From eggs to adulthood: sustained effects of early developmental temperature and corticosterone exposure on physiology and body size in an Australian lizard

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ABSTRACT

As global temperatures continue to rise due to climate change, developing animals may be increasingly exposed to elevated temperatures. Developing vertebrates can be affected by elevated temperatures directly, and indirectly through maternal effects such as increased exposure to prenatal glucocorticoid hormones. Although many studies have examined how elevated temperatures and glucocorticoid exposure during development independently affect vertebrates, fewer studies have tested the combined effects of elevated temperature and glucocorticoids. We tested interactions between incubation temperature and prenatal corticosterone exposure in the delicate skink (*Lampropholis delicata*). We dosed eggs with high or low dosecorticosterone treatments early in development and incubated eggs at either 23°C (cool) or 28°C (warm). We measured the effects of these prenatal treatments on development time, body size, survival from hatching to adulthood and on adult hormone levels (corticosterone, thyroxine, and testosterone in males) and mitochondrial respiration in liver tissue. We found no evidence for interactive effects of incubation temperature and prenatal corticosterone exposure on phenotype. However, incubation temperature and corticosterone treatment each independently decreased body size at hatching and these effects were sustained into the juvenile period and adulthood. We found that lizards exposed to low doses of corticosterone during development had elevated levels of baseline corticosterone as adults. Additionally, we found that lizards incubated at cooler temperatures had higher levels of baseline corticosterone. We found that lizards incubated at cooler temperatures had more efficient mitochondria compared to lizards incubated at warmer temperatures. Our results show that developmental conditions can have sustained effects on morphological and physiological traits in oviparous lizards but suggest that incubation temperature and prenatal corticosterone do not have interactive effects.

INTRODUCTION

Climate change is one of the most ubiquitous anthropogenic disturbances currently experienced by wildlife. With global temperatures increasing at an unprecedented rate (Diffenbaugh and Field, 2013; Tingley and Huybers, 2013), there is an urgent need to understand the physiological capacity of organisms to respond to elevated temperatures, and how such responses affect individual fitness and population dynamics (Chown et al., 2010; Fuller et al., 2010; Helmuth et al., 2005). Developing animals may be particularly sensitive to elevated temperatures associated with climate change because they are generally less able to behaviourally regulate their body temperature compared to adults (but see Du and Shine, 2022). Additionally, developmental conditions can profoundly affect morphology, physiology, and behaviour (Eyck et al., 2019; Monaghan, 2008; Nord and Giroud, 2020). Such developmental effects can affect fitness across life history stages and can be transmitted across generations through intergenerational effects (e.g., Bath et al., 2018; Crino et al., 2014b; Kraft et al., 2021; Mitchell et al., 2013).

Developing animals can be affected by elevated temperatures directly through interactions with their environment, and indirectly through maternal effects. For example, in vertebrates, exposure to stressors or disturbances such as elevated temperature can increase maternal glucocorticoid hormone levels, which can, in turn, have sustained effects on developing animals (reviewed in Crino et al., 2024; Mentesana and Hau, 2022). The independent effects of elevated temperatures and glucocorticoids on developing animals have been well studied (reviewed in Crino and Breuner, 2015; Nesan and Vijayan, 2013; Noble et al., 2018; Seckl and Meaney, 2004; Weeks et al., 2022). However, few studies have tested the combined effects of elevated temperatures and glucocorticoids on developing animals despite the recognition that glucocorticoids are likely to play an important role in shaping individual and population responses to global climate change (Crino et al., 2024; Mentesana and Hau, 2022; Names et al., 2024; Sumasgutner et al., 2023; Taff et al., 2024).

The effects of elevated temperatures on developing animals have been studied extensively in relation to incubation temperature in oviparous reptiles (Booth, 2018; DuRant et al., 2013; Jonsson and Jonsson, 2014; Noble et al., 2018). Incubation temperature is known to affect a range of traits depending on the species, including sex, growth, behaviour, locomotor performance, metabolism, and reproductive success (e.g., Braña and Ji, 2000; De Jong et al., 2023; Esquerré et al., 2014; Kar et al., 2022; Warner and Shine, 2008). For example, in oviparous reptiles, exposure to high temperatures during incubation can accelerate embryonic development, resulting in individuals that hatch quickly but at a smaller

body size than individuals exposed to cooler incubation temperatures (Dayananda et al., 2017; Kar et al., 2024). Elevated incubation temperatures can also have sustained effects on mitochondrial respiration and metabolic enzymes, suggesting that developmental conditions can affect growth at later life history stages through sustained changes in cellular metabolism (Seebacher and Grigaltchik, 2014; Sun et al., 2015).

Similar to incubation temperature, exposure to glucocorticoids during development can affect many aspects of physiology, behaviour, and performance (reviewed in Crino and Breuner, 2015; Eyck et al., 2019; McGowan and Matthews, 2018; Nesan and Vijayan, 2013). Glucocorticoids are steroid hormones that play important roles in vertebrate metabolism and stress responses (McEwen and Wingfield, 2003; Picard et al., 2014; Wingfield and Kitaysky, 2002). Glucocorticoids promote physiological and behavioural responses that allow animals to cope with disturbances and are thus considered mediators of adaptive responses to environmental conditions (Sapolsky et al., 2000). Developing animals can be exposed to glucocorticoids from maternal sources (during gestation, *in ovo*, and from breastmilk in mammals) and from their own endogenous production in response to postnatal disturbances (e.g., food restriction, environmental conditions, parental interactions; Crino and Breuner, 2015; Monaghan and Haussmann, 2015). Exposure to elevated glucocorticoids during development affects a range of phenotypic traits, including cellular metabolism, growth and development, body condition, immune function, and reproductive strategies (Blas et al., 2007; Casagrande et al., 2020; Crino et al., 2014a; Crino et al., 2014b; Grindstaff and Merrill, 2017; MacLeod et al., 2018). Additionally, exposure to glucocorticoids during development can have sustained effects on the neuroendocrine pathway that regulates the production of glucocorticoids (the hypothalamic-pituitary-adrenal or HPA axis), resulting in the secretion of higher levels of glucocorticoids later in life (e.g., Crino et al., 2022; Pakkala et al., 2016; Spencer et al., 2009). In this way, developmental conditions that change HPA axis function can indirectly affect traits at later life history stages that are influenced by glucocorticoids (e.g., mitochondrial function and sexual trait expression; Crino et al., 2022).

Many studies have examined how elevated temperatures and glucocorticoid exposure during development independently affect vertebrates. However, their combined effects have not been rigorously tested despite the fact that they can be biologically linked. In ectotherms, elevated environmental temperatures have been associated with increased glucocorticoid levels in adults (Liu et al., 2020; Racic et al., 2020). Maternal glucocorticoids can be transmitted to developing offspring in both oviparous (Uller et al., 2009) and viviparous lizards (Itonaga et al., 2011) and affect phenotypic traits with possible consequences for

fitness (De Fraipont et al., 2000; Vercken et al., 2007). Thus, during development, oviparous animals could be exposed to both elevated levels of glucocorticoids via maternal transmission and elevated temperatures during incubation. These developmental effects could be sustained across life history stages if exposure to glucocorticoids during development changes HPA axis function resulting in elevated secretion of glucocorticoids across life. Glucocorticoids affect metabolism and thermal tolerance through interactions with thyroid hormones and mitochondria (Debonne et al., 2008; Picard et al., 2014). Sustained changes in glucocorticoid seretion could thus play important roles in regulating phenotypic responses to temperatures through changes in mitochondrial function. Multiple 'stressors' can have additive, synergistic, or antagonistic effects on traits (Kaunisto et al., 2016; Padda and Stahlschmidt, 2022; Todgham and Stillman, 2013). Therefore, evaluating interactions between environmental factors (e.g., developmental temperature and maternal glucocorticoids) can be valuable for understanding the impact of complex environmental disturbances on animal life history and physiology (Padda and Stahlschmidt, 2022).

Here, we tested the long-term effects of prenatal exposure to elevated incubation temperature and corticosterone (the main glucocorticoid in lizards) on body size and growth, hormone responses, mitochondrial bioenergetics, and survival in the delicate skink (*Lampropholis delicata*). We exposed lizards to one of two corticosterone treatments (high corticosterone, low corticosterone) or a control treatment *in ovo* to mimic elevated levels of maternal corticosterone. We then incubated eggs at either low (23º C) or high (28º C) incubation temperatures (representing the approximate range of incubation temperatures in natural nests; Cheetham et al., 2011). We measured body size and condition in response to developmental treatments at hatching and two additional time points over a ~1.5-year period. After 1.5 years, we measured hormone levels (corticosterone, thyroxine, and testosterone – males only) and mitochondrial bioenergetics from liver tissue in adults. We had four main predictions that related to growth and body size, adult endocrine function, adult mitochondrial function, and hatching success and survival:

1. *Growth and body size* – Lizards treated with corticosterone prenatally would be smaller than control lizards at hatching and throughout life because of the sustained effects of corticosterone exposure during development on HPA axis function (see below). Lizards incubated at warmer temperatures would be smaller at hatching than lizards incubated at cool temperatures, but differences in body size would not be present later in life. Further, high incubation temperature would interact

synergistically with corticosterone treatment such that lizards exposed to both these treatments would be smaller than lizards from all other treatments.

2. *Adult endocrine function* – Lizards treated with corticosterone prenatally would have higher baseline corticosterone levels as adults compared to control lizards due to the programmatic effects of corticosterone exposure during development on HPA axis function (Crino and Breuner, 2015; Eyck et al., 2019; Schoech et al., 2011). Males with higher baseline corticosterone levels would have lower testosterone levels because of the suppressive effects of glucocorticoids on sex steroid synthesis (Wingfield and Sapolsky, 2003). Developmental corticosterone treatment would affect adult thyroxine levels because corticotropin releasing factor (a hormone associated with the HPA axis) can stimulate the neuroendocrine pathway that regulates thyroid hormone production (De Groef et al., 2006; Geris et al., 1996). Thyroxine levels would be positively associated with growth among individuals given the role of thyroid hormones in regulating growth and metabolism (Gerwien and Johnalder, 1992; McNabb, 2007).
3. *Mitochondrial bioenergetics in liver tissue and growth* – Similar to past studies, developmental treatments would have sustained effects on adult mitochondrial respiration (Crino et al., 2022; Stier et al., 2022) such that lizards exposed to elevated corticosterone during development have less efficient mitochondria as adult Additionally, we predicted that lizards incubated at higher temperatures would exhibit changes in mitochondrial function that would enable them to meet the heightened metabolic demands imposted by elevated temperature (Seebacher and Grigaltchik, 2014; Sun et al., 2015).
4. *Survival* – Lizards treated with corticosterone during development would have lower survival than control lizards and high incubation temperatures and corticosterone treatment would interact to further decrease survival.

Our research builds on recent research that examines the sustained effects of prenatal exposure to high temperatures on whole animal metabolic rate and growth (De Jong et al., 2023; Kar et al., 2024) by testing the joint effects of elevated temperatures and corticosterone treatments on hormone levels, mitochondrial bioenergetics, and phenotypic and survival outcomes. Additionally, our study tests physiological mechanisms (mitochondrial bioenergetics and corticosterone levels) that may link maternal and developmental effects to sustained responses to elevated temperatures.

MATERIALS AND METHODS

Lizard husbandry and housing

This study was conducted from November 2021 – June 2023 using a colony of delicate skinks at The Australian National University (Canberra, Australia). Delicate skinks are native to eastern Australia, occupy various habitats, and are commonly found in human-altered and urban areas (Cooger, 2014; Wilson and Swan, 2013). Delicate skinks reach sexual maturity at one year of age, are oviparious, and range in life span from ~2 – 4 years (Forsman and Shine, 1995; Greer, 1989; Heatwole and Taylor, 1987). They are easily housed and bred in captivity and are a highly tractable species for empirical studies that test the long-term effects of developmental conditions (e.g., De Jong et al., 2023; Kar et al., 2022).

Lizards were housed communally in terraria (width x length: 40 x 55 cm) in groups of 3 – 4 females with 2 males. Terraria contained non-stick mats as substrate, refuge (eucalyptus bark and half cut PVC pipe), a water container, and a container full of moist vermiculite for egg laying. Terraria were heated by heat chords to provide a thermal gradient (22 – 32°C) to allow lizards to behaviourally thermoregulate and had UV lamps for UVA/UVB exposure. Mean preferred temperatures in *L. delicata* ranges from 26 to 31°C and do not depend on the temperature experienced during development (Anderson et al., 2023; Zhang et al., 2023). Lights were set to a photoperiod of 12:12 h (light/dark). Lizards were provided with water daily, crickets (*Acheta domestica*) every second day, and a calcium and multivitamin supplement once a week. All methods for housing, husbandry, and experimental protocols were approved by The Australian National University Animal Ethics Committee (A2021/56).

Experimental timeline

Lizard enclosures were checked for eggs three days a week. Eggs were treated with hormone solutions the day they were found (~24 – 72 hours after they were laid; Figure 1). Following treatments, eggs were incubated until hatching was recorded. Eggs were checked three days a week. On the day hatching was recorded, lizards were measured for snout-vent length (SVL) to the nearest mm using a ruler and body mass to the nearest mg using a digital balance. After hatching, lizards were moved to solitary enclosures and provided with the same husbandry as lizards in the breeding colony (as above). We collected additional body size measurements when lizards were juveniles (mean days post-hatching = 105.7, SD = 10.8, range = 85 – 123) and when lizards were euthanized as adults at ~1.5 years of age (mean days post-hatching = 466.1, SD = 12.4, range = 440 – 491) at which point they were sexed by hemipene eversion. After euthanising lizards, we collected a blood sample for hormone

analyses and liver tissue to measure mitochondrial bioenergetics. Body condition was calculated at each time point using the scaled mass index derived from SVL and body mass (Peig and Green, 2009). We calculated the growth rate from hatching to the juvenile period and hatching to adulthood as body size measurements as juveniles/adults minus body size at hatching divided by juvenile/adult age (days post-hatching).

Experimental treatments

We exposed eggs to one of six corticosterone/temperature treatments in a fully factorial design. Eggs were assigned to treatment groups in a partially crossed split clutch design such that eggs from a single clutch were randomly assigned across treatment groups. It was not possible to assign an egg from a single clutch to each of the treatment groups because mean clutch size is 3.0 – 4.4 eggs in *L. delicata* (Forsman and Shine, 1995). For hormone treatments, eggs were treated with either a high corticosterone (10 pg/mg), low corticosterone (5 pg/mg), or a control (vehicle) treatment. Corticosterone doses were selected based on published yolk corticosterone concentrations in other oviparous species (Hanover et al., 2019; Lovern and Adams, 2008), estimates of the percentage of steroids that are incorporated into the embryo following topical treatment (Crews et al., 1991; Vassallo et al., 2014), and preliminary measures of untreated eggs. Corticosterone treatments were made by dissolving crystalline corticosterone (Sigma, Cat. No. C2505) in 100% ethanol. To dose eggs, we applied 5µl of solutions to eggshells using a micropipette. Control eggs were treated with 5µl of 100% ethanol. Following treatment with corticosterone solutions, eggs were incubated in covered plastic cups filled with damp vermiculite at either 23°C (hereafter: cool) or 28ºC (hereafter warm; representing the temperature extremes in natural nest sites in this species; Cheetham et al., 2011).

Validation of corticosterone treatments

We measured corticosterone levels in a separate group of eggs to ensure that topical treatments increased corticosterone levels within a biologically relevant range. We dosed eggs with corticosterone treatments as above. We allowed eggs to incubate for 24 ± 2 hours at 28°C prior to removing the egg yolk. We used solid phase extraction (SPE) with silica-bonded vacuum columns (United Chem. Cat. No. CEC18156) to extract corticosterone from yolk samples and Arbor Assay Enzyme Immunoassay (EIA) kits (Cat. No. K014) to measure corticosterone (full methods in Supplemental Materials). Corticosterone treatment increased

mean yolk corticosterone levels 2.54 and 5.95 standard deviations above control eggs for low and high doses (respectively; see Results).

