Effects of prenatal CORT and temperature on the response to a stressor

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## Introduction

Animals constantly face events that can disrupt their homeostasis. These disruptions can be acute, such as a sudden predator attack, or long-term, like prolonged droughts or habitat degradation ([McEwen & Wingfield, 2003](#ref-mcewen_concept_2003); [Sapolsky et al., 2000](#ref-sapolsky_how_2000)). Regardless of the nature of the challenge, organisms must adjust to allostasis to maintain their physiological and behavioural integrity ([Wingfield & Kitaysky, 2002](#ref-wingfield2002endocrine)). In vertebrates, the ability to cope with disruptions in homeostasis is carried out by the hypothalamic-pituitary-adrenal/interrenal (HPA/HPI) axis ([Sapolsky et al., 2000](#ref-sapolsky_how_2000)). This system elicits a physiological response, also known as the stress response, mediated by glucocorticoids (GCs), a group of hormones that facilitate the reallocation of energetic resources to restore homeostasis ([McEwen & Wingfield, 2003](#ref-mcewen_concept_2003); [Sapolsky et al., 2000](#ref-sapolsky_how_2000)). For example, when an animal faces a predatory attack, GCs mobilise energy to support increased locomotor activity, vigilance, or attention to avoid the threat ([Trompeter & Langkilde, 2011](#ref-trompeter2011invader)). In the short term, GCs help organisms cope with acute challenges. However, long-term exposure to GCs can carry significant metabolic costs, leading to immune suppression, impaired growth, or permanent neural alterations ([McEwen, 2017](#ref-mcewen2017neurobiological); [Picard & McEwen, 2014](#ref-picard_mitochondria_2014)). Furthermore, reacting to stressors can reduce the time and energy available for essential activities such as foraging, thermoregulation, or social interactions ([Belliure & Clobert, 2004](#ref-belliure2004behavioral); [Martı́n et al., 2024](#ref-martin2024blind)). As such, animals are predicted to evolve mechanisms to appropriately respond to acute stressors while avoiding the costs in the long term. For example, by regulating their responses to homotypic (repeated and similar) stressors, particularly those that are predictable and non-lethal ([Grissom & Bhatnagar, 2009](#ref-grissom2009habituation)). Habituation - the reduction of physiological responses elicited by exposure to a homotypic stressor ([Pfister, 1979](#ref-pfister1979glucocorticosterone)) - can be crucial to avoid the long-term costs of chronic stress without compromising the response to acute stressors.

Importantly, the process of physiological habituation shares key functional characteristics with the cognitive process of habituation - a form of non-associative learning where individuals reduce their responsiveness to a repeated stimulus ([Thompson & Spencer, 1966](#ref-thompson1966habituation)). In both cases, organisms are repeatedly exposed to a stimulus/homotypic stressor and progressively decrease their response toward it ([Grissom & Bhatnagar, 2009](#ref-grissom2009habituation)). In fact, both behavioural and physiological habituation often co-occur. For example, rhesus macaques (*Macaca mulatta*) showed a decline in blood cortisol concentrations across consecutive days of restraint, accompanied by decreased vocalizations and behavioral agitation ([Ruys et al., 2004](#ref-ruys2004behavioral)). How both types of habituation interplay with each other depends on the nature of the stressor. Physiological habituation does not occur when animals are faced with systemic stressors like hypoglycemia or ether exposure, whereas habituation to processive stressors — those involving primarily psychological qualities, such as restraint or simulated predation - is frequent ([Grissom & Bhatnagar, 2009](#ref-grissom2009habituation)). However, physiological habituation alone is insufficient to explain the behavioural patterns observed when animals are repeatedly exposed to a homotypic processive stressor ([Grissom & Bhatnagar, 2009](#ref-grissom2009habituation); [Jaferi & Bhatnagar, 2006](#ref-jaferi2006corticosterone)). For example, adrenalectomised rats - incapable of physiological habituation - still exhibit cognitive habituation ([Jaferi & Bhatnagar, 2006](#ref-jaferi2006corticosterone)), suggesting a degree of independence between the two processes. While the HPA/HPI axis is paramount for physiological habituation, cognitive habituation also involves neural mechanisms related to sensory processing, learning, and behavioural control, such as the limbic system, the superior colliculus, or the optic tectum ([Dutta & Gutfreund, 2014](#ref-dutta2014saliency); [Grissom & Bhatnagar, 2009](#ref-grissom2009habituation)). Habituation to processive stressors is therefore likely to involve complex interactions between physiological and cognitive processes.

Crucially, the effectiveness of either system depends on how well the underlying neural structures develop and integrate. Many of these brain regions mature concurrently and can be highly plastic during early stages of development ([Amiel et al., 2017](#ref-amiel_effects_2017); [Coomber et al., 1997](#ref-coomber_independent_1997); [Zhu et al., 2004](#ref-zhu_prenatal_2004)). Early-life conditions can shape the structure and function of both neural systems, with long-term consequences for how individuals respond to acute and chronic stressors ([Crino et al., 2020](#ref-crino_under_2020); [Crino et al., 2024](#ref-crino2024eggs); [Van Bodegom et al., 2017](#ref-van2017modulation); [Vargas et al., 2016](#ref-vargas2016early); [Zito et al., 2017](#ref-zito2017early)). One of the key mediators of early-life effects is GC exposure. Elevations in GCs during development exert potent effects on HPA/HPI axis function, altering the response to stressors later in life ([Costantini et al., 2011](#ref-costantini2011meta); [Crino et al., 2024](#ref-crino2024eggs); [Vargas et al., 2016](#ref-vargas2016early); [Zito et al., 2017](#ref-zito2017early)). For example, zebra finches (*Taeniopygia guttata*) nutritionally stressed during early-life had higher levels of CORT than controls at post-hatching day 25, while periodic maternal separation increased basal corticosterone levels in rats (*Rattus norvegicus*) ([Vargas et al., 2016](#ref-vargas2016early); [Zito et al., 2017](#ref-zito2017early)). In parallel, developmental GC exposure can impact brain regions involved in learning, memory, and behavioural flexibility, affecting neural processes relevant to cognitive habituation ([Costantini et al., 2011](#ref-costantini2011meta); [Lemaire et al., 2000](#ref-lemaire_prenatal_2000); [Zhu et al., 2004](#ref-zhu_prenatal_2004)). Therefore, early-life exposure to GCs can have long-lasting effects on the response to both acute and chronic stressors, potentially leading to maladaptive responses in adulthood.

