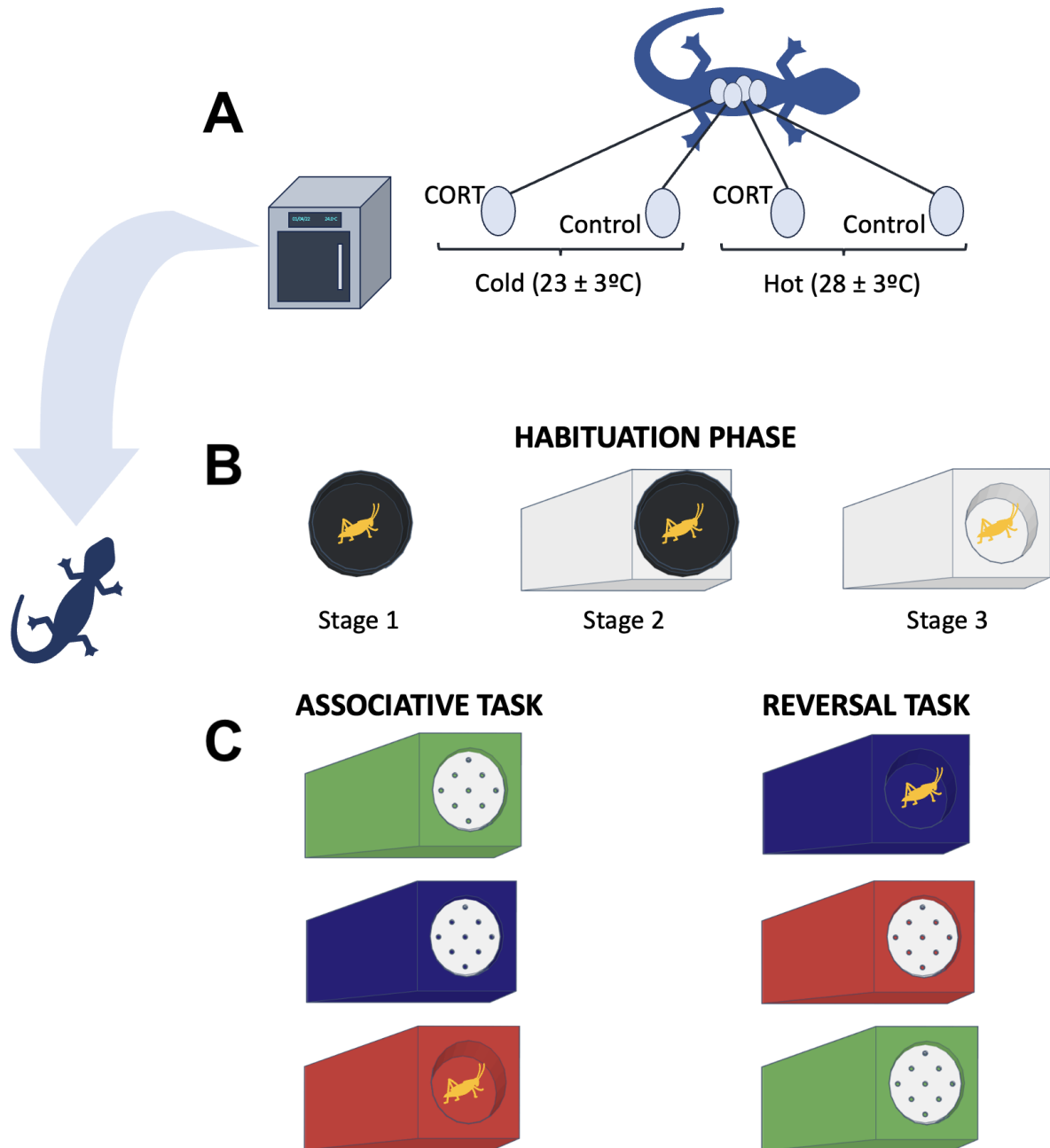


Fig 1— Experimental design of early environment manipulation and learning tasks. Panel A represents the early environment manipulation for both species. Panel B shows the habituation phase with the respective three different stages. And panel C represents the associative and reversal tasks; white lids show the ramps where the food reward was not accessible.

## 172 *Statistical analyses*

173 We performed the analyses for each species separately. We fitted two different Bayesian  
 174 multilevel models using the `brm` function from the *brms* package (Bürkner, 2017) using an R  
 175 extension (version 2.8.2) (R Core Team, 2021). We ran four parallel MCMC chains of 3000

iterations for each model, with a warmup period of 1000 iterations. We modelled correct choices [correct (1) or not (0)] as the response variable, and trial, hormone (CORT versus Control), incubation temperature (Cold versus Hot), and their interaction as fixed factors. We included a random intercept and slope (trial) for each lizard in our models. We also incorporated the clutch identity as a random factor. Learning can also be age-dependent (see Noble et al. (2014)), and given that lizards incubated at different temperatures hatch at different times we explored the effects of age on learning. Indeed, lizards' age at the beginning of the experiment ranged from 41 to 148 days old in *L. delicata* and 48 to 132 in *L. guichenoti*. However, when this variable was included in the models we did not find any significant effect of age (see Supplementary material). As such, we present models without age as a fixed effect. We used the posterior distributions of parameters from these models to test for differences in learning rate between treatments and species. Learning slopes were obtained using the 'trial' estimates and its interaction with hormone and temperature treatments. Slope estimates greater than zero were considered as evidence of learning, while those less or equal to zero were not. We calculated the probability that slopes or contrasts between slopes differed from zero ( $p_{\text{mcmc}}$ ) using the posterior distributions. We considered statistical significance if  $p_{\text{mcmc}} < 0.05$ .

