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

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## Abstract

Behavioural flexibility is crucial for animals adapting to and colonizing new environments. However, novel environmental conditions experienced during early development can profoundly impact behavioural flexibility making it unclear how invasive populations establish in new locations. Given the advantages associated with behaving flexibly during the invasion process we predict that behavioural flexibility in invasive species should be less susceptible to the impacts of early environmental conditions. Here, we investigated the interactive effects of prenatal corticosterone (CORT) and temperature on behavioural flexibility in two lizard species, *Lampropholis delicata* and *L. guichenoti*, with distinct histories of invasion. We manipulated prenatal CORT levels and incubation temperature in a 2x2 factorial design, and then assessed behavioural flexibility through a reversal learning task. Contrary to our expectations, we found no significant differences in the reversal learning rate between treatments or species. Behavioural flexibility appears to be robust to prenatal temperature and CORT which may be one reason why *L. delicata* has been so successful as an invasive species. However, the lack of difference in reversal learning between *L. delicata* and *L. guichenoti* suggests that other processes are likely more influential in invasion success than behavioural flexibility itself.

## 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](#ref-brown2010behavioral)). It comprises a suite of behaviours, including problem-solving or innovation ([Brown & Tait, 2010](#ref-brown2010behavioral)), that can be beneficial for adapting to novel circumstances ([Szabo et al., 2020](#ref-szabo2020can); [Szulkin et al., 2020](#ref-szulkin2020urban)). 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](#ref-chapple2012can); [Szabo et al., 2020](#ref-szabo2020can); [Szulkin et al., 2020](#ref-szulkin2020urban); [Wright et al., 2010](#ref-wright2010behavioral)). 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](#ref-webb2014behavioural)); while succesful colonisation of new environments by birds is related to higher frequency of foraging innovations ([Sol & Lefebvre, 2000](#ref-sol2000behavioural)). 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](#ref-amiel_smart_2011); [Sol et al., 2008](#ref-sol2008brain); [Sol & Lefebvre, 2000](#ref-sol2000behavioural)). 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. In this sense, the brain is considered especially sensitive to environmental changes during the early stages of development ([Zhu et al., 2004](#ref-zhu_prenatal_2004)), creating uncertainty about how the conditions faced during early life might impact behavioural flexibility.

The conditions in which the organisms develop can be altered by the circumstances faced by the parents. For instance, exposing threespine sticklebacks females to a model predator while yolking eggs affected offspring tedency to use social cues ([Feng et al., 2015](#ref-feng2015effect)). 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](#ref-sapolsky_how_2000)). Glucocorticoids can be transmitted to offspring and influence their phenotype and development through transgenerational effects ([Crino et al., 2023](#ref-Crino_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](#ref-kolbe2002impact)). This way, parental effects like transmitted GCs, are predicted to interact with other factors from the early environment to influence the offspring phenotype. Both GCs and temperature are known to exert potent effects on brain development ([Amiel et al., 2017](#ref-amiel_effects_2017); [Coomber et al., 1997](#ref-coomber_independent_1997); [Jonson et al., 1976](#ref-jonson1976effect); [Zhu et al., 2004](#ref-zhu_prenatal_2004)) and cognition ([Clark et al., 2014](#ref-clark_colour_2014); [Lui et al., 2017](#ref-lui2017chronic)), but little is known about how their ineraction could affect behavioural flexibility. Since behaving flexibly is essential for facing novel circumstances, 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](#ref-Crino_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](#ref-chapple_know_2011), [2014](#ref-chapple_biology_2014)), but only *Lampropholis delicata* has been successful in colonising different areas around the globe ([Baker, 1979](#ref-baker1979rainbow); [Chapple et al., 2013](#ref-chapple2013divergent), [2015](#ref-chapple2015deliinvLHI)). The differences in invassion success between both skinks may be driven by disparities in behavioural flexibility, a prominent quality in other invassive species ([Amiel et al., 2011](#ref-amiel_smart_2011); [Chapple et al., 2012](#ref-chapple2012can); [Sol et al., 2008](#ref-sol2008brain); [Sol & Lefebvre, 2000](#ref-sol2000behavioural); [Wright et al., 2010](#ref-wright2010behavioral)). However, the relative impact of early environments on behavioural flexibility remains unexplored. Early environment affects suites of morphological and behavioural traits differently in each species ([Carrasco et al., 2023](#ref-carrasco2023maternal); [Kar et al., 2023](#ref-kar2023heritability)), but we are uncertain about the interactive role of maternal CORT and early temperatures on behavioural flexibility. We stigate here the effect of early environment on behavioural flexibility in both species by manipulating CORT and incubation temperature and then subjecting hatchlings to a reversal learning task. Since invassive species are expected to behave more flexibly ([Amiel et al., 2011](#ref-amiel_smart_2011); [Chapple et al., 2012](#ref-chapple2012can); [Sol et al., 2008](#ref-sol2008brain); [Sol & Lefebvre, 2000](#ref-sol2000behavioural); [Wright et al., 2010](#ref-wright2010behavioral)), we predict that *L. delicata*, on average, will perform more proficiently in the task compared to *L. guichenoti*. Furthermore, given that behavioural flexibility can be advantageous during different stages of invasion ([Chapple et al., 2012](#ref-chapple2012can); [Szabo et al., 2020](#ref-szabo2020can); [Wright et al., 2010](#ref-wright2010behavioral)), and that these phases can involve more than one generation, we anticipate that *L. guichenoti* will be more impacted by the prenatal environment than *L. delicata*. Finally, we predic that both cold incubation temperatures and CORT treatment will impair behavioural flexibility ([Clark et al., 2014](#ref-clark_colour_2014); [Lui et al., 2017](#ref-lui2017chronic)), and that the combination of both treatments to have the most detrimental effect on behavioural flexibility.

