Early environmental conditions do not impact associative learning in two lizard species

Pablo Recio1, , Dalton C. Leibold1, Ondi L. Crino 1,2, Kristoffer H. Wild1,3, Chris Friesen4, Basile Mauclaire1,5, Amelia Y. Peardon 1, Daniel W.A. Noble1

1 Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia  
2 Flinder’s University, College of Science and Engineering, Bedford Park, SA 5042, Australia  
3 Melbourne University, Melbourne, VIC 3000, Australia  
4 University of Wollongong, Wollongong, NSW 2500, Australia  
5 Université de Lille, Lille 59000, France  
 Corresponding author: pablo.reciosantiago@anu.edu.au

ORCID:

Pablo Recio ORCID: 0000-0002-5890-0218  
Dalton C. Leibold ORCID: 0000-0001-9645-2033  
Ondi L. Crino ORCID: 0000-0001-5700-1387  
Kristoffer H. Wild ORCID: 0000-0001-6714-3311  
Chris Friesen ORCID: 0000-0001-5338-7454  
Basile Mauclaire ORCID:  
Amelia Y. Peardon ORCID: 0009-0005-6227-8379  
Daniel W.A. Noble ORCID: 0000-0001-9460-8743

## Abstract

Animals must acquire new information through learning to adjust their behaviour adaptively. However, learning ability can be constrained by the conditions experienced during early stages of development, when the brain is especially susceptible to environmental inputs. Temperature is a pervasive mechanism of phenotypic plasticity in ectotherms, exerting direct effects on growth, metabolism, or learning. In vertebrates, abrupt thermal changes can trigger a chronic stress, which is mediated by glucocorticoids that can be transmitted to the offspring, potentially impacting their learning abilities. Glucocorticoids and the thermal environments are therefore predicted to have interactive effects on the development of learning functions in animals. Here, we investigated the combined effects of corticosterone (CORT) - the main GC in reptiles - and elevated temperature during development on learning in juveniles of two species of lizards, *Lampropholis delicata* and *L. guichenoti*. We manipulated prenatal CORT levels and incubation temperature in a 2x2 factorial design, and then subjected juveniles to a colour-associative learning task. We predicted that elevated CORT and low temperatures would impair associative learning. **However, both species learned the task at the same rate independently of treatment. This suggests these two species may have evolved mechanisms to maintain learning abilities despite prenatal challegnes. We also found that lizards did not learn when food rewards were associated with blue-coloured ramps, underscoring the need to carefully select the color used in cognitive tests involving visual stimuli.**

## Introduction

Cognition is a set of processes by which animals gather, preserve, and use information from their environment through perception, learning, memory, and decision making ([Shettleworth 2010](#ref-shettleworth)). These processes are fundamental to foraging, mate selection, antipredatory strategies, and social behaviours, all of which are crucial for survival and reproduction ([Dukas 2004](#ref-dukas_evolutionary_2004)). Particularly, learning - the acquisition of new information ([Dukas 2004](#ref-dukas_evolutionary_2004)) - is essential for coping with environmental changes by enabling individuals to create new associations between events ([Dukas 2004](#ref-dukas_evolutionary_2004); [Leal and Powell 2012](#ref-leal_behavioural_2012); [Buchanan et al. 2013](#ref-buchanan_condition_2013)). However, the capacity to acquire information varies among individuals, shaped by factors such as age, sex, and the developmental environment ([Szuran et al. 1994](#ref-szuran_water_1994); [Lemaire et al. 2000](#ref-lemaire_prenatal_2000); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Amiel and Shine 2012](#ref-amiel_hotter_2012); [Amiel et al. 2014](#ref-amiel_egg_2014); [Carazo et al. 2014](#ref-carazo_sex_2014); [Noble et al. 2014](#ref-noble_age-dependent_2014)). These variations can further affect population dynamics, as differences in learning ability may lead to unequal responses to environmental pressures ([Ward-Fear et al. 2016](#ref-ward2016ecological); [Welklin et al. 2024](#ref-welklin2024spatial)). For example, fast learners may better exploit novel resources or avoid new dangerous stimuli, while those with lower learning capabilities might struggle to adapt to environmental changes. This could affect their survival and reproductive output, ultimately influencing population growth rates and stability ([Ward-Fear et al. 2016](#ref-ward2016ecological); [Welklin et al. 2024](#ref-welklin2024spatial)). Therefore, understanding the factors that shape learning is crucial to predict how populations will respond to novel circumstances.

Developmental conditions are especially relevant in shaping learning abilities because the brain is highly susceptible to environmental inputs during early stages of life ([Zhu et al. 2004](#ref-zhu_prenatal_2004)). Impacts on brain development can have long-lasting effects on cognition, potentially influencing an individual’s ability to learn and adapt to new environments ([Lemaire et al. 2000](#ref-lemaire_prenatal_2000); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Amiel and Shine 2012](#ref-amiel_hotter_2012); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020)). For example, the state of the mother can influence offspring phenotype beyond genetic transmission through nest-site selection, provisioning, or the transmission of hormones or nutrients (i.e., ‘maternal effects’ [Moore et al. 2019](#ref-moore_mothers_2019)); and the developing brain is not exempt of these effects.

Glucocorticoids (GCs) are a class of steroid hormone that are particularly relevant in this context. In vertebrates, GCs regulate metabolism and maintain homeostasis in response to disturbances (i.e., ‘the stress response’ [Sapolsky et al. 2000](#ref-sapolsky_how_2000)). Under stressful situations, animals react by instigating adaptive physiological and behavioural adjustments mediated by GCs. These GCs can be transmitted directly from the parents to their offspring with different effects on offspring phenotype ([Crino et al. 2023](#ref-Crino_2023)). Elevations in GCs during early stages of development typically result in altered neurogenesis, brain structure, and metabolic activity that in most cases are related to decreased learning abilities ([Lemaire et al. 2000](#ref-lemaire_prenatal_2000); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Du et al. 2009](#ref-du_dynamic_2009); [Eaton et al. 2015](#ref-eaton2015mild); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn), [2016](#ref-farrell_developmental_2016)). For instance, prenatal stress in rats (*Rattus norvegicus*) induced reduction of neurogenesis in the dentate gyrus associated to impairments in hippocampal-related spatial tasks ([Lemaire et al. 2000](#ref-lemaire_prenatal_2000)). However, factors like sex or the nature of the learning task can affect the direction and magnitude of the effects of prenatal exposure to elevated GCs ([Szuran et al. 1994](#ref-szuran_water_1994); [Crino et al. 2014](#ref-crino_corticosterone_2014-learn); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn), [2016](#ref-farrell_developmental_2016); [Bebus et al. 2016](#ref-bebus_associative_2016)). Because the effects of prenatal GCs on learning can be context-dependent, it is crutial to conduct studies across diverse taxa and experimental conditions to fully understand these effects.

In addition to the environments experienced by parents, offspring also experience potentially stressful environmental conditions that can interact with or amplify parental effects. In ectotherms, early thermal environment is a pervasive mechanism of phenotypic plasticity, influencing a broad spectrum of traits including growth, metabolism, or learning abilities ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Amiel et al. 2014](#ref-amiel_egg_2014); [Dayananda and Webb 2017](#ref-dayananda_incubation_2017); [Noble et al. 2018](#ref-noble_developmental_2018); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020)). For instance, Port Jackson sharks (*Heterodontus portusjacksoni*) incubated at 23.6ºC took fewer days to reach a learning criterion in a numerical learning task than those incubated at 20.6ºC ([Vila Pouca et al. 2019](#ref-vila_pouca_quantity_2019)). In skinks, high incubation temperatures are generally associated with faster learning rates ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014)); but velvet geckos (*Amalosia lesueurii*) incubated at temperatures beyond their natural thermal range are worse learners than those incubated at colder temperatures ([Dayananda and Webb 2017](#ref-dayananda_incubation_2017); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020)). The effects of incubation temperature in reptiles are likely mediated by changes in neural structure and function, as high temperatures increase neural density and metabolic activity in the brain ([Coomber et al. 1997](#ref-coomber_independent_1997); [Sakata et al. 2000](#ref-sakata_neural_2000); [Amiel et al. 2017](#ref-amiel_effects_2017); [Beltrán et al. 2021](#ref-beltran_are_2021)).