#### *Ethical note*

We collected subjects from a breeding colony kept in the lab since 2019. Both the breeding animals and the ones used in this experiment were provided humane laboratory housing, with thermoregulatory opportunities, light (UV and heat) and moderate levels of humidity (see above for details). Lizards in the colony received food ad libitum while the subjects in the experiment were feed one cricket daily during the experiment. 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 until they were humanely euthanized following approved procedures.

## **Results**

We started with 96 lizards, 48 per species and 12 per treatment per species. However, our final sample size was 81 due to natural mortality ( $n = 11$ ), failure to complete the training stage ( $n = 1$ ), or because the number of failed tasks (see above) was higher than 15 ( $n = 3$ ). The final sample sizes per treatment and species are listed in Fig. 2.

Both species learnt across all treatments (Fig. 2 B, D), but we did not find any difference between treatments or species in the rate of learning. For *L. delicata*, hormone treatment (Control - CORT = 0.005,  $p_{\text{mcmc}} = 0.873$ ), incubation temperature (Hot - Cold = -0.001,  $p_{\text{mcmc}} = 0.989$ ), or the interaction  $[(\text{Control-Hot} - \text{CORT-Hot}) - (\text{Control-Cold} - \text{CORT-Cold})] = -0.034$ ,  $p_{\text{mcmc}} = 0.062$ ) did not affect learning (see Fig. 2 A, B). We similarly found no significant effects of hormone (Control - CORT = 0.005,  $p_{\text{mcmc}} = 0.810$ ), incubation temperature (Hot - Cold = 0.009,  $p_{\text{mcmc}} = 0.629$ ), or the interaction  $[(\text{Control-Hot} - \text{CORT-Hot}) - (\text{Control-Cold} - \text{CORT-Cold})] = -0.016$ ,  $p_{\text{mcmc}} = 0.529$ ) on learning in *L. guichenoti* (see Fig. 2 C, D).

