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Highlights

- Behavioural flexibility is key for adapting to environmental changes.
- Prenatal stress and incubation temperature were predicted to affect flexibility.
- Flexibility remained robust under both prenatal conditions in both species.
- No significant differences in reversal learning were found between species.

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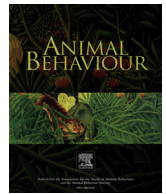
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Early environmental conditions do not impact behavioural flexibility in an invasive and noninvasive lizard species

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

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Behavioural flexibility describes the ability of individuals to adjust behaviour adaptively in response to changes in the internal or external environment (Brown & Tait, 2010). It comprises a suite of behaviours, including problem-solving or innovation (Brown & Tait, 2010) that can be beneficial for adapting to novel circumstances (Szabo et al., 2020; Szulkin et al., 2020). Species with greater behavioural flexibility are expected to be more resilient to environmental changes because behaving flexibly is predicted to be crucial for exploiting new resources and facing new challenges

(Chapple et al., 2012; Szabo et al., 2020; Szulkin et al., 2020; Wright et al., 2010). For example, the successful colonization of new environments by birds is related to a higher frequency of foraging innovations (Sol & Lefebvre, 2000). Although there is limited understanding of the physiological mechanisms involved in animals' ability to behave flexibly, relative brain size is a good predictor of behavioural flexibility in mammals, birds, reptiles and amphibians (Amiel et al., 2011; Sol et al., 2008; Sol & Lefebvre, 2000). This suggests that behavioural flexibility is likely to be underpinned by complex neural mechanisms and that alterations in these mechanisms may impact animals' ability to respond to novel situations. The brain is considered especially sensitive to environmental change during the early stages of development (Zhu et al., 2004),

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creating uncertainty about how the conditions faced during early life might impact behavioural flexibility.

The circumstances faced by their parents can alter the conditions in which organisms develop. For instance, exposing female three-spined stickleback, *Gasterosteus aculeatus*, females to a model predator during ovulation affected their offspring's tendency to use social cues (Feng et al., 2015). Under stressful situations, animal sensory systems detect and transmit environmental signals to instigate adaptive physiological and behavioural adjustments, responses that are mediated by glucocorticoids (GCs; Sapolsky, Romero, & Munck, 2000). GCs can be transmitted to offspring and influence their phenotype and development through transgenerational effects (Crino et al., 2023). In addition to the environments experienced by parents, offspring also experience potentially stressful environmental conditions that can interact with or amplify parental effects. For example, human disturbance alters snapping turtles' nest-site choice, ultimately impacting the temperature at which eggs are incubated (Kolbe & Janzen, 2002). Animals' responses to abrupt temperature changes are mediated by GCs (Crino et al., 2023), which can influence animals' decision-making while nesting (Kolbe & Janzen, 2002). As a result, GC transmission and early thermal environment are expected to interact and shape offspring traits. Both GCs and temperature are known to exert potent effects on brain development (Amiel et al., 2017; Coomber et al., 1997; Jonson et al., 1976; Zhu et al., 2004) and cognition (Clark et al., 2014; Lui et al., 2017), but little is known about how they may interact to affect behavioural flexibility. Since behaving flexibly is essential for dealing with novel environments, understanding how early developmental environments collectively impact cognition is crucial for predicting the consequences of environmental change.

Here, we studied the effects of prenatal temperature and corticosterone (CORT; the main GC in reptiles; Crino et al., 2023) on behavioural flexibility in two species of reptiles, the delicate skink, *Lampropholis delicata*, and the common garden skink, *Lampropholis guichenoti*. Both species have similar life histories and overlapping distributions (Chapple et al., 2011, 2015), but only *L. delicata* has been successful in colonising different areas around the globe (Baker, 1979; Chapple et al., 2015; Chapple, Miller, et al., 2013). Previous studies exploring behavioural differences between the two species have found *L. delicata* to be more exploratory than *L. guichenoti* (Chapple et al., 2011), but no differences in learning were observed between the skinks in an associative learning task (Bezzina et al., 2014). Disparities in behavioural flexibility may be driving the differences in invasion success between both skinks, a prediction supported in other invasive species (Amiel et al., 2011; Chapple et al., 2012; Sol et al., 2008; Sol & Lefebvre, 2000; Wright et al., 2010). However, the relative impact of early environments on behavioural flexibility remains unexplored. Early environments are known to affect suites of morphological and behavioural traits differently in each species (Carrasco et al., 2024; Kar et al., 2024). We manipulated CORT and incubation temperature in both species and then subjected hatchlings to a reversal learning task. Reversal learning is a widely employed tool to measure behavioural flexibility (Gapp et al., 2014; Hurlbise & Howland, 2017), as it assesses an individual's ability to reverse a previously learnt behaviour, providing researchers with a clear indicator of their ability to adjust to new conditions (Brown & Tait, 2010).