## Methods

#### Husbandry

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

*Eggs collection and incubation* – Between mid-October 2022 to the end of February 2023, we provided females with a place to lay the 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 length and width of eggs with a digital caliper to the nearest 0.1 mm and weighed them with a (OHAUS, Model spx123) digital scale ± 0.001g error, and we recorded the clutch and egg identities. Then 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.

*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 (OHAUS, Model spx123) digital scale ± 0.001g 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 water every day and received 3-6 small *A. domestica* crickets three times a week. All care otherwise follows similar protocols to adults (see above).

Two weeks before starting the training phase (see below), lizards were moved to the experimental arena for acclimatation. The arenas were individual medium size (41 L x 29.7 W x 22 H cm) plastic containers with a shelter (9 L x 6 W x 1.5 H cm) on one of the extremes and a water dish on the other. These new enclosures were placed in two rooms in 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 acclimatation 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 22-24ºC.

#### 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 – 23ºC ± 3ºC or Hot – 30ºC ± 3ºC) in a 2x2 factorial design ([Fig. 1](#fig-Methods) A). We first allocated eggs onto one of two different treatments: in the CORT treatment, eggs were topically supplied with 5µL of CORT dissolved in 100% Ethanol (vehicle) at a final (10 pg CORT/mL) concentration; in the Control treatment eggs received an equal volume of the vehicle. CORT concentration employed in the CORT treatment represents 2 standard deviations above the mean natural concentration obtained in eggs from both species (non-published data). Then, eggs were incubated in one of the two previously mentioned temperature regimes (Cold or Hot) until hatching. This temperatures represent the upper and lower limit of the natural incubation temperatures ([Cheetham et al., 2011](#ref-cheetham2011embryonic); [Qualls & Shine, 2000](#ref-qualls2000post)). The number of eggs per clutch assigned to each hormone and temperature treatment were counterbalanced in both species.

#### Quantifying Behavioural Flexibility

Briefly, 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](#fig-Methods) 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](#fig-Methods) 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](#fig-Methods) 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](#fig-Methods) 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. [Fig. 1](#fig-Methods) Habituation phase, Stage 3). Trials in every stage lasted one hour, begining 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 we decided not to use the lizard in the next phases because it did not pass the aqcuisition criterium.

In the colour association (Associative task in Fig. [Fig. 1](#fig-Methods)) and reversal task (Reversal task in [Fig. 1](#fig-Methods) B), we used three ramps that were identical in shape and size but differ 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](#ref-Baden_Osorio_2019_Vert_vision)). 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 6th of March until the 26th 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 and those trials were scored as NA. Here, we analysed the data of the reversal task as a measure of behavioural flexibility ([Brown & Tait, 2010](#ref-brown2010behavioral)).

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| 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. |

#### Statistical analyses

We performed the analyses for each species separately. We fitted two different Bayesian multilevel models using the brm function from the *brms* package ([Bürkner, 2017](#ref-burkner2017brms)) using an R extension (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 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. 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 (pmcmc) using the posterior distributions. We considered statistical significance if pmcmc < 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 thermorregulation opportunity, light and humidity conditions (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 for until they were euthanized following standard humane procedures.