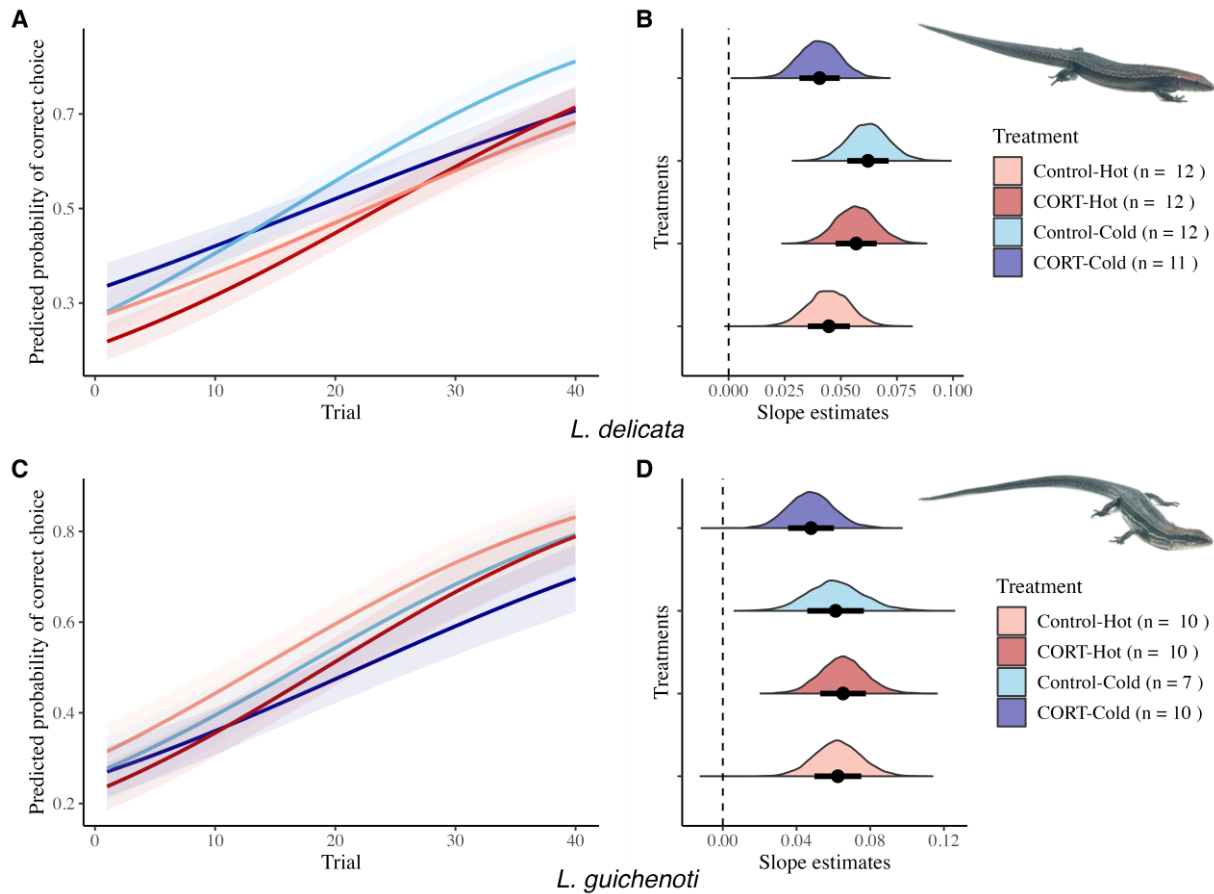


Fig 2— Results for *L. delicata* (A,B) and *L. guichenoti* (C, D). Panels A and C show the predicted probability of choosing the correct feeder first over trials. The lines represent the mean predicted probability of choosing the correct feeder first on each trial, and the shaded areas indicate the standard deviation of the mean; both were obtained by using the slope and intercept estimates from the posterior distributions. The different colours indicate the different treatments. Panels B and D show the distribution of the estimates of slopes per each treatment. The x-axis represents the slope estimate, and in the y-axis are 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.

Finally, when all individuals from all the treatments were pooled together and species were compared, there were not significant differences in the estimated slopes (mean of learning slope in *L. delicata* = 0.051, 95% CI = [0.027, 0.075]; mean learning of slope in *L. guichenoti* = 0.059, 95% CI = [0.030, 0.088]; *L. delicata* - *L. guichenoti* = -0.008,  $p_{\text{mcmc}} = 0.636$ ).

## Discussion

This study aimed to determine whether prenatal temperature and GCs impact behavioural flexibility in two closely related species, the delicate skink (*Lampropholis delicata*) and the common garden skink (*L. guichenoti*). We expected early environment to influence behavioural flexibility in both species. We also predicted that *L. delicata* would be more flexible than *L. guichenoti* and less affected by the early environment. Nevertheless, we did not find significant

differences between treatments or species when we compared their performance in a reversal task. Our results show that, on average, all the lizards from the four treatments and the two species learnt the reversal task - indicated by estimated slopes that are significantly different from zero - and that they learn, on average, at the same rate. Furthermore, these results were consistent when only those individuals who performed better in the previous associative task were included (see Supplementary material).

Behavioural flexibility appears to be robust to early exposure to maternal GCs in both *L. delicata* and *L. guichenoti*. We predicted that prenatal CORT treatment would decrease reversal learning abilities. As such, our results contrast to previous studies that show high GCs levels impaired performance in different types of learning tests (Farrell et al., 2016; Lemaire et al., 2000; Zhu et al., 2004). Our results are, however, consistent with other experiments where GCs did not impact learning abilities (Bebus et al., 2016; Szuran et al., 1994). One possible reason for why GCs did not affect learning in our study may be because GCs can have hormetic effects on brain structure and learning. For example, in Sprague–Dawley rats, exposure to high doses of CORT, but not low doses, reduced the density of immature neurons and cell proliferation in the dentate gyrus (Brummelte & Galea, 2010). It is possible such dose-dependent effects exist and our concentrations failed to elicit changes to the brain that would result in impaired behavioural flexibility. We find this possibility unlikely since, in a previous experiment, we observed that a similar dose affected a multitude of other traits including growth and mortality in *L. delicata* (unpublished data). Nonetheless, we cannot dismiss this possibility because dose effects can differ between tissues (Lemaire et al., 2000). Conversely, the effects of GCs early in development may vary depending on the cognitive task, as evidenced by studies showing variations in the impact of prenatal GCs on different brain regions (Lemaire et al., 2000). Prenatal GCs can have programmatic effects on HPA axis sensitivity, potentially impacting the lizards' sensitivity to stressors (Crino et al., 2014). This altered stress response may influence learning dynamics through hormetic effects of CORT (Du et al., 2009), particularly in stressful contexts of which our experiment was not (Taborsky et al., 2021). These hypotheses are not mutually exclusive and could be tested in future experiments using multiple doses of prenatal CORT treatment, assessing learning using multiple cognitive tasks, and testing the effect of stressors during the learning process.

More striking is the absence of an effect of incubation temperature on behavioural flexibility because most studies have reported better performance in different associative learning and motor tasks when incubated at higher temperatures (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014). Studies that have reported cold-incubated lizards to learning faster (Abayarathna & Webb, 2020; Dayananda & Webb, 2017) used incubation temperatures far beyond the natural range in their hot treatments and it is unclear how such conditions relate to wild environments. Our results suggest that behavioural flexibility is robust to temperature in both species of skinks - at least in this study. It could be argued that the differences in our incubation temperatures were not extreme enough to elicit significant differences in learning performance. However, the mean difference between the temperatures used in our experiment were similar to those in previous studies (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014), and are aligned with natural variation at the extremes of incubation conditions in the wild (Cheetham et al., 2011; Qualls & Shine, 2000). In lizards, impacts on learning from early thermal environments are associated with changes in neural density (Amiel et al., 2017). However, several studies have shown that the effects of temperature on brain structure and physiology are