We predicted that both cold incubation temperatures and CORT treatment would impair behavioural flexibility (Clark et al., 2014; Lui et al., 2017), and the combination of both treatments would have the most detrimental effect on behavioural flexibility. Since invasive species are expected to behave more flexibly (Amiel et al., 2011; Chapple et al., 2012; Sol et al., 2008; Sol & Lefebvre, 2000; Wright et al., 2010), we predicted that *L. delicata*, on average, will

perform more proficiently in the task compared with *L. guichenoti* and that behavioural flexibility in *L. delicata* will be more robust to early environmental conditions.

METHODS

Husbandry

Breeding colony

We tested juveniles from a breeding colony we established in the laboratory in 2019. A total of 270 and 180 adults of *L. delicata* and *L. guichenoti*, respectively, were established in containers (41.5 L × 30.5 W × 21 H cm) with six lizards (two males and four females) per enclosure. Enclosures had nonstick matting, a shelter and several small water dishes. Water was given daily and they were fed approximately 40 mid-size crickets, *Acheta domestica*, per enclosure 3 days a week. Crickets were dusted with calcium weekly and multivitamins and calcium biweekly. Using a heat cord and a heat lamp, we created a temperature gradient and kept the lights on a 12:12 h light:dark (LD) cycle. We also provided UVA/UVB lighting. Room temperatures were set to 22 °C–24 °C and the warm side of enclosures reached 32 °C.

Eggs collection and incubation

Between mid-October 2022 and the end of February 2023, we provided females with a place to lay eggs using small boxes (12.5 L × 8.3 W × 5 H cm) with moist vermiculite inside that were placed on one side of the communal enclosures (see above). We checked for eggs in the boxes 3 days a week. After collection, we measured the length and width of eggs with a digital calliper to the nearest 0.1 mm and weighed them with a digital scale with a ± 0.001 g error. We also assigned a unique number to the clutch and each egg. Eggs were then treated with CORT or vehicle (see CORT and temperature manipulation below) and were placed in individual cups (80 mL) with moist vermiculite (12 parts water to 4 parts vermiculite). The cups were covered with cling wrap to retain moisture and left in different 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 hatching, we measured juveniles' SVL and tail length with a ruler to the nearest mm and weighed them with a digital scale with a ± 0.001 g error. We then placed hatchlings in individual enclosures (18.7 L × 13.2 W × 6.3 H cm) and provided them with nonstick matting, a shelter and a small water dish. During this period, they were sprayed with water every day and received three to six small *A. domestica* crickets three times a week. All care otherwise followed similar protocols to adults (see above). Two weeks before starting the training phase (see below), lizards were moved to an experimental arena for acclimatisation. The arenas were individual, medium-sized (41 L × 29.7 W × 22 H cm) plastic containers with a shelter (9 L × 6 W × 1.5 H cm) on one of the sides and a water dish on the other. These new enclosures were placed in two rooms across seven different racks that were monitored by seven different closed circuit television (CCTV) systems (device model DVR-HP210475) that allowed us to record their behaviour during the experiment (see details below). Conditions in these rooms were identical to the main room, but 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 on the rack. During acclimatization 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 using

heat cords and heat lamps in a LD 12:12 h cycle. The temperature of both rooms was set to between 22 °C and 24 °C.

Manipulating Early Thermal and CORT Environments

To empirically test the effect of early environment, we manipulated CORT concentration in eggs and incubated them under one of two temperature regimes (cold, 23 °C ± 3 °C or hot, 30 °C ± 3 °C) in a 2 × 2 factorial design (Fig. 1a). We first allocated eggs into one of two different treatments: in the CORT treatment, eggs were topically supplied with 5 µl of CORT dissolved in 100% ethanol (vehicle) at a final concentration of 10 pg CORT/mL; in the control treatment, eggs received an equal volume of the vehicle. This method has been validated before in *L. delicata* (Crino et al., 2024), increasing CORT concentration in eggs by approximately 2 standard deviations above the mean natural concentration. After the hormone treatment, eggs were incubated in one of the two previously mentioned temperature regimes (cold or hot) until hatching. These temperatures represent the upper and lower limits of the natural incubation temperatures (Cheetham et al., 2011; Qualls & Shine, 2000).

We counterbalanced the number of eggs assigned to each hormone and temperature treatment using a partial split-clutch design. We assigned at least one egg per clutch to each treatment

and the remaining eggs were randomly assigned to one of the treatments. When the eggs per clutch were less than four eggs, we assigned each egg randomly to one of the treatments. The final total number of clutches per species is included in the Results.