Mitochondrial bioenergetics

Lizards were fasted for 72 ± 4 hours prior to euthanasia. Lizards were euthanized via an injection of Alfaxan (10 mg/mL) followed by rapid decapitation. Immediately following decapitation, whole livers were removed, rinsed twice in 1 mL of ice-cold 1M phosphate buffered solution, and stored in 1 mL of ice-cold isolation buffer (250 mM sucrose, 1 mM EGTA, 20 mM Tris HCL, pH 7.4 with KOH) prior to further processing (>30 minutes). We used differential centrifugation to isolate mitochondria (Lampl et al., 2015; Pallotti and Lenaz, 2001). Liver tissue was homogenized on ice with 3 – 4 gentle hand passes using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 4ºC, 750 x g for 10 minutes. The supernatant was transferred to a clean Eppendorf tube and centrifuged for a second time at 4ºC, 750 x g for 10 minutes. The supernatant was then transferred again to a clean Eppendorf tube and centrifuged at 4ºC, 10,000 x g for 10 minutes. The resulting supernatant was removed and the pellet containing isolated mitochondria was resuspended in 500 µl of MiR05 respiration media [0.5 mM EGTA, 3mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM Hepes, 110 mM sucrose, free fatty acid bovine albumin (1 g/L), pH 7.1 with KOH].

We measured mitochondrial oxygen consumption (pmol O_2/sec) using Oxygraph-2K high-resolution respirometers (Oroboros Instruments, Innsbruck, Austria) based on established methods (Brand et al., 1993; Doerrier et al., 2018; Salin et al., 2018) with minor modifications. Immediately following preparation, we added resuspended mitochondria to 1.5 mL of respiration media equilibrated at 30º C in one respiration chamber. We applied a series of mitochondrial substrates and inhibitors to measure oxygen consumption at five states: basal (state 2), maximal (state 3), leak (state 4), and residual oxygen consumption. Basal respiration was measured following the addition of pyruvate (5 mM), malate (2 mM), and succinate (10 mM) that support electron entry into the electron transport system via complexes I and II. Maximal respiration was induced with the addition of ADP (2 mM). Leak respiration was induced by adding oligomycin (2.5 µM), which inhibits ATP synthase. Oxygen consumption following the addition of oligomycin is attributed to proton leak across the inner mitochondrial membrane. Finally, we added antimycin A (2.5 µM), which inhibits mitochondrial complex III and allows for measurements of non-mitochondrial oxygen consumption. After administering antimycin A, we collected a 1 mL aliquot of the

mitochondrial suspension from the respiration chamber to determine sample concentration. These samples were stored at -20 ºC until assayed using Coomassie Plus (Bradford) assays (Thermo Scientific, Cat. No. 23236; Supplemental Materials).

Oxygen consumption values for basal, OXPHOS (state 3), and leak (state 4) respiration were corrected for non-mitochondrial oxygen consumption (following the addition of antimycin A) and protein content, yielding values in pmol O_2. $sec^{-1}.\mu g^{-1}$ of mitochondrial protein. We estimated mitochondrial efficiency as the respiratory control ratio (RCR), which is calculated as the ratio of oxygen consumed to drive the phosphorylation of ADP to ATP (OXPHOS) to oxygen consumed to offset proton leak across the inner mitochondrial membrane (Brand and Nicholls, 2011). A high RCR indicates that mitochondria have a high respiratory capacity for ATP production relative to the respiration require to offset proton leakage (Brand and Nicholls, 2011).

Blood collection and plasma hormone analysis

We collected blood samples immediately after euthanizing and decapitating lizards. Blood was collected from the trunk using heparinized microcapillary tubes within six minutes of disturbing lizards. The average time to collect blood samples was 2.4 ± 0.8 minutes (range = 1.5 - 5.8 minutes). In endothermic animals, glucocorticoids levels generally increase above baseline within three minutes of disturbance (Romero and Reed, 2005; Small et al., 2017). However, glucocorticoid levels in reptiles generally remain at baseline 5 – 15 minutes following disturbance (Cockrem, 2013; Tylan et al., 2020). We found no effect of the time to collect blood samples on plasma corticosterone levels ($p = 0.55$, $F_{1,75} = 0.36$). Blood samples were kept on ice (<1 hour) and then centrifuged at 7,000 rpm for seven minutes to separate plasma from red blood cells. The isolated plasma was frozen at -20°c prior to conducting hormone assays.

We measured corticosterone, thyroxine, and testosterone (males only) from 5 µl of plasma. All hormones were measured from raw plasma diluted to [1:100] using Arbor Assay Enzyme Immunoassay kits (Cat. No. K014, K050, K032). All samples and standards were run in triplicate and all plates were read on a FLUOstar Omega microplate reader at 450 nm (full details in Supplemental Materials).

Statistical analysis

Data were analyzed in R version 4.4.0 using the lme4 (1.1.35.3), emmeans (1.10.2), performance (0.11.0), and car packages (3.1.2; Barton, 2009; Fox and Weisberg, 2019; Lenth,

2024; Ludecke et al., 2021). We used a general linear mixed effects model (GLMM) with a guassian error distribution to test the effects of corticosterone treatments on yolk corticosterone levels. We also used GLMMs to test the effects of developmental treatments on incubation duration, body size and condition at hatching and across life, adult hormone levels, and adult mitochondrial bioenergetics. To control for lizards originating from the same clutch, we included clutch of origin as a random effect in all models. Initial models included an interaction term between temperature and corticosterone treatments. However, there were no interactions between temperature and corticosterone treatments for all analyses and the interaction term was removed from the final models (Table S1). We tested associations between growth and physiological parameters using GLMMs with growth of mass or SVL as dependent variables, thyroxine levels, corticosterone levels, and mitochondrial respiration parameters as covariates, and sex as a fixed factor. We ensured that the underlying statistical assumptions of models were not violated by visually inspecting QQ plots, homogeneity of variance, variance inflation factors, and model residuals using the 'check_model' function (Zuur et al., 2009). We tested differences between corticosterone treatments with pairwise comparison using 'emmeans,' corrected with the Tukey method. We conducted Pearson's Chi-squared test to determine the effects of developmental treatments on post-hatch survival across the duration of our study. Means are provided with one standard deviation unless indicated otherwise. Full model details and outputs and figures showing raw data are found in Supplemental Materials.

RESULTS

Hormone treatment effects on yolk corticosterone levels

Topical corticosterone treatment affected yolk corticosterone levels ($p = 0.002$, $F_{2,22} = 7.98$; Figure 2, Supplemental Figure S1). Eggs treated with high doses of corticosterone had higher levels of yolk corticosterone than control eggs ($p = 0.002$; $mean_{high\_corticosterone} = 11.42 \pm 8.44$ pg/mg, $mean_{control} = 3.09 \pm 1.40$ pg/mg). Eggs treated with low doses of corticosterone had yolk corticosterone levels intermediate between the high dose and control treatments ($mean_{low\_corticosterone} = 6.64 \pm 4.92$ pg/mg). There were no differences in yolk corticosterone levels between eggs treated with high and low doses of corticosterone ($p = 0.11$) and low doses of corticosterone and the control treatment ($p = 0.21$).

Developmental treatments across life – effects on incubation time, body size and condition

Lizards incubated at warm temperatures hatched faster than lizards incubated at cool temperatures ($p < 0.001$; $F_{1,115} = 1008.42$; average days to hatch: warm = 30.9 ± 4.8 and cool = 48.3 ± 8.4). There was no effect of corticosterone treatment on the time for lizards to hatch ($p = 0.52$, $F_{2,115} = 1.31$; average days to hatch: high corticosterone = 39.5 ± 11.0, low corticosterone = 40.6 ± 11.5, control = 39.8 ± 11.0).

Incubation temperature did not affect SVL at hatching ($p = 0.99$, $F_{1,123} = 0.01$; Supplemental Figures S2 and S3) but did affect body mass such that lizards incubated at warm temperatures weighed less than lizards incubated at cool temperatures ($p = 0.017$, $F_{1,123} = 5.71$; Figure 3, Figure S4) and had lower body condition ($p = 0.03$, $F_{1,123} = 4.31$). As juveniles, lizards incubated at warm temperatures during development had smaller SVLs ($p = 0.01$, $F_{1,101} = 6.42$) and weighed less compared to lizards incubated at cooler temperatures ($p < 0.001$, $F_{1,101} = 10.73$), but there were no differences in body condition ($p = 0.91$, $F_{1,101} = 0.01$). The effects of incubation temperature on body size that we observed at early ages were not present in adults. Incubation temperature did not affect adult SVL ($p = 0.35$, $F_{1,80} = 0.88$), body mass ($p = 0.10$, $F_{1,80} = 2.63$), or body condition ($p = 0.91$, $F_{1,80} = 0.01$).

Corticosterone treatment affected both SVL (Supplemental Figures S2 and S3) and body mass at hatching ($p < 0.001$, $F_{2,123} = 13.40$; $p = 0.011$, $F_{2,123} = 8.93$; Figure 3, Supplemental Figure S4) and had non-significant effects on body condition ($p = 0.06$, $F_{2,123} = 5.43$). Lizards treated with high doses of corticosterone had smaller SVLs than those treated with low doses of corticosterone ($p = 0.01$) and control lizards ($p = 0.003$). Lizards treated with high doses of corticosterone weighed less than control lizards ($p = 0.01$) but did not differ in body mass compared to those treated with low doses of corticosterone ($p = 0.80$). There were no differences in SVL or body mass measurements between lizards treated with low doses of corticosterone and control lizards ($p = 0.87$ and 0.06). Corticosterone treatment during development affected juvenile body mass ($p = 0.03$, $F_{2,101} = 6.99$) such that lizards exposed to high doses of corticosterone during development weighed less than control lizards ($p = 0.04$) but were not different from lizards treated with low doses of corticosterone ($p = 0.12$). There were no differences in body mass between lizards treated with low doses of corticosterone and control lizards ($p = 0.81$). Corticosterone treatment during development did not affect juvenile SVL ($p = 0.20$, $F_{2,101} = 3.15$) or body condition ($p = 0.0.71$, $F_{2,101} = 0.69$). In adults, corticosterone treatment during development affected SVL ($p = 0.03$, $F_{2,80} = 6.86$), with adults exposed to high doses of corticosterone during development having smaller SVLs as adults compared to control lizards ($p = 0.042$) but not lizards with low doses of corticosterone ($p = 0.12$). Additionally, there was no difference in adult SVL between lizards

that received low doses of corticosterone during development and control lizards ($p = 0.95$). Corticosterone treatment did not affect adult mass ($p = 0.20$, $F_{2,80} = 3.20$) or body condition ($p = 0.34$, $F_{1,80} = 2.11$).

Lizards incubated at warmer temperatures grew less in SVL from hatching to the juvenile period compared to lizards incubated at cooler temperatures ($p < 0.001$, $F_{1,102} = 12.83$) and hatching to adulthood ($p < 0.001$, $F_{1,81} = 11.69$) but did not differ in growth of body mass from hatching to the juvenile period ($p = 0.26$, $F_{1,102} = 1.26$) or hatching to adulthood ($p = 0.06$, $F_{1,81} = 3.52$). Corticosterone treatment during development negatively affected growth of body mass from hatching to the juvenile period ($p = 0.042$, $F_{2,102} = 3.15$). Lizards exposed to high doses of corticosterone grew more slowly than control lizards ($p = 0.046$) but did not differ from low dose lizards ($p = 0.17$). There was no difference in body mass gain between low dose and control lizards from hatching to the juvenile period ($p = 0.79$). Corticosterone treatment during development did not affect the growth of SVL from hatching to the juvenile period ($p = 0.68$, $F_{2,102} = 0.76$) or hatching to adulthood ($p = 0.57$, $F_{2,81} = 1.11$) and did not affect change in body mass from hatching to adulthood ($p = 0.19$, $F_{2,81} = 3.28$).

Sustained effects of developmental treatments into adulthood – effects on hormones and mitochondrial bioenergetics

Corticosterone treatment during development affected adult baseline corticosterone levels ($p = 0.045$, $F_{2,68} = 3.25$; Figure 4). Lizards treated with low doses of corticosterone had higher levels of baseline corticosterone compared to control lizards but did not differ from lizards treated with high doses of corticosterone (control ~ low: $p = 0.01$, low ~ high: $p = 0.13$). There was no difference in baseline corticosterone levels between lizards treated with high doses of corticosterone and control lizards ($p = 0.72$). Corticosterone treatment did not affect thyroxine ($p = 0.75$, $F_{2,67} = 0.56$) or testosterone levels (Kruskal-Wallis chi-squared = 0.19, $p = 0.91$, $n_{control} = 12$, $n_{low} = 16$, $n_{high} = 12$).

Incubation temperature affected baseline corticosterone levels in adult lizards (Figure 4A; $p = 0.04$, $F_{1,68} = 4.29$). Lizards incubated at a cooler temperature had higher baseline corticosterone compared to lizards incubated at a warmer temperature ($cool_{mean} = 5.39 \pm 0.130$, $warm_{mean} = 4.98 \pm 0.106$). In our experiment, lizards incubated at cooler temperatures were larger compared to lizards incubated at warmer temperatures (see above). Glucocorticoids are linked to energy demands in endotherms (e.g., Jimeno et al., 2020; Rubalcaba and Jimeno, 2022) and it is possible that cooler incubation temperatures indirectly

affect baseline corticosterone levels through changes in body size. We conducted a post-hoc analysis to test the potential effects of increased energy demands associated with larger body size on baseline corticosterone levels. When adult body mass was included as a covariate, we found that baseline corticosterone levels were positively correlated with adult body mass ($p = 0.01$, $F_{1,67} = 6.33$) and did not differ between incubation treatments ($p = 0.21$, $F_{1,65} = 1.54$). Incubation temperature treatment did not affect adult thyroxine ($p = 0.99$, $F_{1,67} = 0.001$) or testosterone levels (Kruskal-Wallis chi-squared = 0.06, $p = 0.81$, $n_{cool} = 21$, $n_{warm} = 22$).

Mitochondrial efficiency (i.e., RCR) was affected by incubation temperature ($F_{1,77} = 4.40$, $p = 0.041$, Supplemental Table S2) but not corticostrone treatment ($F_{2,77} = 2.14$, $p = 0.34$; Supplemental Table S2). Lizards incubated at colder temperatures had greater mitochondrial efficiency as indicated by higher RCRs than lizards incubated at warmer temperatures (Figure 5). Developmental treatments did not affect basal, OXPHOS, or leak respiration (Supplemental Table S2). Basal and OXPHOS respiration were positively associated with adult body mass ($p = 0.04, 0.04$; $F_{1,75} = 4.21, 4.02$; Figure 6a and b) and leak respiration showed a near significant relationship with body mass ($p = 0.05$, $F_{1,75} = 3.69$; Figure 6c). There was no association between RCR and body mass ($p = 0.97$, $F_{1,75} = 0.01$; Figure 6d).

Overall, females had higher baseline corticosterone levels ($p = 0.003$, $F_{1,68} = 8.5$) and higher thyroxine levels than males ($p = 0.02$, $F_{1,67} = 5.36$). Males had higher oxygen consumption than females for basal ($p < 0.001$, $F_{1,75} = 15.35$), OXPHOS respiration ($p = 0.004$, $F_{1,75} = 8.14$), and leak respiration ($p < 0.001$, $F_{1,75} = 14.60$). However, there was no difference between males and females in RCR values ($p = 0.21$, $F_{1,75} = 1.58$).

Associations between growth, mitochondrial bioenergetics, and hormone levels

There were no associations between growth in body mass or SVL and mitochondrial respiration parameters from hatching to the juvenile period or hatching to adulthood (Supplemental Tables S4 and S5). Growth in body mass from hatching to adulthood was positively associated with corticosterone levels for all models with mitochondrial respiration parameters, but these effects were not significant (basal: $F_{1,67} = 3.36$, $p = 0.07$; OXPHOS: $F_{1,67} = 3.06$, $p = 0.08$; leak: $F_{1,67} = 3.56$, $p = 0.06$; RCR: $F_{1,67} = 3.52$, $p = 0.06$). There were no associations between growth in SVL from hatching to adulthood and corticosterone levels for all models (basal: $F_{1,67} = 1.41$, $p = 0.23$; OXPHOS: $F_{1,67} = 1.08$, $p = 0.30$; leak: $F_{1,67} = 1.66$, $p = 0.20$; RCR: $F_{1,67} = 1.57$, $p = 0.21$). Body mass and SVL growth were lower for males than

females in all models (Supplemental Tables S4 and S5). There were no associations between growth in body mass or SVL and thyroid hormone levels (Supplemental Tables S4 and S5).