Other environmental factors may also shape stress-related phenotypes, either independently or in combination with GCs. In ectotherms, early thermal conditions play a critical role in cognitive development ([Abayarathna & Webb, 2020](#ref-abayarathna_effects_2020); [Amiel et al., 2017](#ref-amiel_effects_2017)). Incubation temperature affects cognitive abilities such as learning, memory, or perception, likely through changes in brain function and structure ([Abayarathna & Webb, 2020](#ref-abayarathna_effects_2020); [Amiel et al., 2011](#ref-amiel_smart_2011), [2017](#ref-amiel_effects_2017); [Amiel & Shine, 2012](#ref-amiel_hotter_2012); [Dayananda & Webb, 2017](#ref-dayananda_incubation_2017)). In addition, early thermal environment can also affect the development of the HPA/HPI axis ([Crino et al., 2020](#ref-crino_under_2020); [Crino et al., 2024](#ref-crino2024eggs)). For example, in the delicata skink (*Lampropholis delicata*), individuals incubated at cooler temperatures showed increased baseline CORT concentration in the blood ([Crino et al., 2024](#ref-crino2024eggs)). Although these differences are believed to be driven by size rather than direct endocrine effects ([Crino et al., 2024](#ref-crino2024eggs)), they can still have important long-term consequences for how individuals respond to acute and chronic stressors. Importantly, incubation temperature and prenatal CORT may not act independently ([Crino et al., 2023](#ref-Crino_2023); see [Wingfield, 2008](#ref-wingfield_comparative_2008)), and their interaction could produce complex, trait-specific outcomes that shape both physiological and behavioural responses to stress. Yet, the interactive effects of these early-life conditions on stress responses remain largely unexplored.

In this study, we investigate how prenatal CORT and incubation temperature shape the response to a stressor in two species of skinks: the delicate skink (*Lampropholis delicata*) and the common garden skink (*L. guichenoti*). We focus on the effects of these early-life conditions on the behavioural responses to a processive stressor. Specifically, we examine how these factors influence the acute (i.e., the first exposure) and the chronic response (i.e., repeated exposures over time) responses to a simulated predatory attack. Finally, we assessed body mass change across the stress period as a potential indirect cost, while controlling for food intake. We predicted that prenatal CORT exposure would heighten acute stress reactivity and slow habituation, while warmer incubation temperatures would reduce acute responses and enhance habituation. Furthermore, we predicted higher temperatures would buffer the effects of prenatal CORT on stress responses, potentially leading to more flexible behavioural adjustment. Finally, we expected that less flexible stress responses would lead to higher decreases in mass.

## Methods

### Subjects

We used two species of skinks, the delicate skink (*Lampropholis delicata*) and the common garden skink (*L. guichenoti*). These are oviparous, generalist, small (∼35-55 mm snout-vent length (SVL)) lizards that are sympatric in suburban areas throughout south-eastern Australia ([Chapple et al., 2011](#ref-chapple_know_2011), [2014](#ref-chapple_biology_2014)). However, *L. delicata* has been reported as a successful invader in New Zealand and Hawaii, while there are no such reports for *L. guichenoti* ([Chapple et al., 2011](#ref-chapple_know_2011), [2014](#ref-chapple_biology_2014)). Invassiveness does not seem to be related to differences in cognitive abilities ([Bezzina et al., 2014](#ref-bezzina2014does); [Recio et al., 2025a](#ref-recio2025early), [2025a](#ref-recio2025early)), but seems to be associated with differences in personality ([Chapple et al., 2011](#ref-chapple_know_2011)), which may be linked to differences in their ability to respond to acute and chronic processive stressors ([Koolhaas et al., 1999](#ref-koolhaas1999coping)).

### Collection and housing

*Breeding colony* - Lizards came from a breeding colony established in the laboratory since 2019. This colony consisted of 270 adults of *L. delicata* and 180 adults of *L. guichenoti* housed in plastic containers (41.5 L x 30.5 W x 21 H cm) with two males and four females per enclosure. Enclosures were provided with shelter, nonstick matting, and several small water dishes. The lizards were given water daily and were fed approximately 40 mid-size crickets (*Acheta domestica*) per enclosure three days a week. The crickets were dusted with calcium weekly and multivitamins and calcium biweekly. Room temperature was set to 22-24 ºC, but we also provided the enclosures with a heat cord and a heat lamp following a 12 h light:12 h dark cycle, keeping the warm side of the enclosures at 34 ºC.

*Eggs collection and incubation* - Eggs were collected between mid-October 2022 to the end of February 2023. We placed a small box (12.5 L x 8.3 W x 5 H cm) with moist vermiculite on one side of the communal enclosures to provide females with a place to lay the eggs. These boxes were checked three days a week. After egg collection, we measured length and width with a digital caliper to the nearest 0.1 mm and weighed the eggs with a (OHAUS, Model spx123) digital scale with an accuracy of ± 0.001 g error. 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 two incubators at two different temperatures (see CORT and temperature manipulation below) until hatching.

*Hatchlings* - Incubators were checked three times a week for hatchlings. Lizards were measured and weighed immediately after hatching. Snout-vent length (SVL) and tail length (TL) were measured to the nearest millimeter, and weight was recorded using a (OHAUS, Model spx123) digital scale with an accuracy of ± 0.001 g. Hatchlings were then placed in individual enclosures (18.7L x 13.2W x 6.3H cm) with nonstick matting and a small water dish. All care otherwise follows similar protocols to adults (see above).

