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. Additionally, elevated temperatures could affect developing animals through indirect effects such as increased exposure to maternal glucocorticoid hormones. Exposure to elevated levels of glucocorticoids during development can have immediate and sustained effects on physiological and behavioural traits. Although many studies have examined how elevated temperatures and glucocorticoid exposure during development independently affect animals, far fewer studies have tested the combined effects of elevated temperatures and glucocorticoids. We tested interactions between incubation temperature and prenatal corticosterone exposure in the delicate skink (*Lampropholis delicata*). Eggs were dosed with one of three corticosterone treatments (high dose corticosterone, low dose corticosterone, or control) and incubated at either 23°C (cool) or 28°C (warm). We measured the effects of these prenatal treatments on development time, body size and condition, growth, and survival from hatching to adulthood. Additionally, we measured the effects of developmental treatments 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 had independent effects on body size at hatching that were sustained into the juvenile period and adulthood. Additionally, we found that prenatal corticosterone treatment affected adult baseline corticosterone levels. We found no direct effects of developmental treatments on adult mitochondrial respiration. However, regardless of treatment, we found positive associations between baseline corticosterone levels and growth rate, suggesting that developmental treatments can have sustained effects on phenotype through effects on corticosterone secretion.

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1. INTRODUCTION

Climate change is one of the most ubiquitous anthropogenic disturbances currently experienced by wildlife. With global temperatures increasing at an unprecedented rate (Diffenbaugh & Field 2013; Tingley & 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 (Helmuth, Kingsolver & Carrington 2005; Chown *et al.* 2010; Fuller *et al.* 2010). Compared to adults, developing animals may be particularly sensitive to elevated temperatures associated with climate change because behavioural avoidance is not possible and developmental conditions can profoundly affect morphology, physiology, and behaviour (Monaghan 2008; Eyck *et al.* 2019; Nord & Giroud 2020). Such developmental effects can be sustained across life history stages and influence behavioural strategies, fitness, and can be transmitted across generations through intergenerational effects (Mitchell, Warner & Janzen 2013; Crino *et al.* 2014; Bath, Morimoto & Wigby 2018). 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 temperatures can increase maternal glucocorticoid hormone levels, which can, in turn, have sustained effects on developing animals (reviewed in Mentesana & Hau 2022; Crino *et al.* 2024). The independent effects of elevated temperatures and glucocorticoids on developing animals have been well studied (reviewed in Seckl & Meaney 2004; Nesan & Vijayan 2013; Crino & Breuner 2015; Noble, Stenhouse & Schwanz 2018; 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 (Mentesana & Hau 2022; Sumasgutner *et al.* 2023; Crino *et al.* 2024; Names *et al.* 2024; Taff *et al.* 2024).

The effects of elevated temperatures on developing animals have been studied extensively in relation to incubation temperature in oviparous reptiles (reviewed in DuRant *et al.* 2013; Jonsson & Jonsson 2014; Noble, Stenhouse & Schwanz 2017; Booth 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 & Ji 2000; Warner & Shine 2008; Esquerré, Keogh & Schwanz 2014; Kar, Nakagawa & Noble 2022; De Jong *et al.* 2023). 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, Penfold & Webb 2017; Kar, Nakagawa & Noble 2024). Elevated incubation temperatures also increase mitochondrial respiration and metabolic enzymes in developing ectotherms suggesting that developmental conditions can affect growth at later life history stages through sustained changes in cellular metabolism (Seebacher & 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 Nesan & Vijayan 2013; Crino & Breuner 2015; McGowan & Matthews 2018; Eyck *et al.* 2019). Glucocorticoids are steroid hormones that play important roles in vertebrate metabolism and stress responses (Wingfield & Kitaysky 2002; McEwen & Wingfield 2003; Picard, Juster & McEwen 2014). In response to stressors or disturbances, vertebrates activate the

hypothalamic-pituitary-adrenal (HPA) axis, the neuroendocrine pathway that regulates the release of glucocorticoid hormones. Glucocorticoids promote physiological and behavioural responses that allow animals to cope with disturbances and stressors and are thus considered mediators of adaptive responses to environmental conditions (Sapolsky, Romero & Munck 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). Exposure to elevated glucocorticoids during development affects a range of phenotypic traits including cellular metabolism, growth and development, body condition, and immune function, and decreased survival (e.g., Blas *et al.* 2007; Crino, Driscoll & Breuner 2014; Grindstaff & Merrill 2017; MacLeod *et al.* 2018; Casagrande *et al.* 2020). Additionally, exposure to glucocorticoids during development can have sustained effects on the HPA axis resulting in the secretion of higher levels of glucocorticoids later in life (e.g., Spencer, Evans & Monaghan 2009; Crino *et al.* 2022). Such sustained effects of developmental conditions on HPA axis function can affect other physiological and behavioural traits such as mitochondrial function that underly phenotypic development, including sexual trait expression (Crino *et al.* 2022).

Although many studies have examined how elevated temperatures and glucocorticoid exposure during development independently affect animals, the combined effects of elevated temperatures and glucocorticoids have not been rigorously tested. In ectotherms, elevated environmental temperatures have been associated with increased glucocorticoid levels in adults (Liu, Cain & Schwanz 2020; Racic, Tylan & Langkilde 2020). Maternal glucocorticoids can be transmitted to developing offspring in both oviparous (Uller *et al.* 2009) and viviparous lizards (Itonaga, Wapstra & Jones 2011) and affect phenotypic traits with possible consequences for fitness (De Fraipont *et al.* 2000; Vercken *et al.* 2007). Thus,

in environments experiencing elevated temperatures, oviparous animals could be exposed to both elevated temperatures during development and elevated levels of glucocorticoids via maternal transmission. Glucocorticoids have direct effects on metabolism and thermal tolerance via interactions with mitochondria and thus could play important roles in regulating phenotypic responses to temperatures through changes in mitochondrial function (Picard, Juster & McEwen 2014; Picard *et al.* 2018; Weeks *et al.* 2022). In this way, exposure to glucocorticoids during development can interact with incubation temperatures to influence development and growth. These effects could be sustained across multiple life history stages if exposure to glucocorticoids during development results in sustained changes to HPA axis function resulting in elevated secretion of glucocorticoids.

Here, we tested the long-term effects of early exposure to elevated incubation temperature and corticosterone on body size and growth, hormone responses, mitochondrial bioenergetics, and survival in the delicate skink (*Lampropholis delicata*). Delicate skinks are native to eastern Australia, occupy various habitats, and are commonly found in human-altered and urban areas (Wilson & Swan 2013; Cooger 2014). Delicate skinks reach sexual maturity at one year of age and range in life span from ~2 – 4 years (Greer 1989). Delicate skinks are oviparous, and females lay clutches of ~3 – 4 eggs (Heatwole & Taylor 1987; Forsman & Shine 1995). 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., Kar, Nakagawa & Noble 2022; De Jong *et al.* 2023). We exposed skinks to one of two corticosterone treatments (high corticosterone, low corticosterone) or a control treatment *in ovo* and 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 then measured body size and condition in response to developmental treatments at hatching and three additional time points and survival 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. We had four main predictions that related to growth and body size, adult endocrine function, adult mitochondrial function, and hatching success and survival:

1. *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.
2. *Growth and body size* – Lizards treated with corticosterone during development would be smaller than control lizards at hatching and throughout life. 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 with corticosterone treatment such that lizards exposed to both these treatments would be smaller than lizards from all other treatments.
3. *Adult endocrine function* – Lizards treated with corticosterone would have higher baseline corticosterone levels as adults because of the sustained effects on the HPA axis due to prenatal corticosterone exposure. Males with higher baseline corticosterone levels would have lower testosterone levels because of the suppressive effects of glucocorticoids on sex steroid synthesis (Wingfield & Sapolsky 2003). Developmental treatment would not affect adult thyroxine levels because no evidence suggests that exposure to glucocorticoids during development affects adult thyroid hormone axis function. We predicted that thyroxine and corticosterone levels would be positively associated with growth among individuals given their roles in regulating metabolism.

4. *Mitochondrial function in liver tissue and growth* – Similar to past studies, developmental treatments would have sustained effects on adult mitochondrial respiration (Crino *et al.* 2022; Stier, Monaghan & Metcalfe 2022). Mitochondrial respiration would be postively associated with growth rate because liver tissue plays an important metabolic role through gluconeogenesis (Sapolsky, Romero & Munck 2000; Picard, Juster & McEwen 2014).