GCs can play a pivotal role in determining vertebrate responses to elevated temperatures ([Crino et al. 2023](#ref-Crino_2023)) potentially fostering natural interactions between temperature and GCs. Additionally, GCs and temperatures can act upon similar physiological mechanisms ([Coomber et al. 1997](#ref-coomber_independent_1997); [Sakata et al. 2000](#ref-sakata_neural_2000); [Lemaire et al. 2000](#ref-lemaire_prenatal_2000); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Du et al. 2009](#ref-du_dynamic_2009); [Amiel et al. 2017](#ref-amiel_effects_2017); [Beltrán et al. 2021](#ref-beltran_are_2021)). This implies that the effects of GCs and temperature could be interdependent, with the impact of one factor being modulated by the other. However, the interactive effects of GCs and temperature on learning abilities remain unexplored.

Here, we investigated the combined effects of prenatal corticosterone (CORT) - the main GC in reptiles ([Crino et al. 2023](#ref-Crino_2023)) - and incubation temperature on learning in two species of skinks, the delicate skink (*Lampropholis delicata*) and the common garden skink (*L. guichenoti*). We manipulated CORT levels in the eggs and then incubated them at two different temperatures in a 2X2 factorial design. Post-incubation, juveniles were subjected to a colour-associative task to assess their learning abilities. We hypothesized that prenatal CORT levels and thermal environment will induce sustained effects on brain’s physiology, ultimately impacting learning of the association task. We predicted that individuals exposed to high levels of CORT or low temperatures will learn slower than control individuals or those incubated at higher temperatures ([Zhu et al. 2004](#ref-zhu_prenatal_2004); [Amiel and Shine 2012](#ref-amiel_hotter_2012); [Eaton et al. 2015](#ref-eaton2015mild); [Amiel et al. 2017](#ref-amiel_effects_2017)). Additionally, we predicted that incubation at high temperatures will mitigate the impact of CORT on skinks’ performance, while cold incubation temperatures were expected to enhance the effects of CORT. This may occur for two reasons that are not mutually exclusive: first, an increase in temperature leads to an overall rise in neural density ([Amiel et al. 2017](#ref-amiel_effects_2017)), thereby counteracting the impact of CORT (see [Lemaire et al. 2000](#ref-lemaire_prenatal_2000); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Eaton et al. 2015](#ref-eaton2015mild)); and second, the elevated metabolic rate associated with higher temperatures can accelerate CORT metabolism, resulting in embryos being exposed to CORT for a shorter time.

## Methods

#### Subjects

\_Lampropholis delicata and *L. guichenoti* are small [∼35-55 mm snout-vent length (SVL)], oviparous, and generalist skinks that usually share the same habitat in suburban areas throughout south-eastern Australia ([Chapple et al. 2011](#ref-chapple_know_2011)). Both species have similar breeding periods, but with some differences in reproductive output: while *L. delicata* lays 1-6 eggs in only one clutch per season, *L. guichenoti* clutches are smaller (1-5 eggs per clutch) and they usually lay two clutches per season ([Joss and Minard 1985](#ref-joss1985reproductive); [Chapple et al. 2011](#ref-chapple_know_2011), [2014](#ref-chapple_biology_2014)). Previous studies exploring behavioral differences between the two species have found *L. delicata* to be more exploratory than *L. guichenoti* ([Chapple et al. 2011](#ref-chapple_know_2011)). However, no differences in learning were observed between the skinks in an associative learning task ([Bezzina et al. 2014](#ref-bezzina2014does)).

#### Husbandry

*Breeding colony* – We tested juveniles from a 2019-established lab breeding colony. A a total of 270 and 180 adults of *L. delicata* and *L. guichenoti* respectively, were housed in plastic containers (41.5 L x 30.5 W x 21 H cm) with six lizards (2 males and 4 females) per enclosure. Enclosures were lined with non-stick matting, shelter, and several small water dishes. Water is given daily, and they were fed approximately 40 mid-size crickets (*Acheta domestica*) per enclosure three days a week. Crickets were dusted with calcium weekly and multivitamin and calcium biweekly. To ensure a temperature gradient, we used a heat chord and a heat lamp following a 12 h light:12 h dark cycle. Room temperatures were set to 22-24 ºC, and the warm side of enclosures was usually at 32-34 ºC.

*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) containing moist vermiculite. These boxes were placed in one side of the communal enclosures (see above). We checked for the presence of eggs in the boxes three days a week (Monday, Wednesday, and Friday). After collection, we measured length and width of eggs with a digital caliper to the nearest 0.1 mm, and mass using a digital scale (OHAUS, Model spx123; ± 0.001g error). Then we assigned individual IDs to the clutch and each egg. Eggs were 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. Eggs were then checked three times a week for hatchlings.

*Hatchlings* – After hatching, we measured juveniles’ SVL and Tail Length (TL) with a ruler to the nearest mm, and mass using a digital scale (OHAUS, Model spx123; ± 0.001g error). Hatchlings were then housed individually in small enclosures (18.7L x 13.2W x 6.3H cm) provided with non-stick matting, shelter, and a water dish. All care otherwise follows similar protocols to adults (see above).

Two weeks prior to the training phase (see details below), lizards were moved to the experimental arena for acclimatisation. 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 sides and a water dish on the other. These arenas were placed in two rooms in 7 different racks that were monitored by 7 different CCTV systems (device model DVR-HP210475) that allowed us to record their behaviour during the experiment (see details below). Although the conditions in the experimental rooms were identical to the colony room, the number of lizards per species and treatment in each rack was counterbalanced to control for any potential effect of the room or the position of the lizard in the rack. During acclimatisation and throughout the experiment, lizards were fed with only one cricket per day dusted with calcium and multivitamin, 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.

#### CORT and Temperature Manipulation

To empirically test the effect of early environment we manipulated CORT concentration in eggs and incubated them under one of two temperature regimes (‘Cold’ - 23ºC ± 3ºC or ‘Hot’ - 28ºC ± 3ºC) in a 2x2 factorial design ([Fig. 1](#fig-Methods) A). We topically dosed eggs with a CORT solution (10pg/mL) or a control treatment (100% ethanol). Corticosterone treatments were made by dissolving crystalline corticosterone (Sigma, Cat. No. C2505) in 100% ethanol. To dose eggs, we applied 5µl of solutions to eggshells using a micropipette. We selected these doses based on previous studies publishing yolk CORT concentrations in other oviparous reptiles ([Lovern and Adams 2008](#ref-lovern2008effects); [Hanover et al. 2019](#ref-hanover2019corticosterone)), while also validating that it fell within the range of CORT concentrations in eggs within our population ([Crino et al. 2024](#ref-crino2024eggs)). CORT treatment increased mean yolk CORT levels ~3.7x higher than control eggs ([Crino et al. 2024](#ref-crino2024eggs)). After the treatment, the eggs were incubated in one of the two previously mentioned temperature regimes (‘Cold’ or ‘Hot’) until hatching. The number of eggs per clutch assigned to each hormone and temperature treatment were counterbalanced in both species.