#### CORT and Temperature manipulation

To test the interactive effects of CORT and incubation temperature, we manipulated CORT concentrations in eggs and incubated them at cold (23 ± 3 ºC) or hot (28 ± 3 ºC) conditions (see [Fig. 1](#fig-Methods) A). We used a partial split clutch design where eggs from a given clutch were distributed equally across the four treatments when clutch sizes were larger than four and randomly across treatments when less than four. Eggs were topically supplied with either 5 µL of crystalline corticosterone (Sigma, Cat. No. C2505) dissolved in 100% ethanol at a final 10 pg CORT/mL concentration (CORT treatment), or an equal volume of 100% Ethanol (Control treatment). We selected doses based on our previous study, where CORT treatment increased mean yolk CORT levels by approximately 2 standard deviations above the mean natural concentration ([Crino et al., 2024](#ref-crino2024eggs)). Mean incubation temperatures represent the lower and upper limits of the natural range of nest temperatures in *L. delicata* [mean nesting temperatures = 27.4 ºC; Cheetham et al. ([2011](#ref-cheetham2011embryonic))]. Nonetheless, the higher temperature treatment (28 ºC) was above the thermal optima estimated for *L. delicata* [Topt = 25 ºC; Pettersen et al. ([2023](#ref-pettersen2023maternal))].

#### Response to acute and long-term stressors

Three weeks before the start of the behavioural tests, we adapted the lizard enclosures to the experimental setup. The enclosures used for the stress response trials were the same as those where the lizards had previously been housed, but modified to include only a single shelter (9 × 6 × 1.5 cm) placed at one end and a water dish in the centre. All other shelter materials and the matting substrate were removed to allow continuous monitoring. The enclosures were then relocated to the experimental rooms, each containing six racks equipped with their own CCTV system (model DVR-HP210475). The number of lizards per treatment was counterbalanced across racks to control for potential effects of room or rack position. In these three weeks, lizards were fed one calcium- and multivitamin-dusted cricket per day, and water was provided *ad libitum*. A temperature gradient was maintained using heat cords and heat lamps on a 12:12 h light–dark cycle, with room temperatures kept between 22–24 °C.

To test the effects of prenatal conditions on the response to a stressor, we simulated a predatory attack - a standardized processive stressor ([Thaker et al., 2009](#ref-thaker2009acute); [Trompeter & Langkilde, 2011](#ref-trompeter2011invader)) - once daily over multiple days. Each trial began with one of the researchers (PR) removing the water dish from the enclosure, followed by the shelter. The lizard was then chased for 60 seconds using a soft paintbrush, simulating the predatory attack. Immediately after the chase, the shelter — but not the water dish — was returned to its original position, marking the start of the behavioural observation period. The lizard’s behaviour was then recorded for one hour. All videos were later analysed by MD, who was blinded to the lizards’ treatment. From each video, we recorded three behavioural variables: i) latency to move — the time spent immobile immediately after the simulated attack; ii) latency to shelter — the time it took the lizard to hide under the shelter after resuming movement; and iii) emergence — whether the lizard emerged from the shelter during the 40 minutes following hiding (1 = emerged, 0 = did not emerge). A lizard was considered to have resumed movement if it moved continuously for at least five seconds, and to be under shelter if no limbs were visible. This procedure was repeated daily for eight consecutive days to assess the effects of long-term exposure to a processive stressor. All trials were conducted between 1000–1400 h, when lizards were most active.

Both before and after the completion of the behavioural tests, lizard mass was measured to the nearest 0.001 g using a digital scale. Change in mass was calculated as the difference between the final minus the initial mass. Change in mass was used as a proxy for the metabolic cost of repeated stress exposure, where mass loss or lower growth was considered as higher costs. To control for hunger levels and potential effects of food ingestion on mass change, we also recorded the number of crickets ingested by each lizard during the acclimation period. To do so, we placed a known number of crickets in each enclosure after each of the trials, and counted the number of crickets remaining after 24 hours. The total number of crickets ingested during the eight days of tests was then used as a covariate in the analyses of mass change. The experimental design is summarised in [Fig. 1](#fig-Methods) B.

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| Fig. 1— Scheme of our experimental design. In panel A, the early environment manipulations leading to four experimental conditions. In panel B, the timeline of the postnatal stress-response assay: on Day 0 and 9, lizard mass was recorded; from Days 1–8, lizards were subjected daily to a simulated predator attack (chased with a soft paintbrush for 1 minute), followed by a 1-hour behavioural recording. Variables measured the latency to move, the latency to shelter, and the probability of emergening from the shelter in 40 minutes after the stressor. |

### Statistical analyses

We performed the analyses for each species separately. We fitted a total of eight models using the *brm* package ([Bürkner, 2017](#ref-burkner2017brms)), which fits Bayesian multilevel models with *Stan* ([Stan Development Team, 2024](#ref-stan)) with R version 4.4.0 ([R Core Team, 2021](#ref-R)). We ran four parallel MCMC chains of 10000 iterations for each model, with a warmup period of 4000 iterations.

We used as the response variables the latency to move, the latency to shelter, and the emergence probability, as well as the change in mass after the eight days of stress exposure. We fitted separate models for each response variable. For the three behavioural variables, we included the prenatal condition (Control-Cold, CORT-Cold, Control-Hot, CORT-Hot), the day of the trial, and their interaction as fixed effects. To facilitate interpretation of the model intercepts, trial day was transformed so that day 1 in the experiment corresponds to day 0 in the model (i.e., the intercept reflects lizard behaviour in response to the first exposure to the stressor). For the variable mass, we included the prenatal condition and the total number of crickets ingested per each individual during the eight days of stress exposure as predictors.

We included each lizard’s random intercept and slope (trial) as a random factor in the behavioural models, but not when mass was the response. For all models, we included the clutch as a random factor. *L. delicata* lays one clutch per year, while *L. guichenoti* lays two ([Chapple et al., 2011](#ref-chapple_know_2011); [Chapple et al., 2015](#ref-chapple2015deliinvLHI)). 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., 2023](#ref-kar2023heritability)). 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.

We used the posterior distributions of model parameters to test for differences between prenatal conditions. For behavioural variables, differences in the model intercepts between conditions were interpreted as differences in the acute response to a stressor (i.e., the first day of exposure), while differences in the slopes were interpreted as differences in behavioural adjustment across repeated exposures (i.e., chronic stress response). For mass change, between-treatments differences reflect differences in the energetic cost of the chronic stressor. We used the 95% Highest Posterior Density Intervals (95% HPDI) using the hdi function in bayestestR ([Makowski et al., 2019](#ref-bayestestR)) to test if the contrasts between treatments or the slopes for each treatment were different from zero.