Quantifying Behavioural Flexibility

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

The habituation phase was divided into three stages: in the first stage, the reward, a small, frozen cricket, *A. domestica*, was placed in an opaque Petri dish (3D × 1.6 H cm; Fig. 1 habituation phase, stage 1); in the second stage, the Petri dish with the cricket was placed on top of the white 3D-printed ramps (Fig. 1 habituation phase, stage 2); and in the third stage, the cricket was left inside a well (3D × 1.75 H cm) on top of the ramp (Fig. 1 habituation phase, stage 3). Trials in every stage lasted 1 h, beginning when the feeding

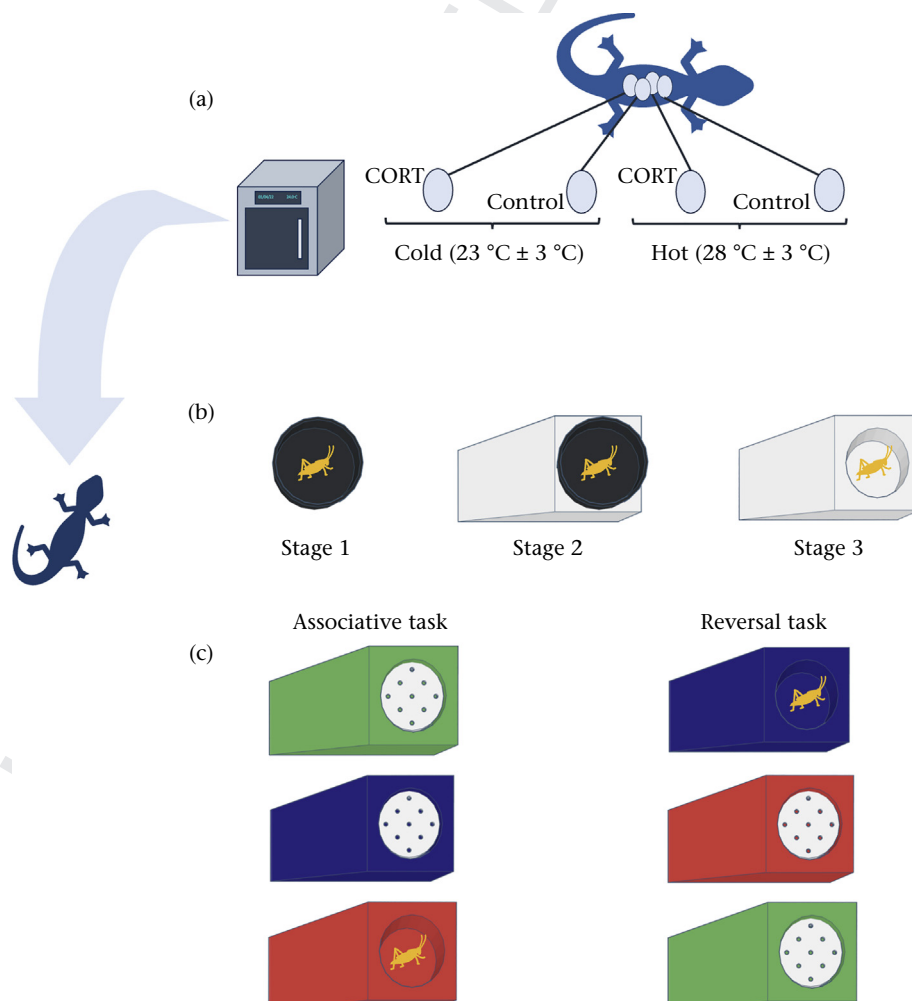


Figure 1. Experimental design of early environment manipulation and learning tasks. (a) Represents the early environment manipulation for both species. (b) Shows the habituation phase with the respective three different stages. (c) Represents the associative and reversal tasks; white lids show the ramps where the food reward was not accessible.

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 complete when the lizards ate the crickets in at least five out of six trials. This phase lasted 38 days, and only in one case a lizard did not progress to the next phase because it did not pass the acquisition criteria.

In the colour association (associative task in Fig. 1) and reversal (reversal task in Fig. 1b), we used three ramps that were identical in shape and size but different in colour. The colours of these ramps were green, red and blue, as previous studies demonstrate that squamates can discriminate between these colours (Baden & Osorio, 2019). However, to control for potential colour biases, we assigned one group of lizards within each treatment to associate food in the blue ramp followed by the red ramp in the reversal, while in the other group, the order was reversed. As in the last stage of the habituation phase, we placed the cricket inside the wells of the ramps, but then two of them were covered with 3D-printed lids (3D × 0.5 H cm) so prey was only accessible on one ramp. The food reward was placed on 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 6 March and 26 June 2023. We conducted tests between 1100 and 1200 hours 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 when the head of the lizard was inside the well of one of the ramps. We considered a trial failed when there was no choice in 1 h of recording. Those trials were scored as NA. Here, we analysed the data of the reversal task as a measure of behavioural flexibility (Brown & Tait, 2010).