region dependent (Amiel et al., 2017; Coomber et al., 1997; Sakata et al., 2000). It is possible that the temperatures used in our experiment did not affect the brain regions involved in coding reversal learning. Future studies should explore the effects of thermal environment on brain structure and function in these species to understand the mechanisms underlying the effects of temperature on learning. Furthermore, testing learning across different cognitive tasks could provide a more comprehensive understanding of the influence of temperature on cognition. In our analyses, sex was not included as a factor because lizards were not considered sexually mature, and because previous studies have found weak evidence for sex-dependent learning in many cognitive domains (Szabo et al., 2019). Indeed, the impacts of temperature on learning was not sex-dependent for the Eastern three-lined skink (*Bassiana dupeyerrii*) (Amiel & Shine, 2012; Clark et al., 2014). However, some studies find evidence for interactive effects of incubation temperature and sex on brain physiology in other species of reptiles (Coomber et al., 1997; Sakata et al., 2000). In addition, *B. dupeyerrii* are known to sex-reverse - some individuals can have female chromosomes but male phenotypes - under cold temperatures (Dissanayake et al., 2021). Such effects were not easily accounted for in these experiments making it challenging to understand if sex or temperature were the major drivers of learning differences (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014). Future studies should explore the possible interactive effects of sex and incubation temperature on cognitive performance. However, such studies will be challenging given the large sample sizes required to execute these studies rigorously.

Despite sharing life-history traits, only *L. delicata* has proven successful in colonising various global regions (Baker, 1979; Chapple, Miller, et al., 2013; Chapple et al., 2015), and we predicted this may relate to higher behavioural flexibility. Furthermore, we expected behavioural flexibility in this species to be less susceptible to the effect of early environment since this trait can be adaptive in several stages of invasion involving more than one generation. While it will be important to explore replicate invasion events (both successful and unsuccessful) such information is seldom available. Nonetheless, there is precedence in the literature to expect differences between these two species (Chapple et al., 2011; Chapple, Whitaker, et al., 2013). Regardless, while we did not find differences in reversal learning between *L. delicata* and *L. guichenoti*, previous studies have shown that *L. delicata* more readily explores novel environments than *L. guichenoti*, which can be advantageous during colonisation (Chapple et al., 2011). However, Bezzina et al. (2014) found no differences in learning between the two skinks in an associative learning task. Our results align with the latter study and suggest that behavioural flexibility does not differ between these species either. Our results along with others' (Bezzina et al., 2014) suggest that other aspects of their biology are more relevant for *L. delicata*'s success as invaders than learning and behavioural flexibility. Differences in exploratory behaviour is likely to be the key factor explaining why *L. delicata* and not *L. guichenoti* is better at colonising new areas (Chapple et al., 2011).

In conclusion, our results indicate that lizards performed the reversal learning task, a measure of behavioural flexibility, at similar rates, with no discernible differences between treatments or species. These findings contrast with our initial predictions and suggest that behavioural flexibility in both species is robust to insults (at least for temperature and GCs) during early stages of development. Furthermore, our results indicate that other aspects of the biology of *L. delicata* may be more influential for their success as invaders than behavioural flexibility alone. Nevertheless, future studies should investigate the potential influence of prenatal CORT and

temperature on cognition, considering other factors such as the type of cognitive task. It is also imperative to explore the effects of these treatments on brain function comprehensively. Additionally, further research is essential to determine which aspects of *L. delicata*'s biology contribute more significantly to their success as invaders than behavioral flexibility alone. Investigating these areas will provide a better understanding of the cognitive and biological mechanisms underlying the adaptability and invasive potential of both species.

## **Data accessibility**

All data, data description, and R code are available in public repository [https://github.com/Pablo-Recio/CORT\\_Temp\\_Behavioural\\_flexibility](https://github.com/Pablo-Recio/CORT_Temp_Behavioural_flexibility)

## **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**

P.R.: conceptualization, methodology, data collection, data curation, formal analysis, writing—original draft, writing—review and editing; D.C.L.: data collection, writing—review and editing; O.C.: conceptualization, methodology, writing—review and editing; K.H.W.: conceptualization, writing—review and editing; C.F.: conceptualization, methodology, funding acquisition, writing—review and editing; B.M.: data collection, writing—review and editing; A.Y.P.: data collection, writing—review and editing; D.N.: 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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Supplementary Material

