**Maternal investment and early thermal environment interact to affect antipredator responses in two Australian lizard species**

Maider Iglesias Carrasco1\*, Jiayu Zhang2, Daniel W.A. Noble2

1 Evolution and Ecology of Sexual Interactions group. Doñana Biological Station-CSIC

2 Division of Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, Australia

\*Corresponding author: miglesias15@gmail.com

**Abstract:**

**Keywords:**

**Introduction**

Gradual and sudden changes in temperature due to anthropogenic activities pose a challenge to organisms, having important consequences on phenotype and fitness. Ectotherms are especially vulnerable to altered thermal conditions, and exposure to increased temperatures during early development have been shown to lead to phenotypic plasticity in morphology, physiology and behaviour, that can impact fitness in a range of taxonomic groups (Dang et al., 2015; Dayananda & Webb, 2017; Raynal et al., 2022; Ślipiński et al., 2021; Valenzuela & Lance, 2004). While research has focused mainly on the effects of early life temperature on individual development, little work has addressed the multifaceted role that environments play across generations. Environmental stress (e.g., changes in thermal or resource conditions) experienced by mothers is expected to cascade to affect offspring through maternal effects, and this may interact in complex ways with environments experienced by offspring.

Maternal effects are important contributors to offspring phenotypic variation (Bernardo, 1996; Noble et al., 2014; Wolf & Wade, 2009), and may moderate the effects of the environment experienced during development. Mothers can adjust their reproductive behaviour or differentially invest in energy and resources that are deposited in eggs in response to changes in the environmental conditions they experience (Carter et al., 2018; Huang et al., 2013; Rutstein et al., 2005). For example, nutrient deprived mothers alter their reproductive allocation compared to mothers fed with normal quality food, leading to changes in offspring phenotype and sex (Warner et al., 2007). Similarly, maternal diet can affect hormone deposition in eggs, which is known to influence hatching success, and offspring phenotype and fitness (e.g., Rosenfeld and Roberts 2004; Warner and Lovern 2014; Rutstein et al. 2005; Huang et al. 2013; Carter et al. 2018). Therefore, maternal effects can exacerbate, dampen or counteract negative phenotypic or fitness effects of early life environments experienced by offspring, and may play an important role in explaining the diversity of phenotypic responses observed within and across species (e.g., Noble et al. 2018). Nonetheless, the interaction between maternal and offspring environments on key fitness traits is seldom explored in detail to better understand the multifaceted role environments will have on phenotypic development and fitness.

Maternal and offspring environments that impact upon behaviour are likely to have important consequences in shaping how organisms interact with their environment. Such behavioural changes can have a direct link to fitness. This is the case with antipredator responses, including predator escape and refuge seeking, since the inability to escape from predatory attacks are inevitably associated with mortality. Thermal developmental conditions and maternal effects are known to affect antipredator strategies. For instance, exposure to high temperatures during early development (e.g., eggs or juveniles) has been shown to affect sprint velocity and a range of antipredator behaviours such as predator avoidance and hiding time (Brodie & Russell, 1999; Dalesman & Rundle, 2010; McDonald & Schwanz, 2018; Webb et al., 2001). Similarly, maternal effects can pre-adapt offspring responses to the prevalent predatory conditions by changing egg composition (Sharda et al., 2021). Such effects are likely mediated by changes in morphology (e.g., size, body condition etc), which in turn can influence the antipredator strategies of individuals (Lancaster et al., 2010; Mcghee et al., 2012; Räsänen et al., 2005). Clearly, thermal conditions and maternal effects independently have the potential to influence a range of fitness-related behaviours, however, whether the two interact to shape offspring responses remains unknown.

Whether and to what extent maternal investment and thermal developmental conditions interact to affect offspring performance and behaviour is expected to be associated with specific evolutionary trajectories and life-history traits (e.g., Manenti et al. 2021, but see Overgaard et al. 2014). To test this, we explore how development temperatures interact with changes in maternal resource investment to shape integrated sets of morphological and behavioural traits related to antipredator responses in two closely related lizard species with different life-histories, *Lampropholis delicata* and *L. guichenoti*. We apply ‘phenotypic engineering’ methods (Sinervo XX) to manipulate both maternal investment in eggs and offspring temperature in a fully factorial design. We predicted that: 1) embryos experiencing high temperatures early in development would be smaller in size with longer tails and have reduced performance (e.g., (Sanger et al., 2018; Tiatragul et al., 2017). As a result, we expect them to be risk adverse relative to embryos experiencing cold temperatures; 2) lower maternal investment in eggs should exacerbate the effects of temperature; 3) *Lampropholis delicata* will be more strongly impacted by temperature and reduced maternal investment because of the smaller egg size and larger clutches compared to *L. guichenoti (REFS)*.

**Methods**

*Study species and housing*

We used two sympatric skink species. The delicate skink (*Lampropholis delicata*) is a small lizard (max. SVL 51 mm) native to south-eastern Australia (Wilson & Swan, 2010). Females lay a single clutch of 3-6 small eggs each year. It has been widely used in experiments to explore how different environments affect the morphology and performance of individuals (Bilcke et al., 2006; Downes & Hoefer, 2007). In addition, it is an important invasive species in some islands (Chapple, 2016; Chapple et al., 2014), which suggests that the species has the potential to respond and become used to novel environmental conditions. The garden skink (*Lampropholis guichenoti*) is a small lizard (max. SVL 48 mm) and widespread across south-eastern Australia (Wilson & Swan, 2010). Females lay one or two clutches of 2-4 large eggs a year. The garden skink has been used in previous studies exploring the effect of rearing temperatures and humidity conditions on offspring phenotypic plasticity (Booth et al., 2000; Qualls & Shine, 1998).