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) using an R extension (version 2.8.2; R Core Team, 2021). We ran four parallel MCMC chains of 3000 iterations for each model, with a warmup period of 1000 iterations. We modelled correct choices [correct (1) or not (0)] as the response variable and trial, hormone (CORT versus control) and incubation temperature (cold versus hot), along with the three-way interaction between trial, hormone and temperature as predictors. If early environments impact learning, then we would predict that the rate of learning (i.e. trial slope) varies by treatment as captured by the interactions. The error structure was modelled 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. *Lampropholis delicata* lays one clutch per year, while *L. guichenoti* lays two (Chapple et al., 2011, 2015). Since eggs were collected during half of the breeding season, clutches likely come from different mothers. Additionally, previous research has shown that clutches are generally sired by a single male, but sperm storage can occur (Kar et al., 2024). Given our partial split-clutch design and the fact that maternal effects are expected to be stronger than paternal effects in these species,

including the clutch as a random factor should account for the effects of parental identity.

Learning can also be age-dependent (see Noble et al., 2014), and given that lizards incubated at different temperatures hatch at different times, we explored the effects of age on learning. Indeed, lizards' age at the beginning of the experiment ranged from 41–148 days old in *L. delicata* and 48–132 in *L. guichenoti*. However, when this variable was included in the models, we did not find any significant effect of age (see Supplementary Material). As such, we present models without age as a fixed effect.

We used the posterior distributions of parameters from these models to test for differences in learning rate between treatments and species. Learning slopes were obtained using the 'trial' estimates and its interaction with hormone and temperature treatments. Slope estimates >0 were considered as evidence of learning, while those ≤0 were not. p_{mcmc} were used to test the hypothesis that slopes and slope contrasts are different from zero. We considered an effect statistically significant when $p_{\text{mcmc}} < 0.05$.

Ethical Note

We collected subjects from a breeding colony kept in the laboratory since 2019. Both the breeding animals and the ones used in this experiment were provided humane laboratory housing, with thermoregulatory opportunities, light (UV and heat) and moderate levels of humidity (see above for details). Lizards in the colony received food ad libitum while the subjects in the experiment were fed 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). Animals born in captivity cannot be released into the wild as stringent biosafety protocols across the states prohibit this. It is also not possible to rehome such a large number of animals. Therefore, upon completion of experimental trials, lizards were kept in their enclosure until they were humanely euthanized following approved procedures. To euthanize animals, we used an injection of sodium pentobarbital (i.e. Lethobarb; diluted in 1:4 Ringer's solution) at 60–100 mg/kg, injected intrapleuroperitoneally. We monitored the animals to ensure there was no irritation from the agent, as indicated by distressed animals. Gently pinching the toes was used to confirm the absence of a response before the animal was disposed of.

Crickets were maintained in big communal enclosures (68.5 L × 49 W × 39 H cm) in the same room where the big colony is and at the same temperature and light conditions. They were provided vegetables and water ad libitum, and they were given several egg carton shelters for refuge. All lizards were fed alive crickets except during the experiment when we used crickets frozen at −21 °C for 24 h.

Results

We started with 96 lizards, 48 per species and 12 per treatment per species. However, our final sample size was 84 due to natural mortality ($N = 11$) or failure to complete the training stage ($N = 1$). The final sample sizes per treatment and species are listed in Fig. 2. These animals came from a total of 36 clutches in *L. delicata* and 33 in *L. guichenoti*.

Both species learnt across all treatments (Fig. 2b–d), but we did not find any difference between treatments or species in the rate of learning [mean slopes (denoted as β throughout) per treatment for both species provided in Table S1 in Supplementary Material]. For