Differences between species were tested by comparing the posterior distributions of the intercepts and slopes of the different treatments. We used the posterior distributions of the intercepts and slopes to test for differences between species in their response to an acute stressor (intercept) and a chronic stressor (slope). 95% HPDI were used to test the hypothesis that the contrasts differed from zero.

All the estimated parameters in the results were transformed back to original units employing appropriate formulas depending on the distribution employed in the models. The error structure for latency to move, latency to shelter, or mass change followed a lognormal distribution [family = lognormal()], while the probability of emerging from the shelter was modeled using a Bernoulli distribution with a logit link function [family = Bernoulli(link = ‘logit’)]. Change in mass was also rescaled by adding the minimum change in mass recorded before running the models to avoid negative values but transformed back to original values for the presentation of the results.

## Results

We started with 96 lizards, 48 per species and 12 per treatment per species. However, our final sample size was 85 due to natural mortality (n = 9). The final sample sizes per treatment and species are listed in [Fig. 2](#fig-results_beh_deli), [Fig. 3](#fig-results_beh_guich), and [Fig. 4](#fig-results_mass). These animals came from a total of 36 clutches in *L. delicata* and 34 in *L. guichenoti*.

#### Do prenatal conditions affect the behavioural response to an acute stressor?

*Lampropholis delicata*: Incubation at cold temperatures decreased the latency to move after a simulated attack on the first day (Intercept (denoted hereafter as I)Hot - ICold = 52.793; 95% Highest Posterior Density Intervals (95% HPDI) = [-5.229, 136.528]), while neither CORT or the interaction have any effect on latency to shelter or emergence (see [Fig. 2](#fig-results_beh_deli) A, D, G and Table S1 in *Supplementary Material*). We did not find any effects of prenatal temperature, CORT or their interaction on latency to shelter or emergence probability after an acute stressor (see [Fig. 2](#fig-results_beh_deli) A, D, G and Table S1 in *Supplementary Material*).

*Lampropholis guichenoti*: Neither prenatal CORT, temperature, nor their interaction had any effect on the behavioural response to an acute stressor on *L. guichenoti* (see [Fig. 3](#fig-results_beh_guich) A, D, G and Table S2 in *Supplementary Material*).

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| Fig. 2— Behavioural response of Lampropholis delicata to an stressor. |

#### Do prenatal conditions affect the behavioural response to a chronic stressor?

*Lampropholis delicata*: CORT-treated lizards incubated at cold temperatures increased their latency to move over time (Slope (denoted hereafter as β) = 0.183; 95% HPDI = [0.074, 0.282]), while the slopes were not different from zero in the rest of the treatments (see [Fig. 2](#fig-results_beh_deli) B, C and Table S3 in *Supplementary Material*). However, constrasts between between treatments showed no effect of prenatal conditions on the latency to move througout the experiments (see Table S1 in *Supplementary Material*). Lizards from all treatments increased the probability of emerging from the shelter throughout the experiment (β > 0 in all cases, see [Fig. 2](#fig-results_beh_deli) H, I and Table S3 in *Supplementary Material*), with no differences between treatments (see Table S1 in *Supplementary Material*). In contrast, the latency to shelter did not change over time (β = 0 in all cases, see [Fig. 2](#fig-results_beh_deli) E, F and Table S3 in *Supplementary Material*). The contrasts between treatments showed no differences in the slopes (see Table S1 in *Supplementary Material*).

*Lampropholis guichenoti*: Behavioral responses towards a stressor did not change over time in *L. guichenoti* (see [Fig. 3](#fig-results_beh_guich) B, C, E, F, H, I and Table S3 in *Supplementary Material*). There were no significant differences in the behavioural response of *L. guichenoti* to the repeated exposure to an stressor (see [Fig. 3](#fig-results_beh_guich) B, C, E, F, H, I and Table S2 in *Supplementary Material*).

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| Fig. 3— Behavioural response of Lampropholis guichenoti to an stressor. |

#### Do prenatal conditions influence the costs of repeated stress exposure?

*Lampropholis delicata*: Overall, animals grew over time (Median Δmass = 63.993 mg; 95% HDPI = [-6.871 ,161.539]), with no effect of temperature, CORT, or their interaction (see [Fig. 4](#fig-results_mass) A and Table S4 in *Supplementary Material*).

*Lampropholis guichenoti*: Mass of lizards increased over time (Median Δmass = 89.479 mg; 95% HDPI = [58.264 ,139.138]), and the contrasts between treatments showed no differences in the change in mass (see [Fig. 4](#fig-results_mass) B and Table S4 in *Supplementary Material*).

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| Fig. 4— Change in mass of *Lampropholis delicata* and *Lampropholis guichenoti* after eight days of repeated stress exposure. |

#### Do both species differ in their response to an stressor?

*Acute stressor*: The probability of emerging from the shelter after an acute stressor was lower in cold incubated *L. delicata* than *L. guichenoti* for both control (Idelicata - Iguichenoti = -0.858; 95% HDPI = [-0.994, -0.465]) and CORT-treated (Idelicata - Iguichenoti = -0.527; 95% HDPI = [-0.884, -0.124]) lizards, but not in hot incubated animals (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))). There were no differences between species in the latency to move or the latency until sheltering after an acute stressor (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))).

*Chronic stressor*: Over time, *L. delicata* CORT-treated lizards incubated at cold temperatures increased latency to move more than *L. guichenoti* (β~L. deli~ - β~L. guich~ = 0.256; 95% HDPI = [0.088, 0.413]), while the rest of the treatments did not differ between species (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))). The probability of emerging from the shelter increased over time in *L. delicata* more than in *L. guichenoti* (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))), but those differences were significant only in the Control-Cold treatment (β~L. deli~ - β~L. guich~ = 0.778; 95% HDPI = [0.316, 1.313]), while the rest of the treatments did not differ between species (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))). There were no significant differences in the changes on latency to shelter between species (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))).

*Mass change*: increases in mass were not different between species in any of the treatments (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))).