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, Nakagawa & Noble 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.

2 MATERIALS AND METHODS

2.1 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). 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 and had UV lamps for UVA/UVB exposure. 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

195 week. All methods for housing, husbandry, and experimental protocols were approved by
196 The Australian National University Animal Ethics Committee (A2021/56).

197 **2.2 Experimental timeline**

198 Lizard enclosures were checked for eggs three days a week. Eggs were treated with
199 hormone solutions the day they were found. Following treatments, eggs were incubated until
200 hatching was recorded. Eggs were checked three days a week. On the day hatching was
201 recorded, lizards were measured for snout-vent length (SVL) to the nearest mm using a ruler
202 and body mass to the nearest mg using a digital balance and moved to solitary enclosures. We
203 collected additional body size measurements when lizards were juveniles (mean days post-
204 hatching = 105.7, SD = 10.8, range = 85 – 123) and when lizards were euthanized as adults at
205 ~1.3 years of age (mean days post-hatching = 466.1, SD = 12.4, range = 440 – 491) at which
206 point they were sexed by hemipene eversion. After euthanising lizards, we collected a blood
207 sample for hormone analyses and liver tissue to measure mitochondrial bioenergetics. Body
208 condition was calculated at each time point using the scaled mass index derived from SVL
209 and body mass (Peig & Green 2009). We calculated the growth rate from hatching to the
210 juvenile period and hatching to adulthood as body size measurements as juveniles/adults
211 minus body size at hatching divided by juvenile/adult age (days post-hatching).

212 **2.3 Experimental treatments**

213 We exposed eggs to one of six corticosterone/temperature treatments in a fully
214 factorial design. For hormone treatments, eggs were treated with either a high corticosterone
215 (10 pg/mg), low corticosterone (5 pg/mg), or a control (vehicle) treatment. Corticosterone
216 treatments were made by dissolving crystalline corticosterone (Sigma, Cat. No. C2505) in
217 100% ethanol. To dose eggs, we applied 5µl of solutions to eggshells using a micropipette.
218 Control eggs were treated with 5µl of 100% ethanol. Following treatment with corticosterone

219 solutions, eggs were incubated in covered plastic cups filled with damp vermiculite at either
220 23° C (hereafter: cool) or 28º C (hereafter warm; representing the temperature extremes in
221 natural nest sites in this species; Cheetham *et al.* 2011).

222 **2.4 Validation of corticosterone treatments**

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

230 **2.5 Mitochondrial bioenergetics**

231 Lizards were fasted for 72 ± 4 hours prior to euthanasia. Lizards were euthanized via
232 an injection of Alfaxan (10 mg/mL) followed by rapid decapitation. Immediately following
233 decapitation, whole livers were removed, rinsed twice in 1 mL of ice-cold 1M phosphate
234 buffered solution, and stored in 1 mL of ice-cold isolation buffer (250 mM sucrose, 1 mM
235 EGTA, 20 mM Tris HCL, pH 7.4 with KOH) prior to further processing (> 30 minutes).
236 Liver tissue was homogenized on ice with 3 – 4 gentle hand passes using a Potter-Elvehjem
237 homogenizer. The homogenate was centrifuged at 4ºC, 750 x g for 10 minutes. The
238 supernatant was transferred to a clean Eppendorf tube and centrifuged for a second time at
239 4ºC, 750 x g for 10 minutes. The supernatant was transferred to a clean Eppendorf tube and
240 centrifuged at 4ºC, 10,000 x g for 10 minutes. The resulting supernatant was removed and the
241 pellet containing isolated mitochondria was resuspended in 500 µl of MiR05 respiration
242 media [0.5 mM EGTA, 3mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM

243 KH_2PO_4, 20 mM Hepes, 110 mM sucrose, free fatty acid bovine albumin (1 g/L), pH 7.1 with
244 KOH].

245 We measured mitochondrial oxygen consumption (pmol O_2/sec) using Oxygraph-2K
246 high-resolution respirometers (Oroboros Instruments, Innsbruck, Austria). Immediately
247 following preparation, we added resuspended mitochondria to 1.5 mL of respiration media
248 equilibrated at 30º C in one respiration chamber. We applied a series of mitochondrial
249 substrates and inhibitors to measure oxygen consumption at five states: basal (state 2),
250 maximal (state 3), leak (state 4), and residual oxygen consumption. Basal respiration was
251 measured following the addition of pyruvate (5 mM), malate (2 mM), and succinate (10 mM)
252 that support electron entry into the electron transport system via complexes I and II. Maximal
253 respiration was induced with the addition of ADP (2 mM). Leak respiration was induced by
254 adding oligomycin (2.5 µM), which inhibits ATP synthase. Oxygen consumption following
255 the addition of oligomycin is attributed to proton leak across the inner mitochondrial
256 membrane. Finally, we added antimycin A (2.5 µM), which inhibits mitochondrial complex
257 III and allows for measurements of non-mitochondrial oxygen consumption. After
258 administering antimycin A, we collected a 1 mL aliquot of the mitochondrial suspension from
259 the respiration chamber to determine sample concentration. These samples were stored at -20
260 ºC until assayed using Coomassie Plus (Bradford) assays (Thermo Scientific, Cat. No. 23236;
261 Supplemental Materials).

262 Oxygen consumption values for basal, OXPHOS (state 3), and leak (state 4)
263 respiration were corrected for non-mitochondrial oxygen consumption (following the
264 addition of antimycin A) and protein content, yielding values in pmol O_2. $sec^{-1}.\mu g^{-1}$ of
265 mitochondrial protein. We estimated mitochondrial efficiency as the respiratory control ratio
266 (RCR), which is calculated as the ratio of oxygen consumed to drive the phosphorylation of
267 ADP to ATP (OXPHOS) to oxygen consumed to offset proton leak across the inner

mitochondrial membrane (leak; Brand & Nicholls 2011). A high RCR indicates that mitochondria have a high respiratory capacity for ATP production relative to the respiration require to offset the proton leakage.

2.6 Plasma hormone levels

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 EIA kits. All samples and standards were run in triplicate and all plates were read on a FLUOstar Omega microplate reader at 450 nm (Supplemental Materials).

2.7 Statistical analysis

Data were analyzed in R version 4.2.2 using the lme4, emmeans, performance, and car packages (Barton 2009; Fox & Weisberg 2019; Ludecke *et al.* 2021; Lenth 2024). We used a general linear effects model (GLM) to test the effects of corticosterone treatments on yolk corticosterone levels. We used GLMs 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. 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. We tested associations between growth and physiological parameters using GLMs 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

292 'emmeans,' corrected with the Tukey method. We conducted Kruskal-Wallis tests to
293 determine the effects of developmental treatments on survival across the duration of our
294 study. Means are provided with one standard deviation unless indicated otherwise. Full model
295 details and outputs are found in Supplemental Materials.

296 **3 RESULTS**

297 **3.1 Hormone treatment effects on yolk corticosterone levels**

298 Topical corticosterone treatment affected yolk corticosterone levels ($p = 0.002$, $F_{2,22} =$
299 7.98; Figure 1). Eggs treated with high doses of corticosterone had higher levels of yolk
300 corticosterone than control eggs ($p = 0.002$; $mean_{high\_corticosterone} = 11.42 \pm 8.44$ pg/mg,
301 $mean_{control} = 3.09 \pm 1.40$ pg/mg). Eggs treated with low doses of corticosterone had yolk
302 corticosterone levels intermediate between the high dose and control treatments
303 ($mean_{low\_corticosterone} = 6.64 \pm 4.92$ pg/mg). There were no differences in yolk corticosterone
304 levels between eggs treated with high and low doses of corticosterone ($p = 0.11$) and low
305 doses of corticosterone and the control treatment ($p = 0.21$).

306 **3.2 Developmental treatments across life – effects on incubation time, body size and**
307 **condition, and survival**

308 Lizards incubated at warm temperatures hatched faster than lizards incubated at cool
309 temperatures ($p < 0.001$; $F_{1,115} = 184.64$; average days to hatch: warm = 30.9 ± 4.8 and cool =
310 48.3 ± 8.4). There was no effect of corticosterone treatment on the time for lizards to hatch (p
311 $= 0.88$, $F_{2,115} = 0.13$; average days to hatch: high corticosterone = 39.5 ± 11.0, low
312 corticosterone = 40.6 ± 11.5, control = 39.8 ± 11.0). Incubation temperature did not affect
313 mortality across the duration of our study (cool temperature: n = 6 of 56 deceased; warm
314 temperature: n = 9 of 54 deceased; Kruskal-Wallis chi-squared = 0.62, d.f. = 1, $p = 0.43$).
315 Similarly, corticosterone treatment did not affect mortality (high corticosterone: n = 5 of 33

316 deceased; low corticosterone: n = 5 of 38 deceased; control: n = 5 of 39 deceased; Kruskal-
317 Wallis chi-squared = 0.07, d.f. = 2, p = 0.97).