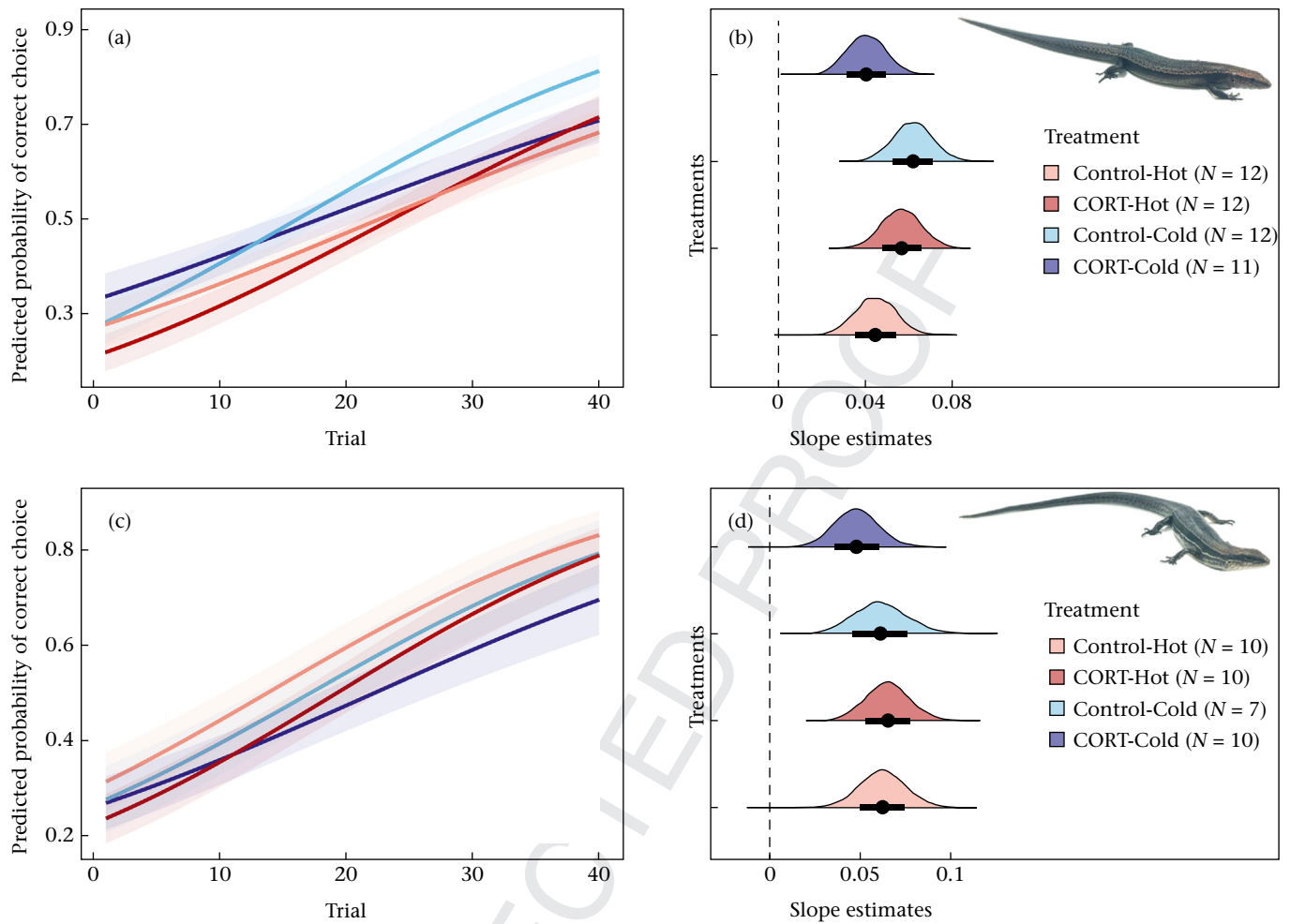


Figure 2. Results for (a), (b) *L. delicata* and (c), (d) *L. guichenoti*. (a) and (c) show the predicted probability of choosing the correct feeder first over trials. The lines represent the mean predicted probability of choosing the correct feeder first on each trial, and the shaded areas indicate 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. (b) and (d) show the distribution of the estimates of slopes per each treatment. The x-axis represents the slope estimate and the y-axis represents the density of the estimates. Different colours indicate the different treatments. Points and bars represent the mean and standard deviation of the mean of the estimates, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

L. delicata, hormone treatment ($\beta_{\text{Control}} - \beta_{\text{CORT}} = 0.005$, 95% CI = $[-0.034, 0.043]$, $p_{\text{mcmc}} = 0.873$), incubation temperature ($\beta_{\text{Hot}} - \beta_{\text{Cold}} = -0.001$, 95% CI = $[-0.039, 0.038]$, $p_{\text{mcmc}} = 0.989$) or the interaction ($[(\beta_{\text{Control-Hot}} - \beta_{\text{CORT-Hot}}) - (\beta_{\text{Control-Cold}} - \beta_{\text{CORT-Cold}})] = -0.034$, 95% CI = $[-0.070, 0.002]$, $p_{\text{mcmc}} = 0.062$) did not affect learning (see Fig. 2a and b). We similarly found no significant effects of hormone ($\beta_{\text{Control}} - \beta_{\text{CORT}} = 0.005$, 95% CI = $[-0.034, 0.046]$, $p_{\text{mcmc}} = 0.810$), incubation temperature ($\beta_{\text{Hot}} - \beta_{\text{Cold}} = 0.009$, 95% CI = $[-0.033, 0.048]$, $p_{\text{mcmc}} = 0.629$) or the interaction ($[(\beta_{\text{Control-Hot}} - \beta_{\text{CORT-Hot}}) - (\beta_{\text{Control-Cold}} - \beta_{\text{CORT-Cold}})] = -0.016$, 95% CI = $[-0.069, 0.035]$, $p_{\text{mcmc}} = 0.529$) on learning in *L. guichenoti* (see Fig. 2c and d).