We captured gravid females *L. delicata* and *L. guichenoti* in semiurban parks in Sydney (Australia). Animals were brought to the laboratory at The Australian National University (ANU, Canberra) where they were housed in single-species groups of five in indoor terraria (3-4 females – width x length: 40x55 cm) to allow them to lay eggs. Terraria were filled with approximately 8 cm deep of soil, refuge, a water container, and a container full of vermiculite for egg laying. Terraria were heated by a lamp and had a UV lamp for UVA/UVB exposure. The heat lamp was situated at one end to ensure a temperature gradient. Lights were set to a photoperiod of 12:12 h (light/dark). Animals were provided with water every day (both spraying the soil and filling the water container) and with crickets dusted in calcium and multivitamin every second day. Females were kept in the laboratory for around two weeks for egg-laying and were then released at their capture locations.

*Experimental design*

To explore how incubation temperature and maternal investment interact to affect performance and antipredator behaviour of juveniles, we designed a fully-factorial experiment where eggs of the two skink species were exposed to two levels of temperature [cold (23 + 3° C) or hot (28 + 3° C)] and yolk removal (yolk content reduced or a sham-control) to simulate changes in maternal investment in eggs. Incubation temperatures were selected to mimic extreme temperatures measured in natural nests of *L. delicata* (Cheetham et al., 2011).

Enclosures were checked daily for eggs. We randomly allocated one egg from a clutch to each of our four treatments: 23°C sham-control eggs; 23°C yolk reduced eggs; 28°C sham-control eggs; and 28°C yolk reduced eggs. Our design was a partial split-clutch design as it was not possible to allocate eggs from a given clutch to each of the four treatments (i.e., a split-clutch design) given the small clutch sizes of some individuals. Eggs allocated to the yolk reduced treatment were weighed (to the nearest mg), and then pierced with a sterilised needle to extract part of the yolk. Eggs were weighted again, and the difference in weight pre- and post-extraction was used as an approximation of the percentage of yolk extracted. Following methods in Sinervo, 1990 (Sinervo, 1990) we aimed for around 15% yolk removal (mean =- SD = 12.49% + 2.64). Control eggs were weighted and pierced with a needle, but we did not remove any yolk. Eggs were then placed in a container filled with four grams of vermiculite dampened with 12 g of water and covered with Glad Wrap cling-wrap to avoid dehydration. Each egg was then placed in an incubator at the corresponding temperature.

*Measures of morphological traits, performance, and antipredator behaviour*

We checked the eggs every day for hatchlings and each hatched lizard was individually housed in terraria (20 x 35 cm) heated by a heat cord. All animals also had UV lighting. Enclosures contained paper as substrate, a water container, and a refuge. All the animals were housed in the same laboratory conditions. Juveniles were feed every second day with crickets.

Juveniles were measured and their behaviour tested when they were 3-5 weeks old. We ensured that there was equal representation from each of the four treatment combinations and species (e.g., eggs in cold treatment had longer incubation times) during each measurement session (groups of 48 per day, see below). Body size and mass can influence performance and behaviour (Baxter-Gilbert et al., 2018; Huey & Hertz, 1984). As such, we measured weight, snout-vent length, tail length and total length at each measurement to control for these during the analysis. Given the small size of hatchlings, we weighed lizards (to the nearest mg) using an Ohaus scale and took a ventral photo of each lizard. From this photo we later measured the snout-vent length (SVL) as the distance (in mm) from the snout to the cloaca, and the tail length as the distance from the cloaca to the tip of the tail using imageJ (Abràmoff et al., 2004).

We set up 12 CCTV cameras with each recording four individual terraria simultaneously. Each week we selected 48 juveniles from the correct age window – a mix of the two species and four treatment combinations. We weighted and photographed the animals, and then placed them randomly across the shelves to avoid any biases associated with the location in the laboratory. The terraria for the assays were opaque to avoid lizards viewing each other which could influence their behaviour. In addition, the terraria were separated from the walking corridors by thick-opaque curtains to avoid the presence of the researchers to influence lizard behaviour. All enclosures had a refuge and a water container. The heat lamp was placed on one side of the enclosure to ensure a thermal gradient of at least 6 degrees between the lamp and the refuge. The same group of animals were housed in these terraria during the 6 days of the trials.

Over the six days animals were under cameras we took three measurements of performance and antipredator behaviour for each animal with one day between subsequent measurements. More specifically, we collected the following behavioural variables:

1. *Running velocity (in seconds)*: We measured running performance as the total time needed to run the one-metre-long straight racetrack. We also recorded burst speed as the fast 25 cm section (the racetrack had a detector and time tracker every 25cm). For analysis, we used the total time used to cover the full one meter and the fastest time taken to run a 25 cm interval. Fifteen minutes before each performance measure, lizards were placed in an incubator at 28°C to ensure constant body temperature across lizards. After the trial, each animal was placed back in their corresponding terraria. We repeated the running trial 3 times for each individual, on alternate days.
2. *Activity*: We measured the distance travelled (in cm) as a proxy for the activity level of individuals. To record activity, we removed the refuge and the water container from the terraria to avoid animals hiding. We then switched on the camera and left the animals to behave and move freely in their terrariums for 20 minutes. The distance covered was later calculated using the software EthoVision XT (vers. 12.0).
3. *Antipredator behaviour*. Immediately after the activity trial, we replaced the refuge. After 30 minutes, we simulated a predatory attack. To do this, we approached the terraria and tapped the animals with a painting brush near the tail until they took refuge. The same person (JZ) performed all the predatory attacks. The only identification in the terraria was the randomly allocated ID number provided to the eggs at the beginning of the experiment such that JZ was blind to the treatment. After the simulated attack, we recorded each lizard’s response for 90 minutes. From the videos we calculated (1) the time (in seconds) each lizard took to seek refuge since the first tape in the tail (hereafter “time to hide”) (2) the time between the moment the animal took refuge (time to hide) until the animal’s head appeared at the entrance and was clearly visible in the videos (hereafter “hiding time”, in seconds) and (3) the time elapsed since the animal took refuge (time to hide) to the moment the animal left the refuge to start their normal activity after the predatory attack (hereafter “time to activity”). We consider this to be the moment when the back forelimbs left the refuge. We repeated the activity and antipredator assays three times in alternate days from performance trails.