318 Incubation temperature did not affect SVL at hatching ($p = 0.79$, $F_{1,123} = 0.07$;
319 Supplemental Figure S1) but did affect body mass such that lizards incubated at warm
320 temperatures weighed less than lizards incubated at cool temperatures ($p = 0.046$, $F_{1,123} =$
321 4.08; Figure 2) and had lower body condition ($p = 0.045$, $F_{1,123} = 4.10$). As juveniles, lizards
322 incubated at warm temperatures during development had smaller SVLs ($p = 0.001$, $F_{1,101} =$
323 10.69) and weighed less compared to lizards incubated at cooler temperatures ($p < 0.001$,
324 $F_{1,101} = 18.21$), but there were no differences in body condition ($p = 0.35$, $F_{1,101} = 0.87$). The
325 effects of incubation temperature on body size that we observed at early ages were not
326 present in adults. Incubation temperature did not affect adult SVL ($p = 0.49$, $F_{1,80} = 0.47$),
327 body mass ($p = 0.13$, $F_{1,80} = 2.35$), or body condition ($p = 0.15$, $F_{1,80} = 2.11$).

328 Corticosterone treatment affected both SVL (Figure S1) and body mass at hatching (p
329 $= 0.004$, $F_{2,123} = 5.68$; $p = 0.048$, $F_{2,123} = 3.11$; Figure 2), but not body condition ($p = 0.08$,
330 $F_{2,123} = 2.63$). Lizards treated with high doses of corticosterone had smaller SVLs than those
331 treated with low doses of corticosterone ($p = 0.02$) and control lizards ($p = 0.006$). Lizards
332 treated with high doses of corticosterone weighed less than control lizards ($p = 0.047$) but did
333 not differ in body mass compared to those treated with low doses of corticosterone ($p = 0.77$).
334 There were no differences in SVL or body mass measurements between lizards treated with
335 low doses of corticosterone and control lizards ($p = 0.92$ and 0.19). Corticosterone treatment
336 during development also affected juvenile body mass ($p = 0.04$, $F_{2,101} = 3.39$) such that
337 lizards exposed to high doses of corticosterone during development weighed less than control
338 lizards ($p = 0.04$) but were not different from lizards treated with low doses of corticosterone
339 ($p = 0.10$). There were no differences in body mass between lizards treated with low doses of
340 corticosterone and control lizards ($p = 0.91$). Corticosterone treatment during development

did not affect juvenile SVL ($p = 0.22$, $F_{2,101} = 1.56$) or body condition ($p = 0.32$, $F_{2,101} = 2.00$). In adults, corticosterone treatment during development affected SVL ($p = 0.04$, $F_{2,80} = 3.42$), with adults exposed to high doses of corticosterone during development having smaller SVLs as adults compared to lizards that received low doses of corticosterone ($p = 0.048$) but not control lizards ($p = 0.08$). 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 ($p = 0.10$, $F_{2,80} = 2.32$) or body condition ($p = 0.20$, $F_{1,80} = 1.66$).

Lizards incubated at warmer temperatures grew less in SVL from hatching to the juvenile period compared to lizards incubated at cooler temperatures ($p < 0.001$, $F_{1,102} = 12.84$) and hatching to adulthood ($p = 0.005$, $F_{1,81} = 8.46$) but did not differ in growth of body mass from hatching to the juvenile period ($p = 0.20$, $F_{1,102} = 1.67$) or hatching to adulthood ($p = 0.09$, $F_{1,81} = 2.96$). Corticosterone treatment during development negatively affected growth of body mass from hatching to the juvenile period ($p = 0.047$, $F_{2,102} = 3.15$). Lizards exposed to high doses of corticosterone grew more slowly than control lizards ($p = 0.04$) but did not differ from low dose lizards ($p = 0.15$). There was no difference in body mass gain between low-dose and control lizards ($p = 0.82$) from hatching to the juvenile period. Corticosterone treatment during development did not affect the growth of SVL from hatching to the juvenile period ($p = 0.70$, $F_{2,102} = 0.35$) or hatching to adulthood ($p = 0.47$, $F_{2,81} = 0.76$) and did not affect change in body mass from hatching to adulthood ($p = 0.10$, $F_{2,81} = 2.40$).

3.3 Developmental treatments in adults – effects on hormones and mitochondrial bioenergetics

Corticosterone treatment during development affected adult baseline corticosterone levels ($p = 0.045$, $F_{2,68} = 3.25$; Figure 3). Lizards treated with low doses of corticosterone had

higher levels of baseline corticosterone compared to lizards treated with high doses and control treatments, but post-hoc pairwise comparisons revealed no significant differences between treatment groups (control ~ low: $p = 0.06$, control ~ high: $p = 0.99$, low ~ high: $p = 0.10$). Incubation temperature did not affect baseline corticosterone levels in adult lizards ($p = 0.19$, $F_{1,68} = 1.77$). Males had higher baseline corticosterone levels than females, but the difference was non-significant ($p = 0.057$, $F_{1,68} = 3.74$). Neither incubation temperature nor corticosterone treatment affected adult thyroxine levels ($p = 0.95$, $F_{1,67} = 0.004$; $p = 0.88$, $F_{2,67} = 0.13$, respectively). Females had higher thyroxine levels than males ($p = 0.02$, $F_{1,67} = 5.60$). Testosterone levels in males were not affected by incubation temperature (Kruskal-Wallis chi-squared = 0.06, $p = 0.81$, $n_{cool} = 21$, $n_{warm} = 22$) or corticosterone treatment (Kruskal-Wallis chi-squared = 0.19, $p = 0.91$, $n_{control} = 12$, $n_{low} = 16$, $n_{high} = 12$).

Mitochondrial respiration was not affected by incubation temperature or corticosterone treatment during development (Supplemental Table S1) or baseline corticosterone levels (Supplemental Table S2). Basal and OXPHOS respiration were positively associated with adult body mass ($p = 0.03$, 0.04; $F_{1,75} = 4.94$, 4.54; Figure 4a and b) and leak respiration was non-significantly associated with body mass ($p = 0.054$, $F_{1,75} = 3.82$; Figure 4c). There was no association between RCR and body mass ($p = 0.56$, $F_{1,75} = 0.34$; Figure 4d). Males had higher oxygen consumption than females for basal respiration ($p < 0.001$, $F_{1,75} = 15.78$), OXPHOS respiration ($p = 0.007$, $F_{1,75} = 7.82$), and leak respiration ($p < 0.001$, $F_{1,75} = 12.70$). However, there was no difference between males and females in RCR values ($p = 0.40$, $F_{1,75} = 0.73$).

3.4 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

389 (Supplemental Tables S3 and S4). Growth in body mass from hatching to adulthood was
390 positively associated with corticosterone levels for all models with mitochondrial respiration
391 parameters (basal: $F_{1,67} = 5.03$, $p = 0.03$; OXPHOS: $F_{1,67} = 4.60$, $p = 0.04$; leak: $F_{1,67} = 5.58$, p
392 $= 0.02$; RCR: $F_{1,67} = 5.25$, $p = 0.03$; Figure 4). There were no associations between growth in
393 SVL from hatching to adulthood and corticosterone levels for all models (basal: $F_{1,67} = 2.39$,
394 $p = 0.13$; OXPHOS: $F_{1,67} = 2.01$, $p = 0.16$; leak: $F_{1,67} = 2.75$, $p = 0.10$; RCR: $F_{1,67} = 2.36$, $p =$
395 0.13). Body mass and SVL growth were lower for males than females in all models (Tables
396 S3 and S4). There were no associations between growth in body mass or SVL and thyroid
397 hormone levels (Supplemnetal Tables S3 and S4).