Developmental temperature and corticostrone effects on mortality

Incubation temperature did not affect mortality across the duration of our study (cool temperature: n = 6 of 56 deceased; warm temperature: n = 9 of 54 deceased; chi-squared = 0.27, d.f. = 1, p = 0.60). Similarly, corticosterone treatment did not affect mortality (high corticosterone: n = 5 of 33 deceased; low corticosterone: n = 5 of 38 deceased; control: n = 5 of 39 deceased; chi-squared = 0.07, d.f. = 2, p = 0.97).

DISCUSSION

Developing animals can be affected by elevated temperatures associated with climate change directly through interactions with their environment, and indirectly through maternal effects such as increased exposure to maternally derived glucocorticoids (Crino et al., 2024; Mentesana and Hau, 2022). Developing animals may be particularly affected by elevated temperatures associated with climate change because developmental conditions can have long term effects on physiological traits (e.g., Cossin-Sevrin et al., 2022; Crino et al., 2022; Stier et al., 2022). We found evidence that incubation temperatures and exposure to prenatal corticosterone affect body size and physiological traits (baseline corticosterone and mitochondrial function) across lifespan. Contrary to our predictions, we found no evidence for interactive effects between incubation temperature and prenatal corticostorone exposure. Our results are consistent with past studies showing that incubation temperature can have sustained effects on physiological traits in oviparous vertebrates. Our results are the first to show that prenatal conditions corticosterone exposure can cause sustained changes to HPA axis function in lizards.

Early thermal environment and corticosterone do not have interactive effects but independently affect physiology and growth

We found that incubation temperature and corticosterone treatment had independent effects on body size. Lizards incubated at warm incubation temperatures (28°C) or exposed to high levels of corticosterone prenatally were smaller compared to lizards incubated at cooler temperatures (23°C) and lizards not exposed to corticosterone (control). These treatment effects on body size were present at hatching and the juvenile period (temperature and corticosterone) and in adults (corticosterone treatment only). In oviparous lizards,

elevated incubation temperatures generally decreases incubation duration and can result in smaller hatchlings in some species (Booth, 2018; Noble et al., 2018). The effects of incubation temperature on growth in oviparous lizards are likely due to the effects of temperature on energy metabolism (see discussion on mitochondrial function; Angilletta et al., 2002; Dowd et al., 2015; Salin et al., 2016). In our experiment, elevated temperature had sustained but not lifelong effects on body size, suggesting that lizards compensate for early developmental effects with changes in postnatal growth. Such compensatory growth has been linked to elevated production of reactive oxygen species (ROS), oxidative damage, and faster senescence (Metcalfe and Monaghan, 2001; Monaghan et al., 2009). Compensatory growth has also been linked to elevated production of antioxidants that can mitigate the damaging effects of ROS (De Block and Stoks, 2008; Noguera et al., 2015). We found no effect of elevated incubation temperature on mortality across the duration of our study but did not measure ROS or antioxidant production. Future studies that assess the effects of incubation temperature and compensatory growth on ROS and antioxidant production could uncover mechanisms that shape long-term effects of developmental conditions.

We predicted that incubation temperature would affect the metabolism of yolk corticosterone and result in interactive effects between incubation temperature and corticosterone treatment because elevated incubation temperatures can increase embryonic metabolism (e.g., Angilletta et al., 2006; Booth et al., 2000). However, we did not see interactive effects between incubation temperature and corticosterone treatment that would support this prediction. It is possible that incubation temperature and corticosterone treatment did not have interactive effects because they affect developing embryos through different physiological pathways and/or across different timescales. In viviparous lizards, placental tissue metabolizes corticosterone which potentially buffers developing embryos from elevated levels of maternal corticosterone (Painter and Moore, 2005). Much less is known about how maternal corticosterone affects developing embryos in oviparous lizards. However, in birds, maternal corticosterone is metabolized into 5β-corticosterone and 20β-corticosterone in the extraembryonic membrane early in development (Vassallo et al., 2019; Vassallo et al., 2014). For example, studies showed that embryonic chickens (*Gallus gallus*) and Japanese quail (*Cortunix japonica*) metabolized ~100% of yolk corticosterone during the first 4 - 6 days of development (Harders et al., 2024; Vassallo et al., 2014). To our knowledge, no study to date has characterized the rate of corticosterone metabolism by embryonic lizards. However, in red-eared slider turtles (*Trachemys scripta*), embryos metabolized ~50% of topically applied estradiol during the first nine days of development

(Crews et al., 1991). Together these studies suggest that lizard embryos in our experiment were exposed to elevated corticosterone levels for a short period following treatment whereas temperature treatments affected lizards until hatching.

Incubation temperature and prenatal corticosterone affect baseline corticosterone as adults

Exposure to elevated levels of glucocorticoids during development can affect HPA axis function across lifespan (reviewed in Gans and Coffman, 2021; Matthews and McGowan, 2019; Seckl and Meaney, 2004). We showed that prenatal exposure to corticosterone affects baseline corticosterone in adult lizards (average age = 466.1 days post-hatching). Lizards exposed to low doses of corticosterone had higher levels of baseline corticosterone compared to control lizards (Figure 4B). However, we found no differences in corticosterone levels between lizards treated with high doses of corticosterone and lizards treated with low doses of corticosterone or the control treatment. The effects of glucocorticoid exposure during development on HPA axis function later in life are complex and vary by the dose or magnitude of glucocorticoid exposure, the longevity of effects, species, sex, and postnatal conditions (Chaby, 2016; Gans and Coffman, 2021; Majer et al., 2023; Monaghan and Haussmann, 2015). For example, glucocorticoids can have biphasic dose-dependent effects where low doses elicit one response that is reversed at higher doses (resulting in an inverted U-shaped response curve; Gopi and Rattan, 2019; Lupien et al., 2005; Pratsinis et al., 2006). Such dose-dependent effects of glucocorticoids on HPA axis function could increase survival by priming glucocorticoid responses to match developing animals to their postnatal environment (a process called hormesis; Costantini et al., 2010; Monaghan and Haussmann, 2015). The duration of such glucocorticoid-mediated changes in HPA axis function has important implications for understanding the power of these developmental effects to shape evolutionary responses. For example, exposure to developmental glucocorticoids in one generation can affect future generations through intergenerational effects when glucocorticoid exposure during development has sustained effects on HPA axis function that persist until sexual maturity (Crino et al., 2024).

Environmental temperatures experienced during development can influence thermoregulation and temperature tolerance at later life-history stages (Esquerré et al., 2014; Goodman and Walguarnery, 2007; Nord and Giroud, 2020). Such developmental effects can be mediated through changes in the HPA axis and the thyroid hormone axis (hypothalamic-pituitary-thyroid axis), which plays an important role in behavioural and physiological

thermoregulation (Debonne et al., 2008; Loyau et al., 2015; Wilsterman et al., 2015). Consistent with this idea, we found that lizards incubated at cooler temperatures had higher levels of baseline corticosterone compared to lizards incubated at warmer temperatures (Figure 4A). Glucocorticoids regulate physiological processes that increase circulating levels of glucose and lipids by increasing hepatic glucose production (through gluconeogenesis) and by reducing glucose uptake by skeletal muscles (Picard et al., 2014; Sapolsky et al., 2000). As such, glucocorticoids are often linked to metabolic demands (Astheimer et al., 1992; Jimeno et al., 2018; Remage-Healey and Romero, 2000). In our experiment, lizards incubated at cooler temperatures were larger than lizards incubated at warmer temperatures at hatching and during the juvenile period (Figure 3, Supplemental Figure 2). We found that larger lizards had higher levels of baseline corticosterone when body mass was included in a post-hoc analysis (and no effect of incubation treatment). We also found no difference in thyroxine levels in adult lizards incubated at warm and cool temperatures. Together, these results suggest that incubation temperature does not have programmatic effects on endocrine mechanisms. Rather, incubation temperature drives sustained effects on body size and, hence, metabolic demands as reflected by difference in corticosterone levels in our experiment.

Mitochondrial bioenergetics are affected by incubation temperature and differ between sexes

We found that incubation temperature had sustained effects on the efficiency of mitochondrial respiration (i.e., RCR; Figure 5). The mitochondrial RCR is calculated as a ratio of oxygen consumed during State 3 respiration (OXPHOS; when ATP is synthesized) to oxygen consumed during State 4 respiration (leak; when protons flux across the inner mitochondrial membrane into the matrix without producing ATP; Gnaiger, 2012). Mitochondrial respiratory control is considered one of the best metrics of mitochondrial function in isolated mitochondria because it is affected by numerous biochemical factors and captures a biologically relevant metric of mitochondrial efficiency (Brand and Nicholls, 2011). High mitochondrial RCRs indicate that mitochondria have a high capacity for substrate oxidation and ATP turnover relative to a low loss of potential energy (as heat) due to proton leak (Brand and Nicholls, 2011).

In our experiment, lizards incubated at cooler temperatures had higher mitochondrial efficiency (higher RCR) as adults compared to lizards incubated at warmer temperatures. The liver plays a central role in glucose and lipid metabolism (Han et al., 2016; Jones, 2016). As such, higher efficiency mitochondria may explain why we observed larger body size in cold

611 animals because more ATP is expected be available early in development for growth and
612 somatic mainatence. Our results also suggest that temperatures experienced during early
613 development can have sustained effects on the efficiency of hepatic mitochondria, possibly
614 through changes in membrane fluidity and/or lipid profile differences that can affect
615 mitochondrial leakage. Membrane fluidity is affected by membrane phospholipid
616 characterics, varies in response to temperature, and is considered an important mechanism
617 that promotes thermal adaptation in ectotherms (i.e., homeoviscous adaptation; Chung and
618 Schulte, 2020; Cooper et al., 2014; Dahlhoff and Somero, 1993). Work in *Drosophilia* has
619 shown that the ability to plastically change phospholipid composition in response to thermal
620 environments varies across populations (Cooper et al., 2014), which can have implications
621 for 'leakiness' to protons in the mitochondria. Exploring how lipid membranes change in
622 response to temperature (if at all) would be a fruitful future endeavour to test a potential
623 mechanism through which temperature affects mitochondrial efficiency.
624 Independent of developmental treatments, in our experiment males had higher
625 mitochondrial oxygen consumption than females for basal, OXPHOS, and leak respiration
626 (Figure 6). Oxygen consumed during OXPHOS respiration is used to drive the
627 phosphorylation of ADP to ATP while leak respiration is a measure of oxygen consumption
628 used to offset proton loss across the inner mitochondrial membrane and is reflective of energy
629 loss (Brand and Nicholls, 2011). Our result suggest that males have a greater ability to
630 produce ATP but also require more energy to offset proton leak than females. However, we
631 found no difference between RCR values between males and females indicating no difference
632 between capacity for energy production relative to energy loss, suggesting that males have
633 overall higher mitochondrial function compared to females despite their smaller body size
634 and slower growth rate. An enhanced ability of mitochondria to produce ATP may account
635 for variation in metabolically costly processes and traits other than growth such as thermal
636 tolerance, reproduction, and sexual displays and ornaments (Chung and Schulte, 2020; Hill,
637 2014; Koch and Hill, 2018).
638
639 **Conclusion**
640 Understanding the short-term and sustained effects of developmental conditions on
641 growth, body condition, and survival is essential for understanding how developmental
642 effects drive population-level responses. However, the interaction between physiological
643 systems and environmental conditions likely entails physiological tradeoffs that constrain
644 phenotypic expression and ultimately affect life history strategies. For this reason, it is critical

to understand how developmental conditions interact during sensitive periods like prenatal development. Oviparous animals could be affected by elevated temperatures associated with global climate change through direct effects on incubation temperature and maternal effects such as increased exposure to glucocorticoids. Studies that track the physiological changes to elevated temperatures and glucocorticoids during development across lifespan will provide a more holistic understanding of the multigenerational consequences of elevated temperatures associated with global climate change.

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AUTHOR CONTRIBUTIONS

O.C., C.F., D.N., and K.S. conceived the ideas and designed the experiment. O.C., D.L., N.L., A.P., P.R., and D.N. collected the data. K.W., D.N., and O.C. analyzed the data and K.W. made the figures. O.C. wrote the initial draft of this manuscript and all authors provided feedback and approved the final draft.

DATA AVAILABILITY

The data associated with this manuscript will be made available on the Dryad Digital Repository prior to publication.

COMPETING INTERESTS

The authors declare they have no competing interests.

DIVERSITY AND INCLUSION STATEMENT

Our study brings together researchers from several countries, including researchers based in the country where the study was conducted. We included researchers from a diversity of

678 career stages in this project. Additionally, we cited literature published by scientists from a
679 diversity of career stages whenever possible.
680
681 **REFERENCES**

682 **Anderson, R. O., Goulet, C. T. and Chapple, D. G.** (2023). Acclimation of thermal
683 physiology to new basking regimes in a widespread Australian skink. *Journal of Thermal*
684 *Biology* **113**.
685 **Angilletta, M. J., Lee, V. and Silva, A. C.** (2006). Energetics of lizard embryos are
686 not canalized by thermal acclimation. *Physiological and Biochemical Zoology* **79**, 573-580.
687 **Angilletta, M. J., Niewiarowski, P. H. and Navas, C. A.** (2002). The evolution of
688 thermal physiology in ectotherms. *Journal of Thermal Biology* **27**, 249-268.
689 **Astheimer, L. B., Buttemer, W. A. and Wingfield, J. C.** (1992). Interactions of
690 Corticosterone with Feeding, Activity and Metabolism in Passerine Birds. *Ornis*
691 *Scandinavica* **23**, 355-365.
692 **Barton, K.** (2009). Multi-Model Inference. *R package version 0.12.2/r18, URL*
693 *http://R-Forge.R-project.org/projects/mumin/*.
694 **Bath, E., Morimoto, J. and Wigby, S.** (2018). The developmental environment
695 modulates mating-induced aggression and fighting success in adult female. *Functional*
696 *Ecology* **32**, 2542-2552.
697 **Blas, J., Bortolotti, G. R., Tella, J. L., Baos, R. and Marchant, T. A.** (2007). Stress
698 response during development predicts fitness in a wild, long lived vertebrate. *Proceedings of*
699 *the National Academy of Sciences of the United States of America* **104**, 8880-8884.
700 **Booth, D. T.** (2018). Incubation temperature induced phenotypic plasticity in
701 oviparous reptiles: Where to next? *Journal of Experimental Zoology Part a-Ecological and*
702 *Integrative Physiology* **329**, 343-350.
703 **Booth, D. T., Thompson, M. B. and Herring, S.** (2000). How incubation
704 temperature influences the physiology and growth of embryonic lizards. *Journal of*
705 *Comparative Physiology B-Biochemical Systemic and Environmental Physiology* **170**, 269-
706 276.
707 **Braña, F. and Ji, X.** (2000). Influence of incubation temperature on morphology,
708 locomotor performance, and early growth of hatchling wall lizards (Podarcis muralis).
709 *Journal of Experimental Zoology* **286**, 422-433.
710 **Brand, M. D., Harper, M. E. and Taylor, H. C.** (1993). Control of the Effective
711 P/O Ratio of Oxidative-Phosphorylation in Liver-Mitochondria and Hepatocytes.
712 *Biochemical Journal* **291**, 739-748.
713 **Brand, M. D. and Nicholls, D. G.** (2011). Assessing mitochondrial dysfunction in
714 cells. *Biochemical Journal* **435**, 297-312.
715 **Casagrande, S., Stier, A., Monaghan, P., Loveland, J. L., Boner, W., Lupi, S.,**
716 **Trevisi, R. and Hau, M.** (2020). Increased glucocorticoid concentrations in early life cause
717 mitochondrial inefficiency and short telomeres. *Journal of Experimental Biology* **223**.
718 **Chaby, L. E.** (2016). Why are there lasting effects from exposure to stress during
719 development? An analysis of current models of early stress. *Physiology & Behavior* **164**,
720 164-181.
721 **Cheetham, E., Doody, J. S., Stewart, B. and Harlow, P.** (2011). Embryonic
722 mortality as a cost of communal nesting in the delicate skink. *Journal of Zoology* **283**, 234-
723 242.

