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

## Introduction

Animals facing stressors/disruptors and examples.  
Mechanisms involved in the response to stressors/disruptors: endocrinology. Consequences of stressors/disruptors on animals: short-term and long-term.

Predation as a stressor/disruptor.

Can early environment influence the response to stressors/disruptors: physiological AND behavioural effects of GC exposure. Effects on antipredatory behaviour.

Can early environment influence the response to stressors/disruptors: other aspects. Why is temperature important? How is related to antipredatory behaviour?

Hypothesis PRESENTATION:

Both temperature and CORT influence the development of proactive-reactive personality types, associated at the same time with different HPA axis reactivity. In this sense, both increases in prenatal CORT and lower temperatures are related to more reactive individuals [**REFs**], which are more sensitive to stressors. As such, I predict individuals incubated at low temperature and/or treated with CORT to be more reactive, and thus to spent more time frozen after chasing them and not coming out from the shelter. At the same time I expect these individuals to have increased phisiological costs (mass lost) when submitted to a ‘chronic’ stressor.

## 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 not such reports for *L. guichenoti* ([Chapple et al., 2011](#ref-chapple_know_2011), [2014](#ref-chapple_biology_2014)). Invassivenes does not seem to be related to differences in cognitive abilities ([Bezzina et al., 2014](#ref-bezzina2014does); [Recio et al., 2025](#ref-recio2025early), [2025](#ref-recio2025early)), but seems to be associated with differences in personality [**REFpers**], which may be linked to differences in their stress response ([Koolhaas et al., 1999](#ref-koolhaas1999coping)).

### Subjects treatments, 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 approx. 40 mid-size crickets (*Acheta domestica*) per enclosure three days a week. The crickets were dusted with calcium weekly and multivitamin and calcium biweekly. Room temperature was set to 22-24 ºC, but we also provided the enclosures with a heat chord and a heat lamp following a 12 h light:12 h dark cycle keeping warm side of 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 in 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 weighted 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; ([**pettersen2023maternal?**](#ref-pettersen2023maternal))] and reached more stressful temperatures daily as fluctuations occurred (i.e., 31 ºC).

#### 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 once daily over multiple days [**insert relevant reference here**]. 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 a 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 **MATHEO**, who was blinded to the lizards’ treatment. From each video, we recorded three behavioural variables: i) latency to move — the time spent immobile (frozen) 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 mid-term exposure to a stressor. All trials were conducted between 1000–1400 h, when lizards are 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 final minus the initial mass and then rescaled by adding the smallest mass change observed in the dataset, to avoid negative values. This rescaled change in mass was used as a proxy for the physiological cost of repeated stress exposure. 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 resulting in four experimental conditions. In panel B, the timeline of the postnatal stress-response assay. On Day 0, 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 included freezing duration (latency to move), latency to shelter, and emergence from shelter. Final mass was measured on Day 9. |

### Statistical analyses

We performed the analyses for each species separately. We fitted all 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, latency to shelter, and emergence from the shelter after the simulated attack, 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, the total number of crickets ingested during the eight days of stress exposure, and their interaction as predictors.

Lizard identity was included as a random factor in the behavioural models, but it was not included 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); [**chapple2015deliinvLHI?**](#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 ([**kar2023heritability?**](#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 difference reflect differences in energetic cost of the chronic stressor. We used the 95% Highest Posterior Density Intervals (95% HPDI) using the hdi function in bayestestR ([**bayestestR?**](#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).