## Results

Originally, 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 if the number of failed tasks (see above) was higher than 15 (n = 3). Final sample sizes per treatment and species are listed in [Fig. 2](#fig-results).

Both species learnt across all treatments ([Fig. 2](#fig-results) 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, pmcmc = 0.873), incubation temperature (Hot - Cold = -0.001, pmcmc = 0.989), or the interaction ([(Control-Hot - CORT-Hot) - (Control-Cold - CORT-Cold)] = -0.034, pmcmc = 0.062) did not affect learning (see [Fig. 2](#fig-results) A, B). We similarly found no significant effects of hormone (Control - CORT = 0.005, pmcmc = 0.810), incubation temperature (Hot - Cold = 0.009, pmcmc = 0.629), or the interaction ([(Control-Hot - CORT-Hot) - (Control-Cold - CORT-Cold)] = -0.016, pmcmc = 0.529) on learning in *L. guichenoti* (see [Fig. 2](#fig-results) C, D).

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| 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, pmcmc = 0.636).

## Discussion

This study aimed to determine the interactive effects of prenatal temperature and GCs on behavioural flexibility in two closely related species, the delicate skink (*Lampropholis delicata*) and the common garden skink (*L. guichenoti*).

Our early environment manipulation did not affect the lizards’ performance in the task. We predicted that prenatal CORT treatment would decrease reversal learning abilities and that high temperatures would minimise these effects. However, our results show no significant differences between treatments in both species. In contrast to previous studies that show high GCs levels impaired performance in different types of learning tests ([Farrell et al., 2016](#ref-farrell_developmental_2016); [Lemaire et al., 2000](#ref-lemaire_prenatal_2000); [Zhu et al., 2004](#ref-zhu_prenatal_2004)), we did not find any effect of CORT on the speed at which lizards learn. Our results are, however, consistent with other experiments where GCs did not impact learning abilities ([Bebus et al., 2016](#ref-bebus_associative_2016); [Szuran et al., 1994](#ref-szuran_water_1994)). In some systems, exposure to CORT during development has hormetic effects on learning and neural structures ([Du et al., 2009](#ref-du_dynamic_2009); [McEwen, 2012](#ref-mcewen_brain_2012)). For example, in Sprague–Dawley rats, exposure to high doses of CORT, but not low doses, reduced density of immature neurons and cell proliferation and in the dentate gyrus ([Brummelte & Galea, 2010](#ref-DOSEneurobrummelte2010chronic)). The CORT treatment employed here is known to impact other aspects such as growth or mortality in these species (unpublished data), but the effect of the same dose can differ between tissues (Lemaire et al. ([2000](#ref-lemaire_prenatal_2000))). More striking is the absence of an effect of incubation temperature since most of the studies in reptiles reported better performance when incubated at higher temperatures in different associative learning and motor tasks ([Amiel et al., 2014](#ref-amiel_egg_2014); [Amiel & Shine, 2012](#ref-amiel_hotter_2012); [Clark et al., 2014](#ref-clark_colour_2014)). Furthermore, those studies that reported cold-incubated lizards learn faster ([Abayarathna & Webb, 2020](#ref-abayarathna_effects_2020); [Dayananda & Webb, 2017](#ref-dayananda_incubation_2017)) used incubation temperatures far beyond the natural range in their hot treatments. We know that our prenatal treatments affect other traits in both species of lizards used in this experiment (unpublished data). However, it is possible that the treatments affected tissues differently. An alternative hypothesis is that the effects of the prenatal treatments are age-dependent (see [Frisone et al., 2002](#ref-frisone2002social)). Here, the 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*, and when this variable was included in the models, we did no find any significant effect of age (see Supplementary material). However, the treatments may have consequences at earlier or later stages of life that are not being captured in our experiment. Conversely, the effects of the early environment may likely vary depending on the cognitive task, as evidenced by studies showing variations in the impact of prenatal GCs and temperature on different brain regions ([Coomber et al., 1997](#ref-coomber_independent_1997); [Lemaire et al., 2000](#ref-lemaire_prenatal_2000); [Sakata et al., 2000](#ref-sakata_neural_2000)). In addition, prenatal GCs can have programmatic effects on HPA axis sensitivity, potentially impacting the lizards’ sensitivity to stressors ([Crino et al., 2014](#ref-crino_corticosterone_2014)). This altered stress response may influence learning dynamics through hormetic effects of CORT ([Du et al., 2009](#ref-du_dynamic_2009)), particularly in stressful contexts ([Taborsky et al., 2021](#ref-taborsky2021towards)); although such effects were not likely observable in our experimental design. 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 at different ages, testing the effect of stress during the learning process, and measuring the neurophysiological effects on different brain nuclei.