Chown, S. L., Hoffmann, A. A., Kristensen, T. N., Angilletta, M. J., Stenseth, N. C. and Pertoldi, C. (2010). Adapting to climate change: a perspective from evolutionary physiology. *Climate Research* **43**, 3-15.

Chung, D. J. and Schulte, P. M. (2020). Mitochondria and the thermal limits of ectotherms. *Journal of Experimental Biology* **223**.

Cockrem, J. F. (2013). Individual variation in glucocorticoid stress responses in animals. *General and Comparative Endocrinology* **181**, 45-58.

Cooger, H. G. (2014). Reptiles and Amphibians of Australia Melbourne, Australia: CSIRO Publishing.

Cooper, B. S., Hammad, L. A. and Montooth, K. L. (2014). Thermal adaptation of cellular membranes in natural populations of. *Functional Ecology* **28**, 886-894.

Cossin-Sevrin, N., Hsu, B. Y., Marciau, C., Viblanc, V. A., Ruuskanen, S. and Stier, A. (2022). Effect of prenatal glucocorticoids and thyroid hormones on developmental plasticity of mitochondrial aerobic metabolism, growth and survival: an experimental test in wild great tits. *Journal of Experimental Biology* **225**.

Costantini, D., Metcalfe, N. B. and Monaghan, P. (2010). Ecological processes in a hormetic framework. *Ecology Letters* **13**, 1435-1447.

Crews, D., Bull, J. J. and Wibbels, T. (1991). Estrogen and Sex Reversal in Turtles - a Dose-Dependent Phenomenon. *General and Comparative Endocrinology* **81**, 357-364.

Crino, O. L., Bonduriansky, R., Martin, L. B. and Noble, D. W. A. (2024). A conceptual framework for understanding stress-induced physiological and transgenerational effects on population responses to climate change. *Evolution Letters* **8**, 161-171.

Crino, O. L. and Breuner, C. W. (2015). Developmental stress: evidence for positive phenotypic and fitness effects in birds. *Journal of Ornithology* **156**, S389-S398.

Crino, O. L., Driscoll, S. C. and Breuner, C. W. (2014a). Corticosterone exposure during development has sustained but not lifelong effects on body size and total and free corticosterone responses in the zebra finch. *General and Comparative Endocrinology* **196**, 123-129.

Crino, O. L., Falk, S., Katsis, A. C., Kraft, F. and Buchanan, K. L. (2022). Mitochondria as the powerhouses of sexual selection: Testing mechanistic links between development, cellular respiration, and bird song. *Hormones and Behavior* **142**.

Crino, O. L., Prather, C. T., Driscoll, S. C., Good, J. M. and Breuner, C. W. (2014b). Developmental stress increases reproductive success in male zebra finches. *Proceedings of the Royal Society B-Biological Sciences* **281**.

Dahlhoff, E. and Somero, G. N. (1993). Effects of Temperature on Mitochondria from Abalone (Genus Haliotis) - Adaptive Plasticity and Its Limits. *Journal of Experimental Biology* **185**, 151-168.

Dayananda, B., Penfold, S. and Webb, J. K. (2017). The effects of incubation temperature on locomotor performance, growth and survival in hatchling velvet geckos. *Journal of Zoology* **303**, 46-53.

De Fraipont, M., Clobert, J., John, H. and Alder, S. (2000). Increased pre-natal maternal corticosterone promotes philopatry of offspring in common lizards. *Journal of Animal Ecology* **69**, 404-413.

De Groef, B., Van der Geyten, S., Darras, V. M. and Kühn, E. R. (2006). Role of corticotropin-releasing hormone as a thyrotropin-releasing factor in non-mammalian vertebrates. *General and Comparative Endocrinology* **146**, 62-68.

De Jong, M. J., Alton, L. A., White, C. R., O'Bryan, M. K., Chapple, D. G. and Wong, B. B. M. (2023). Long-term effects of incubation temperature on growth and thermal physiology in a small ectotherm. *Philosophical Transactions of the Royal Society B-Biological Sciences* **378**.

Debonne, M., Baarendse, P. J. J., Van Den Brand, H., Kemp, B., Bruggeman, V. and Decuypere, E. (2008). Involvement of the hypothalamic-pituitary-thyroid axis and its interaction with the hypothalamic-pituitary-adrenal axis in the ontogeny of avian thermoregulation: a review. *Worlds Poultry Science Journal* **64**, 309-321.

Diffenbaugh, N. S. and Field, C. B. (2013). Changes in Ecologically Critical Terrestrial Climate Conditions. *Science* **341**, 486-492.

Doerrier, C., Garcia-Souza, L. F., Krumschnabel, G., Wohlfarter, Y., Meszaros, A. T. and Gnaiger, E. (2018). High-resolution fluorespirometry and OXPHOS protocols for human cells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. New York, NY: Humana Press.

Dowd, W. W., King, F. A. and Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *Journal of Experimental Biology* **218**, 1956-1967.

Du, W. G. and Shine, R. (2022). The behavioural and physiological ecology of embryos: responding to the challenges of life inside an egg. *Biological Reviews* **97**, 1272-1286.

DuRant, S. E., Hopkins, W. A., Hepp, G. R. and Walters, J. R. (2013). Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biological Reviews* **88**, 499-509.

Esquerré, D., Keogh, J. S. and Schwanz, L. E. (2014). Direct effects of incubation temperature on morphology, thermoregulatory behaviour and locomotor performance in jacky dragons (Amphibolurus muricatus). *Journal of Thermal Biology* **43**, 33-39.

Eyck, H. J. F., Buchanan, K. L., Crino, O. L. and Jessop, T. S. (2019). Effects of developmental stress on animal phenotype and performance: a quantitative review. *Biological Reviews* **94**, 1143-1160.

Forsman, A. and Shine, R. (1995). Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard Lampropholis delicata. *Functional Ecology* **9**, 818-828.

Fox, J. and Weisberg, S. (2019). An R Companion to Applied Regression. Sage, Thousand Oaks CA.

Fuller, A., Dawson, T., Helmuth, B., Hetem, R. S., Mitchell, D. and Maloney, S. K. (2010). Physiological Mechanisms in Coping with Climate Change. *Physiological and Biochemical Zoology* **83**, 713-720.

Gans, I. M. and Coffman, J. A. (2021). Glucocorticoid-Mediated Developmental Programming of Vertebrate Stress Responsivity. *Frontiers in Physiology* **12**.

Geris, K. L., Kotanen, S. P., Berghman, L. R., Kuhn, E. R. and Darras, V. M. (1996). Evidence of a thyrotropin-releasing activity of ovine corticotropin-releasing factor in the domestic fowl (Gallus domesticus). *General and Comparative Endocrinology* **104**, 139-146.

Gerwien, R. W. and Johnalder, H. B. (1992). Growth and Behavior of Thyroid-Deficient Lizards (Sceloporus-Undulatus). *General and Comparative Endocrinology* **87**, 312-324.

Gnaiger, E. (2012). Mitochondrial Pahtways and Respiratory Control: An Introduction to OXPHOS Analysis. Axams, Austria: Steiger Druck GmbH.

Goodman, R. M. and Walguarnery, J. W. (2007). Incubation temperature modifies neonatal thermoregulation in the lizard. *Journal of Experimental Zoology Part a-Ecological Genetics and Physiology* **307a**, 439-448.

Gopi, I. K. and Rattan, S. I. S. (2019). Biphasic Dose-Response and Hormetic Effects of Stress Hormone Hydrocortisone on Telomerase-Immortalized Human Bone Marrow Stem Cells In Vitro. *Dose-Response* **17**.

Greer, A. E. (1989). The Biology and Evolution of Australian Lizards. Sydney, Australia Surrey Beatty.

Grindstaff, J. L. and Merrill, L. (2017). Developmental corticosterone treatment does not program immune responses in zebra finches (Taeniopygia guttata). *Journal of Experimental Zoology Part a-Ecological and Integrative Physiology* **327**, 262-272.

Han, H. S., Kang, G., Kim, J. S., Choi, B. H. and Koo, S. H. (2016). Regulation of glucose metabolism from a liver-centric perspective. *Experimental and Molecular Medicine* **48**.

Hanover, A. M., Husak, J. F. and Lovern, M. (2019). Corticosterone in Lizard Egg Yolk Is Reduced by Maternal Diet Restriction but Unaltered by Maternal Exercise. *Physiological and Biochemical Zoology* **92**, 573-578.

Harders, E. P., Charboneau, C. and Paitz, R. T. (2024). Extraembryonic metabolism of corticosterone protects against effects of exposure. *General and Comparative Endocrinology* **347**, 114439.

Heatwole, H. and Taylor, J. (1987). Ecology of Reptiles. Surrey Beatty: Chipping Norton.

Helmuth, B., Kingsolver, J. G. and Carrington, E. (2005). Biophysics, physiologicalecology,and climate change: Does mechanism matter? *Annual Review of Physiology* **67**, 177-201.

Hill, G. E. (2014). Cellular respiration: The nexus of stress, condition, and ornamentation. *Integrative and Comparative Biology* **54**, 645-657.

Itonaga, K., Wapstra, E. and Jones, S. M. (2011). Evidence for placental transfer of maternal corticosterone in a viviparous lizard. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **160**, 184-189.

Jimeno, B., Hau, M. and Verhulst, S. (2018). Corticosterone levels reflect variation in metabolic rate, independent of 'stress'. *Scientific Reports* **8**.

Jimeno, B., Prichard, M. R., Landry, D., Wolf, C., Larkin, B., Cheviron, Z. and Breuner, C. (2020). Metabolic Rates Predict Baseline Corticosterone and Reproductive Output in a Free-Living Passerine. *Integrative Organismal Biology* **2**.

Jones, J. G. (2016). Hepatic glucose and lipid metabolism. *Diabetologia* **59**, 1098-1103.

Jonsson, B. and Jonsson, N. (2014). Early environment influences later performance in fishes. *Journal of Fish Biology* **85**, 151-188.

Kar, F., Nakagawa, S. and Noble, D. W. A. (2022). Impact of developmental temperatures on thermal plasticity and repeatability of metabolic rate. *Evolutionary Ecology* **36**, 199-216.

Kar, F., Nakagawa, S. and Noble, D. W. A. (2024). Heritability and developmental plasticity of growth in an oviparous lizard. *Heredity* **132**, 67-76.

Kaunisto, S., Ferguson, L. V. and Sinclair, B. J. (2016). Can we predict the effects of multiple stressors on insects in a changing climate? *Current Opinion in Insect Science* **17**, 55-61.

Koch, R. E. and Hill, G. E. (2018). Behavioural mating displays depend on mitochondrial function: a potential mechanism for linking behaviour to individual condition. *Biological Reviews* **93**, 1387-1398.

Kraft, F. L. H., Crino, O. L. and Buchanan, K. L. (2021). Developmental conditions have intergenerational effects on corticosterone levels in a passerine. *Hormones and Behavior* **134**.

Lampl, T., Crum, J. A., Davis, T. A., Milligan, C. and Moore, V. D. (2015). Isolation and Functional Analysis of Mitochondria from Cultured Cells and Mouse Tissue. *Jove-Journal of Visualized Experiments*.

Lenth, R. (2024). Estimated marginal means, aka Least-squares. *R package version 1.10.1* <https://CRAN.R-project.org/package=emmeans>.

Liu, G., Cain, K. and Schwanz, L. (2020). Maternal temperature, corticosterone, and body condition as mediators of maternal effects in jacky dragons (Amphibolurus muricatus). *Physiological and Biochemical Zoology* **93**, 434-449.

Lovern, M. B. and Adams, A. L. (2008). The effects of diet on plasma and yolk steroids in lizards (*Anolis carolinensis*). *Integrative and Comparative Biology* **48**, 428-436.

Loyau, T., Bedrani, L., Berri, C., Métayer-Coustard, S., Praud, C., Coustham, V., Mignon-Grasteau, S., Duclos, M. J., Tesseraud, S., Rideau, N. et al. (2015). Cyclic variations in incubation conditions induce adaptive responses to later heat exposure in chickens: a review. *Animal* **9**, 76-85.

Ludecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P. and Makowski, D. (2021). performance: An R package for assessment, comparison and testing of statistical models. *Jounral of Open Source Software* **6**, 3139.

Lupien, S. J., Buss, C., Schramek, T. E., Maheu, F. and Pruessner, J. (2005). Hormetic influence of glucocorticoids on human memory. *Nonlinearity in Biology, Toxicology, Medicine* **3**, 23-56.

MacLeod, K. J., Sheriff, M. J., Ensminger, D. C., Owen, D. A. S. and Langkilde, T. (2018). Survival and reproductive costs of repeated acute glucocorticoid elevations in a captive, wild animal. *General and Comparative Endocrinology* **268**, 1-6.

Majer, A. D., Paitz, R. T., Tricola, G. M., Geduldig, J. E., Litwa, H. P., Farmer, J. L., Prevelige, B. R., McMahon, E. K., McNeely, T., Sisson, Z. R. et al. (2023). The response to stressors in adulthood depends on the interaction between prenatal exposure to glucocorticoids and environmental context. *Scientific Reports* **13**.

Matthews, S. G. and McGowan, P. O. (2019). Developmental programming of the HPA axis and related behaviours: epigenetic mechanisms. *Journal of Endocrinology* **242**, T69-T79.

McEwen, B. S. and Wingfield, J. C. (2003). The concept of allostasis in biology and biomedicine. *Hormones and Behavior* **43**, 2-15.

McGowan, P. O. and Matthews, S. G. (2018). Prenatal stress, glucocorticoids, and developmental programming of the stress response. *Endocrinology* **159**, 69-82.

McNabb, F. M. A. (2007). The hypothalamic-pituitary-thyroid (HPT) axis in birds and its role in bird development and reproduction. *Critical Reviews in Toxicology* **37**, 163-193.

Mentesana, L. and Hau, M. (2022). Glucocorticoids in a warming world: Do they help birds to cope with high environmental temperatures? *Hormones and Behavior* **142**.

Mitchell, T. S., Warner, D. A. and Janzen, F. J. (2013). Phenotypic and fitness consequences of maternal nest-site choice across multiple early life stages. *Ecology* **94**, 336-345.

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B-Biological Sciences* **363**, 1635-1645.

Monaghan, P. and Haussmann, M. F. (2015). The positive and negative consequences of stressors during early life. *Early Human Development* **91**, 643-647.

Names, G. R., Grindstaff, J. L., Westneat, D. F. and Heidinger, B. J. (2024). Climate change and its effects on body size and shape: the role of endocrine mechanisms. *Philosophical Transactions of the Royal Society B-Biological Sciences* **379**.

Nesan, D. and Vijayan, M. M. (2013). Role of glucocorticoid in developmental programming: Evidence from zebrafish. *General and Comparative Endocrinology* **181**, 35-44.

Noble, D. W. A., Stenhouse, V. and Schwanz, L. E. (2018). Developmental temperatures and phenotypic plasticity in reptiles: a systematic review and meta-analysis. *Biological Reviews* **93**, 72-97.

Nord, A. and Giroud, S. (2020). Lifelong Effects of Thermal Challenges During Development in Birds and Mammals. *Frontiers in Physiology* **11**.

Padda, S. S. and Stahlschmidt, Z. R. (2022). Evaluating the effects of water and food limitation on the life history of an insect using a multiple-stressor framework. *Oecologia* **198**, 519-530.

Painter, D. L. and Moore, M. C. (2005). Steroid hormone metabolism by the chorioallantoic placenta of the mountain spiny lizard as a possible mechanism for buffering maternal-fetal hormone exchange. *Physiological and Biochemical Zoology* **78**, 364-372.

Pakkala, J. J., Norris, D. R., Sedinger, J. S. and Newman, A. E. M. (2016). Experimental effects of early-life corticosterone on the hypothalamic-pituitary-adrenal axis and pre-migratory behaviour in a wild songbird. *Functional Ecology* **30**, 1149-1160.

Pallotti, F. and Lenaz, G. (2001). Isolation and subfractionation of mitochondria from animal cells and tissue culture lines. *Methods in Cell Biology, Vol 65* **65**, 1-35.

Peig, J. and Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos* **118**, 1883-1891.

Picard, M., Juster, R. and McEwen, B. S. (2014). Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. *Nature Reviews Endocrinology* **10**, 303-310.