## 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 = 10). 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 - I Cold = 0.959; 95% Highest Posterior Density Intervals (95% HPDI) = [0.035, 1.904]), 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*: Latency to move after the first exposure in Control-Hot incubated *L. delicata* was higher than for *L. guichenoti* (I~L. deli~ - I~L. guich~ = 0.964; 95% HDPI = [0.021, 1.920]), but there were no differences in Latency to move between the rest of the treatments (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))). In contrast, the probability of emerging from the shelter after an acute stressor was lower in cold incubated *L. delicata* than *L. guichenoti* for both control (I~L. deli~ - I~L. guich~ = -6.322; 95% HDPI = [-9.690, -3.174]) and CORT-treated (I~L. deli~ - I~L. guich~ = -3.750; 95% HDPI = [-6.451, -1.214]) lizards, but not in hot incubated animals (see ([**table\_contrasts\_species?**](#ref-table_contrasts_species))). There were no differences between species in 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 | Control-Cold | 0.168 | [-0.893, 1.210] | -0.001 | [-0.188, 0.178] |
|  | CORT-Cold | -0.524 | [-1.453, 0.421] | **0.256** | **[0.088, 0.413]** |
|  | Control-Hot | **0.964** | **[0.021, 1.920]** | -0.055 | [-0.221, 0.105] |
|  | CORT-Hot | 0.752 | [-0.177, 1.701] | 0.152 | [-0.006, 0.313] |
| Latency to shelter | Control-Cold | -0.041 | [-1.215, 1.180] | -0.071 | [-0.299, 0.156] |
|  | CORT-Cold | 0.026 | [-1.061, 1.089] | -0.015 | [-0.226, 0.184] |
|  | Control-Hot | 0.170 | [-0.928, 1.196] | -0.040 | [-0.242, 0.169] |
|  | CORT-Hot | 0.513 | [-0.562, 1.580] | -0.191 | [-0.393, 0.015] |
| Emergence | Control-Cold | **-6.322** | **[-9.690, -3.174]** | **0.778** | **[0.316, 1.313]** |
|  | CORT-Cold | **-3.750** | **[-6.451, -1.214]** | 0.373 | [-0.033, 0.789] |
|  | Control-Hot | -2.399 | [-5.136, 0.150] | 0.193 | [-0.203, 0.620] |
|  | CORT-Hot | -0.973 | [-3.602, 1.466] | 0.260 | [-0.127, 0.634] |
| Δmass | Control-Cold | -0.229 | [-0.733, 0.282] | - | - |
|  | CORT-Cold | 0.091 | [-0.399, 0.589] | - | - |
|  | Control-Hot | -0.312 | [-0.802, 0.170] | - | - |
|  | CORT-Hot | -0.315 | [-0.834, 0.215] | - | - |

## Discussion

## 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 | **0.959** | **[0.035, 1.904]** | -0.087 | [-0.264, 0.083] |
|  | Hormone | -0.313 | [-1.263, 0.599] | -0.141 | [-0.313, 0.037] |
|  | Interaction | -0.123 | [-1.424, 1.187] | 0.103 | [-0.104, 0.309] |
| Latency to shelter | Temperature | -0.065 | [-1.205, 1.124] | 0.002 | [-0.211, 0.218] |
|  | Hormone | -0.498 | [-1.625, 0.706] | 0.111 | [-0.105, 0.328] |
|  | Interaction | 0.614 | [-0.821, 2.054] | 0.04 | [-0.259, 0.343] |
| Emergence | Temperature | 0.868 | [-1.73, 3.597] | -0.211 | [-0.723, 0.23] |
|  | Hormone | -1.174 | [-3.803, 1.498] | 0.196 | [-0.256, 0.688] |
|  | Interaction | -0.58 | [-4.242, 3.162] | -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 | -0.107 | [-1.144, 1.012] | -0.008 | [-0.204, 0.187] |
|  | Hormone | -0.771 | [-1.869, 0.294] | 0.093 | [-0.1, 0.29] |
|  | Interaction | 0.357 | [-1.075, 1.839] | 0.053 | [-0.203, 0.328] |
| Latency to shelter | Temperature | -0.445 | [-1.827, 1.003] | 0.081 | [-0.189, 0.327] |
|  | Hormone | -0.282 | [-1.731, 1.117] | 0.061 | [-0.189, 0.331] |
|  | Interaction | 0.89 | [-0.801, 2.512] | -0.167 | [-0.458, 0.135] |
| Emergence | Temperature | -2.36 | [-5.836, 0.792] | 0.128 | [-0.368, 0.629] |
|  | Hormone | 0.63 | [-2.423, 4.123] | 0.049 | [-0.487, 0.501] |
|  | Interaction | -1.726 | [-5.809, 2.362] | 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 | -0.231 | [-0.957, 0.466] |
|  | Hormone | 0.021 | [-0.686, 0.747] |
|  | Interaction | 0.308 | [-0.59, 1.248] |
| *L. guichenoti* | Temperature | 0.012 | [-0.224, 0.243] |
|  | Hormone | 0.186 | [-0.05, 0.414] |
|  | Interaction | -0.014 | [-0.328, 0.314] |

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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