Checking the models plots

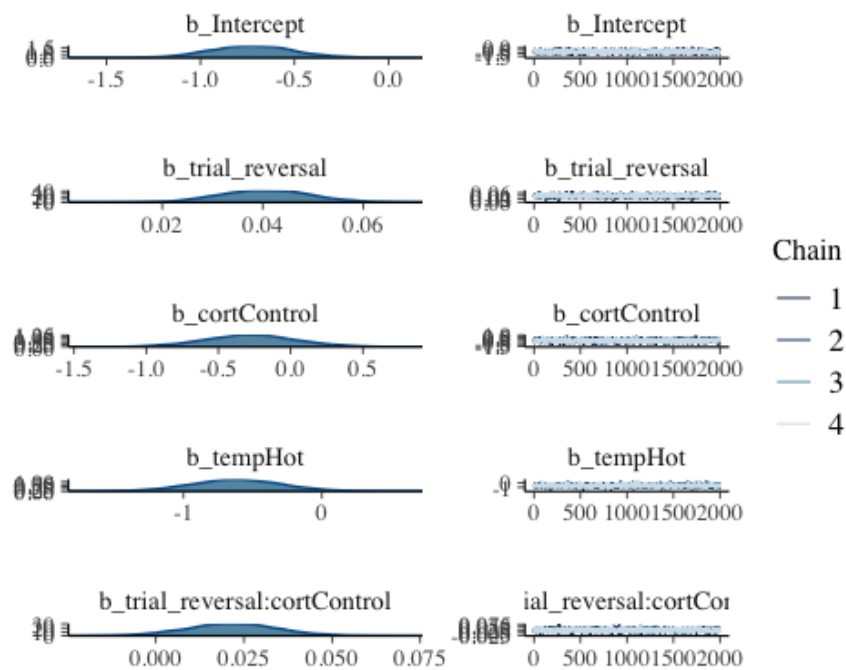
Model formula for the reversal task is:

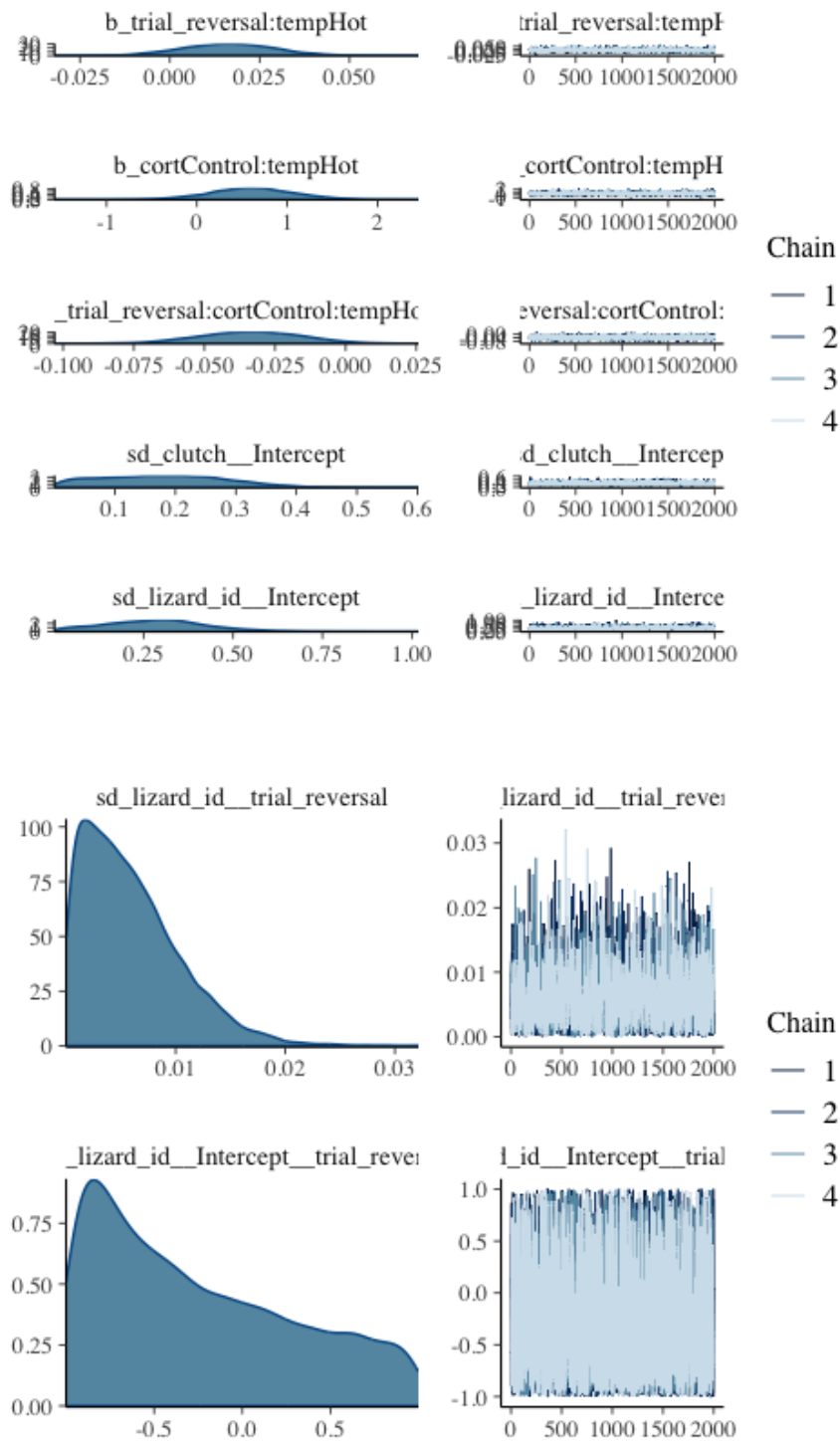
Choice ~ trial\_reversal $\text{corttemp}$  + (1 + trial\_reversal|lizard\_id)