Pratsinis, H., Tsagarakis, S., Zervolea, I., Stathakos, D., Thalassinos, N. and Kletsas, D. (2006). The unexpected anabolic phenotype and extended longevity of skin fibroblasts after chronic glucocorticoid excess. *Dose-Response* **4**, 133-144.

Racic, A., Tylan, C. and Langkilde, T. (2020). Effects of temperature on plasma corticosterone in a native lizard. *Scientific Reports* **10**.

Remage-Healey, L. and Romero, L. M. (2000). Corticosterone and insulin interact to regulate glucose and lipid concentrations during stress in starlings undergoing molt. *American Zoologist* **40**, 1187-1188.

Romero, L. M. and Reed, J. M. (2005). Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **140**, 73-79.

Rubalcaba, J. G. and Jimeno, B. (2022). Biophysical models unravel associations between glucocorticoids and thermoregulatory costs across avian species. *Functional Ecology* **36**, 64-72.

Salin, K., Auer, S. K., Anderson, G. J., Selman, C. and Metcalfe, N. B. (2016). Inadequate food intake at high temperatures is related to depressed mitochondrial respiratory capacity. *Journal of Experimental Biology* **219**, 1356-1362.

Salin, K., Villasevil, E. M., Anderson, G. J., Auer, S. K., Selman, C., Hartley, R. C., Mullen, W., Chinopoulos, C. and Metcalfe, N. B. (2018). Decreased mitochondrial metabolic requirements in fasting animals carry an oxidative cost. *Functional Ecology* **32**, 2149-2157.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* **21**, 55-89.

Schoech, S. J., Rensel, M. A. and Heiss, R. S. (2011). Short- and long-term effects of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: A review. *Current Zoology* **57**, 514-530.

Seckl, J. R. and Meaney, M. J. (2004). Glucocorticoid programming. *Biobehavioral Stress Response: Protective and Damaging Effects* **1032**, 63-84.

Seebacher, F. and Grigaltchik, V. S. (2014). Embryonic Developmental Temperatures Modulate Thermal Acclimation of Performance Curves in Tadpoles of the Frog Limnodynastes peronii. *Plos One* **9**.

Small, T. W., Bebus, S. E., Bridge, E. S., Elderbrock, E. K., Ferguson, S. M., Jones, B. C. and Schoech, S. J. (2017). Stress-responsiveness influences baseline glucocorticoid levels: Revisiting the under 3 min sampling rule. *General and Comparative Endocrinology* **247**, 152-165.

Spencer, K. A., Evans, N. P. and Monaghan, P. (2009). Postnatal Stress in Birds: A Novel Model of Glucocorticoid Programming of the Hypothalamic-Pituitary-Adrenal Axis. *Endocrinology* **150**, 1931-1934.

Stier, A., Monaghan, P. and Metcalfe, N. B. (2022). Experimental demonstration of prenatal programming of mitochondrial aerobic metabolism lasting until adulthood. *Proceedings of the Royal Society B-Biological Sciences* **289**.

Sumasgutner, P., Cunningham, S. J., Hegemann, A., Amar, A., Watson, H., Nilsson, J. F., Andersson, M. N. and Isaksson, C. (2023). Interactive effects of rising temperatures and urbanisation on birds across different climate zones: A mechanistic perspective. *Global Change Biology* **29**, 2399-2420.

Sun, B. J., Li, T., Gao, J., Ma, L. and Du, W. G. (2015). High incubation temperatures enhance mitochondrial energy metabolism in reptile embryos. *Scientific Reports* **5**.

Taff, C. C., Baldan, D., Mentesana, L., Ouyang, J. Q., Vitousek, M. N. and Hau, M. (2024). Endocrine flexibility can facilitate or constrain the ability to cope with global change. *Philosophical Transactions of the Royal Society B-Biological Sciences* **379**.

Tingley, M. P. and Huybers, P. (2013). Recent temperature extremes at high northern latitudes unprecedented in the past 600 years. *Nature* **496**, 201-+.

Todgham, A. E. and Stillman, J. H. (2013). Physiological Responses to Shifts in Multiple Environmental Stressors: Relevance in a Changing World. *Integrative and Comparative Biology* **53**, 539-544.

Tylan, C., Camacho, K., French, S., Graham, S. P., Herr, M. W., Jones, J., McCormick, G. L., O'Brien, M. A., Tennessen, J. B., Thawley, C. J. et al. (2020). Obtaining plasma to measure baseline corticosterone concentrations in reptiles: How quick is quick enough? *General and Comparative Endocrinology* **287**.

Uller, T., Hollander, J., Astheimer, L. and Olsson, M. (2009). Sex-specific developmental plasticity in response to yolk corticosterone in an oviparous lizard. *Journal of Experimental Biology* **212**, 1087-1091.

Vassallo, B. G., Litwa, H. P., Haussmann, M. F. and Paitz, R. T. (2019). metabolism and yolk glucocorticoid concentration interact to influence embryonic glucocorticoid exposure patterns. *General and Comparative Endocrinology* **272**, 57-62.

Vassallo, B. G., Paitz, R. T., Fasanello, V. J. and Haussmann, M. F. (2014). Glucocorticoid metabolism in the

environment modulates exposure to maternal corticosterone in Japanese quail embryos (*Coturnix japonica*). *Biology Letters* **10**.

Vercken, E., de Fraipont, M., Dufty, A. M. and Clobert, J. (2007). Mother's timing and duration of corticosterone exposure modulate offspring size and natal dispersal in the common lizard (Lacerta vivipara). *Hormones and Behavior* **51**, 379-386.

Warner, D. A. and Shine, R. (2008). The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451**, 566-U5.

Weeks, B. C., Klemz, M., Wada, H., Darling, R., Dias, T., O'Brien, B. K., Probst, C. M., Zhang, M. Y. and Zimova, M. (2022). Temperature, size and developmental plasticity in birds. *Biology Letters* **18**.

Wilson, S. and Swan, G. (2013). A Complete Guide to Reptiles of Australia. Sydney, Australia: Reed New Holland.

Wilsterman, K., Mast, A. D., Luu, T. H. and Haussmann, M. F. (2015). The timing of embryonic exposure to elevated temperature alters stress endocrinology in domestic chickens (Gallus gallus). *General and Comparative Endocrinology* **212**, 10-16.

Wingfield, J. C. and Kitaysky, A. S. (2002). Endocrine responses to unpredictable environmental events: Stress or anti-stress hormones? *Integrative and Comparative Biology* **42**, 600-609.

Wingfield, J. C. and Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology* **15**, 711-724.

Zhang, R. Y., Wild, K. H., Pottier, P., Carrasco, M. I., Nakagawa, S. and Noble, D. W. A. (2023). Developmental environments do not affect thermal physiological traits in reptiles: an experimental test and meta-analysis. *Biology Letters* **19**.

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A. and Smith, G. M. (2009). Mixed Effect Models and Extensions in Ecology with R. New York: Springer.

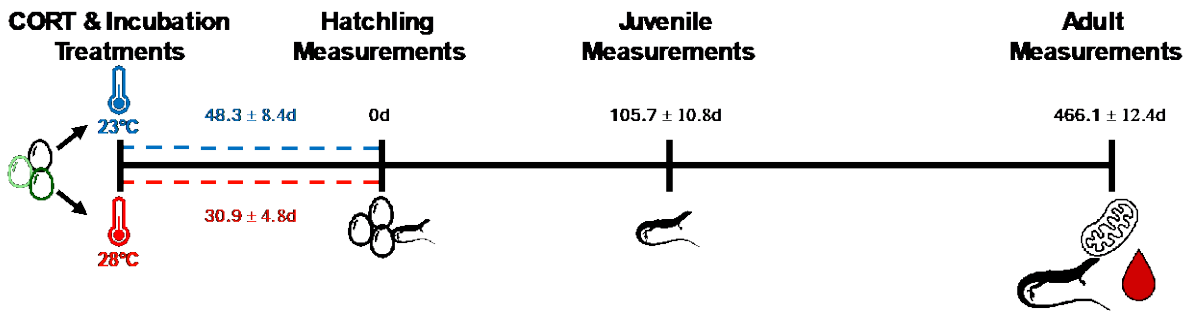


Figure 1. Timeline of experimental protocol. Eggs were dosed with one of three corticosterone (CORT) treatments (control - black, low - light green, and high -dark green) and then incubated at 23 or 28 °C until hatching. We collected morphometric measurements (SVL and mass) following hatching and when lizards were juveniles (~105.7 days post-hatch) and adults(~466.1 days post-hatch). We measured hormone levels (corticosterone, thyroxine, and testosterone (males only) and mitochondrial function from liver tissue in adult lizards.

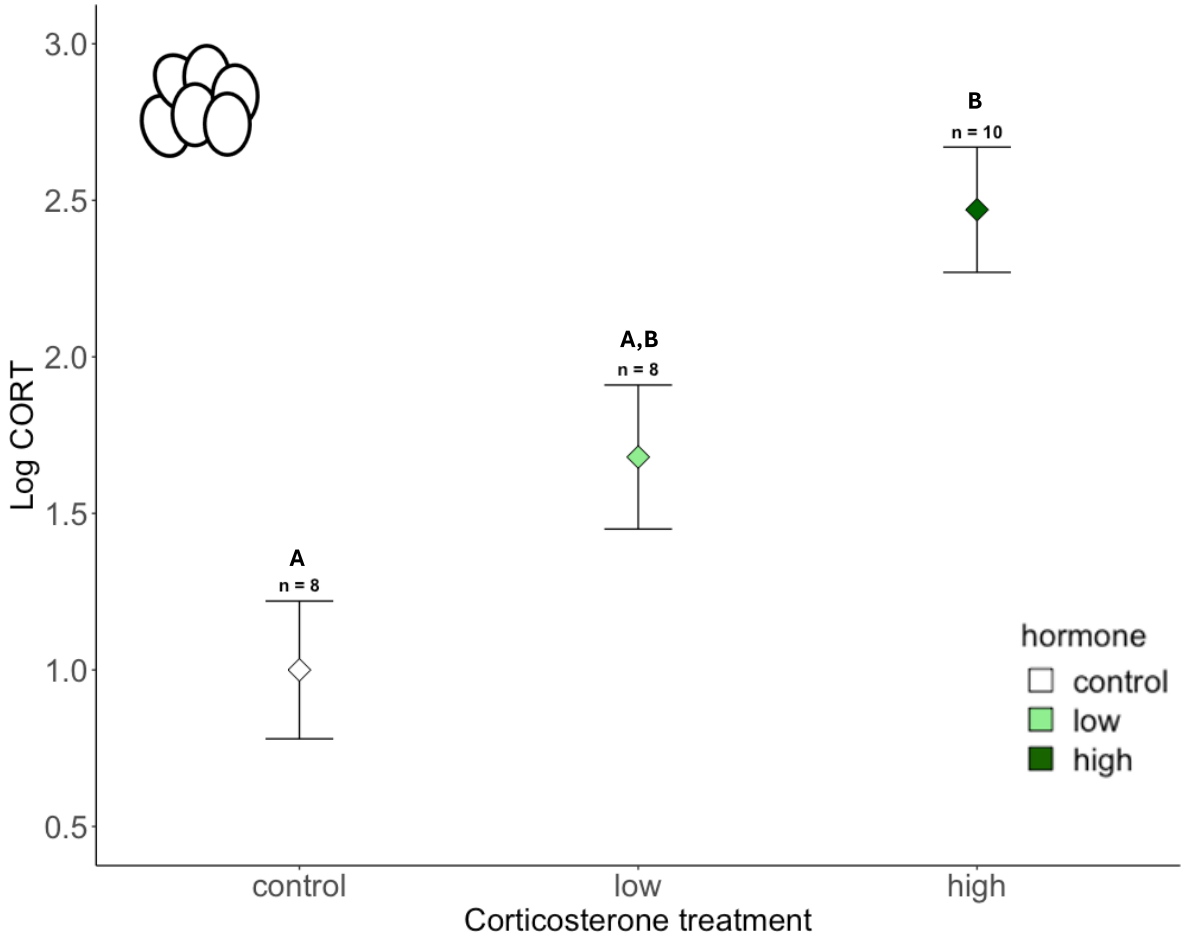


Figure 2. Log-transformed yolk corticosterone levels (log CORT) following treatment with control, low dose corticosterone, and high dose corticosterone treatments. Marginalized mean estimates (mean and SE) are provided based on a model that accounted for corticosterone developmental treatment and test plate effects on yolk corticosterone levels. Significant differences ($p < 0.05$) from post hoc tests are indicated by different letters. Sample sizes (n) for each treatment are indicated error bars.

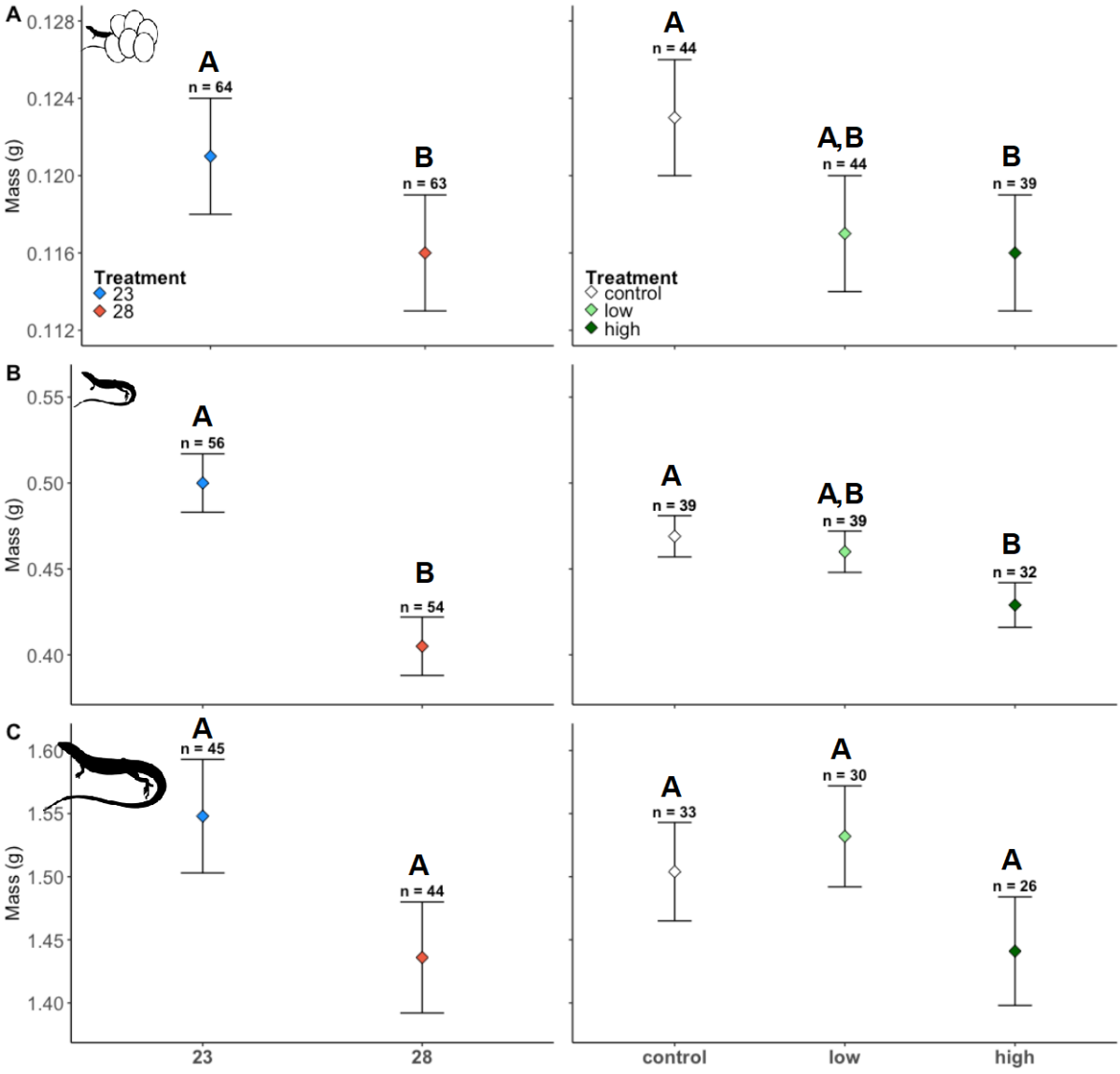


Figure 3. Body mass at hatching (A), the juvenile period (B), and adulthood (C) in lizards exposed to incubation treatments (left panels) and prenatal corticosterone treatments (right panels). Significant differences ($p < 0.05$) from main effects of incubation temperature and post hoc tests for differences between corticosterone treatments are indicated by different letters and sample sizes (n) for each treatment are indicated above. Marginalized mean estimates (mean and SE) are provided based on a model that accounted for incubation temperature, hormone treatment, body size, age, and clutch ID as a random factor.