All trials took place between 9 and 12 am, during the period when activity was the highest. We recorded 22 individuals per species and treatment. We discarded from the analysis any individual that lost their tail during the experiment (n = 5 out of 176) to avoid any bias associated with impaired running ability. The final sample sizes were 22 for hot-control *L. delicata*, and 23:C and 28:R *L. guichenoti*, and 21 for the rest of the combinations.

*Statistical analysis*

We used Bayesian Multivariate Mixed Effects Models using *rstan* () in the package *brms* (version?) to explore whether incubation temperature and maternal investment impacted morphology (tail length, SVL and weight), performance (running velocity and activity level) and antipredator behaviour. We also estimated the correlations between the variables measured at the between- and within-individual level. For all models, we ran four MCMC chains, with each chain being run for 4000 iterations with a warmup of 1000 and used default priors. We retained each sample (thinning of 1) from each chain. We checked that MCMC chains were mixing well by visualising trace plots, checked that all chains had converged (Rhat < 1.01), and that the effective sample size for each parameter was greater than 1000.

We ran two separate multivariate mixed models for each species, one with morphological traits, and the other with performance and antipredator behaviour as response variables. We separated morphology from performance and behaviour because the latter variables were measured 3 times allowing us to decompose between and within-individual variation (O’Dea et al. 2021). Models contained fixed effects (explanatory variables) of incubation temperature and maternal investment treatment along with their interaction. When the interaction between temperature and maternal treatments was non-significant, we refit the models without the interaction to interpret the main effects. We also included individual and clutch identity as random effects (intercepts). In the morphology model, only clutch was added as random effect given that we only had a single measurement for each individual. Given that incubation temperature impacts hatching time hot incubated animals were more likely to be older. To correct for possible age effects on morphology, we added the age scaled to mean of 0 (using the function *scale*) as a covariate in the model. In the behaviour model, SVL was included as covariate to control for any potential effect of body size on the traits measured. We repeated the behaviour/performance model without SVL as covariate to explore for any indirect effect of temperature and maternal treatments on behaviour that might have been influenced by body size. Most of the performance and antipredator variables showed moderate to high repeatability between the three different measures taken (Table S1).

**Results**

*Early thermal environment, but not maternal investment, affect morphology in L guichenoti but not L delicata*

We did not find any effect of temperature, maternal investment, or their interaction on morphology in *Lampropholis delicata* [tail length: (Fig1a), snout-vent-length (SVL, Fig 1b) or weight (Fig 1c) (See Table 1)].

In contrast, maternal investment and temperature treatment interacted to affect weight in *L guichenti* (Table 1). The weight difference between control and yolk removal treatment was larger in the cold incubation temperature compared to the difference under warm incubation temperatures for *L guichenoti* (Table 1). We also found a statistically significant effect of incubation temperature in juvenile size of *L. guichenoti* (Table 1). Generally, individuals coming from eggs incubated at hotter temperatures had longer tails (estimate = 6.347, 95% CI = 9.619– 3.079, Table 1, Fig 1d), larger SVL (estimate = 2.973, 95% CI = 4.715 – 1.241, Table 1, Fig 1e) and were heavier (estimate = 0.112 , 95% CI = 0.207– 0.019, Table1, Fig1f) than those hatched from eggs at colder temperatures.

In both species, there was a positive correlation between SVL, tail length and body mass, with individuals with larger bodies also being heavier and having longer tails (Supp Info Table S2).

**Table** **1–** Posterior means and 95% credible intervals for the interaction between temperature (Temp) and maternal investment (Invest) along with the main effects of temperature and maternal investment on morphological traits for *Lampropholis delicata* and *Lampropholis guichenoti*. Main effects are pooled posterior means over each level of the second predictor variable (either temperature or maternal investment treatments depending on the focal variable). Posterior distributions are estimated from a multi-response model that accounts for the correlation between morphological traits. Bold indicates significant effects.

| **Species** | **Trait** | **Term** | **Estimate** | **2.5%** | **97.5%** | **pMCMC** |
| --- | --- | --- | --- | --- | --- | --- |
| *L. delicata* | SVL | Interaction [(C23 - A23) - (C28 - A28)] | -0.198 | -1.651 | 1.288 | 0.790 |
|  |  | Temp (23-28) | -0.192 | -1.327 | 0.913 | 0.738 |
|  |  | Invest (C-A) | 0.404 | -0.663 | 1.442 | 0.438 |
|  | Weight | Interaction [(C23 - A23) - (C28 - A28)] | 0.010 | -0.035 | 0.057 | 0.665 |
|  |  | Temp (23-28) | -0.006 | -0.042 | 0.031 | 0.728 |
|  |  | Invest (C-A) | 0.002 | -0.031 | 0.038 | 0.918 |
|  | Tail | Interaction [(C23 - A23) - (C28 - A28)] | -2.898 | -6.238 | 0.516 | 0.089 |
|  |  | Temp (23-28) | -1.299 | -4.979 | 2.128 | 0.565 |
|  |  | Invest (C-A) | -0.777 | -4.348 | 2.475 | 0.768 |
| *L. guichenoti* | SVL | Interaction [(C23 - A23) - (C28 - A28)] | 1.324 | -0.501 | 3.124 | 0.150 |
|  |  | **Temp (23-28)** | **-2.973** | **-4.715** | **-1.241** | **0.000** |
|  |  | Invest (C-A) | 0.348 | -1.394 | 2.072 | 0.739 |
|  | Weight | **Interaction [(C23 - A23) - (C28 - A28)]** | **0.089** | **0.006** | **0.172** | **0.035** |
|  |  | **Temp (23-28)** | **-0.112** | **-0.207** | **-0.019** | **0.012** |
|  |  | Invest (C-A) | 0.014 | -0.080 | 0.108 | 0.862 |
|  | Tail | Interaction [(C23 - A23) - (C28 - A28)] | 2.174 | -1.420 | 5.776 | 0.241 |
|  |  | **Temp (23-28)** | **-6.347** | **-9.619** | **-3.079** | **0.000** |
|  |  | Invest (C-A) | 0.902 | -2.361 | 4.147 | 0.627 |