#### Learning

To estimate learning skills, we tested each lizard’s ability to locate a food reward in a colour-associative learning task ([Fig. 1](#fig-Methods) C, D). First, we performed a training phase where lizards had to learn to eat from white 3D-printed PLA ramps (9 L x 4 W x 5 H cm) identical in size and shape to the ones used during the associative task. We divided the training phase into three stages: the first stage where lizards had to eat a small, frozen cricket (*A. domestica*) from an opaque petri dish (3D x 1.6H cm) placed in the middle of their enclosure ([Fig. 1](#fig-Methods) C, Stage 1); the second stage where the petri dish with the cricket was placed on top of the white 3D printed ramps ([Fig. 1](#fig-Methods) C, Stage 2); and the third where the cricket was left inside a well (3D x 1.75H cm) on the top of the ramp ([Fig. 1](#fig-Methods) C, Stage 3). Every trial began when we left the feeding block (petri dish, ramp, or both) inside the enclosure and finished one hour later, when we took it away. At the end of each trial, we recorded whether the cricket had been consumed or not. A trial was considered successful if the lizard could locate and consume the reward, while completion of each stage required the lizards to eat the crickets in at least 5 out of 6 trials to ensure lizards were feeding consistently. This phase lasted 38 days until all the lizards learned to eat from the ramp; only in one case did we decide not to use the lizard because its behaviour was not consistent over the course of the training phase.

During the associative learning phase, we trained lizards to associate colour with a food reward ([Fig. 1](#fig-Methods) D). This test was like the third stage of the training phase, but here lizards were presented with three 3D printed ramps that differed in colour. Ramps were green, red, and blue, as previous studies demonstrate that squamates can discriminate between these colours ([Baden and Osorio 2019](#ref-Baden_Osorio_2019_Vert_vision)). The food reward (small, frozen, *A. domestica* crickets) was placed inside the wells of the three ramps, covering two of the crickets with 3D-printed lids (3D x 0.5H cm) so prey was only accessible in “the correct” ramp. This way, we controlled for the use of prey chemical cues, as the lids had a series of small holes on the top to allow the release of those chemicals. To control for potential colour preference that could bias our results, we split the subjects into two groups counterbalanced by treatment and species: in one group the correct choice (i.e., the ramp with the non-covered frozen cricket) was blue, while the other group was assigned the red ramp as correct. In all trials, the position of the ramps was changed randomly to ensure subjects were using colour rather than spatial cues for the association. Lizards were tested in this task once a day for 35 days.

|  |
| --- |
| Fig 1— Experimental design of the experiment. Panel A shows the early environment manipulation (see methods for ). In panel B, the measurements of the 3D-printed ramps employed in the habituation and learning tasks. Panel C shows the habituation process with the three different stages. And in panel D, the associative task with the three different 3D printed ramps. White lids in D show the ramps where the food reward was not attainable. |

The experiment took place between the 6th of March to the 17th of May 2023, and tests were performed between 11 AM to 12 PM when the lizards were active. Trials in the learning phase were recorded with 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 to have failed if there was no choice after one hour of recording. These trials were scored as NA. We excluded from our analyses those individuals that did not perform consistently as defined as not choosing in less than 20 out of 35 trials (~57%). For each lizard, we considered their first trial to be the first one where a choice was made.

#### Statistical analyses

We performed analyses for species separately. Preliminary analyses showed a significant effect of the colour. As such, we decided to split the data by colour (blue or red). Therefore, we ran a total of four different Bayesian multilevel models using the *brm* function from the brms package ([Bürkner 2017](#ref-burkner2017brms)) in R (version 2.8.2) ([R Core Team 2021](#ref-R)). We ran four parallel MCMC chains of 3000 iterations for each model, with a warmup period of 1000 iterations. We checked that all MCMC chains converged (Rhat < 1.2) and were mixing effectively to ensure we had >1000 effective samples from the posterior distribution.

We modelled correct choice [correct (1) or not (0)] as the response variable, and trial, incubation temperature (Cold versus Hot), hormone (CORT versus Control), and the three-way interaction as fixed factors. The error structure was modeled using a Bernoulli distribution with a logit link function [family = Bernoulli(link = ‘logit’)]. We included a random intercept and slope (trial) for each lizard in our models. We also incorporated the clutch identity as a random factor. *L. delicata* lays one clutch per breeding season while *L. guichenoti* lays up to two ([Joss and Minard 1985](#ref-joss1985reproductive); [Chapple et al. 2011](#ref-chapple_know_2011), [2014](#ref-chapple_biology_2014)). Since egg collection was done during half of the breeding season, each clutch likely came from a unique mother, and so, clutch identity captures potential maternal effects. In addition, previous studies indicate that clutches are typically fertilized by a single male, although sperm storage can occur ([Kar et al. 2024](#ref-kar2024heritability)). Considering our partial split-clutch design and the expectation that maternal effects are likely to be more pronounced than paternal effects in these species, incorporating clutch as a random factor should effectively account for parental condition.

Learning in lizards can be age-dependent ([Noble et al. 2014](#ref-noble_age-dependent_2014)), and given that incubation temperature will affect hatching time ([Zhang et al. 2023](#ref-zhang2023developmental)), we explored the effects of age on learning by including it as a predictor. Lizard ages 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 to test for differences in learning rate between treatments and species. 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. pmcmc test the hypothesis that slopes and slopes contrasts are different from zero. We considered statistical significant if pmcmc < 0.05.

## Results

Originally, we started with 96 lizards, 48 per species and 12 per treatment per species. However, due to natural mortality (n = 11), incompletion of the training stage (n = 1), or no motivation during the learning tasks (n = 3), we had a final sample size of 81 lizards. Final sample sizes per treatment and species are listed in [Fig. 2](#fig-deli) and [Fig. 3](#fig-guich) (figures for both species with the raw data are included in the *Supplementary Material*). Mean slopes (denoted as β throughout) per treatment for both species are provided in Table 1 in the *Supplementary Material*.

The probability of choosing correctly in the first trial when the correct ramp was blue not different to the probability of choosing correctly when the correct ramp was red for *L. delicata* (mean PBlue = 0.610, 95% CI PBlue = [0.321 , 0.867]; mean PRed = 0.273, 95% CI PRed = [0.062 , 0.552]; Contrast between probabilities: PBlue - PRed = 0.337, pmcmc =0.12, see [Fig. 2](#fig-deli)); neither for *L. guichenoti*, the probability of choosing correctly was similar between blue or red ramps (mean PBlue = 0.487, 95% CI PBlue = [0.187 , 0.815]; mean PRed = 0.195, 95% CI PRed = [0.024 , 0.485]; Contrast between probabilities: PBlue - PRed = 0.292, pmcmc =0.11, see [Fig. 3](#fig-guich)). **However, the P>0.33 in which cases: Table Suppl Mat**

There was no significant effect of the colour of the correct ramp in *L. delicata* (mean βBlue = 0.023, 95% CI βBlue = [-0.041 , 0.090]; mean βRed = 0.085, 95% CI βRed = [0.030 , 0.143]; Contrast between slopes: βBlue - βRed = -0.062, pmcmc =0.12, see [Fig. 2](#fig-deli)), nor in *L. guichenoti* (mean βBlue = 0.063, 95% CI βBlue = [-0.030 , 0.149]; mean βRed = 0.103, 95 CI βRed = [0.032 , 0.180]; βBlue - βRed = -0.041, pmcmc =0.46, see [Fig. 3](#fig-guich)). **However, because of the potential bias towards blue in the initial choice that could be affecting the estimated slopes we analyzed the differences between treatments for each ramp colour separately.**

Decision making in *L. delicata* was not impacted by the hormone (βControl - βCORT = 0.064, pmcmc =0.66), incubation temperature (βHot - βCold = 0.110, pmcmc =0.51) or their interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = -0.175, pmcmc =0.38) when red ramps were the correct choice (see [Fig. 2](#fig-deli) A). There was no significant effect of hormone (βControl - βCORT = 0.034, pmcmc =0.83), temperature (βHot - βCold = -0.165, pmcmc =0.36) or their interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = -0.171, pmcmc =0.43) when blue ramps were the correct choice (see [Fig. 2](#fig-deli) D).