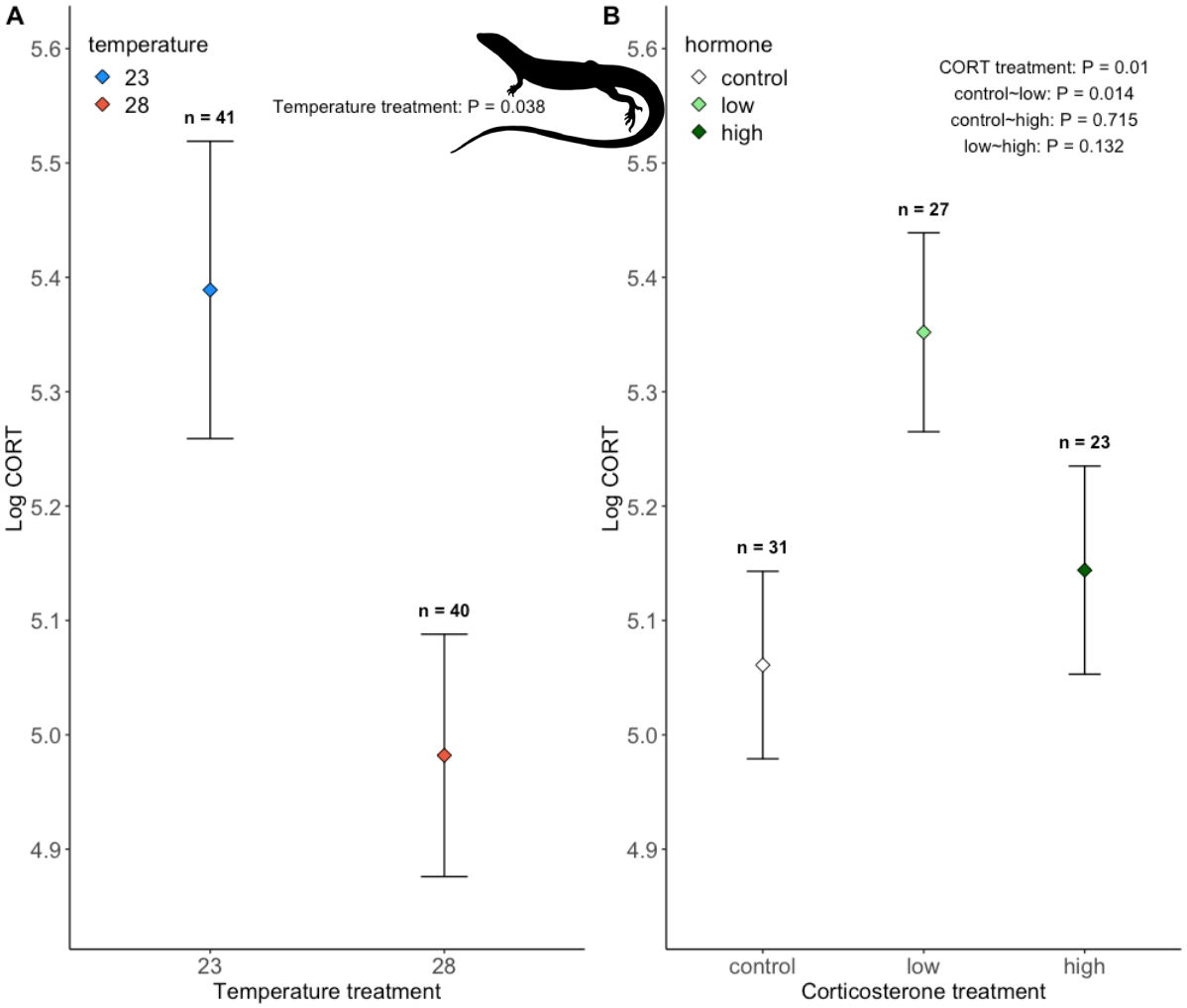


Figure 4. Corticosterone treatments during development affected baseline corticosterone levels in adults. Data are from model results from emmean model, with mean and SE. Sample sizes (n) for each treatment are indicated above. Marginalized mean estimates (mean and SE) are provided based on a model that accounted for incubation temperature, hormone treatment, sex, age, test plate, and clutch ID as a random factor.

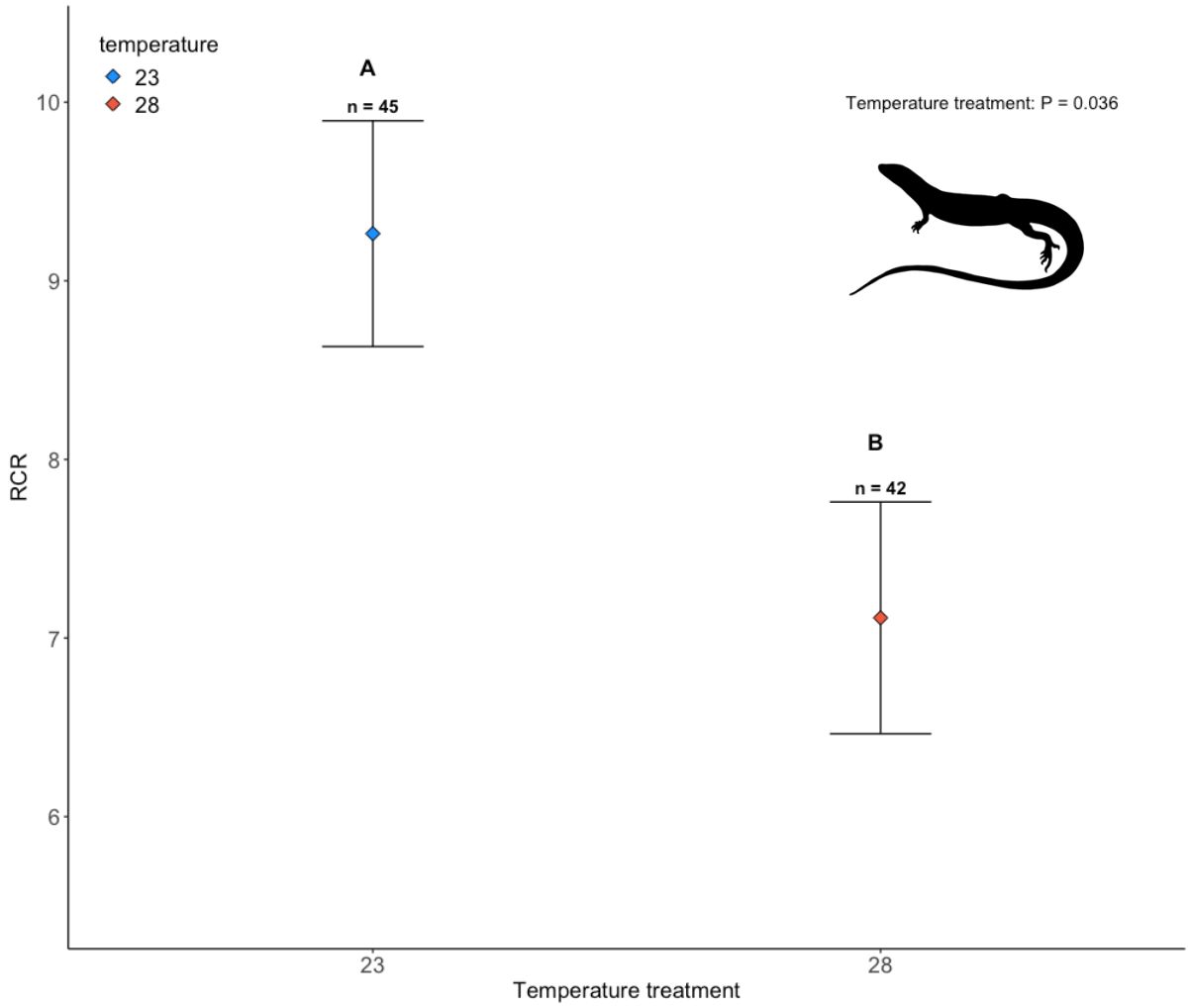


Figure 5. The relationship between adult RCR values and the developmental temperature. The model accounted for hormone, sex, body size and age. Clutch was treated as a random factor. Data are marginalized means and standard error of developmental temperature used in our model.

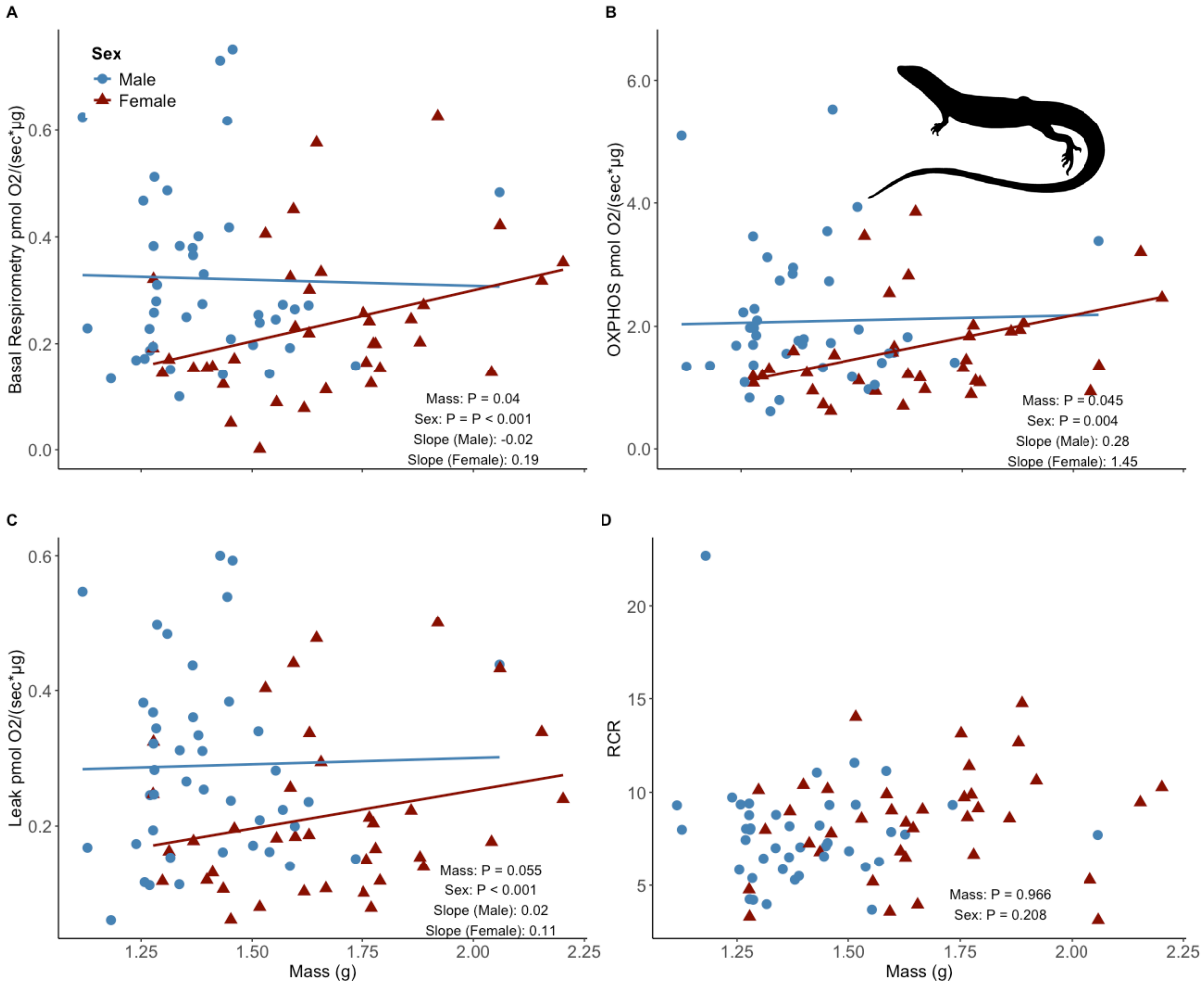


Figure 6. Associations between adult body mass (g) and mitochondrial respiration parameters, including basal respiration (A), OXPHOS respiration (B), leak respiration (C), and RCR (D). Colors indicate sex and P values in the bottom right corners indicate sex differences between treatments.

SUPPLEMENTAL MATERIALS

Measurements of yolk corticosterone levels

To remove the egg yolk, we made a lateral incision with a razor blade on the eggshell. We used dissection scissors to widen the incision and removed the egg yolk using a small spatula. We used KimWipes to remove any albumin that surrounded the egg yolk. Egg yolks were weighed to the nearest in 0.001 mg using a Sartorius microbalance, diluted with 1 mL of doubly distilled water, vortexed thoroughly, and stored at -20º C until assayed.

We used solid phase extraction (SPE) with silica bonded vacuum columns (United Chem. Cat. No. CEC18156) to extract corticosterone from yolk samples. We prepared columns by washing them twice with 5 mL of doubly deionized water. Diluted yolk samples were run through the columns, followed by a wash with 5 mL of 40% methanol to remove lipids. Columns were then soaked with 5 mL of 100% methanol for 2 minutes before samples were eluted with vacuum filtration. Samples were dried under nitrogen at 35º C and then stored at -20º C until assayed. To determine extraction efficiency, we pooled yolk samples and used SPE to extract corticosterone from an aliquot that was spiked with 1,000 pg of corticosterone and an aliquot that was not spiked. We calculated one value of extraction efficiency for each group of samples that was assayed on a hormone plate with an average extraction efficiency across all plates of 25.2%.

We measured yolk corticosterone levels using Arbor Assay Enzyme Immunoassay (EIA) kits (Cat. No. K014). Following extraction, samples were reconstituted in 600 µL of assay buffer, vortexed thoroughly, and mixed on a test tube shaker for 20 minutes. An external standard of 500 pg/mL was run on every plate and used to calculate inter-plate variation. All samples and standards were run in triplicate. Plates were read on FLUOstar Omega microplate readers at 450 nm. Corticosterone levels were calculated from a four parameter nine-point standard curve ranging from 39.063 to 10,000 pg/mL. Intra- and inter-plate variation was 2.05 and 6.05% respectively. We tested assay parallelism by serially diluting a pooled yolk extract and comparing the slope of the antibody binding to that of the standard supplied with the EIA kits. The serial dilution curve was parallel to the standard curve ($p = 0.22$, $F_{1,7} = 1.83$).

Determining protein concentration of mitochondrial suspensions

We used Coomassie Plus (Bradford) assays (Thermo Scientific, Cat. No. 23236) to determine the protein concentration of liver mitochondria suspensions. Sample concentration

was determined from 400 µL aliquots of mitochondrial suspension. We separated mitochondria from the respiration media prior to assays. Briefly, we centrifuged aliquots at 4ºC, 10,000 x g for 10 minutes. We removed and discarded the supernatant and resuspended the pelleted mitochondria in 400 µL of doubly distilled water. We centrifuged the re-suspended aliquots at 4ºC, 10,000 x g for 10 minutes. We removed and discarded the supernatant and dried the pelleted mitochondria under nitrogen (~20 minutes). We re-suspended the dried pellet in 100 µL of doubly distilled water. Samples were assayed in triplicate from 20 µl aliquots of the washed mitochondrial suspension. A pooled sample of washed mitochondrial suspension was run on every plate and used to calculate inter-plate variation. Plates were read on a FLUOstar Omega microplate reader at 595 nm. Protein values were determined from a 10-point standard curve (7.8125 – 1000 ug/mL) and corrected by the dilution factor. Intra- and inter-plate variation for was 3.76 and 8.99% respectively.