Despite sharing life-history traits, only *L. delicata* has proven successful in colonising various global regions ([Baker, 1979](#ref-baker1979rainbow); [Chapple et al., 2013](#ref-chapple2013divergent), [2015](#ref-chapple2015deliinvLHI)), 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. Nevertheless, we did not find significant differences between treatments or species when we compared their performance in a reversal task. We found that, on average, all the lizards from the four treatments and the two species learned 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).

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](#ref-chapple_know_2011)). However, Bezzina *et al.* ([Bezzina et al., 2014](#ref-bezzina2014does)) 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. In addition, we did not find any effect of the treatments on either species. Both results contrast our original predictions and may indicate that other aspects of their biology are more relevant for *L. delicata*’s success as invaders than behavioural flexibility. For instance, the mentioned differences in exploratory behaviour can be the key factor explaining why *L. delicata* and not *L. guichenoti* is better at colonising new areas, since exploring more can also enhance the ability to effectively locate novel resources in *L. delicata* ([Chapple et al., 2011](#ref-chapple_know_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 our initial predictions and suggest that other aspects of *L. delicata* biology may be more influential for their success as invaders than behavioural flexibility alone. The present study also indicates that prenatal CORT and temperature do not affect learning abilities in these species. Nevertheless, it remains possible that the effects of the early environment are task-dependent, and future studies should explore performance across different cognitive tasks and the effects of both treatments on brain function.

## Data accessibility

All data, data description, and R code are availabe in public repository 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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## References

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

Amiel, J. J., Bao, S., & Shine, R. (2017). The effects of incubation temperature on the development of the cortical forebrain in a lizard. *Animal Cognition*, *20*(1), 117–125. <https://doi.org/10.1007/s10071-016-0993-2>

Amiel, J. J., Lindström, T., & Shine, R. (2014). Egg incubation effects generate positive correlations between size, speed and learning ability in young lizards. *Animal Cognition*, *17*(2), 337–347. <https://doi.org/10.1007/s10071-013-0665-4>

Amiel, J. J., & Shine, R. (2012). Hotter nests produce smarter young lizards. *Biology Letters*, *8*(3), 372–374. <https://doi.org/10.1098/rsbl.2011.1161>

Amiel, J. J., Tingley, R., & Shine, R. (2011). Smart Moves: Effects of Relative Brain Size on Establishment Success of Invasive Amphibians and Reptiles. *PLoS ONE*, *6*(4), e18277. <https://doi.org/10.1371/journal.pone.0018277>

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

Baker, J. K. (1979). *The rainbow skink, lampropholis delicata, in hawaii*.

Bebus, S. E., Small, T. W., Jones, B. C., Elderbrock, E. K., & Schoech, S. J. (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>

Bezzina, C. N., Amiel, J. J., & Shine, R. (2014). Does invasion success reflect superior cognitive ability? A case study of two congeneric lizard species (lampropholis, scincidae). *PLoS One*, *9*(1), e86271.

Brown, V. J., & Tait, D. S. (2010). Behavioral flexibility: Attentional shifting, rule switching and response reversal. *Encyclopedia of Psychopharmacology, Springer-Verlag: Berlin*, 209–213.

Brummelte, S., & Galea, L. A. (2010). Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience*, *168*(3), 680–690.

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

Carrasco, M. I., Zhang, J., & Noble, D. W. (2023). *Maternal investment and early thermal conditions affect performance and antipredator responses*.

Chapple, D. G., Miller, K. A., Chaplin, K., Barnett, L., Thompson, M. B., & Bray, R. D. (2014). Biology of the invasive delicate skink (Lampropholis delicata) on Lord Howe Island. *Australian Journal of Zoology*, *62*(6), 498. <https://doi.org/10.1071/ZO14098>

Chapple, D. G., Miller, K. A., Chaplin, K., Barnett, L., Thompson, M. B., & Bray, R. D. (2015). Biology of the invasive delicate skink (lampropholis delicata) on lord howe island. *Australian Journal of Zoology*, *62*(6), 498–506.