398

399 **4 DISCUSSION**

400 We found evidence that incubation temperature and corticosterone exposure during
401 development had short-term and sustained effects on lizards. However, we found no evidence
402 for interactive effects of incubation temperature and corticosterone exposure during
403 development. As expected, we found that skinks incubated at warmer temperatures hatched
404 more quickly and at a smaller body size compared to skinks incubated at cooler temperatures.
405 Incubation temperatures had sustained but not permanent effects on body size (mass and
406 SVL) such that juveniles incubated at warmer temperatures were smaller than juveniles
407 incubated at cooler temperatures, but these differences were not present in adults.
408 Corticosterone treatment during development did not affect the time for skinks to hatch, but
409 high doses of corticosterone decreased body size from hatching until adulthood.
410 Corticosterone treatment during development affected adult baseline corticosterone levels.
411 We found positive associations between adult corticosterone levels and growth, suggesting
412 that developmental conditions can have sustained effects on growth through changes in

endocrine function. However, developmental treatments did not affect mitochondrial bioenergetics of liver tissue in adult skinks and there were no associations between mitochondrial bioenergetics and growth. Males had higher OXPHOS and leak mitochondrial respiration than females.

4.1 Effects of early thermal environment and corticosterone do not interact but have independent effects on physiology and growth

We found no evidence of interactive effects between incubation temperature and corticosterone treatment. 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 rapidly metabolizes corticosterone which potentially buffers developing embryos from elevated levels of maternal corticosterone (Painter & Moore 2005). In oviparous lizards, much less is known about how glucocorticoids affect developing embryos and how long they remain biologically active in the yolk. In red-eared slider turtles (*Trachemys scripta*), topical application of radiolabeled estradiol to eggs resulted in peak levels of embryonic estradiol 50 hours after application with levels halved 216 hours after application (Crews, Bull & Wibbels 1991). In our experiment, it is possible that exogenous corticosterone affected embryos for part of the incubation period while incubation temperature affected embryos for the entire period, resulting in less time for treatments to have interactive effects on embryos. However, it seems likely that incubation temperature would have affected the metabolism of exogenous corticosterone with warmer temperatures increasing the rate of clearance. Alternatively, warmer incubation temperatures could have extended the exposure of exogenous corticosterone through to the postnatal period if lizards incubated at warmer temperatures hatch with greater amounts of internalized residual yolk (Murphy *et al.* 2020). Future studies could clarify potential interactions between incubation

438 temperature and maternally derived or exogenous yolk hormones using radiolabeled
439 hormones to test interactions between incubation temperature and yolk hormone metabolism.

440 Although we found no interactive effects of developmental treatments, incubation
441 temperature and corticosterone treatment had independent effects on body size such that
442 skinks incubated at warm incubation temperatures (28°C) or exposed to high levels of
443 corticosterone prenatally were smaller compared to skinks incubated at cooler temperatures
444 (23°C) or not exposed to corticosterone (control). These treatment effects on body size were
445 present at hatching and the juvenile period (temperature and corticosterone) and in adults
446 (corticosterone treatment only). In oviparous lizards, elevated incubation temperatures
447 generally decrease incubation duration and can result in smaller hatchlings in some species
448 (reviewed in Noble, Stenhouse & Schwanz 2017; Booth 2018). The effects of incubation
449 temperature on growth in oviparous lizards are likely due to the effects of temperature on the
450 rate of biochemical reactions (Angilletta, Niewiarowski & Navas 2002; Dowd, King &
451 Denny 2015). In our experiment, elevated temperature had sustained but not lifelong effects
452 on body size, suggesting that skinks compensate for early developmental effects with changes
453 in postnatal growth. Such compensatory growth has been linked to elevated production of
454 reactive oxygen species (ROS) and faster senescence (Metcalfe & Monaghan 2001).

455 **4.2 Early corticosterone affects baseline corticosterone as adults**

456 Across taxa, past studies have shown that exposure to elevated levels of
457 glucocorticoids during development can affect HPA axis function at later life history stages
458 (reviewed in Seckl & Meaney 2004; Matthews & McGowan 2019; Gans & Coffman 2021).
459 We showed that prenatal exposure to corticosterone has sustained effects on baseline
460 corticosterone in skinks. In some species, exposure to elevated glucocorticoids during
461 development can have sustained but not lifelong effects on HPA axis function (Crino,

462 Driscoll & Breuner 2014). Our experiment measured corticosterone levels in adult skinks
463 (average days post-hatching = 466.1) that could have been near the end of their lifespan (2 - 4
464 years; Greer 1989). Palacios *et al.* (2023) found associations between maternal corticosterone
465 levels and offspring corticosterone levels in neonate western terrestrial garter snakes
466 (*Thamnophis elegans*) that were not detectable in one-year-old juveniles. Similarly, we may
467 have detected stronger effects of corticosterone treatment on HPA axis function at earlier life
468 stages. The duration of glucocorticoid-mediated changes in HPA axis function has important
469 implications for understanding the power of these developmental effects to shape
470 evolutionary responses. For example, if exposure to elevated glucocorticoids during
471 development has sustained effects on HPA axis function that persist until sexual maturity,
472 exposure to developmental glucocorticoids in one generation can affect future generations
473 through intergenerational effects (Crino *et al.* 2024).

474 **4.3 Effects of corticosterone on growth**

475 Exposure to elevated levels of corticosterone during development is associated with
476 reduced growth and development and smaller postnatal body size (Meylan & Clobert 2005;
477 Vercken *et al.* 2007; Crino, Driscoll & Breuner 2014). In adult animals, glucocorticoids
478 regulate physiological processes that increase circulating levels of glucose and lipids by
479 increasing hepatic glucose production (through gluconeogenesis) and by reducing glucose
480 uptake by skeletal muscles (Sapolsky, Romero & Munck 2000; Picard, Juster & McEwen
481 2014). However, chronic elevation or high doses of glucocorticoids can impair glucose
482 metabolism through effects on mitochondrial function (Picard, Juster & McEwen 2014; Kuo
483 *et al.* 2015), whereas low doses of glucocorticoids enhance mitochondrial function (Du *et al.*
484 2008). In developing animals, exposure to elevated levels of glucocorticoids has been linked
485 to decreased mitochondrial efficiency (Casagrande *et al.* 2020; Crino *et al.* 2022), suggesting

a pathway that links elevated glucocorticoids levels to reduced growth via effects on mitochondrial function.

Although prenatal corticosterone treatment reduced body size, we found positive associations between baseline corticosterone in adults and growth. If baseline corticosterone levels in adults are reflective of baseline corticosterone levels across life, our results suggest that changes in HPA axis function during development can have sustained effects on growth. Glucocorticoids play central roles in many metabolic pathways, including supporting gluconeogenesis in the liver (Kuo *et al.* 2015). Although high levels of glucocorticoids are often associated with decreased growth and body condition in developing animals and low body condition in adults (Crino, Driscoll & Breuner 2014; Crino *et al.* 2017; Crino *et al.* 2018; Kraft *et al.* 2019), moderately elevated baseline levels of glucocorticoids may allow animals to meet energy requirements of metabolically demanding processes (e.g., growth, parental care, migration; Bonier, Moore & Robertson 2011; Rivers *et al.* 2012; Jimeno *et al.* 2020). In our experiment, lizards had access to *ad libitum* food which may have facilitated the effects of glucocorticoids on growth. Free-living animals are likely to be comparatively energy-limited and developmentally induced changes in glucocorticoids may be beneficial in some postnatal environments but not others. Studies that track the effects of developmental conditions over the lifespan of free-living animals under different environmental contexts (e.g., food limitation) or that house animals in semi-natural conditions would help elucidate the fitness consequences of glucocorticoid-mediated developmental effects.