*Maternal investment and/or temperature impact performance in both species.*

Both when and without controlling for body size, we found a statistically significant two-way interaction between maternal yolk investment and incubation temperature in the 25cm burst of juveniles of *L. guichenoti* (SVL as covariate: estimate + SE = -0.41 + 0.20, 95% CI = -0.81– -0.01, Fig 2d, Tables S4-S5). While in the cold incubation temperature individuals coming from the yolk control treatment tended to have slower burst running speed, in the hot incubation treatment the tendency was in the opposite direction, with control individuals being faster at running 25 cm. However, contrasts created using the posterior distribution of parameters did not yield any significant comparison, so we interpret this interaction with caution.

In *L. delicata*, we found that juveniles from the yolk removal treatment took longer to run the 1m long track than those from yolk control treatment (SVL as covariate: estimate + SE = -0.27 + 0.1, 95% CI = -0.46– -0.08, Fig 2a, Tables S3 and S5), both when body size was included as covariate and when not. However, we did not find any effect of incubation temperature, maternal investment or the 2-way interaction between them in neither the 25 cm burst speed or the distance travelled (Fig2a-c, Tables S3, S5).

In both species, trials where individuals had a faster burst speed also took shorter to travel the full 1m rack (*L. delicata*: within-individual correlation + SE = 0.42 + 0.06, 95% CI = 0.29–0.54, Table S3; *L. guichenoti*: within-individual correlation + SE = 0.51 + 0.06, 95% CI = 0.4–0.62, Table S4).

*Antipredator behaviour is affected by early thermal environment and maternal investment in L. delicata but not in L. guichenoti*

The time to activity after a simulated predatory attack depended on both incubation temperature and maternal yolk investment treatment in *L. delicata* (interaction estimate + SE = -1008.81 + 503.67, 95% CI = -2023.9– -22.82, Table S3) after controlling for body size. While in the yolk control treatment individuals reared at both hot and cold temperatures showed similar activity time, in the yolk reduced treatment individuals reared at hot temperatures took longer to be active again after a simulated predatory attack (Figure 3b). However, the comparison of the posteriors was non-significant (HPDI: -9.024 – 26.115), so we interpret this 2-way interaction with caution. When body size was removed from the model, incubation temperature had a significant effect on hiding time and time to activity: individuals reared at hot temperatures spent more time hiding (estimate + SE = 486.62 + 229.06, 95% CI = 39.17– 940.23, Table S5) and took longer to be active again after a predatory attack (estimate + SE = 656.5 + 250.09, 95% CI = 173.99–1150.97, Table S5). This suggested that treatment effects on these behavioural traits are driven by the impacts they have on body size. Antipredator responses of *L. guichenoti* (Tables S4-S5) were not influenced by early incubation temperature, maternal investment or their interaction between these factors neither when and without correcting for body size (Fig3c,d, Table S4).

In *L. delicata*, we found a correlation at the between-individual level between the distance travelled and time to activity. More active individuals were faster to become active after a simulated predatory attack (estimate + SE = -0.2 + 0.09, 95% CI = -0.37- -0.01). However, we did not find any correlation between running performance and anti-predator behaviour in either of the two species (Table S4), indicating that individuals who ran slower on average did not also exhibit risk-adverse behaviours.

**Table** **2 *–*** Posterior means and 95% credible intervals for the interaction between temperature (Temp) and maternal investment (Invest) along with the main effects of temperature and maternal investment on behavioural and performance traits for *Lampropholis delicata* and *Lamprpholis guichenoti*. Main effects are pooled posterior means over each level of second predictor variable. Estimates are from a Bayesian multivariate (multi-response) model not controlling for SVL. See Supplement for model with SVL controlled. Bold estimates are significant and italics indicated effects with less than a 10% chance of being observed.