Finally, when all individuals from all the treatments were pooled together and species were compared, there were no 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 = $[0.030, 0.088]$; $\beta_{L. delicata} - \beta_{L. guichenoti} = -0.008$, 95% CI = $[-0.043, 0.026]$, $p_{\text{mcmc}} = 0.636$). Figures for both species with the raw data are included in the Supplementary Material.

DISCUSSION

This study aimed to determine whether prenatal temperature and GC levels impact behavioural flexibility in two closely related species, the delicate skink, *L. delicata* and the common garden skink, *L. guichenoti*. We expected the early environment to influence behavioural flexibility in both species. We also predicted that *L. delicata* would be more flexible than *L. guichenoti* and less affected by prenatal conditions. Nevertheless, we did not find significant differences between treatments or species when we compared their performance in a reversal task. Our results show that, on average, all the lizards from the four treatments and the two species learnt the reversal task, indicated by estimated slopes that are significantly different from zero, and that they learn, on average, at the same rate. Furthermore, these results were consistent when only those individuals who performed better in the previous associative task were included (see Supplementary Material).

Behavioural flexibility appears to be robust to early exposure to maternal GCs in both *L. delicata* and *L. guichenoti*. We predicted that prenatal CORT treatment would decrease reversal learning abilities.

As such, our results contrast with previous studies that show high GC levels impaired performance in different types of learning tests (Farrell et al., 2016; Lemaire et al., 2000; Zhu et al., 2004). Our results are, however, consistent with other experiments where GC levels did not impact learning abilities (Bebus et al., 2016; Szuran et al., 1994). One possible reason why GC levels did not affect learning in our study may be because GCs can have hormetic effects on brain structure and learning. For example, in Sprague–Dawley rats, exposure to high doses of CORT, but not low doses, reduced the density of immature neurones and cell proliferation in the dentate gyrus (Brummelte & Galea, 2010). Such dose-dependent effects may exist, and our concentrations failed to elicit changes in the brain that would result in impaired behavioural flexibility. We find this possibility unlikely since, in a previous experiment, we observed that a similar dose affected a multitude of other traits, including growth and baseline CORT levels in *L. delicata* (Crino et al., 2024). Nonetheless, we cannot dismiss this possibility because dose effects can differ between tissues (Lemaire et al., 2000). Conversely, the effects of GCs early in development may vary depending on the cognitive task, as evidenced by studies showing variations in the impact of prenatal GC levels on different brain regions (Lemaire et al., 2000). Prenatal GCs can have programmatic effects on hypothalamic–pituitary–adrenal (HPA) axis sensitivity, potentially impacting the lizards' sensitivity to stressors (Crino et al., 2014). This altered stress response may influence learning dynamics through the hormetic effects of CORT (Du et al., 2009), particularly in stressful contexts, of which our experiment was not one (Taborsky et al., 2021). These hypotheses are not mutually exclusive and could be tested in future experiments using multiple doses of prenatal CORT treatment, assessing learning using multiple cognitive tasks and testing the effect of stressors during the learning process.

More striking is the absence of an effect of incubation temperature on behavioural flexibility because most studies have reported better performance in different associative learning and motor tasks when incubated at higher temperatures (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014). However, studies that have reported cold-incubated lizards to learn faster (Abayarathna & Webb, 2020; Dayananda & Webb, 2017) used incubation temperatures far beyond the natural range in their hot treatments. As such, it is unclear how such conditions relate to wild environments. Our results suggest that behavioural flexibility is robust to temperature in both species of skinks, at least in this study. It could be argued that the differences in our incubation temperatures were not extreme enough to elicit significant differences in learning performance. However, the mean differences between the temperatures used in our experiment were similar to those in previous studies (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014) and are aligned with natural variation at the extremes of incubation conditions in the wild (Cheetham et al., 2011; Qualls & Shine, 2000). In lizards, impacts on learning from early thermal environments are associated with changes in neural density (Amiel et al., 2017). However, several studies have shown that the effects of temperature on brain structure and physiology are region-dependent (Amiel et al., 2017; Coomber et al., 1997; Sakata et al., 2000). It is possible that the temperatures used in our experiment did not affect the brain regions involved in coding reversal learning. Future studies should explore the effects of the thermal environment on brain structure and function in these species to understand the mechanisms underlying the effects of temperature on learning. Furthermore, testing learning across different cognitive tasks could provide a more comprehensive understanding of the influence of temperature on cognition. In our analyses, sex was not included as a factor because lizards were not considered sexually mature and because previous studies have found weak evidence for sex-