4.4 Mitochondrial bioenergetics are not affected by developmental conditions but vary between sexes

We found no evidence that developmental treatments affect mitochondrial respiration in liver tissue in adult skinks and no associations between mitochondrial respiration and

growth. Mitochondrial respiration can vary widely across tissues because of tissue-specific metabolic needs (Salin *et al.* 2016), across life-history stages to meet variable energy demands that accompany life stage transitions (Hood *et al.* 2018; Koch *et al.* 2021; Hood 2024), and as animals age (Dai & Rabinovitch 2009; Ehinger *et al.* 2024). It is possible that our developmental treatments affected tissues other than the liver or affected liver mitochondrial function early in life, but these effects were no longer present when we measured mitochondrial function in adult skinks. Alternatively, incubation temperature and prenatal corticosterone treatment may not affect mitochondrial function in the liver directly (as suggested by our data) but have indirect effects that are mediated through developmentally induced changes in pathways that regulate mitochondrial function, such as the HPA axis (Crino *et al.* 2022). We measured mitochondrial respiration from isolated mitochondria to test if developmental conditions affected metabolic phenotype through changes intrinsic to mitochondrial function (e.g., altered activity of electron transport chain complexes or inner membraine permeability; Divakaruni & Jastroch 2023). In contrast, measuring mitochondrial function from intact or permeabilized cells preserves interactions that mitochondria share with other organelles and captures changes in function that may be influenced by multiple organismal processes (Koch *et al.* 2021; Divakaruni & Jastroch 2023). We found no associations between baseline corticosterone levels and respiration of isolated mitochondria. However, mitochondria interact with corticosterone through glucocorticoid receptors (GRs; Kokkinopoulou & Moutsatsou 2021) that have a low affinity for glucocorticoids (in contrast to high-affinity mineralcorticoid receptors (MRs); Lattin *et al.* 2012). Thus, GRs are thought to generally regulate physiological processes in response to elevated or stress-induced levels of glucocorticocids while MRs are activated by baseline levels of glucocorticoids (Deviche *et al.* 2017). Our data suggest that baseline corticosterone levels do not have direct regulatory effects on the electron transport chain in delicate skinks

535 but do not address potential glucocorticoid-mediated changes in mitochondrial function
536 regulated at the cellular level.

537 Males had higher mitochondrial oxygen consumption than females for basal,
538 OXPHOS, and leak respiration. Oxygen consumed during OXPHOS respiration is used to
539 drive the phosphorylation of ADP to ATP while leak respiration is a measure of oxygen
540 consumption used to offset proton loss across the inner mitochondrial membrane and is
541 reflective of energy loss (Brand & Nicholls 2011). Our result suggest that males have a
542 greater ability to produce ATP but also require more energy to offset proton leak than
543 females. However, we found no difference between RCR values between males and females
544 indicating no difference between capacity for energy production relative to energy loss,
545 suggesting that males have overall higher mitochondrial function compared to females
546 despite their smaller body size and slower growth rate. An enhanced ability of mitochondria
547 to produce ATP may account for variation in metabolically costly processes and traits other
548 than growth such as thermal tolerance, reproduction, and sexual displays and ornaments (Hill
549 2014; Koch & Hill 2018; Chung & Schulte 2020).

550

551 **5 CONCLUSIONS**

552 Understanding the short-term and sustained effects of developmental conditions on
553 growth, body condition, and survival is essential for understanding how developmental
554 effects drive population-level responses. However, the interaction between physiological
555 systems and environmental conditions likely entails physiological tradeoffs that constrain
556 phenotypic expression and ultimately affect life history strategies. For this reason, it is critical
557 to understand how developmental conditions interact during sensitive periods like prenatal
558 development. Oviparous animals could be affected by elevated temperatures associated with

559 global climate change through direct effects on incubation temperature and maternal effects
560 such as increased exposure to glucocorticoids. Studies that track the physiological changes to
561 elevated temperatures and glucocorticoids during development across lifespan will provide a
562 more holistic understanding of the multigenerational consequences of elevated temperatures
563 associated with global climate change.

564

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570

571 **COMPETING INTERESTS**

572 The authors declare they have no competing interests.

573

574 **AUTHOR CONTRIBUTIONS**

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

579

580 **DATA ACCESSIBILITY**

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

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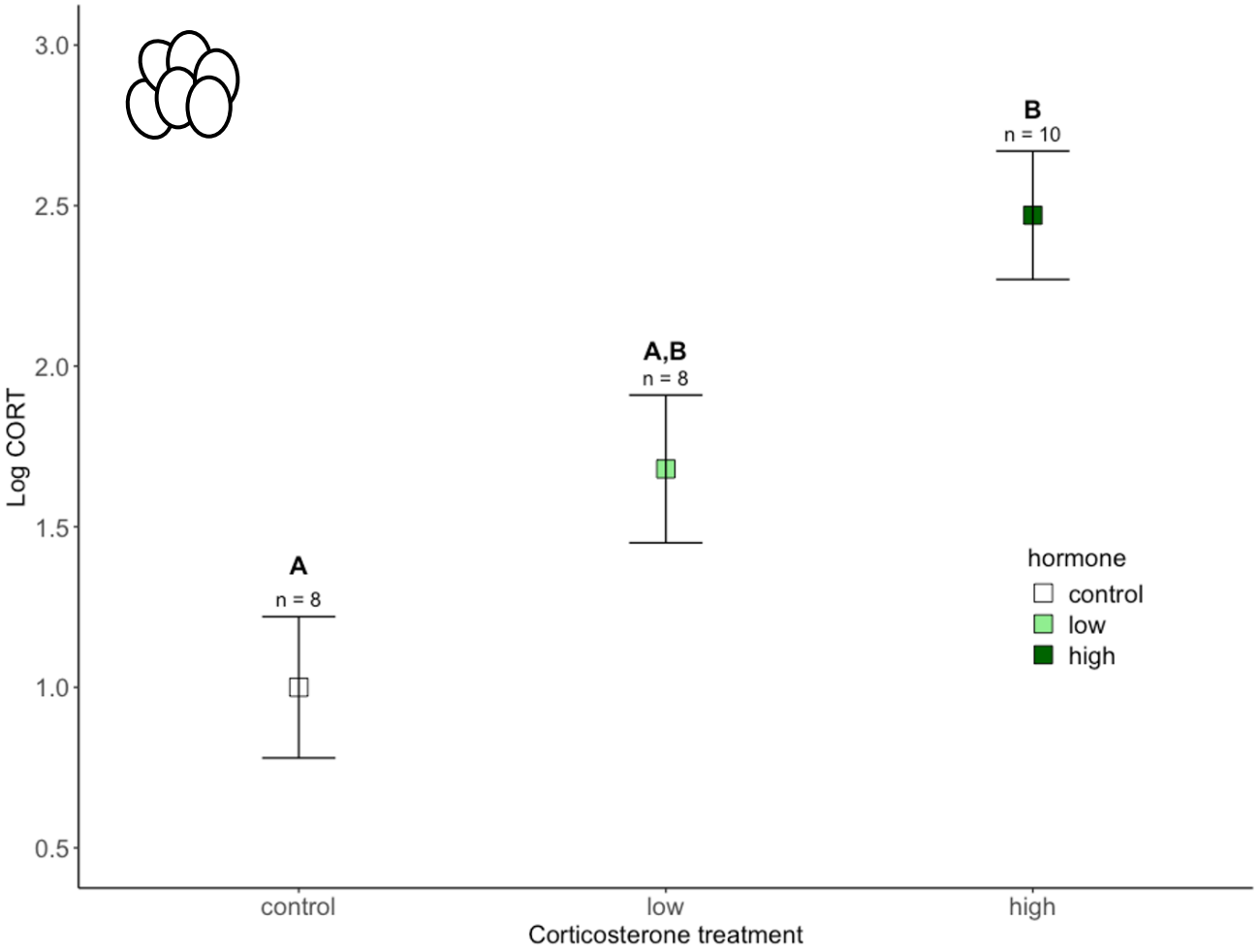

854 **Figure 1.** Log-transformed yolk corticosterone levels following treatment with control, low
855 dose corticosterone, and high dose corticosterone treatments. Data are from model results
856 from emmean model, with mean and SE. Significant differences ($p < 0.05$) from post hoc
857 tests are indicated by different letters and sample sizes (n) for each treatment are indicated
858 above.