Learning slopes in *L. delicata* assigned to red ramps did not show significant effects of the hormone (βControl - βCORT = -0.014, pmcmc =0.76), temperature (βHot - βCold = -0.004, pmcmc =0.94), or the interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = 0.061, pmcmc =0.17) ([Fig. 2](#fig-deli) B, C). Similarly, those assigned to blue were not afected by CORT (βControl - βCORT = 0.000, pmcmc =0.98), temperature (βHot - βCold = 0.040, pmcmc =0.32), or the interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = 0.041, pmcmc =0.41) ([Fig. 2](#fig-deli) E, F).

|  |
| --- |
| Fig 2— Results for Lampropholis delicata for both colour groups red (A, B, C) and blue (D, E , F). Panels A and D show the predicted probability of choosing the correct ramp in the first trial (Decision first trial). In panels B and E, the distribution of the estimates of slopes per each treatment. In all A, B, D, and E 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. Dashed lines indicate value 0.33 (the probability of choosing correctly by chance) in panesl A, D, and 0 in panels B, E. Panels C and F show the predicted probability of choosing the correct ramp first over trials. The lines represent the mean predicted probability of choosing the correct ramp for 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. |

Decision making in *L. guichenoti* when the red ramps are the correct choice, was not affected by the hormone (βControl - βCORT = 0.084, pmcmc =0.47), temperature (βHot - βCold = 0.127, pmcmc =0.31) or their interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = -0.071, pmcmc =0.71) ([Fig. 3](#fig-guich) A). The same was observed when blue ramps were the correct choice (βHot - βCold = 0.006, pmcmc =0.97; βControl - βCORT = 0.138, pmcmc =0.57) ([Fig. 3](#fig-guich) D).

Learning slopes in *L. guichenoti* was not influenced by hormone (βControl - βCORT = -0.078, pmcmc =0.18), temperature (βHot - βCold = -0.032, pmcmc =0.49), or the interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = 0.026, pmcmc =0.66) when red ramps was the correct choice ([Fig. 3](#fig-guich) B, C). Temperature (βHot - βCold = 0.040, pmcmc =0.38), or the hormone-temperature interaction ([(βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)] = 0.016, pmcmc =0.79) were not significant for *L. guichenoti* assigned to blue ramps. However, CORT-treated lizards showed lower learning slopes than controls (βControl - βCORT = -0.134, pmcmc < 0.05) when the blue ramps were the correct choice (see [Fig. 3](#fig-guich) E, F).

|  |
| --- |
| Fig 3— Results for Lampropholis guichenoti for both colour groups red (A, B, C) and blue (D, E , F). Panels A and D show the predicted probability of choosing the correct ramp in the first trial (Decision first trial). In panels B and E, the distribution of the estimates of slopes per each treatment. In all A, B, D, and E 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. Dashed lines indicate value 0.33 (the probability of choosing correctly by chance) in panesl A, D, and 0 in panels B, E. Panels C and F show the predicted probability of choosing the correct ramp first over trials. The lines represent the mean predicted probability of choosing the correct ramp for 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. |

Overall, decision making did not differ between species when the red ramps were the correct choice (mean β*L. delicata* = 0.273, 95% CI β*L. delicata* = [0.062 , 0.552]; mean β*L. guichenoti* = 0.195, 95% CI β*L. guichenoti* = [0.024 , 0.485]; β*L. delicata* - β*L. guichenoti* = 0.078, pmcmc =0.53) or when the blue ramps were the correct choice (mean β*L. delicata* = 0.610, 95% CI = [0.321 , 0.867]; mean β*L. guichenoti* = 0.487, 95% CI β*L. guichenoti* = [0.187 , 0.815]; β*L. delicata* - β*L. guichenoti* = 0.123, pmcmc =0.56).

When we average learning rates across treatments, we did not find any significant differences when we compared between species when the red ramp was the correct choice (mean β*L. delicata* = 0.077, 95% CI β*L. delicata* = [0.026 , 0.135]; mean β*L. guichenoti* = 0.092, 95% CI β*L. guichenoti* = [0.023 , 0.171]; β*L. delicata* - β*L. guichenoti* = -0.016, pmcmc =0.72) neither when the blue ramps were the correct choice (mean β*L. delicata* = 0.023, 95% CI β*L. delicata* = [-0.041 , 0.090]; mean β*L. guichenoti* = 0.063, 95% CI β*L. guichenoti* = [-0.030 , 0.149]; β*L. delicata* - β*L. guichenoti* = -0.040, pmcmc =0.40).

## Discussion

Associative learning in *L. delicata* and *L. guichenoti* is robust to elevations in prenatal CORT and incubation temperature. We predicted that elevating CORT levels in eggs or incubating them at colder temperatures would decrease learning slopes, and that warmer incubation temperatures would relax the effects of CORT elevations. In contrast to our predictions, both species learned to associate a colour with a food reward at the same rate regardless of the experimental treatments. This suggests that these species may have evolved mechanisms to buffer against early environmental stressors and maintain learning performance despite prenatal challenges.

In addition, we found that the learning rates varied depending on the colour assigned to the correct choice, at least for *L. delicata*, with lizards assigned to blue ramps showing lower learning slopes than in the red group. This outcome manifests the need to consider the potential effects of colour biases in the design of cognitive tasks.

#### Effect of prenatal CORT on learning

Prenatal CORT was predicted to have a negative effect on learning ([Lemaire et al. 2000](#ref-lemaire_prenatal_2000); [Zhu et al. 2004](#ref-zhu_prenatal_2004); [Eaton et al. 2015](#ref-eaton2015mild); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn); [Bebus et al. 2016](#ref-bebus_associative_2016)). However, we did not find any significant difference between hormone treatments in either of the two species. Our results are consistent with other experiments that showed no influence of prenatal GCs on learning abilities ([Szuran et al. 1994](#ref-szuran_water_1994); [Bebus et al. 2016](#ref-bebus_associative_2016); [Farrell et al. 2016](#ref-farrell_developmental_2016)) and suggest that *L. delicata* and *L. guichenoti* may have evolved some strategies to buffer the impacts of prenatal stress.

Alternatively, our observations could be obscured by other variables that are known to influence the extent and direction of these outcomes. ([Szuran et al. 1994](#ref-szuran_water_1994); [Crino et al. 2014](#ref-crino_corticosterone_2014-learn); [Farrell et al. 2015](#ref-farrell_developmental_2015-learn), [2016](#ref-farrell_developmental_2016); [Bebus et al. 2016](#ref-bebus_associative_2016)). GCs are known to have hormetic effects. Generally, low elevations or short exposure to GCs improve the rate at which animals learn while high concentrations or exposure to GCs during long periods have the opposite effects ([Du et al. 2009](#ref-du_dynamic_2009); [McEwen 2012](#ref-mcewen_brain_2012)). When using the same dose and application methods in eggs of *L. delicata*, Crino et al. ([2024](#ref-crino2024eggs)) found CORT treatment had sustained effects on mass and mitochondrial activity, but not baseline CORT. In contrast, lower CORT concentration did not affect mass or mitochondrial activity, but increased CORT baseline levels ([Crino et al. 2024](#ref-crino2024eggs)). This suggests that high elevations in CORT could affect learning through permanent changes in brain function (programmatic effects) while lower doses can result in lifelong elevation of baseline CORT, affecting learning through activational effects. Our findings indicate that associative learning in *L. delicata* and *L. guichenoti* is robust to the programmatic effects of high CORT elevations, but we cannot exclude the possibility that lower concentrations may affect learning abilities.