Plasma hormone levels

Immediately following euthanasia and decapitation, trunk blood was collected using heparinized microcapillary tubes and kept on ice until processing (>1 hour). Blood was centrifuged at 7,000 rpm for 7 minutes and the isolated plasma was removed and stored at -20ºC until assayed. We were unable to collect 5 µl of plasma from n = 7 individuals and instead assayed samples of 2 – 4 µl of plasma. These samples were diluted to [1:100] and assayed as per samples of 5µl of plasma. Prior to reading the plate, we were able to distinguish the samples assayed from small volumes due to color differences, suggesting that there was sampling error associated with small volumes. Although these samples were assayed, they were excluded prior to statistical analyses to avoid error associated with small volumes.

Plasma corticosterone levels were quantified using methods described above for measuring yolk corticosterone levels. Intra- and inter-plate variation for plasma corticosterone assays was 2.25 and 3.63% respectively. Thyroxine (T4) levels were quantified with Arbor Assay kits (Cat. No. K050). An external standard of 1,000 ng/mL was run on every plate and used to calculate inter-plate variation. Thyroxine levels were calculated from a 4 parameter six-point standard curve ranging from 0.625 to 20 ng/mL. Intra- and inter-plate variation for thyroxine assays was 3.91 and 7.0% respectively. Testosterone levels in males were quantified with Arbor Assay kits (Cat. No. K032). An external standard of 400 pg/ml was run on every plate and used to calculate inter-plate variation. Testosterone levels were calculated from a linear six-point standard curve ranging from 40.96 to 10,000 pg/mL. A

linear fit was used for the standard curve to calculate high values that were not captured with a 4-parameter fit. We used rank order statistics to analyze testosterone data (see below) because linear standard curves may underestimate high hormone values. Intra- and inter-plate variation for testosterone assays was 1.30 and 3.35% respectively.

Statistical analysis

We used a general linear effects model (GLM) to test differences in yolk corticosterone levels following treatment with log transformed corticosterone levels as the dependent variable and corticosterone treatment and assay plate ID as fixed factors. Sample sizes were n = 10 for high corticosterone, n = 8 for low corticosterone, and n = 8 for control. One yolk treated with the high corticosterone treatment had yolk a corticosterone level over two standard deviations greater than mean corticosterone levels. We conducted separate GLMs with and without this datum. The results were statistically equivalent, and it was included in the final model.

We used generalized linear mixed effects models (GLMMs) using the *lme4* package (1.1.35.3), in R version 4.4.0. In all models, we included clutch of origin as a random effect to account for lizards originating from the same mother. To test the effects of developmental treatments on body size and condition we used SVL, body mass, or body condition as the dependent variables (all assumed to follow a Gaussian error distribution with identity link function) and temperature treatment and corticosterone treatment as fixed factors. For models that tested treatment effects post-hatching, we also included a covariate of the days post-hatching to account for the variation in the ages of the lizards. We included sex as a fixed factor in models that tested the effects of treatments on adult body size measurements. We tested treatment effects on growth using growth scores for SVL and mass measurements as dependent variables and temperature and corticosterone treatment and sex (adults only) as fixed factors.

We also used GLMMs to test the effects of developmental treatments on adult corticosterone and thyroxine levels. We first fit GLMMs to test the effects of the time to collect blood samples and the assay plate ID (to account for inter-assay variation) on log transformed hormone levels. The time to collect blood samples did not affect corticosterone or thyroxine levels ($p = 0.77$, $F_{1,75} = 0.09$; $p=0.29$, $F_{1,71} = 1.12$, respectively), but did positively affect testosterone levels ($p = 0.04$, $F_{1,40} = 4.21$). Plate ID affected corticosterone levels ($p = 0.007$, $F_{4,75} = 19.6$) but not thyroxine or testosterone levels ($p = 0.29$, $F_{3,71} = 1.12$;

$p = 0.22$, $F_{1,40} = 1.49$). Significant factors were included in models to test for treatment effects on hormone levels, but if were not significant were removed from models to ease their interpretation. We used log transformed corticosterone or thyroxine levels as dependent factors, temperature and incubation treatments and sex as fixed factors, and scaled age as a covariate. Again, we used a Gaussian error distribution with an identity link function. We used residuals of testosterone level regressed against blood collection time to test effects of developmental treatments on testosterone levels in males using Kruskal-Wallis tests.

To test the effects of developmental treatments on mitochondrial bioenergetics, we created GLMMs with each respiration state (basal, OXPHOS, and leak) and the RCR as the dependent variables and incubation temperature, corticosterone treatment, sex, and respirometer chamber nested in respirometer identity as fixed factors and scaled age as a covariate. We compared models with and without respirometry chamber to determine if variance in each dependent variable was driven by chamber variation. In all cases, it was not important, so we removed this factor from models to ease interpretation. We tested associations between mitochondrial bioenergetics and endogenous corticosterone, thyroxine, and testosterone (males only) levels using GLMMs with respiration state and RCR as dependent variables, log transformed corticosterone and thyroxine levels as covariates, and sex and respirometer chamber nested in respirometer identity as fixed factors.

We tested associations between growth and physiological parameters using GLMMs with growth of mass or SVL as dependent variables, thyroxine levels, corticosterone levels, and mitochondrial respiration parameters as covariates, and sex as a fixed factor. We used log transformed corticosterone and thyroxine levels in GLMMs. We did not account for handling time to collect blood samples or plate identity in this set of GLMs because these factors did not affect hormone levels in this subset of data. We constructed separate GLMMs for each mitochondrial respiration state because basal, OXPHOS, and leak respiration were highly correlated ($p < 0.001$, $r > 0.81$ for all).

Supplemental Tables and Figures

Table S1 Model comparisons between main effects (temperature + hormone) and interaction model (temperature * hormone interaction) using AIC for the different response variables measured. Note that the AIC of models containing the interaction are subtracted from the AIC of models containing just the main effects. Positive values indicate main effects models had lower AIC and were therefore better supported. If models were within 2 AIC units of

each other we simplified to the model with fewer parameters (i.e., main effects model). All models were fit using maximum likelihood for model selection. For details on the specific models fit for each response variable see the Statistical Analysis section.

Variable	Delta_AIC
Incubation time (days)	3.83
Hatchling Snout-vent Length (SVL) (mm)	3.58
Hatchling Mass (g)	3.47
Juvenile SVL (mm)	1.75
Adult SVL (mm)	0.76
Juvenile Mass (g)	3.01
Adult Mass (g)	2.58
Hatchling to Juvenile Growth SVL (mm)	2.25
Hatchling to Juvenile Growth Mass (g)	3.08
Hatchling to Adult Growth SVL (mm)	0.37
Hatchling to Adult Growth Mass (g)	2.97
Basal respiration (pmol/sec/ng)	3.77
OXPHOS respiration (pmol/sec/ng)	3.97
LEAK respiration (pmol/sec/ng)	3.22
Respiratory Control Ratio (RCR)	1.95

Table S2. Summary of GLMs testing the effects of developmental treatments on mitochondrial respiration (basal, OXPHOS, leak, RCR). Significant terms ($p<0.05$) are highlighted in bold.

Test	term	statistic	df	p.value
Basal	hormone	2.929	2	0.231
	scale(adult_age)	1.262	1	0.261
	scale(adult_mass_g)	**4.213**	**1**	**0.040**
	sex	**15.358**	**1**	**0.000**
	temp	0.232	1	0.630
LEAK	hormone	2.185	2	0.335
	scale(adult_age)	2.522	1	0.112
	scale(adult_mass_g)	3.691	1	0.055
	sex	**14.608**	**1**	**0.000**
	temp	1.496	1	0.221
OXPHOS	hormone	3.917	2	0.141
	scale(adult_age)	0.086	1	0.769
	scale(adult_mass_g)	**4.025**	**1**	**0.045**
	sex	**8.140**	**1**	**0.004**
	temp	1.291	1	0.256
RCR	hormone	2.139	2	0.343
	scale(adult_age)	3.730	1	0.053
	scale(adult_mass_g)	0.002	1	0.966
	sex	1.585	1	0.208

Test	term	statistic	df	p.value
	temp	**4.399**	**1**	**0.036**

137

Table S3. Summary of GLMs testing the effects of baseline corticosterone levels on mitochondrial respiration (Basal, OXPHOS, leak, and RCR). Mitochondrial parameters were adjusted for individual mass, and CORT and T4 were log-transformed to meet assumptions for normality. Significant terms ($p<0.05$) are highlighted in bold.

Test	term	statistic	df	p.value
Basal	basal_corrected_pmol	0.445	1	0.505
	log(CORT_Final_Hormone_ng_mL)	3.356	1	0.067
	log(T4_corrected_ng_mL)	0.784	1	0.376
	sex	**24.879**	**1**	**0.000**
Leak	log(CORT_Final_Hormone_ng_mL)	3.560	1	0.059
	log(T4_corrected_ng_mL)	0.773	1	0.379
	oligo_corrected_pmol	0.521	1	0.470
	sex	**25.123**	**1**	**0.000**
OXPHOS	adp_corrected_pmol	1.158	1	0.282
	log(CORT_Final_Hormone_ng_mL)	3.068	1	0.080
	log(T4_corrected_ng_mL)	0.586	1	0.444
	sex	**26.272**	**1**	**0.000**
RCR	RCR	0.008	1	0.927
	log(CORT_Final_Hormone_ng_mL)	3.524	1	0.060
	log(T4_corrected_ng_mL)	0.799	1	0.371
	sex	**25.096**	**1**	**0.000**

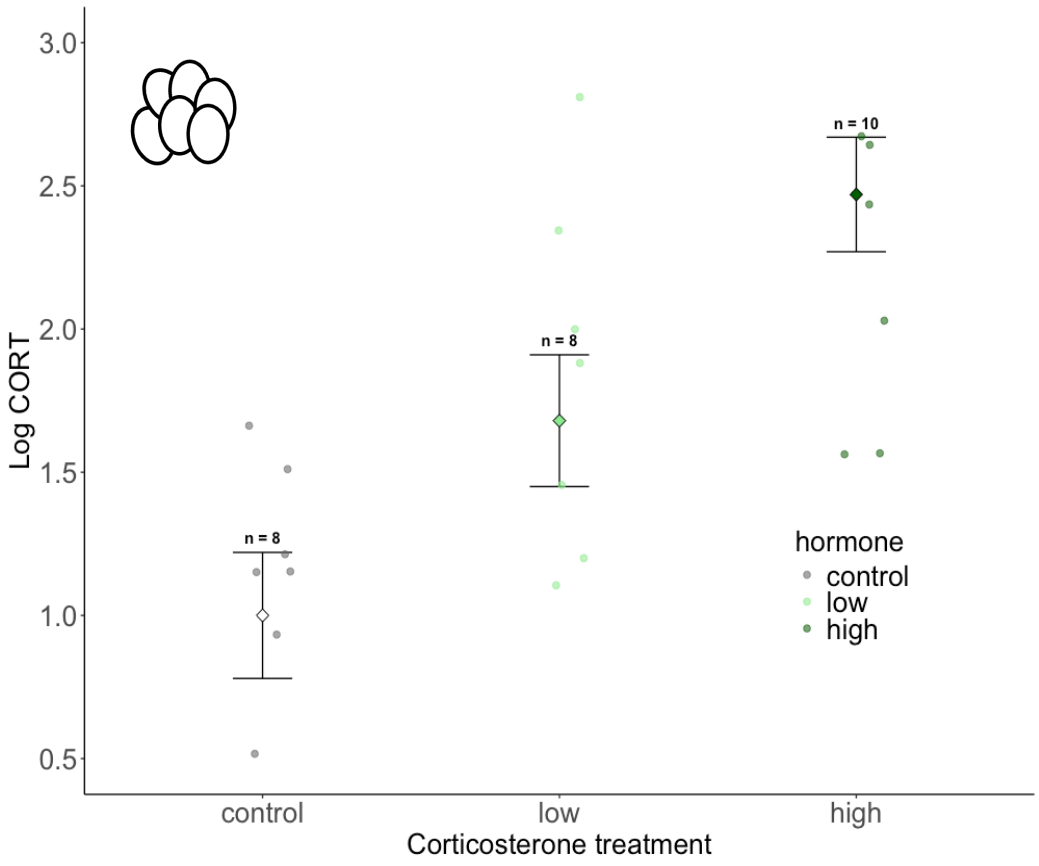
Table S4. Summary of GLMs testing the effects of mitochondrial respiration (basal, OXPHOS, leak, and RCR), sex, baseline corticosterone levels, and thyroxine levels on growth rate (change in mass). Corticosterone and thyroxine levels were log transformed prior to analyses. Significant terms ($p<0.05$) are highlighted in bold.

Test	term	statistic	df	p.value
Growth mass (mg/d)	basal_corrected_pmol	1.104	1	0.293
	log(CORT_Final_Hormone_ng_mL)	1.410	1	0.235
	log(T4_corrected_ng_mL)	0.093	1	0.761
	sex	**8.288**	**1**	**0.004**
Growth mass (mg/d)	log(CORT_Final_Hormone_ng_mL)	1.662	1	0.197
	log(T4_corrected_ng_mL)	0.074	1	0.785
	oligo_corrected_pmol	0.981	1	0.322
	sex	**8.196**	**1**	**0.004**
Growth mass (mg/d)	adp_corrected_pmol	2.761	1	0.097
	log(CORT_Final_Hormone_ng_mL)	1.088	1	0.297
	log(T4_corrected_ng_mL)	0.023	1	0.880
	sex	**9.351**	**1**	**0.002**
Growth mass (mg/d)	RCR	0.338	1	0.561
	log(CORT_Final_Hormone_ng_mL)	1.567	1	0.211
	log(T4_corrected_ng_mL)	0.026	1	0.871
	sex	**6.745**	**1**	**0.009**

Table S5. Summary of GLMs testing the effects of mitochondrial respiration (basal, OXPHOS, leak, and RCR) corrected for protein content, sex, baseline corticosterone levels, and thyroxine levels on growth rate (change in SVL). Corticosterone and thyroxine levels were log transformed prior to analyses. Significant terms are highlighted in bold.

Test	term	statistic	df	p.value
Growth SVL (mm/d)	log(CORT_Final_Hormone_ng_mL)	0.081	1	0.776
	log(T4_corrected_ng_mL)	0.195	1	0.659
	sex	**11.073**	**1**	**0.001**
Growth SVL (mm/d)	log(CORT_Final_Hormone_ng_mL)	0.286	1	0.593
	log(T4_corrected_ng_mL)	0.009	1	0.923
	sex	**12.797**	**1**	**0.000**
Growth SVL (mm/d)	log(CORT_Final_Hormone_ng_mL)	0.256	1	0.613
	log(T4_corrected_ng_mL)	0.425	1	0.515
	sex	**9.526**	**1**	**0.002**
Growth SVL (mm/d)	log(CORT_Final_Hormone_ng_mL)	1.008	1	0.315
	log(T4_corrected_ng_mL)	2.039	1	0.153
	sex	0.379	1	0.538

156


3.0
2.5
2.0
1.5
1.0
0.5
Log CORT
n = 8
n = 8
n = 10
hormone
control
low
high
control
low
high
Corticosterone treatment

157

158 **Figure S1**. Raw data with log-transformed yolk corticosterone levels (log CORT) following
159 treatment with control, low dose corticosterone, and high dose corticosterone treatments.
160 Marginalized mean estimates (mean and SE) are provided based on a model that accounted
161 for CORT developmental treatment and test plate effects. Significant differences ($p < 0.05$)
162 from post hoc tests are indicated by different letters. Sample sizes (n) for each treatment are
163 indicated error bars.