| **Species** | **Trait** | **Term** | **Estimate** | **Q2.5** | **Q97.5** | **pMCMC** |
| --- | --- | --- | --- | --- | --- | --- |
| *L. delicata* | log Emergence Time (s) | *Interaction [(C23 - A23) - (C28 - A28)]* | *-873.199* | *-1,862.572* | *85.763* | *0.074* |
|  |  | Temp (23-28) | -672.019 | -1,697.909 | 319.779 | 0.239 |
|  |  | Invest (C-A) | 240.400 | -823.755 | 1,223.509 | 0.717 |
|  | log Time Snout Out (s) | Interaction [(C23 - A23) - (C28 - A28)] | -720.770 | -1,617.964 | 180.714 | 0.111 |
|  |  | Temp (23-28) | -498.062 | -1,396.534 | 387.565 | 0.333 |
|  |  | Invest (C-A) | 120.330 | -797.114 | 982.923 | 0.817 |
|  | Distance Moved (cm) | Interaction [(C23 - A23) - (C28 - A28)] | 34.728 | -172.455 | 253.488 | 0.760 |
|  |  | Temp (23-28) | 33.924 | -124.793 | 187.184 | 0.656 |
|  |  | Invest (C-A) | -49.849 | -205.973 | 110.220 | 0.526 |
|  | log 1m Speed (cm/s) | *Interaction [(C23 - A23) - (C28 - A28)]* | *0.331* | *-0.035* | *0.699* | *0.074* |
|  |  | Temp (23-28) | -0.028 | -0.409 | 0.349 | 0.922 |
|  |  | Invest (C-A) | -0.263 | -0.642 | 0.111 | 0.223 |
|  | log Burst Speed (cm/s) | Interaction [(C23 - A23) - (C28 - A28)] | 0.210 | -0.172 | 0.600 | 0.278 |
|  |  | Temp (23-28) | -0.060 | -0.394 | 0.270 | 0.738 |
|  |  | Invest (C-A) | -0.096 | -0.431 | 0.233 | 0.600 |
| *L. guichenoti* | log Emergence Time (s) | Interaction [(C23 - A23) - (C28 - A28)] | 0.000 | -0.653 | 0.658 | 1.000 |
|  |  | Temp (23-28) | -0.022 | -0.482 | 0.435 | 0.922 |
|  |  | Invest (C-A) | 0.036 | -0.420 | 0.498 | 0.882 |
|  | log Time Snout Out (s) | Interaction [(C23 - A23) - (C28 - A28)] | 0.025 | -0.912 | 0.927 | 0.948 |
|  |  | Temp (23-28) | -0.148 | -0.798 | 0.503 | 0.656 |
|  |  | Invest (C-A) | 0.025 | -0.627 | 0.667 | 0.939 |
|  | Distance Moved (cm) | Interaction [(C23 - A23) - (C28 - A28)] | 131.108 | -187.723 | 453.171 | 0.412 |
|  |  | Temp (23-28) | 7.628 | -247.054 | 268.843 | 0.962 |
|  |  | Invest (C-A) | -24.269 | -279.812 | 235.172 | 0.852 |
|  | log 1m Speed (cm/s) | Interaction [(C23 - A23) - (C28 - A28)] | -0.186 | -0.567 | 0.198 | 0.337 |
|  |  | Temp (23-28) | 0.116 | -0.204 | 0.441 | 0.495 |
|  |  | Invest (C-A) | -0.027 | -0.346 | 0.294 | 0.875 |
|  | log Burst Speed (cm/s) | Interaction [(C23 - A23) - (C28 - A28)] | **-0.478** | **-0.866** | **-0.087** | **0.017** |
|  |  | Temp (23-28) | 0.134 | -0.336 | 0.606 | 0.775 |
|  |  | Invest (C-A) | -0.035 | -0.504 | 0.434 | 0.951 |

**Discussion**

Environmental conditions experienced by offspring are expected to interact in complex ways with the environments experienced by their parents. Anthropogenic climate change and other human-associated stressors will simultaneously alter temperatures while impacting other factors such as maternal food availability and stress levels that could affect the maternal investment in eggs. In ectotherms, temperature conditions during early life are known to have important effects on individuals (Noble et al. 2018; While et al. 2018; Singh et al., 2020), but little work has explored the interaction between maternal investment and temperatures; even though there have been suggestions of these factors explaining variation thermal effects (e.g., Noble et al. 2018). Understanding how maternal effects interact with offspring environments to affect development and fitness is important for ascertaining whether climate induced changes are likely to be exacerbated or dampened by impacts in parental generations and help to explain why responses vary so dramatically across populations and species with respect to early thermal conditions. Here we explored whether cold and hot incubation temperatures lead to different responses in morphology, performance and antipredator behaviour in two sympatric skink species (*L. delicata* and *L. guichenoti*), and whether a controlled manipulation in the amount of yolk in eggs moderated such responses. We found that hot incubation temperatures affect morphology, and that temperature interacts with maternal yolk treatment to moderate performance and the associated antipredator responses. Effects we observed appear to be species-specific possibly the result of different life-history strategies.

*Effect of Early Life Thermal Conditions and Maternal Investment on Morphology*

We found that juveniles of *L. guichenoti* emerging from eggs incubated at hot temperatures were larger and heavier than those reared at cold temperatures, however incubation temperature did not significantly affect morphology in *L. delicata*. To test whether temperature had an effect on the morphology of recently hatched individuals, or instead the effect of incubation temperature observed in *L. guichenoti* appeared later during the juvenile development, we performed an *ad hoc* analysis, exploring the effect of incubation temperature on morphology on the day of hatching (data collected for another study). We found that in both species, individuals from the hot and cold incubation treatments were similar in size the day of hatch. This suggests that the incubation temperatures selected for our study have no effect on the morphology of *L. delicata*, while the difference in body size between juveniles coming from cold and hot incubation regimes in *L. guichenoti* appeared during juvenile development on the 3-5 weeks elapsed between hatching and the behavioural tests. Our results suggest that rather than a change in metabolic and developmental rates during embryo growth of this species, incubation temperature programmed a post-hatching metabolic and growth physiology, potentially affecting feeding and growing rates, as observed in previous studies in lizards exposed to high temperatures (Singh et al., 2020).

We did not find any effect of the maternal investment treatment in either *L. guichenoti* or *L. delicata* morphology. In both species, juveniles from the yolk reduced treatment were generally smaller at hatching than those from the control (see also e.g., Warner and Lovern 2014). However, maternal investment effects on morphology seem to have disappeared by the age of the behavioural tests. This agrees with previous studies that have found that maternal investment impact on a range of morphological and behavioural traits on young juveniles, effect that often disappears as the individuals age (McDonald & Schwanz, 2018; Pearson & Warner, 2016). The fact that juveniles of both yolk reduced and control eggs attain a similar body size a few weeks after hatch suggests that individuals can, to some extent, compensate the poor start in life by accelerating their growing rates during the first few weeks, probably by increasing their feeding rates. This might be key, since in a range of species, larger juvenile body size has been associated with better survival (e.g., (Einum & Fleming, 2000; Webb et al., 2006), although this relationship is sometimes complex and dependant of other ecological and biological factors (Langkilde & Shine, 2005; Sinervo et al., 1992; Warner & Andrews, 2002). However, such growth compensation might be associated with changes in the allocation of resources and trade-offs with other fitness-associated traits during development, such as the observed slower running speed of juveniles of *L. delicata* hatched from yolk-reduced eggs and of *L. guichenoti* hatched from yolk-reduced and hot incubated eggs. This result suggests that even though the animals from poor development conditions are able to morphologically catch up with individuals from more beneficial early-life conditions, this might come at a cost regarding performance. This is in accordance with previous studies that find that, for example, in the lizard *Sceloporus undulatus*, clutches with individuals with fast grow rates, but slow runners, had lower survival rates than slow growing but fast runners (Warner & Andrews, 2002).