Plots for the different models of the associative task:

1.- *L. delicata*

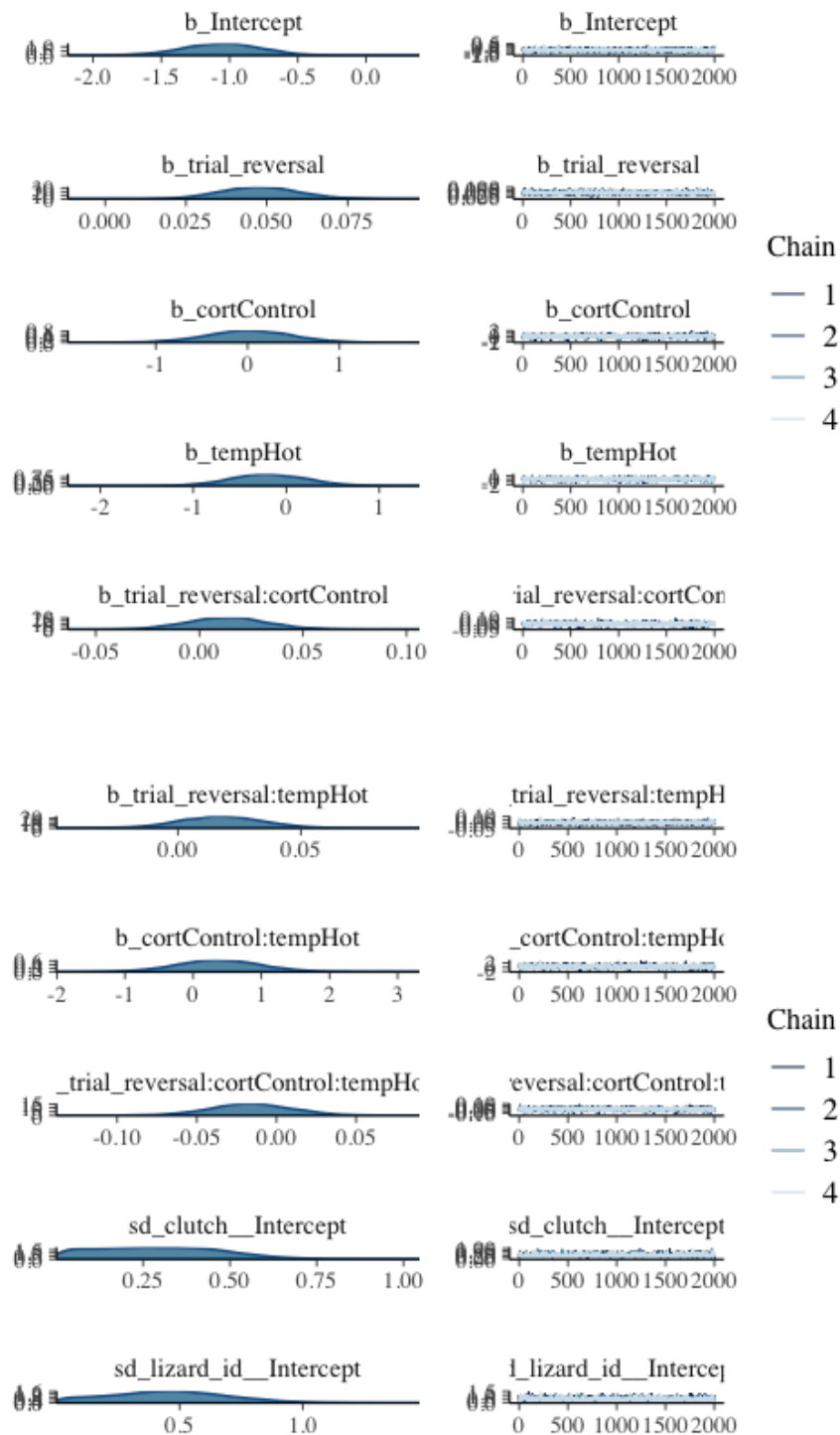
	Estimate	Est.Error	Q2.5	Q97.5
R2	0.1040341	0.01243751	0.07984544	0.1290352