Chapple, D. G., Miller, K. A., Kraus, F., & Thompson, M. B. (2013). Divergent introduction histories among invasive populations of the delicate skink (l ampropholis delicata): Has the importance of genetic admixture in the success of biological invasions been overemphasized? *Diversity and Distributions*, *19*(2), 134–146.

Chapple, D. G., Simmonds, S. M., & Wong, B. B. (2012). Can behavioral and personality traits influence the success of unintentional species introductions? *Trends in Ecology & Evolution*, *27*(1), 57–64.

Chapple, D. G., Simmonds, S. M., & Wong, B. B. M. (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*(3), 278–289. <https://doi.org/10.1002/ece3.22>

Cheetham, E., Doody, J. S., Stewart, B., & Harlow, P. (2011). Embryonic mortality as a cost of communal nesting in the delicate skink. *Journal of Zoology*, *283*(4), 234–242.

Clark, B. F., Amiel, J. J., Shine, R., Noble, D. W. A., & Whiting, M. J. (2014). Colour discrimination and associative learning in hatchling lizards incubated at “hot” and “cold” temperatures. *Behavioral Ecology and Sociobiology*, *68*(2), 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*(3), 409–421. <https://doi.org/10.1002/(SICI)1096-9861(19970414)380:3<409::AID-CNE9>3.0.CO;2-6>

Crino, O. L., Bonduriansky, R., Martin, L. B., & Noble, D. W. A. (2023). A conceptual framework for understanding stressinduced physiological and transgenerational effects on population responses to climate change. *Evolution Letters*.

Crino, O. L., Driscoll, S. C., & Breuner, C. W. (2014). Corticosterone exposure during development has sustained but not lifelong effects on body size and total and free corticosterone responses in the zebra finch. *General and Comparative Endocrinology*, *196*, 123–129. <https://doi.org/10.1016/j.ygcen.2013.10.006>

Dayananda, B., & Webb, J. K. (2017). Incubation under climate warming affects learning ability and survival in hatchling lizards. *Biology Letters*, *13*(3), 20170002. <https://doi.org/10.1098/rsbl.2017.0002>

Du, J., Wang, Y., Hunter, R., Wei, Y., Blumenthal, R., Falke, C., Khairova, R., Zhou, R., Yuan, P., Machado-Vieira, R., McEwen, B. S., & Manji, H. K. (2009). Dynamic regulation of mitochondrial function by glucocorticoids. *Proceedings of the National Academy of Sciences*, *106*(9), 3543–3548. <https://doi.org/10.1073/pnas.0812671106>

Farrell, T. M., Morgan, A., & MacDougall-Shackleton, S. A. (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), 1–14. <https://doi.org/10.1007/s10071-015-0908-7>

Feng, S., McGhee, K. E., & Bell, A. M. (2015). Effect of maternal predator exposure on the ability of stickleback offspring to generalize a learned colour–reward association. *Animal Behaviour*, *107*, 61–69.

Frisone, D. F., Frye, C. A., & Zimmerberg, B. (2002). Social isolation stress during the third week of life has age-dependent effects on spatial learning in rats. *Behavioural Brain Research*, *128*(2), 153–160.

Jonson, K. M., Lyle, J. G., Edwards, M. J., & Penny, R. H. (1976). Effect of prenatal heat stress on brain growth and serial discrimination reversal learning in the guinea pig. *Brain Research Bulletin*, *1*(1), 133–150.

Kar, F., Nakagawa, S., & Noble, D. W. (2023). Heritability and developmental plasticity of growth in an oviparous lizard. *Heredity*, 1–10.

Kolbe, J. J., & Janzen, F. J. (2002). Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. *Ecology*, *83*(1), 269–281.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences*, *97*(20), 11032–11037. <https://doi.org/10.1073/pnas.97.20.11032>

Lui, E., Salim, M., Chahal, M., Puri, N., Marandi, E., Quadrilatero, J., & Satvat, E. (2017). Chronic corticosterone-induced impaired cognitive flexibility is not due to suppressed adult hippocampal neurogenesis. *Behavioural Brain Research*, *332*, 90–98.