Impaired performance was expected to impact the antipredator response of individuals to compensate for a potential increased vulnerability to predators. In contrast to our prediction, we did not find any effect of the incubation treatments in the antipredator behaviour of *L. guichenoti*. Interestingly, however, we found that juvenile *L. delicata* from the yolk-reduced treatments, and especially when reared at hot temperatures (i.e., temperature had a significant effect when body size was not included as covariate), took longer to resume activity after a simulated predatory attack than those from the control treatment. Although we must be cautious with the interpretation because within-individual response in the model did not show any correlation between performance and antipredator responses, our result could suggest that a reduction in running speed might impose a survival cost in *L. delicata*. In consequence, individuals with impaired running speed can alter their antipredator behaviour and hide for longer to successfully avoid a predatory attack according to their perceived higher vulnerability (e.g., (Cooper, 2007; Iglesias-Carrasco et al., 2016; Martin & López, 1999). Increased hiding time can be beneficial in terms of predator avoidance, however, the time spent avoiding predators is expected to trade-off with other fitness-enhancing activities such as feeding and mating (Cooper, 1999; Lima & Dill, 1990; Sih, 1992). From our experiment we cannot know whether the change in antipredator response observed confers a fitness advantage or instead would lead to costs in terms of for instance, prey capture ability and growth in the wild, so further experiments would benefit from studying how incubation temperature and maternal investment interact to affect behaviour, and the consequent fitness payoffs, in a more natural setting.

The two skink species studied differed in their morphological, performance and antipredator responses to the incubation temperature and maternal investment treatment. These differences could be in part associated with some life-history traits, such as the size of the egg. Eggs of *L. delicata* are smaller than those of *L. guichenoti* (), which might make these eggs more sensitive to small alterations in the incubation environment, strongly impacting the phenotype and behaviour of juveniles, as observed in our study. While this result suggests that environmental changes in early thermal environment coupled with reduced maternal investment will impact *L delicata* more negatively compared to *L guichenoti* we caution over interpretation because that mayThe*L delicata’s*

*Conclusions*

We have shown that exploring the complex interaction between offspring and maternal environments is critical to predict how anthropogenic activities will affect individual performance and ultimately fitness. Although maternal yolk investment did not buffer the effects of higher incubation temperature in all the morphological and behavioural traits measured, the general pattern suggests that a reduction in the resources allocated by mothers to eggs exacerbates the response triggered by warmer temperatures. Future studies will benefit from studying whether such plastic responses are adaptive in the novel environment conditions, whether more extreme temperatures predicted by climate change will exert stronger reactions, and whether species sensitivity depends on species-specific life-history traits.

**Acknowledgements:** We thank Kai Dewar, Eoin Noble and Rose Zhang for help in the laboratory. Funding was provided by an ARC Discovery Project to DWAN (DP210101152). Lizards were captured under the NSW SL102296 and ACT LT201917 permits, and the handling and procedures used with animals complied with the ethics approval ARA2019/17 from the Australian National University.

**Author contribution:** MI-C and DN conceived and designed the experiment; MI-C and JZ collected the data; MI-C and DN analysed the data. MI-C drafted the first version of the manuscript; all authors contributed to the draft and gave final approval for publication.

**References**

Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with imageJ. *Biophotonics International*, *11*(7), 36–41.

Baxter-Gilbert, J., Riley, J. L., & Whiting, M. J. (2018). Runners and fighters: clutch effects and body size drive innate antipredator behaviour in hatchling lizards. *Behavioral Ecology and Sociobiology*, *72*(6). https://doi.org/10.1007/s00265-018-2505-7

Bernardo, J. (1996). Maternai effects in animal ecology. *American Zoologist*, *36*(2), 83–105. https://doi.org/10.1093/icb/36.2.83

Bilcke, J., Downes, S., & Büscher, I. (2006). Combined effect of incubation and ambient temperature on the feeding performance of a small ectotherm. *Austral Ecology*, *31*(8), 937–947. https://doi.org/10.1111/j.1442-9993.2006.01663.x

Booth, D. T., Thompson, M. B., & Herring, S. (2000). How incubation temperature influences the physiology and growth of embryonic lizards. *Journal of Comparative Physiology - B Biochemical, Systemic, and Environmental Physiology*, *170*(4), 269–276. https://doi.org/10.1007/s003600000097

Braña, F., & Ji, X. (2000). Influence of incubation temperature on morphology, locomotor performance, and early growth of hatchling wall lizards (*Podarcis muralis*). *Journal of Experimental Zoology*, *286*(4), 422–433. https://doi.org/10.1002/(SICI)1097-010X(20000301)286:4<422::AID-JEZ10>3.0.CO;2-D

Brodie, E. D., & Russell, N. H. (1999). The consistency of individual differences in behaviour: Temperature effects on antipredator behaviour in garter snakes. *Animal Behaviour*, *57*(2), 445–451. https://doi.org/10.1006/anbe.1998.0990

Carter, A. W., Bowden, R. M., & Paitz, R. T. (2018). Evidence of embryonic regulation of maternally derived yolk corticosterone. *Journal of Experimental Biology*, *221*(22). https://doi.org/10.1242/jeb.182600

Chapple, D. G. (2016). Origin, Spread and Biology of the Invasive Plague Skink (*Lampropholis delicata*) in New Zealand. In *New Zealand Lizards* (pp. 341–360). https://doi.org/10.1007/978-3-319-41674-8

Chapple, D. G., Miller, K. A., Chaplin, K., Barnett, L., Thompson, M. B., & Bray, R. D. (2014). Biology of the invasive delicate skink (*Lampropholis delicata*) on Lord Howe Island. *Australian Journal of Zoology*, *62*(6), 498–506. https://doi.org/10.1071/ZO14098