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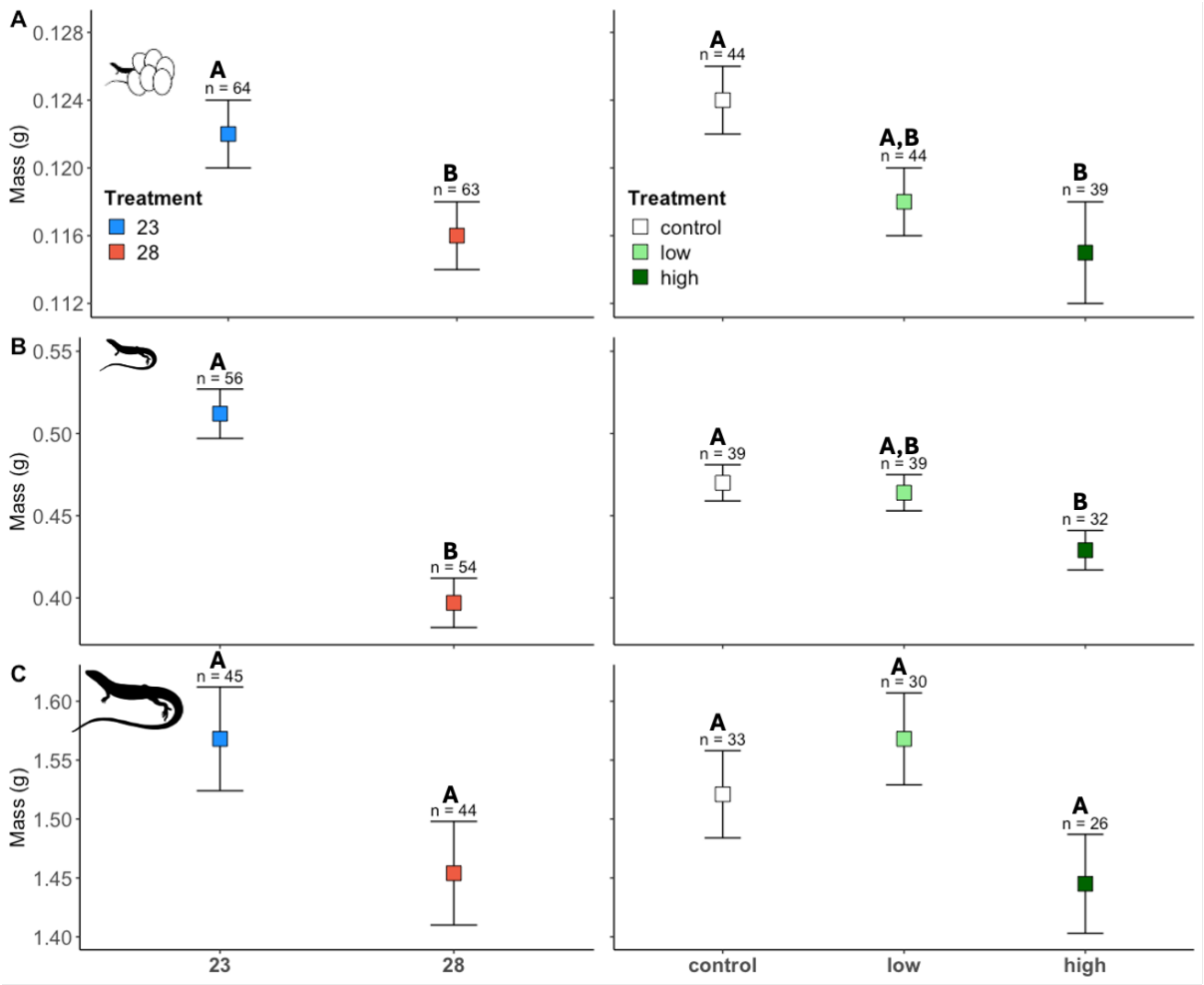
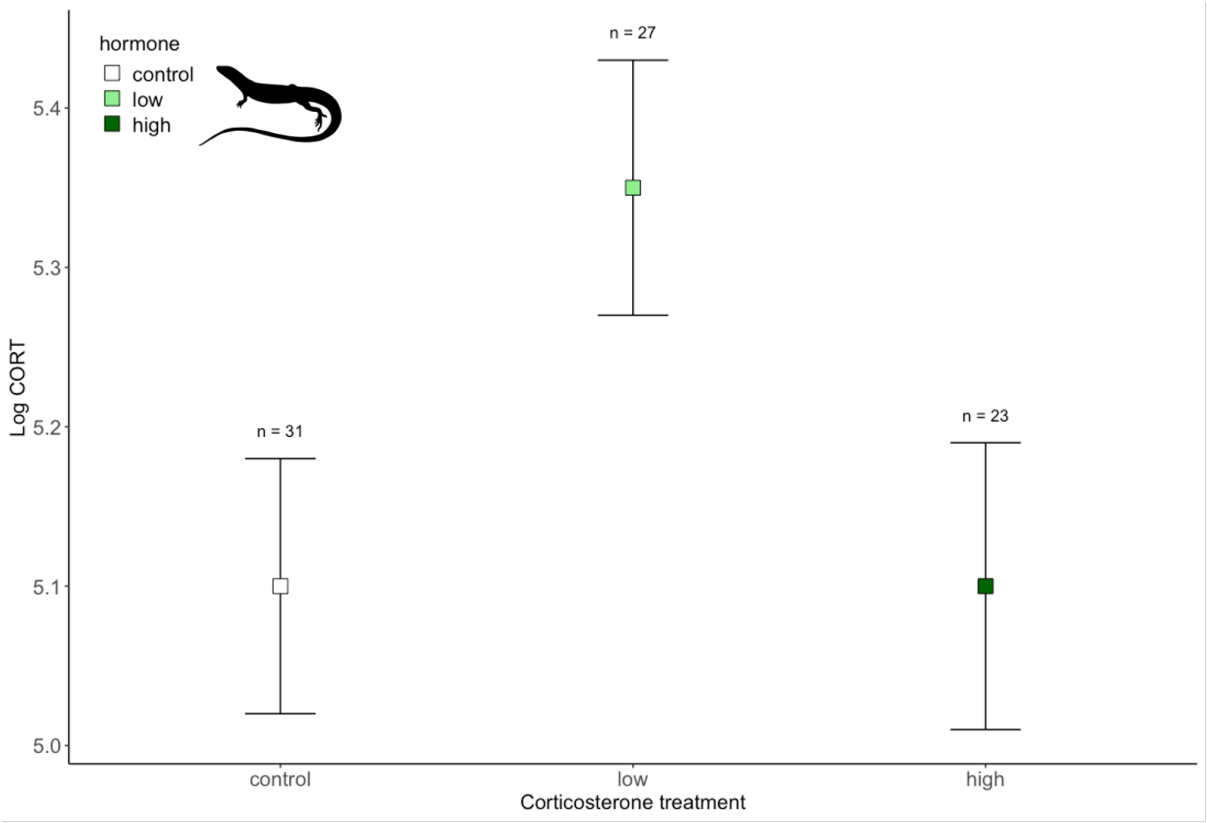


Figure 2. Body mass at hatching (A), the juvenile period (B), and adulthood (C) in lizards exposed to incubation treatments (left panels) and prenatal corticosterone treatments (right panels). Significant differences ($p < 0.05$) from main effects of incubation temperature and post hoc tests for differences between corticosterone treatments are indicated by different letters and sample sizes (n) for each treatment are indicated above.



871

872 **Figure 3.** Corticosterone treatments during development affected baseline corticosterone
873 levels in adults. Data are from model results from emmean model, with mean and SE. Sample
874 sizes (n) for each treatment are indicated above.