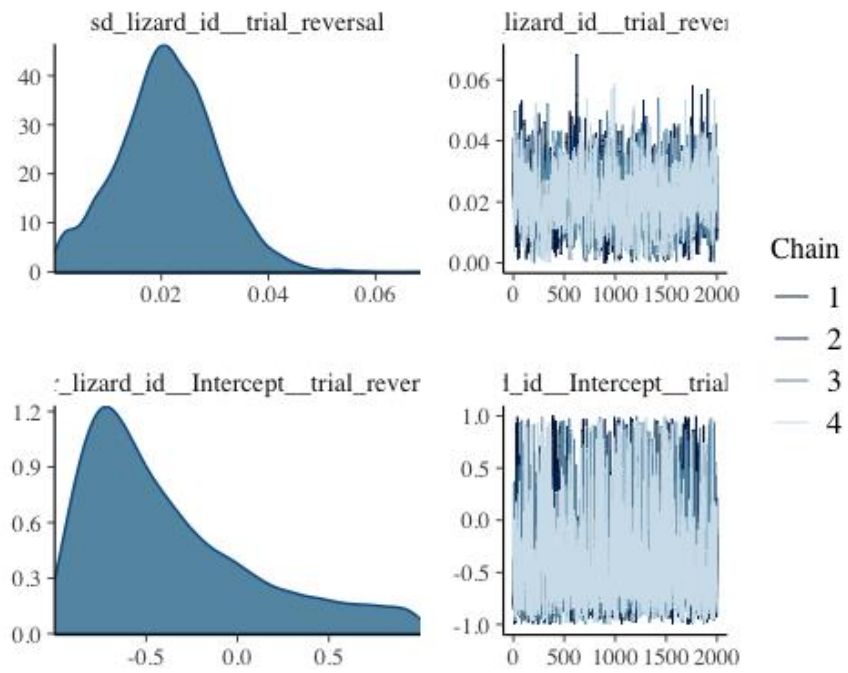




498 2.- *L. guichenoti*

499	Estimate	Est.Error	Q2.5	Q97.5
500	R2	0.1464713	0.0153245	0.1158186 0.1763292







502 Table 1. Estimates of Reversal learning slope for all the different treatments per each task,  
 503 species, and group. Mean shows the arithmetic means of the estimates obtained from the  
 504 posteriors of the model, and 95% CI indicates the 95% confidence interval of the mean. All  
 505 pmcmc tested the hypothesis that the mean equals zero. In bold, those values that are significant  
 506 (pmcmc <0.05)

Specie	Treatment	Mean	95% CI	pmcmc
<i>L. delicata</i> N = 47 Obs = 1880	CORT-Cold (n = 11)	<b>0.041</b>	<b>0.023 , 0.058</b>	<b>&lt; 0.001</b>
	Control-Cold (n = 12)	<b>0.062</b>	<b>0.044 , 0.081</b>	<b>&lt; 0.001</b>
	CORT-Hot (n = 12)	<b>0.057</b>	<b>0.039 , 0.075</b>	<b>&lt; 0.001</b>
	Control-Hot (n = 12)	<b>0.045</b>	<b>0.026 , 0.063</b>	<b>&lt; 0.001</b>
<i>L. guichenoti</i> N = 37 Obs = 1480	CORT-Cold (n = 10)	<b>0.048</b>	<b>0.024 , 0.073</b>	<b>&lt; 0.001</b>
	Control-Cold (n = 7)	<b>0.061</b>	<b>0.032 , 0.093</b>	<b>&lt; 0.001</b>
	CORT-Hot (n = 10)	<b>0.065</b>	<b>0.041 , 0.090</b>	<b>&lt; 0.001</b>
	Control-Hot (n = 10)	<b>0.062</b>	<b>0.038 , 0.088</b>	<b>&lt; 0.001</b>

508 Table 2. Estimates of Reversal learning slope for all the different treatments per each task,  
 509 species, and group. Here we included only those individuals who made the right choice in 8 out  
 510 of the last 10 trials in the previous associative task. Mean shows the arithmetic means of the  
 511 estimates obtained from the posteriors of the model, and 95% CI indicates the 95% confidence  
 512 interval of the mean. All pmcmc tested the hypothesis that the mean equals zero. In bold, those  
 513 values that are significant (pmcmc <0.05)

Specie	Treatment	Mean	95% CI	pmcmc
<i>L. delicata</i>	CORT-Cold (n = 4)	<b>0.052</b>	<b>0.021 , 0.084</b>	<b>&lt; 0.05</b>
	N = 19			
	Control-Cold (n = 5)	<b>0.067</b>	<b>0.038 , 0.096</b>	<b>&lt; 0.001</b>
	Obs = 760			
	CORT-Hot (n = 3)	<b>0.073</b>	<b>0.036 , 0.113</b>	<b>&lt; 0.001</b>
	Control-Hot (n = 7)	<b>0.046</b>	<b>0.023 , 0.070</b>	<b>&lt; 0.001</b>
<i>L. guichenoti</i>	CORT-Cold (n = 3)	<b>0.068</b>	<b>0.012 , 0.124</b>	<b>&lt; 0.05</b>
	N = 18			
	Control-Cold (n = 3)	<b>0.086</b>	<b>0.032 , 0.147</b>	<b>&lt; 0.05</b>
	Obs = 720			
	CORT-Hot (n = 5)	<b>0.072</b>	<b>0.030 , 0.115</b>	<b>&lt; 0.05</b>
	Control-Hot (n = 7)	<b>0.066</b>	<b>0.031 , 0.104</b>	<b>&lt; 0.001</b>

515 Checking the effect of age on the learning rate in the associative task.

516 Model formula: Choice ~ age.start + trial\_associative \* cort \* temp + (1 +  
517 trial\_associative|lizard\_id) + (1|clutch)