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

Cooper, W. E. (1999). Tradeoffs between courtship, fighting, and antipredatory behavior by a lizard, Eumeces laticeps. *Behavioral Ecology and Sociobiology*, *47*(1–2), 54–59. https://doi.org/10.1007/s002650050649

Cooper, W. E. (2007). Compensatory changes in escape and refuge use following autotomy in the lizard Sceloporus virgatus. *Canadian Journal of Zoology*, *85*(1), 99–107. https://doi.org/10.1139/Z06-200

Dalesman, S., & Rundle, S. D. (2010). Influence of rearing and experimental temperatures on predator avoidance behaviour in a freshwater pulmonate snail. *Freshwater Biology*, *55*(10), 2107–2113. https://doi.org/10.1111/j.1365-2427.2010.02470.x

Dang, W., Zhang, W., & Du, W. G. (2015). Incubation temperature affects the immune function of hatchling soft-shelled turtles, Pelodiscus sinensis. *Scientific Reports*, *5*, 1–9. https://doi.org/10.1038/srep10594

Davidson, A., Jennions, M., & Nicotra, A. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive ? A meta-analysis. *Ecology Letters*, *14*, 419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x

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

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Cost and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, *13*(97), 77–81. https://doi.org/10.1111/j.1558-5646.2009.00647.x

Downes, S., & Hoefer, A. M. (2007). An experimental study of the effects of weed invasion on lizard phenotypes. *Oecologia*, *153*(3), 775–785. https://doi.org/10.1007/s00442-007-0775-2

Einum, S., & Fleming, I. A. (2000). Selection against late emergence and small offspring in Atlantic salmon (Salmo salar). *Evolution*, *54*(2), 628–639. https://doi.org/10.1111/j.0014-3820.2000.tb00064.x

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768). https://doi.org/10.1098/RSTB.2018.0174

Huang, V., Bowden, R. M., & Crews, D. (2013). Yolk-albumen testosterone in a lizard with temperature-dependent sex determination: Relation with development. *General and Comparative Endocrinology*, *186*, 67–71. https://doi.org/10.1016/j.ygcen.2013.02.019

Huey, R. B., & Hertz, P. E. (1984). Effects of body size and slope on scceleration of a lizard (Stellio stellio). *Journal of Experimental Biology*, *110*(1), 113–123. https://doi.org/10.1242/jeb.110.1.113

Iglesias-Carrasco, M., Head, M. L., & Cabido, C. (2016). Habitat dependent effects of experimental immune challenge on lizard anti-predator responses. *Behavioral Ecology and Sociobiology*, *70*(11). https://doi.org/10.1007/s00265-016-2199-7

Lancaster, L. T., McAdam, A. G., & Sinervo, B. (2010). Maternal adjustment of egg size organizes alternative escape behaviors, promoting adaptive phenotypic integration. *Evolution*, *64*(6), 1607–1621. https://doi.org/10.1111/j.1558-5646.2009.00941.x

Langkilde, T., & Shine, R. (2005). Different Optimal Offspring Sizes for Sons Versus Daughters May Favor the Evolution of Temperature-Dependent Sex Determination in Viviparous Lizards. *Evolution*, *59*(10), 2275. https://doi.org/10.1554/05-239.1

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, *68*(4), 619–640. https://doi.org/10.1139/z90-092

Manenti, T., Kjærsgaard, A., Schou, T. M., Pertoldi, C., Loeschcke, V., & Moghadam, N. N. (2021). Responses to developmental temperature fluctuation in life history traits of five drosophila species (Diptera: Drosophilidae) from different thermal niches. *Insects*, *12*(10), 1–16. https://doi.org/10.3390/insects12100925

Martin, J., & López, P. (1999). When to come out from a refuge: Risksensitive and state-dependent decisions in an alpine lizard. *Behavioral Ecology*, *10*(5), 487–492. https://doi.org/10.1093/beheco/10.5.487

McDonald, S., & Schwanz, L. E. (2018). Thermal parental effects on offspring behaviour and their fitness consequences. *Animal Behaviour*, *135*, 45–55. https://doi.org/10.1016/j.anbehav.2017.11.007

Mcghee, K. E., Pintor, L. M., Suhr, E. L., & Bell, A. M. (2012). Maternal exposure to predation risk decreases offspring antipredator behaviour and survival in threespined stickleback. *Functional Ecology*, *26*(4), 932–940. https://doi.org/10.1111/j.1365-2435.2012.02008.x

Noble, D. W. A., Mcfarlane, S. E., Keogh, J. S., & Whiting, M. J. (2014). Maternal and additive genetic effects contribute to variation in offspring traits in a lizard. *Behavioral Ecology*, *25*(3), 633–640. https://doi.org/10.1093/beheco/aru032

Noble, D. W. A., Stenhouse, V., & Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews*, *93*(1), 72–97. https://doi.org/10.1111/brv.12333

Oostra, V., Saastamoinen, M., Zwaan, B. J., & Wheat, C. W. (2018). Strong phenotypic plasticity limits potential for evolutionary responses to climate change. *Nature Communications 2018 9:1*, *9*(1), 1–11. https://doi.org/10.1038/s41467-018-03384-9

Pearson, P. R., & Warner, D. A. (2016). Habitat- And season-specific temperatures affect phenotypic development of hatchling lizards. *Biology Letters*, *12*(10). https://doi.org/10.1098/rsbl.2016.0646

Qualls, F. J., & Shine, R. (1998). Geographic variation in lizard phenotypes: Importance of the incubation environment. *Biological Journal of the Linnean Society*, *64*(4), 477–491. https://doi.org/10.1006/bijl.1998.0236

Räsänen, K., Laurila, A., & Merilä, J. (2005). Maternal investment in egg size: Environment- and population-specific effects on offspring performance. *Oecologia*, *142*(4), 546–553. https://doi.org/10.1007/s00442-004-1762-5