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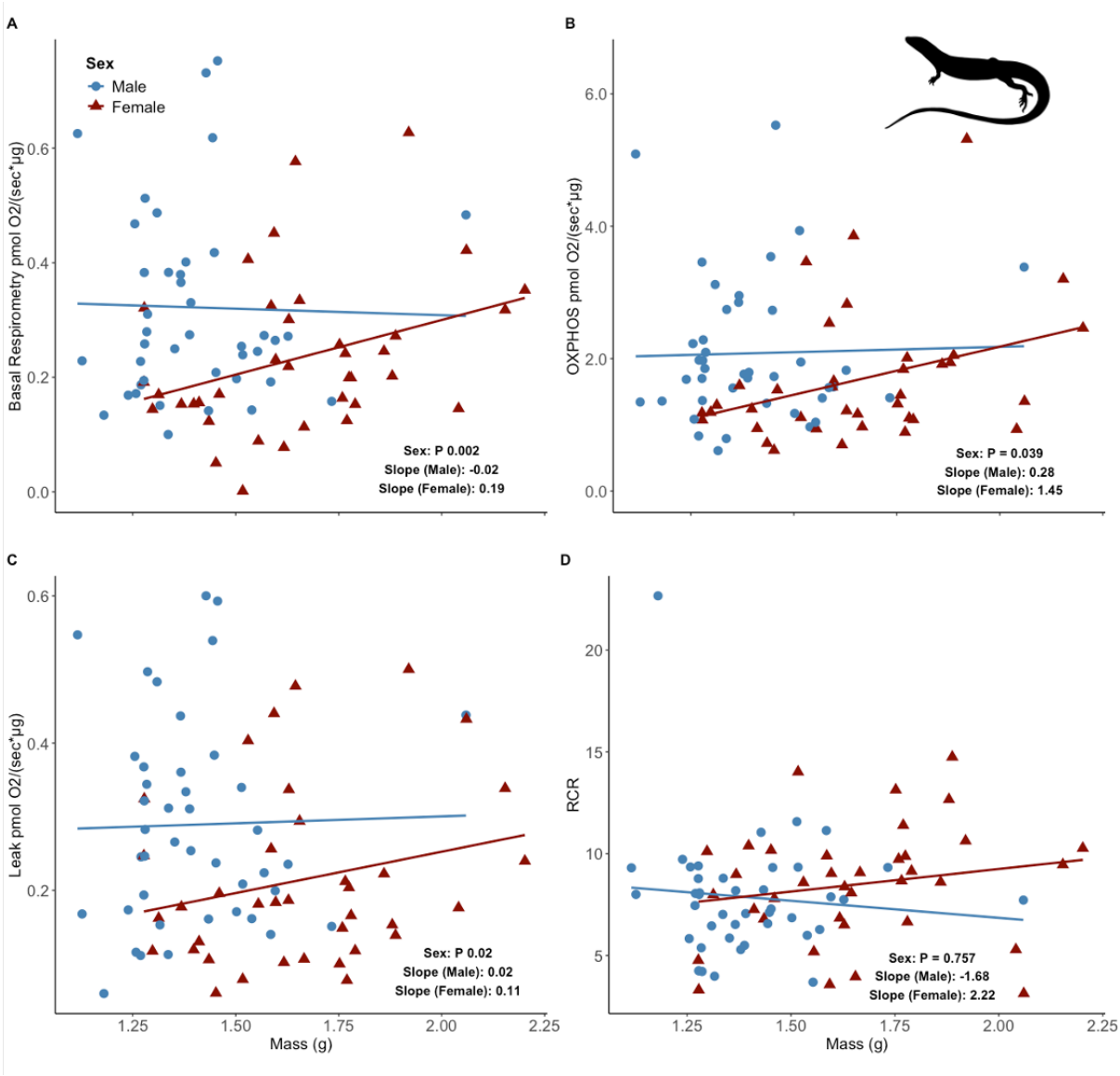
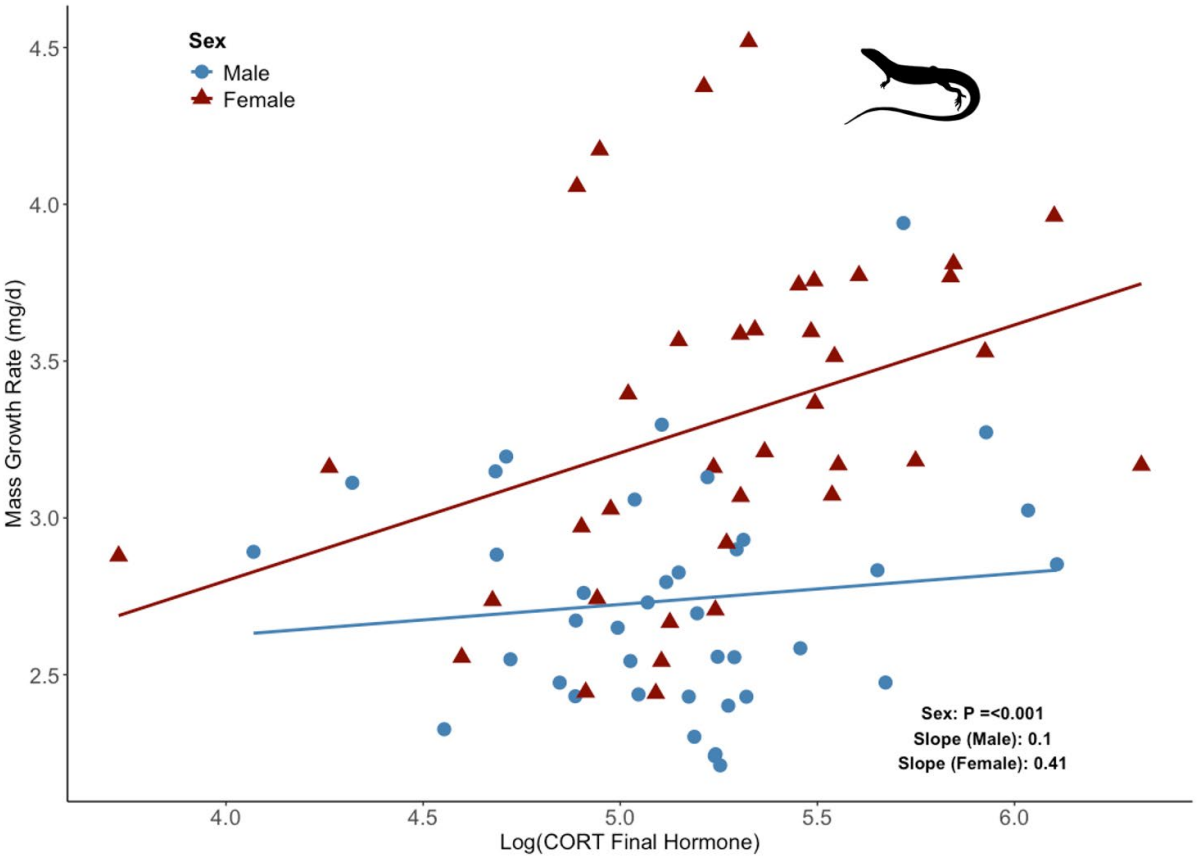


Figure 4. Associations between adult body mas (g) and mitochondrial respiration parameters including basal respiration (A), OXPHOS respiration (B), leak respiration (C), and RCR (D). Colors indicate sex and P value in the top right corner indicates sex differences between treatments.



885

886 **Figure 5.** Growth rate (mg/d) in relation to baseline corticosterone. Growth rates were
887 calculated by dividing the change in mass between hatching and adulthood by adult age (days
888 post-hatching). Colors indicate sex and P value in the top right corner indicates sex
889 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. 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.

To test the effects of developmental treatments on body size and condition we used SVL, body mass, or body condition as the dependent variables 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 used GLMs to test the effects of developmental treatments on adult corticosterone and thyroxine levels. We conducted preliminary GLMs 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 ($p = 0.55$, $F_{1,75} = 0.36$; $p=0.41$, $F_{1,71} = 0.98$, respectively), but did positively affect testosterone levels ($p = 0.03$, $F_{1,40} = 5.13$). Plate ID affected corticosterone levels ($p = 0.007$, $F_{4,75} = 3.83$) but not thyroxine or testosterone levels ($p = 0.41$, $F_{3,71} = 0.98$; $p = 0.34$, $F_{1,40} = 0.94$). Significant factors were included in models to test for treatment effects on hormone levels. 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. 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 GLMs 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 tested associations between mitochondrial bioenergetics and endogenous

corticosterone, thyroxine, and testosterone (males only) levels using GLMs 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 GLMs 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 GLMs. 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 GLMs for each mitochondrial respiration state because basal, OXPHOS, and leak respiration were highly correlated ($p < 0.001$, $r > 0.81$ for all).

Supplemental Table S1. 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	sumsq	df	statistic	p.value
Basal	temp	0.002	1	0.100	0.752
	hormone	0.088	2	1.917	0.154
	scale(Adult_Age)	0.017	1	0.746	0.390
	sex	**0.363**	**1**	**15.775**	**0.000**
	adult_mass_g	**0.114**	**1**	**4.937**	**0.029**
	oroboros	0.012	1	0.523	0.472
	oroboros:chamber	0.006	1	0.271	0.604
	Residuals	1.726	75		
OXPHOS	temp	1.768	1	1.391	0.242
	hormone	5.238	2	2.061	0.135
	sex	**9.932**	**1**	**7.815**	**0.007**
	scale(Adult_Age)	0.278	1	0.219	0.641
	adult_mass_g	**5.774**	**1**	**4.543**	**0.036**
	oroboros	0.000	1	0.000	0.995
	oroboros:chamber	0.162	1	0.128	0.722
	Residuals	95.319	75		
Leak	temp	0.011	1	0.643	0.425
	hormone	0.044	2	1.307	0.277
	sex	**0.214**	**1**	**12.702**	**0.001**
	scale(Adult_Age)	0.019	1	1.123	0.293
	adult_mass_g	0.064	1	3.817	0.054
	oroboros	0.002	1	0.138	0.711
	oroboros:chamber	0.003	1	0.203	0.654
	Residuals	1.263	75		
RCR	temp	26.721	1	3.140	0.080
	hormone	14.284	2	0.839	0.436

Test	term	sumsq	df	statistic	p.value
	sex	6.217	1	0.731	0.395
	scale(Adult_Age)	26.230	1	3.082	0.083
	adult_mass_g	2.923	1	0.343	0.560
	oroboros	0.315	1	0.037	0.848
	oroboros:chamber	5.958	1	0.700	0.405
	Residuals	638.264	75		

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Supplemental Table S2. 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. Finally, respirometer and respirometer chamber were accounted for in the model. Significant terms ($p<0.05$) are highlighted in bold.