Conversely, the absence of significant effects of prenatal CORT on learning could be due to the cognitive task employed, as some studies show that the impact of GCs can vary between brain regions ([Lemaire et al. 2000](#ref-lemaire_prenatal_2000)). GCs action involve genomic and nongenomic mechanisms that implicate different types of receptors that can be distributed unevenly in the various regions of the brain ([McEwen 2012](#ref-mcewen_brain_2012)), and may be related to the learning performance in distinct tasks. For instance, in european starlings (*Sturnus vulgaris*), males stressed during early stages of development performed worse in a visual associative learning task, but show no differences with control birds when the stimuli tested was acoustic ([Farrell et al. 2015](#ref-farrell_developmental_2015-learn)). Similarly, scrub-jays (*Aphelocoma coerulescens*) that have lower CORT levels as nestlings performed better on an associative learning test as adults but not on a reversal-learning task ([Bebus et al. 2016](#ref-bebus_associative_2016)). We must acknowledge the possibility that prenatal CORT does not affect the brain regions involved in coding associative learning in *L. delicata* and *L. guichenoti*, and future studies should focus on examining the effect of early-life stress on different cognitive domains.

#### Effect of incubation temperature on learning

We also predicted hot-incubated lizards to perform better in the associative learning task, since most of the studies demonstrate enhanced learning abilities when eggs are incubated at higher temperatures ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014)). Furthermore, the opposite pattern was found only in studies that employed temperatures far above the natural thermal range of the species in their hot treatments ([Dayananda and Webb 2017](#ref-dayananda_incubation_2017); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020)), and it is unclear how such conditions relate to wild environments. In *L. delicata* and *L. guichenoti*, associative learning appears to be robust to incubation temperature. Our results, therefore, do not align with the outcome observed in previous experiments. However, the effect of prenatal temperature on cognition, and brain physiology and structure has been investigated only in a small number of species (see [Coomber et al. 1997](#ref-coomber_independent_1997); [Sakata et al. 2000](#ref-sakata_neural_2000); [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); [Dayananda and Webb 2017](#ref-dayananda_incubation_2017); [Abayarathna and Webb 2020](#ref-abayarathna_effects_2020)), limiting our understanding on how thermal early environment can affect cognitive abilities. According to our observations, the impact of incubation temperature on learning may not be as widespread across reptiles as we may think.

Alternatively, other factors, such as sex-dependent effects, could be affecting our findings. We did not include sex as a predictor for several reasons: first, lizards were tested before sexual maturation ([Joss and Minard 1985](#ref-joss1985reproductive)); and second, previous studies have found weak evidence for sex-dependent learning in reptiles ([Szabo 2019](#ref-szabo_sex_meta)). In fact, in the eastern three-lined skink (*Bassiana dupeyerri*) the effect of incubation temperature on learning is not sex-dependent ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014)). However, there is previous evidence for interactive effects of incubation temperature and sex on brain physiology in other species of reptiles ([Coomber et al. 1997](#ref-coomber_independent_1997); [Sakata et al. 2000](#ref-sakata_neural_2000)). Notably, in *B. dupeyerri* exposure to cold temperatures during incubation can induce sex-reversal, where individuals under cold incubation temperatures develop male phenotypes while retaining female genotypes ([Quinn et al. 2009](#ref-quinn2009isolation)). As a result, even when examining the interactive effects of sex and temperature in this species, the results could be confounded by the mismatch between genotype and phenotype ([Amiel and Shine 2012](#ref-amiel_hotter_2012); [Clark et al. 2014](#ref-clark_colour_2014); [Amiel et al. 2014](#ref-amiel_egg_2014)). Although sex-reversal does not occur in *L. delicata* or *L. guichenoti*, not accounting for sex in our analyses could be masking potential interactions between temperature and sex in shaping learning abilities (see [Coomber et al. 1997](#ref-coomber_independent_1997); [Sakata et al. 2000](#ref-sakata_neural_2000)). Future research should investigate the potential interactive impacts of sex and incubation temperature on cognitive abilities. Nevertheless, conducting such studies poses challenges due to the substantial sample sizes necessary.

#### Effect of colour on learning

The colour of the ramp associated with the attainable cricket (i.e. the correct choice) influences learning slopes in *L. delicata*, but not *L. guichenoti*. *L. delicata* learned the task when the reward was associated to the red ramp but not when the ramp was blue. In contrast, *L. guichenoti* showed similar results despite the colour of the correct ramp. This indicates that *L. delicata* were not able to associate the blue ramp with the reward. However, our results could be a consequence of an initial bias in lizards’ choice. The analyses presented in the Supplemenatry Material show that, overall, the estimated probability of choosing the correct ramp in the first trial was higher than expected by chance for those animals assigned to the blue ramp. These lizards show learning slopes not different from zero, but high probabilities of choosing the correct ramp also in the first trials (see [Fig. 2](#fig-deli)).

Because there was a possibility that this bias towards blue ramps was a byproduct of the habituation process, we compared the light spectrum and the perceived chromatic differences between the ramps used in the associative task and the ones used during habituation (see *Supplementary Material*). We found no similarities between the white and blue ramps, so we consider that preference towards blue ramps did not arise as a derivate from trining the lizards with the white ramps. Rather, it seems likely that lizards innately prefer the blue ramps. Some animals bias their attention towards colours they are familiar with ([Putman et al. 2017](#ref-putman2017fear) - the ‘species confidence hypothesis’). For instance, dark and light blue T-shirts were associated with lower flight initiation distances and higher capture rates in Western fence lizards where blue is used in intraspecific communication ([Putman et al. 2017](#ref-putman2017fear)). Nevertheless, to the best of our knowledge, there has been no reported colour bias in *L. delicata* or *L. guichenoti* before; and blue colouration is not considered to be involved in intraspecific communication in these species ([Torr and Shine 1996](#ref-torr1996patterns); [Chapple et al. 2014](#ref-chapple_biology_2014)). Regardless, our results demonstrate that *L. delicata* has a bias toward blue colours and highlights the need to consider the colours used in associative learning paradigms.

#### Learning rates between species

We did not see any significant difference in learning rates when both species were compared. This result supports previous studies that found similar learning abilities in *L. delicata* and *L. guichenoti* ([Bezzina et al. 2014](#ref-bezzina2014does)). In the experiment conducted by Bezzina et al. ([2014](#ref-bezzina2014does)), both species failed to complete the associative learning task under the authors’ criterion, while in our experiment both species completed the task, exhibiting similar learning rates. Complexity, experimental design, or the criterion employed to define learning could be the major cause of the discrepancies between ours and Bezzina et al. ([2014](#ref-bezzina2014does)). Developing common strategies and approaches to assess learning in animals could help understand how learning abilities are shaped in different taxa or under different environments.

#### Conclusion

Our results revealed that associative learning abilities in *L. delicata* and *L. guichenoti* are resilient to prenatal CORT and temperature. This outcome contrasts with our initial predictions, indicating that the learning skills of these lizards may be more robust than anticipated under varying early life conditions. We also found significant effects of the colour employed in the task on learning rates in *L. delicata*, but not in *L. guichenoti*. These results seem to be a consequence of an innate colour bias and highlights the importance of carefully selecting the colour employed when testing cognition using visual stimuli.