*Table 1. Contrasts of posterior distributions for intercepts and slopes of behavioral variables across treatments, comparing Lampropholis delicata and L. guichenoti. Contrast represent differences in median parameters between species (L. delicata - L. guichenoti) for each treatment. 95% Highest Posterior Density Intervals (95% HPDIs) indicate the probability that contrasts differ from zero.*

|  | | Acute (Intercept) | | Chronic (Slope) | |
| --- | --- | --- | --- | --- | --- |
| Variable | Treatment | Contrast | 95% HPDI | Contrast | 95% HPDI |
| Latency to move (s) | Control-Cold | 4.467 | [-27.869, 35.133] | -0.001 | [-0.188, 0.178] |
|  | CORT-Cold | -25.777 | [-84.765, 24.465] | **0.256** | **[0.088, 0.413]** |
|  | Control-Hot | 43.888 | [-4.652, 102.742] | -0.055 | [-0.221, 0.105] |
|  | CORT-Hot | 55.059 | [-21.544, 146.961] | 0.152 | [-0.006, 0.313] |
| Latency to shelter (s) | Control-Cold | -2.510 | [-95.905, 78.755] | -0.071 | [-0.299, 0.156] |
|  | CORT-Cold | 3.363 | [-168.062, 163.408] | -0.015 | [-0.226, 0.184] |
|  | Control-Hot | 11.912 | [-72.511, 96.112] | -0.040 | [-0.242, 0.169] |
|  | CORT-Hot | 36.959 | [-45.713, 139.245] | -0.191 | [-0.393, 0.015] |
| Emergence | Control-Cold | **-0.858** | **[-0.994, -0.465]** | **0.778** | **[0.316, 1.313]** |
|  | CORT-Cold | **-0.527** | **[-0.884, -0.124]** | 0.373 | [-0.033, 0.789] |
|  | Control-Hot | -0.173 | [-0.525, 0.032] | 0.193 | [-0.203, 0.620] |
|  | CORT-Hot | -0.115 | [-0.506, 0.177] | 0.260 | [-0.127, 0.634] |
| Δmass (mg) | Control-Cold | -40.797 | [-119.454, 51.802] | - | - |
|  | CORT-Cold | 15.628 | [-64.353, 114.095] | - | - |
|  | Control-Hot | -53.569 | [-125.769, 27.889] | - | - |
|  | CORT-Hot | -45.108 | [-106.031, 29.058] | - | - |

## Discussion

Our study shows that early-life conditions can influence how skinks respond to a processive stressor, but the effects differ between species, the nature of th stressor, and the behaviour evaluated. *Lampropholis delicata* incubated at warmer temperatures were less reactive to a simulated predator attack in the first trial, suggesting that early thermal conditions can shape the behavioural response to an acute stressor. In contrast, *L. guichenoti* showed no treatment effects. Habituation patterns also differed. *Lampropholis delicata* increased the probability of emerging from the shelter, suggesting some habituation to the stressor. However, none of the treatments showed habituation in latency to move or go to shelter. No behavioural change was detected in *L. guichenoti* either. Finally, we found no evidence of physiological costs: individuals generally maintained or gained body mass, regardless of treatment. Together, these findings suggest that incubation temperature and prenatal hormone exposure shape stress-related behaviour in a species-specific manner, potentially reflecting differences in flexibility or stress physiology.

#### Cold incubation temperature increases the response to an acute stressor in *L. delicata* but not in *L. guichenoti*

Our results demonstrate that incubation temperature modulates the behavioural response to an acute stressor in *L. delicata* but not in *L. guichenoti*. Hot-incubated *L. delicata* spent less time immobile following a simulated predatory attack, suggesting a faster recovery from acute stress. **This result is consistent with the idea that shorter immobility duration (i.e., shorter freezing time) reflects a less reactive behavioural response, often interpreted as reduced perceived risk or more efficient coping [REFs].** Thus, the reduced latency to move of hot-incubated individuals may indicate reduced stress reactivity. In *L. delicata*, individuals incubated at 23 ºC exhibit elevated baseline CORT ([Crino et al., 2024](#ref-crino2024eggs)), potentially enhancing sensitivity to stress. Therefore, the heightened acute response in cold-incubated individuals may be explained by elevated endogenous CORT levels. For example, **E.G. about CORT and response**.

In contrast, early thermal environment had no effect on *L. guichenoti*’s response to an acute stressor. The species-specific response also warrants discussion. L. delicata exhibited treatment-dependent variation in response to acute stress, while L. guichenoti did not. This difference may reflect distinct developmental plasticity or stress-coping strategies between species. Given that L. delicata is a successful invasive species, greater sensitivity to early-life thermal environments and a more flexible behavioural response to acute threats may confer adaptive advantages in novel environments [REFs]. In contrast, the more canalised stress response in L. guichenoti could reflect reduced behavioural plasticity, potentially limiting its adaptability in changing environments.

Strikingly, we found no effects of prenatal CORT treatment on the acute stress response in either species. Glucocorticoid elevation during development are known to modulate stress-related phenotypes, including behavioural responsiveness to processive stressors [**REF…before?? I might have another in the intro**]. Previous studies have shown that

#### Habituation to stressors varies between species and treatments

Across repeated exposures to the simulated predatory attack, only L. delicata individuals from the CORT-Cold treatment increased their latency to move. Rather than indicating habituation, this pattern may reflect accumulating allostatic load. Habituation is typically inferred from a reduction in stress-related behaviours over time (e.g., decreased latency to resume activity), whereas an increase in latency could reflect mounting stress or impaired coping ([Grissom & Bhatnagar, 2009](#ref-grissom2009habituation)). Thus, the behavioural trajectory of the CORT-Cold group may suggest that these animals became less, rather than more, tolerant to the repeated stressor.

Why did this pattern emerge only in the CORT-Cold L. delicata group? While our CORT treatment did not significantly elevate baseline hormone levels across groups, previous findings indicate that cold incubation alone raises baseline CORT ([Crino et al., 2024](#ref-crino2024eggs)). The combination of prenatal CORT and cold temperature may therefore have produced a cumulative or synergistic effect on HPI axis development and reactivity. This effect might not have been detectable in our acute response measures, but could become apparent over repeated exposures as stress accumulates.