McEwen, B. S. (2012). Brain on stress: How the social environment gets under the skin. *Proceedings of the National Academy of Sciences*, *109*(supplement\_2), 17180–17185. <https://doi.org/10.1073/pnas.1121254109>

Qualls, F. J., & Shine, R. (2000). Post-hatching environment contributes greatly to phenotypic variation between two populations of the australian garden skink, lampropholis guichenoti. *Biological Journal of the Linnean Society*, *71*(2), 315–341.

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>

Sakata, J. T., 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, R. M., Romero, L. M., & Munck, A. U. (2000). *How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*. *21*(1).

Sol, D., Bacher, S., Reader, S. M., & Lefebvre, L. (2008). Brain size predicts the success of mammal species introduced into novel environments. *The American Naturalist*, *172*(S1), S63–S71.

Sol, D., & Lefebvre, L. (2000). Behavioural flexibility predicts invasion success in birds introduced to new zealand. *Oikos*, *90*(3), 599–605.

Szabo, B., Damas-Moreira, I., & Whiting, M. J. (2020). Can cognitive ability give invasive species the means to succeed? A review of the evidence. *Frontiers in Ecology and Evolution*, *8*, 187.

Szulkin, M., Munshi-South, J., & Charmantier, A. (2020). *Urban evolutionary biology*. Oxford University Press, USA.

Szuran, T., Zimmermann, E., & Welzl, H. (1994). Water maze performance and hippocampal weight of prenatally stressed rats. *Behavioural Brain Research*, *65*(2), 153–155. <https://doi.org/10.1016/0166-4328(94)90100-7>

Taborsky, B., English, S., Fawcett, T. W., Kuijper, B., Leimar, O., McNamara, J. M., Ruuskanen, S., & Sandi, C. (2021). Towards an evolutionary theory of stress responses. *Trends in Ecology & Evolution*, *36*(1), 39–48.

Webb, J. K., Letnic, M., Jessop, T. S., & Dempster, T. (2014). Behavioural flexibility allows an invasive vertebrate to survive in a semi-arid environment. *Biology Letters*, *10*(2), 20131014.

Wright, T. F., Eberhard, J. R., Hobson, E. A., Avery, M. L., & Russello, M. A. (2010). Behavioral flexibility and species invasions: The adaptive flexibility hypothesis. *Ethology Ecology & Evolution*, *22*(4), 393–404.

Zhu, Z., Li, X., Chen, W., Zhao, Y., Li, H., Qing, C., Jia, N., Bai, Z., & Liu, J. (2004). Prenatal stress causes gender-dependent neuronal loss and oxidative stress in rat hippocampus. *Journal of Neuroscience Research*, *78*(6), 837–844. <https://doi.org/10.1002/jnr.20338>

# Suplementary Material

#### Checking the models plots

Model formula for the reversal task is:

Choice ~ trial\_reversal*cort*temp + (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

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2.- *L. guichenoti*

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

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

**?(caption)**

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

**?(caption)**

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

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

Results for *L. delicata*:

| 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\_reversal | 0.04 | 0.01 | 0.02 | 0.06 | 1.00 | 3033.03 | 5261.65 |
| cortControl | -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\_reversal:cortControl | 0.02 | 0.01 | -0.00 | 0.05 | 1.00 | 2939.03 | 4497.28 |
| trial\_reversal:tempHot | 0.02 | 0.01 | -0.01 | 0.04 | 1.00 | 2759.31 | 4331.14 |
| cortControl:tempHot | 0.64 | 0.46 | -0.24 | 1.56 | 1.00 | 2786.33 | 4315.03 |
| trial\_reversal:cortControl:tempHot | -0.03 | 0.02 | -0.07 | 0.00 | 1.00 | 2849.12 | 4135.58 |

**?(caption)**

Results for *L. guichenoti*:

| 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\_reversal | 0.05 | 0.01 | 0.02 | 0.07 | 1.00 | 4170.12 | 4876.09 |
| cortControl | -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\_reversal:cortControl | 0.01 | 0.02 | -0.02 | 0.05 | 1.00 | 3933.61 | 4885.22 |
| trial\_reversal:tempHot | 0.02 | 0.02 | -0.02 | 0.05 | 1.00 | 4123.86 | 4555.44 |
| cortControl:tempHot | 0.35 | 0.63 | -0.93 | 1.58 | 1.00 | 3791.58 | 4842.61 |
| trial\_reversal:cortControl:tempHot | -0.02 | 0.03 | -0.07 | 0.03 | 1.00 | 3623.25 | 4622.64 |

**?(caption)**

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