dependent learning in many cognitive domains (Szabo et al., 2019). Indeed, the impacts of temperature on learning were not sex-dependent for the Eastern three-lined skink, *Bassiana dupeyrieri*, (Amiel & Shine, 2012; Clark et al., 2014). However, some studies show the interactive effects of incubation temperature and sex on brain physiology in other species of reptiles (Coomber et al., 1997; Sakata et al., 2000). In addition, *B. dupeyrieri* are known to be sex-reversed; some individuals can have female chromosomes but male phenotypes under cold incubation temperatures (Dissanayake et al., 2021). Such effects were not easily accounted for in these experiments, making it challenging to understand whether sex or temperature were the major drivers of learning differences (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014). Further research should focus on the possible interactive effects of sex and incubation temperature on cognitive performance. However, such studies will be challenging, given the large sample sizes required to execute these studies rigorously.

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

We acknowledge that sample size may be limiting our ability to detect the small effect sizes in our experiment given the uncertainty around slope contrasts between treatments (see 95% CI of contrasts in results). We analysed the behaviour of 84 individuals coming from a total of 36 unique clutches in *L. delicata* and 33 in *L. guichenoti*, yet it is challenging to do more animals than this given the logistical constraints associated with running such detailed cognitive trials. However, it is noteworthy that our effect sizes are very small and likely of little biological significance. For example, the slopes of CORT-treated animals are 100.094% the slope of controls in *L. delicata* and 96.865% in *L. guichenoti*, while the slope in hot-incubated lizards is 111.582% the slope of those incubated at cold temperatures in *L. delicata* and 127.384% in *L. guichenoti*.

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

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

Author Contributions

Amelia Y. Peardon: Writing – review & editing Data curation, **Basile Mauclaire:** Writing – review & editing, Data curation. **Christopher R. Friesen:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Dalton C. Leibold:** Writing – review & editing, Data curation. **Daniel W.A. Noble:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Kristoffer H. Wild:** Writing – review & editing, Conceptualization. **Ondi L. Crino:** Writing – review & editing, Methodology, Conceptualization. **Pablo Recio:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Data Availability

All data, data descriptions and R code are available in the public repository https://github.com/Pablo-Recio/CORT_Temp_Behavioural_flexibility.

Declaration of Interest

The authors declare no conflict of interest.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at <https://doi.org/10.1016/j.anbehav.2025.123106>.