Alternatively, cognitive explanations are also plausible. The cold-CORT combination may impair neural development in regions critical for behavioural flexibility and non-associative learning. Although our prior work found no significant impact of this combination on associative learning (e.g., colour discrimination and reversal learning; Recio et al. ([2025b](#ref-recio2025cognitive)); Recio et al. ([2025a](#ref-recio2025early))), cognitive processes underpinning habituation may rely on different neural substrates. The hippocampus and prefrontal cortex, implicated in behavioural inhibition and decision-making, may be differentially affected by early-life CORT and temperature exposure. Region-specific developmental trajectories could account for divergent effects across learning types.

In contrast, L. guichenoti showed no evidence of behavioural change across repeated stress exposures, regardless of treatment. This further supports the notion of reduced behavioural flexibility or a more rigid stress-response phenotype in this species. It is noteworthy that L. delicata displayed treatment-specific changes even when these did not conform to classical habituation. Such responsiveness may reflect a greater capacity to modulate behaviour based on environmental cues, a trait often associated with invasive potential [REFs].

Interestingly, across all treatments, L. delicata increased their probability of emerging from shelter within 40 minutes of the stressor as the experiment progressed, suggesting that overall risk perception decreased over time. This pattern is consistent with habituation at the level of decision-making rather than immediate post-threat responses (i.e., latency to move). L. guichenoti, in contrast, exhibited no such trend. This species difference in shelter-emergence behaviour provides further support for differential flexibility in stress evaluation and risk assessment. The divergence may again be rooted in differences in developmental plasticity or evolutionary history, with L. delicata potentially possessing a more labile stress-response system suited to fluctuating environments.

The absence of time effects in latency to move or go to shelter for the other treatments in L. delicata suggests that habituation was limited and potentially context- or trait-dependent. Taken together, our findings highlight the complexity of stress habituation and the need to disentangle multiple behavioural and physiological components to fully understand coping strategies.

#### Physiological costs of the stressor were low and did not differ between treatments or species

Despite repeated exposure to a stressor over several days, we found no clear evidence of physiological costs in either species. Most individuals gained mass over the course of the experiment, and there were no treatment or species differences in mass change. This suggests that the imposed stressor, while sufficient to elicit behavioural responses, did not impair growth or energy balance under our conditions. This result is somewhat surprising, given the literature linking chronic stress to impaired growth, particularly in juvenile ectotherms [REFs].

One possible explanation is that the stressor we employed, while ecologically relevant, was not sufficiently intense or frequent to produce measurable physiological effects. In natural settings, stressors may be more variable and compound with other challenges (e.g., food scarcity, thermal extremes), resulting in more pronounced physiological consequences. Our controlled environment, consistent food availability, and short stress exposure duration may have buffered such effects.

Another consideration is that mass change may not fully capture the physiological costs of stress. Other endpoints, such as oxidative stress markers, telomere attrition, or immune function, may be more sensitive to sublethal chronic stress effects [REFs]. Future studies incorporating these physiological markers would provide a more comprehensive picture of the costs associated with repeated stress.

Finally, it is important to note that our behavioural findings suggest differences in stress evaluation and habituation across treatments and species, even in the absence of overt physiological changes. This dissociation underscores the importance of measuring multiple dimensions of the stress response and not relying solely on growth or condition as proxies for stress burden.

#### Conclusion

Our study demonstrates that early-life environments—specifically incubation temperature and prenatal glucocorticoid exposure—shape behavioural responses to acute and repeated stressors in skinks. These effects were species-specific, with L. delicata showing greater responsiveness to early-life conditions than L. guichenoti. Cold incubation increased acute stress reactivity in L. delicata, and the combination of cold and prenatal CORT impaired habituation, potentially via elevated baseline CORT or neural alterations. In contrast, L. guichenoti appeared less flexible in both acute and repeated stress responses.

These findings contribute to our understanding of how early developmental environments shape adult stress phenotypes. They also highlight potential mechanisms underlying behavioural plasticity in invasive species, where adaptability to repeated stressors may facilitate persistence in novel environments. Future work should aim to link behavioural and physiological responses across ontogeny and explore neural mechanisms that underpin different forms of learning and habituation.

#### Cold incubation temperature increases the response to an acute stressor in *L. delicata* but not in *L. guichenoti*

**CHECK PROPER UNITS** Hot-incubated *L. delicata* spent less time frozen after the simulated attack. We did not see any differnces betwen treatments in any other variable recorded. No treatment effect on the response to an acute stressor in *L. guichenoti*. - Why do we consider less time to be less reactive? - Why differences in temp? –> Cold animals had higher baseline CORT levels…but CORT treated too…at least mean treatment but not high. - Why not differences based on CORT? - Why is temperature in *L. delicata* more important for this than in *L. guichenoti*?? Invassiveness??

#### Habituation to stressors varies between species and treatments

Only CORT-Cold *L. delicata* increase the latency to move over time, suggesting allostatic load more than habituation, as we would expect animals to resume their activity earlier with habituation. However, the analyses did not show significant differences between the contrasts. - WHY those resuls? Power? - Why CORT-Cold –> higher baseline CORT levels?? CORT at the levels we used increases slightly baseline CORT blood levels in juveniles of *L. delicata*, this difference was not significant compared to the controls, but it could be enough for increasing at cold temps when CORT levels are already higher than at warm temps ([Crino et al., 2024](#ref-crino2024eggs)). This seems to make CORT-treated *L. delicata* incubated at cold temperatures as reactive to acute stress as Cold-Controls, but less prone to habituation. Alternatively, this response could be due to lower cognitive abilities as expected by CORT + cold. However, this hasn’t been seen in other learning abilities ([Recio et al., 2025b](#ref-recio2025cognitive), [2025a](#ref-recio2025early)). Nevertheless, the effects of temperature and CORT are expectd to be region-dependent, so there could be effects in non-associative learning while not in associative types of learning (i.e., colour association or its reveresal)  
- Why not in *L. guichenoti*?? The latency to move and to go to shelter did not change over time in any other treatment in *L. delicata*. All *L. delicata* increase the probability of emerging in 40 min after the attack, suggesting that the risk perception decreased during the experiment. This did not happen in *L. guichenoti*  
- Risk perception seems the same both at the beginning and during the experiment for all treatments despite the frozen behaviour being higher in Cold-incubated lizards. Why?? - Why differences between species?? Is *L. delicata* more flexible in evaluating risks?? –> Relationship with invasiveness