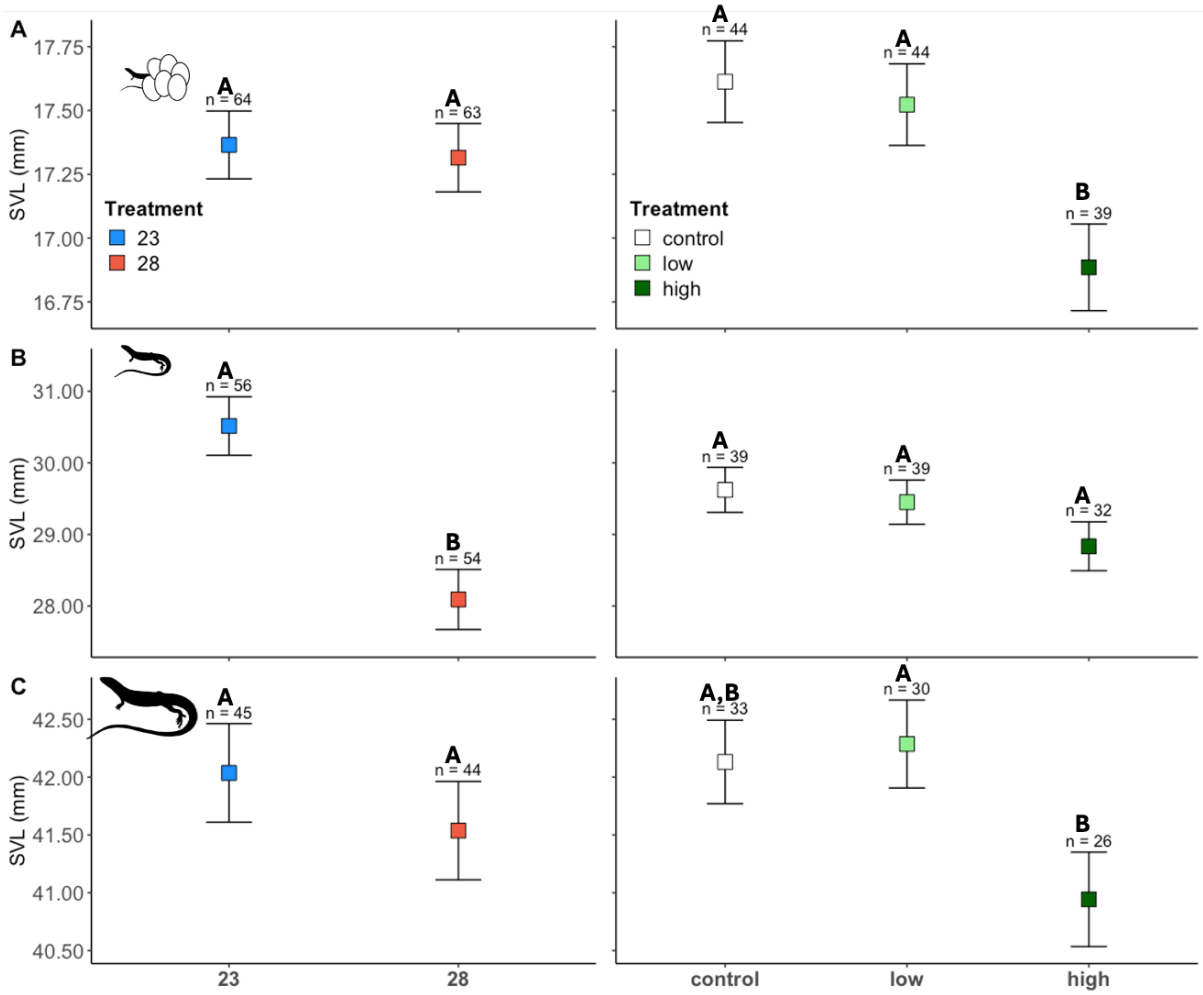


Figure S2. Summarised data of snout vent length (SVL) at hatching (A), the juvenile period (B), and adulthood (C) in lizards exposed to incubation treatments (left panels) and prenatal corticosterone treatments (right panels). Significant differences ($p < 0.05$) from main effects of incubation temperature and post hoc tests for differences between corticosterone treatments are indicated by different letters and sample sizes (n) for each treatment are indicated above. Marginalized mean estimates (mean and SE) are provided based on a model that accounted for incubation temperature, hormone treatment, body size, age, and clutch ID as a random factor.

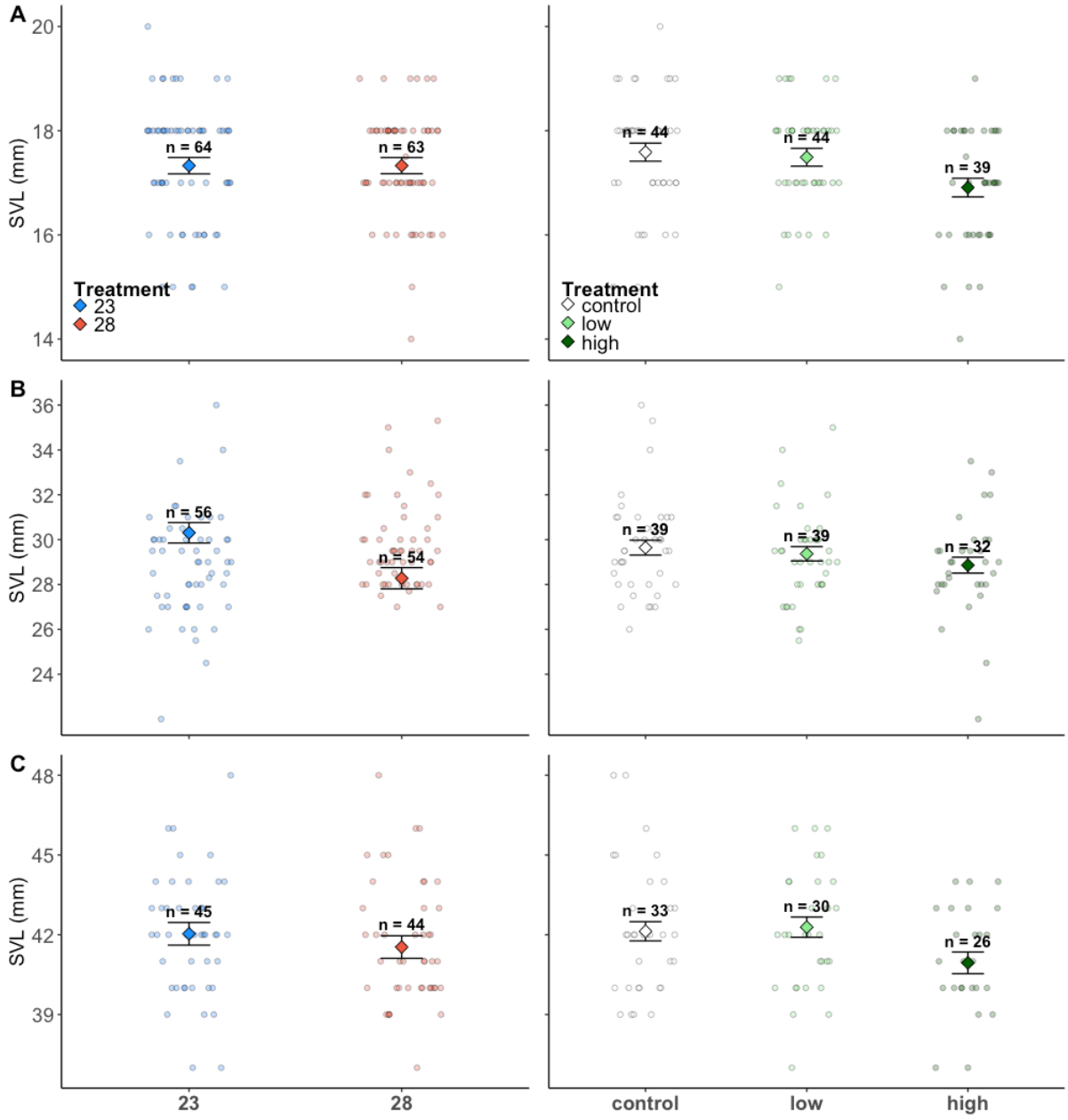


Table S3. Raw data of snout vent length (SVL) at hatching (A), the juvenile period (B), and adulthood (C) in lizards exposed to incubation treatments (left panels) and prenatal corticosterone treatments (right panels). Significant differences ($p < 0.05$) from main effects of incubation temperature and post hoc tests for differences between corticosterone treatments are indicated by different letters and sample sizes (n) for each treatment are indicated above. Marginalized mean estimates (mean and SE) are provided based on a model that accounted for incubation temperature, hormone treatment, body size, age, and clutch ID as a random factor.

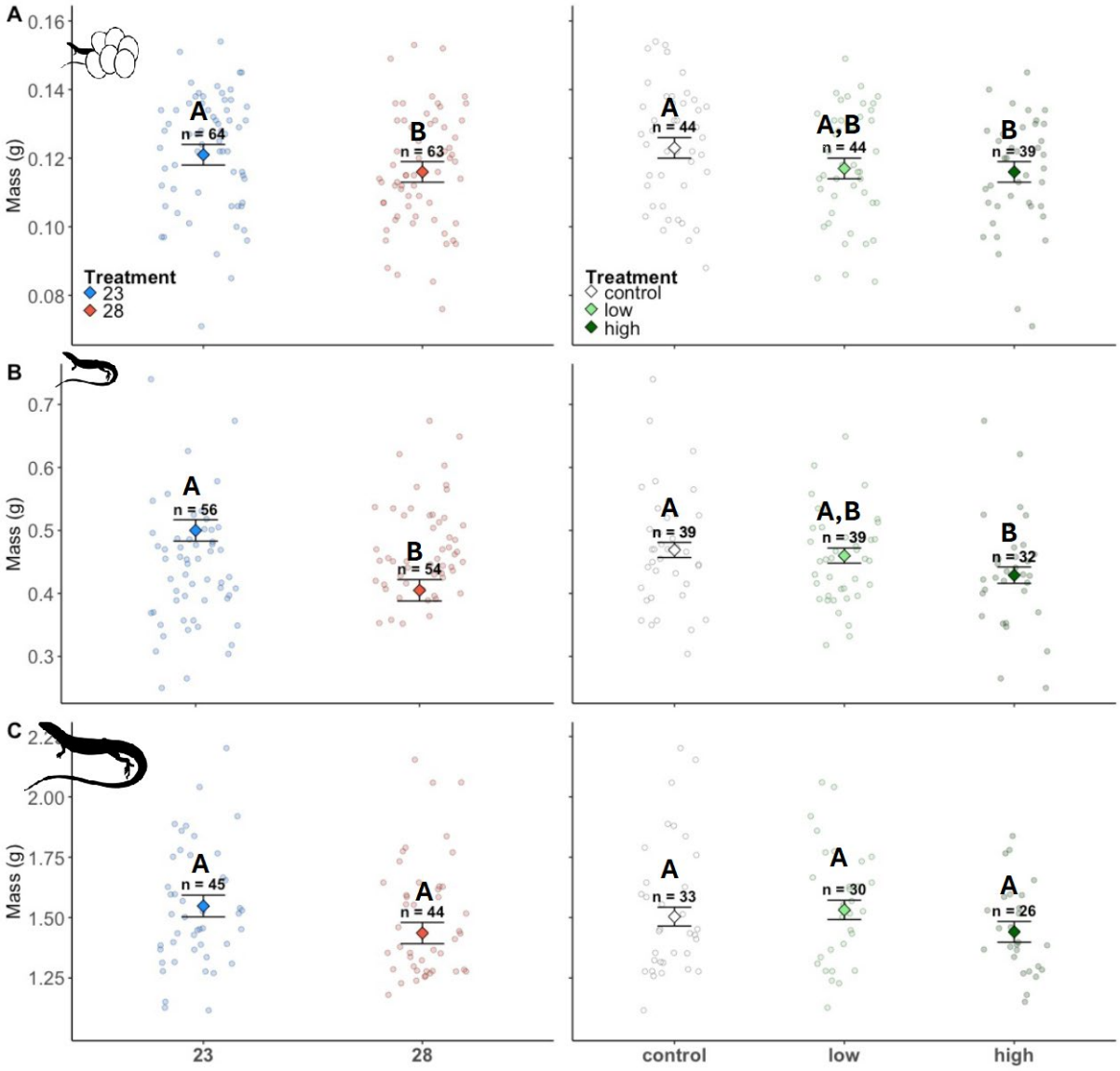
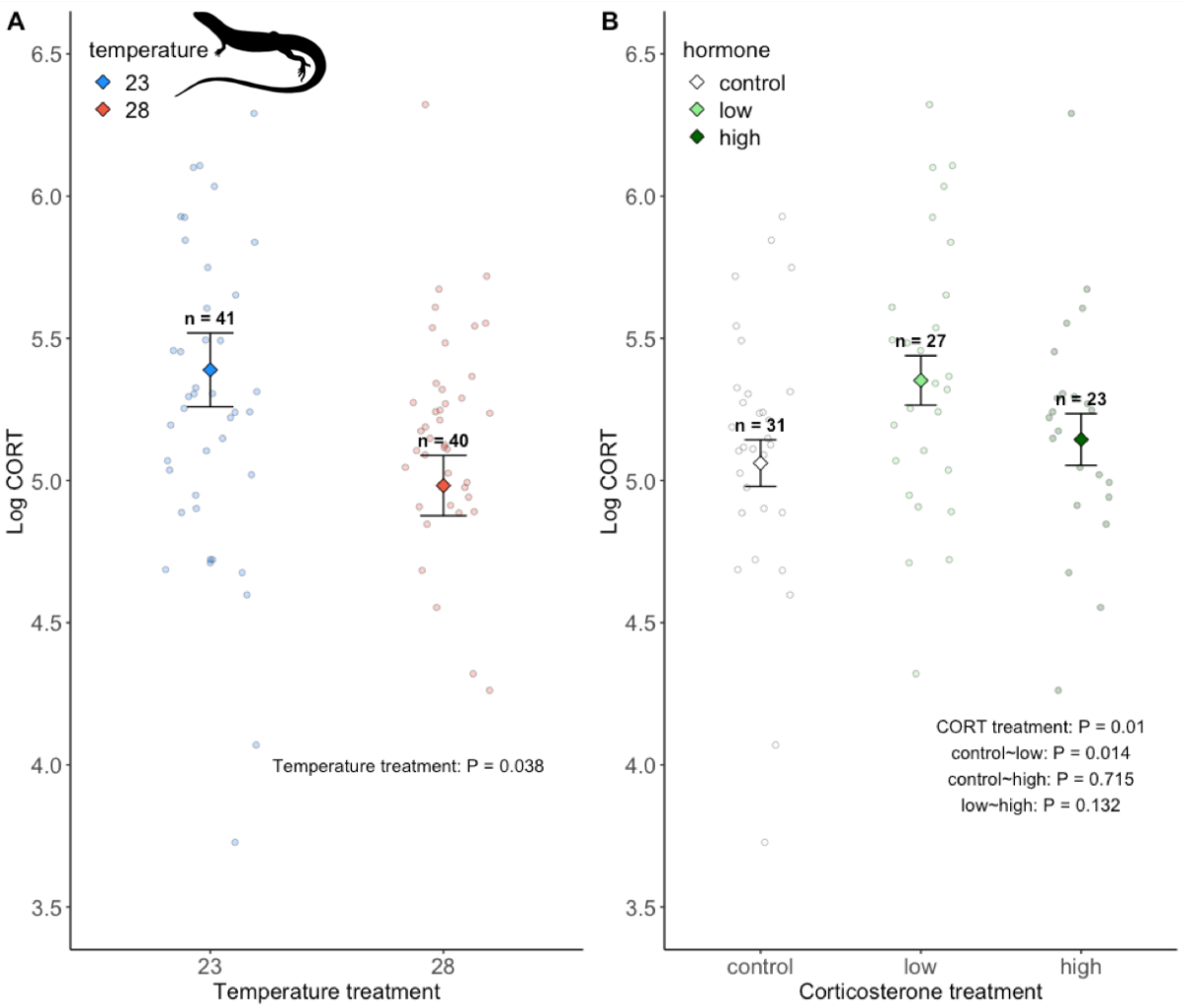


Figure S4. Raw data of body mass at hatching (A), the juvenile period (B), and adulthood (C) in lizards exposed to incubation treatments (left panels) and prenatal corticosterone treatments (right panels). Significant differences ($p < 0.05$) from main effects of incubation temperature and post hoc tests for differences between corticosterone treatments are indicated by different letters and sample sizes (n) for each treatment are indicated above. Marginalized mean estimates (mean and SE) are provided based on a model that accounted for incubation temperature, hormone treatment, body size, age, and clutch ID as a random factor.



194

195 **Figure S5.** Corticosterone treatments during development affected baseline corticosterone
196 levels in adults. Data are from model results from emmean model, with mean and SE. Sample
197 sizes (n) for each treatment are indicated above. Marginalized mean estimates (mean and SE)
198 are provided based on a model that accounted for incubation temperature, hormone treatment,
199 sex, age, test plate, and clutch ID as a random factor.