Raynal, R. S., Noble, D. W. A., Riley, J. L., Senior, A. M., Warner, D. A., While, G. M., & Schwanz, L. E. (2022). Impact of fluctuating developmental temperatures on phenotypic traits in reptiles: a meta-analysis. *Journal of Experimental Biology*, *225*. https://doi.org/10.1242/jeb.243369

Rosenfeld, C. S., & Roberts, R. M. (2004). Maternal diet and other factors affecting offspring sex ratio: A review. *Biology of Reproduction*, *71*(4), 1063–1070. https://doi.org/10.1095/biolreprod.104.030890

Rutstein, A. N., Gilbert, L., Slater, P. J. B., & Graves, J. A. (2005). Sex-specific patterns of yolk androgen allocation depend on maternal diet in the zebra finch. *Behavioral Ecology*, *16*(1), 62–69. https://doi.org/10.1093/beheco/arh123

Sanger, T. J., Kyrkos, J., Lachance, D. J., Czesny, B., & Stroud, J. T. (2018). The effects of thermal stress on the early development of the lizard Anolis sagrei. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *329*(4–5), 244–251. https://doi.org/10.1002/jez.2185

Sharda, S., Zuest, T., Erb, M., & Taborsky, B. (2021). Predator-induced maternal effects determine adaptive antipredator behaviors via egg composition. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(37). https://doi.org/10.1073/pnas.2017063118

Sih, A. (1992). Prey uncertainty and the balancing of antipredator and feeding needs. *The American Naturalist*, *139*(5), 1052–1069.

Sinervo, B. (1990). The evolution of maternal investment in lizards: an experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution*, *44*(2), 279–294.

Sinervo, B., Doughty, P., Huey, R. B., & Zamudio, K. (1992). Allometric engineering: a causal analysis of natural selection on offspring size. *Science*, *258*, 1927–1930.

Singh, S. K., Das, D., & Rhen, T. (2020). Embryonic temperature programs phenotype in reptiles. *Frontiers in Physiology*, *11*, doi.org/10.3389/fphys.2020.00035. https://doi.org/10.3389/fphys.2020.00035

Ślipiński, P., Trigos-Peral, G., Maák, I., Wojciechowska, I., & Witek, M. (2021). The influence of age and development temperature on the temperature-related foraging risk of Formica cinerea ants. *Behavioral Ecology and Sociobiology*, *75*(7). https://doi.org/10.1007/s00265-021-03029-w

Tiatragul, S., Kurniawan, A., Kolbe, J. J., & Warner, D. A. (2017). Embryos of non-native anoles are robust to urban thermal environments. *Journal of Thermal Biology*, *65*(March), 119–124. https://doi.org/10.1016/j.jtherbio.2017.02.021

Valenzuela, N., & Lance, V. A. (2004). *Temperature-Dependent Sex Determination in Vertebrates*. Smithsonian Books.

Van Damme, R., Bauwens, D., Braña, F., & Verheyen, R. (1992). Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard Podarcis muralis. *Herpetologica*, *48*, 220–228.

Warner, D. A., & Andrews, R. M. (2002). Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biological Journal of the Linnean Society*, *76*(1), 105–124. https://doi.org/10.1046/j.1095-8312.2002.00054.x

Warner, D. A., & Lovern, M. B. (2014). The maternal environment affects offspring viability via an indirect effect of yolk investment on offspring size. *Physiological and Biochemical Zoology*, *87*(2), 276–287. https://doi.org/10.1086/674454

Warner, D. A., Lovern, M. B., & Shine, R. (2007). Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 883–890. https://doi.org/10.1098/rspb.2006.0105

Webb, J. K., Brown, G. P., & Shine, R. (2001). Body size, locomotor speed and antipredator behaviour in a tropical snake (Tropidonophis mairii, colubridae): The influence of incubation environments and genetic factors. *Functional Ecology*, *15*(5), 561–568. https://doi.org/10.1046/j.0269-8463.2001.00570.x

Webb, J. K., Shine, R., & Christian, K. A. (2006). The adaptive significance of reptilian viviparity in the tropics: testing the maternal manipulation hypothesis. *Evolution*, *60*, 115. https://doi.org/10.1554/05-460.1

Wilson, S., & Swan, G. (2010). *A complete guide to reptiles of Australia* (3rd ed.). Reed New Holland, Sydney.

Wolf, J. B., & Wade, M. J. (2009). What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1520), 1107–1115. https://doi.org/10.1098/rstb.2008.0238

Yeh, P. J., & Price, T. D. (2004). Adaptive phenotypic plasticity and the successful colonization of a novel environment. *American Naturalist*, *164*(4), 531–542. https://doi.org/10.1086/423825

**Figures**

Figure 1. Effect of temperature (cold, 23 °C vs hot, 28 °C) and maternal resource investment (yolk reduced vs control) in morphological traits of *L. delicata* (a, b and c) and *L. guichenoti* (d, e and f). n.s. = non-significant. The horizontal line represents the interactions or main effects that had a statistically significant effect.

A graph of different colored shapes

Description automatically generated

Figure 2. Effect of temperature (cold, 23 °C vs hot, 28 °C) and maternal resource investment (yolk reduced vs control) in lizard performance: distance moved and running velocity (25 cm burnst and 1 m). *L. delicata* panels a, b and c, and *L. guichenoti* panels d, e and f. Note: 3 datapoints (raw data > 10s and < 0.01s) in the 25 burst of *L. delicata* were removed for visualization reasons. n.s. = non-significant. The horizontal line represents the interactions and/or main effects that had a statistically significant effect.

A graph of different colored lines

Description automatically generated

Figure 3. Effect of temperature (cold, 23 °C vs hot, 28 °C) and maternal resource investment (yolk reduced, control) in lizard antipredator behaviour: time hiding (time, in seconds, since the lizard hided in the refuge until the head was visible) and time to active (time before resuming activity). *L. delicata* panels a and b, and *L. guichenoti* panels c and d. n.s. = non-significant. The horizontal line represents the interactions or main effects that had a statistically significant effect.

A graph of time and time

Description automatically generated