**CHECK TABLE FOR SPP COMPARISSONS**

#### Physiological costs of the stressor were low and did not differ between treatments or species

There were no “physiological costs” - Most of the individuals grew up…How’s the growth compared to other studies where they were not subjected to a stressor?? What are the usual “costs”?? - Caveats of the methods: other costs

## References

## Supplementary material

*Table S1. Contrasts of the posterior distributions of the predictors for each behavioural variable modelled for L. delicata. The contrasts are between the intercepts and slopes of the different treatments. 95% Highest Posterior Density Intervals (95% HPDI) test the hypothesis that the contrasts are different from zero.*

|  | | Acute (Intercept) | | Chronic (Slope) | |
| --- | --- | --- | --- | --- | --- |
| Variable | Predictor | Contrast | 95% HPDI | Contrast | 95% HPDI |
| Latency to move | Temperature | **52.793** | **[-5.229, 136.528]** | -0.087 | [-0.264, 0.083] |
|  | Hormone | -16.63 | [-111.546, 39.596] | -0.141 | [-0.313, 0.037] |
|  | Interaction | -24.662 | [-129.774, 67.339] | 0.103 | [-0.104, 0.309] |
| Latency to shelter | Temperature | -5.626 | [-148.113, 99.028] | 0.002 | [-0.211, 0.218] |
|  | Hormone | -43.731 | [-179.983, 64.821] | 0.111 | [-0.105, 0.328] |
|  | Interaction | 57.713 | [-89.317, 222.164] | 0.04 | [-0.259, 0.343] |
| Emergence | Temperature | 0.026 | [-0.072, 0.202] | -0.211 | [-0.723, 0.23] |
|  | Hormone | -0.034 | [-0.206, 0.062] | 0.196 | [-0.256, 0.688] |
|  | Interaction | -0.049 | [-0.254, 0.106] | -0.156 | [-0.812, 0.464] |

Contrasts were done by:  
- *Temperature*: βHot - βCold  
- *CORT*: βCORT - βControl  
- *Interaction*: (βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)

*Table S2. Contrasts of the posterior distributions of the predictors for each behavioural variable modelled for L. guichenoti. The contrasts are between the intercepts and slopes of the different treatments. 95% Highest Density Intervals (95% HPDI) test the hypothesis that the contrasts are different from zero.*

|  | | Acute (Intercept) | | Chronic (Slope) | |
| --- | --- | --- | --- | --- | --- |
| Variable | Predictor | Contrast | 95% HPDI | Contrast | 95% HPDI |
| Latency to move | Temperature | -4.167 | [-63.389, 40.131] | -0.008 | [-0.204, 0.187] |
|  | Hormone | -29.909 | [-86.165, 13.848] | 0.093 | [-0.1, 0.29] |
|  | Interaction | 16.753 | [-52.079, 85.672] | 0.053 | [-0.203, 0.328] |
| Latency to shelter | Temperature | -33.326 | [-187.719, 79.288] | 0.081 | [-0.189, 0.327] |
|  | Hormone | -20.692 | [-179.784, 89.621] | 0.061 | [-0.189, 0.331] |
|  | Interaction | 76.887 | [-70.31, 251.413] | -0.167 | [-0.458, 0.135] |
| Emergence | Temperature | -0.525 | [-0.914, 0.073] | 0.128 | [-0.368, 0.629] |
|  | Hormone | 0.153 | [-0.37, 0.707] | 0.049 | [-0.487, 0.501] |
|  | Interaction | -0.322 | [-0.945, 0.383] | 0.316 | [-0.22, 0.902] |

Contrasts were done by:  
- *Temperature*: βHot - βCold  
- *CORT*: βCORT - βControl  
- *Interaction*: (βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)

*Table S3. Values of the slopes for the different treatments for each behavioural variable modelled for both species. 95% Highest Density Intervals (95% HPDI) test the hypothesis that the slopes are different from zero.*

| Species | Variable | Treatment | Median | 95% HPDI |
| --- | --- | --- | --- | --- |
| *L. delicata* | Latency to move | Control-Cold | -0.010 | [-0.111, 0.093] |
|  |  | **CORT-Cold** | **0.183** | **[0.074, 0.282]** |
|  |  | Control-Hot | -0.046 | [-0.149, 0.054] |
|  |  | CORT-Hot | 0.042 | [-0.062, 0.149] |
|  | Latency to shelter | Control-Cold | -0.035 | [-0.191, 0.108] |
|  |  | CORT-Cold | -0.126 | [-0.281, 0.019] |
|  |  | Control-Hot | -0.014 | [-0.162, 0.133] |
|  |  | CORT-Hot | -0.145 | [-0.302, 0.008] |
|  | Emergence | **Control-Cold** | **0.740** | **[0.390, 1.156]** |
|  |  | **CORT-Cold** | **0.461** | **[0.193, 0.762]** |
|  |  | **Control-Hot** | **0.442** | **[0.140, 0.778]** |
|  |  | **CORT-Hot** | **0.318** | **[0.052, 0.597]** |
| *L. guichenoti* | Latency to move | Control-Cold | -0.008 | [-0.161, 0.142] |
|  |  | CORT-Cold | -0.073 | [-0.204, 0.048] |
|  |  | Control-Hot | 0.009 | [-0.116, 0.139] |
|  |  | CORT-Hot | -0.110 | [-0.234, 0.009] |
|  | Latency to shelter | Control-Cold | 0.036 | [-0.136, 0.208] |
|  |  | CORT-Cold | -0.111 | [-0.252, 0.034] |
|  |  | Control-Hot | 0.025 | [-0.114, 0.170] |
|  |  | CORT-Hot | 0.046 | [-0.091, 0.178] |
|  | Emergence | Control-Cold | -0.037 | [-0.353, 0.272] |
|  |  | CORT-Cold | 0.088 | [-0.208, 0.377] |
|  |  | Control-Hot | 0.249 | [-0.000, 0.504] |
|  |  | CORT-Hot | 0.058 | [-0.203, 0.320] |