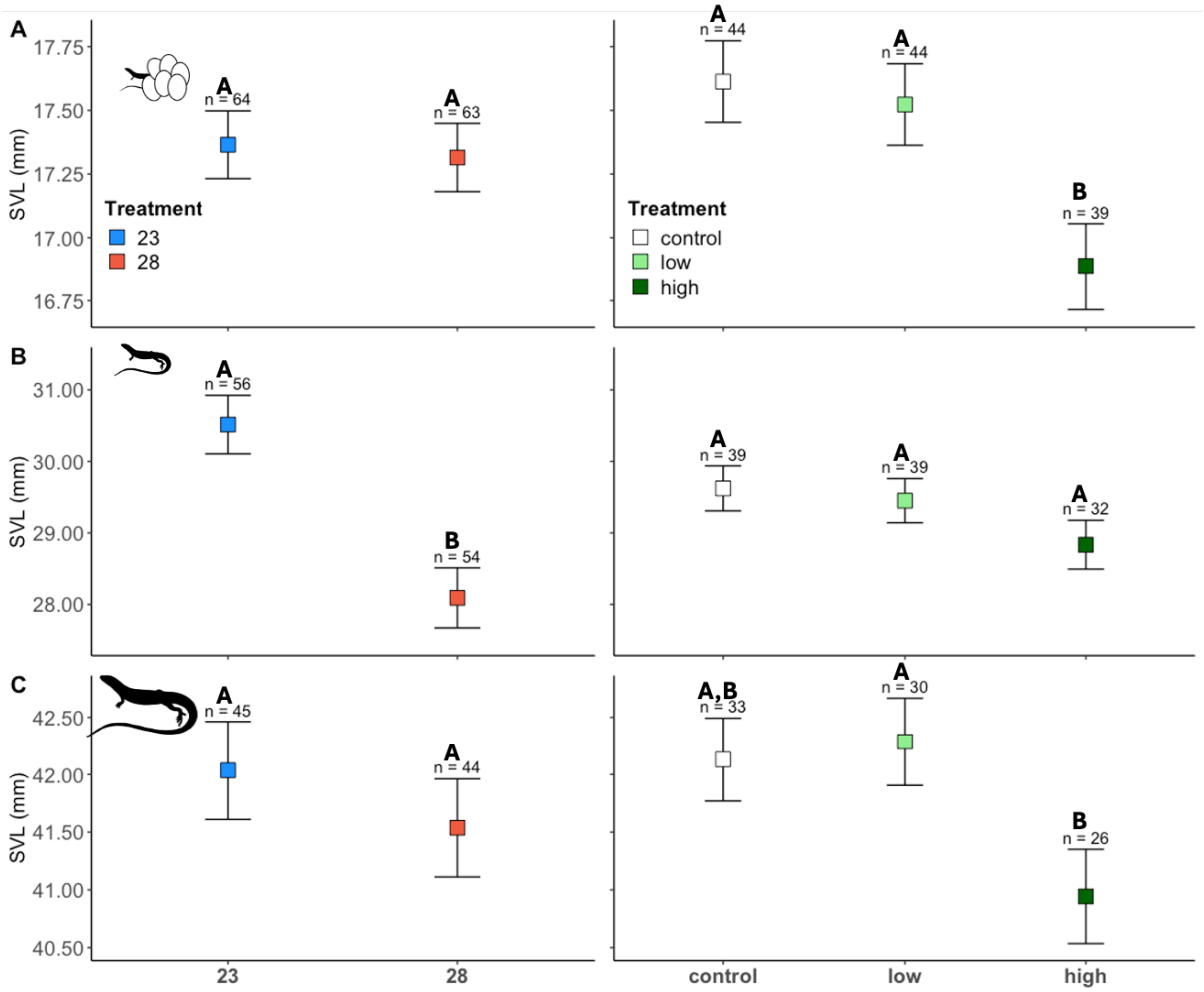
Test	term	sumsq	df	statistic	p.value
Basal	log(CORT_ng_mL)	0.003	1	0.198	0.658
	log(T4_ng_mL)	0.002	1	0.188	0.666
	sex	**0.137**	**1**	**10.790**	**0.002**
	oroboros	0.006	1	0.451	0.504
	oroboros:chamber	0.000	1	0.003	0.957
	Residuals	0.873	69		
OXPHOS	log(CORT_ng_mL)	0.211	1	0.312	0.578
	log(T4_ng_mL)	0.346	1	0.512	0.477
	sex	**6.434**	**1**	**9.525**	**0.003**
	oroboros	0.263	1	0.389	0.535
	oroboros:chamber	0.023	1	0.034	0.853
	Residuals	46.613	69		
Leak	log(CORT_ng_mL)	0.003	1	0.281	0.598
	log(T4_ng_mL)	0.000	1	0.010	0.921
	sex	**0.096**	**1**	**10.720**	**0.002**
	oroboros	0.000	1	0.019	0.891
	oroboros:chamber	0.000	1	0.009	0.925
	Residuals	0.618	69		
RCR	log(CORT_ng_mL)	10.120	1	1.184	0.280
	log(T4_ng_mL)	19.785	1	2.315	0.133
	sex	0.828	1	0.097	0.757
	oroboros	5.148	1	0.602	0.440
	oroboros:chamber	0.354	1	0.041	0.839
	Residuals	589.690	69		

Supplemental Table S3. 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	sumsq	df	statistic	p.value
	basal_corrected_pmol	0	1	0.612	0.437
	sex	**0**	**1**	**20.292**	**0.000**
Growth in mass (mg/day)	**log(CORT_ng_mL)**	**0**	**1**	**5.025**	**0.028**
	log(T4_ng_mL)	0	1	0.187	0.667
	Residuals	0	67		
	adp_corrected_pmol	0	1	1.615	0.208
	sex	**0**	**1**	**21.928**	**0.000**
Growth in mass (mg/day)	**log(CORT_ng_mL)**	**0**	**1**	**4.602**	**0.036**
	log(T4_ng_mL)	0	1	0.085	0.771
	Residuals	0	67		
	leak_corrected_pmol	0	1	0.447	0.506
	log(CORT_ng_mL)	**0**	**1**	**5.583**	**0.021**
Growth in mass (mg/day)	log(T4_ng_mL)	0	1	0.163	0.688
	sex	**0**	**1**	**19.972**	**0.000**
	Residuals	0	67		
	RCR	0	1	0.194	0.661
	sex	**0**	**1**	**19.765**	**0.000**
Growth in mass (mg/day)	**log(CORT_ng_mL)**	**0**	**1**	**5.249**	**0.025**
	log(T4_ng_mL)	0	1	0.101	0.751
	Residuals	0	67		

Supplemental Table S4. 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	sumsq	df	statistic	p.value
Growth in SVL (mm/day)	basal_corrected_pmol	0.000	1	0.612	0.437
	sex	**0.000**	**1**	**20.292**	**0.000**
	log(CORT_ng_mL)	**0.000**	**1**	**5.025**	**0.028**
	log(T4_ng_mL)	0.000	1	0.187	0.667
	Residuals	0.000	67		
Growth in SVL (mm/day)	adp_corrected_pmol	0.000	1	2.149	0.147
	log(CORT_ng_mL)	0.000	1	2.011	0.161
	log(T4_ng_mL)	0.000	1	0.030	0.864
	sex	**0.000**	**1**	**7.455**	**0.008**
	Residuals	0.002	67		
Growth in SVL (mm/day)	leak_corrected_pmol	0.000	1	0.482	0.490
	sex	**0.000**	**1**	**6.265**	**0.015**
	log(CORT_ng_mL)	0.000	1	2.750	0.102
	log(T4_ng_mL)	0.000	1	0.002	0.968
	Residuals	0.002	67		
Growth in SVL (mm/day)	RCR	0.000	1	0.800	0.374
	sex	**0.000**	**1**	**5.682**	**0.020**
	log(CORT_ng_mL)	0.000	1	2.363	0.129
	log(T4_ng_mL)	0.000	1	0.040	0.843
	Residuals	0.002	67		



Supplemental Figure S1. 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.