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), Article e18277. <https://doi.org/10.1371/journal.pone.0018277>
- Bürkner, P.-C. (2017). brms: An R package for Bayesian multilevel models using stan. *Journal of Statistical Software*, 80(1), 1–28. <https://doi.org/10.18637/jss.v080.i01>
- Baden, T., & Osorio, D. (2019). The retinal basis of vertebrate color vision. *Annual Review of Vision Science*, 5, 177–200. <https://doi.org/10.1146/annurev-vision-091718-014926>
- Baker, J. K. (1979). The rainbow skink, *Lampropholis delicata*, in Hawaii. *Pacific Science*, 33(2), 207–212. <https://doi.org/hdl.handle.net/10125/1470>
- 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), Article e86271. <https://doi.org/10.1371/journal.pone.0086271>
- Brown, V. J., & Tait, D. S. (2010). Behavioral flexibility: Attentional shifting, rule switching and response reversal. In I. P. Stolorman, & L. H. Price (Eds.), *Encyclopedia of Psychopharmacology* (pp. 209–213). Berlin: Springer-Verlag. <https://doi.org/10.1007/978-3-540-68706-1>
- 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. <https://doi.org/10.1016/j.neuroscience.2010.04.023>
- Carrasco, M. I., Zhang, J., & Noble, D. W. (2024). Maternal investment and early thermal conditions affect performance and antipredator responses. *Behavioral Ecology*, 35(4), Article arae035. <https://doi.org/10.1093/beheco/arae035>
- 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. <https://doi.org/10.1071/ZO14098>
- Chapple, D. G., Miller, K. A., Kraus, F., & Thompson, M. B. (2013). Divergent introduction histories among invasive populations of the delicate skink (*Lampropholis delicata*): Has the importance of genetic admixture in the success of biological invasions been overemphasized? *Diversity and Distributions*, 19(2), 134–146. <https://doi.org/10.1111/j.1472-4642.2012.00919.x>
- 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? *Ecology and Evolution*, 1(3), 278–289. <https://doi.org/10.1002/ece3.22>
- 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. <https://doi.org/10.1016/j.tree.2011.09.010>
- Chapple, D. G., Whitaker, A. H., Chapple, S. N., Miller, K. A., & Thompson, M. B. (2013). Biosecurity interceptions of an invasive lizard: Origin of stowaways and human-assisted spread within New Zealand. *Evolutionary Applications*, 6(2), 324–339. <https://doi.org/10.1111/eva.12002>
- 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. <https://doi.org/10.1111/j.1469-7998.2010.00764.x>
- 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>
- Coomer, 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. *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](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 stress induced physiological and transgenerational effects on population responses to climate change. *Evolution Letters*, 8(1), 161–171. <https://doi.org/10.1093/evlett/qrada037>
- 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>
- Crino, O. L., Wild, K. H., Friesen, C. R., Leibold, D. C., Laven, N., Peardon, A. Y., Recio, P., Salin, K., & Noble, D. W. A. (2024). From eggs to adulthood: Sustained effects of early developmental temperature and corticosterone exposure on physiology and body size in an Australian lizard. *Journal of Experimental Biology*, 227(24), Article jeb249234. <https://doi.org/10.1242/jeb.249234>
- Dayananda, B., & Webb, J. K. (2017). Incubation under climate warming affects learning ability and survival in hatchling lizards. *Biology Letters*, 13(3), Article 20170002. <https://doi.org/10.1098/rsbl.2017.0002>
- Dissanayake, D. S., Holleley, C. E., & Georges, A. (2021). Effects of natural nest temperatures on sex reversal and sex ratios in an Australian alpine skink. *Scientific Reports*, 11(1), Article 20093. <https://doi.org/10.1038/s41598-021-99702-1>
- 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. <https://doi.org/10.1016/j.anbehav.2015.05.024>
- Gapp, K., Soldado-Magraner, S., Alvarez-Sánchez, M., Bohacek, J., Vernaz, G., Shu, H., Franklin, T. B., Wolfer, D., & Mansuy, I. M. (2014). Early life stress in fathers improves behavioural flexibility in their offspring. *Nature Communications*, 5(1), 5466. <https://doi.org/10.1038/ncomms6466>
- Hurtubise, J. L., & Howland, J. G. (2017). Effects of stress on behavioral flexibility in rodents. *Neuroscience*, 345, 176–192. <https://doi.org/10.1016/j.neuroscience.2016.04.007>
- 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. [https://doi.org/10.1016/0361-9230\(76\)90056-3](https://doi.org/10.1016/0361-9230(76)90056-3)
- Kar, F., Nakagawa, S., & Noble, D. W. (2024). Heritability and developmental plasticity of growth in an oviparous lizard. *Heredity*, 132(2), 67–76. <https://doi.org/10.1038/s41437-023-00660-3>
- 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. [https://doi.org/10.1890/0012-9658\(2002\)083\[0269:IONSSO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0269:IONSSO]2.0.CO;2)
- 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. <https://doi.org/10.1016/j.bbr.2017.05.060>
- Noble, D. W. A., Byrne, R. W., & Whiting, M. J. (2014). Age-dependent social learning in a lizard. *Biology Letters*, 10(7), Article 20140430. <https://doi.org/10.1098/rsbl.2014.0430>
- 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. <https://doi.org/10.1111/j.1095-8312.2000.tb01260.x>
- 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*, 55(3), 139–151. <https://doi.org/10.1159/000006648>
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21(1), 55–89. <https://doi.org/10.1210/edrv.21.1.0389>
- Sol, D., Bacher, S., Reader, S. M., & Lefebvre, L. (2008). Brain size predicts the success of mammal species introduced into novel environments. *American Naturalist*, 172(S1), S63–S71. <https://doi.org/10.1086/588304>
- Sol, D., & Lefebvre, L. (2000). Behavioural flexibility predicts invasion success in birds introduced to New Zealand. *Oikos*, 90(3), 599–605. <https://doi.org/10.1034/j.1600-0706.2000.900317.x>
- Szabo, B., Damas-Moreira, L., & 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. <https://doi.org/10.3389/fevo.2020.00187>
- Szabo, B., Whiting, M. J., & Noble, D. W. (2019). Sex-dependent discrimination learning in lizards: A meta-analysis. *Behavioural Processes*, 164, 10–16. <https://doi.org/10.1016/j.beproc.2019.04.002>
- Szulkin, M., Munshi-South, J., & Charmantier, A. (2020). *Urban evolutionary biology*. Oxford University Press.
- 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](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. <https://doi.org/10.1016/j.tree.2020.09.003>
- 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. <https://doi.org/10.1080/03949370.2010.505580>
- 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>