Future research should continue exploring the potential effects of prenatal corticosterone (CORT) and temperature on cognitive function, but with a focus on interactions between early environmental factors and sex, as well as the type of cognitive tasks employed. Furthermore, it is crucial to explore how these treatments influence brain function at a neurological level. Investigating these aspects will help us understand these species’ cognitive and physiological mechanisms underpinning adaptability and offer insights into how early developmental factors shape long-term cognitive outcomes.

## Data accessibility

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

## Satements and declarations

#### Competing Interests

We declare we have no competing interests

#### Conflict of Interest

We declare we have no conflict of interest

#### Ethical Approval

All experimental procedures and laboratory housing complied with Australian law and were approved by the Australian National University Animal Experimentation Ethics Committee (A2022\_33).

## 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.

## Funding

This work was supported by a National Australian University fellowship (P.R.), the Australian Research Council (grant no. DP210101152) to D.N. and C.F., and the ACT Herpetological Association grant to P.R.

## Acknowledgements

We thank the help and assistance of our lab technicians Benjamin Durant and Michelle Stephens for taking care of the lizards. We are also grateful to ACTHA for the grant for the 3D printed ramps, and we also thank ANU MakerSpace, where we designed and built the prototypes of the 3D printed ramps. Finally, we wish to acknowledge the anonymous reviewers for their valuable feedback on the manuscript.

## References

Abayarathna T, Webb JK (2020) Effects of incubation temperatures on learning abilities of hatchling velvet geckos. Animal Cognition 23:613–620. <https://doi.org/10.1007/s10071-020-01365-4>

Amiel JJ, Bao S, Shine R (2017) The effects of incubation temperature on the development of the cortical forebrain in a lizard. Animal Cognition 20:117–125. <https://doi.org/10.1007/s10071-016-0993-2>

Amiel JJ, Lindström T, Shine R (2014) Egg incubation effects generate positive correlations between size, speed and learning ability in young lizards. Animal Cognition 17:337–347. <https://doi.org/10.1007/s10071-013-0665-4>

Amiel JJ, Shine R (2012) Hotter nests produce smarter young lizards. Biology Letters 8:372–374. <https://doi.org/10.1098/rsbl.2011.1161>

Baden T, Osorio D (2019) The retinal basis of vertebrate color vision. Annual Review of Vision Science 177–200

Bebus SE, Small TW, Jones BC, et al (2016) Associative learning is inversely related to reversal learning and varies with nestling corticosterone exposure. Animal Behaviour 111:251–260. <https://doi.org/10.1016/j.anbehav.2015.10.027>

Beltrán I, Herculano-Houzel S, Sinervo B, Whiting MJ (2021) Are ectotherm brains vulnerable to global warming? Trends in Ecology & Evolution 36:691–699. <https://doi.org/10.1016/j.tree.2021.04.009>

Bezzina CN, Amiel JJ, Shine R (2014) Does invasion success reflect superior cognitive ability? A case study of two congeneric lizard species (lampropholis, scincidae). PLoS One 9:e86271

Buchanan KL, Grindstaff JL, Pravosudov VV (2013) Condition dependence, developmental plasticity, and cognition: Implications for ecology and evolution. Trends in Ecology & Evolution 28:290–296. <https://doi.org/10.1016/j.tree.2013.02.004>

Bürkner P-C (2017) Brms: An r package for bayesian multilevel models using stan. Journal of statistical software 80:1–28

Carazo P, Noble DWA, Chandrasoma D, Whiting MJ (2014) Sex and boldness explain individual differences in spatial learning in a lizard. Proceedings of the Royal Society B: Biological Sciences 281:20133275. <https://doi.org/10.1098/rspb.2013.3275>

Chapple DG, Miller KA, Chaplin K, et al (2014) Biology of the invasive delicate skink (Lampropholis delicata) on Lord Howe Island. Australian Journal of Zoology 62:498. <https://doi.org/10.1071/ZO14098>

Chapple DG, Simmonds SM, Wong BBM (2011) Know when to run, know when to hide: Can behavioral differences explain the divergent invasion success of two sympatric lizards?: Invasion Success of Two Sympatric Lizards. Ecology and Evolution 1:278–289. <https://doi.org/10.1002/ece3.22>

Clark BF, Amiel JJ, Shine R, et al (2014) Colour discrimination and associative learning in hatchling lizards incubated at “hot” and “cold” temperatures. Behavioral Ecology and Sociobiology 68:239–247. <https://doi.org/10.1007/s00265-013-1639-x>

Coomber P, Crews D, Gonzalez-Lima F (1997) Independent effects of incubation temperature and gonadal sex on the volume and metabolic capacity of brain nuclei in the leopard gecko (Eublepharis macularius), a lizard with temperature-dependent sex determination. The Journal of Comparative Neurology 380:409–421. <https://doi.org/10.1002/(SICI)1096-9861(19970414)380:3<409::AID-CNE9>3.0.CO;2-6>

Crino OL, Bonduriansky R, Martin LB, Noble DWA (2023) A conceptual framework for understanding stressinduced physiological and transgenerational effects on population responses to climate change. Evolution Letters

Crino OL, Driscoll SC, Ton R, Breuner CW (2014) Corticosterone exposure during development improves performance on a novel foraging task in zebra finches. Animal Behaviour 91:27–32. <https://doi.org/10.1016/j.anbehav.2014.02.017>

Crino O, Wild KH, Friesen CR, et al (2024) From eggs to adulthood: Sustained effects of early developmental temperature and corticosterone exposure on physiology and body size in an australian lizard

Dayananda B, Webb JK (2017) Incubation under climate warming affects learning ability and survival in hatchling lizards. Biology Letters 13:20170002. <https://doi.org/10.1098/rsbl.2017.0002>

Du J, Wang Y, Hunter R, et al (2009) Dynamic regulation of mitochondrial function by glucocorticoids. Proceedings of the National Academy of Sciences 106:3543–3548. <https://doi.org/10.1073/pnas.0812671106>

Dukas R (2004) Evolutionary Biology of Animal Cognition. Annual Review of Ecology, Evolution, and Systematics 35:347–374. <https://doi.org/10.1146/annurev.ecolsys.35.112202.130152>

Eaton L, Edmonds E, Henry T, et al (2015) Mild maternal stress disrupts associative learning and increases aggression in offspring. Hormones and behavior 71:10–15

Farrell TM, Morgan A, MacDougall-Shackleton SA (2016) Developmental stress impairs performance on an association task in male and female songbirds, but impairs auditory learning in females only. Animal Cognition 19:1–14. <https://doi.org/10.1007/s10071-015-0908-7>

Farrell TM, Neuert MAC, Cui A, MacDougall-Shackleton SA (2015) Developmental stress impairs a female songbird’s behavioural and neural response to a sexually selected signal. Animal Behaviour 102:157–167. <https://doi.org/10.1016/j.anbehav.2015.01.018>

Hanover AM, Husak JF, Lovern M (2019) Corticosterone in lizard egg yolk is reduced by maternal diet restriction but unaltered by maternal exercise. Physiological and Biochemical Zoology 92:573–578

Joss J, Minard J (1985) On the reproductive cycles of lampropholis guichenoti and l. Delicata (squamata: Scincidae) in the sydney region. Australian Journal of Zoology 33:699–704

Kar F, Nakagawa S, Noble DW (2024) Heritability and developmental plasticity of growth in an oviparous lizard. Heredity 132:67–76

Leal M, Powell BJ (2012) Behavioural flexibility and problem-solving in a tropical lizard. Biology Letters 8:28–30. <https://doi.org/10.1098/rsbl.2011.0480>