518 Table 3. Results for *L. delicata* when the model included the age:

Predictors	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
Intercept	-0.68	0.23	-1.14	-0.22	1.00	3099.14	5364.68
age.start	0.00	0.00	-0.00	0.01	1.00	7524.39	6689.19
trial_rever sal	0.04	0.01	0.02	0.06	1.00	3033.03	5261.65
cortContro l	-0.34	0.32	-0.99	0.30	1.00	2845.28	4978.49
tempHot	-0.69	0.33	-1.33	-0.05	1.00	2868.81	4595.09
trial_rever sal:cortCo ntrol	0.02	0.01	-0.00	0.05	1.00	2939.03	4497.28
trial_rever sal:tempH ot	0.02	0.01	-0.01	0.04	1.00	2759.31	4331.14
cortContro l:tempHot	0.64	0.46	-0.24	1.56	1.00	2786.33	4315.03
trial_rever sal:cortCo ntrol:temp Hot	-0.03	0.02	-0.07	0.00	1.00	2849.12	4135.58

519

520 Table 4. Results for *L. guichenoti* when the model included the age:

Predictors	Estimate	Est.Error	l-95% CI	u-95% CI	Rhat	Bulk_ESS	Tail_ESS
Intercept	-0.94	0.32	-1.59	-0.32	1.00	4613.34	5435.92
age.start	0.01	0.01	-0.01	0.02	1.00	7490.80	6179.03
trial_rever sal	0.05	0.01	0.02	0.07	1.00	4170.12	4876.09
cortContro l	-0.01	0.46	-0.92	0.92	1.00	4008.70	4602.77
tempHot	-0.33	0.45	-1.19	0.57	1.00	4124.09	4611.95
trial_rever sal:cortCo ntrol	0.01	0.02	-0.02	0.05	1.00	3933.61	4885.22
trial_rever sal:tempH ot	0.02	0.02	-0.02	0.05	1.00	4123.86	4555.44
cortContro l:tempHot	0.35	0.63	-0.93	1.58	1.00	3791.58	4842.61
trial_rever sal:cortCo ntrol:temp Hot	-0.02	0.03	-0.07	0.03	1.00	3623.25	4622.64

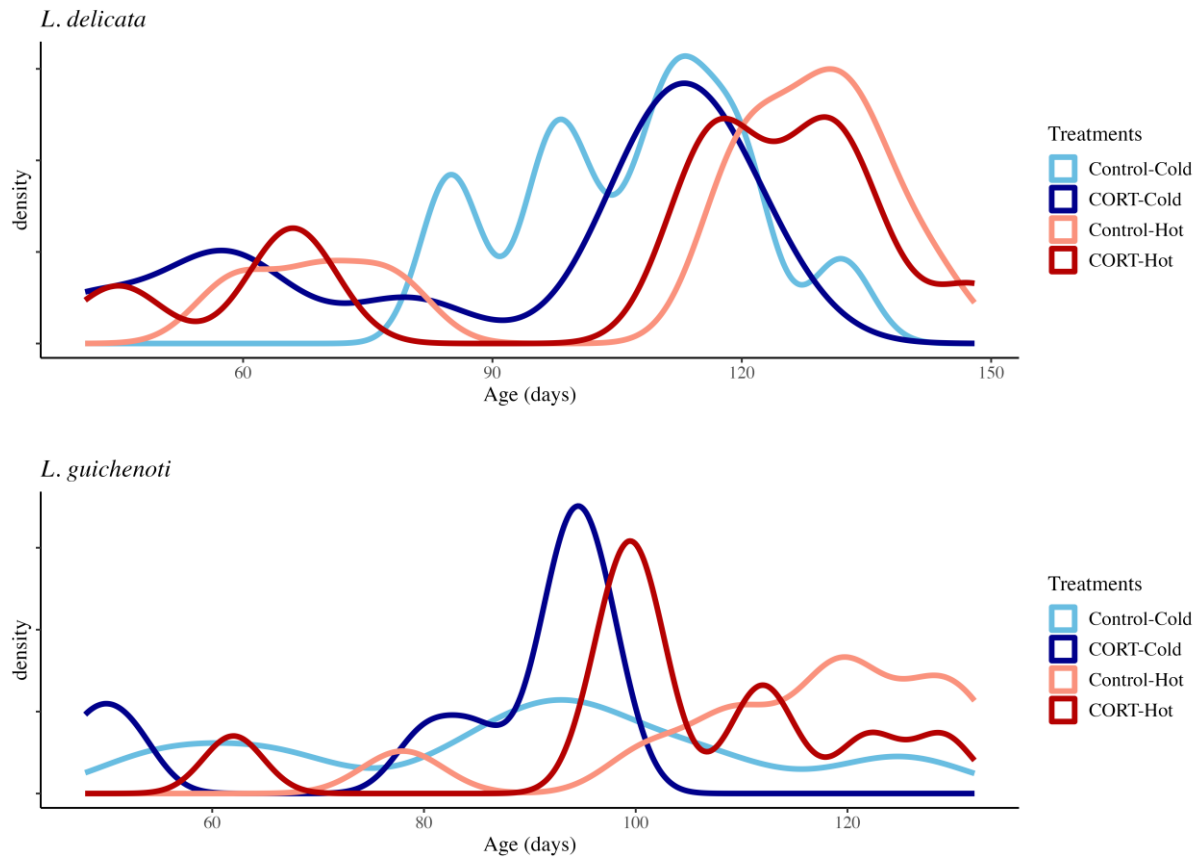


Fig 3— Distribution of the age of the lizards by treatment and species