*Table S4. Contrasts for mass modelled for both species. 95% Highest Density Intervals (95% HPDI) test the hypothesis that the contrasts are different from zero.*

| Variable | Predictor | Contrast | 95% HPDI |
| --- | --- | --- | --- |
| *L. delicata* | Temperature | -34.865 | [-152.725, 74.644] |
|  | Hormone | 3.136 | [-113.478, 116.911] |
|  | Interaction | 45.658 | [-102.047, 193.677] |
| *L. guichenoti* | Temperature | 2.1 | [-42.765, 45.023] |
|  | Hormone | 33.805 | [-9.91, 77.064] |
|  | Interaction | -2.306 | [-63.221, 56.543] |

Contrasts were done by:  
- *Temperature*: βHot - βCold  
- *CORT*: βCORT - βControl  
- *Interaction*: (βControl-Hot - βCORT-Hot) - (βControl-Cold - βCORT-Cold)

*Table S5. Estimates of each predictor for each variable modelled for L. delicata. 95% Highest Density Intervals (95% HPDI) test the hypothesis that the estimates are different from zero.*

| Variable | Predictor | Median | CI |
| --- | --- | --- | --- |
| move | **b\_Intercept** | **3.361** | **[2.720, 4.030]** |
|  | b\_day | -0.010 | [-0.111, 0.093] |
|  | **b\_day:trtCORTMCold** | **0.192** | **[0.046, 0.337]** |
|  | b\_day:trtCORTMHot | 0.053 | [-0.097, 0.197] |
|  | b\_day:trtControlMHot | -0.036 | [-0.182, 0.105] |
|  | b\_trtCORTMCold | 0.264 | [-0.622, 1.218] |
|  | **b\_trtCORTMHot** | **1.288** | **[0.354, 2.202]** |
|  | b\_trtControlMHot | 0.907 | [-0.005, 1.820] |
| shelter | **b\_Intercept** | **4.092** | **[3.405, 4.833]** |
|  | b\_day | -0.035 | [-0.191, 0.108] |
|  | b\_day:trtCORTMCold | -0.092 | [-0.297, 0.123] |
|  | b\_day:trtCORTMHot | -0.110 | [-0.329, 0.105] |
|  | b\_day:trtControlMHot | 0.020 | [-0.182, 0.232] |
|  | b\_trtCORTMCold | 0.801 | [-0.209, 1.826] |
|  | b\_trtCORTMHot | 0.426 | [-0.604, 1.447] |
|  | b\_trtControlMHot | 0.240 | [-0.737, 1.222] |
| emergence | **b\_Intercept** | **-4.421** | **[-6.763, -2.381]** |
|  | **b\_day** | **0.740** | **[0.390, 1.156]** |
|  | b\_day:trtCORTMCold | -0.279 | [-0.749, 0.196] |
|  | b\_day:trtCORTMHot | -0.424 | [-0.913, 0.012] |
|  | b\_day:trtControlMHot | -0.300 | [-0.806, 0.188] |
|  | b\_trtCORTMCold | 0.893 | [-1.758, 3.743] |
|  | b\_trtCORTMHot | 2.062 | [-0.443, 4.837] |
|  | b\_trtControlMHot | 0.596 | [-2.230, 3.480] |
| mass | **b\_Intercept** | **5.066** | **[4.602, 5.542]** |
|  | **b\_food\_ingested** | **0.060** | **[0.031, 0.088]** |
|  | b\_trtCORTMCold | 0.128 | [-0.537, 0.758] |
|  | b\_trtCORTMHot | -0.263 | [-0.934, 0.407] |
|  | b\_trtControlMHot | -0.082 | [-0.749, 0.536] |

*Table S6. Estimates of each predictor for each variable modelled for L. guichenoti. 95% Highest Density Intervals (95% HPDI) test the hypothesis that the estimates are different from zero.*

| Variable | Predictor | Median | CI |
| --- | --- | --- | --- |
| move | **b\_Intercept** | **3.192** | **[2.382, 4.026]** |
|  | b\_day | -0.008 | [-0.161, 0.142] |
|  | b\_day:trtCORTMCold | -0.066 | [-0.263, 0.132] |
|  | b\_day:trtCORTMHot | -0.102 | [-0.303, 0.089] |
|  | b\_day:trtControlMHot | 0.017 | [-0.183, 0.217] |
|  | b\_trtCORTMCold | 0.957 | [-0.135, 2.027] |
|  | b\_trtCORTMHot | 0.705 | [-0.357, 1.752] |
|  | b\_trtControlMHot | 0.107 | [-0.956, 1.209] |
| shelter | **b\_Intercept** | **4.133** | **[3.162, 5.073]** |
|  | b\_day | 0.036 | [-0.136, 0.208] |
|  | b\_day:trtCORTMCold | -0.147 | [-0.364, 0.084] |
|  | b\_day:trtCORTMHot | 0.009 | [-0.205, 0.230] |
|  | b\_day:trtControlMHot | -0.012 | [-0.230, 0.218] |
|  | b\_trtCORTMCold | 0.741 | [-0.502, 1.937] |
|  | b\_trtCORTMHot | -0.124 | [-1.357, 1.083] |
|  | b\_trtControlMHot | 0.037 | [-1.157, 1.239] |
| emergence | b\_Intercept | 1.902 | [-0.499, 4.343] |
|  | b\_day | -0.037 | [-0.353, 0.272] |
|  | b\_day:trtCORTMCold | 0.126 | [-0.307, 0.549] |
|  | b\_day:trtCORTMHot | 0.095 | [-0.313, 0.502] |
|  | b\_day:trtControlMHot | 0.288 | [-0.115, 0.687] |
|  | b\_trtCORTMCold | -1.694 | [-4.859, 1.283] |
|  | **b\_trtCORTMHot** | **-3.261** | **[-6.514, -0.242]** |
|  | **b\_trtControlMHot** | **-3.328** | **[-6.361, -0.289]** |
| mass | **b\_Intercept** | **5.295** | **[5.109, 5.486]** |
|  | **b\_food\_ingested** | **0.029** | **[0.020, 0.039]** |
|  | b\_trtCORTMCold | -0.193 | [-0.440, 0.042] |
|  | b\_trtCORTMHot | -0.176 | [-0.427, 0.050] |
|  | b\_trtControlMHot | 0.002 | [-0.251, 0.243] |

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