Lemaire V, Koehl M, Le Moal M, Abrous DN (2000) Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proceedings of the National Academy of Sciences 97:11032–11037. <https://doi.org/10.1073/pnas.97.20.11032>

Lovern MB, Adams AL (2008) The effects of diet on plasma and yolk steroids in lizards (anolis carolinensis). Integrative and comparative biology 48:428–436

McEwen BS (2012) Brain on stress: How the social environment gets under the skin. Proceedings of the National Academy of Sciences 109:17180–17185. <https://doi.org/10.1073/pnas.1121254109>

Moore MP, Whiteman HH, Martin RA (2019) A mother’s legacy: The strength of maternal effects in animal populations. Ecology Letters 22:1620–1628. <https://doi.org/10.1111/ele.13351>

Noble DWA, Byrne RW, Whiting MJ (2014) Age-dependent social learning in a lizard. Biology Letters 10:20140430. <https://doi.org/10.1098/rsbl.2014.0430>

Noble DWA, Stenhouse V, Schwanz LE (2018) Developmental temperatures and phenotypic plasticity in reptiles: A systematic review and meta-analysis: Incubation temperature and plasticity. Biological Reviews 93:72–97. <https://doi.org/10.1111/brv.12333>

Putman BJ, Drury JP, Blumstein DT, Pauly GB (2017) Fear no colors? Observer clothing color influences lizard escape behavior. PLoS One 12:e0182146

Quinn AE, Radder RS, Sarre SD, et al (2009) Isolation and development of a molecular sex marker for bassiana duperreyi, a lizard with XX/XY sex chromosomes and temperature-induced sex reversal. Molecular Genetics and Genomics 281:665–672

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Sakata JT, Coomber P, Gonzalez-Lima F, Crews D (2000) Functional connectivity among limbic brain areas: Differential effects of incubation temperature and gonadal sex in the leopard gecko, eublepharis macularius. Brain, Behavior and Evolution 139–151

Sapolsky RM, Romero LM, Munck AU (2000) How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. 21:

Shettleworth SJ (2010) Cognition, evolution, and behaviour, 2nd edn. Oxford University Press

Szabo W B. (2019) Sex-dependent discrimination learning in lizards: A meta-analysis. Behavioural processes 164:10–16

Szuran T, Zimmermann E, Welzl H (1994) Water maze performance and hippocampal weight of prenatally stressed rats. Behavioural Brain Research 65:153–155. <https://doi.org/10.1016/0166-4328(94)90100-7>

Torr GA, Shine R (1996) Patterns of dominance in the small scincid lizard lampropholis guichenoti. Journal of Herpetology 230–237

Vila Pouca C, Gervais C, Reed J, et al (2019) Quantity discrimination in Port Jackson sharks incubated under elevated temperatures. Behavioral Ecology and Sociobiology 73:93. <https://doi.org/10.1007/s00265-019-2706-8>

Ward-Fear G, Pearson D, Brown G, et al (2016) Ecological immunization: In situ training of free-ranging predatory lizards reduces their vulnerability to invasive toxic prey. Biology letters 12:20150863

Welklin JF, Sonnenberg BR, Branch CL, et al (2024) Spatial cognitive ability is associated with longevity in food-caching chickadees. Science 385:1111–1115

Zhang RY, Wild KH, Pottier P, et al (2023) Developmental environments do not affect thermal physiological traits in reptiles: An experimental test and meta-analysis. Biology Letters 19:20230019

Zhu Z, Li X, Chen W, et al (2004) Prenatal stress causes gender-dependent neuronal loss and oxidative stress in rat hippocampus. Journal of Neuroscience Research 78:837–844. <https://doi.org/10.1002/jnr.20338>

# Suplementary Material

#### Estimates of reversal learning slopes for all the different treatments per each task, specie, and group.

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

|  | | Red | | | Blue | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Specie | Treatment | Mean | 95% CI | pmcmc | Mean | 95% CI | pmcmc |
| *L. delicata* | CORT-Cold | **0.101** | **[0.055 , 0.152]** | **< 0.001** | 0.013 | [-0.035 , 0.064] | 0.60 |
|  | Control-Cold | **0.056** | **[0.018 , 0.096]** | **< 0.05** | -0.007 | [-0.056 , 0.042] | 0.77 |
|  | CORT-Hot | **0.066** | **[0.023 , 0.110]** | **< 0.05** | 0.032 | [-0.013 , 0.083] | 0.17 |
|  | Control-Hot | **0.082** | **[0.037 , 0.131]** | **< 0.001** | **0.053** | **[0.002 , 0.107]** | **< 0.05** |
| *L. guichenoti* | CORT-Cold | **0.119** | **[0.054 , 0.190]** | **< 0.05** | **0.070** | **[0.012 , 0.129]** | **< 0.05** |
|  | Control-Cold | **0.097** | **[0.032 , 0.171]** | **< 0.05** | 0.015 | [-0.058 , 0.091] | 0.65 |
|  | CORT-Hot | **0.074** | **[0.014 , 0.139]** | **< 0.05** | **0.102** | **[0.036 , 0.177]** | **< 0.05** |
|  | Control-Hot | **0.078** | **[0.016 , 0.145]** | **< 0.05** | **0.064** | **[0.004 , 0.125]** | **< 0.05** |

**?(caption)**

#### Colour preference

To test if lizards were biased towards the assigned colour as our preliminary analyses suggested, we employed the intercepts from our posterior distributions. We first estimated the predicted probability of choosing the correct ramp first in the first trial, by using the formula:

Second, we tested the hypothesis that the estimated probability was higher than 0.33 (the probability expected by chance of choosing the correct ramp) using pmcmc. If the estimated probability is above 0.33 we consider it as an indication that there was a preference towards that colour that could be affecting learning slopes. The results per treatment are summarized in Table 2.

Table 2. Probability of choosing the correct ramp in the first trial when the correct ramp was blue (Prob Blue) or red (Prob Red) for each species and each treatment. pmcmc tested the hypothesis that the probability is >0.33. In bold, those values that are significant (pmcmc <0.05)

| Specie | Treatment | Prob Red | pmcmc Red | Prob Blue | pmcmc Blue |
| --- | --- | --- | --- | --- | --- |
| *L. delicata* | CORT-Cold | 0.142 | 0.98 | **0.633** | **< 0.05** |
|  | Control-Cold | 0.294 | 0.67 | **0.752** | **< 0.001** |
|  | CORT-Hot | 0.340 | 0.51 | **0.553** | **< 0.05** |
|  | Control-Hot | 0.317 | 0.58 | 0.502 | 0.08 |
| *L. guichenoti* | CORT-Cold | 0.072 | 1.00 | 0.355 | 0.46 |
|  | Control-Cold | 0.191 | 0.91 | **0.614** | **< 0.05** |
|  | CORT-Hot | 0.235 | 0.83 | 0.482 | 0.14 |
|  | Control-Hot | 0.283 | 0.69 | 0.498 | 0.10 |

**?(caption)**

On average, we found that, for both species, the proportion of correct choices in the first trial was significantly above chance when the correct ramp was blue for *L. delicata* (mean Prob choice = 0.610, pmcmc < 0.05) but not for *L. guichenoti* (mean Prob choice = 0.487, pmcmc 0.18). When the correct choice was red, it was not significant for neither species (*L. delicata*: mean Prob choice = 0.273, pmcmc 0.68; *L. guichenoti*: mean Prob choice = 0.273, pmcmc 0.68).

#### Light spectrum

To test if the bias towards blue was something acquired during training, we compared the light spectrum of the ramps used in the associative task and the white ones used during habituation. We took three measurements of ten ramps per colour with a spectophotometer, and then analysed the spectrum and the perceived differences in colour using the package pavo. The spetrum of each type of ramp are shown in [Fig. 4](#fig-spectrum); the perceived chromatic constrasts between ramps are shown in [Fig. 5](#fig-perceived1) and [Fig. 6](#fig-perceived2).

|  |
| --- |
| Fig 4— Light spectrum of the ramps used in the associative task and the white ones used during habituation. The different colours represent the different ramps. |

|  |
| --- |
| Fig 5— Perceived chromatic contrasts between ramps. |

|  |
| --- |
| Fig 6— Perceived achromatic contrasts between feeders. |

#### Checking the models plots

Model formula for task is:  
Choice ~ Trial*cort*temp + (1 + Trial|lizard\_id)  
Plots for the different models of the associative task:  
1.- *L. delicata*  
1.a.- Red

Estimate Est.Error Q2.5 Q97.5  
R2 0.1695644 0.02196313 0.1255778 0.2110525

|  |
| --- |
|  |

|  |
| --- |
|  |

|  |
| --- |
|  |

1.b.- Blue

Estimate Est.Error Q2.5 Q97.5  
R2 0.07757601 0.02088415 0.03926128 0.1200952

|  |
| --- |
|  |

|  |
| --- |
|  |

|  |
| --- |
|  |

2.- *L. guichenoti*  
2.a.- Red

Estimate Est.Error Q2.5 Q97.5  
R2 0.1858621 0.02466494 0.1359576 0.2330882

|  |
| --- |
|  |

|  |
| --- |
|  |

|  |
| --- |
|  |

2.b.- Blue

Estimate Est.Error Q2.5 Q97.5  
R2 0.1367205 0.02551682 0.08796974 0.1865906

|  |
| --- |
|  |

|  |
| --- |
|  |

|  |
| --- |
|  |

#### Preliminary analyses

#### Models with age included

Table 3. Results for *L. delicata* assigned to red ramps when the model included the age:

| Predictors | Estimate | Est.Error | l-95% CI | u-95% CI | Rhat | Bulk\_ESS | Tail\_ESS |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Intercept | -2.16 | 0.66 | -3.49 | -0.89 | 1.00 | 3402.24 | 3887.20 |
| age.start | 0.00 | 0.02 | -0.03 | 0.04 | 1.00 | 5418.98 | 5776.45 |
| trial\_associative | 0.10 | 0.02 | 0.06 | 0.15 | 1.00 | 3692.82 | 4207.48 |
| cortControl | 1.09 | 0.86 | -0.60 | 2.83 | 1.00 | 3201.98 | 3890.41 |
| tempHot | 1.28 | 0.93 | -0.47 | 3.15 | 1.00 | 2934.34 | 3471.54 |
| trial\_associative:cortControl | -0.05 | 0.03 | -0.11 | 0.01 | 1.00 | 3572.26 | 4634.15 |
| trial\_associative:tempHot | -0.04 | 0.03 | -0.10 | 0.03 | 1.00 | 3574.66 | 4356.48 |
| cortControl:tempHot | -1.26 | 1.21 | -3.73 | 1.08 | 1.00 | 3074.88 | 4021.52 |
| trial\_associative:cortControl:tempHot | 0.06 | 0.04 | -0.03 | 0.15 | 1.00 | 3628.87 | 4183.50 |

**?(caption)**

Table 4. Results for *L. delicata* assigned to blue ramps when the model included the age:

| Predictors | Estimate | Est.Error | l-95% CI | u-95% CI | Rhat | Bulk\_ESS | Tail\_ESS |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Intercept | 0.50 | 0.58 | -0.64 | 1.64 | 1.00 | 3044.27 | 4311.87 |
| age.start | -0.00 | 0.01 | -0.01 | 0.01 | 1.00 | 5860.40 | 5710.61 |
| trial\_associative | 0.01 | 0.02 | -0.04 | 0.06 | 1.00 | 3068.62 | 4168.88 |
| cortControl | 0.70 | 0.82 | -0.88 | 2.34 | 1.00 | 2870.11 | 3815.82 |
| tempHot | -0.34 | 0.79 | -1.93 | 1.22 | 1.00 | 2598.17 | 3477.53 |
| trial\_associative:cortControl | -0.02 | 0.04 | -0.09 | 0.05 | 1.00 | 2915.98 | 3893.50 |
| trial\_associative:tempHot | 0.02 | 0.03 | -0.05 | 0.09 | 1.00 | 2593.93 | 3764.25 |
| cortControl:tempHot | -0.98 | 1.13 | -3.23 | 1.25 | 1.00 | 2537.70 | 3924.03 |
| trial\_associative:cortControl:tempHot | 0.04 | 0.05 | -0.06 | 0.14 | 1.00 | 2642.40 | 4091.36 |

**?(caption)**

Table 5. Results for *L. guichenoti* assigned to red ramps when the model included the age:

| Predictors | Estimate | Est.Error | l-95% CI | u-95% CI | Rhat | Bulk\_ESS | Tail\_ESS |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Intercept | -3.06 | 0.84 | -4.87 | -1.55 | 1.00 | 2641.91 | 2963.70 |
| age.start | -0.00 | 0.02 | -0.04 | 0.03 | 1.00 | 5914.10 | 5309.44 |
| trial\_associative | 0.12 | 0.04 | 0.06 | 0.20 | 1.00 | 2411.12 | 2897.00 |
| cortControl | 1.28 | 1.15 | -0.97 | 3.62 | 1.00 | 2479.35 | 3043.41 |
| tempHot | 1.65 | 1.14 | -0.57 | 3.97 | 1.00 | 2336.65 | 2802.60 |
| trial\_associative:cortControl | -0.02 | 0.05 | -0.13 | 0.08 | 1.00 | 2444.48 | 3222.97 |
| trial\_associative:tempHot | -0.05 | 0.05 | -0.15 | 0.04 | 1.00 | 2253.20 | 2466.35 |
| cortControl:tempHot | -1.03 | 1.53 | -4.17 | 1.91 | 1.00 | 2353.37 | 3274.21 |
| trial\_associative:cortControl:tempHot | 0.03 | 0.07 | -0.11 | 0.17 | 1.00 | 2319.80 | 3341.04 |

**?(caption)**

Table 6. Results for *L. guichenoti* assigned to blue ramps when the model included the age:

| Predictors | Estimate | Est.Error | l-95% CI | u-95% CI | Rhat | Bulk\_ESS | Tail\_ESS |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Intercept | -0.61 | 0.73 | -2.07 | 0.85 | 1.00 | 4476.16 | 4928.86 |
| age.start | 0.01 | 0.01 | -0.02 | 0.03 | 1.00 | 5957.56 | 5181.70 |
| trial\_associative | 0.07 | 0.03 | 0.01 | 0.13 | 1.00 | 4004.25 | 4029.03 |
| cortControl | 1.32 | 1.00 | -0.70 | 3.29 | 1.00 | 3729.95 | 4199.07 |
| tempHot | 0.38 | 0.94 | -1.61 | 2.22 | 1.00 | 4055.41 | 4399.38 |
| trial\_associative:cortControl | -0.05 | 0.05 | -0.14 | 0.04 | 1.00 | 3437.88 | 3686.90 |
| trial\_associative:tempHot | 0.03 | 0.04 | -0.05 | 0.12 | 1.00 | 3667.39 | 4011.10 |
| cortControl:tempHot | -1.23 | 1.34 | -3.86 | 1.52 | 1.00 | 3524.34 | 4053.85 |
| trial\_associative:cortControl:tempHot | 0.02 | 0.06 | -0.12 | 0.14 | 1.00 | 3340.33 | 4029.47 |

**?(caption)**

|  |
| --- |
| Fig 7— Distribution of the age of the lizards by treatment and species |